The Stress Radionuclide Assessment of Diastolic Function

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A Thesis submitted for the Degree of Doctor of Medicine

To

The University of Glasgow

2009

Abstract

Background

Many patients are referred from primary care with suspected heart failure and are found to have preserved systolic function. These patients may be labelled as having normal ejection fraction or diastolic heart failure, the diagnosis of which is both controversial and difficult. Previous work has identified a large proportion of these patients to have an alternative, pre-existing diagnosis. This thesis prospectively assesses the prevalence of undiagnosed ischaemic heart disease and respiratory disease in this patient group and assess diastolic function using multiple methods. The central hypothesis being tested was that first third fractional filling, a radionuclide ventriculogram (RNVG) parameter previously used to assess diastolic function at rest, would identify diastolic dysfunction more accurately under stress conditions.

Methods

Patients were recruited from an open access echocardiography service. Echocardiography, including tissue Doppler assessment, was carried out independently by 2 experienced observers. Confounding diagnoses including coronary artery disease and respiratory disease were actively sought by myocardial perfusion imaging and spirometry. N-terminal proBNP was measured. List mode radionuclide ventriculography was performed at rest supine and during upright bicycle exercise with simultaneous measurement of VO_2 max.

Validation of the reliability and reproducibility of first third fractional filling, peak filling rate, time to peak filling and other radionuclide parameters of systolic and diastolic function was undertaken. This demonstrated that it was possible to measure both first third fractional filling and peak filling rate with the short acquisition times necessary for assessment during stress. Time to peak filling was poorly reproducible under these conditions.

A normal range for first third fractional filling at rest and during exercise was established. Due to a strong inverse relationship between heart rate and first third fractional filling, a continuous reference range was constructed using an exponential model. This unique approach enables the calculation of the lower limit of normal at any heart rate. A more conventional mean ± 2 standard deviations was used for the other RNVG parameters.

Results

Eighty three patients were recruited and completed an extensive multi-modality assessment of systolic and diastolic function. As with previous work in this field, the patients were predominantly female (82%) and elderly (mean age 66.7). Mild left ventricular systolic dysfunction as determined by RNVG was missed by echocardiography in one third of patients. Systolic dysfunction more significant than this was not observed. N-terminal proBNP was elevated in 21 of 82 patients where it was available with no significant difference in left ventricular ejection fraction between those with normal and elevated levels. Myocardial perfusion scanning was normal in 46 of 83 patients and showed significant ischaemia in 20 of 83. Spirometry was normal in 58 of 82 patients, with mild airflow obstruction in 20 patients and moderate obstruction in 4. In only one patient were no alternative diagnoses present.

There was poor correlation between indices of diastolic function at rest including first third fractional filling, echocardiographic parameters and NT-proBNP. The assessment of diastolic function using stress radionuclide ventriculography did not improve the correlation between measured indices. On stress, however, low first third fractional filling predicted exercise intolerance as an inability to reach anaerobic threshold.

Conclusions

Alternative diagnoses to diastolic dysfunction are present almost universally in patients with suspected normal ejection fraction heart failure. This is true even where these diagnoses are not previously established. This thesis underlines the need to fully assess this patient group to allow appropriate targeting of therapy. It is also clear that echocardiography alone is potentially misleading and it is suggested that it is better placed within a tiered assessment process.

The assessment of diastolic function using stress radionuclide ventriculography, although an appealing concept, does not improve diagnostic accuracy within this patient group. The marked heterogeneity of this patient group is likely to have played a role in this and it may be of interest to reassess stress radionuclide ventriculography in a more acute heart failure population.

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Acknowledgements

I am indebted to the following people for their invaluable support in the preparation of this thesis:

My supervisor Professor Stuart Cobbe, Professor of Cardiology, Glasgow Royal Infirmary, for his help and encouragement from inception to conclusion of this thesis and for the time he gave reading this manuscript whilst reducing his commitments elsewhere.

My co-supervisor Dr William Martin, Principle Physicist, Department of Nuclear Cardiology, Glasgow Royal Infirmary whose initial concept this thesis developed from and without whose help and departmental resources, this thesis would not have been possible.

Dr Nicholas Goodfield, Consultant Cardiologist, Stobhill Hospital, Glasgow, for his support and insightful comments at various stages and for providing an additional source of patient recruitment.

Mrs Adele Rice, Mr Mark McDade and Gayle MacIntosh, Nuclear Technologists, Department of Nuclear Cardiology, Glasgow Royal Infirmary, for their hard work in performing all the nuclear scans.

Dr Roger Carter for his assistance in pulmonary function testing and VO_2 analysis.

Mr John Jarvie, Chief Cardiac Physiologist, for his hard work in providing second observer echocardiograms.

Dr Roy Gardner, Consultant Cardiologist, Glasgow Royal Infirmary and Dr Theresa M^{c}Donagh, Consultant Cardiologist, Royal Brompton Hospital, London for providing the NT-proBNP sample analysis. Dr Ian Morton for performing those analyses.

Publications Arising From This Work

Data from this thesis has been presented in the form of 4 abstracts. There have been no full peer-reviewed papers arising from this work to date.

"Undiagnosed respiratory and ischaemic heart disease is common in open access echo patients with suspected heart failure and preserved systolic function"
A C M^{c}Culloch, W Martin, S M Cobbe
Scottish Society of Physicians, Aberdeen, October 2004

"Systolic and diastolic parameters of ventricular function: a comparison of list mode and multiple gated acquisition"
A C M^{c}Culloch, A Small, W Martin, S M Cobbe
British Nuclear Cardiology Society, London, December 2004

"The Effect of Ischaemia on Diastolic Function in Suspected Heart Failure With Preserved Systolic Function"
A C M^{c}Culloch, W Martin, S M Cobbe
British Nuclear Medicine Society, Manchester, March 2005

"Generation of a Heart Rate Dependant Normal Range for First Third Fractional Filling"
A C McCulloch, W Martin, N E R Goodfield, S M Cobbe
ICNC 9, Barcelona, May 2009

Author's Declaration

Study design, patient recruitment and follow-up, data collection, analysis of data and preparation of the manuscript were performed by the author.

The analysis of radionuclide ventriculography images was performed by the author and Mrs Adele Rice. Quantitative analysis of thallium perfusion images was performed by Dr William Martin. NT-proBNP analysis was performed by Dr Ian Morton.

No work referred to in this thesis has been submitted in support of an application for another degree or qualification in this or any other university.

Signed

Definitions

ACE – Angiotensin Converting Enzyme
BMI – Body Mass Index
BNP – B-type Natriuretic Peptide
COPD – Chronic Obstructive Pulmonary Disease
DT – Deceleration Time
ECG – Electrocardiogram
EDC – End Diastolic Counts
EF – Ejection Fraction
ESC – End Systolic Counts
FTFF – First Third Fractional Filling
IVRT – Isovolumetric Relaxation Time
IVC – Inferior Vena Cava
IVS – Interventricular Septum
LA – Left Atrium
LAO – Left Anterior Oblique
LAVI – Left Atrial Volume Index
LV – Left Ventricle
LVH – Left Ventricular Hypertrophy
MI – Myocardial Infarction
MUGA – Multiple Gated Acquisition
NT-proBNP – N-terminal proBNP
NYHA – New York Heart Association
PCWP – Pulmonary Capillary Wedge Pressure
PER – Peak Emptying Rate
PFR – Peak Filling Rate
RLCB – Rate Limiting Calcium Channel Blocker
RNVG – Radionuclide Ventriculogram
ROI – Region of Interest
RV – Right Ventricle

1. Introduction

1.1 Diastolic Dysfunction

Diastolic dysfunction is an area of cardiology that has become of increasing interest over the last two decades. It has progressed from a subject confined to academic research studies to a disease entity in its own right to which a substantial disease burden has been attributed, with drug trials and endpoint data beginning to emerge, enabling rational treatment strategies to be devised. It is an area which has created much controversy, from the technicalities of how to define it and best measure it, to whether diastolic dysfunction is a genuine clinical entity in its own right, or whether it is something which is dependent on the presence of other structural heart disease and should not be diagnosed in the absence of this.

Part of this debate is fuelled by the limitations inherent in the methods used to assess diastolic function, particularly non-invasively. It has been argued that since many of the parameters in use are non-specific, being affected by factors other than those inherent to the left ventricle, and are more commonly found with increasing age, what is often being measured is in fact part of the normal aging process. Where this normal aging process ends and pathology begins has been difficult to define, particularly because many of the "abnormalities" which can be measured are not infrequently found where there is no clinical evidence of heart failure. Similarly, measurements can be normal or ambiguous even in the presence of what is thought to be heart failure.

Another fundamental problem when dealing with diastolic dysfunction is one of definitions and terminology. There are two main terms in use to describe the syndrome, these being 'diastolic dysfunction' and 'heart failure with preserved systolic function'. Although the terms are superficially similar and often used synonymously, in fact they imply very different things. The importance of this distinction will be explored further.

1.1.1 The Concept of Diastolic Dysfunction

With the development of echocardiography and angiocardiography, both radionuclide and x-ray contrast, allowing visualisation of the failing heart and quantification of systolic function, a subset of patients emerged who did not have systolic impairment, valvular

dysfunction or pulmonary hypertension/cor pulmonale, yet had been regarded as having heart failure. Although alternative non-cardiac diagnoses will account for a proportion of these, a subset of patients remain in whom the clinical syndrome of heart failure exists but systolic and valvular function are preserved. Attention therefore turned to the part of the cardiac cycle which had received relatively little attention, namely diastole.

The concept underlying diastolic dysfunction is that due to an abnormality either of structure or function of the left ventricle there is impairment of left ventricular filling. This reduction in filling will lead to a decrease in stroke volume and cardiac output unless left ventricular filling pressures are increased to compensate. This increase in left atrial and intra-pulmonary pressures, as measured by pulmonary capillary wedge pressure, mirrors the process which follows a rise in left ventricular diastolic pressures due to systolic dysfunction with all its consequences in terms of clinical signs and symptoms. In effect two separate but related pathologies come together to form a single common pathway responsible for the clinical syndrome seen.

Although the concept itself is attractive with a clear flow of logic from a putative structural or functional problem to its sequelae and the resulting clinical syndrome, the process of demonstrating the underlying problem or its haemodynamic consequences reliably has been far from easy.

1.1.2 Diastolic Dysfunction versus Heart Failure with a Normal Ejection Fraction

Defining what is mean by diastolic heart failure is fairly fundamental, but surprisingly something which is confused by two different terms which, although used interchangeably, differ fundamentally in terms of what pathology is covered by the definition and where this pathology is located.

Heart failure with preserved systolic function is a looser term which lacks precision and specificity, although it is simpler to apply in routine clinical practice, indeed being used in a major clinical trial, CHARM-Preserved(1). The term refers to the clinical syndrome of heart failure where left ventricular systolic function has been assessed as preserved, i.e. at a sufficient level where it should not be expected to account for heart failure. However, this does not automatically mean that there is an abnormality of diastolic function to account

for the clinical findings. The syndrome encompassed by this term can very likely include signs and symptoms which, although clinically taken for heart failure, are not cardiac in origin at all. If a clinical syndrome is to be attributed to cardiac causes, it is surely imperative that an abnormality of cardiac structure or function can be demonstrated. A patient with dyspnoea and ankle oedema could easily be interpreted as having heart failure but, as these are non-specific and, in the case of dyspnoea, subjective findings, to make such a decision without actually demonstrating abnormal cardiac function intuitively seems wrong.

More recently, terminology has evolved further. Heart failure with a normal ejection fraction (HFNEF) has become the term of choice for this condition(2). This takes into account the observation that abnormalities of left ventricular longitudinal systolic function may be demonstrable before the ejection fraction becomes abnormal(3, 4).

Diastolic dysfunction on the other hand, specifically implies that there is a measurable abnormality in diastolic function which is responsible for the signs and symptoms observed. If one is to be more specific still, it implies that the abnormality is intrinsic to the ventricle rather than a reflection of some factor external to the ventricle. It is here that much of the problem in diagnosing diastolic heart failure arises – the difficulty in differentiating intrinsic and extrinsic factors using measurements which can be affected by either or both simultaneously.

Whilst such a distinction may appear to be unnecessary pedantry, it is of major significance both in routine clinical practice where medication must be prescribed appropriately for cardiac dysfunction, rather than inappropriately for non-cardiac disorders, and in the context of clinical trials. Where the entry criteria for a trial are sufficiently loose to allow inclusion of patients with non-cardiac disease, the magnitude of any treatment effect from the drug under study is at risk of being diluted. This can be further compounded if the drug in question has pleiotropic effects. Such a potential limitation can be seen in CHARM-Preserved(1) where diastolic function is not itself measured, instead the distinction is made on the basis of impaired or ‘preserved’ systolic function with an ejection fraction cut point of 40%.

The necessity of measuring diastolic function has itself been questioned. Zile et al put forth this point of view in 2001(5). In a group of 63 patients who met Framingham criteria for heart failure but with normal left ventricular systolic function on a

contemporaneous echocardiogram, 58 had elevated left ventricular end diastolic pressure at cardiac catheterisation. Using a combination of E/A ratio, isovolumetric relaxation time and mitral deceleration time, all patients could be demonstrated to have abnormal diastolic function by at least one of these criteria. However, these measures in isolation performed poorly – the best correlation was with deceleration time which was abnormal in 64%. E/A ratio performed less well at 48% abnormal, even although the criterion for abnormality was set at <1.0, with only 38% having diastolic dysfunction by IVRT criteria. One critical factor with this group of patients is that they were required to have left ventricular hypertrophy, a condition one would expect to result in impaired diastolic function. In a less rigorously defined group, such as is seen in routine practice, the percentage of patients with diastolic dysfunction validated on invasive study could be expected to be significantly less. Perhaps the argument here should not be that we should not measure diastolic function at all, but rather we should endeavour to use methods other than standard transmitral Doppler which has been repeatedly shown to be unreliable(6).

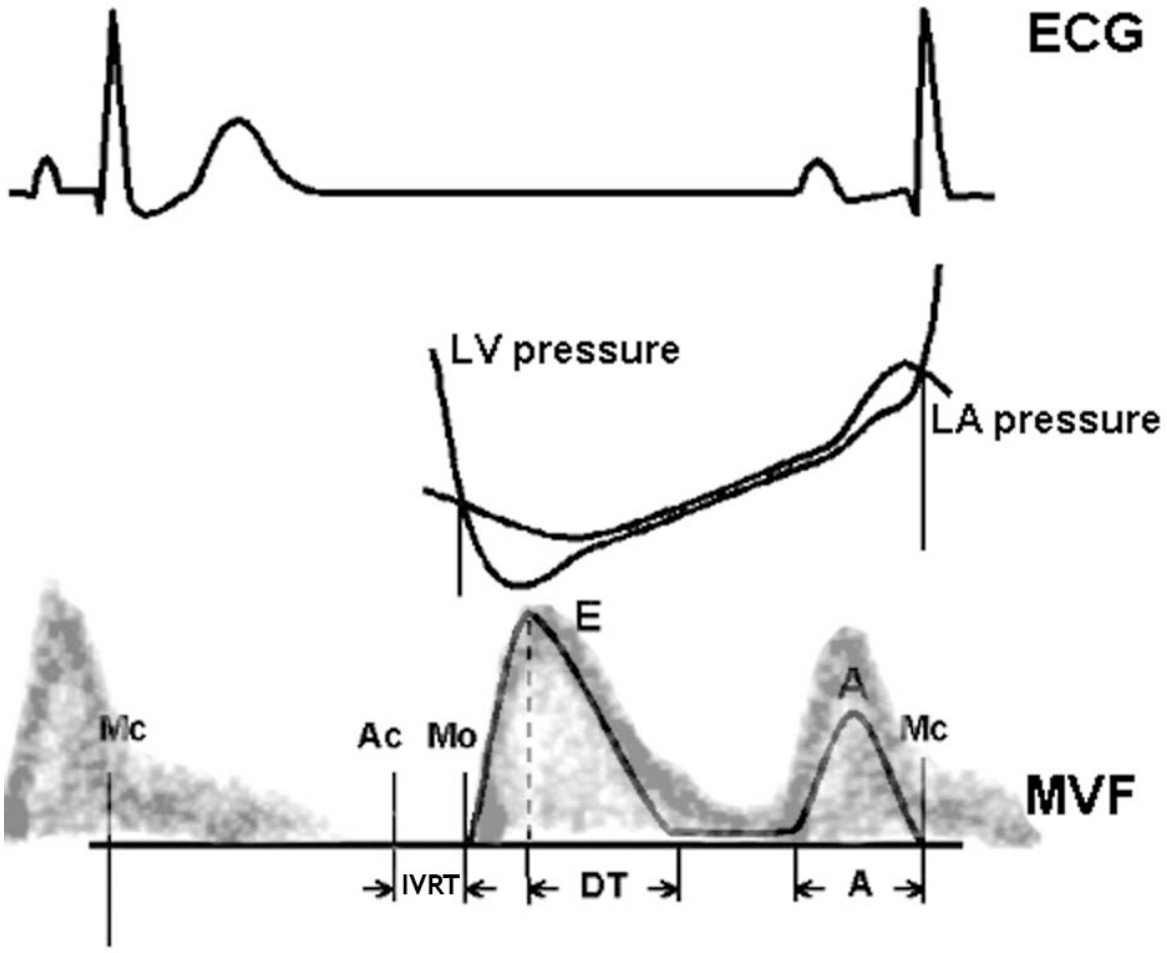
1.2 Defining Diastole - The Components of Diastolic Function

To understand what processes and pathologies can lead to diastolic dysfunction it is important to first understand the complex processes which combine to produce diastole.

When regarded from a flow mechanics viewpoint, diastole begins with a phase of isovolumetric relaxation following the closure of the aortic valve as left ventricular pressure falls below aortic pressure. At this point the mitral valve is also closed. As left ventricular pressure continues to fall, it decreases below left atrial pressure allowing the mitral valve to open, marking the end of isovolumetric relaxation and the beginning of passive ventricular filling. As ventricular filling progresses, left ventricular pressure will begin to equilibrate with left atrial pressure with a period of diastasis following if the pressures in the two chambers equalise. With the onset of atrial systole, left atrial pressure again exceeds left ventricular pressure and a second, active wave of filling begins. The duration of this active phase of filling is determined less by left atrial pressure than its primary determinant, the atrio-ventricular delay. Once the wave of depolarisation exits the atrioventricular node and depolarises the ventricles, the far greater bulk of the ventricle almost immediately overcomes left atrial pressure and the mitral valves closes, ending

diastole. These processes are perhaps best understood when visualised with Doppler echocardiography (Figure 1-1).

Figure 1-1 – Left ventricular and atrial pressure traces in relation to mitral inflow and surface ECG



Mc = Mitral valve closure, Mo = Mitral valve opening, Ac = Aortic valve closure, IVRT = isovolumetric relaxation time, DT = deceleration time, MVF = mitral valve flow

From a clinical perspective, diastolic function can be broken down into a series of 4 processes which combine what is happening at a cellular level as well as a mechanical level. These processes are closely interrelated and an abnormality in one will have consequences further down the chain.

1.2.1 Relaxation

For diastole to commence, the active contraction of systole must cease and the changes in the ventricle brought about by this must be reversed; the ventricle must begin to relax. It

would be easy to regard diastole as a primarily passive process given that much of the driving force comes from the pressure gradient between the left atrium and left ventricle that exists at the end of systole as a result of passive filling of the left atrium by pulmonary venous return. This simplified view of the process neglects key aspects of the process that are far from passive.

At a cellular level, systole ends with the hydrolysis of ATP and unlinking of the actin-myosin cross bridges responsible for systolic contraction. In order for this to occur, the high intracellular level of calcium necessary for excitation-contraction coupling must be lowered by active reuptake of calcium into the sarcoplasmic reticulum. This energy dependent process fuelled by ATP underlines the fact that diastole is both an active and passive process.

1.2.2 Suction

As the ventricle relaxes, the elastic recoil exhibited by the myocardium in early diastole results in a suction effect. This actively draws blood into the ventricular cavity by enhancing the pressure gradient that exists between the atrium and ventricle. Although not itself requiring any energy, elastic recoil should be regarded as an active process as it is the driving force behind the suction effect rather than being the consequence of ventricular filling.

1.2.3 Filling

Although ventricular filling is the overall result of diastole, its raison d'être, it is the result of several processes happening in synchrony. Two of these have already been discussed, relaxation and suction, both active processes. A substantial component to left ventricular filling is passive, occurring by virtue of venous return through the pulmonary veins. Much of what is measured as diastolic function is this passive component.

Pulmonary venous return to the left atrium is determined by several components. It is highly dependent on pre-load and many studies of diastolic function have focussed on manipulation of this, either by pharmacological means such as venous dilatation with nitrates, volume loading with intravenous fluids, or by using the Valsalva manoeuvre to manipulate venous return to the heart.

The left atrium is a thin walled chamber which, under normal conditions, is able to expand to accommodate varying filling patterns. Left atrial volume, indexed to body surface area. has been found to correlate well with diastolic function, this being independent of systolic function(7).

1.2.4 Atrial Contraction

As the heart ages, the contribution of atrial contraction to ventricular filling becomes increasingly important. This is commonly seen as first an equalisation, then a reversal in the E/A ratio of mitral inflow using Doppler echocardiography. This has the potential to be over-interpreted as a pathological process and, hence, as diastolic dysfunction. An increase in the relative contribution of atrial contraction to ventricular filling has been shown to be a normal finding in the healthy elderly population(8), and hence a normal part of the ageing process. The precise point at which this changes from normal to pathological is a grey area. For this reason, multiple age stratified normal ranges for the E/A ratio have been published(9-13), none of which have been shown to perform robustly(6).

The importance of atrial contraction is recognised in the current European Society guidelines for the diagnosis of diastolic heart failure(14). Atrial fibrillation is considered equal to demonstrable abnormalities of standard Doppler parameters for supporting a diagnosis of diastolic heart failure where tissue Doppler is equivocal.

1.3 Defining Heart Failure

The definition of what we mean by heart failure, the physical signs, symptoms and abnormal investigations we associate with heart failure, is essential before we can define the patient group we are addressing. Most important in this is precisely which and how many of these clinical features we require to fulfil a diagnosis of heart failure. The criteria adopted for a study will have major implications not only in terms of overall prevalence of the condition, but also in the exclusion of valid cases of heart failure by overly strict criteria or the inappropriate inclusion of subjects who do not have heart failure due to lax criteria. Several different systems have been developed which range from the easily applied Framingham criteria which specify major and minor criteria to the more complex Boston system which attributes a score to various signs and symptoms, giving an overall probability to the diagnosis. The commonly used systems are summarised in Table 1-1,

Table 1-2, Table 1-3 and Table 1-4. Although many clinical trials use these standard criteria, or minor modifications of them, a large number use either their own criteria or are not specific.

Table 1-1 – Framingham Criteria for Diagnosing Heart Failure(15)

Major Criteria	Minor Criteria
Paroxysmal nocturnal dyspnoea	Bilateral ankle oedema
Neck vein distension	Nocturnal cough
Rales	Dyspnoea on ordinary exertion
Radiographic cardiomegaly	Hepatomegaly
Acute pulmonary oedema	Pleural effusion
S3 gallop	Decrease in vital capacity by one third from maximal value recorded
Central venous pressure >16 cm H_2O	Tachycardia (rate ≥ 120 beats/min)
Circulation time ≥ 25 sec	
Hepatojugular reflux	
Pulmonary oedema, visceral congestion, or cardiomegaly at autopsy	
Weight loss 4.5 kg in 5 days in response to treatment of congestive heart failure	

Requires simultaneous fulfilment of 2 major or 1 major and 2 minor criteria

Table 1-2 – European Society of Cardiology criteria for diagnosing heart failure(16)

Appropriate Symptoms	Dyspnoea, fatigue, fluid retention
Clinical Signs of Fluid Retention	Pulmonary or peripheral
Abnormal Cardiac Structure or Function	Necessary but not sufficient for diagnosis

Response to treatment confirms if doubt

Table 1-3 – Boston Criteria for diagnosing heart failure(17)

Category I: History		
Rest dyspnoea	4	
Orthopnoea	4	
Paroxysmal nocturnal dyspnoea	3	
Dyspnoea walking on level area	2	
Dyspnoea while climbing	1	
Category II: Physical examination		
Heart rate abnormality		Maximum of 4 points from each category – possible maximum 12 points.
91 to 110 beats per minute	1	
>110 beats per minute	2	
Jugular venous elevation		
> 6 cm H_2O	2	
> 6 cm H_2O + hepatomegaly or oedema)	3	8-12 – Definite heart failure
Lung crackles		5-7 – Possible
Basilar	1	≤ 4 – Unlikely
More than basilar	2	
Wheezing	3	
Third heart sound	3	
Category III: Chest x-ray		
Alveolar pulmonary oedema	4	
Interstitial pulmonary oedema	3	
Bilateral pleural effusion	3	
Cardiothoracic ratio > 0.5	3	
Upper zone flow redistribution	2	

Table 1-4 – Cardiovascular Health Study criteria(18)

Symptoms Dyspnoea, fatigue, orthopnoea, PND **Physical Signs** Oedema, rales, tachycardia, gallop rhythm, displaced apex beat **Any of the following sufficient but not necessary** Cardiomegaly, pulmonary oedema on chest x-ray, dilated ventricle, global or segmental wall motion abnormalities with impaired systolic function

1.4 The Misdiagnosis of Heart Failure

The signs and symptoms of heart failure are unfortunately insensitive and non-specific(19). In patients referred with suspected heart failure, dyspnoea only had an 18% positive

predictive value for left ventricular systolic dysfunction. Even a history of oedema combined with dyspnoea had sensitivity of 46% and specificity of 52%.

1.4.1 Noncardiogenic Pulmonary Oedema

Even pulmonary oedema, the hallmark of acute heart failure, is not specific for demonstrable cardiac dysfunction. Both clinical and radiological pulmonary oedema can result from a range of conditions unrelated to either fixed or transient cardiac dysfunction. Noncardiogenic pulmonary oedema usually results from an increase in pulmonary capillary permeability, allowing protein rich fluid to leak into the alveoli. This situation may arise in several common clinical conditions such as pneumonia, sepsis, aspiration pneumonitis and with major trauma particularly with large volume blood transfusion(20). It is not uncommon for patients presenting with infective exacerbations of chronic obstructive pulmonary disease to be treated with both antibiotics and loop diuretics due to diagnostic doubt in the acute situation. Some of the subsequent clinical improvement may then be attributed to treatment of "heart failure" by the diuretic rather than an improving pneumonic process. When preserved left ventricular systolic function is subsequently demonstrated, it is all too easy to arrive at a diagnosis of diastolic heart failure. This is particularly true in the elderly in whom a degree of systolic or valvular dysfunction are more common and the risk factors associated with diastolic dysfunction are often present.

Neurogenic pulmonary oedema is a poorly understood and uncommon cause of pulmonary oedema usually seen in the context of significant brain injuries such as subarachnoid haemorrhage or trauma which result in elevated intracranial pressure. It has also been reported in up to one third of patients with status epilepticus and may also be seen with non-haemorrhagic stroke. The mechanism appears to be a combination of changes in pulmonary capillary permeability and haemodynamic effects resulting from an increase in sympathetic tone. This results in pulmonary venoconstriction which increases capillary hydrostatic pressure and can result in pulmonary oedema in the absence of elevated left heart pressures. It may also result in elevated left heart pressures through a combination of increased venous return, systemic hypertension and direct impairment of left ventricular function by the catecholamine excess.

Despite the difficulties in distinguishing clinically between cardiogenic and noncardiogenic pulmonary oedema, a number of radiological features have been identified

which may aid in this(21, 22). Pleural effusions, Kerley B lines and peribronchial cuffing are usually present with cardiogenic pulmonary oedema but not so with noncardiogenic. Whilst an increased cardiothoracic ratio is supportive of cardiogenic pulmonary oedema due to left ventricular systolic dysfunction, a normal cardiac size would not be regarded as confirming a non cardiac cause as this is still compatible with diastolic heart failure.

1.4.2 Dependent oedema

Dependent oedema, whilst being commonly seen in inadequately treated patients with left ventricular systolic dysfunction, may also be caused by several other processes such as venous or lymphatic insufficiency, obesity, arthritis or other joint trauma, hypoproteinaemia, hypo or hyperthyroidism, renal or hepatic dysfunction. It is also associated with several classes of drugs most noticeably calcium channel blockers with the dihydropyridine group being most commonly associated with this. Clinically detectable peripheral oedema has been reported in 33% of patients in response to treatment with amlodipine and symptoms of leg swelling in 64% overall(23). Other common pharmacological causes of oedema include both steroidal and non-steroidal drugs, tricyclic antidepressants and antipsychotic drugs such as chlorpromazine.

The response of oedema to diuretics should not be taken as evidence that heart failure was the underlying cause as the salt and intravascular volume depletion caused by these drugs, particularly loop diuretics, will result in significant fluid shifts from the interstitium to the vascular compartment regardless of cause.

1.4.3 Dyspnoea

Many disease processes include dyspnoea as part of their symptomatology. Respiratory disease in the form of either reversible or fixed large airways disease, interstitial lung disease or destruction of the alveoli by an emphysematous process is the most obvious cause of dyspnoea. A recent UK survey found an overall COPD prevalence of 13% in those aged over 35. This rose to 15% in ex-smokers and 20% in current smokers over the whole age group. In current smokers over the age of 65, the prevalence of COPD approached 40%(24). In 80% of those with COPD defined by abnormal spirometry, no prior respiratory diagnosis had been made.

Although the number of smokers in the UK is declining and will eventually result in a reduced incidence of COPD, another condition, obesity, is on the rise. In England and Wales in 2003 65% of men and 56% of women were either overweight or obese(25). Obesity can cause dyspnoea by several mechanisms. Firstly, the simple physical effort of carrying the additional body mass may result in dyspnoea either by exceeding the exercise capacity in an otherwise healthy individual or by unmasking subclinical disease in other systems. Obesity can also have a direct effect on respiratory function by increasing the intrathoracic pressures required to achieve adequate ventilation. This can be seen on pulmonary function testing as a restrictive defect with a decreased expiratory reserve volume being characteristic.

The anginal equivalent is a well recognised clinical entity where myocardial ischaemia results not in chest pain, but in dyspnoea. Since the development of pain occurs further down the cascade of myocardial ischaemia than the development of impaired diastolic function or wall motion abnormalities, it is easy to see how ischaemia below the threshold required to cause angina could result in dyspnoea. This atypical presentation of angina is more common in the elderly(26).

Not only are there multiple causes of dyspnoea, the problem is compounded by the fact that dyspnoea is a highly subjective symptom. Several scales attempt to quantify dyspnoea in different settings – the New York Heart Association (NYHA) scale (27)stratifies heart failure by symptoms, the BORG scale(28), and the Medical Research Council (MRC) dyspnoea score(29) is used to assess those with chronic respiratory disease. Although the NYHA and MRC scales apply a degree of objectivity on the part of the assessor by relating dyspnoea to the extent of limitation of normal daily activities, the underlying subjective nature of dyspnoea still exerts an influence.

What does not appear to be in doubt is that dyspnoea is an important symptom which has prognostic significance. Self reported dyspnoea as a symptom has been shown to be an independent predictor of both cardiac and all-cause mortality in a group of nearly 18,000 patients undergoing myocardial perfusion imaging(30). As might be expected, the level of inducible ischaemia was higher in patients with known coronary artery disease. However, it was similar in patients with dyspnoea and asymptomatic individuals without known coronary disease despite a fourfold increase in cardiac death in the dyspnoeic group. The study is limited by the fact it is derived from a nuclear cardiology database including

symptoms and demographic data documented at the time of scanning. It therefore does not include any assessment of respiratory pathology or diastolic function. It should be noted that this patient group were not classified on the presence of clinical heart failure, the presence of dyspnoea itself was sufficient to confer an adverse prognosis.

1.5 Assessing Diastolic Function

The various methods devised to assess diastolic function reflect the fact that there is not one single parameter that fully encompasses this complex entity. Many of the parameters measured do not assess ventricular relaxation directly but instead assess the haemodynamic effects of ventricular relaxation by way of patterns of ventricular filling and techniques to estimate ventricular filling pressures. This is due to the fact that the accurate measurement of myocardial relaxation and stiffness by non-invasive means remains an elusive goal. For the routine assessment of diastolic function in a high volume of patients, invasive measurements are neither feasible nor desirable. In clinical practice, therefore, one is limited to non-invasive measures. Each of these has its own pitfalls and caveats partly related to the parameter itself, its reproducibility and dependence on intrinsic factors such as pre-load and after-load, and limitations of the modality used to measure the parameter. A further complicating factor is the fact that many of these parameters behave differently with preserved versus impaired systolic function. Mitral inflow Doppler parameters, whilst showing good predictive power for left ventricular filling pressure in the presence of impaired systolic function, are unreliable in isolation with preserved systolic function due to significant variation in filling pressures in this patient group. The net effect of this is that one non-invasive parameter alone cannot adequately assess diastolic function – multiple parameters must be measured and integrated to grade diastolic function.

1.5.1 Echocardiography

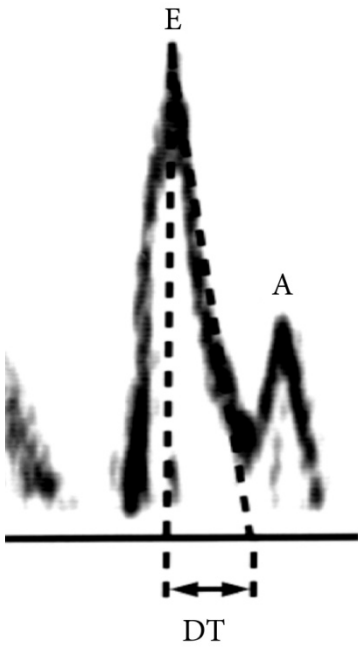
Since echocardiography is universally available, non-invasive, and relatively easy to perform to at least a basic standard, it is an obvious modality of choice for assessing diastolic function. Numerous different parameters have been employed ranging from basic pulsed wave Doppler to new applications of colour flow mapping and new developments in the form of tissue Doppler(31). The grading of diastolic dysfunction from grade 1 (normal) to grade 4 using the mitral E/A ratio, deceleration time and the response of these

to the Valsalva manoeuvre is appropriate to subjects with an ejection fraction less than 50%. Where systolic function is preserved, these measures must be supplemented with tissue Doppler assessment and left atrial volume. Pulmonary vein Doppler and mitral inflow propagation velocity may be used to provide additional evidence of elevated filling pressures.

1.5.1.1 Mitral Doppler

By placing a pulsed wave Doppler envelope in the left ventricular cavity just beyond the tips of the mitral leaflets parallel to flow, the velocity time profile of ventricular filling can be measured (Figure 1-2).

Figure 1-2 – Transmitral flow – E, A and DT



The E wave of early ventricular filling represents passive ventricular filling. In theory it provides an index of the pressure gradient between the left atrium and left ventricle at the onset of diastole as well as a measure of how this pressure gradient decays over time, measured as the deceleration time (DT), the time taken from peak E wave velocity to intercept the X axis.

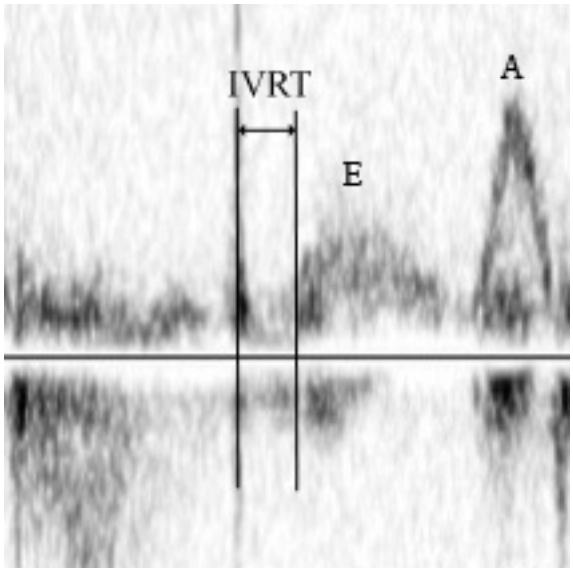
The A wave represents atrial contraction. The ratio between E and A waves indicates the relative contribution of passive and active filling. As passive filling is impaired by diastolic dysfunction, there is a shift from passive to active filling as the force of atrial

contraction is required to overcome a stiff ventricle. As filling pressures increase, pseudonormalisation of the E/A ratio occurs (grade 2 dysfunction). At this stage, the underlying abnormality can be unmasked with pre-load alteration such as the Valsalva manoeuvre. As diastolic dysfunction progresses further, the E/A ratio becomes fixed with a short DT indicating restrictive physiology.

1.5.1.2 Isovolumetric Relaxation Time

If a pulsed wave Doppler sample envelope is placed adjacent to the left ventricular outflow tract such that it overlaps mitral inflow and aortic outflow, an additional parameter can be measured, isovolumetric relaxation time (IVRT). This is the period immediately following the end of systole and aortic valve closure where the ventricle has begun to relax but left ventricular intracavity pressure has not yet fallen below left atrial pressure. This is illustrated in Figure 1-3 where the artefact of aortic valve closure can be clearly seen. IVRT is measured from this point to the beginning of the mitral E wave, the onset of ventricular filling.

Figure 1-3 – Isovolumetric relaxation time



In theory, a ventricle which has impaired relaxation i.e. diastolic dysfunction, will manifest this as a prolongation in this period. However, IVRT is influenced by multiple factors which confound it as a solitary measure of diastolic function:

- Active ventricular relaxation (Ca^{2+} transport, ATP dependent)
- Elastic ventricular recoil
- Pre-load – any factor which increases pre-load will increase left atrial pressure, which in the absence of any change in the ventricle itself, decreases the atrio-ventricular pressure gradient and shortening the time taken for left ventricular pressure to fall below atrial and allow mitral valve opening. Conversely, a reduction in preload reduces left atrial pressure and increases IVRT.
- After-load – the resistance which the left ventricle must pump against in the form of afterload is determined by systemic vascular tone. Its mechanism of influence on IVRT can be seen by the interaction between diastolic blood pressure, predominantly influenced by systemic vascular resistance, and left ventricular end systolic pressure. Assuming that left ventricular end systolic pressure and its rate of decay with ventricular relaxation remains the same, a rise in diastolic blood pressure reduces the pressure gradient between the aorta and left ventricle, thereby shortening the time to aortic valve closure and prolonging IVRT.
- Aortic regurgitation. An incompetent aortic valve allows ventricular filling to occur retrogradely during what should be an isovolumetric period. Left ventricular intracavity pressure will therefore decay more slowly than it otherwise would and increase the time taken for this to fall below left atrial pressure and permit mitral valve opening. Although in this instance what is being measured is not strictly speaking IVRT, the time period is still easy to measure and is usually still referred to as IVRT. As the severity of aortic regurgitation increases, IVRT becomes progressively less reliable as an isolated measure of diastolic function.

1.5.1.3 Pulmonary Venous Doppler

By placing a pulsed wave Doppler envelope in either the left or right upper pulmonary vein it is possible to profile left atrial inflow as shown in Figure 1-4. As can be seen by comparing this tracing from a real patient with the idealised representation of Figure 1-5, the quality of the tracings obtained can make analysis difficult. It should be possible to

obtain interpretable data in up to 80% of subjects(32). This is very dependent on echogenicity of the subject but may be improved with the use of contrast agents such as SonoVue™.

Three distinct waves occur in pulmonary venous flow; 2 antegrade and one retrograde. The only retrograde wave, PVa, occurs with atrial contraction and corresponds to the P wave on the surface ECG. The first antegrade wave occurs with ventricular systole and may be biphasic, something more commonly seen in older patients(33). The first part of this wave, PVs_1, begins immediately upon mitral valve closure and is primarily due to rapid filling of the atrium with the blood which has backed up in the pulmonary veins during atrial systole. As ventricular contraction begins in earnest, longitudinal contraction of the heart towards its apex creates a suction force drawing blood into the left atrium resulting in the PVs_2 wave. This flow slows as the point of peak ventricular contraction is passed and the pressure gradient between the left atrium and the pulmonary veins falls.

Figure 1-4 – Pulsed valve pulmonary venous Doppler – acquisition and typical waveform

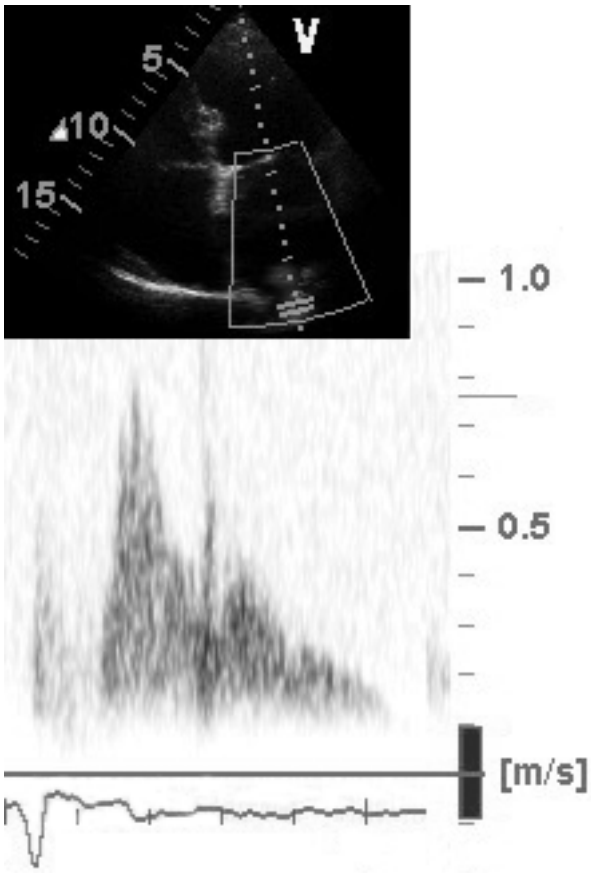
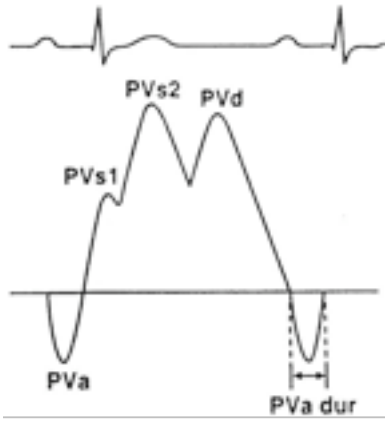


Figure 1-5 – Idealised pulmonary venous waveform



With the onset of diastole, the mitral valve opens and passive ventricular filling begins – the E wave of mitral inflow. This re-establishes the pressure gradient between atrium and pulmonary vein resulting in the PVd wave.

It has been shown that in subjects with normal left atrial pressure measured as pulmonary capillary wedge pressure, systolic flow is the predominant wave in the pulmonary veins. In subjects with elevated left atrial pressure, diastolic flow is predominant(34). This, however must be interpreted with caution as impaired left ventricular systolic function, as assessed by fractional area shortening in this study, confounded this relationship. It should also be noted that in this, and other studies of pulmonary venous Doppler and left ventricular filling(35), transoesophageal echocardiography was used. This is not the case in routine clinical practice where transthoracic echocardiography is typically used. Transoesophageal echocardiography allows the Doppler sample envelope to be more reliably placed within the pulmonary vein, rather than at its ostium. This may result in a lower rate of detection of atrial reversal (PVa) (36) than transoesophageal echocardiography as atrial, rather than pulmonary venous, flow is being sampled.

The duration of the pulmonary venous A wave in relation to the duration of the mitral inflow A wave has been shown to correlate to left ventricular end diastolic pressure(37). PVa duration exceeding mitral A duration by more than 30ms has a high specificity for elevated LVEDP.

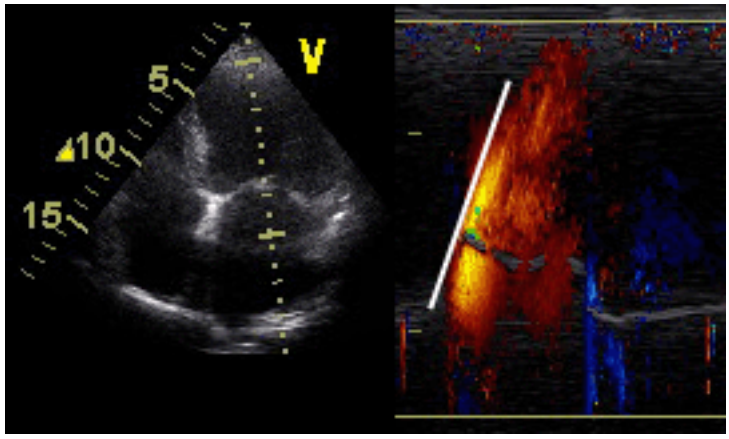
1.5.1.4 Colour Flow M-Mode

The limitations of peak transmitral velocities having been recognised, attention turned to alternative ways of assessing early ventricular filling. The use of colour M-Mode to

measure flow propagation through the mitral valve into the left ventricle was first proposed by Brun in 1992(38). It was subsequently validated in animals and humans that Propagation Velocity (v_p) correlated well with tau, the time constant of isovolumetric pressure decay. Further work by Garcia et al(39) demonstrated that v_p was largely independent of alterations in preload in anaesthetised dogs undergoing caval clamping and humans undergoing partial cardiopulmonary bypass. It showed good correlation with tau when lusitropic conditions were altered with dobutamine and esmolol. Overall the relationship could be described by the equation tau = $775.63(v_p)^{-0.7718}$, with a correlation coefficient of 0.78.

In an apical 4 chamber view, colour flow Doppler is placed over mitral inflow, extending to the left ventricular apex. An M-mode cursor is than placed through this and a suitable sweep speed chosen to maximise the size of jet being measured, and hence, minimise measurement error. The slope of the leading edge of the inflow jet is measured to give a propagation velocity (Figure 1-6).

Figure 1-6 – Colour flow M-mode mitral inflow propagation – technique and typical appearance

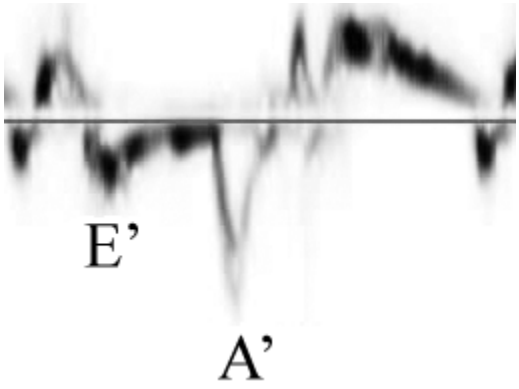


However, the technique is limited by the fact that there are methodological differences between different authors who have employed this technique(40, 41) who disagree on exactly how and where to measure the slope of the propagation wave(42). Depending on how the aliasing velocity is configured and whether the propagation wave is measured at its leading edge or at the first aliasing velocity, the propagation velocity will be different, limiting general applicability until a standard approach can be agreed.

1.5.1.5 Tissue Doppler

Tissue Doppler, also referred to as Tissue Velocity Imaging (TVI), represents a relatively new development in echocardiography which allows quantification of the low velocity motion of the myocardium and related structures (typically velocities <20cm/s). This is achieved by inverting the low pass filters applied to standard Doppler and only allow through signals from structures travelling at low velocity. The technique was first utilised to examine subtle abnormalities in systolic and diastolic function in ischaemic heart disease(43).

Figure 1-7 – Pulsed wave spectral tissue Doppler obtained from mitral annulus



For the assessment of mitral annular velocities, tissue Doppler is obtained in the apical 4 chamber view. A pulsed wave sample volume is placed over either the lateral or medial mitral annulus and, with tissue Doppler mode enabled rather than standard Doppler, a signal like than in Figure 1-7 is obtained. Care should be taken not to have the Doppler gain set too high as this can lead to an overestimate of the peak velocity due to artefact being interpreted as part of the waveform. It can be seen that the waveform is relatively broad and a range of possible velocities may be measured; the innermost edge, middle or outermost edge. A similar waveform may also be derived from colour coded rather than spectral tissue Doppler. This has a systematically lower value as it measures the mean value of the waveform, rather than the outermost, true peak, value measured by tissue Doppler. The published studies of left ventricular filling pressure have used spectral tissue Doppler and it is therefore not possible to make direct comparison with values obtained by colour coded tissue Doppler(44).

Filling of the left ventricle in diastole results in movement of the mitral annulus away from the relatively fixed apex. The velocity of this movement can be measured with pulsed

wave tissue Doppler. In sinus rhythm this movement occurs in two phases which correspond to the E and A waves seen on mitral inflow Doppler. These are usually referred to as E' and A', although they can also be labelled Ea, Em and Am, Aa. In addition to peak velocity, the time to peak velocity and duration of each of these waves can also be measured although there has been no clinical application of these additional parameters as yet.

The measurement of most interest is E', the peak velocity of the mitral annulus with early passive diastolic filling. This has been shown to be less dependent on filling conditions than traditional transmitral and pulmonary Doppler measurements(45). E' itself is not a direct measurement of diastolic relaxation as its correlation with τ is intermediate ($r = 0.46$ in one series(32). One reason for this that in addition to ventricular relaxation, another key determinant of E' is the peak velocity of mitral inflow. The ratio of E' to peak early transmitral velocity (E) as the E/E' ratio corrects for this influence and achieves far better correlation with invasively measured indices of relaxation and filling pressure.

The E/E' ratio should not be regarded as a single value, however. Mitral annular velocity can be measured in four different positions – lateral and septal on the apical 4 chamber view, and inferior and anterior on the 2 chamber view. Of these, only the lateral and septal velocities have been routinely measured. Neither should these four measurements be regarded as necessarily being identical. The structures surrounding and thus influencing the movement of the septal portion of the mitral annulus are markedly different to those related to the lateral annulus. The relative superiority of these positions has not yet been clearly established – the septal velocity has been shown to correlate better with LVEDP than the lateral velocity(32) (the mean of the two showed the same degree of correlation however). Despite this, another series used only the lateral annular velocity to look for correlation with pulmonary capillary wedge pressure(46). It should also be noted that any regional wall motion abnormalities may have a profound impact on individual measurements and in this instance either a measurement from an unaffected segment or a mean of several segments may be more appropriate to yield a result representative of global function.

Currently the most useful application of the E/E' ratio is in the assessment of mean left ventricular diastolic pressure (mLVDP). Although LV end diastolic pressure is commonly used as an index of left ventricular filling pressures, in fact it correlates relatively poorly

with mean left atrial pressure. It has been shown that mLVDP correlates significantly better with mean left atrial pressure (r=0.94) than LVEDP does (r=0.82) when left atrial pressure is measured directly via transseptal puncture(47).

In a series of 100 patients Ommen et al(32) found that all patients with a septal E/E' ratio of ≥ 15 were found to have an elevated mLVDP (>12mmHg having been defined as abnormal). Although the majority of patients falling into this category had left ventricular systolic dysfunction (ejection fraction <50%), it was also observed in a small number of patients with preserved systolic function. Of 27 patients with an E/E' ratio of <8, only 4 of these were found to have an elevated mLVDP giving a negative predictive value of 85%. Two of these patients had preserved systolic function. The cut-off values of 8 and 15 were derived via ROC analysis which gave an area under the curve of 0.82 for the septal annulus and a definition of >12mmHg as abnormal. However, a substantial number of patients fell into the indeterminate region with ratios between 8 and 15. Other techniques for differentiating normal from elevated filling pressures must therefore be employed in this group.

The utility of the E/E' ratio for non-invasive estimation of pulmonary capillary wedge pressure is less clear and various series have yielded seemingly contradictory results. Nagueh et al(48) found E' to be a preload independent index of left ventricular relaxation. Using the lateral mitral annulus, the E/E' ratio correlated well with invasively measured pulmonary wedge pressure with the formula PCWP = 1.9 + 1.24(E/E') giving an r value of 0.87. Further work by this group validated the E/E' ratio in sinus tachycardia of up to 132 beats per minute(49). By combining this patient group with their previous group, a unified formula covering a patient group of 180 was produced: $PCWP = 2 + 1.3(E/E'_{lateral})$. The E/E' ratio was found to be relatively independent of heart rate with no change in the predictive accuracy for PCWP when heart rate was included in stepwise regression analysis(49).

Sundereswaran et al(46) reported on a series of fifty cardiac transplant recipients undergoing simultaneous right heart catheterisation and Doppler echocardiographic studies, including tissue Doppler analysis. Left ventricular systolic function was generally well preserved with a mean ejection fraction of 56% (range 20% - 70%). It was found that an E/E' ratio of >8 detected a wedge pressure of ≥15mmHg with a sensitivity of 87% and

specificity of 81%. Little additional clinical data is given, however, which may limit its general application.

In contrast Firstenberg and colleagues found no correlation between the E/E' and pulmonary wedge pressure(50). Seven normal healthy subjects of mean age 37 with no history of cardiovascular disease underwent simultaneous right heart catheterisation and echocardiography at rest and during preload manipulation by lower body negative pressure and saline infusion. All subjects had normal wedge pressures at baseline, rising to a maximum of 20mmHg with volume loading. Although the septal E' and peak E velocities correlated strongly with wedge pressure, when combined as the E/E' ratio there was no statistically significant relationship with wedge pressure. However, the authors have also demonstrated that the response of the E' velocity, which in this group of normal subjects increased with increasing preload and wedge pressure and so maintained an unchanged E/E' ratio, is dependent on underlying diastolic function(51). In dogs with impaired relaxation induced by esmolol, as shown by an increase in tau, the effect of preload on E' was diminished giving rise to the relationship between E/E' and wedge pressure seen elsewhere.

1.5.1.6 Left Atrial Volume

The fact that the left atrium dilates in response to chronic pressure overload has been recognised for a number of years(52). It is only more recently that the utility of this in assessing diastolic function has been appreciated. In much the same way as glycosylated haemoglobin is used to assess medium term control in diabetes mellitus, left atrial volume may be used to assess left atrial pressure in the medium to long term.

A number of methods may be used to measure left atrial volume. Simpson's rule of discs, most commonly use to calculate left ventricular volumes and ejection fraction, may also be applied to the left atrium. The left atrial border, excluding the orifices of the pulmonary veins, is traced round in the apical 4 and 2 chamber views at ventricular end systole, immediately prior to mitral value opening. The other commonly used technique is the area length method. Here atrial volume is calculated from only 3 measurements – left atrial length and width on the apical 4 chamber view, and anteroposterior diameter on the parasternal long axis view. As real-time 3 dimensional echocardiography becomes more widely used, it too will have a role to play. It has been shown that 3D echo yields higher

volumes than 2D, this perhaps being the reason for its superior correlation with clinical events(53).

It is necessary to index left atrial volume to body surface area (left atrial volume index, LAVI) as, like other echocardiographic measurements such as left ventricular mass, left atrial volume is directly related to body surface area. Failure to correct for this will diminish its correlation with left atrial pressure. Reference ranges for LAVI have previously been published (54). The 95th centile in females is 30ml/m^2, and 33ml/m^2 in males.

One limitation of left atrial volume is that it is affected by valvular heart disease, particularly mitral valve disease. This will result in atrial dilatation even in the absence of a primary left ventricular abnormality. A diagnosis of valvular, rather than diastolic, heart failure is more appropriate here. Left atrial volume is also affected by arrhythmias, particularly atrial fibrillation. It has been shown than, in patients with chronic lone atrial fibrillation, there is progressive left atrial dilatation from an initially normal size(55).

1.5.2 Radionuclide Ventriculography

1.5.2.1 Resting RNVG

Prior to the advent of cardiac MRI, radionuclide ventriculography was regarded as the most accurate and reproducible means of calculating left ventricular ejection fraction. Given the limited availability, higher costs and limitations in individuals with metallic foreign bodies including pacemakers and defibrillators, radionuclide ventriculography (RNVG), commonly referred to as MUGA (MUltiple Gated Acquisition), still has a major role to play. RNVG uses an intravenously injected radioactive tracer, technetium-99m, to create an area of activity within the chambers of the heart, either by following a first-pass bolus through the heart or by attaching the tracer to red blood cells to create a stable blood pool. Since the activity detected within the left ventricle is proportional to the blood volume, the activity-time curve is equivalent to a volume-time curve thus allowing calculation of ejection fraction and other parameters of ventricular emptying and filling.

Using the LV volumes obtained during contrast ventriculography at cardiac catheterisation, it has been shown that peak filling rate (PFR) is significantly reduced in patients with coronary artery disease compared with normal subjects(56). It was suggested that this was

due to decreased compliance or impaired relaxation, in this case secondary to ischaemia. However, once these patients are controlled for age it is not possible to distinguish them from normal subjects due to considerable overlap between the two groups. There is an age related decline in PFR in otherwise healthy subjects(57) highlighting the need for age specific normal ranges. This relationship between diastolic function and age is seen in other imaging modalities such as echocardiography(58).

PFR, however, has a disadvantage in that it assesses ventricular filling at a variable point in diastole. Although there is not an age related increase in Time to PFR, each individual is likely to have a different point in diastole assessed. Filling in specific parts of diastole can be assessed by arbitrarily dividing diastole into thirds and quantifying the proportion of total ventricular filling occurring during each third. Early passive ventricular filling in the form of First Third Fractional Filling (FTFF) has attracted most interest. Abnormalities have been demonstrated in FTFF at rest in patients with ischaemic heart disease and normal systolic function(59) with an increase in diastolic dysfunction induced by exercise.

The utility of assessing diastolic function on exercise has been shown in two other ways. Although improvements in diastolic function have been observed following angioplasty, one group of investigators found that this was only demonstrable during exercise rather than at rest(60). Diastolic function seems to be an important determinant of exercise capacity, with PFR on exercise being the most important determinant of exercise capacity in patients with ischaemic heart disease and left ventricular systolic dysfunction(61).

Other processes such as left ventricular hypertrophy and hypertension may also result in relaxation abnormalities. RNVG offers a non-invasive method of determining these potentially useful values. It is also likely to be more accurate given it does not involve the direct injection of contrast into the left ventricle with the haemodynamic and arrhythmogenic effects this has. It should also be borne in mind that calculation of left ventricular volumes from 2D data relies on geometric assumptions and uniformity of function. RNVG does not make these assumptions as it samples a 3D dataset.

1.5.2.2 Exercise RNVG

The demonstration in 1958 that, in patients with coronary artery disease and normal resting left ventricular function, exercise induced an increased in pulmonary capillary wedge pressure and by implication, left ventricular dysfunction (62), offered an alternative route

to the diagnosis of ischaemic heart disease. The observation that a decrease in left ventricular ejection fraction as measured by contrast angiography occurred on exercise in patients with ischaemic heart disease whilst it increased in normal subjects (63) could be put into routine clinical use as a non invasive tool for the diagnosis of ischaemic heart disease when nuclear cardiology developed sufficiently to allow this to be reproduced using radionuclide ventriculography(64).

Although the initial studies reported sensitivities and specificities of over 90% (65), superior to those of exercise electrocardiography, they involved highly selected patient groups with a high prevalence of previous myocardial infarction or more severe coronary artery disease. The control subjects used tended to be fitter than the population who were to undergo the procedure as a diagnostic test for ischaemic heart disease. Once exercise RNVG was put in to routine use as a diagnostic tool in patient groups more representative of normal clinical practice, it became clear that it did not live up to the initial hopes. Whilst its sensitivity was within an acceptable range, its specificity dropped to as low as 50%(66) and a negative stress RNVG was associated with an annual event rate of up to 3%(67). This compares to rates of less than 1% for myocardial perfusion scintigraphy or stress echo(68). An abnormal left ventricular ejection fraction response to exercise is not exclusive to coronary artery disease – it can be observed in several conditions where myocardial function is abnormal such as aortic regurgitation where, although resting systolic and diastolic function is preserved, the left ventricle's reserve capacity to respond to exercise is impaired. These false positives further limited the utility of exercise RNVG as a tool for the initial diagnosis of coronary artery disease.

Techniques

There are two different techniques for acquiring the RNVG data – the first pass technique and the equilibrium method.

First-pass angiography refers to the method where technetium not fixed to any constituent of the patients' blood is observed on its first pass through the heart following bolus administration. The theoretical advantage of this is that it will allow wall motion and ejection fraction to be calculated for a very specific point in time – of significant benefit in exercise RNVG where the timing of this may be critical. It has been shown that the ejection fraction may recover to, or rebound above, pre-test values if the acquisition is delayed by as little as 30-60 seconds post peak exercise even in patients with

angiographically proven coronary disease whose peak exercise ejection fractions would have yielded a positive result (69).

However, there are problems with this approach. The technique by which the bolus is administered is critical. If the tracer is administered or flushed slowly this will result in a prolonged delivery of tracer to the heart with the consequence that temporal resolution of the tracer between the two ventricles will be lost and no calculations will be possible. This is because no positioning of the camera to achieve septal separation prior to injection is possible and images are typically acquired in an anterior view resulting in spatial overlap of the right and left ventricles.

The second problem is that of equipment. The high tracer activity levels and count rates required to obtain adequate data in such a small time frame (typical time from entry of tracer into RV to exit from LV is 30 seconds) are not suitable for the single crystal gamma cameras in routine use due to their count rate response being non-linear at these levels. For accurate calculation of ejection fraction these studies have to be carried out with multi-crystal cameras.

Equilibrium angiography has been regarded as the less technically demanding of the two techniques. Here the red blood cells of the patient are labelled with technetium, typically by means of stannous pyrophosphate prior to commencing image acquisition. Image data are acquired over a period of several minutes until a predetermined number of counts or cardiac cycles have been acquired, or a set time elapsed. This in effect gives a time-activity curve which is a mean of usually several hundred cardiac cycles and allows data from ectopic beats to be excluded from analysis. In atrial fibrillation it ensures that the data accepted for analysis is representative of overall ventricular function, rather than the very limited number of beats acquired with the first pass technique whose cycle lengths may be extreme values for that patient.

Exercise versus pharmacological stress

There has been debate as to whether dynamic exercise or a pharmacological agent is the optimal choice for stress testing not only with exercise RNVG but also latterly with myocardial perfusion imaging.

There are some aspects of pharmacological stress which are clearly superior to dynamic exercise. First is the virtual elimination of motion artefact – the drug can be infused with

the patient at rest under the camera. Second is the standardisation that such a protocol allows. With dynamic exercise the duration and intensity of stress achieved will vary markedly between patients. With a standard dose of stressor drug, normally calculated by body weight, identical levels of stress can, in theory, be induced in every patient. In practice, patients will vary in their response to a specific dose of a stressor. This may be due to the influence of drugs such as beta blockers or, in the case of dipyridamole or adenosine stress, theophyllines or other methylxanthines such as caffeine. While these may be withheld prior to stress, the biological half-life of these drugs may mean they are still present in sufficient quantities to attenuate the response to the stressor. Disease states may also influence the response to a stressor. Myocardial beta adrenoceptors, for instance, are downregulated in heart failure(70), resulting in a reduced chronotropic and inotropic response to stress(71).

There are drawbacks however. Given that dobutamine will tend to increase ejection fraction unless substantial coronary disease is present, the sensitivity of dobutamine stress RNVG for the diagnosis of coronary heart disease is poor, with values as low as 33% for induction of wall motion abnormalities reported in one study (72) and 14% for an abnormal ejection fraction response in another once patients with resting wall motion abnormalities were excluded (73). Dipyridamole requires even more severe disease than dobutamine to yield a positive result and thus has even lower sensitivity, albeit with high specificity (74). It should be noted that these agents perform substantially better when used in myocardial perfusion imaging (72). Perhaps more important is the information obtained from the exercise itself. A patient's exercise capacity and their haemodynamic response to that exercise provides important diagnostic and prognostic information which would otherwise not be obtained(75).

Erect versus supine exercise

Both erect and supine positions can be used for stress RNVG. Supine exercise is technically easier to perform and the degree of patient motion is minimised compared to upright exercise. Although the left ventricular ejection fraction and wall motion responses are no different in erect and supine exercise(76), the same is not necessarily true of diastolic function. In this group, in the absence of coronary artery disease, left ventricular end-diastolic volume remained static during supine exercise but increased by 25% during upright exercise. Equal ejection fraction responses were the result of a greater decrease in end-systolic volume during supine exercise.

1.5.2.3 RNVG Parameters of Systolic and Diastolic Function

A variety of parameters derived from RNVG have been used to describe systolic and diastolic function. The most common of these are ejection fraction, peak filling rate, time to peak filling and first third fractional filling. These parameters and their derivation are summarised below.

Parameter	Abbreviation	Normalisation	Units
Ejection fraction	EF	None	%
First third fractional filling	FTFF	None	%
Second third fractional filling	STFF	None	%
Peak filling rate	PFRrr	RR interval	% of EDC per cardiac cycle
	PFRdt	Diastole duration	% of EDC per diastolic period
Time to first third full	ttFTFs	None	Seconds
	ttFTFrr	RR interval	Cardiac cycles
	ttFTFdt	Diastole duration	Diastolic periods
Time to peak filling	ttPFs	None	Seconds
	ttPFrr	RR interval	Cardiac cycles
	ttPFdt	Diastole duration	Diastolic periods
Peak emptying rate	PERrr	RR interval	% of EDC per cardiac cycle
	PERst	Systole duration	% of EDC per systolic period
Time to peak emptying	ttPEs	None	Seconds
	ttPErr	RR interval	Cardiac cycles
	ttPEst	Systole duration	Systolic periods
Time to first third empty	ttFTEs	None	Seconds
	ttFTErr	RR interval	Cardiac cycles
	ttFTEst	Systole duration	Systolic periods

In the calculations for the indices derived from the activity-time curve, the following definitions are used:

t = time	t_f = frame time
f = frame	d is at end-diastole
c = count	s is at end-systole
R = RR interval	a is at start of atrial filling

Normalisation – Volume

Both filling and emptying rates must be normalised to a unit of volume. Here both have been normalised to end diastolic counts, this being proportional to end diastolic volume. Whilst this is the most practical means of normalising these indices, it does have drawbacks. Unlike an index expressed in terms of absolute volume or flow rates, indices normalised to end diastolic counts are subject to change if the end diastolic volume changes. This introduces a degree of error when there is a change in ventricular volumes or loading conditions between serial studies and limits comparability between patients who may have markedly different ventricular volumes.

Ideally, these indices should be normalised to absolute volumes. However, this requires the drawing of a blood sample to determine the activity level in each millilitre of blood. Using this information, the activity-time curve can be converted into a volume-time curve. The drawbacks of this approach are the infection control and radiation protection issues involved in the processing of a radioactive blood sample.

Normalisation - Temporal

Any parameter which describes either a period of time or rate requires a unit of time to be expressed in. Although the simplest and easiest unit of time to deal with is the second and its divisions such as milliseconds, in this case it is not necessarily the most useful. The parameters being measured in terms of their rate or time of occurrence are themselves contained within an event, the cardiac cycle, which is itself of variable duration. Any parameter which is measured in terms of absolute time cannot be reliably compared between different heart rates unless one can be absolutely sure that it is linearly related to any changes in heart rate. Normalisation is the process whereby these parameters are adjusted so that they are expressed relative to the cardiac cycle length or definable portions thereof.

Parameters such as ejection fraction, first third fractional filling and second third fractional filling are either not expressed in terms of time in the case of ejection fraction, or are already expressed in terms of their relative duration within the cardiac cycle in the case of FTFF and STFF.

RR Interval

The simplest and most intuitive form of normalisation is to the cardiac cycle length. Instead of times being expressed as an absolute time in seconds from the beginning of the cardiac cycle, they are expressed as a number of cardiac cycles. This is achieved by dividing the time by RR interval duration thus:

Equation 1-1 – Normalisation to cardiac cycle length

$$\text{Cardiac cycles} = \left(\frac{\text{Time (s)}}{\text{RR interval (S)}} \right)$$

As the times being dealt with are all less than 1 cardiac cycle it can be useful in some circumstances to express them as a percentage of the cardiac cycle. This is done by multiplying the result of Equation 1-1 by 100.

Diastole Duration

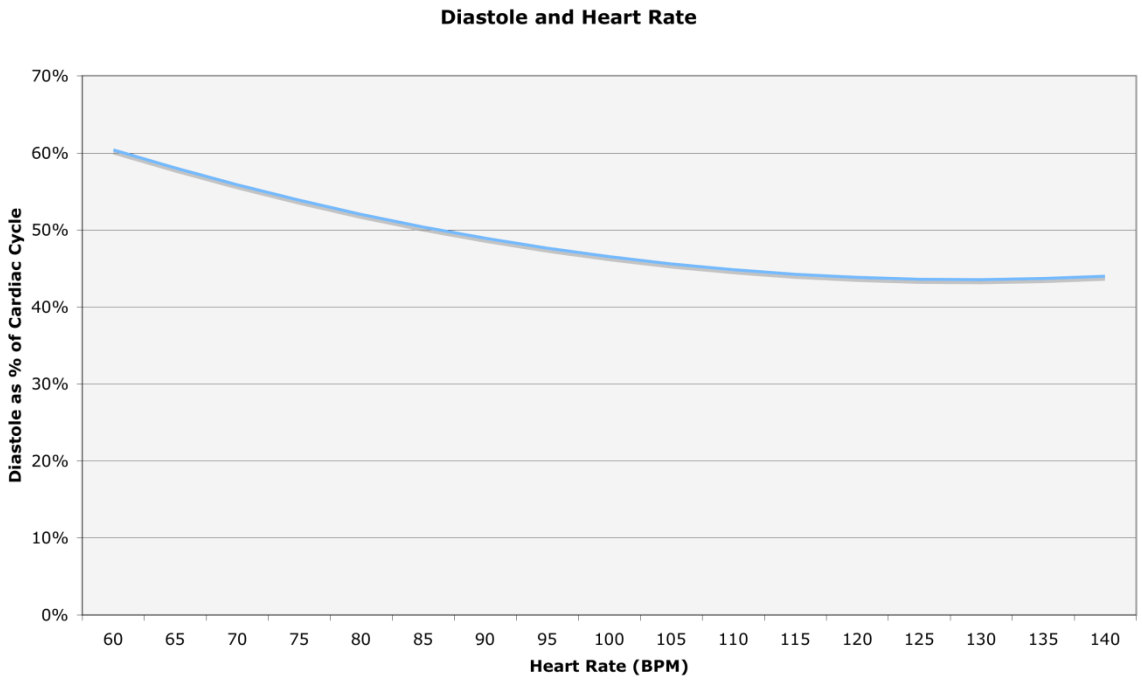
With increasing heart rate there are changes in the relative proportions of the cardiac cycle occupied by systole and diastole. It might therefore be beneficial to look at the timing of events within diastole and normalise not only for heart rate but to remove the additional factor of how systole and diastole interact with alterations in heart rate. Although electrical and mechanical systole have a relatively linear relationship with heart rate(77), diastole does not. The relationship of diastole duration and the echo Doppler derived components of this – the E wave duration, diastasis and A wave duration – have had their response to heart rate change with supine bicycle exercise measured(78). The following equation has been derived to predict mechanical diastole duration (MDD) with an r^2 value of 0.98:

Equation 1-2 – Mechanical diastole duration

$$\text{MDD} = -549 + (2.13 \times \text{HR}) + \frac{61500}{\text{HR}}$$

This can be used to model the change in diastole duration over a range of heart rates with the result expressed as a percentage of cardiac cycle duration (Figure 1-8)

Figure 1-8 – Plot of percentage diastole duration predicted by the model in Equation 1-2



As can be seen the proportion of the cardiac cycle occupied by diastole initially falls linearly with heart rate but becomes non-linear at about 90bpm. Above heart rates of 120 the equation predicts an essentially fixed diastole duration. This is due to the E and A wave durations showing only minimal relationship to heart rate with only an 18% shortening of E duration for a 100% increase in heart rate. The majority of shortening of diastole occurs in the diastasis period – once this is zero, further shortening of diastole will be minimal. Given this complex relationship, it seemed reasonable to attempt to look at diastole in isolation.

To normalise to diastole duration, the absolute time is divided by the duration of diastole. Diastole duration is defined here as the time from end systole as defined by the minimum point on the activity-time curve to the end of the cardiac cycle. The absolute duration of diastole can be calculated by multiplying the number of frames from end systole to the end of the cardiac cycle by the frame duration in milliseconds. This is either predetermined in the case of fixed frame acquisition or can be calculated by dividing the cycle length by the number of frames per cardiac cycle used in variable frame width acquisition. The resulting units are therefore Diastole Periods in the case of measures of time and counts per diastolic

period in the case of rates. As with normalisation to heart rate, the unit of time Diastole Duration can be multiplied by 100 to be expressed as a percentage of diastole.

Systole Duration

As diastole diminishes as a proportion of the cardiac cycle with increasing heart rate, so must systole increase even though its absolute duration decreases.

The duration of systole can be determined by the QT interval, although it is important to note that this is electrical, rather than mechanical systole. The duration of mechanical systole is, on average, 3-5% shorter than electrical systole (79). The relationship of the QT interval to heart rate has been extensively studied (80). Correction of the QT interval for heart rate is an essential tool to allow this important marker to be compared between individuals and compared with a standard range in order to risk stratify arrhythmogenic potential. Three equations have been used to correct the QTc interval in routine practice:

Equation 1-3 – Bazett(81)

$$QTc = \frac{QT}{\sqrt{RR(s)}}$$

Equation 1-4 – Fridericia(82)

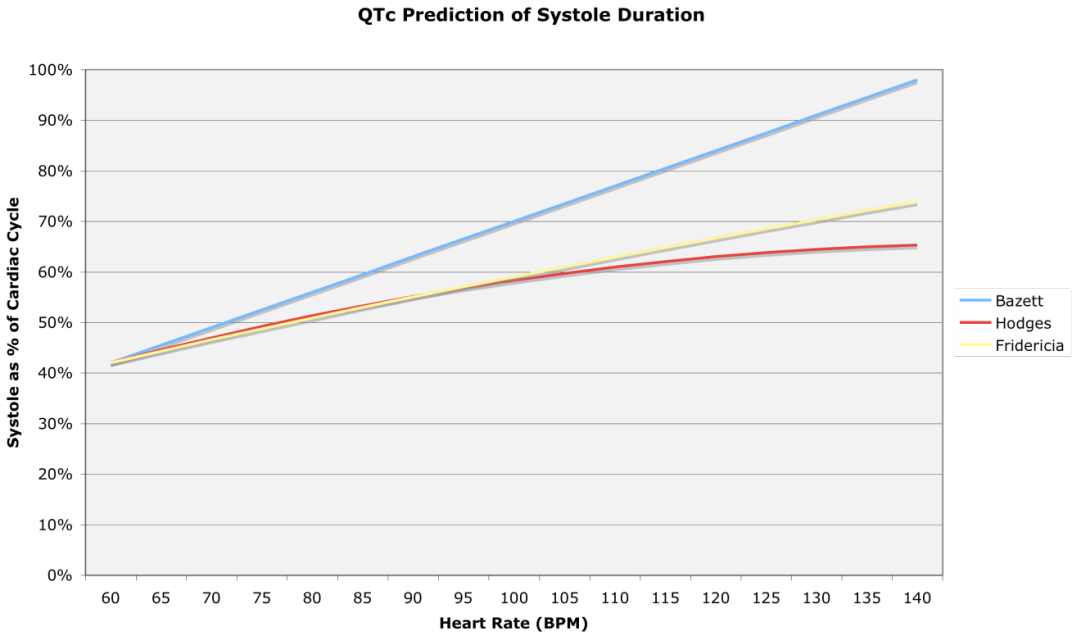
$$QT_c = \frac{QT}{\sqrt[3]{RR(s)}}$$

Equation 1-5 – Hodges(83)

$$QTc = QT + 1.75(HR - 60)$$

These equations can be manipulated to determine what the QT interval and, by inference, the duration of systole will be at different heart rates. These predicted QT durations can then be used to determine the proportion of the cardiac cycle systole will occupy at a given heart rate.

Figure 1-9 – Prediction of systole duration derived from QTc models of Bazett, Fredericia and Hodges.



The accuracy of the most commonly used formula for correction of the QT interval, the Bazett equation which dates back to 1920, has been questioned(83), particularly at the extremes of heart rate. In our model above (Figure 1-9), Bazett predicts that at a heart rate of 140 systole will occupy 98% of the cardiac cycle. Clearly, this cannot be so. In fact, the curve which most closely mirrors the observed data on systole duration is the Hodges formula.

As with normalisation to diastole duration, normalisation to systolic duration is achieved by dividing absolute time by duration of systole, systolic duration being defined from the activity-time curve as the period from the beginning of the activity-time curve to its minimum point.

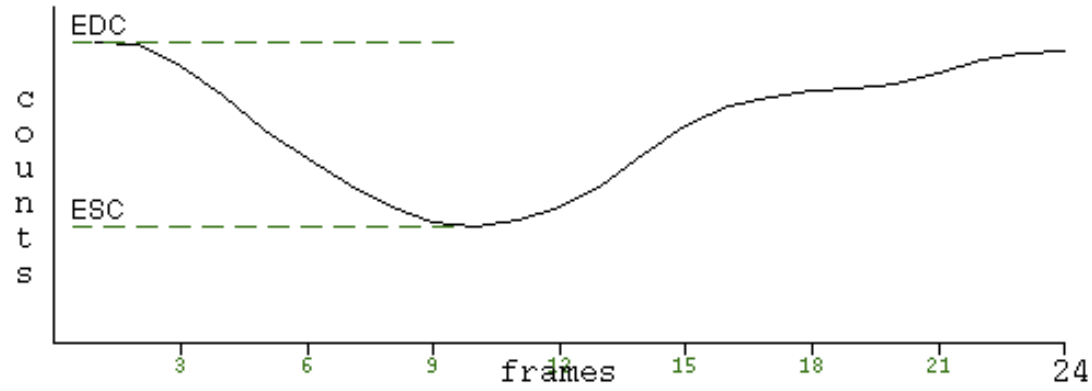
Ejection Fraction

Left ventricular ejection fraction is the most widely used parameter of cardiac function and can be calculated using several imaging modalities in addition to radionuclide ventriculography including echocardiography and cardiac magnetic resonance imaging. Its strength is the prognostic information it imparts and the fact that so much of the risk stratification and clinical decision making process is based around this single measure.

Radionuclide ventriculography has the advantages of superior reproducibility to echocardiography and a lack of reliance on the patient being a good imaging subject. It also makes no assumptions whatsoever about uniformity of ventricular regional wall motion which can lead to inaccuracies in assessment of ejection fraction even with biplane measurements on echocardiography.

From a background corrected activity-time curve, ejection fraction is calculated by Equation 1-6 where end diastolic counts (C_d) are defined as the initial curve maximum and end systolic counts (C_s)as the minimum point on the curve. These points are determined automatically by the software. Due to limitations in the software, additional steps are required to ensure correct automatic determination of end systolic counts when using activity-time curves generated from fixed-frame width acquisitions. These steps are described in section 3.1.2.4.

Figure 1-10 – Calculation of ejection fraction from activity-time curve



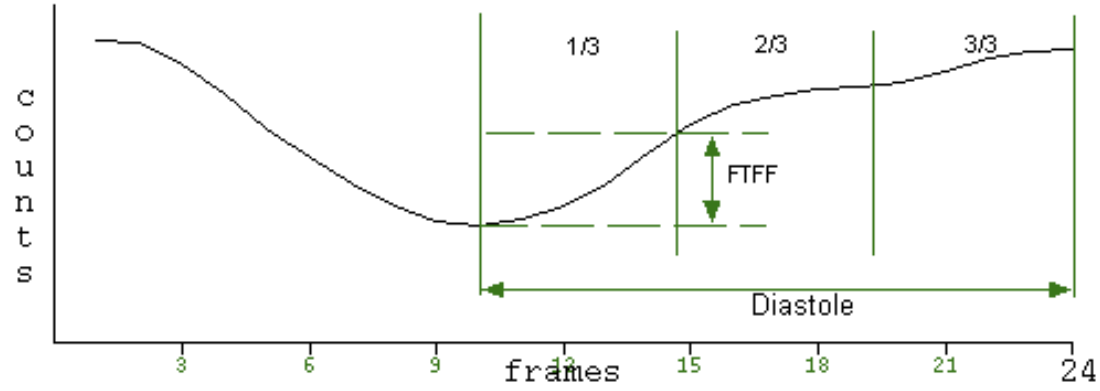
Equation 1-6 – Ejection Fraction

$$EF = \frac{C_d - C_s}{C_d} \times 100$$

First Third Fractional Filling

First third fractional filling is defined as the proportion of total ventricular filling which takes place within the first third of diastole and is typically expressed as a percentage. Following determination of the end systolic frame from the minimum point of the activity-time curve, the number of frames to the end of the cardiac cycle is counted. This is divided by 3 and this number to divide diastole into equal thirds.

Figure 1-11 – First third fractional filling



The value of the activity-time curve at the first of these three points is used in the following equation to calculate FTFF.

Where $c_{1/3}$, the counts one third of the way into diastole, is defined as:

$c_{1/3} = (1\text{-}b)c_a + bc_{a+1}$ where a is the integer part and b is the fractional part of $(f_d - f_s)/3$ and f_d and f_s are the end diastolic and end systolic frames respectively, first third fractional filling is calculated by Equation 1-7.

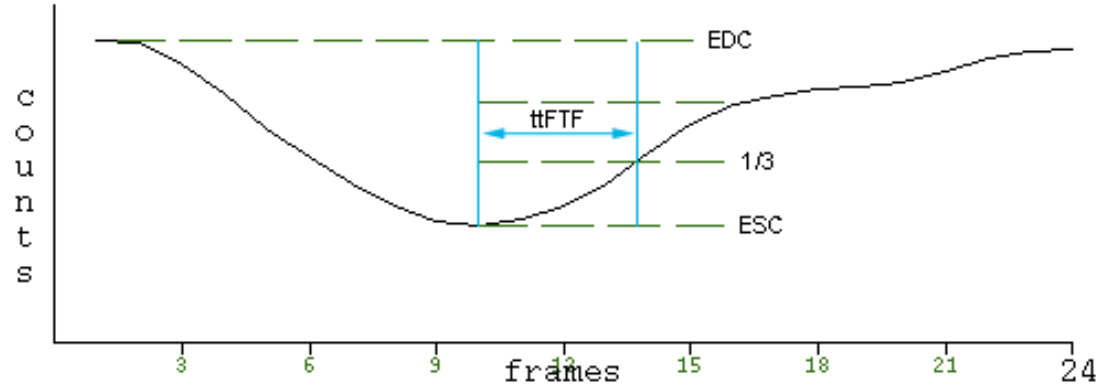
Equation 1-7 – First Third Fractional Filling

$$FTFF = \frac{c_{1/3} - c_s}{c_d - c_s} \times 100$$

Time to First Third Full

In addition to determining the proportion of ventricular filling occurring in a specified time, it is also possible to determine the time taken to achieve a specified proportion of ventricular filling. Instead of dividing the diastolic time period into thirds, end systolic and end diastolic counts are determined as before. The difference between these is divided into thirds and the time taken from end systole to achieve this increase in counts is measured. The initial measurement is made in frames, the basic unit of time of the activity-time curve. This is then converted to an absolute time by multiplying by the frame duration and can be normalised either to cardiac cycle length or diastole duration as previously described.

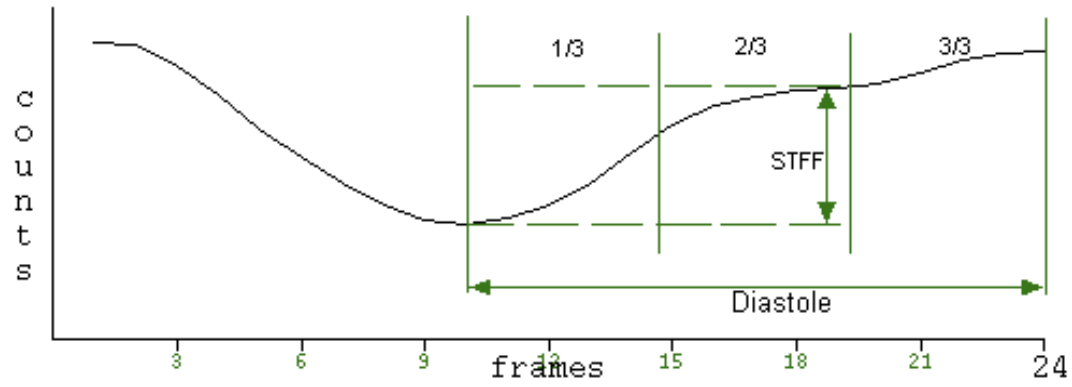
Figure 1-12 – Time to first third full



Second Third Fractional Filling

Second third fractional filling refers to the proportion of total ventricular filling which has occurred by the end of the second third of diastole. Similarly to FTFF it is calculated by dividing diastole into thirds and then determining counts at the end of the second third. This value is used in essentially the same equation as FTFF. It should be noted that the value generated is not only the proportion of ventricular filling occurring in the second third of diastole, it also includes that which has already taken place in the first third. First third fractional filling must be calculated separately and subtracted if one wishes to know the proportion of filling occurring solely during the second third of diastole.

Figure 1-13 – Second third fractional filling



Peak Filling Rate

Using both the raw activity-time curve and 3 point linear regression, the slope of the diastolic filling curve can be examined to determine the maximal rate of positive change in counts. The accuracy of this determination will be limited by two main factors. Firstly, the temporal resolution of the curve and portion of the curve being examined i.e. the

number of frames per cardiac cycle and the number of frames within diastole, has a significant impact. With greater temporal resolution more data points are available on the activity-time curve and more subtle changes in filling rate can be determined. Even with acceptable temporal resolution, a very short diastolic period may contain too few frames to accurately determine peak filling.

Secondly, the overall count statistics for the activity-time curve are important. All activity-time curves generated by radionuclide imaging are subject to a degree of artefact either due to motion artefact or random variations in radioactive decay. This is tempered to a degree by some smoothing of the curves prior to analysis. The degree of smoothing must, however, be limited as overly aggressive smoothing of the activity-time curve will distort any values ultimately derived from it.

These two factors interact with each other. If the number of frames per cardiac cycle is increased to improve temporal resolution, a trade-off must take place with count statistics. Unless the acquisition time is increased, the total number of counts available for generation of the activity-time curve must remain the same. For an increase in frame rate from 24 to 32 frames per cycle, this fixed quantity of data is divided into smaller bins resulting in fewer counts per frame.

Equation 1-8 – Peak Filling Rate

$$\chi_{\max} = \frac{\max[c_i - c_{i+1}]_{i=s}^{d_1}}{c_d t_f} \times 100$$

Where c_i = counts in frame i, d_1 = end diastolic frame, s = end systolic frame, c_d = counts at end diastole, and t_f = frame duration.

By regression, intermediate points between the acquired frames may be interpolated. Peak filling rate may be calculated using these interpolated points to improve temporal resolution.

Time to Peak Filling

The time from end systole to the point at which peak filling occurs is calculated by:

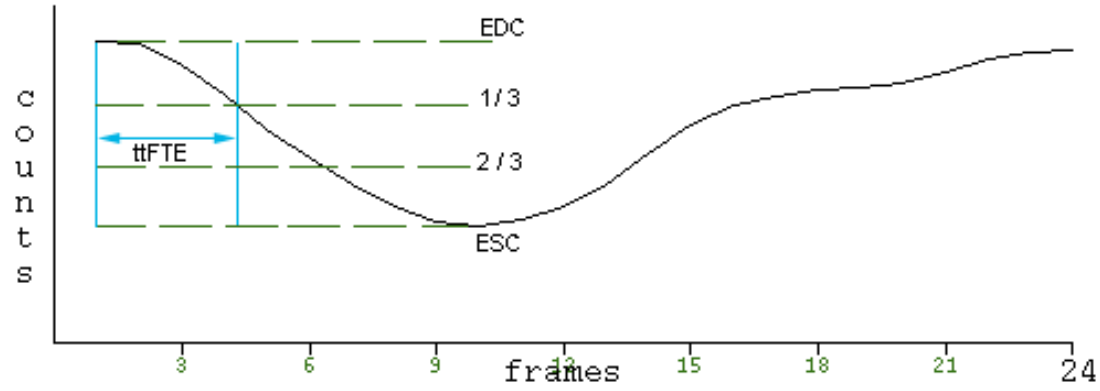
$$t_{\chi\max} = (i_{\max} - f_s + 0.5)t_f$$

where i_{max} is the frame number giving the maximum difference in Equation 1-8. The initial units are seconds. This can then be normalised to either the RR interval or diastole.

Time to First Third Empty

Similarly to Time to First Third Full, Time to First Third empty can be calculated be determining the time for the activity-time curve to fall by one third the total value of the difference between end systolic and end diastolic counts. Its basic units are the same as ttFTF and following conversion to an absolute time, can be normalised to either cardiac cycle duration (RR interval) or systole duration.

Figure 1-14 – Time to first third empty



Peak Emptying Rate

Without using regression to refine the activity-time curve beyond the resolution of the number of frames per cardiac cycle, the peak emptying rate, ε_{max}, is calculated by Equation 1-9. Without any normalisation it is expressed as the percentage of end diastolic counts per second.

Equation 1-9 – Peak Emptying Rate

$$\varepsilon_{max} = \frac{\max[c_i - c_{i+1}]_{i=d}^{s}}{c_d t_f} \times 100$$

As with peak filling rate, additional points may be interpolated by regression to improve temporal resolution.

Time to Peak Emptying

The time from end diastole to the point at which the peak emptying rate occurs is calculated by:

$$t_{\varepsilon max} = (i_{max} - f_d + 0.5)t_f$$

where i_{max} is the frame number giving the maximum difference in Equation 1-9. This is initially calculated in absolute time and can then be normalised as required.

1.5.3 Magnetic Resonance Imaging

Regarded by many as the gold standard in non-invasive cardiac imaging, MRI has predominantly been used to assess systolic function and structure. However, it has also been used in the assessment of diastolic function. Many of the techniques described to date simply replicate those which can be achieved with other imaging modalities. Only one technique, myocardial tagging, exploits the unique capabilities of MRI. Cardiac MRI does have significant advantages when compared with alternative imaging techniques. However, it also has several disadvantages.

1.5.3.1 Advantages of MRI

Foremost in the advantages of MRI is its superlative image quality. Its spatial resolution far exceeds that of nuclear techniques, either perfusion scintigraphy or ventriculography, and either transthoracic or transoesophageal echocardiography. Unlike nuclear techniques it does not involve exposure to ionising radiation. Unlike echocardiography it is not limited by the adequacy of acoustic windows either in terms of image quality or achievable imaging plane.

MRI is also exceptionally versatile, allowing an increasingly diverse range of methods of cardiac assessment. From simple visual assessment of cardiac motion, through quantitative assessment of systolic and diastolic myocardial motion, assessment of perfusion and scarring with the use of gadolinium to magnetic resonance spectroscopy allowing examination of tissues at the molecular level, MRI has been hailed as a ‘one stop shop’ for cardiac assessment.

1.5.3.2 Disadvantages of MRI

Such a wide range of possible scanning techniques comes at a cost: the more comprehensive an examination undertaken, the longer that examination takes. Whilst a comprehensive cardiac MRI examination should not take longer than the equivalent examination by a combination of other modalities, a disadvantage unique to MRI becomes more problematic. Current MRI scanners are enclosed devices, far more so than CT scanners or the gamma cameras used in nuclear cardiology. As a result, a proportion of patients are unable to undergo even brief MRI examinations due to claustrophobia. As imaging time increases, the problem is exacerbated.

MRI also has issues with temporal resolution. Depending on the scanning protocol being used and magnetic field strength, most cardiac MRI has a temporal resolution of 30 to 50 milliseconds. A temporal resolution of 30 milliseconds will yield 33 frames per cardiac cycle at a heart rate of 60 beats per minute, more than adequate for most applications. However, a resolution of 50 milliseconds at 80 beats per minute will result in only 15 frames per cycle. Although this may be adequate for ejection fraction analysis, more advanced volume-time curve analysis may be unreliable. With increasing heart rates this problem is exacerbated and even ejection fractions may be unreliable if true end-systole and end-diastole are missed due to inadequate temporal resolution.

1.5.3.3 Myocardial Tagging

It is possible to locally saturate the magnetisation of the myocardium in such a way that a dark stripe, or tag, is imprinted on the myocardium. As the myocardium moves and deforms, the tag moves with it. This, therefore, provides a means of tracking the magnitude, vector and velocity of myocardial motion. This tagging process is typically carried out on multiple short axis slices with the tag lines appearing black against the grey myocardium, although tagging in any plane is possible. The technique was initially described in 1988(84), applying radial tag lines centred on the left ventricular cavity. The technique was refined, with parallel stripes being created(85), and was referred to as SPAMM, SPAtial Modulation of Magnetisation. Further refinement came with the application of two perpendicular sets of stripes(86), creating a lattice. The technique was primarily used for assessment of regional systolic function.

The assessment of diastolic function was impeded by tag line fading in diastole. The technique was subsequently refined and CSPAMM, Complementary SPAtial Modulation of Magnetisation evolved(87).

Torsion

The spiral arrangement of fibres within the myocardium of the left ventricle means that, as the ventricle contracts, it twists about its base. Conversely, as it relaxes, it untwists. The magnitude and rate of this torsional deformation can be measured with tagging. If a series of radial tags are applied to short axis slices, the torsion angle is measured as the angle between a tag line on any slice and the corresponding tag line on the basal slice. This can be measured throughout the cardiac cycle to give an overall magnitude and instantaneous torsion rates. When studied in a canine model, this technique reveals that the majority of

this untwisting occurs during isovolumetric relaxation, prior to mitral valve opening(88). By doing so, it causes left ventricular pressure to fall below left atrial pressure more rapidly than it would otherwise do. This is measured indirectly as the isovolumetric relaxation time, although this is confounded to a degree by pre-load and after-load.

Strain/Strain Rate

The concept of myocardial strain, defined as fractional tissue deformation in response to applied stress was first described in 1973(89). Along with its derivative strain rate, it offered a quantitative, rather than qualitative, means of describing regional myocardial function. Until the advent of tagged cardiac magnetic resonance imaging, it remained impractical to measure in routine clinical practice, although M-mode echocardiography was proposed (90). The use of myocardial tagging to quantify 3 dimensional deformation, and thereby strain, was first described in 1995(91). Circumferential shortening had been described several years earlier(92).

Although systolic strain and strain rate were well established and used to validate tissue Doppler derived strain in 2002(93), the first utilisation of MRI derived strain to assess diastolic function was in 2004(94). In this canine model of reperfused acute myocardial infarction, persistent diastolic dysfunction was demonstrated despite full recovery of systolic function.

1.5.3.4 Velocity Encoded MRI

Velocity encoded MRI is the technique whereby the velocity of blood flow within vessels or across valves can be measured. It may be performed using several MRI techniques including 'Time of Flight' (TOF) where unsaturated spins moving into a gradient echo slice produce signal enhancement. Phase contrast angiography may also be used. This has several advantages over TOF in that there is complete suppression of stationary tissues – tissues with a short T1 relaxation time such as fat may produce artefact. It may also be acquired with gradient echo cine sequences to produce Velocity Encoded Cine MR, velocity maps with high temporal resolution(95).

Much of the early work with this technique concentrated on measurement of velocities within the aorta and pulmonary arteries, and in quantifying cardiac output(96). Although this was already achievable by means of Doppler echocardiography, the ability to make these measurements with MRI added another facet to the perception of MRI as a 'one stop

shop' for cardiac assessment. In addition, it allowed assessment in patients with poor echo windows and in those with complex anatomy not amenable to standard echo approaches.

It has been shown to be technically possible to assess pulmonary venous and transmitral velocities using MRI(97). The feasibility of this in clinical practice and its accuracy when compared to Doppler echocardiography has been assessed(98). Transmitral flow was assessed in both the vertical and horizontal long axes with 2D and 3D velocity encoded MRI. Mitral E wave velocity had a correlation coefficient of 0.68-0.69 in the four modalities. E/A ratio correlation was similar, other than for 3D horizontal long axis values which had a correlation coefficient of 0.84. Pulmonary venous flow also showed good correlation with coefficients of 0.74 and 0.83 for the X velocity and X/Y ratio respectively. An advantage of MRI becomes apparent here. It was possible to record the Y velocity in all patients with MRI whereas this was not so with echocardiography.

However, there are caveats. The temporal resolution of velocity encoded MRI is poor, particularly with 3D encoding where a frame width in excess of 100 milliseconds results in averaging of data between frames. In comparison, Doppler has a temporal resolution of just a few milliseconds depending on the velocity range being sampled and sample depth. With the young age and relatively low heart rates of Hartiala's normal subjects, this results in a temporal resolution of 16 frames per cardiac cycle. Whilst this is adequate for the E/A ratio, it is inadequate for mitral deceleration time or isovolumetric relaxation time. In an older population with higher heart rates, the number of frames per cycle will be even poorer. This lack of temporal resolution results in a systematic under estimate of velocities. Thus, normal ranges are not directly translatable from echo to MRI.

Another significant limitation of velocity encoded MRI in comparison with Doppler echocardiography is the length of time necessary to acquire the data. Disregarding the time required by both modalities to align the imaging plane correctly, adequate Doppler data can be acquired in less than 10 seconds with a minimum of 3 cardiac cycles samples. Velocity encoded MRI requires 4 to 5 minutes of data acquisition and a longer analysis time.

1.5.3.5 Volume-Time Curves

Cine MRI, particularly when used with a balanced steady state free precession technique, allows high quality images with high blood pool to endocardium contrast to be generated.

By tracing the ventricular cavity area over a series of short axis slices, multiplying this by the slice thickness added to any inter-slice gap(99), the ventricular volume may be calculated over the entire cardiac cycle. Whilst the most common application of this is the calculation of ejection fraction, any parameter which can be derived from the activity-time curve of radionuclide ventriculography may also be calculated from MRI. Peak filling rate calculated in this manner has been used as a parameter of diastolic function in several studies(100-102).

Several key differences should be considered when comparing RNVG and MRI derived ventricular volume curves. Firstly, RNVG makes no geometric assumptions about the left ventricle. The entire ventricle is sampled and, in the absence of any operator error, is included in the derived curve. MRI, when adopting a slice based approach, must make assumptions about the ventricular contours for the entire thickness of the slice and any inter-slice gap. Where slice thickness is small this should be of little or no clinical significance as the far superior spatial resolution of MRI, approximately 1.5 x 2 mm^2 should more than offset this.

Secondly, temporal resolution and possible framing modes are different between RNVG and MRI. As previously discussed, MRI has a fixed temporal resolution of between 30 and 50 milliseconds per frame for most acquisition sequences. As heart rate increases, the number of frames per cardiac cycle decreases. RNVG, however, can be acquired in list mode where raw data is acquired continuously and retrospectively formatted with variable frame durations to maintain a constant number of frames per cycle independent of heart rate. This has an additional benefit in that RNVG is better able to cope with arrhythmias such as atrial fibrillation. In such circumstances, or where patients are unable to perform the breath holds required for routine MRI acquisitions, MRI can be acquired in real time at the expense of temporal and spatial resolution. Typically resolutions of 3 x 3 mm^2 at 90 milliseconds per frame can be achieved(103, 104).

1.5.3.6 Myocardial Velocities and Tissue Tracking

In a technique analogous to tissue Doppler echocardiography, velocity encoded phase contrast MRI can be used to measure instantaneous myocardial velocities by analysing phase shifts in bipolar velocity encoded gradients(105). The velocities obtained can be used to calculate displacement and hence strain and strain rate(106) by tracking tissue over time without the need for myocardial tagging. However, errors in velocity measurement

result in progressive errors as the cardiac cycle progresses, although this can be reduced with improved tracking algorithms(107, 108).

A newer technique, DENSE (Dual Encoding with Stimulated Echoes), solves this problem by using a process similar to tagging to encode displacement information within the phase signal. The tag is applied at the R wave and read back at a point later in the cardiac cycle giving a measurement of displacement relative to end-diastole. The development of cine DENSE techniques(109, 110) have replaced the single cardiac phase that was initially read. A temporal resolution of 34 milliseconds and spatial resolution of 2.5 x 2.5 x 8 mm^3 has recently been achieved(111).

1.5.4 Invasive Assessment of Left Ventricular Filling Pressures

1.5.4.1 Pulmonary Capillary Wedge Pressure

The fact that left heart pressures are transmitted retrogradely through the pulmonary vasculature has been recognised for over 100 years. The measurement of pulmonary capillary wedge pressure (PCWP) was first demonstrated in 1948(112) and, with advances in left heart catheterisation, were taken to be equivalent to left atrial pressure. The key advantage of PCWP was that it involved venous rather than arterial puncture, thereby reducing complication rates. The underlying principle of PCWP is that the balloon isolates the distal pressure sensor from the pulmonary arterial side of the circulation, leaving it solely exposed to the pressure waves transmitted back through the pulmonary capillary bed.

However, the correlation between PCWP and directly measured left atrial pressure is not perfect and significant discrepancies have been shown, particularly at higher pulmonary arterial pressures(113). The reason for this is that the pressure wave must traverse the pulmonary capillary bed. Damage to this bed means that the pressure wave is not transmitted faithfully. This may either be from long standing backpressure from left ventricular dysfunction, left sided valvular disease, or from pulmonary pathology such as interstitial lung disease, thromboembolic disease or intrinsic lung disease.

1.5.4.2 Left Ventricular End Diastolic Pressure

The association between left ventricular dysfunction and elevated left ventricular end diastolic pressure (LVEDP) has been recognised since the early days of left heart catheterisation (114). This has also been observed where systolic function is preserved and a diagnosis of diastolic dysfunction has been made by other criteria (115). The choice of end diastolic pressure, rather than another point during diastole or mean pressure, is one based partly on pragmatism and the limited diagnostic facilities of the time. End diastole is an easily identified point on the left ventricular pressure trace. This, therefore, improves reproducibility between studies and between different operators. Analogue acquisition of the pressure trace also meant that instantaneous, as opposed to mean, pressures were much simpler to measure.

1.5.4.3 Mean Left Ventricular Diastolic Pressure

Although left ventricular end diastolic pressure is convenient and does correlate with filling pressure, it does not necessarily equal mean left atrial pressure(116). Mean left ventricular diastolic pressure, although more cumbersome to calculate, requiring either digitisation of a pressure trace or native digital acquisition, has been shown to correlate more closely with mean left atrial pressure than LVEDP(47).

1.5.4.4 Tau

Tau (τ) is the time constant of isovolumetric pressure decline in the left ventricle and is measured in milliseconds. This is measured by means of a catheter placed in the left ventricle and calculations performed on the acquired pressure tracings. In theory, this goes a step beyond the relatively crude measurement of IVRT by measuring what happens to left ventricular pressure during this period rather than simply its duration. As left ventricular pressure is being measured at a time when no ventricular filling occurs, this has been shown to be independent of preload(117).

Tau may be calculated by several techniques. The original description of tau used a natural log plot of left ventricular pressure against time and assumed a monoexponential pressure decline to zero(118). This was later refined to accommodate a non-zero endpoint of pressure decline by using a negative plot of dP/dt versus left ventricular pressure(119).

The third method, essentially a simplification of the original method, calculates the time for left ventricular pressure to fall by half from peak negative dP/dt(120).

However, tau is not fully load independent. It has been shown that it is affected by afterload in that an increase in afterload increases tau(121). The same has been seen in studies primarily concerned with preload where volume loading was sufficient to also affect afterload(122).

1.5.5 BNP

Brain natriuretic peptide (BNP) and the n-terminal fragment of its precursor proBNP (NT-proBNP) are cardiac neurohormones secreted primarily by the ventricles in response to volume and pressure overload. The apparent misnomer arises from the fact it was initially isolated from preparations of brain tissue. They have been shown to be elevated in patients with left ventricular systolic dysfunction and their levels correlate both with NYHA functional class and prognosis(123-125). It has also been shown that BNP is elevated to an intermediate level in patients presenting with heart failure with preserved systolic function compared to those with systolic dysfunction(126). The level of BNP correlates with grade of diastolic dysfunction with higher levels being seen in patients with a restrictive pattern of mitral inflow(127).

NT-proBNP has several advantages over BNP. It is more stable in patient samples with frozen plasma remaining suitable for analysis at least 4 months after collection(128). NT-proBNP also has a longer plasma half-life than BNP (70 versus 5 minutes) (129). This gives a better indication of average filling pressures and is of more use in acute volume overload where the secretion of natriuretic peptides decreases once their reservoir is exhausted.

BNP is, however, not specific for either systolic or diastolic dysfunction. BNP is known to be elevated in any condition which elevates right ventricular pressures such as pulmonary embolism, pulmonary hypertension, and fluid overload states such as cirrhosis and dialysis dependent renal failure. This is not surprising given that BNP is not specific for the left ventricle and merely indicates volume or pressure overload rather than the aetiology of this. It is therefore predictable that BNP is also raised in conditions that increase left ventricular wall stresses without necessarily causing overt dysfunction such as systemic hypertension or left ventricular hypertrophy.

1.6 Diagnostic Criteria for Diastolic Heart Failure

The biggest problem in diagnosing diastolic heart failure is the lack of standardised criteria which perform reliably in the real world where co-morbidity and confounding diagnoses are common. Several schemes have been proposed for integrating multiple parameters of systolic and diastolic function in an attempt to more reliably diagnose and grade diastolic function. The most widely accepted of these come from the European Study Group on Diastolic Heart Failure(130). Originally published in 1998, these guidelines were updated in 2007 to include recent advances in diagnosing diastolic dysfunction such as tissue Doppler imaging, MRI and neurohormones such as BNP(14).

The 1998 European criteria require three conditions to be met simultaneously for a diagnosis of diastolic heart failure to be made: Signs or symptoms of heart failure; preserved left ventricular systolic function; and evidence of abnormal diastolic function.

Table 1-5 – 1998 European Society of Cardiology criteria for diastolic heart failure

Signs/symptoms	Systolic Function	Diastolic Function
Exertional dyspnoea Orthopnoea Gallop sounds Lung crepitations Pulmonary oedema	LVEF ≥ 45% And LVEDIDI < 3.2cm/m^2 Or LVEDVI <102ml/m^2	IVRT: <30y >92ms 30-50y >100ms >50y >105ms $E/A_{<50y}$ < 1.0 and $DT_{<50y}$ >220ms $E/A_{>50y}$ <0.5 and $DT_{>50y}$ >280ms Pulmonary venous flow: $S/D_{<50y}$ >1.5 $S/D_{>50y}$ >2.5 PV A duration >30ms greater than MV A duration PV A flow > 35cm/s PFR <160ml/s/m^2 or $PFR_{<30}$ <2.0 EDV/s PFR_{30-50} <1.8 EDV/s $PFR_{>50}$ <1.6 EDV/s $LVdP/dt_{min}$ <1100mmHg/s τ > 48ms LVEDP>16mmHg or PCWP>12mmHg Increased LV chamber/muscle stiffness: b >0.27 and/or b' >16

LVEDIDI = left ventricular end-diastolic internal dimension index; LVEDVI = left ventricular end-diastolic volume index

The 2007 revision of the European Society criteria (14) retains this requirement for a triad of signs or symptoms of heart failure, preserved systolic function and objective evidence of

diastolic dysfunction. Tissue Doppler is given a prominent role in establishing diastolic dysfunction with an E/E' ratio of greater than 15 being sufficient to make the diagnosis. An E/E' ratio between 8 and 15 requires either elevated biomarkers or one of the additional markers to be abnormal. Biomarkers play a key role, although elevated BNP or NT-proBNP require confirmation with an additional abnormal parameter. Standard Doppler (E/A < 0.5 and deceleration time >280ms) is relegated to a confirmatory role along with left ventricular hypertrophy, atrial fibrillation, left atrial volume index >40ml/m^2 and pulmonary vein Doppler.

In 2000, Vasan and Levy proposed criteria for diagnosing diastolic heart failure based on the principles of the European criteria but introducing a hierarchy of diagnostic certainty(131). Diastolic heart failure was categorised as definite, probable and possible based on whether normal left ventricular systolic function was documented within 72 hours of an index heart failure event and whether there is objective evidence of abnormal diastolic function (Table 1-6). All three levels of diagnostic certainty require definite evidence of congestive heart failure in the form of clinical signs and symptoms, supportive investigations such as chest X-ray, and a typical clinical response to diuretic treatment.

Table 1-6 – Vasan criteria for the diagnosis of acute diastolic heart failure

Definite DHF	**Probable DHF**	**Possible DHF**
LVEF ≥ 50% within 72hrs	LVEF ≥ 50% within 72hrs	LVEF ≥ 50% outwith 72hrs
and	*but*	*and*
Abnormal LV relaxation, filling or distensibility on cardiac catheterisation	No conclusive evidence on diastolic function	No conclusive evidence on diastolic function

The presence of features such as significant hypertension during the heart failure episode, left ventricular hypertrophy or clinical improvement as a result of treating a cause of diastolic dysfunction such as tachycardia are cited as reasons to upgrade a diagnosis from possible to probable diastolic heart failure.

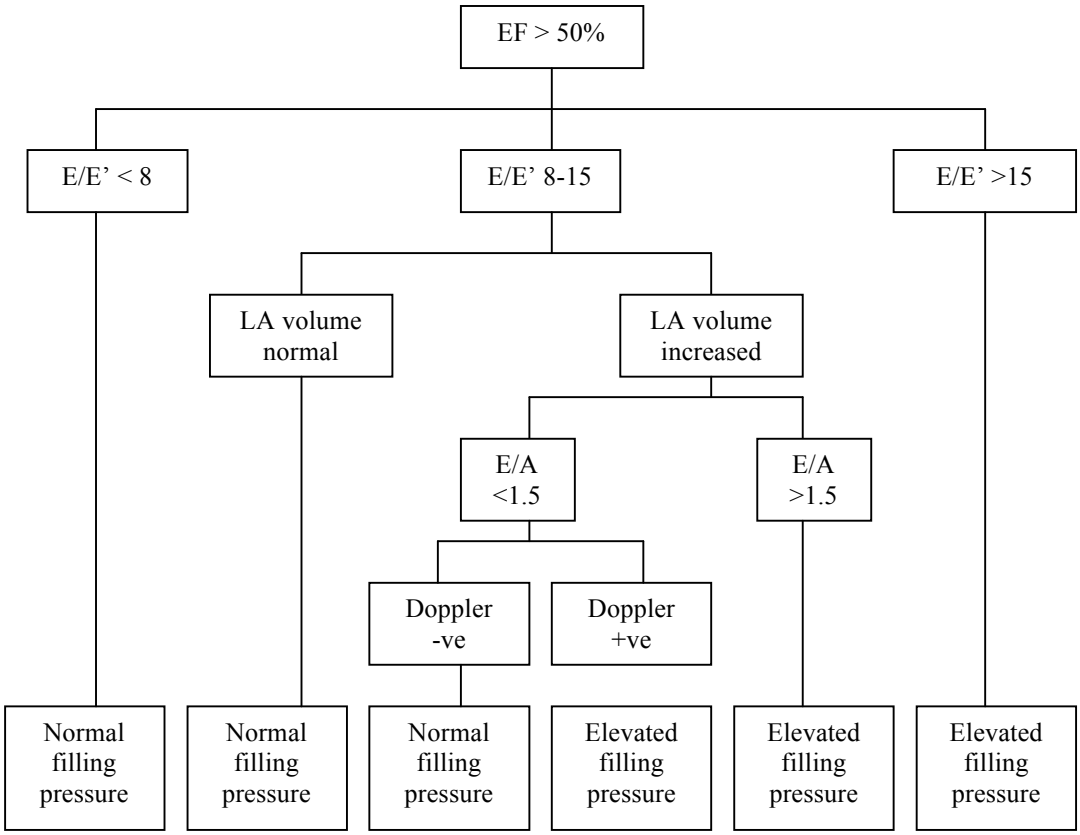
The absence of specific criteria for abnormal diastolic function and the reliance of invasive rather than non-invasive measures limits the usefulness of the Vasan criteria. Ommen and Nishimura(31) provide a useful scheme for differentiating normal from elevated filling pressures in the presence of preserved systolic function (Figure 1-15).

Once abnormal filling pressures are identified, it may be useful to go on to describe the severity of the abnormality in diastolic function. Diastolic dysfunction may be graded as grade I to IV based primarily on the E/A ratio and its response to the Valsalva manoeuvre. To refine this categorisation, cut-off values have been published for other commonly measured echocardiographic parameters (Table 1-7)(132).

Table 1-7 – Echocardiographic parameters in relation to diastolic dysfunction grade

	Normal	Grade I	Grade II	Grade III-IV
E/A	>1	<1	1-2	≥2
DT (ms)	160-210	>220	150-200	<150
IVRT (ms)	70-90	>95	60-95	<60
S/D	1.3-1.5	1.6-2.0	<1	0.4-0.6
E' (cm/s)	>8	<8	<8	<5
Vp (cm/s)	>55	<45	<45	<35
E/E'	<8			>16
E/Vp				>2.5

Figure 1-15 – Scheme for diagnosis of elevated LV filling pressures. Adapted from Ommen and Nishimura(31)



1.7 The Aetiology of Diastolic Heart Failure

Diastolic dysfunction should result from any process which interferes with the normal components of diastolic function.

Left ventricular hypertrophy arises either as a consequence of systemic hypertension, aortic stenosis, as part of hypertrophic cardiomyopathy or as a compensatory change following myocardial infarction. Whatever the underlying disorder, the endpoint and its consequences for diastolic function are the same. The increased myocardial mass itself does not appear to be responsible for impairment of diastolic function. In the physiological hypertrophy seen in athletes, diastolic function is preserved compared with similarly aged subjects with the same degree of left ventricular hypertrophy due to hypertension(133). In pathological LVH, myocyte hypertrophy is accompanied by excess collagen production. Fibrosis occurs – it is this that is responsible for impairing ventricular relaxation by reducing compliance and increasing stiffness. Passive ventricular filling is impaired and, as fibrosis progresses, elastic recoil is impaired, reducing the suction effect.

The active component of ventricular relaxation will be impaired by any process which either reduces the energy available to the myocardium or increases the energy required to drive the calcium exchange required for relaxation. In ischaemic heart disease, ischaemia caused by coronary stenosis results in abnormal relaxation at an earlier point in the ischaemic process than the systolic dysfunction seen with stress echo. The resulting rise in ventricular pressure is transmitted back through the pulmonary veins and may in part account for the clinical syndrome of effort dyspnoea as an “anginal equivalent”.

LVH also results in thickening of coronary artery walls and damage to the microcirculation, reducing coronary flow reserve. Again the athlete’s heart differs from pathological LVH. Using myocardial contrast echo and dipyridamole as a coronary vasodilator, it has been shown that abnormalities in microcirculation can be seen in hypertensive LVH(134). A different pattern is seen in athletes where function is enhanced above that seen in normal control subjects.

1.7.1 Prevalence of Diastolic Heart Failure

Estimating the prevalence of diastolic heart failure, or heart failure with preserved function, is a task which ought to be straightforward but is in fact beset with difficulties ranging from variations in what constitutes preserved systolic function, what constitutes diastolic dysfunction and whether diastolic function even needs to be assessed. Even something as fundamental as the criteria used to define heart failure can vary substantially from series to series.

As part of the MONICA project, Fischer et al(135) examined the prevalence of abnormalities of diastolic function as defined by the European Study Group on Diastolic Heart Failure(130) in a group of 1678 randomly selected individuals. Only 1418 had echocardiographic images of sufficient quality to determine left ventricular ejection fraction and 1274 of these had mitral Doppler signals suitable for analysis. Overall, the prevalence of "abnormal" diastolic function was 11.1% with a strong correlation with age – the prevalence rose from 2.8% in the 25-35 year age group to 15.8% in the 65-75 year age group (subjects aged over 75 years old were not included in the study). The presence of diastolic dysfunction, or diastolic heart failure, was not itself specifically studied but instead was inferred from the use of diuretics and/or left atrial enlargement. Using this flawed inference, the prevalence of diastolic dysfunction was calculated at 3.1%.

The prevalence of abnormal diastolic function was strongly associated with the presence of traditional risk factors, namely hypertension, left ventricular hypertrophy and coronary artery disease. It is therefore not surprising that the prevalence of diastolic abnormalities is significantly higher in males from this population in whom these risk factors, particularly coronary artery disease, are more common. This is in contrast to other studies where patients presenting with a diagnosis of heart failure with preserved systolic function have a striking female preponderance.

In Olmstead County, Minnesota during the year 1991, a total of 216 people from a total population of 106,470 received a new diagnosis of heart failure(136), retrospectively validated with a minor modification of the Framingham criteria. Although only 137 of these had an assessment of left ventricular ejection fraction, systolic function was preserved in 59 patients (43%). Only 5 of these patients had haemodynamically significant valvular heart disease to account for their heart failure. Thus, although diastolic function

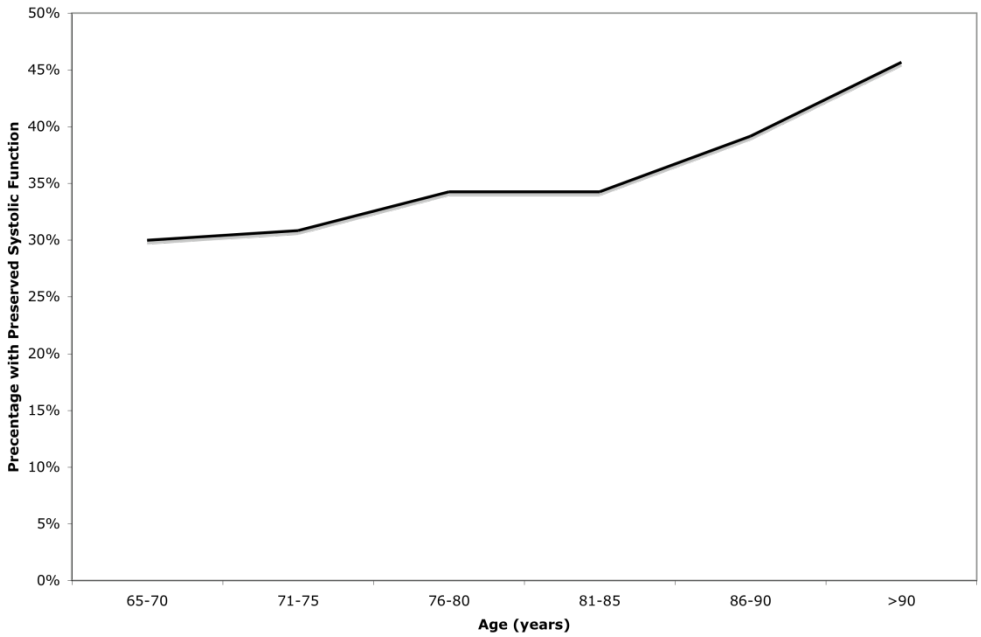
was not specifically measured, the proportion of isolated diastolic dysfunction is an estimated 39.4% of the 216 patients, giving an incidence of 0.8 cases /1000 population / year. 69% of these were female, in contrast to those with a reduced ejection fraction, where only 41% were female.

There is significant variation in the proportion of patients diagnosed with heart failure with preserved systolic function. Cowie et al(137)found only 16% of patients to have normal left ventricular systolic function in a cohort of 332 patients identified via a hospital based rapid access heart failure clinic in a 15 month period. Mean age was 75 with 54% males over the group as a whole with no breakdown given between subgroups defined by LV systolic function. Vasan et al(138) found 51% of a cohort of 73 patients from the Framingham population with a diagnosis of heart failure to have normal left ventricular systolic function with a 65% female preponderance and a mean age of 72 within this group.

It may be that the difference in prevalence of heart failure with preserved systolic function is due to different criteria used to diagnose heart failure. Cowie et al(137) use the European Society criteria for heart failure(16) whereas the Framingham criteria(15) were used by Vasan(138). Similar degrees of variation have been reported by other authors. Gardin(12) reported a 47% prevalence in a community based cohort whereas Madsen(139) reported only a 14% prevalence. Unfortunately, neither author of these two papers report their criteria for diagnosing heart failure, and only Madsen specified the LV ejection fraction cut-off used for preserved function – 53% in this case.

The true figure appears to lie between these two extremes. In the largest series to date, Masoudi et al(140) retrospectively reviewed 19,710 Medicare recipients over the age of 65 admitted to hospital with a validated diagnosis of heart failure. All of these patients had documentation of left ventricular systolic function, with normal being defined here as an ejection fraction ≥ 50%. When the percentage of patients with preserved systolic function is plotted for each 5 year cohort of patients (Figure 1-16), the inexorable rise in this with age is clear, rising from 30% in the 65-70 year group to 45.7% in patients over 90 years of age.

Figure 1-16 – Percentage of patients with heart failure in whom ejection fraction is ≥50% in relation to age (adapted from Masoudi et al.(140))



What is also striking from this data set is the striking female preponderance of heart failure with preserved systolic function. Over the whole group, female gender conferred an unadjusted odds ratio of 2.49. Even when adjusted for medical history, admission characteristics and race (Caucasians have a higher risk compared with non-Caucasians, especially African-Americans), female gender confers a relative risk of 1.71. It has been suggested that the reason for this discrepancy is, in part, a difference in left ventricular response to haemodynamic stress between the sexes, with different responses to aortic stenosis(141) and systemic hypertension(142). In Masoudi's group, adjusting for medical history should have removed the influence of any co-morbidity, assuming the model was robust. Two possibilities remain to explain this. Either factors not accounted for have influenced LV function or female gender itself exerts an influence independent of any underlying pathological processes.

1.7.2 Heart Failure With Preserved Systolic Function

The concept of preserved systolic function comes from the recognition that systolic and diastolic heart failure commonly co-exist and a desire to designate patients as having predominantly systolic or diastolic heart failure based on a cut-off point in systolic function(143). It is unclear whether this designation is in fact valid, given that a group of patients with a normal ejection fraction but abnormal invasively measured parameters of

diastolic filling can be demonstrated, these patients having isolated diastolic dysfunction. While "preserved systolic function" will indeed include a proportion of patients with normal ejection fractions, it will also include a substantial proportion of patients with mild left ventricular systolic dysfunction. Is the primary problem in this subset of patients actually mild systolic dysfunction rather than diastolic dysfunction? If so, is diastolic dysfunction even present?

The cut point for preserved versus impaired systolic function will clearly have a substantial effect on the incidence of diastolic dysfunction if one accepts the definition of predominant diastolic dysfunction rather than isolated diastolic dysfunction. Even when not considering varying imaging modalities and the variation in normal ranges introduced by this, the cut point for preserved systolic function varies significantly in the literature. For radionuclide ventriculography this has ranged from as low as 45%(144) to as high as 53% (139). With echocardiographic assessment of systolic function there is not only the issue of varying definitions of normality, there are also different techniques for assessing systolic function. The commonly used indexes are the subjective eyeball assessment of function, fractional shortening and ejection fraction. Even here there are multiple techniques which can be employed for calculating one index, ejection fraction – Simpson's biplane, the modified Quinones formula(145) and the Teicholtz formula. Although the echocardiographic ejection fraction definition of preserved systolic function is relatively consistent at between 50(146-148) and 55%(149, 150), the variation for fractional shortening, a parameter widely regarded as inferior to measurement of ejection fraction by Simpson's rule but potentially available when image quality precludes this, is more substantial. Fractional shortening values as low as 17% have been regarded as preserved systolic function(151), whilst other series have required values of 25%(152, 153) or even 30%(154).

1.8 Treatment of Diastolic Heart Failure

Before evidence based strategies for treating diastolic dysfunction can be developed it is necessary to develop a robust measure of diastolic function, and the response of this parameter to drug intervention must be measured. As has been discussed previously, the fundamental problem with this to date has been the lack of a universally accepted parameter of diastolic function that can be monitored in the manner and quantity necessary to provide evidence of treatment efficacy, or lack thereof.

As a surrogate for directly measuring diastolic function, it is possible to measure the effect of interventions on the risk factors and disease processes known to cause diastolic dysfunction.

Clearly it is possible to cause regression of left ventricular hypertrophy by adequate long-term control of blood pressure. However, the question remains as to how closely the structural changes of myocardial fibrosis correlate with left ventricular hypertrophy and its regression. Is it sufficient to measure changes in left ventricular hypertrophy and assume that the more challenging to measure indices of myocardial fibrosis have responded in the same manner? Or can changes in myocardial fibrosis be seen even without observable alterations in LVH? Indeed, to what extent is myocardial fibrosis reversible?

Some of these questions have been answered by Brilla et al(155) in a small study involving 35 patients with hypertension and LVH who were randomised to treatment with either lisinopril or hydrochlorothiazide for six months. Patients with left ventricular systolic dysfunction or any cardiovascular disorder other than left ventricular hypertrophy were excluded. Myocardial fibrosis was evaluated biochemically by hydroxyproline concentration from left ventricular endomyocardial biopsies and by collagen volume fraction following histological staining of the endomyocardial biopsies with a collagen-specific dye, Sirius Red. All patients had evidence of myocardial fibrosis at baseline. Left ventricular hypertrophy was determined by left ventricular mass index on echocardiography and by myocyte diameter from biopsy specimens. Despite no significant change in either index of LVH and no change in blood pressure from 24 hour recordings in the lisinopril group, there was a significant decrease in both parameters of fibrosis at 6 months, with the decrease in hydroxyproline concentration being highly statistically significant with a p valve < 0.00001. No significant change in myocardial fibrosis was seen in the hydrochlorothiazide group despite a significant decrease in myocyte diameter and a non-significant trend toward decreased left ventricular mass index.

Diastolic function was assessed by three non-invasive techniques, the E/A ratio, IVRT and transmitral A wave duration, and invasively by the stiffness constant k, calculated from pressure-volume measurements obtained at left ventricular angiography(89). The lisinopril group showed statistically significant increases in E/A ratio and A wave duration and a significant decrease in IVRT compared to baseline whereas these changes were not seen in the hydrochlorothiazide group. In neither group was any significant change seen in the

stiffness constant measured at left ventricular catheterisation raising the possibility that there was no genuine treatment effect on diastolic function. However, the authors highlight several significant methodological weaknesses in the technique which assumes that myocardial structure and function is homogenous, and in their equipment. The use of fluid filled catheters to obtain pressure tracings is potentially inadequate when compared with more sensitive high fidelity pressure wires for detecting small changes over the observation period.

Heart rate

Tachycardia has been recognised as common precipitating factor in diastolic heart failure. Since the relative proportion of the cardiac cycle diastole occupies decreases as heart rate rises, any impairment of diastolic function at rest will be exacerbated at higher heart rates. Clearly pathologically elevated heart rates are deleterious and have been associated with tachycardiomyopathy. In theory by reducing heart rate and increasing diastolic filling time, diastolic function will be improved. The role of rate limiting therapy with heart rates in the normal range is less clear. Improvement in diastolic function with regression of LVH has been observed with verapamil(156). No significant change in heart rate was seen however. This has also been seen where there has been no change in left ventricular mass(157). First third fractional filling does however improve when the heart rate is reduced from 77 to 60 with metoprolol(158). This improvement is lost on exercise, however, despite an albeit attenuated heart rate increase suggesting that despite diastolic function being related to heart rate, rate limiting drugs have complex effects beyond heart rate reduction.

1.8.1 Evidence Based Treatment of Diastolic Heart Failure

The lack of an evidence base in the approach to treating diastolic heart failure is striking. Until recently the only source of evidence was based largely on small scale observational studies and on extrapolation from trials with heart failure and impaired systolic function. Although both the American Heart Association(159) and the European Society of Cardiology(160) have published guidelines in recent years which included expert consensus opinions on the management of diastolic heart failure, data from large scale randomised clinical trials have been sadly lacking.

1.8.1.1 CHARM-Preserved

As a pre-defined substudy of the main CHARM trial(161), CHARM-Preserved(1) assessed the impact of candesartan on the combined end point of cardiovascular mortality or admission to hospital for worsening heart failure. Patients here were diagnosed as having heart failure with preserved systolic function on the basis of symptomatic heart failure (NYHA II-IV), a history of hospital admission for cardiac reasons and an ejection fraction ≥ 40%. Diastolic function itself was not measured either at enrolment or during follow-up and was not used to assess the efficacy of candesartan.

A total of 3025 patients were randomised, with approximately 60% in NYHA class II and 38% NYHA class III heart failure. After a median follow-up of 36.6 months there was no significant difference in either the primary composite endpoint or in cardiovascular death alone. However, there was a significant reduction in single hospitalisation for heart failure (230 vs 279, p=0.017) as well as a trend towards a reduction in the number of patients with multiple admissions. There was no difference in the number of hospital admissions for any cause, however.

The CHARM population were, on the whole, a sick patient group. 68.7% had previously been admitted to hospital for heart failure. Nearly 45% of patients had previously suffered a myocardial infarction with 27.5% suffering angina at enrolment. Prior coronary revascularisation was common – overall 21.6% having had coronary artery bypass grafting and 17.3% with percutaneous revascularisation. Unsurprisingly, the primary cause of heart failure was judged to be ischaemic in 56% of patients. Given that the role of inhibition of the renin-angiotensin system in stable coronary artery disease has been established by the HOPE (162) and EUROPA(163) trials, it is possible that the treatment benefit seen here is due to a beneficial effect of candesartan on coronary artery disease rather than diastolic function.

1.8.1.2 PEP-CHF

The Perindopril in Elderly People with Chronic Heart Failure (PEP-CHF) trial(164), which began in 1999 and was due to report in 2001, was finally presented at the World Congress of Cardiology in Barcelona, 2006. The substantially increased trial duration was necessitated by an event rate lower than predicted in combination with slow recruitment. In addition there was a high rate of cessation of blinded therapy with open-label ACE

inhibitor use. The trial compared perindopril at a target dose of 4mg with placebo in patients aged 70 and over with a diagnosis of diastolic heart failure. To qualify, patients were required to have had a cardiovascular hospitalisation in the 6 months leading up to randomisation and have a left ventricular ejection fraction of greater than 40%. In addition, patients were required to meet at least 3 clinical and 2 echocardiographic criteria from the following: exertional dyspnoea; orthopnoea or paroxysmal nocturnal dyspnoea; ankle swelling; improved dyspnoea with diuretic therapy; increased jugular venous pressure; prior episode of clinical pulmonary oedema; prior MI; cardiothoracic ratio >0.55; and previous radiological pulmonary oedema. Echocardiographic criteria were mild left ventricular systolic dysfunction (ejection fraction 40-50%); left atrial diameter >40mm or 25mm/m^2 body surface area; left ventricular hypertrophy; and one or more abnormal parameters of diastolic function defined by the European Society of Cardiology Study Group on Diastolic Heart Failure(130). Any patients in atrial fibrillation were regarded as meeting this last criterion.

The primary endpoint was a composite of all-cause mortality and unplanned heart failure related hospitalisation. After a mean follow up of 26 months there was no difference between treatment and placebo groups for the primary endpoint. A decrease approaching statistical significance was seen at 12 months (p=0.055) at which point 90% of patients randomised to the active arm were receiving 4mg of perindopril. Thereafter the Kaplan-Meier curves converge. By 18 months only 60% of the treatment group were receiving their study medication and by the end of the study 36% of all patients were receiving open-label ACE inhibitors. Although this may account for the apparent loss of treatment benefit beyond 1 year, it is also possible that the low event rate combined with short follow-up time has exaggerated the treatment benefit at 1 year. The increasing trend for discontinuation of randomised trials for early treatment benefit has attracted criticism partly for this reason(165, 166).

As with other trials looking at diastolic heart failure, the combination of a lack of a gold standard definition of diastolic heart and necessarily loose entry criteria allow inclusion of patients without heart failure but signs and symptoms due to other causes. Such a situation results in a heterogenous group of patients with multiple co-morbidities which will dilute and contaminate the treatment effect of the drug in question.

1.8.1.3 I-Preserve

The I-Preserve trial (167, 168) aimed to build on the encouraging findings on the use of angiotensin receptor blockers seen with candesartan in the CHARM-Preserved trial(1). Here irbesartan was used in a double blind placebo controlled manner in 4128 patients followed up for a mean of 49.5 months. The primary end points were all cause mortality and cardiovascular hospitalisation (a composite of worsening heart failure, unstable angina, myocardial infarction, ventricular or atrial arrhythmia or stroke). The sample size was increased in April 2004 from 3600 due to a lower than anticipated event rate, similar to the problem which befell PEP-CHF.

The key difference from CHARM were the additional criteria required in an attempt to strengthen the diagnosis of diastolic heart failure. Although diastolic function was not itself measured, these criteria aim to add additional weight to the heart failure diagnosis with the implication that in the presence of "preserved" systolic function (here defined as left ventricular ejection fraction ≥ 45%), the diagnosis must be diastolic dysfunction. To be enrolled, patients required either ongoing NYHA class II or worse symptoms with a hospitalisation for heart failure within the previous 6 months or ongoing NYHA III or IV symptoms with at least 1 of chest x-ray evidence of pulmonary congestion, ECG or echocardiographic evidence of moderate or severe left ventricular hypertrophy, left bundle branch block on ECG or an enlarged left atrium in the absence of atrial fibrillation.

There was no difference in the mortality rate between the irbesartan or placebo group (52.6 and 52.3 per 1000 patient years respectively). There was a non statistically significant decrease in the other primary endpoint of cardiovascular hospitalisation in the irbesartan group (70.6 versus 74.3 per 1000 patient years, p=0.44). There was also no significant difference in any of the secondary endpoints which included disease specific quality of life, assessed by the Minnesota Living with Heart Failure scale(169).

1.8.1.4 Hong Kong Diastolic Heart Failure study

This pilot study(170) assessed the efficacy of ramipril and irbesartan in 150 patients of mean age 74. Diastolic heart failure was defined as a clinical history of heart failure supported by a chest x-ray showing pulmonary congestion, and left ventricular ejection fraction of >45% on echo. Patients were randomised to diuretic alone, diuretic plus ramipril, or diuretic plus irbesartan.

With only 50 patients in each subgroup, the study was under-powered to detect any difference between the treatments for one of the primary outcomes, quality of life score measured by the Minnesota Heart Failure Symptom Questionnaire. There was, however, a significant improvement within each subgroup from baseline. There was no significant difference in exercise capacity, with a small but not statistically significant increase in 6 minute walking distance. The other primary outcome, echo derived left ventricular mass and tissue Doppler derived E' and E/E' showed no significant difference at 1 year. An important error in the methodology should be noted with regards the tissue Doppler values. The authors have used colour coded tissue Doppler which yields systematically lower values for E' than spectral Doppler(44), the modality used in the main reference papers(32, 48, 49), one of which is used as a reference by the authors. This results in the E/E' values for the patients in this study being artefactually high when compared to this reference data.

NT-proBNP was measured at baseline and during follow-up. There was a very large spread at baseline with a range of 5-4748pg/ml. Statistically significant reductions were seen in the ramipril and irbesartan subgroups.

1.8.1.5 SWEDIC

The SWEDIC trial(171) evaluated the use of carvedilol in patients with symptoms of heart failure but preserved systolic function as assessed by echocardiography. A total of 97 patients completed the study, having being randomised to either carvedilol (target dose 25mg bd or 50mg bd depending on body weight) or placebo for a total of 6 months. Entry to the trial required one of several markers of diastolic function to be abnormal:

- E/A ratio < age related reference range
- IVRT > age related reference range
- Normal E/A ratio with abnormal pulmonary venous flow

Patients with chronic obstructive pulmonary disease, significant valvular heart disease, unstable angina, atrial fibrillation, restrictive or hypertrophic cardiomyopathy, evidence of conducting system disease and either hypotension or uncontrolled hypertension were excluded.

The results were disappointing. The only parameter that showed any statistically significant change from baseline, albeit borderline, was the E/A ratio which rose from 0.71 to 0.76 in the placebo group and from 0.72 to 0.83 in the treatment group with a quoted p

value of 0.046. More importantly, there was no significant change in patients' symptoms. In fact, there was a trend toward patients doing better on placebo than carvedilol with 12 compared to 2 improving their NYHA class, and 8 compared to 14 deteriorating. The lack of change in patients' status can also be seen in their BNP levels. BNP was 8 pmol/L in both groups at baseline and was unchanged in the placebo group, rising to 12 in the carvedilol group. Perhaps the reason for the lack of any clinical effect relates more to the lack of evidence of heart failure than the lack of efficacy by carvedilol.

1.8.1.6 SENIORS

The SENIORS(172) trial set out to address the issue of the role of beta blockade in the treatment of heart failure in a patient group largely excluded from previous trials, the elderly. To qualify for this placebo controlled trial of nebivolol, a vasodilating β_1 selective blocker, patients had to be aged at least 70 and either have a hospital admission with discharge diagnosis of heart failure within the last year or documented left ventricular ejection fraction within the last month. Patients with heart failure secondary to valvular heart disease and those with coronary artery disease awaiting either surgical or percutaneous revascularisation were excluded. A left ventricular ejection fraction of 35% was used to dichotomise preserved and impaired systolic function, with 35% of patients having preserved function. Diastolic function was not specifically used to include or exclude any patients from the preserved systolic function group but was monitored in an echocardiographic substudy(173).

After a mean follow-up of 20 months, the primary endpoint, a composite of all-cause mortality or hospital admission for cardiovascular cause was seen in 31.1% of patients treated with nebivolol compared to 35.3% given placebo, an absolute risk reduction of 4.2% with a p value of 0.039. No significant difference was seen between those with preserved and those with impaired left ventricular systolic function suggesting that improved outcome is seen with nebivolol in heart failure with preserved systolic function.

However, the diagnosis of diastolic heart failure was never robustly established in this patient group. Although diastolic function was measured as far as the E/A ratio, mitral deceleration time and left atrial area, these parameters were not used to further categorise patients. Baseline parameters were essentially normal with a mean E/A ratio of 0.9 and deceleration time of 200ms. These parameters did not change over a 12 month treatment period. This therefore raises the question as to whether these patients ever had heart

failure, let alone diastolic heart failure. The accuracy of hospital discharge diagnoses must always be regarded with some caution. The fact that this will have been used as a sole entry criterion in all of the patients with "preserved" systolic function and a proportion of those with ejection fractions <35% must be viewed as a weakness. Given that diastolic function as measured could be regarded as probably normal at baseline and no treatment effect could be discerned, it might be reasonable to infer that the improved outcomes were due to beneficial effects on co-morbidities rather than diastolic function.

1.8.1.7 Caldaret (MCC-135)

Caldaret, also known as MCC-135, is a compound modulating sarcoplasmic calcium handling. It has been investigated as an agent for attenuating intracellular calcium overload and reducing infact size in reperfusion injury models(174). In clinical trials, as an adjunct to percutaneous coronary intervention, no benefit was seen(174). The MCC-135-GO1 trial(175) is a phase II randomised double blind placebo-controlled trial in 500 patients with heart failure, 230 of whom have diastolic heart failure. Although data collection has been completed, and the study due to report in 2004, no results have been published to date.

1.9 Background to this Thesis

The concept of the clinical entity of diastolic heart failure has been with us for many years now. However, it is a diagnosis which, although in theory easy to define and therefore apply to patients, is in practice beset with difficulties not only in establishing widely applicable diagnostic criteria but also in applying these criteria in the presence of confounding diagnoses. In 2000 Caruana et al(176) reported on a series of patients referred to an open access echocardiography service with suspected heart failure. In the two thirds of their patients who did not have left ventricular systolic dysfunction diagnoses other than diastolic dysfunction, where found, were assumed to account for symptoms. Although pulmonary function was assessed by means of spirometry and was abnormal in 92% of the group, ischaemic heart disease was not assessed beyond a self reported history and ECG, and diastolic function was not assessed beyond the E/A ratio of mitral inflow.

From this starting point it is clear that when assessing patients with suspected heart failure and preserved systolic function we must consider alternative diagnoses as well as diastolic

dysfunction. However, the presence of these diagnoses should not in themselves be taken as evidence that diastolic dysfunction is not present. To properly characterise this group of patients we need not only to assess the presence of alternative diagnoses formally, but also robustly assess diastolic function using multiple techniques. Another factor we must consider is the suitability of echocardiography in its role as sole arbiter of left ventricular systolic function. It is well recognised that echocardiography becomes more difficult to perform reliably in the presence of obesity and respiratory disease. Yet, it is precisely these conditions that are so predominant in the patients studied by Caruana et al.

Radionuclide ventriculography is well established as a reliable and reproducible means of assessing left ventricular systolic function and is not affected by the factors that make echocardiography difficult. In addition it provides another modality for assessing diastolic function by means of first third fractional filling, peak filling rate and other indices. Stress RNVG has previously been used in the assessment of systolic function and diagnosis of myocardial ischaemia. It has been used to a lesser extent in the assessment of diastolic function. An improvement in diastolic function on exercise following percutaneous coronary revascularisation has been shown by stress RNVG(60). Since the symptoms associated with diastolic dysfunction are, like cardiac ischaemia, largely present on exertion rather than at rest, it would therefore be reasonable to assume that assessment of diastolic function should also be performed under stress conditions. The key question to be answered is whether the assessment of diastolic function on stress will indeed improve our diagnostic accuracy, and whether stress RNVG is the correct tool for this purpose.

Central to this is the reproducibility of the RNVG parameters of interest. This will have a critical influence on the normal ranges which must be defined both at rest and on stress. The interplay between reproducibility and breadth of the normal range will determine which, if any, of the RNVG parameters are of diagnostic utility.

Taking the work of Caruana et al as a starting point, a protocol was developed to identify a similar group of patients in whom diastolic heart failure could be diagnosed on clinical grounds. Detailed characterisation of this patient group beyond that which had previously been undertaken would be achieved by:

- Formal assessment of ischaemic heart disease by myocardial perfusion scanning.
- Spirometry in all patients.

- Use of tissue Doppler to enhance the echocardiographic assessment of diastolic function.
- The assessment of left ventricular ejection fraction by RNVG to validate its echocardiographic evaluation.
- The assessment of diastolic function both at rest and stress by RNVG.
- The addition of VO_2 to stress RNVG to improve the differentiation of respiratory and cardiac limitation to exercise.
- The use of NT-proBNP to provide biochemical evidence of heart failure.

1.10 Hypothesis

The assessment of diastolic function on stress by RNVG is more sensitive and correlates better with other indices of diastolic function than assessment at rest.

In addition, with the support of a successful BHF Project Grant, this thesis sought to answer a further series of questions:

In patients presenting with suspected heart failure and normal left ventricular systolic function:

- In what proportion of patients is the limitation in effort capacity due to cardiac causes? Other causes of limitation, including respiratory disease and lack of physical fitness without evidence of cardiopulmonary abnormalities, will be documented.
- What is the prevalence of left ventricular diastolic dysfunction as measured by radionuclide ventriculography?
- Among patients with exercise induced left ventricular diastolic dysfunction, what proportion will have evidence of reversible myocardial ischaemia?

2. Methods

2.1 Recruitment – Patients

Patients were primarily identified through contact with the local open access echocardiography service following referral for assessment of dyspnoea of suspected cardiac cause. This involved a combination of ongoing prospective identification of patients as well as the retrospective identification and reassessment of patients previously referred to the service. It was also possible to recruit a small number of patients from other sources on an ad-hoc basis.

Inclusion criteria:

- Exertional dyspnoea
- Preserved left ventricular systolic function by visual echo assessment
- Able to exercise using bicycle ergometer
- Pulmonary rales – not an absolute requirement
- Peripheral oedema – not accepted as an entry criterion if present without dyspnoea

Exclusion criteria:

- Established ischaemic heart disease
- Previous myocardial infarction
- Previous coronary revascularisation – either percutaneous or surgical
- Angiographically proven coronary artery disease
- Angina confirmed on stress testing
- Significant valvular heart disease i.e. any valvular stenosis or regurgitation greater than mild in severity
- Morbid obesity with BMI >40
- Chronic obstructive pulmonary disease confirmed by pulmonary function testing

Although parameters of diastolic function were measured as part of the echocardiogram at entry to the study, these did not form any part of inclusion or exclusion criteria.

Ethical approval for the study was granted by the North Glasgow Research Ethics Committee (project number 02MC005).

2.1.1 Prospective Open Access Echo Recruitement

A GP open access echocardiography service has been established at Glasgow Royal Infirmary for many years. Patients are primarily referred for assessment of left ventricular function in suspected heart failure, for assessment of murmurs, and for assessment of hypertensive heart disease. Thirteen slots are available per week across two sessions. Each patient would have an electrocardiogram followed by a transthoracic echocardiogram performed and interpreted by an experienced echocardiographer. From a retrospective audit of the service, approximately 50% of referrals were for assessment of left ventricular function with the vast majority having normal or "preserved" systolic function without significant valvular heart disease. It was therefore estimated that that this source would yield between two and four suitable patients per week

Potentially suitable patients were identified prior to arrival on the basis of referral indication and past medical history provided by the General Practitioner on the referral form. A cardiac and respiratory history was obtained prior to echocardiography and the symptoms of the patient were assessed. Where the symptoms were not consistent with a cardiac cause of dyspnoea, the patient was not assessed further for recruitment purposes. Patients who gave a previously undisclosed cardiorespiratory history at this stage were similarly excluded.

Provided the echocardiogram met the entry criteria on the basis of left ventricular systolic function and valvular function, the patient was invited to participate. Subsequent participation involved a single day visit for all other elements of the study protocol. Where possible, this was arranged with the patient at the time of recruitment. Patients were given an information leaflet that included contact details and were free to withdraw from the study at any point.

2.1.2 Retrospective Open Access Echo Recruitment

Due to patient co-morbidity being higher than anticipated, the number of patients meeting inclusion criteria prospectively from the open access echocardiography service was significantly lower than anticipated. Therefore, it became necessary to augment recruitment from other sources.

Using archived results from Glasgow Royal Infirmary, patients who had attended the open access echocardiography service over the previous three years were assessed for eligibility

for the study. As key exclusion criteria for recruitment to the prospective arm were established diagnoses of ischaemic heart disease or respiratory disease, a screening process was undertaken. The echocardiogram result and referral information were initially reviewed to create a list of patients of potential interest. The clinical history given here was augmented by examining the computerised records of each patient's hospital clinic attendances with particular regards cardiology and respiratory clinics. These records typically extended in excess of 5 years. These clinic attendances were used to enable a targeted search for previous myocardial perfusion scanning and pulmonary function testing

Where a patient had attended a cardiology clinic, the records of nuclear cardiology were searched for any thallium scans relating to that patient. Where the thallium scan was abnormal or no scan was found, the patient was excluded from further consideration. Patients with negative scans subsequently discharged from clinic were considered potentially suitable. Those with more than one clinic visit post scan were disregarded.

For patients who had attended respiratory clinics, a search was made for previous results of pulmonary function testing. Patients with normal spirometry, or full pulmonary function tests if that is what had been performed, were considered potentially suitable. All others were disregarded.

For the remaining cohort of potentially suitable patients, the general practitioner of each patient was contacted via post announcing our intention to contact the patient and invite them to participate. No objections were raised by any general practitioner. Telephone contact was then made with the patient. The nature and purpose of the study were briefly explained to the patient. Provided they were agreeable in principle, their symptoms were verified with respect to whether they were still present, had progressed, and were potentially consistent with a cardiac cause. Medical history was also explored to identify disqualifying medical conditions. Patients remaining suitable at this stage underwent an echocardiogram to update left ventricular systolic function if none had been performed in the last 6 months. If this was within acceptable limits the patient was formally recruited.

A similar open access echocardiography service also exists at Stobhill Hospital. The archived results for the previous two years were screened and patients recruited in a similar fashion to that described above.

2.1.3 Ad-Hoc

Patients thought to have an anginal equivalent, the syndrome of exertional dyspnoea due to myocardial ischaemia but without typical chest pain, were another potential source of patients. Those in whom ischaemia was excluded on perfusion imaging were considered to meet entry criteria provided they had been demonstrated to have normal left ventricular systolic function and either proven normal respiratory function or a low index of suspicion for pulmonary disease. Given that myocardial perfusion imaging was typically further down the line of diagnostic tests than echocardiography or spirometry, this could be assumed for the majority of patients in this category and subsequently verified.

Patients, from any source, whose request form indicated suspected heart failure were screened prior to appointment. These were then scheduled appropriately to enable all the additional tests required for the study to be performed, subject to consent for study participation being obtained and the thallium scan showing no significant abnormality. The stress perfusion images, along with the patient's suitability for stress RNVG, were reviewed immediately after acquisition. Only patients with normal stress perfusion were recruited – those with perfusion defects, even if these were thought likely to be due to tissue attenuation, were not.

2.2 Recruitment – Control Subjects

2.2.1 Exercise RNVG

To enable an in-house reference range for exercise RNVG parameters to be constructed it was necessary to recruit normal subjects of approximately the same age as the patients being recruited. As the sole purpose of these subjects was to generate these reference data, they did not have echocardiography or spirometry performed and did not have their blood taken for NT-proBNP assay. VO_2 max and anaerobic threshold were not required either in these subjects and they therefore did not have their exhaled gases collected during the exercise RNVG.

A similar method to the ad-hoc method of patient recruitment was employed to recruit control subjects for exercise RNVG. Potentially suitable patients were identified on the basis of their referral criteria being either atypical chest pain, or typical angina with angiographically normal coronary arteries

Inclusion criteria:

- No significant perfusion defect on thallium scintigraphy
- Normal resting ECG

Exclusion criteria:

- Established ischaemic heart disease
- Previous myocardial infarction
- Previous coronary revascularisation – either percutaneous or surgical
- Angiographically proven coronary artery disease
- Angina confirmed on stress testing
- Exertional dyspnoea or documented left ventricular dysfunction
- Chronic obstructive pulmonary disease

As before, subjects pre-screened as being potentially suitable had their thallium scan scheduled at an appropriate time to permit exercise RNVG later that day provided they proved to be suitable. Those meeting criteria with normal stress perfusion were recruited as before. Those with perfusion defects thought to be consistent with tissue attenuation had their redistribution perfusion images reviewed. They were considered suitable if the whole stress-redistribution thallium scan showed only tissue attenuation artefact but no significant reversible perfusion defect.

2.2.2 Rest RNVG

An archive of request forms, final reports, demographic, medication and co-morbidity data is maintained on all patients undergoing thallium scanning at Glasgow Royal Infirmary. In addition, an electronic archive is kept of the formatted studies for all thallium scans and RNVGs. This includes the regions of interest used to generate the left and right ventricular ejection fractions issued on the final report. Using similar criteria to those used in the prospective recruitment of control subjects for exercise RNVG, this archive was searched for patients meeting entry criteria. Patients with incomplete data, typically prescribed medication, were excluded. Where necessary the perfusion images were reviewed to resolve any ambiguity in the report.

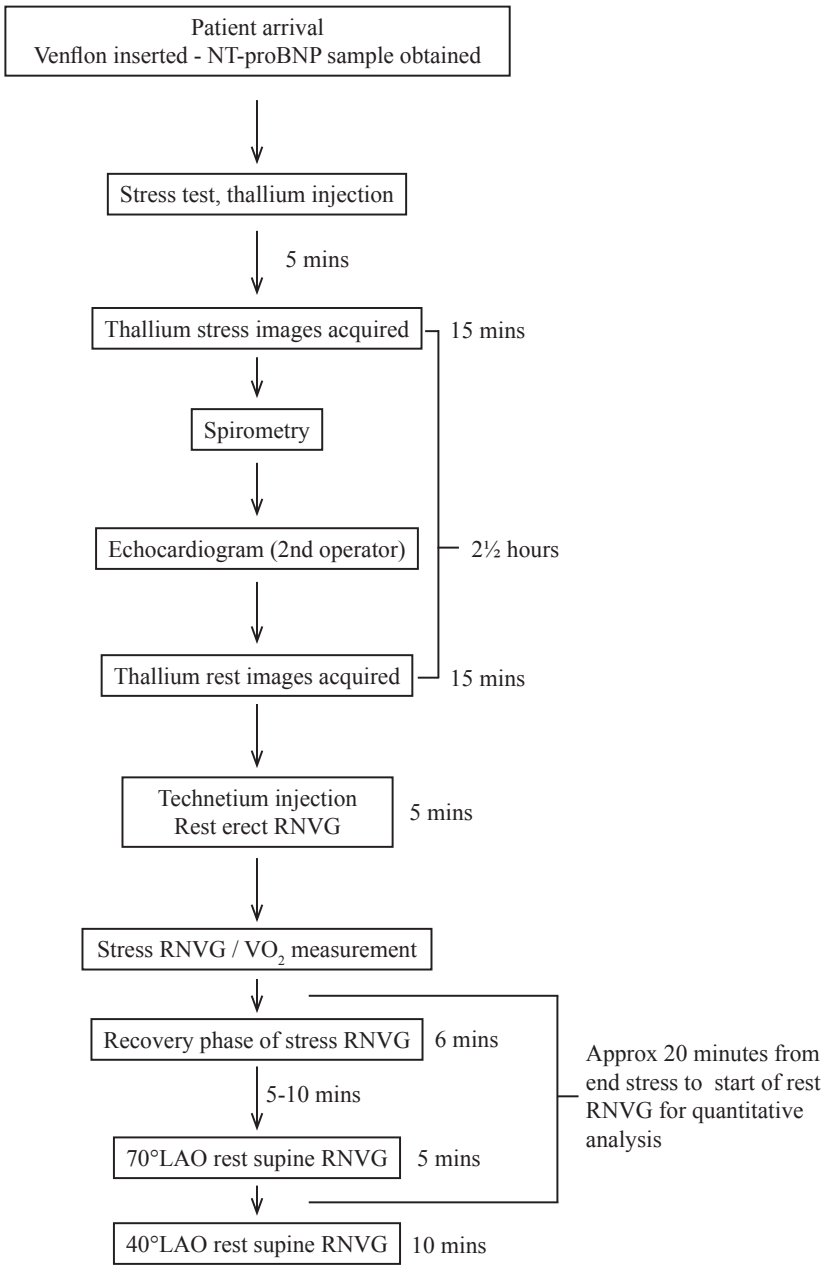
2.3 Study Protocol

For the majority of patients who were recruited at echocardiography, the following tests were scheduled to take place over the course of a single day in order to answer the questions posed by the thesis:

- Myocardial perfusion imaging
- Resting radionuclide ventriculography
- Stress radionuclide ventriculography with VO_2 measurement
- Spirometry
- Repeat echocardiography by a second operator
- N-terminal proBNP measurement

The sequence and timescale of these is illustrated in Figure 2-1. A minimum of 2.5 hours is required between thallium stress and redistribution/rest images. During this window, the second echocardiogram and spirometry were performed.

Figure 2-1 – Typical patient study pathway



2.4 Echocardiography

2.4.1 Equipment

Three echo machines were used in the course of this work. On the Glasgow Royal site, the majority of echos were carried out on a GE Vingmed Vivid7 equipped with a M3S probe. The remainder were carried out on a GE Vingmed Vivid3 using a 3S probe. Both machines were linked to an EchoPac machine enabling a combination of on and off-line image analysis. On the Stobhill site all echos were performed using a Vingmed Vivid5 machine, also linked to EchoPac software.

2.4.2 Operators

All patients had an echocardiogram performed by AM[c]C. In order to assess inter-observer and intra-observer reproducibility of the parameters being used to assess systolic and diastolic function, all patients also had a second echocardiogram performed. These were predominantly carried out by the author, or by one of two British Society of Echocardiography (BSE) accredited sonographers (JJ and LF). Although JJ and LF were not blinded to the participation of the patients in the study, the results of other investigations on the patients were not available to them. Their analysis and interpretation of the second echocardiogram was carried out without reference to the first study.

2.4.3 Protocol

A standard set of 2D views were obtained on all patients, allowing for patient echogenicity. This was based on the minimum BSE dataset and comprised:

- Parasternal long axis (PLAX)
- Parasternal short axis, papillary muscle level (PSAX). Aortic valve, mitral valve and apical levels were also examined and stored.
- Apical 4 chamber (4ch)
- Apical 2 chamber (2ch)
- Subcostal, including view of inferior vena cava

At least one cardiac cycle was acquired in each of the views. Images were stored digitally as raw data, using magneto-optical disks for long term storage to enable retrospective data analysis. Valvular regurgitation was assessed by colour flow Doppler in at least 2 imaging

planes with quantitative evaluation by spectral Doppler in the appropriate plane for the valve concerned. All images were acquired using 2nd harmonic imaging. It has been observed that 2nd harmonic imaging may produce a systematic overestimate of left ventricular mass compared with fundamental imaging(177). This should be kept in mind with all measurements here.

Table 2-1 – Standard 2D and M-Mode Echocardiographic Measurements

Measurement	Preferred Modality	View Used
Left atrial diameter	M-Mode	PLAX
Aortic root diameter	M-Mode	PLAX
RV diastole, systole	M-Mode	PLAX
IVS diastole, systole	M-Mode	PLAX
LV end diastole	M-Mode	PLAX
LV end systole	M-Mode	PLAX
Posterior wall diastole, systole	M-Mode	PLAX
Left ventricular mass index	M-Mode derived	PLAX
LV ejection fraction (Teicholtz)	M-mode derived	PLAX
LV ejection fraction (Simpson's Biplane)	2D planimetry	4ch, 2ch
IVC inspiration, expiration	2D	Subcostal

All M-mode measurements were carried out using the leading edge to leading edge method.

Table 2-2 – Standard Doppler Measurements

Measurement	Modality
Mitral E velocity	Pulsed wave Doppler
Mitral A velocity	Pulsed wave Doppler
Mitral E/A ratio	Pulsed wave Doppler
Mitral deceleration time	Pulsed wave Doppler
Isovolumetric relaxation time	Pulsed wave Doppler
Aortic valve peak velocity	Continuous wave Doppler
Aortic valve velocity time integral	Continuous wave Doppler
Tricuspid valve peak regurgitant velocity	Continuous wave Doppler
Mitral E' velocity – septal, lateral annulus	Pulsed wave tissue Doppler
Mitral E/E' ratio – septal, lateral annulus	Derived

A sweep speed of 100mm/s was used for all measurements. A minimum of sequential 3 cardiac cycles with adequate quality were averaged to obtain values. Extra-systolic and post extra-systolic beats were not used for any measurements.

2.5 Perfusion Imaging

Thallium 201 was used as the myocardial perfusion agent in all patients. A one day stress-redistribution protocol was used in all patients with a single 60MBq dose of thallium administered for the stress images. As a high sensitivity collimator was used for all image acquisition, similar counts could be collected at this reduced dose compared to an 80MBq dose imaged with a general purpose collimator. Redistribution images were obtained after a minimum 2½ hour period, typically around 3 hours. Patients did not receive a second dose of thallium for rest images and there was no-reinjection of thallium in the event of persisting defects.

2.5.1 Stress Protocol

Upright bicycle exercise was employed as the stressing modality of choice. A significant proportion of the department's workload consists of patients unable to exercise adequately on the treadmill. Resting heart rate, blood pressure and 12 lead ECG were obtained in all

patients. Exercise would then commence at an initial workload of 50W. Continuous monitoring of the 12 lead ECG was carried out and blood pressure was measured after 2 minutes at each workload using a manual anaeroid sphygmomanometer. After 3 minutes the workload would be increased by either 25W or 50W based on a judgment of the remaining exercise capacity of the patient. A minimum of 3 minutes exercise at 50W was accepted as adequate stress. Failure to achieve this prompted a switch to pharmacological stress. This threshold was chosen rather than a heart rate target on the basis of previous in-house validation that showed no difference in sensitivity for ischaemia detection compared to dipyridamole stress.

The criteria for terminating exercise were: ECG changes diagnostic of ischaemia, significant chest pain, limiting dyspnoea, significant arrhythmia, fall in systolic blood pressure of $\geq$ 20mmHg or fatigue. Patients were asked to indicate when they felt they were approaching maximal exercise capacity. Thallium was injected at this stage with an appropriate flush. Exercise was continued for a further 30 seconds beyond injection time to permit adequate myocardial uptake. As thallium has a myocardial extraction fraction of approximately 85%, this 30 second period permits 2 passes through the coronary circulation and therefore $\geq$ 95% of the total myocardial uptake of thallium occurring whilst at peak exercise.

For a minority of patients unable to exercise at sufficient intensity to adequately stress the heart, pharmacological stress was undertaken using intravenous dipyridamole. Patients were given a total of 0.56mg/kg dipyridamole over a period of 4 minutes. Following this a 3 minute period of either low intensity cycling or isometric isometric arm lifts using 1kg weights was undertaken. Thirty seconds before the end of this period thallium was injected.

Beta blockers and other heart rate limiting medication was not discontinued for the stress test. This is standard departmental policy on the basis that the symptoms of presumed myocardial ischaemia were present despite beta blockade and should therefore still be detectable on perfusion imaging. There is also a small but non-zero risk in discontinuing beta blockers for stress testing which was a second factor in this policy decision.

2.5.2 Image Acquisition

Both stress and redistribution perfusion images were acquired using one of 3 gamma cameras, these being a General Electrics (GE) CamStar 3000, GE StarCam or GE Optima.

A high sensitivity parallel collimator was used for all image acquisition. Planar images were acquired in 3 views: anterior, 40° LAO, and 70° LAO projections. Raw ECG gated data were acquired in List Mode from the camera using Link MAPS 10000 software running on Sun workstations. This allows accurate reconstruction of a representative cardiac cycle. Images were acquired for a total of 5 minutes in each projection and were formatted after acquisition was complete. Acquisition of all 3 stress images was completed within 25 minutes of injection of thallium.

When processing the raw data, all heart beats regardless of cycle length or likelihood of artefact were accepted. A cine loop of 8 frames per cardiac cycle was created for all stress and rest images to permit wall motion scoring. A single frame comprising all data collected during each image acquisition was generated by summating these 8 frames.

2.5.3 Image Analysis

All image analysis was on a dual reporter basis as is standard practice within our department. Stress and rest perfusion along with wall motion scoring would be undertaken first by one experienced observer. A second observer would later review the images along with the first observer's interpretation of them. Minor modifications could be made to the initial analysis without further review but where a significant difference of opinion remained over interpretation, either the dispute would be resolved by further review and mutual agreement or a third observer would make a determination. In practice significant differences of opinion are uncommon and usually restricted to cases where image quality is suboptimal for either technical reasons or patient factors such as high lung or high subdiaphragmatic uptake of thallium. The latter can be a particular problem with dipyridamole stress where there has been a minimal exercise component or with an unfavourable body habitus.

Images were reported using a standard template depicting all 3 acquired projections with stress and rest images being reported on separate parts of the template. The left ventricle was divided into 5 segments on each of the 3 projections which were from the top right segment in each projection (Figure 2-2 and Table 2-3).

Figure 2-2 – Planar myocardial perfusion assessment scheme

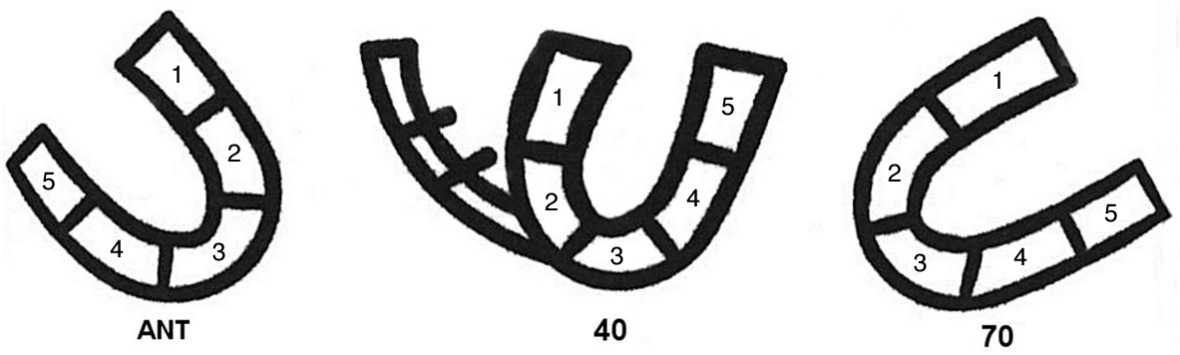


Table 2-3 – Planar myocardial segment designation

Segment	Anterior	40° LAO	70° LAO
1	Basal anterior	Basal septal	Basal anterior
2	Mid/distal anterior	Mid/distal septal	Mid/distal anterior
3	Apical	Apical	Apical
4	Mid/distal infero-septal	Mid/distal lateral	Mid/distal posterior
5	Basal infero-septal	Basal lateral	Basal posterior

Additionally, the right ventricle was divided into 3 segments. Although the right ventricle could not usually be seen on the summated image due to its significantly lower myocardial bulk, it is usually easily discernable when viewing the 8 frame cine images using a sixteen shade greyscale display.

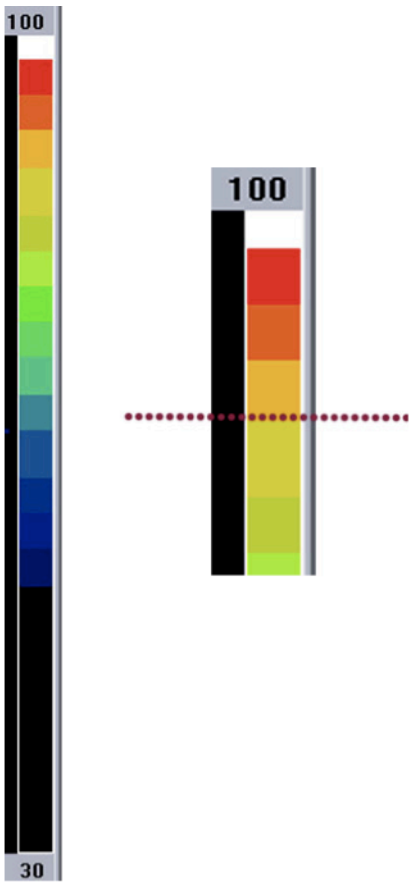
Images were displayed with a default background setting of 30%. Overall gain/saturation was determined automatically by the software based on having a standard number of pixels of maximum intensity. Where another organ or artefact created an object of higher count intensity than the myocardium this would result in the myocardial counts being scaled down. In this case background and gain settings were adjusted to give 3 to 4 pixels within the myocardium of maximum intensity. It should be noted that both the automatic and manual approach rely on the assumption that at least one portion of the myocardium within the field of view has normal perfusion. It is possible that this assumption may not be valid if there is widespread uniform ischaemia. Where this is the case abnormally high lung uptake will usually be present, whether this is due to ischaemia induced lung uptake of thallium or to artefact produced by the scaling changes required to elevate a portion of the myocardium to maximal perfusion.

Perfusion images are displayed using a 16 colour rainbow scale (Figure 2-3). In this example, background has been left at its default setting of 30 causing everything below this

threshold to be displayed as black. The image can be optimised by adjusting either end of the scale, expanding or compressing it accordingly.

The top four colours in the scale are defined as normal perfusion (Figure 2-3, above dotted line). Abnormal perfusion is defined as any segment of myocardium with a lower colour on the scale than this threshold. The lower down the scale, the more severe the perfusion defect.

Figure 2-3 – Myocardial perfusion colour scale with enlargement showing cutoff between normal and abnormal perfusion



Using the template, each segment of myocardium is shaded in according to the displayed images. This provides an objective physical record of what has been seen and how it has been interpreted for future review and co-reporting. A score is calculated from the number of segments not normally perfused with mild defects being scored as ½. Defect size is then graded accordingly.

Defect size and location and degree of reperfusion are then integrated with wall motion for an overall conclusion. The difficulty of tissue attenuation is ameliorated by the information gleaned from wall motion scoring – a fixed defect in a characteristic area with preserved regional wall motion has a high likelihood of being tissue attenuation artefact.

2.6 Radionuclide Ventriculography

2.6.1 Resting RNVG

Equilibrium gated radionuclide ventriculography was routinely carried out immediately following acquisition of redistribution thallium images unless time constraints necessitated stress RNVG be delayed. Thallium 201 has, in addition to its main energy peak at 77 keV, a second energy peak at 140 keV which overlaps that of technetium-99m. The substantially higher counts from technetium at 140 keV means that any contribution from thallium to the counts obtained during RNVG acquisition is, however, negligible.

For patients undergoing both stress and rest RNVG, stress RNVG was performed first. Although it would have been more desirable to perform rest RNVG first to ensure no hangover effect from stress affecting resting ventricular function, the shorter acquisition times needed during each stage of stress imaging required optimal count statistics to generate analysable images. The 15 minutes required for acquisition of resting supine images in addition to the setup time required to reconfigure the gamma camera and move in the stress equipment would result in a drop in activity sufficient to make analysis of the subsequently acquired stress images substantially more difficult.

Two steps were taken to minimise the impact of this approach. Firstly an upright RNVG was acquired immediately prior to any stress, allowing comparison with the later supine images. Secondly, the supine 70° LAO image which is not used for generation of activity-time curves was always acquired first. This results in a gap of at least 10 minutes between stress and rest. In addition, the 10 minute 40°LAO acquisition was formatted into two 5 minute halves as well as in its entirety. Any residual effect of stress will diminish during the acquisition and be detectable as a difference between parameters measured in the first and second five minutes.

2.6.1.1 Image Acquisition

Following in-vivo labelling of red cells with stannous pyrophosphate, each patient was administered 600MBq of technetium-99m. This is lower than the maximum permissible 800MBq due to the increased counts detected by the high sensitivity collimator used. Images were acquired in two projections: 40°LAO adjusted to give best septal separation, and 70°LAO. Although the most common application of RNVG is calculation of ejection

fraction, it also affords an excellent opportunity to evaluate regional wall motion. The 70°LAO projection allows additional assessment and complements the wall motion scoring from thallium scanning, particularly for the infero-posterior wall of the left ventricle.

As with the thallium images a high sensitivity parallel collimator was used. A 20% energy window was used, centred around 140KeV. Data were acquired in List Mode using Link MAPS 10000 software as before. The 70°LAO image was acquired for a total of 5 minutes, the 40°LAO image for 10 minutes. In practice, images of sufficient quality for reliable ejection fraction can be acquired in 5 minutes or less. The additional time improves image quality, making wall motion analysis and ejection fraction calculation easier.

The data were formatted as follows: a histogram showing the cycle lengths of all acquired beats along with the mode of the values was displayed; an appropriate window of cycle lengths accepted for processing was then calculated, typically 10% above and below the mode of the cycle lengths. Images were formatted as 24 frames per cardiac cycle with variable bin widths (List Mode). The final processed image was checked along with the cycle lengths accepted for processing before deletion of the raw data.

2.6.1.2 Image Analysis and Generation of the Activity-Time Curve

The activity-time curve generated by radionuclide ventriculography can be used to generate multiple indices of both systolic and diastolic performance, the most basic and commonly used of these being the ejection fraction. The basic underlying principle of the technique is that the volume of a chamber is directly proportional to the activity, or number of counts, detected by the gamma camera as coming from that chamber. Thus, although the units will be different, one may construct a series of data with essentially the same information as a volume-time curve but without the need for invasive measurements. In principle, if volumetric data are absolutely essential, this can be calculated to a reasonable degree of accuracy by obtaining a blood sample at the time of data acquisition and extrapolating from the number of counts per millilitre of blood.

There are a number of important factors to consider in constructing the activity-time curve from raw data. First, the number of regions of interest used to describe each ventricle will have a significant effect on both the accuracy of our measure of activity within the ventricular region of interest, but will also affect our estimate of background activity. Second, these regions may be either created manually, semi-automatically, or in a fully

automatic manner. Third, the issue of how background activity is estimated and the activity-time curve corrected for this is important.

All activity-time curves in this thesis have been generated using a manual single region of interest technique. This will now be discussed along with the alternative techniques and the effects of this on the resulting curves. Link Medical MAPS 10000 software running on Sun workstations was used in all image and activity-time curve analysis. Custom scripts written in C Shell were used to drive the basic software. This allowed sufficient flexibility to adjust analysis routines to optimise performance and reproducibility as well as extracting data not normally used in routine practice.

Manual Single Region of Interest

This technique has been previously shown to provide the most reproducible measure of left ventricular ejection fraction of all the techniques of RNVG analysis (179). It is this fact that makes it the most appropriate for use where any serial measurement of ventricular function is contemplated, such as in monitoring the response to treatment in heart failure or in detecting subtle early deteriorations as a side effect of cardiotoxic chemotherapy.

With this technique a single region of interest is manually drawn around the left ventricle in end diastole typically using end-systolic, end diastolic, stroke-volume and paradox images (Figure 2-4). It is also possible to use amplitude and phase images here but great care must be taken as these can cause the left ventricular size to be overestimated, thereby leading to an underestimate of ejection fraction. Their main role is in verifying the accuracy of regions of interest, particularly as aneurysmal segments are often best seen on the phase image. The atria are often better seen on the phase image than on the paradox view allowing confirmation that ventricular or background regions of interest have not inadvertently incorporated the atria.

A background region of interest is then created for the left ventricle. This extends in an arc from the left ventricular apex to a point on the lateral wall of the left ventricle half way between the apex and valve plane. This region is typically 2-3 pixels in width.

This process is then repeated for the right ventricle. However, the background region for the right ventricle is often determined by two factors. Firstly is the position of the right atrium which can be of substantial size when right sided pressures are elevated, thus limiting the size of background region which can be created. Secondly, the close anatomical relationship between the inferior surface of the right ventricle and the liver can

cause substantial problems. If the liver is particularly close to the right ventricle it may not be possible to create a background region in the usual area without including part of the liver in this. If this then creates an erroneously high estimate of right ventricular background activity, the calculated right ventricular ejection fraction can be substantially elevated.

It is a matter of routine in our institution that these regions are then immediately drawn for a second time. Although one set of regions (one LV, one RV region of interest and a background region to accompany each) is sufficient to produce background corrected activity-time curves for both left and right ventricles, it is possible that an error can be made in either the ventricular or background regions that is not appreciated on visual inspection. It is unlikely that any substantial error will be duplicated on a second occasion. Therefore, if a second set of regions are drawn, this gives a total of four combinations of ventricular and background region of interest for each ventricle thus: LV1 BG1, LV1 BG2, LV2 BG1 and LV2 BG2 and equivalents for the right ventricle. The 4 background corrected activity-time curves generated from these are then analysed separately, giving 4 possible values for each parameter being measured. A mean and standard deviation are calculated for each value. A high standard deviation for the ejection fraction, typically greater than 2.0, indicates either an error has been made in at least one of the regions, or there is sufficient uncertainty as to where the regions should be placed to cause significant variation in the activity-time curves. In either case the regions are rejected and the process begun again.

Figure 2-4 – Typical images used in RNVG activity-time curve creation

Left and right ventricular, and background regions of interest are shown. ED = end diastole; ES = end systole

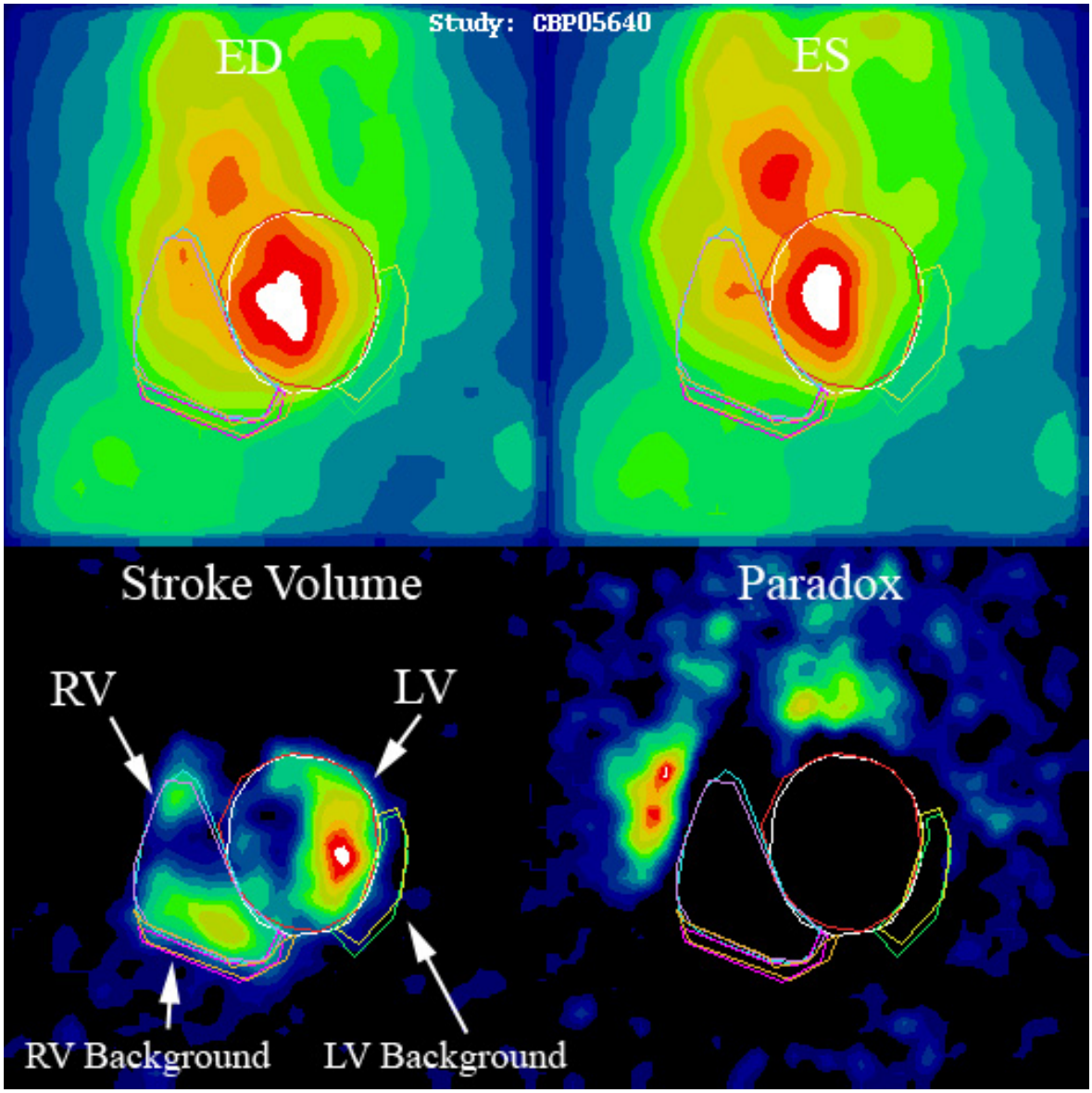
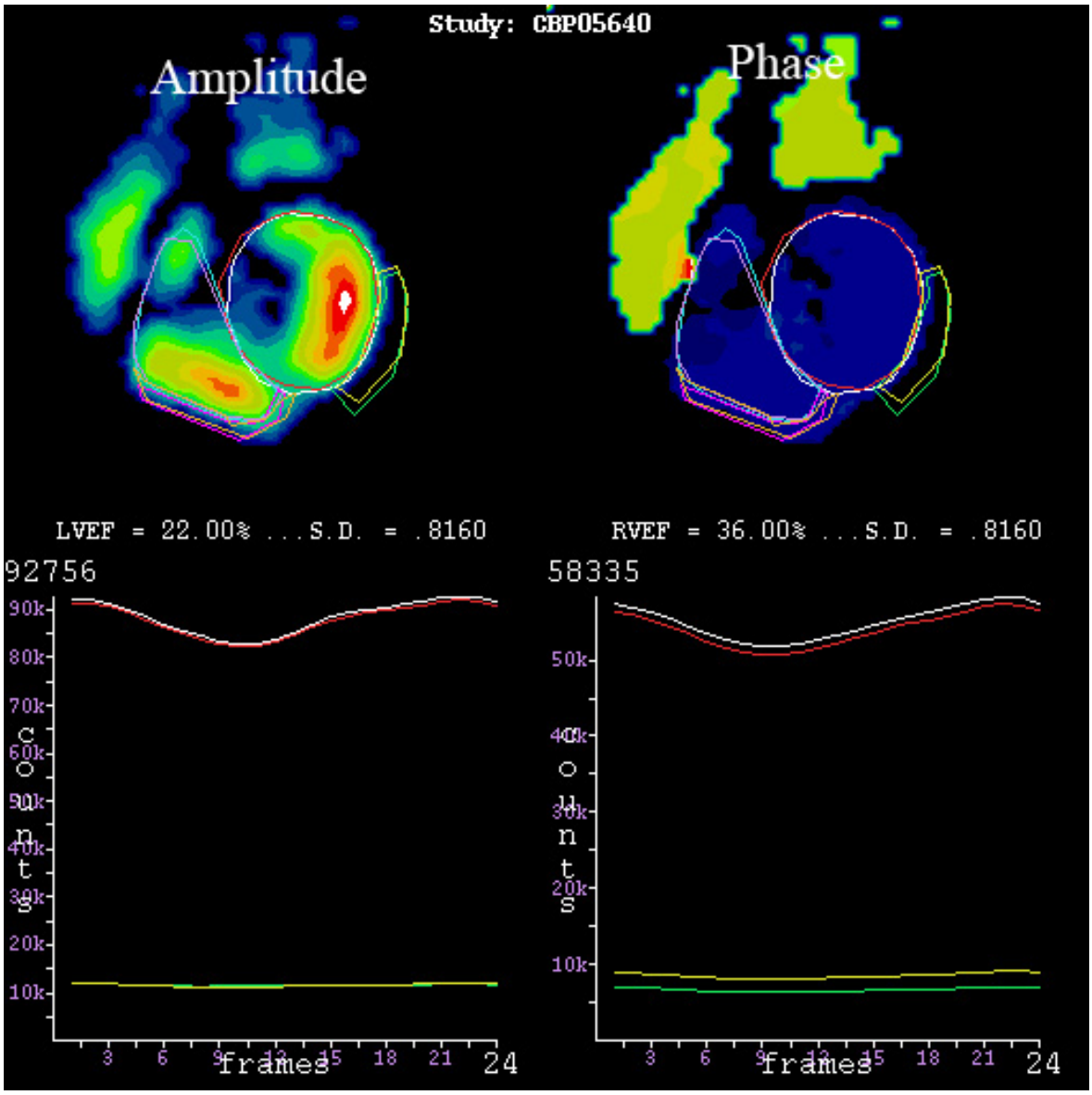


Figure 2-4 shows the typical set of images used in creating activity-time curves. Clockwise from top left are end-diastole, end-systole, paradox and stroke-volume images. Superimposed on each are the regions of interest which have been created.

The second stage of region checking is shown in Figure 2-5. Clockwise from top left are the amplitude, phase, right ventricular activity-time curves and left ventricular activity-time curves with regions of interest superimposed on the amplitude and phase images. Visually these regions are acceptable and standard deviations on both ejection fractions are 0.816 which is within acceptable limits.

Figure 2-5 – RNVG amplitude and phase images with superimposed regions of interest for left and right ventricles, and background regions.

Left and right ventricular ejection fraction results are shown along with activity-time curves for both ventricles.



A third stage of region checking comes when a second observer repeats the visual check of ROI accuracy.

The main consequence of only creating regions around the ventricles in end-diastole is that background activity is systematically underestimated. This is due to the activity sampled adjacent to the ventricles in end-diastole being lower than the true level behind the heart. Background activity behind the heart includes activity from the mediastinum which is higher than the areas of lung which have been sampled. This results in a lower ejection fraction and will also decrease peak emptying and filling rates. Provided a normal reference range is established and quoted, there should be no major difficulties in comparing ejection fractions thus derived with those obtained from other techniques.

Two Regions of Interest

The systematic underestimation of left ventricular ejection fraction by a single region of interest approach can lead to difficulties in comparing results between 2 imaging modalities such as echocardiography and RNVG. It also creates the potential for confusion for those not used to dealing with a different "normal" range. Other analysis techniques are available which do not have this limitation.

If a similar process as is used to generate end diastolic regions of interest is applied to end systole, a pair of ROIs is generated. As the ROI generated at end systole is drawn to reflect the actual size of the ventricle rather than tracking a fixed ROI throughout the cardiac cycle, the overestimate of end systolic counts, and therefore underestimate of ejection fraction, with a single ROI approach is avoided.

However, a two region of interest approach has drawbacks. Firstly, its reproducibility is reduced compared to a single region approach as two ventricular and two background ROIs must be generated thus increasing the potential for error and interaction of errors between the ROIs. Secondly, and more fundamentally for our purposes here, it does not generate an activity-time curve as such. Only two points are determined – end diastolic counts and end systolic counts. Whilst sufficient for ejection fraction analysis, no other parameters can be readily extracted.

Multiple Regions of Interest

In order to generate more familiar ejection fraction results but retain the ability to generate a full activity-time curve and derive the required parameters of systolic and diastolic function, additional regions of interest must be created. For each frame in the cardiac cycle, a ventricular ROI can be created to generate a point on the activity-time curve. To do this manually would not be feasible and so the process is generally either fully or semi-automated where the regions are computer generated using edge detection algorithms and then manually checked and adjusted.

The drawbacks of this technique are firstly that there is either a heavy reliance on automated ROI generation programs or a time consuming ROI checking and alteration process must be used. Secondly, it is more prone to errors than the single region technique. As every data point is generated from a different ROI, each point is subject to random error. With a single region approach any error introduced by an imperfect ROI will be systematic. Therefore although inter-study reproducibility may be affected by such

an error, one can be more certain that the change in counts from point to point on the activity-time curve are reliable.

Background Correction

An essential step in the generation of the activity-time curve and of deriving accurate indices from this is the estimation of background activity. Background regions of interest are created as described in section 2.6.1.2. As these regions are smaller in area than the ventricular ROIs the activity measured within the background region must be corrected for area in every frame of the cycle thus:

Equation 2-1 – Area corrected background activity

Background activity was assumed to be essentially constant throughout the cardiac cycle. Therefore, prior to correction of background activity for ROI area, each curve was smoothed using 100 iterations of a cyclical smoothing algorithm.

2.6.2 Stress RNVG

Stress RNVG was performed immediately after completion of redistribution thallium image acquisition.

2.6.2.1 Equipment

The same gamma camera used in rest supine RNVG imaging was used for stress RNVG. The head of this camera is able to be swung out to the side of the camera gantry to a sufficient degree to allow either a patient to be imaged in a chair or, as in this case, seated on a bicycle ergometer. Erect stress was used exclusively as a supine ergometer was not available.

The same bicycle ergometer used in stress thallium imaging was used. This was placed at an approximate 40° angle to the camera head and several centimetres back so that once seated on the bicycle, the patient would have to lean forward slightly in order to make contact between their chest wall and the camera head. This gap was necessary to allow enough clearance between their left leg and the camera head to permit them to pedal unimpeded. The exact angle the bicycle was placed at was adjusted immediately after administration of technetium in order to obtain best septal separation.

Patients were fitted with a mouth piece and nose clip several minutes prior to commencing exercise to allow collection of baseline gases and permit the patient to become accustomed to the mouthpiece. This is expanded upon in section 2.6.2.5

ECG gating of gamma camera and VO_2 data was by means of single lead ECG. As none of the patients exhibited significant ECG changes during stress thallium testing, something which was reviewed on a per patient basis, it was not felt necessary to carry out full 12 lead ECG monitoring during stress RNVG.

2.6.2.2 Stress Protocol

Patients underwent a symptom limited exercise test using the bicycle ergometer in an upright position as described in section 2.6.2.1. None of the tests had to be terminated for technical or other clinical reasons. Following approximate positioning of the patient using a best guess and the residual thallium activity, the quality of the ECG gating was checked. Firstly electrode position and lead configuration were adjusted to obtain a large dominant R wave on the monitor free of significant interference at rest. This was then tested under exercise conditions by asking the patient to pedal gently against no resistance. If this resulted in sufficient artefact to result in inaccurate gating, the electrodes were replaced following additional skin preparation.

Following this 600MBq of technetium was administered, having been preceded by administration of stannous pyrophosphate 10 to 15 minutes previously. The position of the patient and bicycle were then adjusted to give optimal septal separation and sufficient space on either side of the left and right ventricle to allow for minor degrees of patient movement. The left and right heart borders were marked on the camera header display to enable continuous monitoring of cardiac position without having to refer to the acquisition workstation display. One member of staff was positioned behind the patient and was responsible for maintaining close patient apposition to the camera head and limiting lateral and vertical motion during exercise.

A 5 minute pre-exercise acquisition was then commenced with the mouthpiece and nose clip being applied at the start of this. Following a final check of patient position, exercise commenced. A 3 minute ramping protocol was used, beginning with unladen exercise and increasing by 25W at the end of each stage. The degree of patient fatigue was monitored by asking the patient to make non-verbal signals in response to questions as to whether they felt able to continue with exercise and were able to cope with an increase in workload.

Where patients indicated they would be unable to continue if the workload were to increase, exercise was either discontinued at the end of that 3 minute stage or a workload increment of less than 25W was attempted. Where a patient was unable to complete the full 3 minutes of a workload increment they were vigorously encouraged to complete at least 2 minutes, even if this necessitated a small reduction in workload to achieve. Initial trials of non-study volunteers undergoing RNVG for routine clinical reasons had shown that an RNVG acquisition of less than 2 minutes acquired under these conditions was generally not analysable. This was primarily due to the low count statistics rendering the images needed for ROI creation very difficult to interpret.

Exhaled gases were continuously monitored during exercise and an approximate indication given as to whether and when anaerobic threshold was reached. For the majority of patients who did reach anaerobic threshold, encouragement was given to continue exercise for at least 2 minutes beyond this point in order to create an RNVG image exclusively looking at the post anaerobic threshold exercise phase.

Following completion of exercise the patient was kept in place and data acquisition continued for a further 6 minutes in order to monitor recovery from exercise. Collection of exhaled gases was terminated at the start of this recovery phase.

2.6.2.3 Image Processing

Raw RNVG data were acquired in List Mode into two files that were processed after exercise was complete. The pre-exercise rest phase was acquired into a file labelled R. Exercise and recovery data were acquired as a single continuous acquisition beginning a few seconds after the patient had commenced cycling (allowing their body position to stabilise) and terminated 6 minutes after exercise had ceased. All processing was with Link Medical MAPS 10000 software. This permits the raw data to be processed using specified time windows to effectively create multiple temporally separate acquisitions within the one file. The stress acquisition (S) was divided and formatted thus:

Table 2-4 – Stress RNVG image naming and processing scheme

Label	Start time (mins)	End time (mins)	Description
1	0	3	Unladen exercise
2	3	6	25W exercise
3	6	9	50W exercise
P1	9	12	First 3 minutes of recovery
P2	12	15	Second 3 minutes of recovery
A	AT – 2	AT	2 minutes leading up to AT time
B	AT	AT +2	2 minutes leading on from AT time

AT = time anaerobic threshold is reached

Where patients were able to exercise for longer than 9 minutes, that is to stages 4 and 5, exercise was continued and the time windows for P1 and P2 are adjusted accordingly. Processing of these time windows was achieved with a custom written script which automatically calculated the necessary parameters and relayed these to the actual formatting software once it had been provided with the number of exercise stages, duration of last exercise stage and time of anaerobic threshold. A separate script was used for patients who did not reach anaerobic threshold, which did not attempt to create images A and B.

All images were formatted as 24 frame per cardiac cycle List Mode files (variable bin width). In addition the same raw data were formatted as 32 frame List Mode and 24 frame fixed bin width (Frame Mode) images. These were stored separately and were used to compare the results obtained using different temporal resolutions (24 versus 32 frame) and different bin width modes (variable versus fixed). All 3 formats used identical limits around the mode of heart rate accepted for inclusion in the formatted image at each stage. These limits were generally similar to those applied in routine resting RNVG formatting as heart rate tended to rise only a modest amount during each workload increment. This is by virtue of the fact that the workload increments were 25W at most. Where a very narrow peak was seen on the cycle length histogram these limits were tightened by examining the minimum and maximum cycle length present within the peak.

2.6.2.4 Image Analysis

The same manual single region of interest technique used for routine resting supine RNVG analysis was used for creation of activity-time curves for stress RNVG. The same process of verification of ROI accuracy was also performed. Although movement artefact was minimised, a small degree was inevitable. In addition, any change in left ventricular end

diastolic volume throughout the various stages of exercise and recovery, although unlikely to be of any significant magnitude, would have to be accounted for. For this reason separate regions of interest were created for each stage of exercise and recovery.

For technical reasons a small number of scans were difficult to analyse using the usual method. For these images, custom analysis scripts were written to utilise the amplitude image, phase image or both to provide additional information to guide ROI creation. These images are not used routinely in ROI creation as they have a tendency to overestimate the end diastolic dimensions of the left and right ventricles and hence cause a reduction in the measured ejection fraction. Any other parameter derived from the activity-time curve would also be affected. Any ROIs created using this method were rigorously checked to ensure no gross errors had been made and the ROIs were the best that could be created with the data quality available.

2.6.2.5 VO_2

Exhaled gases were continuously collected during the stress RNVG by means of a mouthpiece and nose clip. Prior to each test the equipment was calibrated for flow rate and the oxygen and carbon dioxide sensors calibrated with reference gases. Exhaled CO_2 and O_2 were measured on a breath to breath basis. The VO_2/VCO_2 slope was continuously plotted and used to determine anaerobic threshold. Peak oxygen consumption rate, VO_2 max, was determined after exercise had been completed. Respiratory rate was calculated in breaths per minute.

2.7 Spirometry

Spirometry was performed on all patients. In addition, all patients had earlobe capillary blood obtained as an approximation to an arterial blood gas sample. This permitted measurement of pO_2, pCO_2, bicarbonate, carbon monoxide and, where the sample obtained was of sufficient volume, haemoglobin concentration.

As a minimum data set the following were measured:

- Forced Vital Capacity (FVC)
- Forced Vital Capacity in 1st second (FEV_1)
- FEV_1/FVC ratio

The expiratory flow was also plotted graphically and flow rates at 50-75% and 75-100% of the expiratory phase were available to allow assessment of small airways disease.

Where a significant abnormality was found, more complete pulmonary function testing was performed including lung volumes and transfer factor.

2.8 NT-proBNP

N-terminal proBNP is significantly more stable than BNP, both in terms of short term biological variability and after the blood sample has been obtained. It is therefore not necessary to ensure that the patient is rested and supine prior to sampling. Upon arrival for myocardial perfusion imaging, the patients were seated and, after approximately five minutes, an intravenous cannula was inserted and 10ml of blood withdrawn into a Vacutainer containing potassium edetate. This was centrifuged and the plasma frozen within 2 hours of the initial blood draw. The samples from all study subjects were processed in a single batch by Ian Morton at the Western Infirmary, Glasgow using a commercially available assay. This was the Roche NT-proBNP assay which was performed on an Elecys (2000) analyser.

3. Normal Ranges and Reproducibility of Resting and Stress Systolic and Diastolic Function by RNVG

3.1 The Effect of Acquisition Mode and Framing Rate on Parameters of Systolic and Diastolic Function

By its very nature, equilibrium RNVG requires a large number of cardiac cycles to be summated to produce an average cycle with sufficient data for analysis. If the heart rate was constant throughout the acquisition, summating the acquired cardiac cycles would be simple. However, with the exception of patients with a permanent pacemaker and in advanced heart failure, there is a significant variation in heart rate in normal sinus rhythm. In atrial fibrillation this variability of heart rate is even more pronounced. In order to acquire an image within a reasonable time frame, a range of cycle lengths, typically 10 to 15% around the mean cycle length, must be accepted. As this variation in cycle length is dealt with differently by the two different acquisition modes commonly in use, list mode and frame mode, there are implications firstly for the comparability of results between modes and secondly for the accuracy of the results.

The variation in heart rate is further complicated by the fact that different phases of the cardiac cycle are affected to a different degree by changing heart rate. Although the P-R interval does show a statistically significant negative linear relationship with heart rate on exercise, very little variability is seen within an individual between 80 and 120 bpm (178).

The duration of systole and diastole also exhibit a negative linear relationship with heart rate. Systole and diastole are not affected to the same degree by changes in heart rate, however. Diastole is shortened proportionally more than systole for the same change in heart rate. How the different methods of acquiring and processing RNVGs are affected by the proportionally greater effect of normal heart rate variation on the duration of diastole therefore becomes important if one wishes to move beyond the assessment of ejection fraction.

Another factor which must be considered when comparing radionuclide ventriculogram data is the number of frames per cardiac cycle the data are divided into. A tradeoff must be made when the number of frames per cardiac cycle is decided. A higher number of

frames will increase temporal resolution at the expense of lower counts per frame and a greater impact of random fluctuation. How this affects the parameters of systolic and diastolic function being measured will be examined here by comparing the standard 24 frame per cardiac cycle list mode acquisition with 16 and 32 frame per cycle acquisitions.

3.1.1 Acquisition Modes

3.1.1.1 Frame Mode

The most commonly used acquisition mode is frame mode. Here the variation in cardiac cycle length is effectively ignored and the heart rate regarded as fixed. Prior to commencing data acquisition, the heart rate is monitored and a mean heart rate is determined. This mean heart rate is used to determine the duration of the individual frames the cardiac cycle will be divided into and the data bins into which data will be acquired for the whole of the acquisition by the formula: Bin Width = Mean Cycle Length / Number of frames. A heart rate of 80 bpm, cycle length 750ms, will yield bin widths of 31.25ms for a 24 frame per cycle acquisition. As the duration of each frame is preset, data can be processed in real time into the data bins thereby minimising the quantity of raw data which must be stored either in the computer's memory or on disk. For cardiac cycles which fall outwith the tolerances set on cycle length, the data from the affected cycle will need to be discarded before processing the following frame as this cannot be identified until the cycle ends. In the early days of radionuclide ventriculography, this was an effective solution to the technical limitations of the systems of the time and expensive, low capacity hard disk drives.

This approach has its drawbacks, however. Cardiac cycles which do not precisely match the predetermined mean cycle length but are still within the tolerance limits will either have too little or too much data to contribute to the image being acquired. Cycles which are shorter than the mean cycle length do not have data to contribute to the terminal frames of the image. The impact of this can be seen in the drop off in the number of counts in the terminal frames of the image, with up to three frames being affected in patients in sinus rhythm, or more in atrial fibrillation. The end of diastole will be additionally distorted by atrial systole of these shorter cycles being superimposed on the end of the passive phase of diastolic filling of the longer frames.

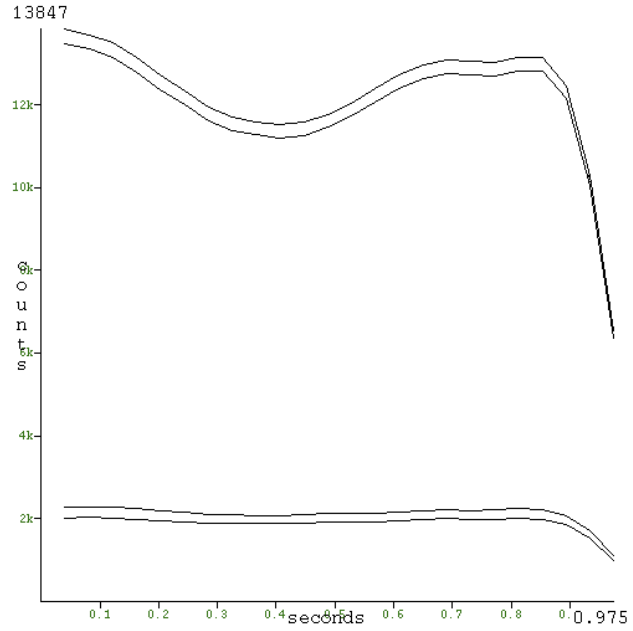
Cycles longer than the mean length will still have data available when the final frame ends which must then be discarded. Data from the passive filling phase of diastole of these

cycles will be counted as part of atrial systole of the final image, with the atrial phase being discarded as there is nowhere to place the data.

The impact this has on systole and, therefore, the calculation of ejection fraction will be minimal given the fact that the duration of systole is relatively fixed compared to diastole and will vary very little within the limits of tolerance which have been set on cardiac cycle length. Diastole is more variable in duration and will be affected by these variations.

The overall effect this has on the activity-time curve can be seen in Figure 3-1 and Figure 3-2. In this 24 frame curve, the decline in counts from both the left ventricle and background regions can be seen beginning at the 21st frame. By the 24th frame there has been a marked drop which has grossly distorted end diastole. When this curve is compared to one generated by List Mode processing from the same raw data, the importance of processing mode becomes clear. But, given that the parameters we wish to measure are typically in early diastole, prior to this drop in counts, does this matter? If the effect is limited to these macroscopic appearances, it should make no difference which mode is used when assessing diastolic function. If, however, the distortion extends beyond this and affects portions of the curve earlier in the cardiac cycle without causing a visible change, the indices of diastolic function will be similarly affected and will be inaccurate.

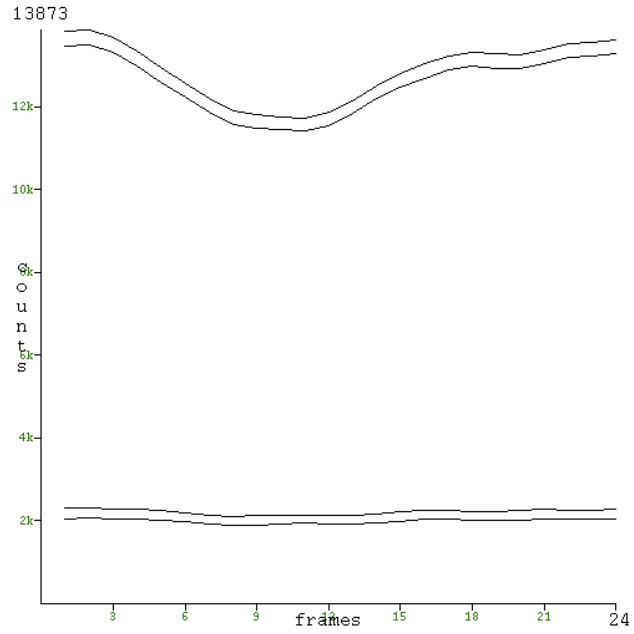
Figure 3-1 – Typical Frame Mode activity-time curve demonstrating tail drop



3.1.1.2 List Mode

List mode takes into account the normal variation in heart rate by not fixing the cycle length and duration of the frames in to which data is divided. Instead, each cardiac cycle which falls within the limits of tolerance is divided into 24 frames of equal duration.

Figure 3-2 – Typical List Mode activity-time curve



3.1.2 Study Design

3.1.2.1 Patient Characteristics

In a group of 67 consecutive patients undergoing routine radionuclide ventriculography, the raw data acquired from a single acquisition were formatted as both List Mode and Frame Mode. Indications for the test were as follows: Ischaemic heart disease (32), Heart failure (26), Chemotherapy (6), Arrhythmia (2), Cardiac transplant follow-up (1). Ethical approval was not sought for this as this section of the study involved reusing anonymised data acquired as a routine part of a clinically indicated study.

The patients each received 600MBq of technetium 99m following administration of stannous pyrophosphate. Raw data were acquired directly from the camera head of a General Electrics Camstar 3000 fitted with a high sensitivity parallel collimator. Data were acquired for 10 minutes in best septal separation. Using Link Medical MAPS 10000 software the raw data were formatted as both List Mode and Frame Mode images using

identical beat limits, set at a maximum of 15% on either side of the average heart rate during the acquisition.

3.1.2.2 Simultaneous Acquisition of Multiple Framing Modes

By acquiring raw data directly from the gamma camera along with an ECG gating signal, the X, Y and Z coordinates of every count detected be stored and retrospectively formatted. The formatting process is highly configurable and can be driven by user designed scripts, or can be performed manually. The raw data are not altered during the formatting process and can be formatted multiple times with different parameters applied to it. Therefore, a single RNVG acquisition can be formatted both as list and frame mode, and at different framing rates. The data here were formatted as 16, 24 and 32 frame per cycle variable frame width (list mode) images, and as 24 frames per cycle fixed frame width (frame mode).

3.1.2.3 Generation of Activity-Time Curves

As each of the sub formats is identical in terms of ventricular position, the approach used in chapter 3, where a set of regions of interest are generated from a reference image and applied across the others, is also valid here. The 24 frame per cycle variable width has been used as the reference image and a manual single region of interest technique used as previously described.

3.1.2.4 Correction of Frame Mode Images

Due to limitations in the processing and analysis software used it was necessary to correct the count drop-off at the end of frame mode curves. The first of these limitations prevents the correct generation of amplitude and phase images when the drop-off is sufficiently large to cause the counts to fall below the value at end systole, which should be the lowest point in the cardiac cycle. The second limitation arises during the calculation of peak emptying and filling rates as the curve analysis program is unable to differentiate between the distorted and undistorted segments of the curve.

Two options exist for dealing with these frames. The first of these is to simply delete the frames which exhibit significant count drop-off. This results in a truncated cardiac cycle. Whilst this is unimportant for the calculation of ejection fraction, any parameter which is expressed as a function of cycle length, such as filling and emptying rates normalised to

cycle length rather than absolute time, will be changed and will no longer be valid. The alternative is to preserve the length of the original cardiac cycle by replacing the affected frames with arbitrary data by duplicating the last frame in the cycle not deemed to be affected by the problem.

A left ventricular activity-time curve was generated using a roughly drawn region of interest. This was then displayed along with the number of counts from each frame along with the percentage decrease from the previous frame. The total number of frames which required correction could therefore be identified. Frames were defined as abnormal if they had ≥ 3% fewer counts than the preceding frame.

Due to statistical fluctuation in the number of counts detected, the standard deviation in counts between two identical acquisitions is approximately 1%. A 95% confidence interval can then defined as ± 2 standard deviations, and any frame at the end of the Frame Mode image falling outwith this defined as abnormal. As the left ventricular regions of interest being used were only rough outlines rather than the final regions used for analysis, this limit was increased to 3% to allow for this error except where there was still clear drop-off seen between 2 and 3%.

3.1.3 Results

3.1.3.1 Comparison of 24 Frame Fixed and Variable Frame Width

There is no significant difference in the values obtained for left ventricular ejection fraction, first third fractional filling, time to peak filling and peak emptying rate between list and frame mode acquisitions (Table 3-1). There is a trend towards higher first third fractional filling with fixed frame width data but this does not reach statistical significance. There is, however, a significant difference with peak filling rate which is an average of 5.75 lower with frame mode data compared with list mode data. As has already been shown, the activity-time curve becomes distorted at end diastole (Figure 3-1). The peak filling rate may occur later in diastole than the period over which first third fractional filling is measured, accounting for the significant difference in one but not the other. This is further confirmed by the absence of any significant difference in the values obtained for peak emptying rate. This is obtained very early in the cardiac cycle before the progressive effect of fixed frame durations can exert a significant effect on the data.

Table 3-1 – Comparison of parameters of left ventricular systolic and diastolic function using 24 frame list mode and 24 frame fixed frame width radionuclide ventriculography

	Mean value (24 frame, list mode)	Difference (list – frame mode)	p (paired samples T-test)
Ejection Fraction (%)	33.1	-0.13	0.24
First Third Fractional Filling (%)	46.8	1.13	0.19
Peak Filling Rate (%EDC/cycle)	135.3	-5.75	0.01
Time to Peak Filling (cycles)	0.21	0	0.99
Peak Emptying Rate (%EDC/cycle)	149.7	0.38	0.7

3.1.3.2 Comparison of 16, 24 and 32 Frame Variable Width (List Mode)

Although left ventricular ejection fraction determined from 32 frame per cycle data is only 0.35% higher than the mean 24 frame per cycle result, this is statistically significant with a p value of 0.001 (paired samples T-test). With 16 frame data, the ejection fraction is a mean 1.21% lower and the difference is highly statistically significant ($p < 0.001$) (Table 3-2). This indicates that a frame rate of 16 per cycle undersamples the data and results in the true trough value of the activity-time curve being missed. First third fractional filling shows a similar pattern with a marginal increase in the value measured with 32 frame data, although not statistically significant, and a decrease with 16 frame data. The difference with 16 frame data is more marked than with ejection fraction and is approaching the level where it may be distinguishable from measurement error on serial studies.

Although peak filling rate is normalised to cycle length and should, therefore, be relatively independent of the number of frames per cycle, both peak filling rate and peak emptying rate show a positive linear correlation with frame rate (Table 3-2).

Table 3-2 – Comparison of parameters of left ventricular systolic and diastolic function using 16, 24 and 32 frame list mode radionuclide ventriculography (paired samples T-test)

	Mean value (24 frame)	Difference (24 – 32 frame)	p	Difference (24 – 16 frame)	p
Ejection Fraction (%)	33.1	-0.35	0.001	1.21	<0.001
First Third Fractional Filling (%)	46.8	-0.31	0.66	3.0	<0.001
Peak Filling Rate (%EDC/cycle)	135.3	-31.6	<0.001	23.0	<0.001
Time to Peak Filling (cycles)	0.21	-0.04	0.001	0.01	0.15
Peak Emptying Rate (%EDC/cycle)	149.7	-11.6	<0.001	15.4	<0.001

3.1.4 Conclusions

Although there is no statistically significant difference between list and frame mode data for the indices of interest with the exception of peak filling rate, there is evidence that data quality is compromised by fixed frame duration. Peak filling rate is systematically overestimated and there is a non-significant trend to underestimate first third fractional filling. Therefore, list mode data should be used in preference to frame mode, particularly for indices of diastolic function.

List mode data with 32 frames per cycle shows minimal difference to that derived from 24 frame data for the two parameters of most interest, ejection fraction and first third fractional filling. Results derived from 16 frame data do show differences that are not only statistically significant, but of sufficient magnitude to be of clinical significance also. Sixteen frame data are, therefore, inadequate and a minimum of 24 frames per cardiac cycle must be used. At this standard acquisition length (10 minutes), there is little to chose between 24 and 32 frame per cycle data. However, problems with low counts per frame will become more pronounced as acquisition time is significantly shortened during stress testing. Therefore 24 frame per cardiac cycle list mode data is required for stress radionuclide ventriculography. The effect of framing rate on peak filling and emptying rates means that these values are not interpretable using reference ranges for other framing rates.

3.2 The Reproducibility of Systolic and Diastolic Function by RNVG

The reproducibility of a test is crucial to its utility as a diagnostic tool. Firstly, in the serial evaluation of a patient where change in the result obtained should be due to a change in the parameter being measured rather than variation intrinsic to the measurement technique. Secondly, in the one-off assessment of patients where there must be confidence that a result is either normal or abnormal to the degree found, and that this would not vary significantly were it to be repeated by the same or a different operator.

3.2.1 Factors Affecting Reproducibility

The reproducibility of a test will be governed by these factors:

- Short term biological variability
- Number of operators carrying out test
- Operator experience
- Difficulty in measuring the variable
- Precision to which variable is measured

3.2.1.1 Short Term Biological Variability

This refers to the minute to minute constancy of the variable being measured. This may be constant such as a patient's weight, it may be independently variable such as heart rate, or it may be variable dependent on another physiological parameter. The majority of parameters of interest in terms of systolic and diastolic function such as ejection fraction and first third fractional filling are dependent on other variables such as heart rate, loading conditions and inotropic-lusiotropic state for their short term variability. In sinus rhythm at rest and in the absence of external manipulation of other factors, biological variability should be minimal and any observed variability largely due to technical factors.

In atrial fibrillation, however, there may be significant beat to beat variation in heart rate. This can result in significant differences in filling times and rates depending how truncated or prolonged the current cardiac cycle is compared to the mean. Parameters of systolic function will be similarly affected – a short preceding beat will result in a reduced stroke volume and vice versa. Whilst this is a significant problem for echocardiography, meaning multiple, typically 5, beats must be measured and averaged, it affects RNVG to a lesser degree. As the majority of RNVG is carried out using the equilibrium method rather than first pass, an intrinsic part of the technique is the averaging of a large number of beats over several minutes. Thus RNVG may give a more accurate estimate of ventricular function in this situation by virtue of averaging more heart beats. The reproducibility will be largely determined by the mean heart rate from one study to the next and the breadth of the heart rate range accepted for analysis.

3.2.1.2 Operator Experience

It is widely recognised that a minimum level of experience is required in order to reliably acquire and interpret a test, whether this is echocardiography, myocardial perfusion

imaging or radionuclide ventriculography. This is reflected in professional accreditation schemes stipulating a minimum level of experience in order to hold the qualification. An inexperienced operator will be more subject to errors in both acquiring or correctly interpreting the data. This will be seen as a changing result where there is no genuine change.

3.2.1.3 Number of Operators

As the number of operators increases, there is a tendency to an increase the degree of variability seen in a test due to systematic differences in acquisition or analysis between operators. One technique commonly employed to limit this source of variability is to limit the number of operators. Whilst this will be of benefit in a research situation where it is desirable to minimise this potential source of variability, it is less feasible in routine clinical practice where it is unlikely that the same operator will carry all serial examinations on a specific patient. This will introduce a degree of uncertainty when a test that has been shown to have good reproducibility under these artificial conditions is used routinely.

Standardised acquisition and analysis protocols aim to limit this source of variability. This aims to limit any systematic error introduced by an experienced operator differing slightly in their technique, but in a consistent manner. An inexperienced operator, however, will vary randomly and thus have more poorly reproducible results.

3.2.1.4 Difficulty in Measuring Variable

Logic dictates that a variable which is technically difficult to measure will be more prone to errors of measurement, and hence variability with repeated measurement, than one which can be measured easily. For example, left ventricular ejection fraction measured by Simpson's biplane rule on echocardiography will be subject to greater variability of measurement than measurement of mitral inflow velocities. This is due to it requiring high quality 2D echocardiography images rather than an easy to acquire Doppler signal.

In terms of RNVG, each parameter is effectively derived from one measurement – the activity-time curve. Anything that compromises the generation of this will reduce the accuracy of any variables measured. Poor ventricular separation, poor image quality or overlapping structures interfering with either ventricular or background regions of interest will globally affect the activity-time curve. It is possible, even with experienced technical

staff, for random and systematic variation to occur in the positioning of the gamma camera. Whether or not this significantly affects either ROI generation or the derived parameters will be assessed.

3.2.1.5 Acquisition Time and Data Quality

For the assessment of systolic and diastolic function during stress to be a worthwhile endeavour, it will be necessary to demonstrate that the short acquisition times required by this do not adversely affect the parameters being measured by virtue of the lower counts obtained. Any parameters which are significantly affected in this way cannot be used to reliably assess systolic or diastolic function.

However, not all parameters are equally affected. Lower count numbers in combination with unavoidable random variation in counts between frames results in an activity-time curve that has sharp bumps. Although this is overcome to an extent by smoothing the curve and using regression techniques to measure some parameters, this does introduce a margin of error for the peak filling and emptying rates and the times at which they occur. If such a bump occurs at the end of the first third of diastole, first third fractional filling will also be affected. The ejection fraction is less susceptible to this as the framing rate is usually sufficient such that the trough seen at end-systole occupies 2 frames and thus can be more reliably smoothed.

To assess the validity of the 2 and 3 minute acquisition times necessary for stress RNVG, values obtained by analysing the first 2 or 3 minutes of a 10 minute continuous acquisition will be compared with the value obtained when analysing the 10 minute acquisition as a whole.

3.2.2 Assessing Reproducibility

Reproducibility can be measured in several different ways:

- One observer, multiple analyses of same data (intra-observer reproducibility)
- Multiple observers, one or more analysis each of same data (inter-observer reproducibility)
- One observer, one or more analysis of multiple data sets (inter-study reproducibility)
- One observer, multiple analyses of data within one study (intra-study reproducibility)
- Multiple observers, one or more analyses of multiple data sets.

The following experiment was designed to assess inter-observer, inter-study and intra-study reproducibility of RNVG derived parameters. Intra-observer reproducibility was not specifically examined as this is carried out as a routine part of ROI creation where a standard deviation of >2 in the ejection fraction result obtained from the 2 ventricular and 2 background ROIs created for each ventricle is taken as inaccurate ROI creation. The process of ROI creation is repeated until this value is <2.

3.2.2.1 Design

Radionuclide ventriculography is performed as a routine adjunct to myocardial perfusion imaging in this institution as well as a standalone investigation for evaluation of ventricular function where echocardiography is not felt to be adequate in terms of reproducibility. The standard protocol involves a 10 minute acquisition in best septal separation (typically 40° LAO) used for ejection fraction as well as wall motion analysis, followed by a 5 minute 70° LAO acquisition solely for wall motion analysis. For the purposes of this study, a second 40° LAO acquisition was then carried out, this time for 5 minutes. For this extra acquisition the camera would be repositioned by a different technician, effectively creating a new study with camera position and short-term biological variability the only factors to have changed.

As previously described, raw List mode data were acquired. These were formatted as our standard 24 frame per cardiac cycle List mode format (24L). In addition, 32 frame per cycle and fixed frame width images were also produced from the same raw data (Table 3-3). This enables reproducibility between frame width (24 frames/cycle vs 32 frames/cycle) and frame mode (variable/list and fixed frame width) to be assessed. In addition it will be possible to determine whether any of these schemes has superior or inferior reproducibility.

Table 3-3 – Summary of framing modes and rates formatted from raw data

	Framing Mode	Framing Rate (frames/cycle)
24L	Variable Width	24
32L	Variable Width	32
24M	Fixed Width	24

Manipulation of the program which formats the 10 minute acquisition allows the data to be formatted not only in their entirety, but also between any two time points in the acquisition. The 10 minute acquisition was therefore divided into two halves – the first

and second five minutes. In order to also examine the effect on reproducibility of shortening acquisition time to the durations to be used during stress RNVG, the first 2 and first 3 minutes of both the 10 minute acquisition and the repositioned 5 minutes acquisition were also formatted. This, therefore, produced the following separate acquisitions from which measurements could be made:

Table 3-4 – Acquisition subformats used in the assessment of reproducibility

Position 1		Position 2	
2	0-2 minutes	**2R**	0-2 minutes
3	0-3 minutes	**3R**	0-3 minutes
5	0-5 minutes	**5R**	0-5 minutes
S	5-10 minutes		
A	0-10 minutes		

3.2.2.2 Patients

Patients undergoing RNVG for any indication were recruited on an ad hoc basis dependent on availability of gamma camera and technician time. No inclusion or exclusion criteria were used. As the study did not involve any additional drug or radioactivity administration, merely being a repeat view of a test they were already undergoing for a clinical indication, and no activity was required of the patient other than to lie on the gamma camera table for an extra five minutes, formal consent was not obtained. Verbal consent was obtained by explaining the purpose of the repeat acquisition and its short duration. No patients declined to participate.

66 patients (35 male, 31 female) were studied with a mean age of 55.3. Clinical indications for the studies were:

Table 3-5 – Reproducibility study patients RNVG indications

Indication	Number	Percentage
Ischaemic Heart Disease	32	48.5%
Heart Failure	19	28.8%
Transplant Follow-up	1	1.5%
Pre-transplant Assessment	6	9.1%
Chemotherapy	6	9.1%
Arrhythmia	2	3%

3.2.2.3 Analysis

Using the standard procedure, ROIs and activity-time curves were generated by two observers (AMC and AR) for both acquisitions. For position 1, ROIs were created using acquisition A; for position 2, acquisition 5R was used. Since acquisitions 2, 3, 5 and S are a subset of acquisition A and have an identical camera and patient position, ROIs valid for acquisition A are also valid for the other 4 sub-acquisitions. The ROIs generated for acquisition A were therefore applied to these to generate activity-time curves. By doing so, an important factor in reproducibility, variability in ROIs, is eliminated and differences between acquisitions 5 and S will more closely reflect variability due to the technique itself. A degree of short term biological variability will remain but will be minimised by the continuous acquisition over a short time with no patient movement. This technique also allows the comparison of sub-acquisitions 2 and 3 with A to solely reflect the impact of reduced counts on the activity-time curve rather than the combined effect of this and region of interest variation.

Paired samples T-tests have been used to assess the degree of agreement between observers and within each study. Bland-Altman plots were generated to assess the degree of intra and inter-observer variation. In all plots the thick dashed line represents the mean difference between the paired values. The thinner dashed lines represent +2 standard deviations and -2 standard deviations.

3.2.2.4 Results

An overview of the results obtained is given below. Additional supporting data are presented in Appendix 1.

Heart Rate

There was no significant difference in heart rate either within the sub-acquisitions of the 10 minute study, or between the first and second studies. There is small decline in mean heart rate between the first and second 5 minutes of the 10 minute acquisition (5-S). This is only 0.258 beats per minute and does not achieve statistical significance. After this initial decline, heart rate remains steady with a mean difference of only 0.02 beats per minute between the S and 5R acquisitions.

Ejection Fraction

Intra-study/Intra-observer Reproducibility

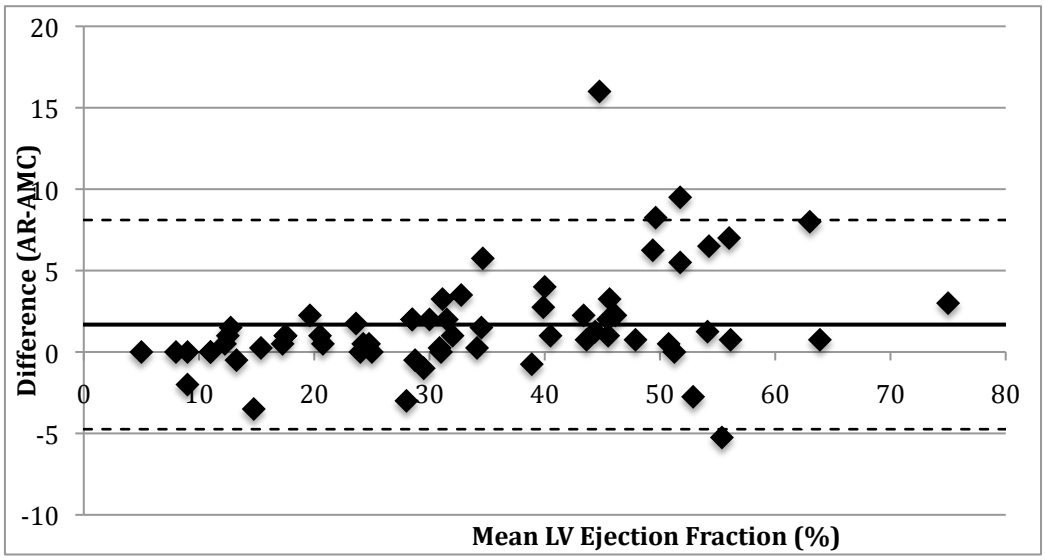
Within the two five minute halves of the 10 minute A acquisition there is no significant difference in left ventricular ejection fraction for either operator. There is near perfect correlation between the two, with a correlation coefficient of 0.99 for all three acquisition modes and both observers. The small and statistically non-significant decrease in heart rate seen between these sub-acquisitions also has no significant effect on ejection fraction (Table Ap1-1). The mean differences in ejection fraction range from 0.004% to 0.2% depending on acquisition mode and framing rate. This represents the absolute limit of reproducibility under optimal conditions, and is far superior to that seen in any real world situation.

Intra-study/Inter-observer Reproducibility

Comparing the ejection fraction obtained for each sub-acquisition between the two operators, there is a significant systematic difference between the two values. Left ventricular ejection fractions obtained by AMC are, on average, 1.7% lower than those obtained by AR (Table Ap1-2). This is seen regardless of acquisition time and acquisition mode. The correlation between results is, however, excellent with a correlation coefficient of 0.98 for all sub-acquisitions.

Bland-Altman analysis reveals a tendency for better correlation at lower ejection fractions and an ejection fraction threshold of around 50% where variability increases (Figure 3-3). In clinical terms this is acceptable as a change in ejection fraction of 5% or more in patients with an ejection fraction of less than 40%, the lower limit of normal for this technique, is greater than the same apparent magnitude of change for patients with ejection fractions well within the normal range. The reason for this difference was examined and was found to be a minor variation in technique where left ventricular ROIs produced by AR were tighter to the ventricle that those of AMC, resulting in a higher estimate of background activity and, hence, higher ejection fraction.

Figure 3-3 – Bland-Altman plot of LV Ejection Fraction - AR-AMC comparison, 24 frame variable width, 10 minute acquisition



Intra-observer/Inter-study Reproducibility

For a single observer analysing serial studies on a patient, there was no significant difference in ejection fraction (Table Ap1-3). This is unaffected by acquisition mode or framing rate. There is an extreme outlier with AR's data where there is an apparent change in ejection fraction of more than 20%. This subject has a similar change in ejection fraction with AMC. This indicates that the apparent change is either a genuine change in the patient's ejection fraction over this time period or is due to technical issues with either the first or second scan rather than an analysis problem.

The agreement between studies is, as expected, less than that within the same acquisition although still excellent with correlation coefficients greater than 0.95 for all sub-acquisitions and formats. A modest improvement in reproducibility can be seen by increasing acquisition time from 2 to 3, then 5 minutes with a small decrease in the mean ejection fraction change and reduction of the standard deviation. This trend could be expected to be even more pronounced when acquisition time is increased to 10 minutes, although a second full 10 minute acquisition was not performed.

There is no significant effect from increasing the number of frames per cardiac cycle to 32 (mean difference -0.59 (8.98, -10.15) or from using fixed frame duration (mean difference -0.25 (8.72, -9.21)

Inter-observer/Inter-study Reproducibility

The most likely scenario in clinical practice is that different observers will analyse serial studies on a patient. There was a significant systematic difference between the two

observers when reanalysing the same data, this is also the case when they independently analyse serial studies (Table Ap1-4). Again, the results obtained by AMC are systematically lower than those of AR. This difference has increased to between 2.1 and 2.5% with the greatest differences being seen with 2 minute acquisitions and the smallest with 5 minute acquisitions. These differences are all significant at a level of < 0.01 (paired samples T-test). The Pearson correlation coefficient is 0.95 (Table Ap1-5), the same as that for intra-observer/inter-study reproducibility.

Short Acquisition Time Effect

With the 24 frame list mode format, there is no significant difference in ejection fraction measured with either 2 or 3 minutes of data compared to the full 10 minute acquisition time (Table Ap1-6). However, this is not the case with either 32 frame list mode or 24 frame fixed width acquisitions. Correlation between results is, again, very high with correlation coefficients for each of the acquisition modes and observers being 0.99 or greater.

Fixed frame mode results in a statistically significant difference in ejection fraction with a 2 minute acquisition for one observer, and a trend for the other observer which is almost significant ($p = 0.069$). The mean difference in ejection fraction is, however, only 0.5% but nonetheless, this suggests that list mode (variable frame width) is the superior of the two when using such limited acquisition times. With an acquisition time of 3 minutes, the difference becomes non-significant for both observers.

The results for 32 frame list mode are interesting. Although no significant difference was seen between sub-acquisitions 5 and S (Table Ap1-1), the ejection fraction is significantly lower when measured using either the 2, 3 or 5 minute sub-acquisitions compared to the full 10 minute A acquisition. The effect becomes more pronounced as acquisition time is reduced further with differences of 1.1%, 0.6% and 0.4% at 2,3 and 5 minutes respectively.

First Third Fractional Filling

Intra-study/Intra-observer Reproducibility

Within the 5 and S sub-acquisitions there is no statistically significant difference in first third fractional filling result. There is however, a large standard deviation and, as can be seen from the Bland-Altman plot (Figure 3-4), a moderate degree of scatter which remains constant across all values of FTFF. As these sub-acquisitions should be identical in all respects other than a 5 minute time difference, the differences seen here partly represent the limitations inherent in this technique. It is likely that this is due to the filling curves

being produced are not smooth enough to be analysed more reproducibly. There are 2 ways to improve this – either by increasing temporal resolution, a trade-off that decreases the counts per frame, or by increasing the acquisition time to increase the number of counts per frame. An increase from 24 to 32 frames per cycle does not improve reproducibility, if anything it decreases it with a greater mean difference and greater standard deviation (Table 3-6 and Table Ap1-7).

It should be remembered that there was a small decrease in heart rate between these two sub-acquisitions. Whilst this did not reach statistical significance or affect ejection fraction, first third fractional filling is heart rate dependent and is likely to be more sensitive to changes in heart rate. This will serve to exaggerate the technical issues of curve smoothness.

Figure 3-4 – Bland-Altman Plot of left ventricular first third fractional filling intra-study/intra-observer reproducibility (24L AMC 5-S acquisitions)

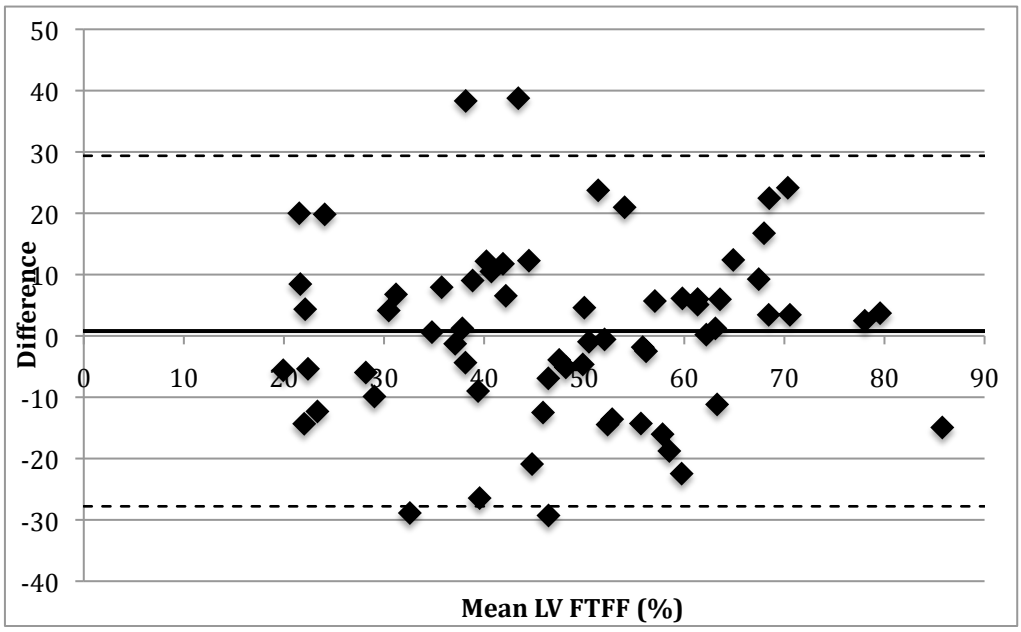


Table 3-6 – Left ventricular first third fractional filling Intra-study/Intra-observer reproducibility (5-S comparison) – Pearson correlation coefficient

	24L	32L	24M
AMC	0.718	0.658	0.718
AR	0.778	0.740	0.683

Intra-study/Inter-observer Reproducibility

The inter-observer reproducibility (Table Ap1-8) is, perhaps surprisingly, superior to the intra-study reproducibility. The correlation coefficient for inter-observer reproducibility is

in excess of 0.93 for all bar one of the sub-acquisitions. This suggests that the magnitude of effect of the heart rate changes between sub-acquisitions is significantly higher than that of re-analysis of the same data by a second observer.

Acquisition duration seems to have only a modest effect on reproducibility. The standard deviation for the measurement pairs for acquisition lengths of 3 minutes or greater is fairly constant at around 5 (Table Ap1-9, Table Ap1-10 and Table Ap1-11). At an acquisition length of 2 minutes, there is a higher degree of variability which is particularly marked with the repeat (R) acquisition where a standard deviation of 12.48 is seen with 32 frame per cycle formatting (Table Ap1-10). This may be explained by the interaction of lower count statistics on the second study and short acquisition time exaggerating any minor differences in region of interest definition between the two observers.

Intra-observer/Inter-study Reproducibility

Inter-study reproducibility initially appears much poorer than inter-observer reproducibility. However, heart rate again plays a factor here. The mean heart rate is 0.27 beats per minute higher in the 5 sub-acquisition versus 5R. Between S and 5R the difference is only 0.02 beats per minute. This has the effect of reducing the standard deviation from 14.04 for the 5-5R comparison to 12.21 for the S-5R comparison.

There is an improvement in reproducibility with increasing acquisition length. There is a progressive improvement in correlation coefficient with increasing acquisition time which is reasonably consistent across the different frame rate / mode combinations (Table Ap1-12). 24 frame list mode data show an increase from a correlation coefficient of 0.55 at 2 minutes, to 0.65 at 3 minutes and 0.67 at 5 minutes. The standard deviation also improves from 16.15 for AMC and 17.57 for AR at 2 minutes to 14.04 and 14.29 respectively at 5 minutes.

The results for 24 frame variable width and 24 frame fixed width acquisition show no statistically significant difference (paired samples T-test) (Table Ap1-13 and Table Ap1-14). Although there is also no significant difference with 32 frame list mode data, the reproducibility of first third fractional filling is poorer (Table Ap1-15) with standard deviations 2 or 3 points higher. This is likely to be due to the temporal resolution / counts per frame trade-off which has already been discussed.

Inter-observer/Inter-study Reproducibility

Correlation here is very similar to that seen when a single observer analyses serial studies. The correlation coefficients for 5 minute acquisitions are 0.66 for 24 frame list mode data,

0.58 for 24 frame fixed width data, and 0.63 for 32 frame list mode data. There is no significant difference between the paired values for any of the formats at either 2, 3 or 5 minutes.

Short Acquisition Time Effect

By reducing the acquisition time to 2 or 3 minutes, there is no significant difference in first third fractional filling when compared to a 10 minute acquisition (Table Ap1-16). This is true for all 3 acquisition modes both at 2 and 3 minutes. Both observers have similar results. There is, however, an improvement in correlation from 0.72 to 0.85 seen with the 3 minute acquisition as opposed to the 2 minute data (Table Ap1-17).

It can be seen that the degree of variability here is lower than the intra-observer/inter-study variability of FTFF. The reduction in data quality from a short acquisition time is outweighed by other sources of variation. It is therefore safe to conclude that values of first third fractional filling from 3 or even 2 minute acquisitions are just as valid as those from longer acquisitions.

Peak Filling Rate (3 point regression)

Intra-study/Intra-observer Reproducibility

There is no significant difference in left ventricular peak filling rate between the two 5 minute sub-acquisitions of the 10 minute study (Table Ap1-18). The mean difference and standard deviation is higher for the 32 frame variable width acquisition than for either of the 24 frame formats. This is reflected in a correlation coefficient of 0.83 to 0.84 for 32 frame data compared to 0.92 to 0.94 for variable and fixed width 24 frame data. A small increase in the degree of variability is seen with increasing peak filling rates (Figure Ap1-1).

Intra-study/Inter-observer Reproducibility

Correlation between both observers is excellent for all acquisition durations and modes with correlation coefficients of between 0.98 and 0.99. There is a small but statistically significant difference in the values between the two observers with AMC obtaining lower values than AR by between 5 and 6 points (Table Ap1-19). This becomes more pronounced at higher peak filling rates (Figure Ap1-2) and also during the repeat 5 minute acquisition.

Intra-observer/Inter-study Reproducibility

Inter-study reproducibility is good with correlation coefficients around 0.88 for 3 and 5 minute acquisitions using 24 frame per cycle variable width format (Table Ap1-20). The

correlation is slightly poorer for the equivalent 2 minute acquisition (0.817 for AMC, 0.834 for AR). The fixed frame width 24 frame format has similar reproducibility at 5 minutes but a dip at 3 minutes. Reproducibility is reduced when 32 frames per cycle are used. Here the correlation coefficient falls to 0.8 even with 5 minute data.

Although there is no statistically significant difference in the values obtained (Table Ap1-21), there is a large standard deviation (30-40) in relation to the values observed, the mean value depending on observer and format being approximately 135. This is seen with all 3 acquisition modes.

Inter-observer/Inter-study Reproducibility

The correlation is good with a coefficient of 0.86 for 24 frame fixed and variable width data using 5 minute acquisitions. For 32 frame list mode data, the correlation is slightly poorer at 0.79 and the values obtained are significantly different (paired samples T-test). The mean difference using 5 minutes of data is 17.2 which compares to a mean difference of 6.0 with 24 frame list mode data, a difference which is not significant.

Short Acquisition Time Effect

It has already been seen that there is a reduction in intra-observer/inter-study reproducibility when acquisition time is reduced to 2 minutes. The correlation between values obtained at 2 minutes compared to a full 10 minute acquisition is also marginally poorer than when 3 minutes of data is acquired (Table Ap1-22). The correlation coefficient for 24 frame list mode data rises from 0.93 with 2 minute data to 0.96 with 3 minutes of data. There is a statistically significant difference between both 2 and 3 minute values and the 10 minute acquisition (Table Ap1-23). The mean difference for 24 frame list mode data is 8.7 at 2 minutes and 5.3 with 3 minute data. For 32 frame list mode data these differences are higher at 15.9 and 11.3 for 2 and 3 minute data respectively.

Time to Peak Filling

Intra-study/Intra-observer Reproducibility

Correlation between the two sub-acquisitions is moderate for 24 frame variable width acquisition (correlation coefficient 0.53 – 0.59) but poor for fixed frame width (0.32) and 32 frame per cycle acquisition (0.29-0.35) despite the superior temporal resolution.

There is no statistically significant difference in left ventricular time to peak filling between the 5 and S sub-acquisitions (paired samples T-test, Table Ap1-24). This is regardless of observer or acquisition mode. The degree of variability, however, is large in

relation to the values observed which are clustered between 0.1 and 0.25 (10-25% of the RR interval). It is possible that some of this variability is accounted for by the change in heart rate between these two sequential acquisitions. However, the parameter is normalised to the RR interval, which should minimise the impact of this.

Intra-study/Inter-observer Reproducibility

The correlation between 2 observers is excellent when the same data are re-analysed (Table Ap1-25). The correlation coefficients for 24 frame list mode data range from 0.79 to 0.97 across the various sub-acquisitions with no relationship to acquisition length. This is significantly better than the intra-study correlation indicating that the poorer correlation there was due to differences in the underlying data rather than region of interest issues. Although it is not possible to be certain, this is likely to be due to poor temporal resolution and an unfavourable trade-off when frame rate is increased. A small change in time to peak filling is translated into a relatively large random fluctuation in the measured value.

The degree of variability is lower here than with the 5-S comparison, indicating that heart rate was indeed playing a significant role despite normalisation. The standard deviation when the same acquisition is analysed by two separate observers is, at 0.03, between one third and one sixth that of the 5-S intra-observer comparison (Table Ap1-26).

Other than the comparison for the 3R sub-acquisition formatted with 24 frame variable width, there is no statistically significant difference between the two observers. This appears to be a statistical aberration rather than a genuine difference, as it is not reproduced with either of the other two formats.

Intra-observer/Inter-study Reproducibility

The degree of variability here is similar to that seen with the 5-S comparison, although the correlation coefficients are slightly lower, particularly with 2 minute acquisition times where a correlation coefficient of 0.24 is seen for 24 frame list mode data, and 0.14 for 32 frame list mode data (Table Ap1-27). This indicates two things. Firstly, heart rate is clearly a significant factor in variability of Time to Peak Filling. Secondly, the impact of a second acquisition, with different regions of interest, is less than the heart rate factor. There is no statistically significant difference between the paired samples with the 24 frame variable width format (Table Ap1-28) or with the other two formats.

Inter-observer/Inter-study Reproducibility

Correlation here is similar to that seen for single observer serial study analysis. For 5 minute 24 frame list mode data the Pearson correlation coefficient is 0.52. However, this

falls to 0.29 with 2 minute data and is also poor with frame mode or 32 frame list mode data. Although there is no statistically significant difference in the paired data, the standard deviation is large (approximately 50%) in relation to the values being measured.

Short Acquisition Time Effect

A reduction in acquisition time to 2 or 3 minutes does not yield statistically different results compared to a 10 minute acquisition. This is unaffected by acquisition mode or observer (Table Ap1-29). The correlation here (Table Ap1-30) is similar to that between the two 5 minute acquisitions, although there is no consistent improvement with increasing from 2 to 3 minute data.

Peak Emptying Rate (RR)

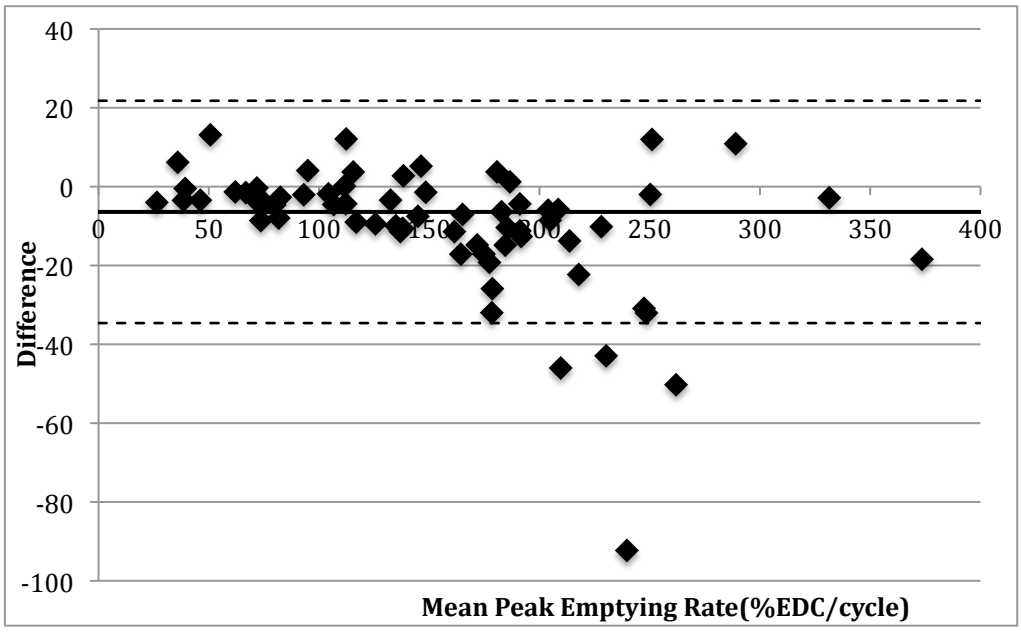
Intra-study/Intra-observer Reproducibility

Correlation is excellent (correlation coefficient of 0.96 for fixed and variable width 24 frame data, and 0.93 for variable width 32 frame data). There is no significant difference in Peak Emptying Rate between the 5 and S sub-acquisitions (Table Ap1-31). The degree of variability is acceptably low with a standard deviation for 24 frame (variable width) of approximately 22 and the majority of measured values between 50 and 200. Peak emptying rate is affected by framing rate with the mean value for 32 frame formats being 10-20 higher than the 24 frame format, a difference which is statistically significant.

Intra-study/Inter-observer Reproducibility

Although correlation remains very high (Table Ap1-32), there is a statistically significant difference between the two observers in all sub-acquisitions and in all 3 formatting modes (Table Ap1-33). The values obtained by AMC are systematically lower than AR, a difference that becomes more pronounced as the peak emptying rate increases (Figure 3-5). The same systematic difference in values was seen with left ventricular ejection fraction. Since ejection fraction and peak emptying rate are related, the same factor arising from the systematic difference in region of interest creation between the 2 observers is likely to be responsible.

Figure 3-5 - Bland-Altman plot of LV Peak Emptying Rate (RR) - AMC-AR comparison (A sub-acquisition)



Intra-observer/Inter-study Reproducibility

Inter-study correlation is very good with Pearson correlation coefficients of 0.87 to 0.92 for both 24 frame per cycle formats (Table Ap1-34). The 32 frame per cycle list mode format performs slightly poorer with correlation coefficients between 0.84 and 0.88. There is a statistically significant difference in peak emptying rate between the two studies, the second study being a mean 8 to 12 points higher for 24 frame fixed and variable width formats (Table Ap1-35 and Table Ap1-36). The magnitude of the mean difference is also high with the 32 frame variable width format with an even higher degree of statistical significance (Table Ap1-37).

This difference is seen with both observers. No such difference was seen with the intra-study/intra-observer comparison. Heart rate may account for this as there was a reduction in heart rate between the 5 and S sub-acquisitions and a further reduction with the 5R sub-acquisition. These heart rate changes were small and it may be that the magnitude of change over the 3 sub-acquisitions is the minimum necessary to produce a significant change.

Inter-observer/Inter-study Reproducibility

Reproducibility is good here with correlation coefficients ranging from 0.84 for 2 minute 32 frame data to 0.91 for 5 minute data (both frame and list mode). This is very similar to the intra-observer/inter-study correlation. However, here there is a statistically significant difference in the values obtained by the two observers ($p < 0.001$, paired samples T-test). The results obtained by AMC were around 20 points lower for 24 frame formats and 30

points lower for 32 frame formats, approximately double the difference seen with inter-observer/intra-study comparsions.

Short Acquisition Time Effect

Although correlation appears very good (Table Ap1-38), significant differences are seen between 2 and 3 minutes sub-acquisitions and the full 10 minute acquisition. This difference is greater with the 2 minute sub-acquisition. The same magnitude and direction of effect is seen for both observers for the 24 frame formats (Table Ap1-39). Again, it is greater for the 32 frame per cardiac cycle format. The increase in magnitude and standard deviation with a reduction from 3 to 2 minutes indicates that data quality is being adversely affected.

Similar differences are seen if, in an attempt to minimise in-patient variation, the 2 and 3 minute sub-acquisitions are compared with the 5 minute sub-acquisition (Table Ap1-40). The standard deviations are of the same magnitude as with the 10 minute comparison, although the mean difference is slightly lower.

The direction of change is the opposite of that seen with the inter-study comparison. The direction of any heart rate change should, however, be the same for both comparisons. Therefore, heart rate is unlikely to be responsible for one or both of the differences seen. The logical conclusion, therefore, is that peak emptying rate is poorly reproducible and should not be used as an index of ventricular function.

3.2.2.5 Summary

The reproducibility data for the 5 parameters of interest are shown in Table 3-7 and Table 3-8. To standardise the comparisons, only data from 5 minute, 24 frame list mode acquisitions were included in this summary rather than the full 10 minutes of the first acquisition.

Left ventricular ejection fraction, peak filling rate and peak emptying rate all show very good correlation between studies and observers. However, both ejection fraction and peak emptying rate showed a significant systematic difference between the two observers. Peak filling rate showed a statistically significant difference for only the inter-observer/inter-study combination. However, the mean difference for the inter-observer/inter-study combination was marginally larger but did not reach statistical significance.

First third fractional filling performed reasonably well in terms of the correlation of results and had no significant difference in the paired values. The inter-observer/inter-study difference was much smaller for first third fractional filling than for ejection fraction (0.3% versus 2.2%).

Time to peak filling performed poorly with an inter-observer/inter-study correlation coefficient of only 0.52. Although there was no significant mean difference, this is more likely to reflect the limited temporal resolution available to describe this parameter.

Table 3-7 – Summary of inter/intra observer and inter/intra study correlation for RNVG parameters of left ventricular systolic and diastolic function (Pearson correlation coefficient)

	Intra-observer Intra-study	Inter-observer Intra-study	Intra-observer Inter-study	Inter-observer Inter-study
Ejection Fraction	0.99	0.98	0.96	0.95
FTFF	0.78	0.97	0.67	0.66
PFR	0.92	0.98	0.89	0.86
ttPF	0.59	0.95	0.54	0.52
PER	0.96	0.98	0.92	0.91

Table 3-8 – Summary of inter/intra observer and inter/intra study mean differences for RNVG parameters of left ventricular systolic and diastolic function

	Intra-observer Intra-study	Inter-observer Intra-study	Intra-observer Inter-study	Inter-observer Inter-study
Ejection Fraction (%)	0.2 (NS)	1.6 ($p < 0.001$)	0.5 (NS)	2.2 ($p = 0.004$)
FTFF (%)	0.2 (NS)	1.1 (NS)	0.8 (NS)	0.3, NS
PFR (%EDC/cycle)	4.0 (NS)	5.9 ($p < 0.001$)	0.1 (NS)	6.0, NS
ttPF (cycles)	0.005 (NS)	0.004 (NS)	0.02 (NS)	0.02, NS
PER (%EDC/cycle)	2.8 (NS)	9.4 ($p < 0.001$)	9.0 (NS)	18.4, ($p < 0.001$)

Values given are in the relevant units for each parameter with the p value (paired samples T-test) if the difference is statistically significant. NS = not significant

The effect of shortening acquisition time to that expected to be used during stress RNVG is summarised in Table 3-9. Left ventricular ejection fraction performs extremely well with near perfect correlation and a small mean difference in values. Peak filling rate and peak emptying rate also show excellent correlation but have significant differences in the mean paired value. First third fractional filling performs strongly with good correlation and almost no difference in the mean paired values. As seen before, time to peak filling performs poorly.

Table 3-9 – Summary of the effect of reducing acquisition time from 10 to 3 minutes on parameters of systolic and diastolic function

	Pearson Correlation Coefficient	Mean Difference	p
Ejection Fraction (%)	0.99	0.14	0.42
FTFF (%)	0.85	0.02	0.98
PFR (%EDC/cycle)	0.96	5.25	0.04
ttPF (cycles)	0.48	0.005	0.69
PER (%EDC/cycle)	0.98	4.87	0.02

24 frame per cycle list mode data are used for all calculations. Mean difference is given in the relevant units for each parameter. Paired samples T-test has been used to calculate the p value for mean difference

3.2.2.6 Conclusion

Left ventricular ejection fraction provides a reproducible measure of left ventricular function even with short acquisition times. Peak filling and emptying rates also perform well but show significant differences when acquisition time is reduced. First third fractional filling performs particularly well under these challenging conditions suggesting that it is at least as good, if not superior to the more commonly used peak filling rate in the assessment of diastolic function. Time to peak filling is a weak, poorly reproducible parameter that is unlikely to be of utility.

3.3 The Effect of Posture on Systolic and Diastolic Function

3.3.1 Background

The majority of tests of cardiac physiology and function are performed with the subject in the supine position. Whilst this is convenient and comfortable for both subject and operator, it must be borne in mind that the assumption of an erect rather than supine posture, and vice versa, has haemodynamic consequences.

Upon standing, an exercise reflex and mechanical squeeze is produced on arterial resistance vessels and venous capacitance vessels which initially outweighs the gravitational pooling of blood in the lower limbs and increases venous return. Squeezing of the arterial resistance vessels increases peripheral resistance and hence blood pressure. The combination of increased venous return leading to increased cardiac output with increased peripheral resistance significantly increases blood pressure. This, however, is

attenuated by a baroreceptor reflex that decreases sympathetic outflow and decreases total peripheral resistance by up to 40%, leading to an overall fall in blood pressure of up to 20mmHg. The heart rate initially increases due to reflex parasympathetic withdrawal and then falls to a nadir by about 20 seconds after posture change. After the initial increase in venous return this declines and sympathetic tone must rise again to increase peripheral resistance and maintain blood pressure, thus achieving a steady state with a largely unchanged blood pressure compared to supine.

When the erect posture is achieved passively, either by sitting or in head-up tilt table testing, the haemodynamic responses differ from active standing as the exercise reflex and mechanical squeeze are significantly less. The gravitational shift of blood from the central to peripheral venous circulation is therefore largely unopposed and the decreased venous return causes stroke volume to decrease by up to 40% initially. The reflex withdrawal of parasympathetic and increase in sympathetic activity that results limits the fall in cardiac output to 20% and maintains blood pressure by increased heart rate and total peripheral resistance.

The net effect of changing from a supine to erect posture could therefore be summarised as a decrease in preload due to decreased venous return and an increase in afterload due to increased sympathetic activity and increased total peripheral resistance. Both preload and afterload have significant effects on many of the indices used to describe diastolic function. The effect of reduced preload on diastolic function is well documented. Where diastolic function is impaired but early diastolic filling is preserved by increased preload causing a pseudonormal E/A ratio, manoeuvres to reduce pre-load such as the Valsalva manoeuvre unmask this and decrease the mitral E wave velocity. Increased afterload affects diastolic function by decreasing the pressure gradient across the aortic valve in systole. This results in left ventricular pressure falling below systemic arterial pressure at an earlier stage in late systole. The isovolumetric relaxation period therefore begins earlier and, assuming preload has remained constant, isovolumetric relaxation time will be prolonged.

Increased sympathetic tone also has an inotropic effect which may have multiple effects on diastolic function both passively through enhanced elastic recoil of the ventricle as a consequence of more vigorous contraction, to increased myocardial oxygen demand and the impairment of active ventricular relaxation due to increased levels of intracellular calcium which requires yet more myocardial energy to return to the sarcoplasmic reticulum and allow relaxation.

The increase in heart rate is also significant. A strong relationship between first third fractional filling and heart rate is demonstrated in later in this chapter (section 3.5.1.4). In addition, two echo Doppler parameters are related to heart rate – isovolumetric relaxation time and mitral deceleration time.

As a consequence of these multiple interacting effects of posture on the heart itself and in the peripheral circulation, it is not safe to assume that measurements taken in one position are interchangeable with those taken in the other. A comparison of diastolic function both whilst supine and erect in a group of patients30 is therefore necessary. Whilst this can be done with the main study population, these represent a specific subgroup of patients in that they all have preserved left ventricular ejection fractions. A more diverse group of patients is therefore required for this purpose.

3.3.2 Methods

35 patients undergoing radionuclide ventriculography either as part of a myocardial perfusion scan or as a standalone investigation were recruited. The patients were not selected or excluded on any criteria other than the availability of the additional gamma camera scanning time necessary and an aim to include patients with a wide range of ejection fractions.

Following acquisition of the normal supine 40°LAO view, each patient was sat upright in a chair and a further 40°LAO view was acquired. Each pair of images was processed in the normal manner and analysed by a single observer to produce paired sets of measurements of systolic and diastolic function in each patient. All data were acquired and formatted in List mode at 24 frames per cardiac cycle.

3.3.3 Patient Characteristics

Patients were drawn from a range of sources and included patients with and without ischaemic heart disease, including prior myocardial infarction and coronary artery bypass grafting. Two patients with end stage heart failure were included and one patient with a previous cardiac transplant. Their details are summarised in Table 3-10.

Categorical variables are shown as absolute numbers in each group (n=35). Continuous variables are shown as mean (minimum, maximum value).

Table 3-10 – Characteristics of patient group assessing the effect of posture on systolic and diastolic function

Demographic	Value
Sex (M/F)	18/17
Age (years)	59 (39, 81)
Height (m)	1.66 (1.47, 1.83)
Weight (kg)	76 (57.6, 110)
BMI (kg/m^2)	27.5 (21.1, 36.4)
Hypertension (Y/N)	15/20
Beta blocker (Y/N)	18/17
RLCB (Y/N)	3/32

RLCB = rate limiting calcium channel blocker

3.3.4 Results

3.3.4.1 Heart Rate

Changing from an erect to a supine posture results in a small but statistically significant decrease in heart rate from a mean of 72.8 to 70.0 beats per minute ($p = 0.005$, paired samples T-test).

3.3.4.2 Left Ventricle

Left ventricular ejection fraction and peak emptying rate both show a statistically non-significant decrease with supine as compared to erect posture. In contrast, parameters of filling in the form of first third fractional filling and peak filling rate are higher when supine. The change in peak filling rate fails to achieve statistical significance regardless of whether 3 point regression is used.

The difference in first third fractional filling is highly statistically significant ($p < 0.001$, paired samples T-test) with a mean erect value of 36.97% and mean supine value of 45.65%.

There is a decrease in time to peak filling from 0.25 to 0.21% of the RR interval when changing from erect to supine. This almost achieves statistical significance with a p value of 0.052.

Table 3-11 – Effect of posture on left ventricular function (paired samples T test)

	Paired Differences – Erect - Supine (Left ventricle)						
	Erect (mean)	Supine (mean)	Mean difference	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
					Lower	Upper	
EF	37.23%	35.35%	1.88	7.47	-0.69	4.44	0.146
FTFF	36.97%	45.65%	-8.68	12.43	-12.95	-4.41	<0.001
PFR3pr (%EDC/cycle)	133.01	140.38	-7.37	38.68	-20.66	5.91	0.267
ttPF (cardiac cycles)	0.25	0.21	0.04	0.12	-0.0003	0.08	0.052
PER (%EDC/cycle)	183.14	165.34	17.80	54.82	-1.03	36.63	0.063

3.3.4.3 Right Ventricle

There is statistically non-significant decrease of approximately 3% in right ventricular ejection fraction when moving from an erect to supine posture. First third fractional filling, which showed a highly statistically significant increase for the left ventricle, also shows an increase with assuming a supine posture. This achieves borderline statistical significance with a mean difference of 7.59% and a p value of 0.051.

Time to peak filling, as was seen with the left ventricle, shows a small decrease which is of borderline significance with a p value of 0.059. Peak filling rate and peak emptying rate both show no significant change with posture.

Table 3-12 – Effect of posture on right ventricular function (paired samples T test)

	Paired Differences – Erect - Supine (Right ventricle)						
	Erect (mean)	Supine (mean)	Mean difference	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
					Lower	Upper	
EF	34.04%	30.88%	3.16	25.08	-5.46	11.77	0.462
FTFF	42.61%	50.2%	-7.59	22.20	-15.22	0.03	0.051
PFR3pr (%EDC/cycle)	116.09	108.39	7.70	90.67	-23.44	38.85	0.618
ttPF (cardiac cycles)	0.23	0.18	0.05	0.15	-0.002	0.10	0.059
PER (%EDC/cycle)	176.73	158.77	17.96	141.55	-30.66	66.58	0.458

3.3.5 Conclusions

Of the parameters of systolic and diastolic function examined here, only left ventricular first third fractional filling shows a statistically significant difference with a change in posture. This can probably be accounted for by the decrease in heart rate seen when adopting a supine posture. As section 3.5 will show, an increase in first third fractional filling with a decrease in heart rate is precisely what one would predict. These data are not

able to exclude an effect from factors other than heart rate on first third fractional filling. It will, therefore, be necessary to construct a reference range which takes account of this.

3.4 Smoothing and RNVG Curve Analysis

3.4.1 Premise

Some of the limitations seen in the reproducibility of the parameters of filling and emptying derived from the activity-time curve stem from the relatively rough texture of the curve. This is inevitable due to the finite number of radioactive counts available for analysis in any given acquisition period and the trade-off that must occur between acceptable acquisition time, adequate temporal resolution and adequate count statistics per frame. In theory, the random fluctuations in the activity-time curve this produces can be minimised by smoothing the curve. However, smoothing has its limitations. The original data are altered with a potential loss of accuracy for any measurements subsequently made. In effect, the data are homogenised, the end point of this being a curve which has been smoothed an infinite number of times, resulting in a flat line. Therefore, a trade-off must be made between a level of smoothing that allows a parameter to be useable from a reproducibility point of view and excessive smoothing compromising the original data.

The effect of smoothing can be seen in this series of figures. Figure 3-6 shows a normally smoothed, background subtracted, 24 frame list mode left ventricular activity-time curve.

Figure 3-6 – Left Ventricular activity-time curve - normal degree of smoothing

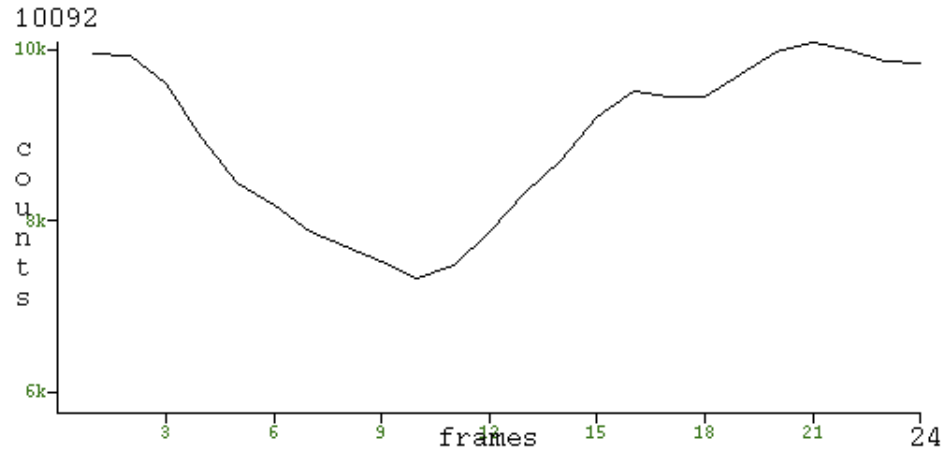


Figure 3-7 shows an activity-time curve which has been smoothed one additional time and one further time in Figure 3-8. The curve is visibly smoother with each step but it should

be noted that the peak number of counts in the curve (the figure at the top left of each curve) decreases progressively from 10092 to 10039 then 10000.

Figure 3-7 – Left ventricular activity-time curve - 1 additional smooth

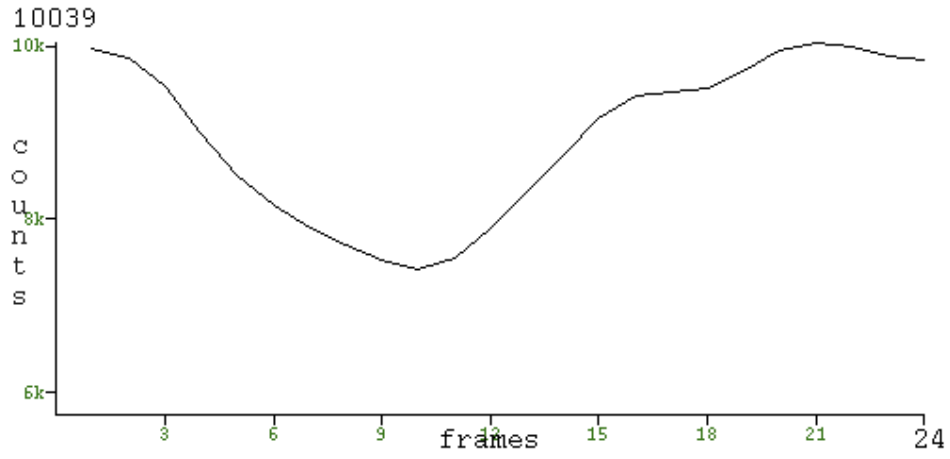
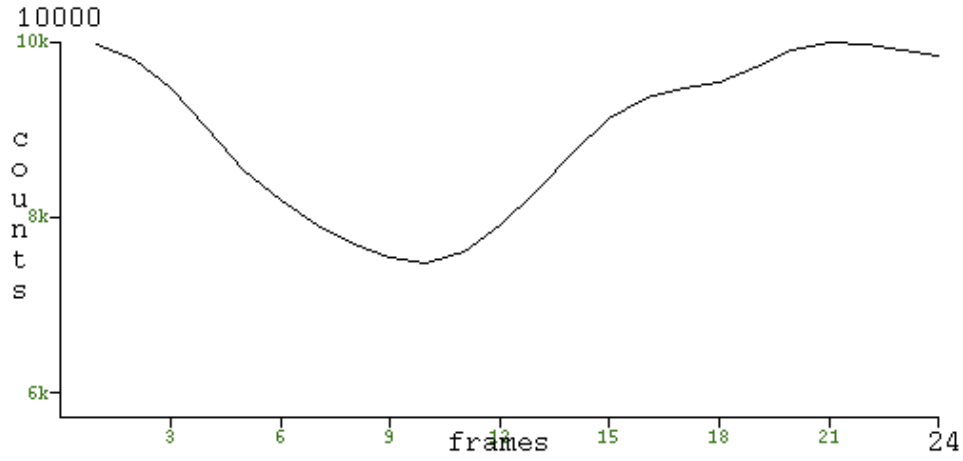


Figure 3-8 – Left ventricular activity-time curve - 2 additional smooths



The level of curve smoothing currently employed is optimal for calculation of ejection fraction. As analysis of other RNVG parameters is not routinely undertaken, it has not been locally validated whether this is an appropriate level of smoothing for this purpose. It is unclear whether the interpolation that smoothing imposes measurement accuracy, or simply homogenises the curve. The following experiment was designed to address this question.

3.4.2 Methods

The data acquired from the patient group used to determine the reproducibility of RNVG activity-time curve parameters described in section 3.2 were utilised again here. Regions of interest from a single observer (the author) were used. As before, one set of ROIs was

generated from the 10 minute acquisition (position 1) and a second set of ROIs from the 5 minute acquisition (position 2). ROIs for the 5 minute subdivisions of the 10 minute acquisition were copied as before rather than being redrawn to eliminate any variability from minor ROI differences. The acquisitions/curves are designated as follows:

A 10 minute acquisition, 1st position
5 1st 5 minutes, 1st position
S 2nd 5 minutes, 1st position
R 5 minute acquisition, 2nd position

Activity-time curves were generated for each acquisition using the normal level of smoothing (n), with 1 extra smooth applied (1s) and with 2 extra smooths applied (2s) to the curve before curve analysis. Three values were therefore generated for each parameter of each acquisition enabling assessment of the effect of additional curve smoothing on the inter- and intra-study reproducibility of each.

Curve smoothing was performed using a cyclical filter with a 1-2-1 pattern. For each group of 3 points the central point is given a weighting of 2 and the other 2 points a weighting of 1 each. These values are summed and divided by 4 to give the new value for the central point. This is progressively stepped through the curve, wrapping around when the end of the curve is reached.

3.4.3 Parameters

The following parameters were calculated for each acquisition:

Parameter	**Abbreviation**
Ejection Fraction	EF
First Third Fractional Filling	FTFF
Peak Emptying Rate	PER
Peak Emptying Rate (3 point regression)	PER3pr
Peak Filling Rate	PFR
Peak Filling Rate (3 point regression)	PFR3pr
Time to First Third Empty (normalised to RR interval)	ttFTErr
Time to First Third Full (normalised to RR interval)	ttFTFrr
Time to Peak Emptying (normalised to RR interval)	ttPErr
Time to Peak Filling (normalised to RR interval)	ttPFrr

The parameter names are then suffixed with the acquisition they have been calculated from and the degree of smoothing which has been applied to the curve. For example, the ejection fraction from acquisition A with 1 extra smooth applied becomes EF_A1s.

3.4.4 Results

3.4.4.1 Ejection Fraction

Effect on Value

With successive smooths, the mean ejection fraction is progressively reduced. The difference between each step is statistically significant using a paired samples T test with all p values being <0.001.

Table 3-13 – Mean left ventricular ejection fraction in 4 sub-acquisitions - effect of progressive activity-time curve smoothing

	A	5	S	R
Normal	33.2%	33.4%	33.4%	33.7%
1 smooth	32.2%	32.3%	32.4%	32.6%
2 smooths	31.5%	31.6%	31.7%	31.8%

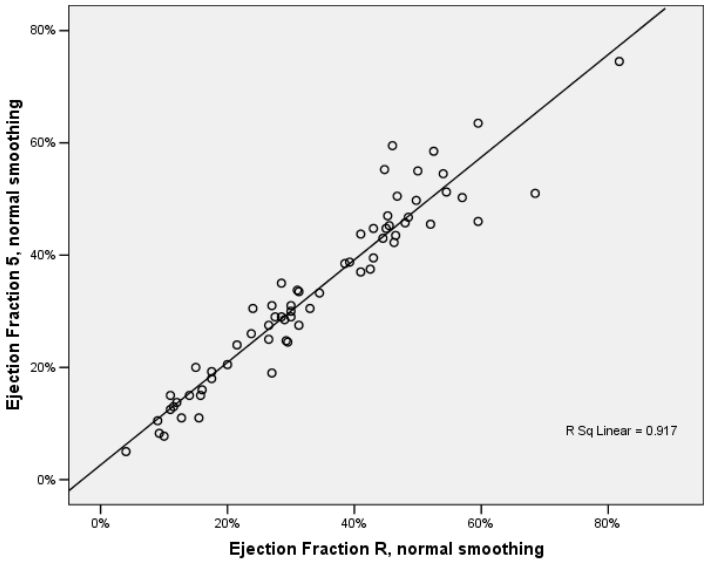
Effect on Ejection Fraction Reproducibility

As can be seen from the R^2 values, there is already excellent inter- and intra-study reproducibility of ejection fraction with the standard level of curve smoothing. Additional smoothing has virtually no effect on this, increasing the R^2 value from 0.917 to 0.92 with 2 additional smoothing steps (Table 3-14). Therefore, although there is no benefit in terms of reproducibility of ejection fraction by additional curve smoothing, if this is undertaken for the sake of other parameters, a lower normal range should be used.

Table 3-14 – Left ventricular ejection fraction intra- and inter-study correlation coefficient - effect of progressive activity-time curve smoothing

	A/R	5/S	5/R
Normal	0.96	0.99	0.96
1 smooth	0.96	0.99	0.96
2 smooths	0.96	0.99	0.96

Figure 3-9 – Correlation between ejection fraction from 5 and R acquisitions, normal smoothing



It can be seen in Figure 3-9 that there is a greater degree of variability seen at higher ejection fraction values. This is not affected with additional smoothing.

3.4.4.2 First Third Fractional Filling

Effect on Value

As with ejection fraction, there is a decrease in first third fractional filling with smoothing (Table 3-15). With the exception of the R acquisition, this is not statistically significant with a single extra smooth applied. When a second extra smooth is applied, the difference from N to 2s is statistically significant with the exception of AN/A2s where the p value of 0.06 is very close to significance.

Table 3-15 – Mean first third fractional filling in 4 sub-acquisitions – effect of progressive smoothing of activity-time curve

	A	5	S	R
Normal	46.5%	46.9%	48.2%	47.6%
1 smooth	46.0%	46.4%	47.6%	46.3%
2 smooths	45.0%	45.4%	46.6%	45.8%

Effect on FTFF Reproducibility

Reproducibility of FTFF between studies (5/R) is moderately good at the default level of smoothing. Within the same study (5/S) the agreement between values is only marginally better showing that the limitation here is not with accuracy of camera positioning or ROI creation. Also, biological variability should be minimal as the two acquisitions are only 5

minutes apart. The main source of variability is the roughness of the activity-time curve and the random fluctuations this introduces. There is an improvement in reproducibility with smoothing but this is not substantial and a significant degree of scatter can still be seen (Table 3-16). Much higher degrees of smoothing would be needed to resolve this but would result in unacceptable distortion of the activity-time curve.

Table 3-16 – First third fractional filling intra- and inter-study correlation (R^2) – effect of progressive smoothing

	A/R	5/S	5/R
Normal	0.74	0.74	0.67
1 smooth	0.78	0.75	0.74
2 smooths	0.82	0.76	0.79

Smoothing has minimal effect on the confidence intervals for the difference between values for the 5 and R studies. Overall, a change of 3% in the value of FTFF should be regarded as a genuine change in the value.

3.4.4.3 Peak Emptying Rate

Effect on Value

With each additional curve smooth there is a statistically significant decrease in PER ($p < 0.001$ paired samples T test). This is the case with or without curve fitting by 3 point regression. The magnitude of effect of this is approximately half that of one iteration of curve smoothing.

Table 3-17 – Peak Emptying Rate – mean value between sub-acquisitions and effect of smoothing

	Normal				3 Point Regression			
	A	**5**	**S**	**R**	**A**	**5**	**S**	**R**
Normal	156.8	160.2	164.0	171.5	150.2	152.5	155.8	161.9
1 smooth	146.1	146.5	149.5	154.1	141.8	142.2	145.0	149.1
2 smooths	138.9	138.7	140.6	144.8	135.4	135.1	137.4	140.9

All values are %End diastolic counts per cardiac cycle

Effect on PER Reproducibility

Reproducibility of peak emptying rate is very good with normal smoothing with only a modest improvement with additional smoothing. The baseline R^2 value of 0.904 for 5/S

indicates there is minimal short term biological variability and the resolution of the activity-time curve is adequate for this parameter. 3 point regression results in a small a small improvement in correlation against no curve fitting, but no improvement with additional curve smoothing.

Table 3-18 – Peak emptying rate intra- and inter-study correlation (R^2) – effect of progressive smoothing

	Normal			3 Point Regression		
	A/R	**5/S**	**5/R**	**A/R**	**5/S**	**5/R**
Normal	0.78	0.90	0.79	0.84	0.91	0.83
1 smooth	0.84	0.94	0.83	0.86	0.94	0.84
2 smooths	0.86	0.95	0.85	0.87	0.95	0.85

All values are %End diastolic counts per cardiac cycle

3.4.4.4 Peak Filling Rate

Effect on Value

With each additional curve smooth there is a statistically significant decrease in PFR both with and without 3 point regression($p < 0.001$ paired samples T test). As with peak emptying rate, 3 point regression has an effect half that of one iteration of curve smoothing.

Table 3-19 – Peak Filling Rate – mean value between sub-acquisitions and effect of activity-time curve smoothing

	Normal				3 Point Regression			
	A	**5**	**S**	**R**	**A**	**5**	**S**	**R**
Normal	144.4	147.1	153.0	146.0	137.0	138.0	141.9	137.0
1 smooth	130.1	130.6	134.8	130.8	125.1	125.2	128.5	125.1
2 smooths	121.2	120.7	125.0	122.3	117.7	116.4	120.0	117.7

All values are %End diastolic counts per cardiac cycle

Effect on PFR Reproducibility

Peak filling rate shows good reproducibility with standard levels of smoothing. There is only a small improvement in the R^2 value with additional smoothing. 3 point regression provides only marginal improvement upon this.

Table 3-20 – Peak filling rate intra- and inter-study correlation (R^2) – effect of progressive activity-time curve smoothing

	Normal			3 Point Regression		
	A/R	**5/S**	**5/R**	**A/R**	**5/S**	**5/R**
Normal	0.79	0.82	0.74	0.79	0.86	0.76
1 smooth	0.81	0.88	0.79	0.83	0.90	0.80
2 smooths	0.83	0.91	0.80	0.84	0.92	0.81

3.4.4.5 Time to First Third Empty

Effect on Value

There is an upward trend for ttFTErr with additional smoothing, although the absolute change is very small. This is statistically significant with the An→A1s, Rn→R1s and 5n→51s stages, although the difference becomes insignificant when a second smooth is applied suggesting that overall, the effect of smoothing is not significant.

Table 3-21 – Time to first third empty – mean value between sub-acquisitions and effect of activity-time curve smoothing

	A	**5**	**S**	**R**
Normal	0.174	0.174	0.174	0.171
1 smooth	0.175	0.175	0.175	0.173
2 smooths	0.175	0.175	0.174	0.173

All units are Cardiac Cycles

Effect on ttFTErr Reproducibility

Reproducibility is already reasonable at baseline with 5n/Rn having an R^2 value of 0.67. There is a small stepwise improvement in inter-study correlation with additional smoothing, with the majority of this being seen after 1 additional smooth. The effect on intra-study correlation is negligible.

Table 3-22 – Time to first third empty intra- and inter-study correlation (R^2) – effect of progressive activity-time curve smoothing

	A/R	**5/S**	**5/R**
Normal	0.67	0.89	0.67
1 smooth	0.77	0.91	0.77
2 smooths	0.81	0.92	0.81

3.4.4.6 Time to First Third Full

Effect on Value

Smoothing has no statistically significant effect on the value of ttFTFrr. There is no discernable effect on A and whereas there is a small drop with 1 smooth of 5 and S, the same process increases the value of R.

Table 3-23 – Time to first third full – mean value between sub-acquisitions and effect of activity-time curve smoothing

	A	5	S	R
Normal	0.173	0.174	0.170	0.167
1 smooth	0.173	0.172	0.167	0.171
2 smooths	0.173	0.172	0.168	0.171

All units are Cardiac Cycles

Effect on ttFTFrr Reproducibility

There is moderate correlation between values with normal smoothing. This improves markedly with smoothing with the majority of this benefit being seen after one iteration. There is benefit from a second iteration for inter-study correlation which is less apparent with intra-study correlation.

Table 3-24 – Time to first third full intra- and inter-study correlation (R^2) – effect of progressive smoothing

	A/R	5/S	5/R
Normal	0.42	0.59	0.37
1 smooth	0.60	0.67	0.51
2 smooths	0.68	0.70	0.62

3.4.4.7 Time to Peak Emptying

Effect on Value

There is a trend to increase ttPErr with additional smoothing. Steps 5n→52s, S1s→S2s, R1s→R2s and Rn→R2s achieve statistical significance with p values of 0.014, 0.049, 0.002 and 0.01 respectively.

Table 3-25 – Time to peak emptying – mean value between sub-acquisitions and effect of activity-time curve smoothing

	A	5	S	R
Normal	0.168	0.167	0.161	0.156
1 smooth	0.165	0.173	0.161	0.160
2 smooths	0.171	0.178	0.167	0.168

All units are Cardiac Cycles

Effect on ttPErr Reproducibility

Reproducibility of ttPErr is poor both between studies and within the same acquisition (5/S). This is not improved with additional smoothing.

Table 3-26 – Time to peak emptying intra- and inter-study correlation (R^2) – effect of progressive activity-time curve smoothing

	A/R	5/S	5/R
Normal	0.16	0.17	0.14
1 smooth	0.23	0.06	0.16
2 smooths	0.23	0.07	0.24

3.4.4.8 Time to Peak Filling

Effect on Value

The steps from An to A2s and 5n to 51s are statistically significant with p values of 0.045 and 0.021 respectively (paired samples T-test) but overall there is no significant effect from smoothing.

Table 3-27 – Time to peak filling – mean value between sub-acquisitions and effect of activity-time curve smoothing

ttPFrr	A	5	S	R
Normal	0.20	0.21	0.21	0.21
1 smooth	0.22	0.20	0.20	0.23
2 smooths	0.23	0.20	0.20	0.22

All units are Cardiac Cycles

Effect on ttPFrr Reproducibility

Time to peak filling is very poorly reproducible not only between studies (5/R) but also within the same study (5/S) where the patient has not moved and the same ROIs have been used. Although smoothing seems to improve this in some cases (A/R normal → 1

smooth), the improvement is not consistent and the correlation deteriorates from A1s/R1s to A2s/R2s. Some of this is due to poor temporal resolution of this parameter with relatively large gaps between possible values.

Table 3-28 – Time to peak filling intra- and inter-study correlation (R^2) – effect of progressive activity-time curve smoothing

	A/R	5/S	5/R
Normal	0.11	0.28	0.12
1 smooth	0.51	0.33	0.22
2 smooths	0.39	0.37	0.15

3.4.5 Summary of Effect of Smoothing on Reproducibility

Ejection fraction, peak emptying rate and peak filling rate, both with and without 3 point regression have very good reproducibility with only modest improvements with additional smoothing. As reproducibility improves with the addition of 3 point regression without otherwise affecting the activity-time curve, PER3pr and PFR3pr should be used in preference to the standard version.

First third fractional filling has only moderate reproducibility compared to ejection fraction. However, the confidence intervals of ± 3 is adequate to allow comparisons to be made between patients.

Time to first third empty has acceptable reproducibility without additional smoothing. Although time to first third full does show an improvement with one additional smooth, it is the only useful parameter to need this and it is probably not justifiable to do this unless it can be measured using a separately smoothed curve.

Time to peak emptying and time to peak filling have very poor reproducibility, even with additional smoothing and are of limited utility.

Table 3-29 – Effect of progressive activity-time curve smoothing on inter-study correlation (R^2) of common indices of systolic and diastolic function

	5n/Rn	51s/R1s	52s/R2s
EF	0.92	0.92	0.92
FTFF	0.46	0.55	0.63
PER	0.79	0.83	0.85
PER3pr	0.83	0.84	0.85
PFR	0.74	0.79	0.80
PFR3pr	0.76	0.80	0.81
ttFTErr	0.67	0.77	0.81
ttFTFrr	0.37	0.51	0.62
ttPErr	0.14	0.16	0.24
ttPFrr	0.12	0.22	0.15

3.5 Normal Ranges of Resting and Stress Systolic and Diastolic Function

3.5.1 Resting Function

For many years at Glasgow Royal Infirmary, RNVG has been a standard component of the myocardial stress perfusion imaging protocol as it provides additional data to improve the sensitivity and specificity of this test. The superior radiological properties of technetium compared to thallium^{201} in terms of photon energy and count density allow better visualisation of ventricular wall motion, allowing better characterisation of fixed perfusion defects. Essentially, if a fixed perfusion defect detected by thallium scanning is akinetic or has significantly impaired wall motion on RNVG, it is likely to represent an infarct. Fixed defects with normal wall motion most likely represent tissue attenuation artefact. This information cannot be discerned as readily using gated SPECT perfusion imaging since assessment of wall motion will be difficult in a segment with very low tracer uptake.

In addition to wall motion, left and right ventricular ejection fractions are calculated for all studies performed regardless of whether this information has been specifically requested or not.

As a result of this and the fact that RNVG image data are preserved indefinitely, a large body of RNVG data exists which can be retrospectively analysed and additional parameters of systolic and diastolic function measured beyond the original measure of

ejection fraction. A substantial amount of data is recorded along with the RNVG and corresponding perfusion scan to characterise these patients in the form of prior cardiac history, medication at the time of scanning, age, sex and the original request form along with the referral indications.

It is therefore possible to utilise this body of data to construct a group of normal patients in order to generate a reference range for the parameters of diastolic and systolic function of interest.

3.5.1.1 Definition of Normal Subjects

The following features were required in order to classify a patient as normal:

- Left ventricular ejection fraction ≥ 37%. The in-house lower limit of normal for this has previously been defined as 40%, with a margin of error of ± 3%, meaning that patients with technically mild impairment of left ventricular systolic function may be found to have a normal result if the scan were to be repeated.
- Right ventricular ejection fraction ≥ 25%. Due to the anatomy of the right ventricle, there is a greater degree of attenuation of counts arising posteriorly from the right ventricle than the left ventricle resulting in an apparently reduced ejection fraction even in the presence of normal wall motion and visually assessed systolic function.
- Normal or borderline normal stress thallium perfusion without evidence of prior myocardial infarction. Patients who had undergone percutaneous intervention were not excluded.
- Drug therapy known, including whether the patient was on any medication which would influence heart rate and any parameters of systolic or diastolic function affected by this.

Exclusion criteria were as follows:

- Any major intraventricular conduction defect i.e. right or left bundle branch block. Minor intraventricular conduction defects were not excluded if the RNVG phase image was normal
- Previous myocardial infarction
- Previous cardiac surgery including CABG and valve replacement.
- Recipients of cardiac transplantation.

- Left ventricular hypertrophy either by ECG criteria or as indicated in the referral information.
- Any rhythm other than sinus.
- A clinical history of heart failure or exertional dyspnoea as indicated in the referral information.

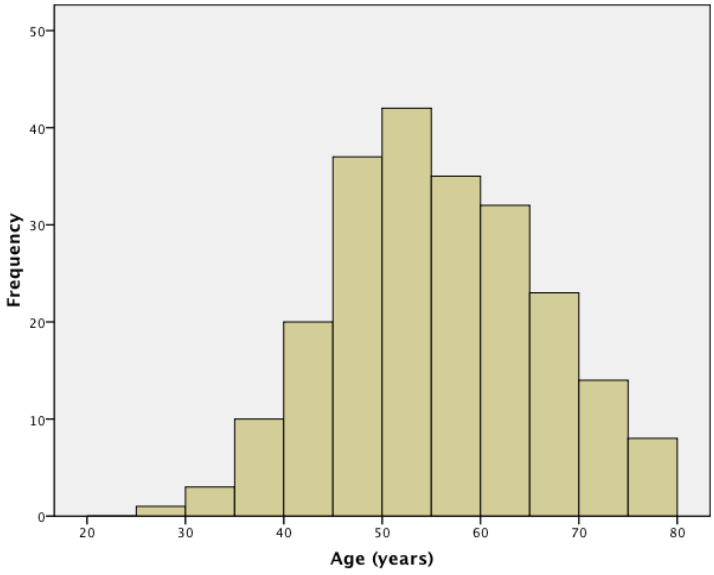
3.5.1.2 Demographics

Using the criteria listed, a total of 225 patients were identified, primarily from studies performed in 2002 and 2007, along with a small cohort of patients that had previously been identified in 1996. Ethical approval was not sought for this study as the data collected were previously obtained as part of routine clinical care and were fully anonymised.

Age

The mean patient age was 55.4, ranging from 28 to 78. This is normally distributed with a skewness statistic of 0.057 and standard error of 0.162. This is illustrated in Figure 3-10.

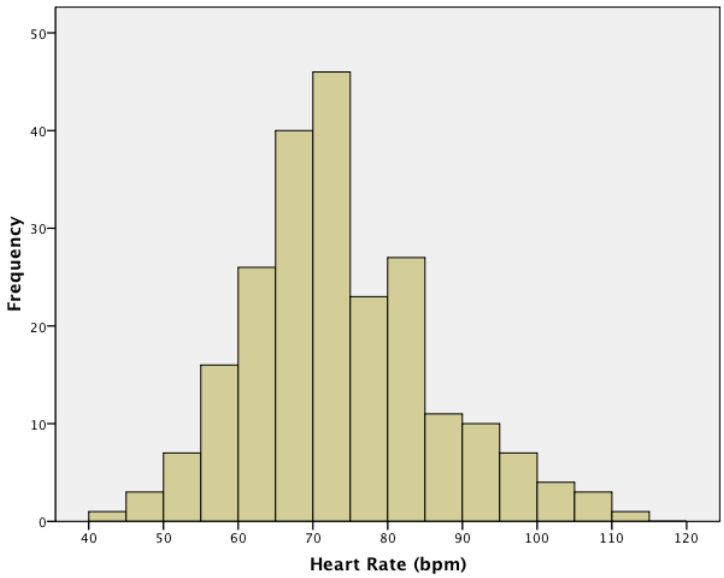
Figure 3-10 – Age distribution of resting RNVG control subjects



Heart Rate and Medication

The mean heart rate was 73.8, range 44.8 to 113.7. The distribution is skewed to the left, skewness statistic 0.549, standard error 0.162. This is illustrated in Figure 3-11.

Figure 3-11 – Heart rate distribution of resting RNVG control subjects



126 patients were taking no heart rate limiting medication. Only one patient was taking both a beta blocker and a rate limiting calcium channel blocker. 63 patients were taking a beta blocker alone, 35 patients were taking a rate limiting calcium channel blocker. Preparations of diltiazem accounted for the vast majority of these.

As one might expect, there is a significant difference between the heart rate between patients prescribed beta blockers and those not (mean heart rate 65.2 vs 77.6, $p< 0.001$, independent samples T test). However, there is no significant difference in resting heart rate between patients prescribed rate limiting calcium channel blockers and those on no heart rate limiting medication (mean heart rate 75.7 vs 77.6, $p=0.422$). The difference between beta blockers and rate limiting calcium channel blockers is also highly statistically significant with a p value of <0.001. In this small sample it is clear that beta blockers are more effective at lowering heart rate at rest compared to rate limiting calcium channel blockers. With the proviso that they represent only 15.6% of this sample, rate limiting calcium blockers appear to have no significant effect on resting heart rate.

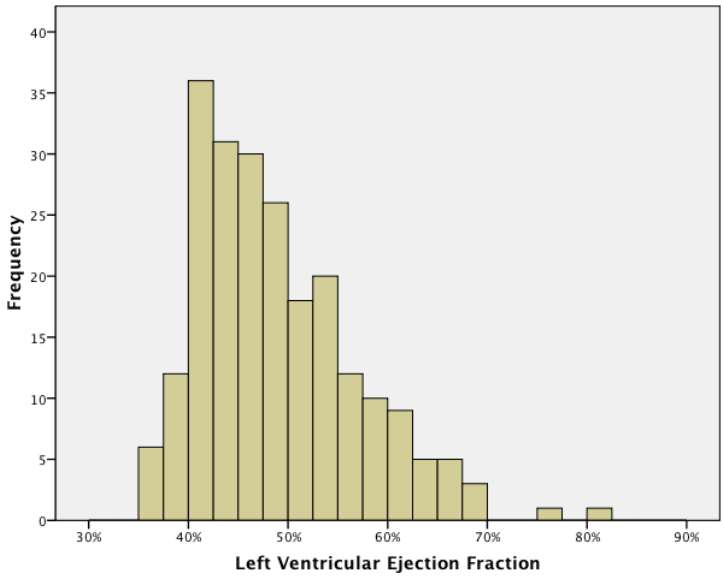
This differential effect between beta blockers and rate limiting calcium channel blockers will clearly need to be accounted for any indices of systolic or diastolic function which are influenced by heart rate. The indices will also require to be examined for any effect of these drugs independent of changes in heart rate.

3.5.1.3 LV Ejection Fraction distribution

The distribution of left ventricular ejection fractions from all 225 patients is shown in Figure 3-12. The mean ejection fraction was 48.8%, ranging from 37% to 81.25% with the median value being 47%.

It can clearly be seen that the results are not normally distributed. The long right tail in the histogram produces a skewness statistic of 0.977 with a standard error of 0.162 indicating a significant departure from symmetrical distribution. This is due to the imposition of a lower limit for inclusion of 37% with no upper limit. If a random sample of all studies performed were to be examined, a far more normal distribution would be expected.

Figure 3-12 – Distribution of left ventricular ejection fraction in all resting control subjects



The few extreme outliers with left ventricular ejection fractions in excess of 65%, the 95th centile for this group, are likely to represent a combination of patients with genuinely hypercontractile ventricles and those in whom technical issues may have artificially elevated the value. If, for example, the left ventricular background region is not truly representative and gives an elevated estimate of background activity, the ejection fraction will be elevated also. Excessively high ejection fractions are also commonly seen in patients with small hypertensive hearts. Although systolic function is within the normal range, it tends to be overestimated as a result of the relatively low resolution of the scan.

Factors Affecting Left Ventricular Ejection Fraction

In this group with left ventricular ejection fractions within the normal range, there was no correlation between either age (Figure 3-13) or heart rate (Figure 3-14) and left ventricular ejection fraction.

Figure 3-13 – Age versus resting LV ejection fraction correlation in control subjects

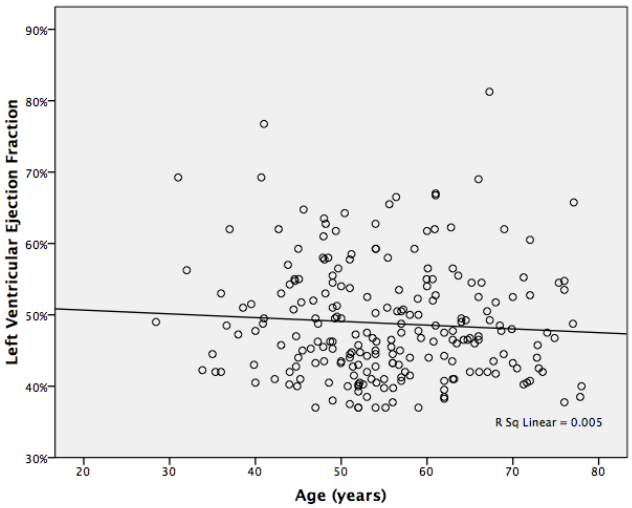
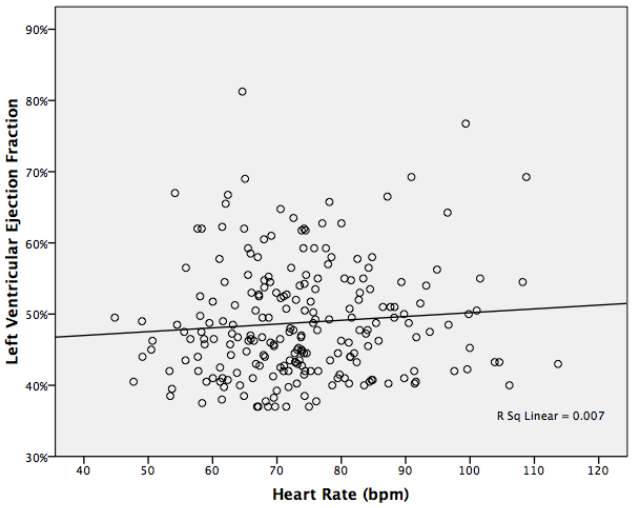
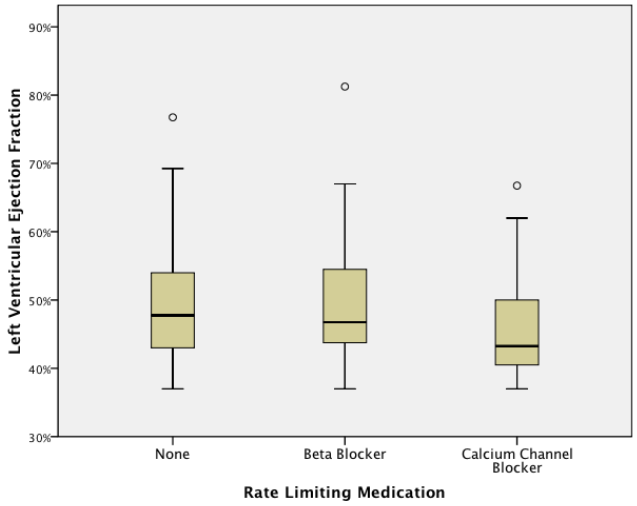


Figure 3-14 – Heart rate versus resting LV ejection fraction correlation in control subjects



Although there is no apparent effect of heart rate on left ventricular ejection fraction, it is still necessary to consider the possibility of an effect from either rate limiting calcium channel blockers or beta blockers independent of the latter's effect on heart rate. The boxplot in Figure 3-15 shows a tendency for patients prescribed rate limiting calcium channel blockers to have a lower ejection fraction (mean 46.2%) than either those on no heart rate limiting therapy (mean 49.35%) or those prescribed a beta blocker (mean 49.26%). This is statistically significant for the comparison with no medication ($p=0.038$, independent samples T-test). There is no difference between those prescribed beta blockers and those on no medication ($p=0.945$).

Figure 3-15 – LV ejection fraction - effect of heart rate limiting medication



3.5.1.4 Correlates of Systolic and Diastolic Function

LVEF and FTFF

Although a close relationship can be demonstrated between left ventricular ejection fraction and peak filling and emptying rates, there is no such relationship between ejection fraction and first third fractional filling. This is not only true for the group here with a preserved ejection fraction (Figure 3-16), the same lack of correlation is seen where systolic function is impaired (Figure 3-17). These data are taken from the patient group used to assess the reproducibility of the RNVG parameters being used (see section 3.2).

Figure 3-16 – LV Ejection Fraction / First Third Fractional Filling correlation at rest in control subjects

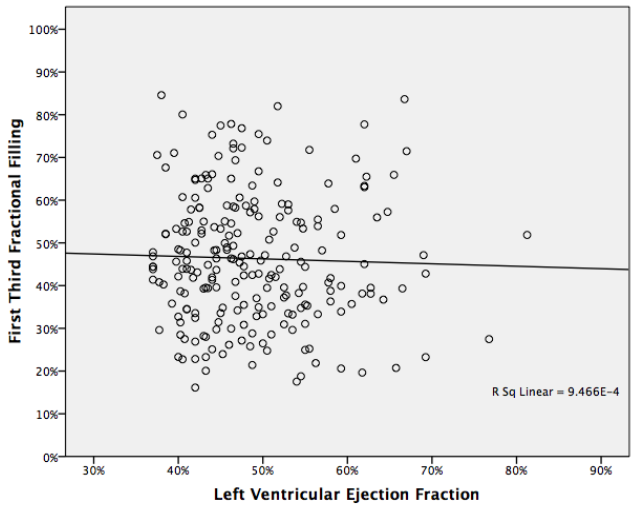
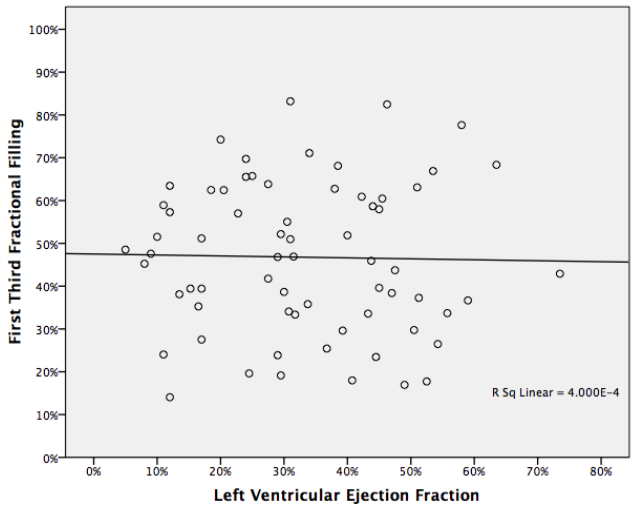


Figure 3-17 – LV Ejection Fraction / First Third Fractional Filling correlation with full range of ejection fractions

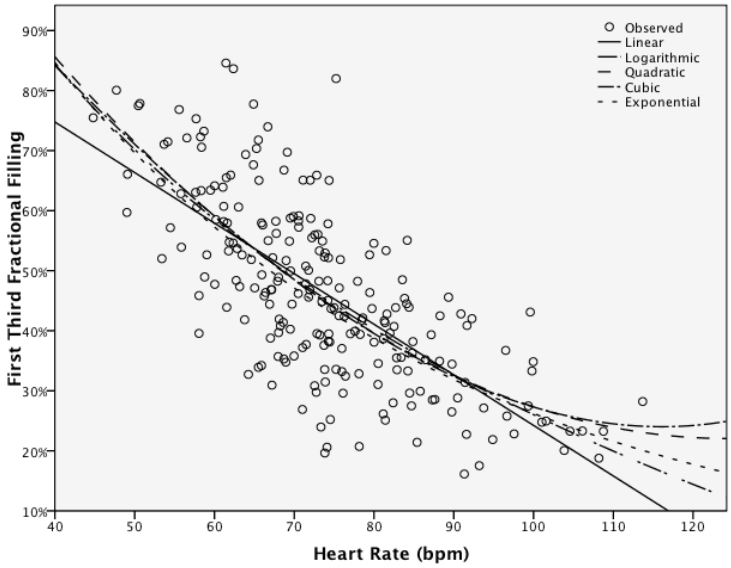


First Third Fractional Filling and Heart Rate

It has been recognised previously that first third fractional filling is related proportionally to heart rate and also that beta blockade appears to have an effect on this independent of its effect on heart rate. The relationship between heart rate and first third fractional filling for the group as a whole is shown in Figure 3-18.

Four different regression models have been used to try to define the relationship between first third fractional filling and heart rate – linear, logarithmic, quadratic and cubic. However, there is a significant degree of scatter about the line of best fit, especially at lower heart rates and it is therefore important to look separately at those whose heart rates have been lowered by either beta blockade or rate limiting calcium channel blockers.

Figure 3-18 – First Third Fractional Filling relationship with heart rate

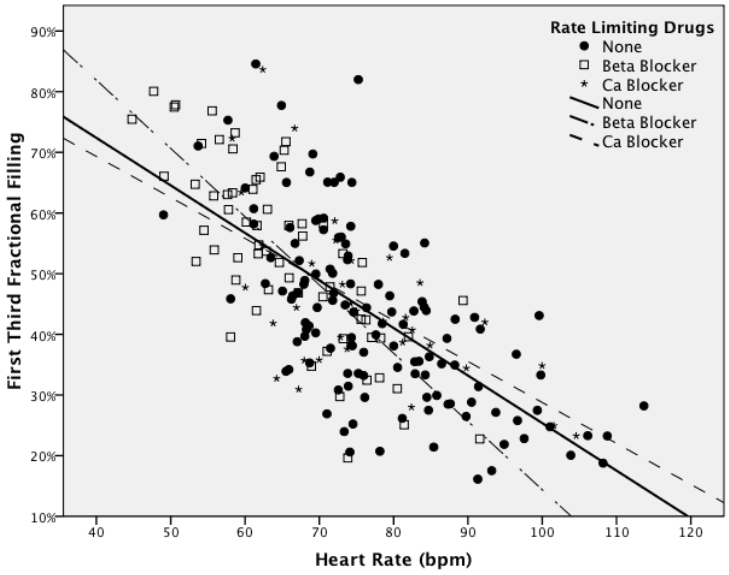


Within subgroups defined by heart rate limiting medication, we have already seen that the resting heart rate of the group on beta blockers was significantly lowered compared to no therapy. Given the close inverse relationship between heart rate and first third fractional filling it is therefore to be expected that those on beta blockers will tend to have higher first third fractional filling than those not. Mean first third fractional filling on no heart rate limiting therapy was significantly lower at 42.96%, compared to 53.552% for the beta blocker group, $p<0.001$ (independent samples t-test).

As with heart rate, there is no significant difference in first third fractional filling between the rate limiting calcium channel blocker and no therapy group (means 45.19 vs 42.96, $p=0.417$). The difference in first third fractional filling between calcium channel blockers and beta blockers was also highly statistically significant ($p=0.006$).

When plotted within the subgroups of no medication, beta blockers and rate limiting calcium channel blockers, very similar relationships can be seen between heart rate and first third fractional filling (Figure 3-19). This suggests that, at least within healthy individuals, there is no significant effect of beta blockers or rate limiting calcium channel blockers on first third fractional filling independent of the effect of these drugs on heart rate.

Figure 3-19 – First Third Fractional Filling / Heart Rate relationship, including linear fit lines, within heart rate limiting medication subgroups



The linear regression equations for these subgroup plots are shown in Table 3-30. In combination with visual inspection of the data, there does not appear to be any significant different difference in first third fractional filling between subgroups of patients on different heart rate limiting drugs outwith the effect on heart rate. It is therefore

unnecessary to consider patients on different cardiac medications separately when assessing FTFF.

Table 3-30 – Linear regression of First Third Fractional Filling and heart Rate by heart rate limiting therapy

	n	Constant	Coefficient (HR)	Correlation Coefficient
All subjects	225	108.4	-0.842	0.71
No therapy	126	103.7	-0.784	0.65
Beta blocker	63	126.9	-1.125	0.77
RLCB	35	96.3	-0.676	0.60

By using regression analysis to model the response of FTFF to changes in heart rate it is possible to attempt to correct FTFF such that effect of heart rate is minimised and patients can be better compared not only to a normal range, but also with themselves on repeated measurements. Four candidate models are described in Table 3-31 and extrapolated beyond the heart rates observed in the control population data in Figure 3-20.

Table 3-31 – Curve regression equations for rest supine First Third Fractional Filling

Model	**R^2**	**Equation**
Linear	0.498	FTFF = 108 - (HR x 0.84).
Logarithmic	0.513	FTFF= 319 - (63.7 x ln(HR)
Quadratic	0.516	FTFF = 160 - (2.22 x HR) + (0.009 x HR^2)
Exponential	0.510	FTFF = 185 x $e^{-0.02xHR}$

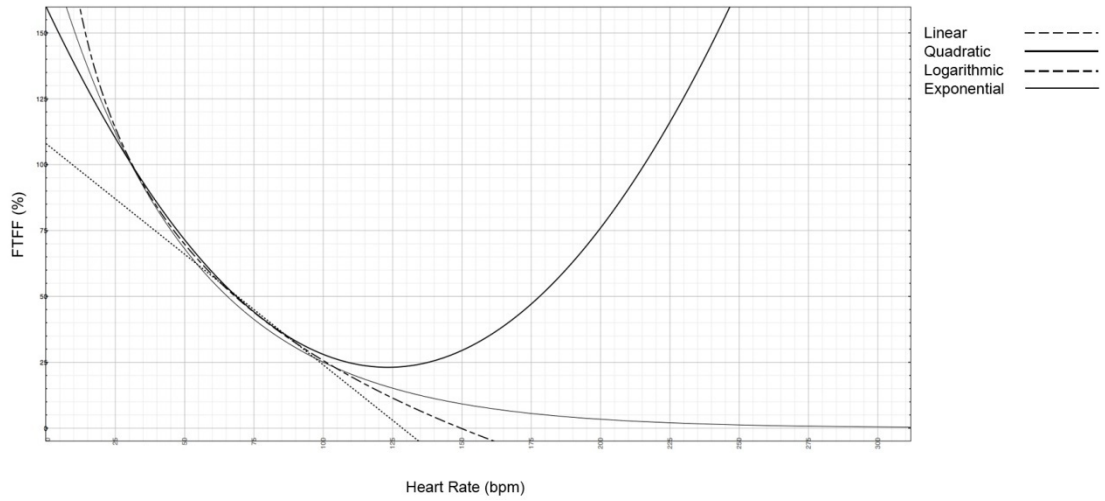
Although linear regression gives a reasonably good fit and an R^2 value of 0.498, the model dictates that at a certain heart rate first third fractional filling will be zero and indeed will be negative above this threshold. In the model generated by this data set, this will occur at a heart rate of 128 beats per minute, a physiologically possible heart rate. This behaviour cannot hold true in the real world.

Logarithmic regression gives a better fit to the observed data but, as with linear regression, this model predicts that first third fractional filling will ultimately fall to zero. This occurs at heart rate of 144, still within the physiological normal range.

The quadratic model more accurately describes what we expect to happen to FTFF in that it will not fall to zero or negative values. It has a superior R^2 value of 0.516 and has a visibly superior fit over the range of heart rates seen in this group. However, the FTFF predicted by this equation begins to rise again after its plateau at a heart rate of 140. This

is not what would be expected and it is clear the model is only valid within the range of resting heart rates observed.

Figure 3-20 – Extrapolated First Third Fractional Filling / Heart Rate curves



Compared to a linear model, the exponential model also has a superior R^2 value at 0.510 and behaves as expected both within the range of physiologically achievable heart rates and beyond. Although this appears an attractive model, it is based on extrapolation from a relatively narrow spectrum of heart rates. In order to create a valid model for how first third fractional filling behaves at levels above normal resting heart rates and to calculate an accurate correction factor and normal range it will be necessary to generate additional data by studying normal subjects during exercise (Section 3.5.2.3).

First Third Fractional Filling and Age

As can be seen from Figure 3-21 there is no relationship between first third fractional filling and age. There is a wide spread of values seen at all ages. The trend line for all patients irrespective of heart rate limiting drug therapy is essentially horizontal. This also holds true when the subjects are divided into 10 year age bands (Figure 3-22).

Figure 3-21 – First Third Fractional Filling versus age in control subjects

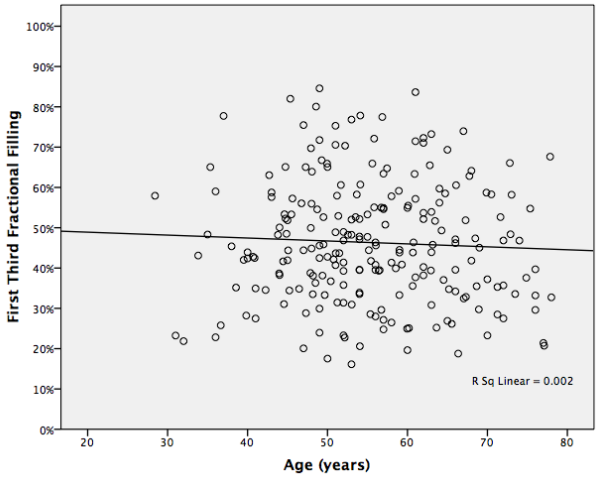
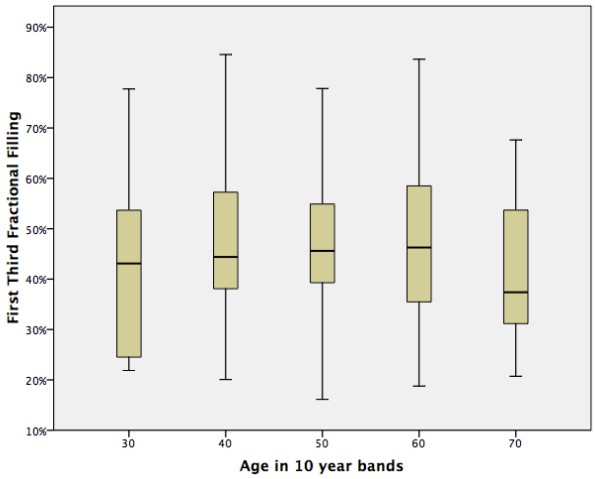


Figure 3-22 – First Third Fractional Filling in control subjects versus age in 10 year bands

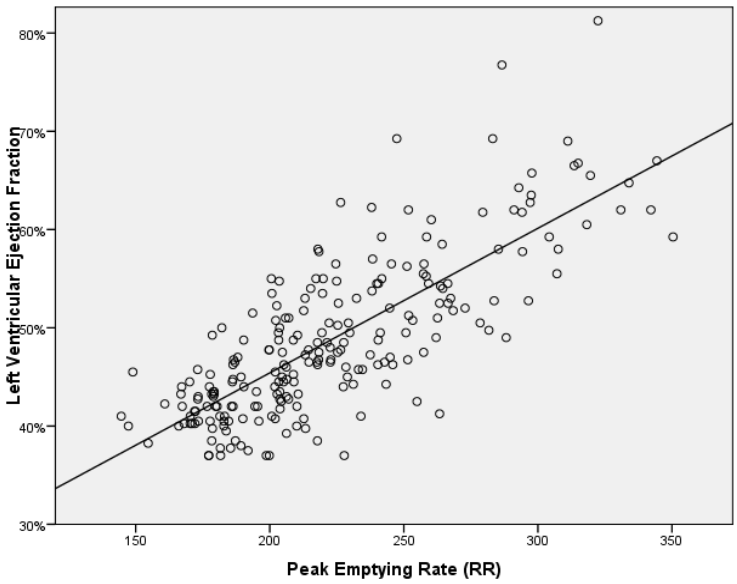


This lack of correlation with age remains when broken down into the drug therapy subgroups. This demonstrates that it is not necessary to calculate an age specific normal range for first third fractional filling

LVEF and PER, PFR

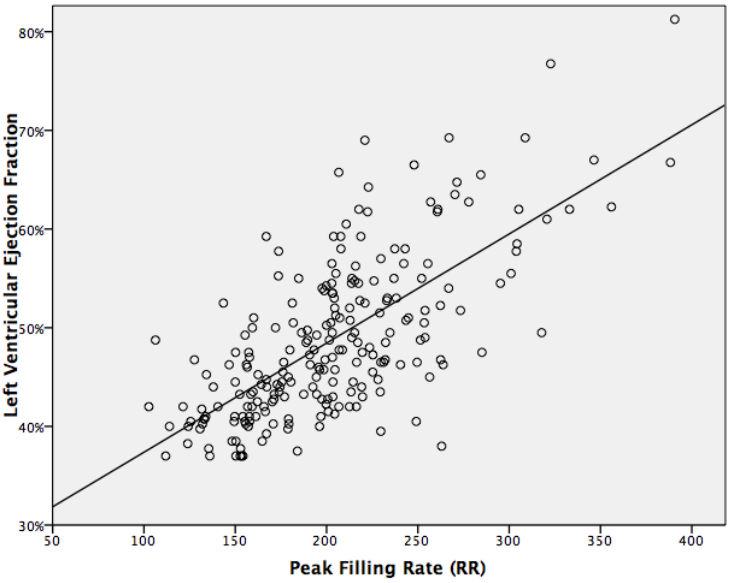
It is reasonable to expect left ventricular ejection fraction to be directly related to peak emptying rate, as confirmed in Figure 3-23 (r^2=0.61, $p < 0.001$). Logically, as peak emptying rate falls the stroke volume must also fall, unless there is a proportional increase in the duration of systole to compensate for this.

Figure 3-23 – Relationship between left ventricular ejection fraction and peak filling rate



The same does not necessarily hold true for peak filling rate. Diastole occupies a greater proportion of the cardiac cycle. It is therefore possible for the same total quantity of filling to allow a "normal" stroke volume to be spread over a greater time, resulting in a lower PFR. Within this normal population, however, this does not occur and PFR has a strong linear relationship with ejection fraction as shown in Figure 3-24 ($r^2 = 0.48$, $p < 0.001$).

Figure 3-24 – Relationship between left ventricular ejection fraction and peak filling rate



PER, PFR and Heart Rate

As the duration of systole shortens in absolute terms but increases as a proportion of the cardiac cycle as heart rate increases, it is not unreasonable to expect that a parameter such as peak emptying rate, which is directly linked to the duration of systole, will show some relationship to heart rate. This can be seen in Figure 3-25 where there is a modest inverse

relationship between PER and heart rate. However, if PER is normalised to the duration of systole (PERst) rather than the entire cardiac cycle, this relationship is lost (Figure 3-26). It may, therefore, be more appropriate to use PERst, particularly when there is a significant difference between heart rates being compared. However, the magnitude of scatter around the linear fit line is such that the heart rate effect with cycle length normalisation will be of no significance in clinical use.

Figure 3-25 – Peak Emptying Rate (normalised to RR interval) versus Heart Rate

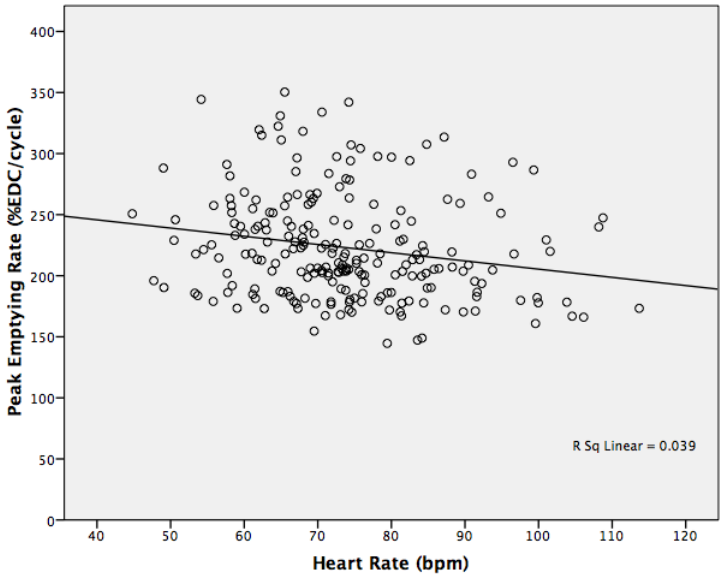
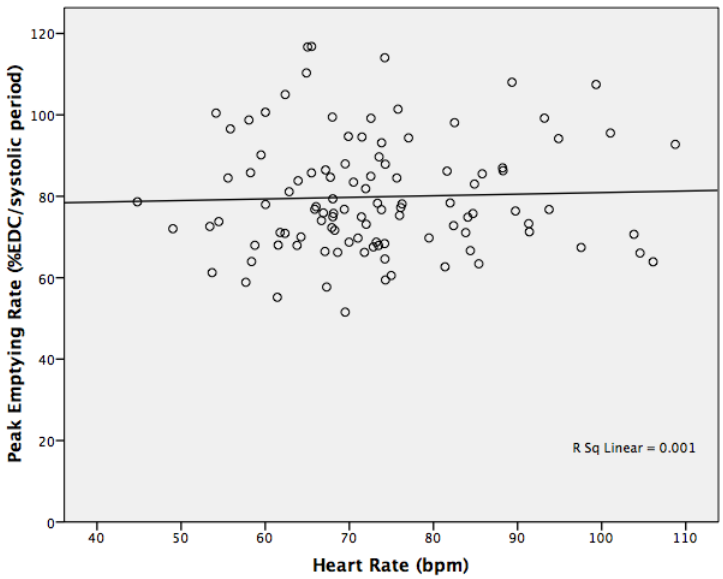


Figure 3-26 – Peak Emptying Rate (normalised to systole duration) versus Heart Rate



This inverse relationship is also seen with peak filling rate (Figure 3-27). However, normalising PFR to diastole (PFRdt) rather than cardiac cycle length does not remove this association. Conversely, it slightly strengthens it with the correlation coefficient rising

from 0.22 to 0.32 (Figure 3-28). As a simple normal range would be independent of heart rate, this increase in heart rate dependency is the opposite of what is required.

Figure 3-27 – Peak Filling Rate (normalised to RR interval) versus Heart Rate

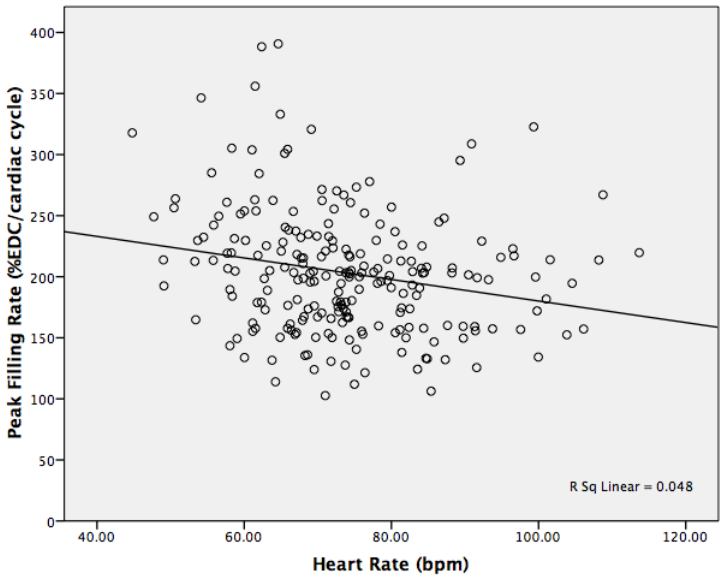
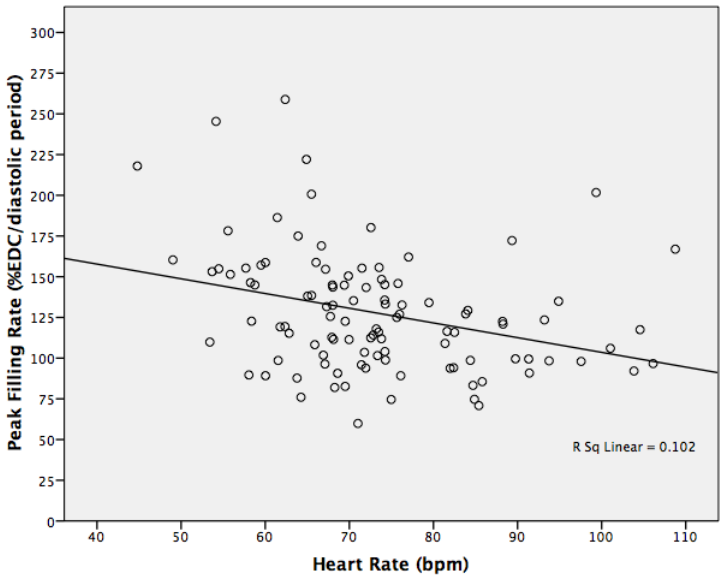


Figure 3-28 – Peak Filling Rate (normalised to diastole duration) versus heart rate



3.5.1.5 Resting Normal Ranges

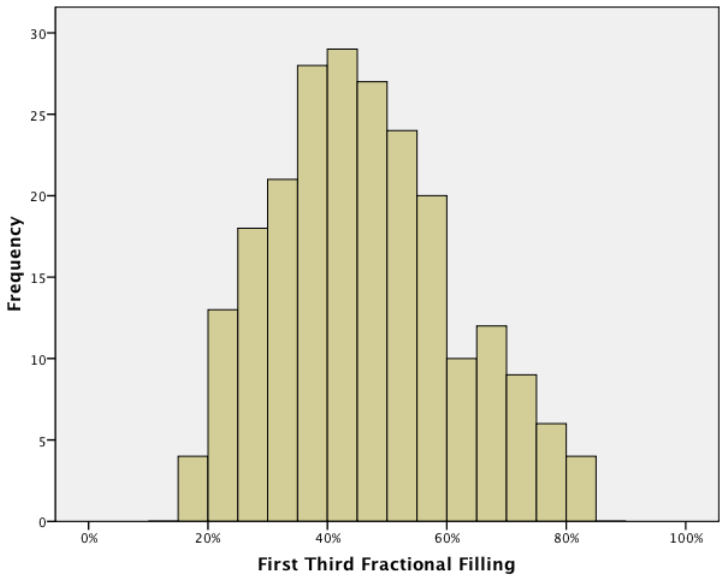
First Third Fractional Filling

If one were to disregard the clear heart rate dependency of first third fractional filling, it should be possible to express the normal range as the mean ± 2 standard deviations from our reference population.

With a mean of 46.33% and median of 44.85% first third fractional filling would appear to be normally distributed. However, there are fewer results in the 60-70% range, resulting in a borderline skewed distribution (Figure 3-29). The skewness statistic is 0.313 which is less than 2 times the standard error of skewness 0.162. The distribution, therefore, is normal and it is therefore appropriate to express the normal range for this as the mean ± 2SD.

By this method, the normal range for first third fractional filling is 46 ± 30.2, i.e. 15.8 to 76.8. This is such a wide range that it is unlikely to have any discriminatory power.

Figure 3-29 – First Third Fractional Filling distribution within normal subjects



Whilst a fixed reference range might be appropriate within a narrow range of heart rates it is clear both from the observed data within the reference population and the extrapolated exponential model that some account of heart rate must be taken. There are three potential approaches to this.

Individual Reference Ranges

Individual reference ranges may be defined for narrow ranges of heart rates. These ranges could be either 10 or 20 beats per minute wide. This approach would have an advantage in that it would be simple to calculate these ranges, but would be likely to suffer from a scarcity of data at the upper and lower limits of what could be considered as a normal resting heart rate in a *normal* patient. In this patient group only 4 subjects had a resting heart rate of <50 and 8 subjects had a heart rate >100. These were merged into the adjacent groups as heart rate <60 and >90 respectively. The tendency towards skewness when the group is taken as a whole disappears when grouped in this way. The results for

these groups are shown in Table 3-32. The normal range is the 5th to 95th centiles using a weighted average.

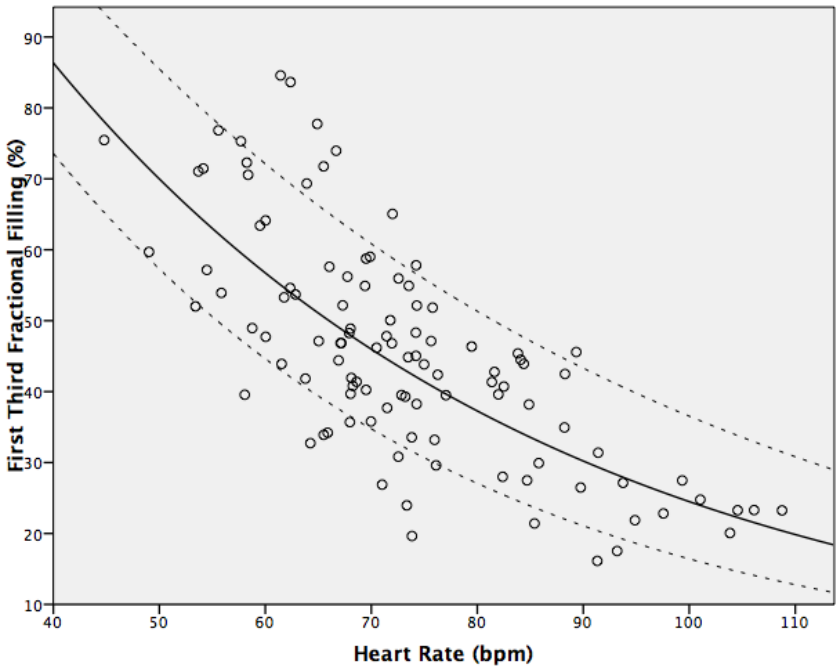
Table 3-32 – Normal ranges for First Third Fractional Filling per 10 beat per minute heart rate interval

HR (bpm)	N	Mean FTFF (%)	SD	Normal Range (%)
<60	27	64.7	10.8	42.1-79.2
60-69	66	53.1	12.5	34.1-76.4
70-79	69	44.5	12.1	22.3-65.1
80-89	38	36.8	7.8	24.9-53.4
>90	25	28.1	8.0	16.6-43.0

Continuous Reference Range FTFF

It has already been shown that the observed reference population data may be best fitted to an exponential curve described by the equation FTFF = 185 x $e^{-0.02xHR}$. By plotting 2 standard deviations on either side of the extrapolated curve a continuous reference range may be constructed. A patient could then be dichotomised as normal or abnormal by plotting their FTFF on this graph. The standard error for this curve was 0.001. In a group of 225 patients this gives a standard deviation of 0.015. The curves for +2SD and -2SD may therefore be expressed as 185 x $e^{-0.017xHR}$ and 185 x $e^{-0.023xHR}$ respectively. These curves are plotted with the reference population data in Figure 3-30. The curves are shown extrapolated to physiologically achievable heart rates with the reference data points omitted in Figure 3-31.

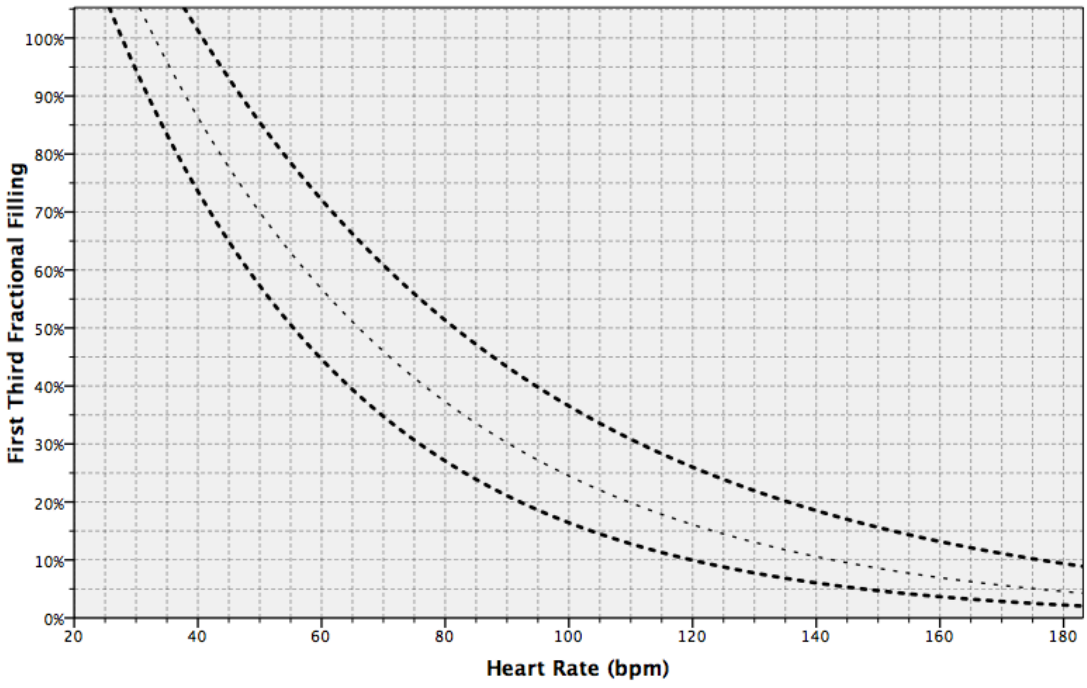
Figure 3-30 – Resting supine first third fractional filling normal range with reference data from normal population



Broken lines represent ± 2 SEM, the upper and lower limit of the normal range

A patient's first third fractional filling result, along with heart rate, may then be plotted on this chart. This will not only allow determination as to whether a patient falls outwith the limits of normality, but also by what extent.

Figure 3-31 – Extrapolated supine resting FTFF continuous reference range



Thick broken lines represent ± 2 SEM, the upper and lower limit of the normal range

Heart Rate Corrected FTFF

A second approach using the reference equations derived above, is to use the equation for minus 2SD (FTFF = 185 x $e^{-0.023xHR}$) to calculate the lower limit of normal for any given heart rate. If a patient's measured FTFF is lower than this calculated value, they can be deemed to be abnormal. This may be less cumbersome than plotting the value on a chart.

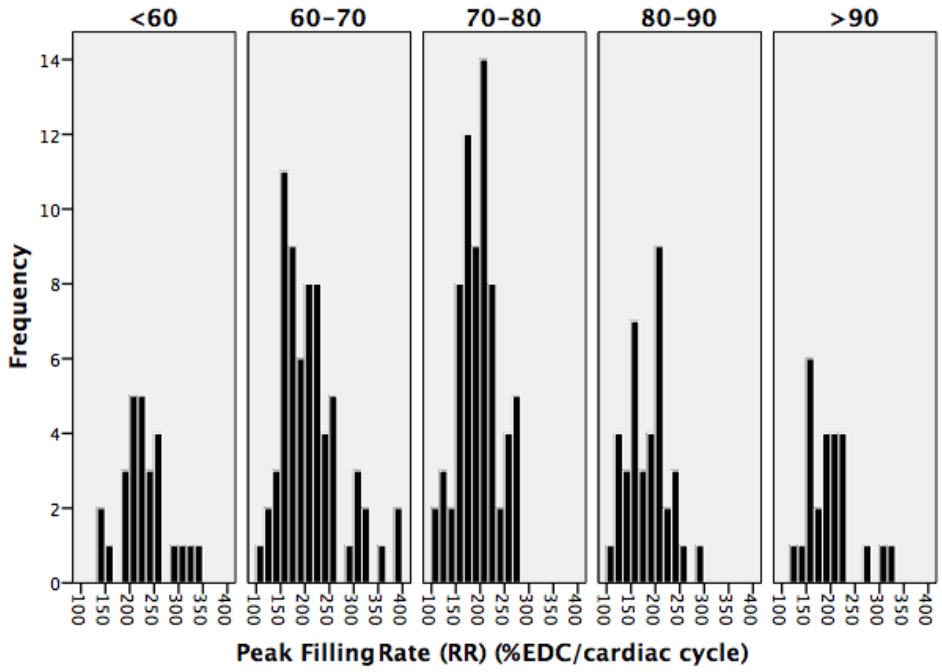
Peak Filling Rate

Peak filling rate is not normally distributed. The whole patient group has a skewness statistic of 0.842 with standard error of 0.162. Mean and 2 standard deviations are therefore not appropriate to define the normal range. This is better represented by the median and interquartile range. For the whole group this is:

Median 201.5 %EDC/cycle, interquartile range 162.2 – 265.1

As peak filling rate shows a weak relationship to heart rate, it may also be appropriate to express its normal range as a series of normal ranges using the same heart rate subgroups used for first third fractional filling. Within the heart rate subgroups, peak filling rate is normally distributed with the exception of the 60-69bpm group where this is skewed by several outliers with very high peak filling rates. This can be seen in Figure 3-32. The skewness statistic for this subgroup is 1.037 with standard error of 0.295.

Figure 3-32 – Supine resting Peak Filling Rate (RR) distribution within heart rate subgroups of normal patients



As with first third fractional filling, the normal ranges shown in Table 3-33 are the 5th and 95th centiles calculated using a weighted average. Median and interquartile range is used for the 60-69 bpm heart rate subgroup.

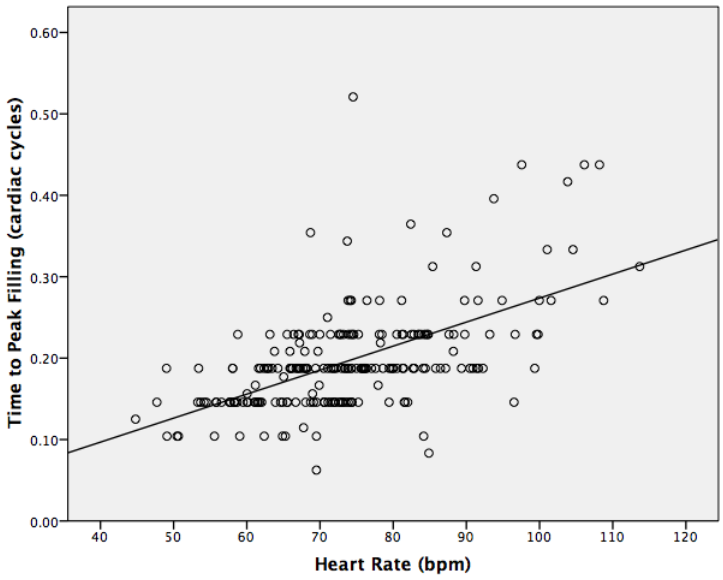
Table 3-33 – Normal ranges for Peak Filling Rate (normalised to RR interval) per 10 beat per minute heart rate interval

HR (bpm)	N	Mean PFR(RR)	SD	Normal Range (%EDC/cycle)
<60	27	230.9	47.9	145.9-335.0
60-69	66	203.2	61.4	163.7-237.6
70-79	69	196.4	40.3	124.5-270.8
80-89	38	184.9	41.8	123.4-258.9
>90	25	199.0	48.5	128.2-318.5

Time to Peak Filling

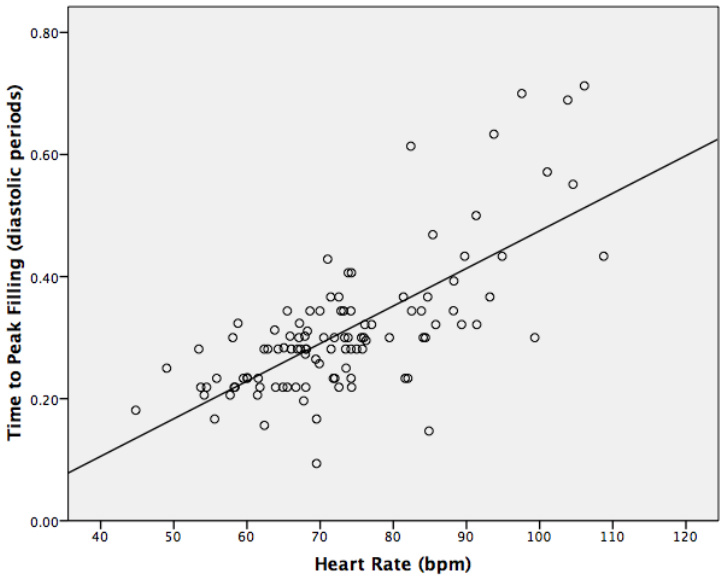
Time to peak filling, expressed as a proportion of the cardiac cycle length, is linearly related to heart rate with a correlation coefficient of 0.57. This is shown in Figure 3-33. It is, therefore, necessary to define normal ranges for Time to Peak Filling using the same heart rate subgroups already employed for first third fractional filling.

Figure 3-33 – Relationship between Time to Peak Filling (RR) and heart rate



As diastole shortens proportionally more than systole with increasing heart rate, one might expect the relationship between time to peak filling and heart rate to weaken if normalised to diastole duration rather than the whole cycle length. However, the relationship is actually strengthened, and has a correlation coefficient of 0.71 (Figure 3-34).

Figure 3-34 – Relationship between Time to Peak Filling (normalised to diastole duration) and heart rate



Within the 69-69 and 70-70 bpm heart rate subgroups, Time to Peak Filling has a skewed distribution. The median and interquartile ranges are therefore used to define the normal ranges for these two subgroups as shown in Figure 3-35.

Figure 3-35 – Normal ranges for Time to Peak Filling (normalised to RR interval) per 10 beat per minute heart rate interval

HR (bpm)	N	Skewness	Std Error Skewness	Mean/Median ttPF(RR)	Normal Range (cardiac cycles)
<60	27	0.702	0.448	0.15	0.10-0.21
60-69	66	0.759	0.295	0.19	0.15-0.19
70-79	69	2.976	0.298	0.19	0.15-0.23
80-89	38	0.53	0.383	0.21	0.10-0.35
>90	25	0.589	0.464	0.28	0.16-0.44

Peak Emptying Rate

Peak emptying rate normalised to the cardiac cycle length (RR interval) shows a weak negative relationship with heart rate (correlation coefficient 0.2). This was shown in Figure 3-25. Normal ranges have, therefore, been calculated within heart rate subgroups and the group as a whole.

Within the whole group, peak emptying rate has a skewed distribution with a skewness of 0.786 (standard error 0.162). The median value is 214.4 %EDC/cycle and interquartile range is 188.6 – 249.1.

Distribution is also skewed within several of the heart rate subgroups (Figure 3-36) and, where appropriate, median and interquartile range is used to define the normal ranges.

Figure 3-36 – Peak Emptying Rate (RR) distribution within heart rate subgroups

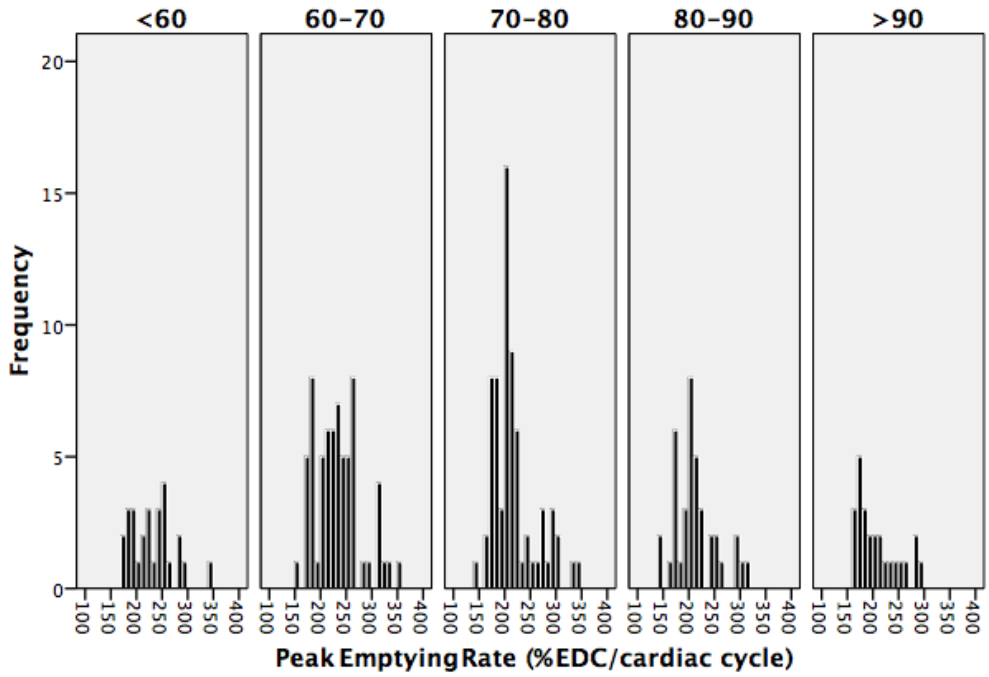


Figure 3-37 – Normal ranges for Peak Emptying Rate (normalised to RR interval) per 10 beat per minute heart rate interval

HR (bpm)	N	Skewness	Std Error Skewness	Mean/Median PER(RR)	Normal Range (%EDC/cycle)
<60	27	0.727	0.448	231.3	175.6 – 323.0
60-69	66	0.606	0.295	231.7	202.9 – 260.7
70-79	69	1.155	0.289	206.2	187.1 – 226.4
80-89	38	0.795	0.383	206.5	187.3 – 232.8
>90	25	0.748	0.464	210.4	162.4 – 291.0

3.5.2 Normal Ranges of Left Ventricular Function by Stress RNVG

3.5.2.1 Patient Characteristics

26 patients were recruited, 18 female, 8 male. The mean age was 57 years, ranging from 47 to 73. 14 patients had a history of hypertension. 3 patients were current smokers, 6 were ex-smokers for greater than 1 year and the remaining 17 had never smoked. No patients were diabetic. The mean BMI was 27.8kg/m^2, ranging from 21.3 to 39.6.

All patients had normal myocardial perfusion, determined by thallium scintigraphy performed on the same day as the stress RNVG. Informed consent was obtained from these patients for this additional test. Ethical approval for this was included in the overall study protocol.

Medication and Heart Rate

14 patients were taking no heart rate limiting medication. 7 patients were taking beta blockers and 5 patients were taking rate limiting calcium channel blockers. The mean heart rates at rest and peak stress are summarised within these groups and within the entire cohort in Table 3-34. Resting heart rate is significantly lower in the beta blocker group compared to the group of patients on no rate limiting medication ($p = 0.013$, independent sample T-test, equal variances demonstrated). There is no significant difference in peak stress heart rates in these two groups ($p = 0.237$) or in change in heart rate from rest to stress on an individual patient basis (mean difference 1.93 bpm, $p = 0.57$). As with supine resting studies (section 3.5.1), this significant difference in heart rate with beta blockade makes it very important to control for heart rate when considering highly heart rate dependent RNVG parameters such as first third fractional filling.

Table 3-34 – Control stress RNVG patients mean heart rates (bpm) at rest and stress

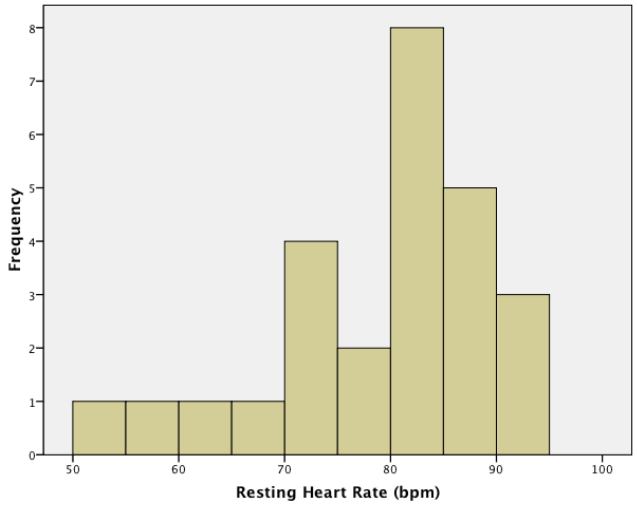
		Rate Limiting Medication		
	Whole group	**None**	**Beta Blocker**	**RLCB**
Rest	79.2	82.3	70.0	83.6
Stress	130.7	135.8	125.4	123.6

RLCB = Rate Limiting Calcium channel Blocker

Resting heart rate has a skewed distribution (skewness statistic -1.187, standard error 0.456, Figure 3-38). As there are only 4 patients with resting heart rates less than 70 beats per minute, the heart rate subgroups initially defined in section 3.5.1.5 are not appropriate and the lower heart groups will be merged as a less than 70bpm group.

Peak stress heart rate is normally distributed around a mean of 130.7 beats per minute. By defining a 20 beat per minute group around this mean heart rate, and one group at either extreme of this range, 3 peak stress heart rate groups are defined. These are < 120 bpm, 120-140 bpm, and ≥ 140 bpm and contain 7, 12 and 7 patients respectively.

Figure 3-38 – Distribution of resting heart rates within control stress RNVG group



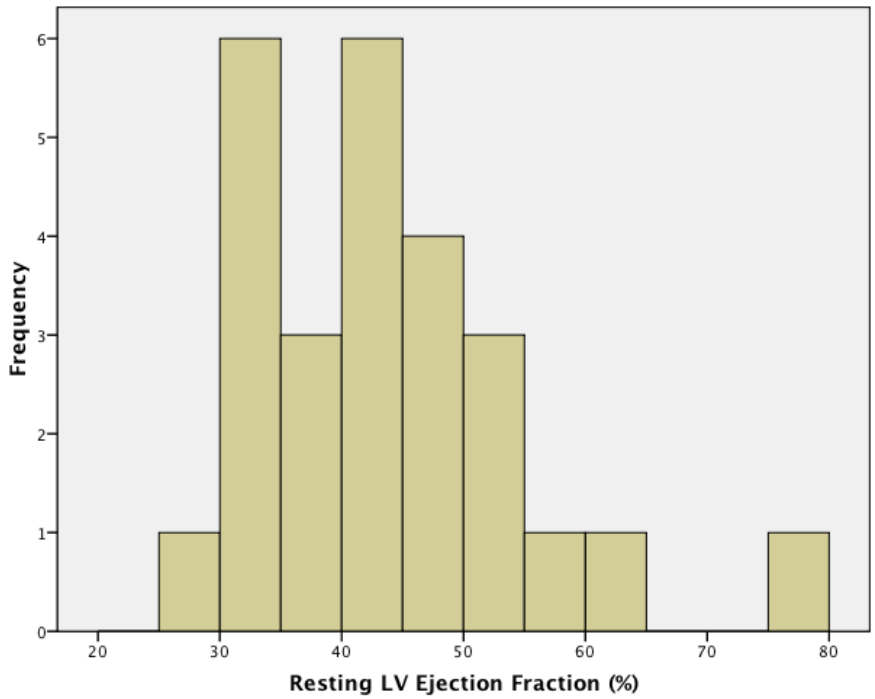
3.5.2.2 Left Ventricular Ejection Fraction

Erect Resting

The mean resting erect left ventricular ejection fraction is 44%, ranging from 27.5% to 79%. The distribution is skewed (skewness statistic 1.174, standard error 0.456). The interquartile range is 33.9% to 50.0%.

10 of the 26 patients have a resting erect left ventricular ejection fraction of less than 40%, the previously defined lower limit of normal for supine ejection fraction. This patient group was defined as normal on a clinical basis in conjunction with normal myocardial perfusion, rather than previously measured ejection fraction. The apparent left ventricular systolic dysfunction may, therefore, represent low normal ejection fractions in otherwise healthy individuals. The spread of erect ejection fractions is shown in Figure 3-39.

Figure 3-39 – Resting left ventricular ejection fraction distribution in stress RNVG control subjects



Peak Stress

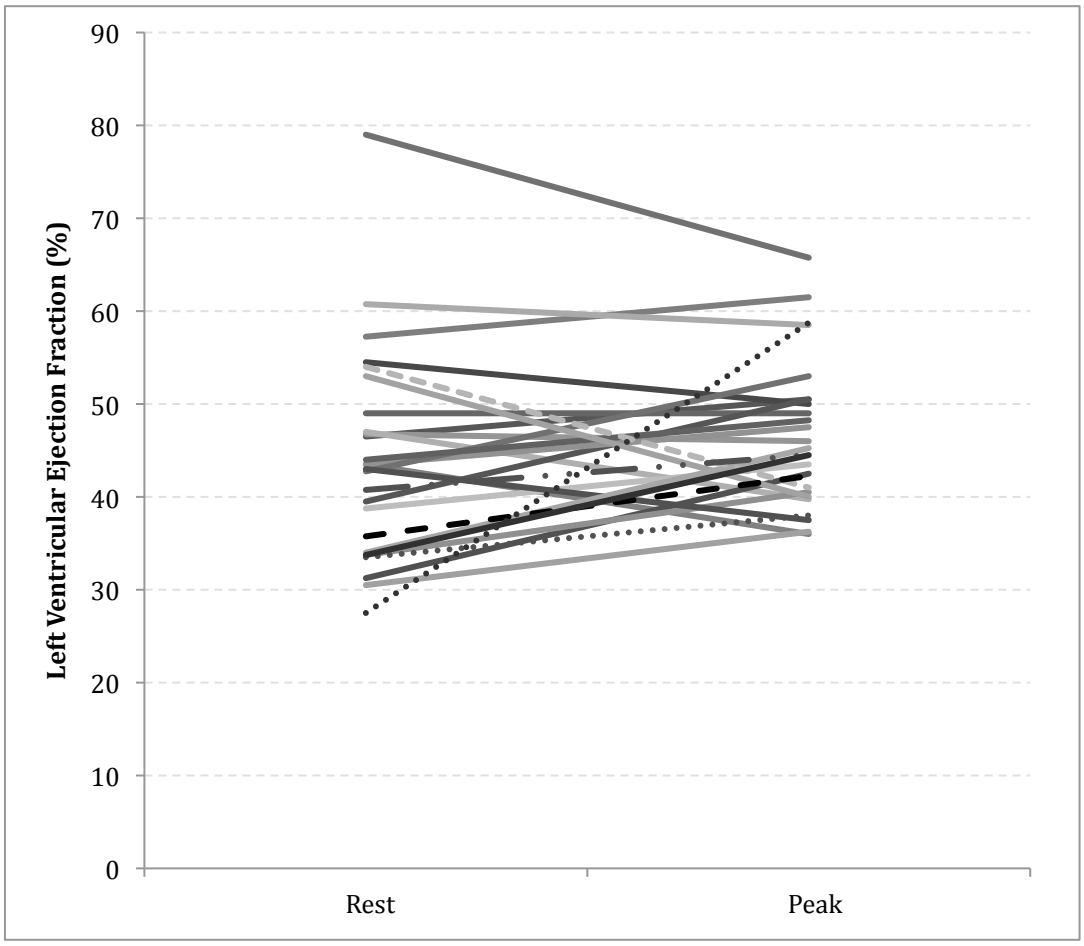
The mean peak stress left ventricular ejection fraction was 46.6%. The interquartile range was 40.4% to 50.5%.

Response to Stress

As a whole, the group showed a statistically non-significant increase in ejection fraction of 2.6% ($p = 0.183$, paired samples T-test). Individual ejection fraction responses can be seen in Figure 3-40. There is a tendency for the highest and lowest resting left ventricular ejection fractions to converge to a midpoint. The patient with the lowest resting ejection fraction showed the greatest rise with exercise, from 27.5% to 58.8%, and has one of the highest peak stress ejection fractions. This would support the assumption that the patient population genuinely is normal, and that regression to the mean is occurring.

A failure to increase ejection fraction with stress, or indeed a fall in ejection fraction, can indicate myocardial ischaemia. This has been excluded here by the use of a more sensitive test, perfusion scintigraphy.

Figure 3-40 – Left ventricular ejection fraction change during stress RNVG in control subjects



3.5.2.3 First Third Fractional Filling

Erect Resting

The relationship between first third fractional filling and heart rate seen with the larger supine control group is also seen in the rest erect subjects. Due to the small numbers in even the reduced number of heart rate subgroups, use of a weighted average to calculate 5th and 95th centiles as the normal range is not possible. The normal ranges quoted in Table 3-35 are, therefore calculated as the mean ± 2 standard deviations. The impact of small patient numbers is seen most clearly in the patients with a resting heart rate greater than 90 beats per minute. The standard deviation is particularly large with respect to the mean value, resulting in a lower limit of normal of only 0.4%.

Table 3-35 – Normal ranges for resting erect First Third Fractional Filling per 10 beat per minute heart rate interval

HR (bpm)	N	Mean FTFF	SD	Normal Range (%)
<70	4	50.8	6.7	37.4 – 64.2
70-79	6	34.7	4.4	25.9 – 43.5
80-89	13	26.9	6.1	14.7 – 39.1
>90	3	26.6	13.1	0.4 – 52.8

Peak Stress

A continued decline in first third fractional filling is seen with increasing heart rate with stress. The mean values and lower limits of normal in Table 3-36 again highlight the problem with the greater than 90 bpm rest erect subgroup.

Table 3-36 – Normal ranges for erect peak stress First Third Fractional Filling by heart rate subgroup

HR (bpm)	N	Mean FTFF	SD	Normal Range (%)
<120	7	28.5	9.0	10.5 – 46.5
120-140	12	25.0	5.7	13.6 – 36.4
>140	7	20.2	5.7	8.8 – 31.6

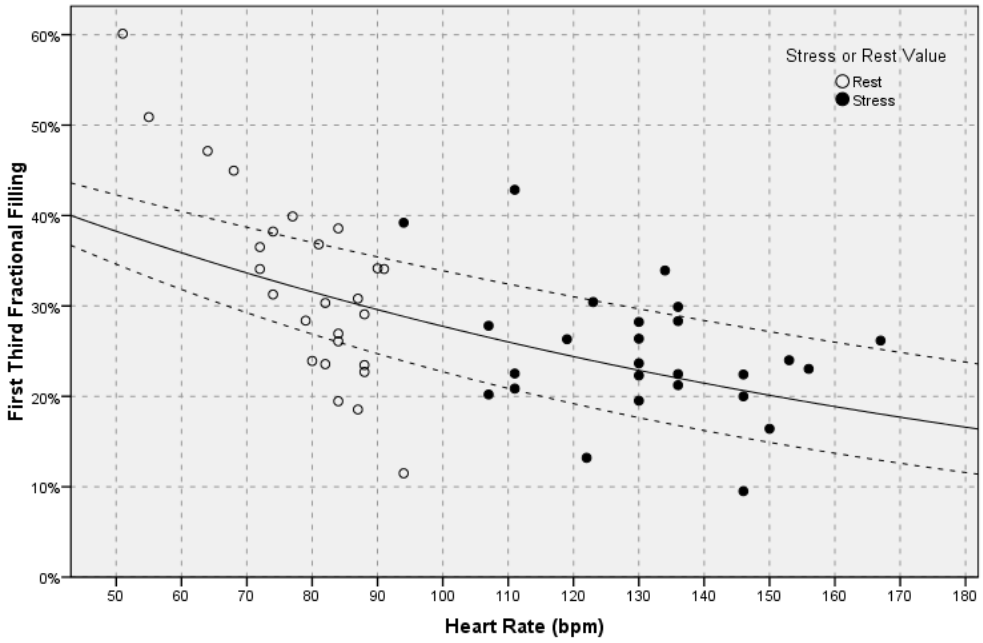
The difference in FTFF between the highest and lowest heart rate groups at peak stress is less than that seen between the groups at rest (Table 3-35). This is precisely what is predicted by the extrapolated exponential curves shown in Table 3-33. If the values obtained for rest and peak stress are considered together, that is as a heart rate/FTFF co-ordinate in one group regardless of whether or not the patient was exercising, the data behaves as predicted by the exponential curves defined in section 3.5.1.4. This is shown in Figure 3-41.

Continuous Reference Range

By considering the group as a whole, enough data points are produced to attempt curve regression analysis to produce the same graphs as those for the supine control subjects. An exponential curve gives a similar correlation coefficient compared to linear regression (r = 0.539 versus r = 0. 585). As previously discussed, an exponential curve behaves in a more biologically plausible manner within physiological heart rates. The regression equation for this patient group is $FTFF = 52.8 \times e^{-0.006 \times HR}$. The standard error was 0.001, giving regression equations of $FTFF = 52.8 \times e^{-0.004 \times HR}$ and $FTFF = 52.8 \times e^{-0.008 \times HR}$ for the upper

and lower limits of normal respectively. These are shown in Figure 3-41 with the reference data included.

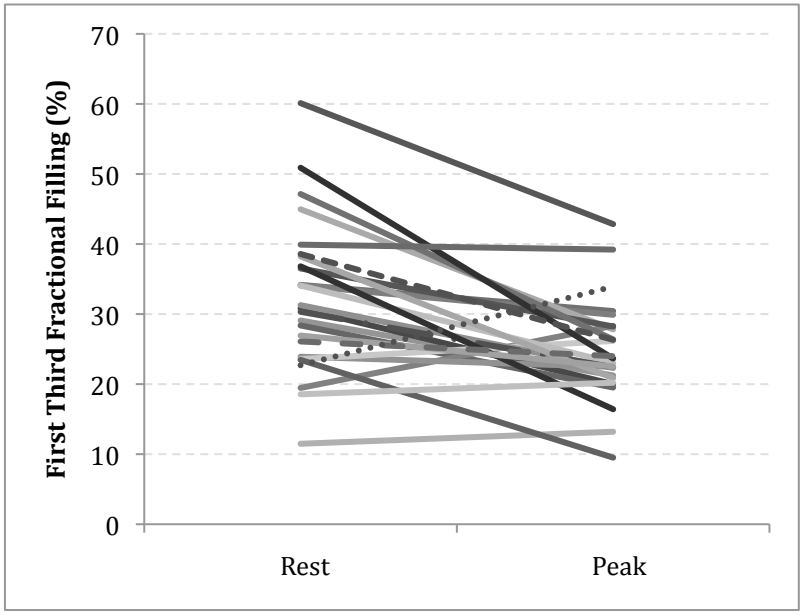
Figure 3-41 – Erect First third fractional filling normal range with combined rest and stress reference data from control subjects. Broken lines represent ± 2 SEM



Response to Stress

As would be expected from the relationship of first third fractional filling to heart rate, and the rise in heart rate which accompanies stress, FTFF falls with stress. With a mean increase in heart rate of 51.4 beats per minute there is a 7.7 point fall in FTFF. The individual responses are shown in Figure 3-42. Although the majority of subjects show the predicted fall in FTFF, a small number, those with the lowest resting values, show a rise in FTFF with exercise. There is a mean decrease in first third fractional filling by 0.143 points per 1 beat per minute rise in heart rate.

Figure 3-42 – Individual control patients' First Third Fractional Filling responses to stress



3.5.2.4 Peak Filling Rate (RR)

Erect Resting

As with the supine control group (section 3.5.1.5), peak filling rate normalised to the RR interval shows a skewed distribution. The skewness statistic is 1.086 with standard error 0.456. The normal range, defined by median and interquartile range is:

Median 148.6 %EDC/cycle, interquartile range 113.9 – 178.4

In view of the weak relationship with heart rate, rest erect PFR(RR) has been analysed in the previously defined heart rate groups for this group. Peak filling rate is normally distributed within each of these subgroups. The normal ranges quoted are the mean ± 2 standard deviations.

Table 3-37 – Normal ranges for erect resting Peak Filling Rate (normalised to RR interval) by heart rate subgroup

HR (bpm)	N	Mean PFR(RR)	SD	Normal Range (%EDC/cycle)
<70	4	179.7	52.6	74.5-284.9
70-79	6	136.2	22.9	90.4-182.0
80-89	13	132.8	33.2	66.4-199.2
>90	3	244.1	39.7	164.7-323.5

Peak Stress

At peak stress, peak filling rate is normally distributed with a skewness statistic of 0.367. The mean peak filling rate is 208.9 and standard deviation 45.8. The normal range, defined by 5th and 95th centiles by weighted average, is 124.2 to 308.2. Within the heart rate subgroups for peak stress, peak filling rate remains normally distributed.

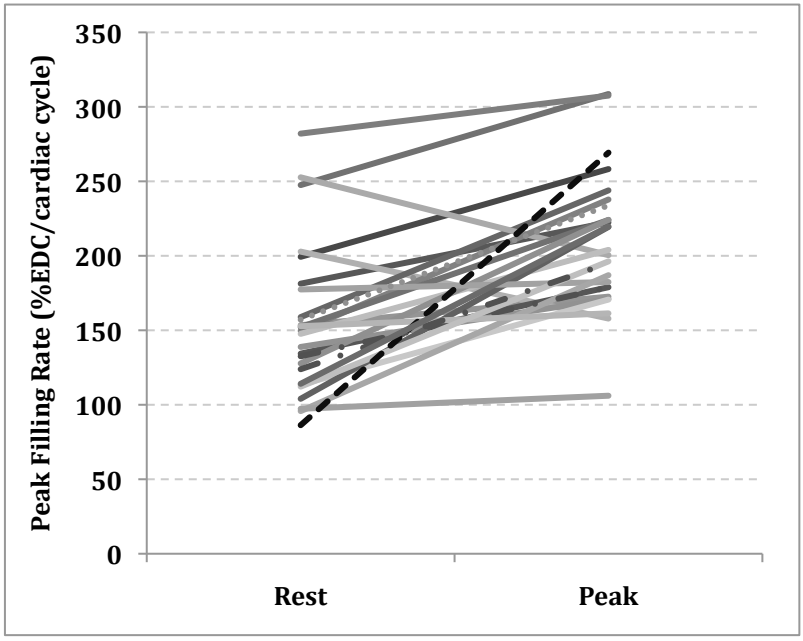
Table 3-38 – Normal ranges for erect peak stress Peak Filling Rate (normalised to RR interval) by heart rate subgroup

HR (bpm)	N	Mean PFR(RR)	SD	Normal Range (%EDC/cycle)
<120	7	187.4	40.3	106.8-267.8
120-140	12	222.9	52.8	117.3-328.5
>140	7	209.1	30.4	148.3-269.9

Response to Stress

With the exception of 2 patients, peak filling rate increases on stress. This is shown in Figure 3-43. For every 1 beat increase in heart rate, PFR increases by 1.14 points.

Figure 3-43 – Individual control patients' Peak Filling Rate responses to stress



3.5.2.5 Time to Peak Filling

Erect Resting

Time to peak filling showed a strong positive correlation with heart rate in the supine control group. It has, therefore, again had normal ranges defined for the heart rate

subgroups seen within this control group at rest. These are shown in Table 3-39 and are calculated as the mean ± 2 standard deviations. The large standard deviations in all subgroups other than the <70bpm group unfortunately render most of the normal ranges of little value.

Table 3-39 – Normal ranges for erect resting Time to Peak Filling (normalised to RR interval) by heart rate subgroup

HR (bpm)	N	Mean ttPF(RR)	SD	Normal Range (cardiac cycles)
<70	4	0.182	0.01	0.162-0.202
70-79	6	0.181	0.067	0.047-0.315
80-89	13	0.271	0.109	0.053-0.489
>90	3	0.278	0.103	0.072-0.481

Peak Stress

The peak stress normal ranges for ttPF at peak stress (Table 3-40) share the problems seen with rest erect. This is due to the combination of an index that has a high inherent degree of variability and a small data set. Time to peak filling is, therefore, unlikely to be either a reproducible or useful parameter.

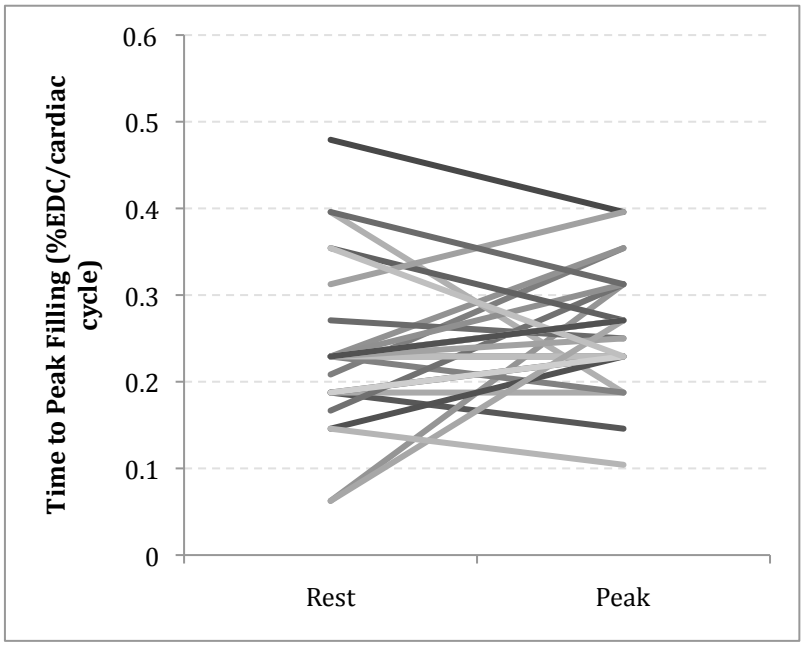
Table 3-40 – Normal ranges for erect peak stress Time to Peak Filling (normalised to RR interval) by heart rate subgroup

HR (bpm)	N	Mean ttPF(RR)	SD	Normal Range (cardiac cycles)
<120	7	0.229	0.096	0.037-0.421
120-140	12	0.274	0.06	0.154-0.394
>140	7	0.271	0.058	0.155-0.387

Response to Stress

As might be predicted from the above figures, the response of ttPF is not consistent. Approximately one third of the subjects show a fall with stress and there is no particular pattern seen with either high or low initial values. This is illustrated in Figure 3-44.

Figure 3-44 – Individual control patients' Time to Peak Filling responses to stress

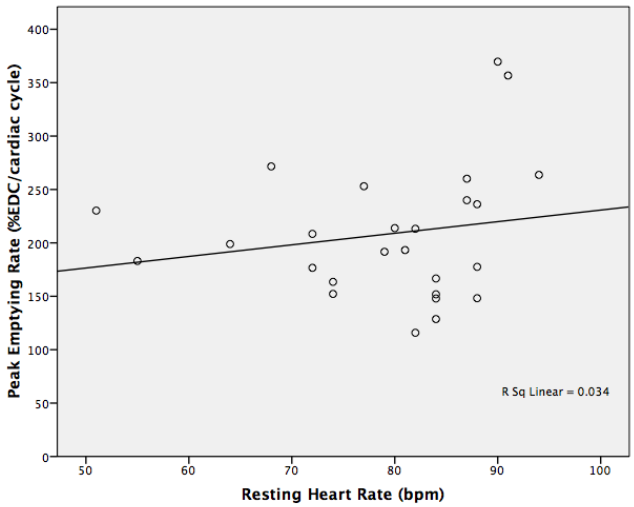


3.5.2.6 Peak Emptying Rate

Erect Resting

Whilst peak emptying rate showed a weak negative correlation with heart rate in the supine position, the opposite is seen whilst erect (Figure 3-45). The correlation coefficient is 0.18. It is possible this is due to the smaller sample size and two outliers with unusually high peak emptying rates and heart rates above 90 bpm, as peak stress shows a very weak negative correlation (correlation coefficient 0.1). Normal ranges for peak emptying rate will therefore be defined for the group as a whole and within heart rate subgroups.

Figure 3-45 – Relationship between rest erect Peak Emptying Rate and heart rate in control subjects



The distribution is slightly skewed (skewness 1.047, standard error 0.456) in the group as a whole. The median and interquartile ranges are, therefore, used to define the normal range in preference to mean ± 2 standard deviations. The median is 196.1 %EDC/cycle, and interquartile range 160.7 – 243.2

Within the heart rate subgroups distribution is normal. The mean ± 2 standard deviations are, therefore used below to define the normal ranges within these subgroups (Table 3-41). The effect of the outliers in the greater than 90bpm heart rate group can be clearly seen.

Table 3-41– Normal ranges for erect resting Peak Emptying Rate (normalised to RR interval) by heart rate subgroup

HR (bpm)	N	Mean PER(RR)	SD	Normal Range (%EDC/cycle)
<70	4	221.0	39.0	143.0-299.0
70-79	6	190.9	36.4	118.1-263.7
80-89	13	184.1	45.8	92.5-275.7
>90	3	330.0	57.8	214.4-445.6

Peak Stress

Peak emptying rate is normally distributed within the group as a whole and within the heart rate subgroups. For the whole group the mean is 175.9 %EDC/cycle and standard deviation is 44.5, giving a normal range of 86.9 – 264.9. The normal ranges for the heart rate subgroups are shown in Table 3-42.

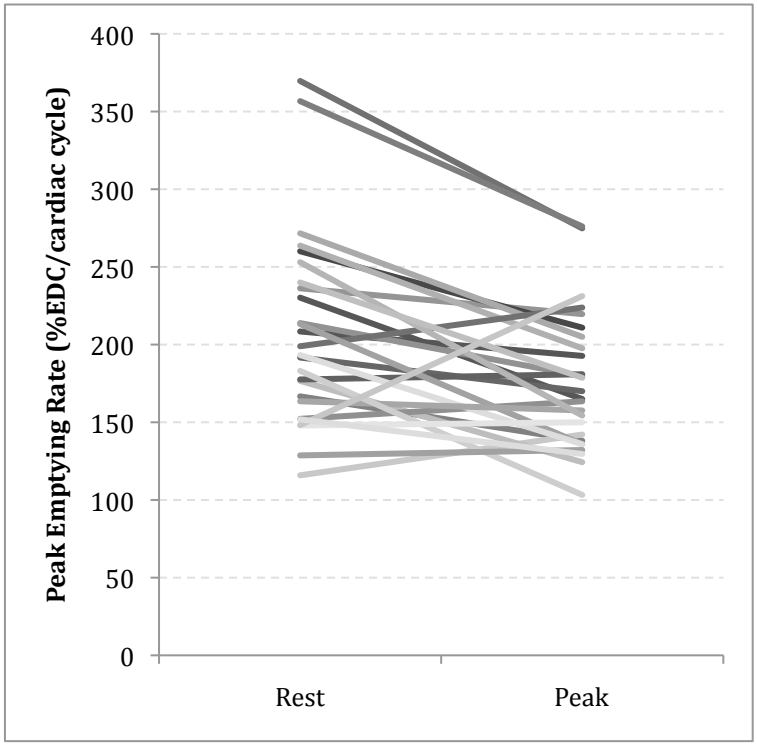
Table 3-42– Normal ranges for erect peak stress Peak Emptying Rate (normalised to RR interval) by heart rate subgroup

HR (bpm)	N	Mean PER(RR)	SD	Normal Range (%EDC/cycle)
<120	7	160.3	26.3	107.7-212.9
120-140	12	190.5	51.5	87.5-293.5
>140	7	154.3	30.7	92.9-215.7

Response to Stress

There is a mean decrease of 32.3 points from rest to peak stress, a decrease of 0.9 per beat per minute increase in heart rate. This is illustrated in Figure 3-46.

Figure 3-46 – Individual control patients' Peak Emptying Rate responses to stress



4. Results

4.1 Demographics

83 patients completed the study protocol. 60 of these patients were recruited prospectively, the remaining 23 by retrospective identification from open access echocardiography sessions. Summary data are presented in Table 4-1. The study population, in keeping with previous studies of diastolic dysfunction is predominantly female, elderly (mean age 66.7), overweight and hypertensive. It is perhaps surprising that nearly 50% had never smoked and, of the 42 patients who had, only 9 of these were still smoking. The most likely explanation for this is selection bias. Patients who are motivated sufficiently about their own health to request investigations for unexplained dyspnoea and to agree to participate in clinical research are presumably those for whom not smoking forms an essential part of their health conscious lifestyle.

Resting ECGs were normal in 61 of the 83 patients. For the summary statistics resting ST/T wave abnormalities and minor intraventricular conduction defects have been classified as a minor abnormality, LVH with or without strain and bundle branch block has been classified as a major abnormality. All patients were in sinus rhythm.

All of the study patients with the exception of 3 had symptoms compatible with NYHA class 2. The remaining 3 (3.6%) patients had class 3 symptoms.

Table 4-1 – Summary demographic details of all study patients

		Percentage
Age (mean, min/max)	66.7 (39 / 80)	
Sex (M/F)	15 / 68	18.1% / 81.9%
BMI (kg/m^2) (mean, min/max)	31.3 (20.3 / 42.0)	
Hypertension (Y/N)	60 / 23	72.3% / 27.7%
Mean Systolic BP	150 mmHg	
Mean Diastolic BP	83 mmHg	
Diabetes (Y/N)	11 / 72	13.3% / 86.7%
Smoker (Never/Ex/Current)	41 / 33 / 9	49.4% / 39.8% / 10.8%
ECG (Normal/Minor/Major abnormality)	61 / 20 / 2	73.5% / 24.1% / 2.4%
NYHA Class (Mean)	2	

Table 4-2 – Summary demographics of prospectively recruited study patients

		Percentage
Age (mean, min/max)	66.6 (39/80)	
Sex (M/F)	10/50	16.7% / 83.3%
BMI (kg/m^2) (mean, min/max)	31.1 (20.3 / 42.0)	
Hypertension (Y/N)	42 / 18	70% / 30%
Mean Systolic BP	151 mmHg	
Mean Diastolic BP	84 mmHg	
Diabetes (Y/N)	6 / 54	10% / 90%
Smoker (Never/Ex/Current)	31 / 24 / 5	51.7% / 40% / 8.3%
ECG (Normal/Minor/Major abnormality)	46 / 12 / 2	76.7% / 20% / 3.3%
NYHA Class (Mean)	2	

4.1.1 Hypertension

Hypertension was both highly prevalent and poorly controlled. 46 patients had systolic blood pressures greater than 140mmHg at rest. This is the threshold for grade 1 hypertension as defined in the 2004 British Hypertension Society guidelines(180). 35 of these patients had an established diagnosis of hypertension suggesting that, on the whole, blood pressure control, even in this motivated patient group, is suboptimal. Of the remaining 11, 3 had systolic blood pressures greater than 160mmHg, a level which if sustained, would mandate treatment under the current British Hypertension Society guidelines.

4.1.2 Resting Heart Rate

To facilitate comparison between patients who were either *relatively* bradycardic or tachycardic at rest, the following cut-offs were defined. Bradycardia was defined as a resting heart rate less than 60 beats per minute. As there were so few patients with a resting heart rate greater than 100, statistical tests on this subgroup would be meaningless. Tachycardia was, therefore, defined as a resting heart rate of 90 beats per minute or greater. Even with this less stringent classification of tachycardia, only 5 patients have resting heart rates of 90 beats per minute or greater. The remaining central group was divided into two groups with heart rates of 60-74 and 75-89 beats per minute.

Table 4-3 – Study Patients Heart Rate Groups

Heart Rate	N	Percent
<60	17	20.5
60-74	36	43.4
75-89	25	30.1
>90	5	6.0
Total	83	

Table 4-4 – Supine Resting Control Patients Heart Rate Groups

Heart Rate	N	Percent
<60	27	12
60-74	112	49.8
75-89	61	27.1
>90	25	11.1
Total	225	

4.1.3 Drug Therapy

A summary of the cardiac and respiratory drugs being taken by the study patients at the time of enrolment is shown in Table 4-5. In keeping with the diagnosis of suspected heart failure, one third of patients were being treated with a loop diuretic in the form of frusemide. One third of patients were receiving either an ACE inhibitor or an angiotensin receptor blocker. No patients were receiving both agents.

Table 4-5 – Cardiorespiratory drugs therapy taken by study patients at time of enrolment

Drug	Number taking (%)
Frusemide	27 (32.5%)
ACE Inhibitor	20 (24.1%)
Angiotensin Receptor Blocker	10 (12%)
Beta Blocker	26 (31.3%)
Calcium Channel Blocker	Non-rate limiting - 11 (13.3%) Rate limiting - 4 (4.8%)
Thiazide Diuretic	27 (32.5%)
Other anti-hypertensive therapy	9 (10.8%)
Aspirin	28 (33.7%)
Oral nitrate	3 (3.6%)
Statin	28 (33.7%)
Bronchodilators	5 (6%)

4.2 Echocardiography

4.2.1 Left Ventricular Dimensions

Complete left ventricular dimension measurements were available on all except 2 patients who were poorly echogenic. In one patient only measurement of diastolic dimensions was possible, in the other patient neither systolic nor diastolic measurements were possible. Left ventricular mass and end diastolic dimensions were, therefore, assessable in 82 of 83 patients.

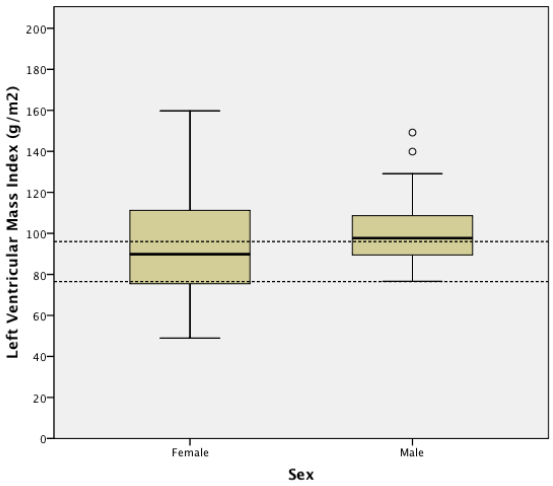
The mean left ventricular end diastolic diameter (LVEDD) was 44.6mm with all patients being within the normal limit of 57mm. 48 patients had left ventricular hypertrophy by wall thickness criteria with either the septum or posterior wall being greater than 11mm in diastole on the parasternal long axis view.

Left ventricular mass index (LVMI) was calculated by dividing left ventricular mass (measured on the parasternal long axis view) by body surface area. Previously published normal ranges(181) were used for males and females. The upper limit of normal for males was 96g/m^2, for females 76.5g/m^2. 8 of 15 (53%) males and 48 of 68 females (70.6%) had left ventricular hypertrophy by these criteria. 43 of those with left ventricular hypertrophy by wall thickness also had hypertrophy by LVMI. In contrast, 14 of the 56 patients with left ventricular hypertrophy by LVMI had wall thicknesses within the normal range. This highlights the fact that, although LVMI is more cumbersome in that it requires a contemporaneous height and weight, approximately 1 in 4 patients with left ventricular hypertrophy will otherwise be missed. Overall 67.5% of the patients had a left ventricular mass index above the upper limit of normal for their sex. This is illustrated in Figure 4-1.

Since hypertension and left ventricular hypertrophy are associated with diastolic dysfunction, it is to be expected that their prevalence is high in a patient group suspected of having diastolic dysfunction.

Figure 4-1 – Distribution of left ventricular mass index by sex

Broken lines represent upper limits of normal for males and females



4.2.2 Left Ventricular Systolic Function

All patients had normal left ventricular systolic function by visual assessment. All except 2 patients had an ejection fraction calculable by the Teicholtz method. Whilst this has its limitations, it is an acceptable measure of left ventricular ejection fraction where left ventricular geometry is normal. It is only valid where there are no regional wall motion abnormalities that will be under or overrepresented by measuring basal wall motion on the parasternal long axis view. Calculation of ejection fraction by Simpson's biplane method was also attempted. As left ventricular opacification contrast was not available, only 26 of the 83 patients had echocardiographic images with sufficiently good endocardial definition to allow calculation of ejection fraction by Simpson's biplane method.

The mean left ventricular ejection fraction by the Teicholtz method was 77% and 63.9% by Simpson's rule. The difference between the two was highly statistically significant ($p < 0.001$, paired samples T-test). There was no significant correlation between left ventricular ejection fraction measured by the two methods (Pearson correlation coefficient 0.11, $p=0.62$). In only 1 patient was the Teicholtz ejection fraction less than 50%. In this patient it was not possible to calculate an ejection fraction by Simpson's rule. The ejection fraction was, however, normal by radionuclide ventriculography.

4.2.3 The Left Atrium

4.2.3.1 Antero-Posterior Diameter

The left atrial antero-posterior (AP) diameter was measured in all 83 patients. The mean was 36.1mm, ranging from 23.9 to 48.6. 16 patients had a left atrial diameter greater than 40mm. Left atrial AP diameter did not correlate significantly with any other index of elevated left atrial pressure (Table 4-6). As continuous variables, however, left atrial diameter did correlate significantly with left ventricular mass index (Pearson correlation coefficient 0.4, $p<0.001$) and left atrial volume index (Pearson correlation coefficient 0.5, $p=0.008$)

Table 4-6 – Correlation between left atrial dilatation by antero-posterior diameter and other indices of left atrial pressure dichotomised as normal or abnormal.

	Spearman Correlation Coefficient	p
Elevated LA Volume Index	0.11	0.59
LVH by LV Mass Index	0.19	0.08
Hypertension	0.18	0.10
E/A Ratio	-0.11	0.32
Abnormal E/E' Septal ratio	0.15	0.17
Abnormal E/E' Lateral ratio	0.13	0.24

4.2.3.2 Left Atrial Volume Index

Left atrial volume index (LAVI) was retrospectively calculated in a subset of 27 patients. The mean LAVI was 26.4ml/m^2, ranging from 8.7 in a patient with an AP diameter of 27mm to 41.8 ml/m^2. In 8 of the 27 patients LAVI was above the upper limit of normal for gender (30ml/m^2 in females, 33ml/m^2 in males). There was no significant correlation between left atrial dilatation by AP diameter and by LAVI (Spearman correlation coefficient 0.11, $p=0.59$). There was a moderate correlation between a dilated left atrium by LAVI and an elevated septal E/E' ratio (Table 4-7).

Table 4-7 – Correlation between left atrial dilatation by left atrial volume index and other indices of left atrial pressure

	Spearman Correlation Coefficient	p
Elevated LA AP Diameter	0.11	0.59
LVH by LV Mass Index	0.23	0.25
Hypertension	-0.1	0.59
E/A Ratio	0.24	0.22
Abnormal E/E' Septal ratio	0.4	0.04
Abnormal E/E' Lateral ratio	0.25	0.2

4.2.4 Indices of Diastolic Function

Mitral E/A ratio, deceleration time and isovolumetric relaxation time were obtained in all patients. Septal diastolic mitral annular velocity (E' sep) was not evaluable in one patient due to poor quality Doppler signals.

Table 4-8 – Mean Echocardiographic Results

	Mean
Ejection Fraction (Teich)	76.2%
LV Mass Index	95.3g/m^2
LA A-P Diameter	36.7mm
LA Volume Index	24.6ml/m^2
E/A Ratio	0.9
E/E' Lateral	11.3
E/E' Septal	14.9
Mitral DT	229ms
IVRT	103ms

4.2.4.1 E/E' Ratio

Using the criteria defined by Ommen et al(32) where mean left ventricular diastolic pressure could be categorised as normal (E/E' ratio <8), equivocal (E/E' 8-15) and elevated (E/E' >15) using the septal E/E' ratio, 36 of the patients have evidence of elevated filling pressures. All patients who had a septal E/E' ratio in the abnormal range, also had an abnormal lateral annulus E/E' ratio.

Table 4-9 – Tissue Doppler E/E' ratios in study patients

	E/E' Ratio			N
	<8	**8-15**	**>15**	
Septal	5	41	36	82*
Lateral	17	55	11	83

***Septal annular velocity was not obtained on 1 patient**

Although both septal and lateral ratios have a large number of subjects within the 8-15 equivocal category, there are a significantly higher number of subjects falling into the abnormal category. The E/E' ratio is significantly higher using the septal ratio by a mean of 3.59 ($p<0.001$, paired samples T-test). The lateral E/E' ratio has a very high proportion of patients which fall into the equivocal range. The lateral mitral annular velocity does, however, have advantages in that it is easier to obtain and is more reproducible than the septal velocity.

The lateral annular ratio has also been used to predict elevated filling pressures with a cut-off ratio of 10 yielding optimal sensitivity of 78% and specificity of 95%(49). By this criterion 48 patients (57.8%) had a pulmonary capillary wedge pressure of >12mmHg.

By combining the Ommen and Nagueh criteria it should be possible to utilise the reproducibility of the lateral ratio with the septal cut-offs that acknowledge the degree of imprecision inherent in the technique. This is summarised in Table 4-10. The results were wholly concordant in 39 of 82 patients (septal E/E' was not available for one patient) with only 5 patients having normal filling pressures by both criteria. Of the 41 patients with equivocal filling pressures by the Ommen criteria (septal E/E' 8-15), 14 of these were confirmed as having elevated filling pressures by the Nagueh criteria. In only 2 patients were the results discordant with septal E/E' being <8 and lateral E/E' >10. This may be explained by the technical disadvantages of the septal annulus measurement. Inadvertent anterior angulation of the echo probe whilst measuring the septal E' velocity may cause the view to move from 4 chamber to 5 chamber with the result that the pulsed wave sample volume partially includes the left ventricular outflow tract.

Overall, 48 patients can be classified as having impaired diastolic function by the E/E' ratio. The only patient in whom a septal E/E' ratio was not recorded on either the first or second echocardiogram had a lateral ratio of 9.13 and is therefore classified as normal.

Table 4-10 – Combined Ommen and Nagueh E/E' Criteria

	Frequency	Percent
Both Positive	34	41.5
Nagueh Positive, Ommen Equivocal	14	17.1
Nagueh Negative, Ommen Equivocal	27	32.9
Both Negative	5	6.1
Nagueh Negative, Ommen Positive	2	2.4
Total	**82**	**100.0**

4.2.4.2 Standard Doppler

Assessment of diastolic function by pulsed wave Doppler alone has partly fallen by the wayside in favour of more sophisticated techniques using tissue Doppler. However, the indices derived from this – mitral E/A ratio, mitral deceleration time and isovolumetric relaxation time, are readily obtained and reported. The E/A ratio, in particular, continues to be quoted as an indicator of diastolic function. The criteria used here (Table 4-11) are those quoted in the 1998 rather than 2007 iteration of the recommendations of the European Study Group on Diastolic Heart Failure. This is due to the shift in focus away from these parameters in the current guidelines. Routine clinical practice, however, has yet to make the same shift and the previous guidelines remain in use. In this patient group no patients were aged less than 30 years old. The guidelines can therefore be simplified by removing the IVRT cutoff of 95ms for the under 30 age group.

Table 4-11 – Simplified 1998 European Study Group criteria for diastolic dysfunction

Age (yrs)	IVRT (ms)	E/A ratio	DT (ms)
30 – 50	>100	<1.0	>220
>50	>105	<0.5	>280

By these criteria only 2 patients had an abnormal E/A ratio, 13 had an abnormal mitral deceleration time and 36 an abnormal IVRT. All 3 parameters were normal in 44 patients. A single parameter was abnormal in 28 patients, 2 of 3 abnormal in 10 patients and all 3 abnormal in 1 patient. If an additional category of equivocal E/A ratio is introduced for those aged over 50 years with an E/A ratio between 0.5 and 1.0, an additional 61 patients can be categorised as having borderline abnormal diastolic function.

This apparent lack of diastolic dysfunction is in stark contrast to the results obtained from tissue Doppler where 48 of the 83 patients had abnormal diastolic function. The poor specificity inherent in these parameters, which has led to their less prominent role in current guidelines, necessitates more extreme cutoff values and a drop in sensitivity.

4.2.4.3 Reproducibility of Echo Criteria

81 of the 83 patients completing investigations underwent a second echocardiogram, primarily by the same second operator (JJ – 57 patients) with a minority by other operators (LF – 10, AMC – 7, others – 7). The operator performing the study carried out the analysis of each echo. The reproducibility of the following measures of diastolic function was assessed along with the left ventricular ejection fraction (Teicholtz method): Lateral and septal E/E' ratio, mitral E wave velocity, mitral E/A ratio, mitral deceleration time and isovolumetric relaxation time. The reproducibility of left ventricular ejection fraction by Simpson's biplane method was not assessed as too few patients were able to have this measured on both studies. The mean time between studies was 69.6 days with a range of 0 to 330.

Table 4-12 – Inter-observer correlation of echocardiographic indices of diastolic and systolic function

		Mean	N	Std. Deviation	Correlation	Sig.
E/E' lat	Operator 1	11.1	60	3.81	0.48	<0.001
	Operator 2	10.4	60	3.95		
E/E' sep	Operator 1	14.7	60	4.78	0.49	<0.001
	Operator 2	12.4	60	4.59		
MV E	Operator 1	0.74m/s	79	0.18	0.64	<0.001
	Operator 2	0.77m/s	79	0.20		
E/A	Operator 1	0.88	79	0.26	0.69	<0.001
	Operator 2	0.93	79	0.28		
MV DT	Operator 1	228ms	77	61.49	0.38	0.001
	Operator 2	226ms	77	57.58		
IVRT	Operator 1	105ms	70	20.10	0.27	0.029
	Operator 2	110ms	70	21.62		
EF Teich	Operator 1	77.0%	76	9.07	0.21	0.08
	Operator 2	72.6%	76	8.02		

It is to be expected that there is a degree of correlation between the two results for each parameter given it is simply a repeat measurement and there was no significant clinical

change observed in any of the patients between the two studies. The strength of correlation here reflects a combination of biological variability in each of the parameters and their ease of measurement. It can be seen from Table 4-12 that the two parameters with the strongest correlation and, hence, greatest inter-study reproducibility are the E/A ratio and mitral E wave velocity. These are the simplest parameters to measure, requiring only a peak velocity to be marked at 2 points on a spectral Doppler trace. The E/E' ratio is also relatively simple to measure although does present more technical difficulties in terms of obtaining an adequate tissue Doppler signal.

The difference between first and second operator is significant for the septal E/E' ratio and left ventricular ejection fraction by the Teicholtz technique (Table 4-13). The difference for isovolumetric relaxation time approaches, but does not achieve, statistical significance (p=0.07)

Table 4-13 – Differences in inter-observer measurements of echocardiographic indices of diastolic and systolic function (paired samples T-test)

	Mean Difference	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
			Lower	Upper	
E/E' lat	0.65	3.98	-0.37	1.68	0.21
E/E' sep	2.35	4.74	1.12	3.57	<0.001
MV E	-0.02m/s	0.16	-0.06	0.01	0.19
E/A	-0.04	0.21	-0.09	0.01	0.12
MV DT	2.78ms	66.5	-12.32	17.88	0.72
IVRT	-5.57ms	25.2	-11.57	0.44	0.07
EF Teich	4.33%	10.8	1.86	6.80	0.001

4.3 NT-proBNP

N-terminal proBNP samples were obtained in 82 of the 83 study patients. These were obtained and stored as previously described. Once all samples were collected, they were processed in a single batch to eliminate the assay as a source of variability within the results. In the one remaining patient, a NT-proBNP sample was obtained in September 2008 in the context of very frequent hospital admissions with dyspnoea and chest pain. The level of this was 45pg/ml, below the reference level of 100pg/ml.

NT-proBNP rises with increasing age and with comorbidities such as hypertension and renal dysfunction that also increase in incidence with advancing age. It can, therefore, be viewed either in terms of dichotomous cut points, or as a continuous variable. A single age cutoff of 75 years has been used here with NT-proBNP levels of 125pg/ml and 450pg/ml being the upper limits of normal for age less than 75 years and age 75 years old or greater, respectively. 66 of 82 patients were aged 75 years or less, 16 patients were 75 years or older.

Using these values, 61 of 82 patients had normal NT-proBNP levels for their age. One of these patients was reassessed in February 2007 and found to have an elevated NT-proBNP level of 224pg/ml. All 21 patients with an elevated NT-proBNP level were aged less than 75 years old and, therefore used the 125pg/ml upper limit of normal. The median NT-proBNP level within this group was 185pg/ml, ranging from 127pg/ml to 871pg/ml. When the patients with a normal NT-proBNP are compared to those with an elevated level by means of an independent samples T-test, most indices one would expect to be abnormal in light of an elevated NT-proBNP show no significant difference (Table 4-14). The E/A ratio is marginally higher in those with an elevated NT-proBNP (1.01 compared to 0.84) although, this is of borderline statistical significance and does not take account of age related changes in the E/A ratio.

However, the resting heart rate is significantly lower in those with an elevated NT-proBNP. This can be explained by the higher proportion of patients with an elevated NT-proBNP who are being treated with beta blockers (13 of 21 versus 13 of 61, $p = 0.001$, X^2 test).

Table 4-14 – Characteristics of patients with elevated or normal NT-proBNP levels

	NT-proBNP	N	Mean	Std. Deviation	Sig.(2-tailed)
Age (years)	Normal	61	66.7	9.11	0.69
	Elevated	21	66.0	5.87	
BMI (kg/m^2)	Normal	61	31.32	5.05	0.98
	Elevated	21	31.29	4.11	
LV Ejection Fraction (%)	Normal	61	45.43	9.85	0.62
	Elevated	21	44.25	7.19	
Mitral E/A ratio	Normal	61	0.84	0.19	0.05
	Elevated	21	1.01	0.37	
E/E' Lateral	Normal	61	10.91	3.85	0.171
	Elevated	21	12.38	5.10	
E/E' Septal	Normal	60	14.75	5.06	0.65
	Elevated	21	15.30	3.93	
LV Mass Index (kg/m^2)	Normal	61	96.32	23.28	0.36
	Elevated	21	90.73	26.50	
Systolic BP (mmHg)	Normal	61	151.34	25.36	0.43
	Elevated	21	146.43	20.56	
Diastolic BP (mmHg)	Normal	61	83.95	11.08	0.18
	Elevated	21	80.24	9.93	
Resting Heart Rate (bpm)	Normal	61	76.64	14.48	0.003
	Elevated	21	66.33	10.01	
LA Volume Index (ml/m^2)	Normal	22	24.52	9.27	0.88
	Elevated	5	25.19	7.99	
LA diameter (mm)	Normal	61	36.49	5.58	0.3
	Elevated	21	35.03	5.14	
LV End Diastolic Diameter (mm)	Normal	60	44.59	5.01	0.96
	Elevated	21	44.52	4.92	
Thallium Reversible Ischaemia Score	Normal	61	4.93	4.24	0.99
	Elevated	21	4.95	4.30	
VO_2 max (ml/kg/min)	Normal	52	9.57	3.21	0.85
	Elevated	19	9.73	3.09	
VO_2 max (% predicted)	Normal	52	52.51	14.22	0.23
	Elevated	19	57.20	15.22	
FEV1 (% predicted)	Normal	60	100.83	18.23	0.29
	Elevated	21	95.90	18.87	
FEV1/FVC (% predicted)	Normal	60	95.05	10.06	0.46
	Elevated	21	93.24	7.88	

In this group, NT-proBNP does not discriminate between normal and abnormal even when these indices of diastolic dysfunction are considered as categorical, rather than continuous, variables. Only an abnormal E/E' lateral ratio shows a relationship with elevated NT-proBNP (Table 4-15) which approaches statistical significance. Here, 16 of 21 patients with elevated NT-proBNP have an abnormal E/E' ratio compared to 32 of 61 patients with

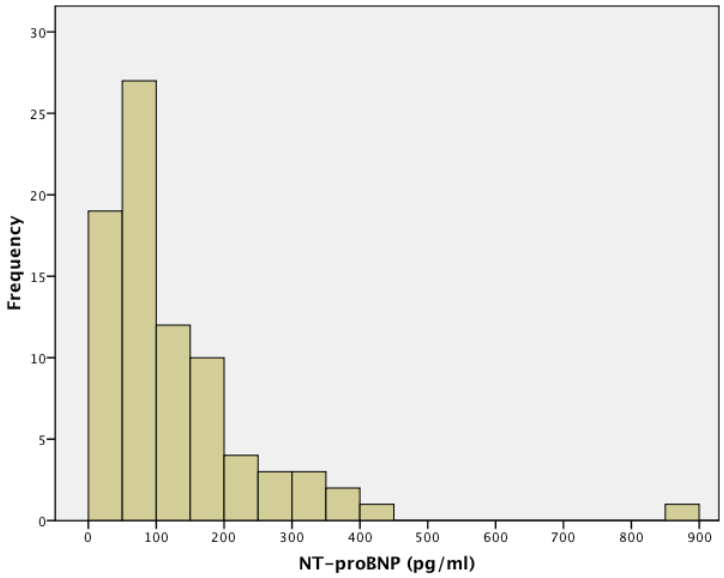
a normal NT-proBNP. It would be reasonable to assume that this would reach significance in a larger group. The relatively poor performance of the septal E/E' ratio is due to the large group of patients with an equivocal result. A trend can be seen, however, within the abnormal NT-proBNP group with 3, 7 and 11 patients having a normal, equivocal or abnormal E/E' septal ratio respectively. The results are summarised in Table 4-15 where the Chi-Square test is used throughout.

Table 4-15 – Association between abnormal NT-proBNP and indices of abnormal diastolic function (Chi-Square test)

	P value
Abnormal Supine FTFF	0.45
LA dilatation (AP diameter)	0.4
LA dilatation (LAVI)	0.57
LVH by LV mass index	0.78
Abnormal E/A ratio	0.19
Abnormal E/E' Lateral	0.06
Abnormal E/E' Septal	0.49

As a continuous variable, NT-proBNP has a skewed distribution with the majority of subjects having a level of less than 100pg/ml and one extreme outlier with a level of 871pg/ml. This is shown in Figure 4-2. Other than resting heart rate, which becomes non-significant when controlled for beta blocker use, there is no statistically significant correlation between NT-proBNP level as a continuous variable and the indices previously examined in Table 4-14. There are, however, two indices which have weak correlations which approach statistical significance. Age has a Pearson correlation coefficient of 0.2 at a significance level of 0.07 indicating a trend towards rising NT-proBNP with increasing age. Body mass index shows a weak negative correlation with NT-proBNP with a correlation coefficient of -0.21 and p value of 0.054. This association has been observed previously in patients without heart failure(182, 183). More recently, reduced natriuretic peptide levels have been demonstrated in individuals with features of the metabolic syndrome, independent of body mass index(184). The direction of causality is not yet clear. Activation of the renin-angiotensin system by low natriuretic peptide levels may cause insulin resistance. Conversely, adipose tissue contains high concentrations of natriuretic peptide clearance receptors(185) which remove natriuretic peptides from the circulation.

Figure 4-2 – Distribution of NT-proBNP levels within study patients



4.4 Spirometry

4.4.1 Definition of COPD

Airflow obstruction, the underlying mechanism for the symptoms of chronic obstructive pulmonary disease, can be described in simple terms using two parameters derived from spirometry. These are the Forced Expiratory Volume in 1 second (FEV_1), usually expressed as a percentage of the value predicted by age and sex; and the FEV_1:FVC ratio with FEV_1 usually being expressed as a percentage of the forced vital capacity. Although these two parameters do not provide the detailed information, such as total lung volume and functional residual capacity, required to characterise the gas trapping and hyperinflation seen in obstructive diseases, and the reduced lung volumes of restrictive diseases such as pulmonary fibrosis, they are sufficient as a screening test.

As a single parameter, FEV_1 may allow respiratory function to be characterised as normal or abnormal to varying degrees. The FEV_1:FVC ratio adds a degree of refinement to this by separating subjects into either obstructive (<70%) or restrictive physiology (ratio in normal range of 75-80%). A number of severity classification scales have been produced with some placing weight on the patients' symptoms. Whilst there were significant differences previously, the more recently issued joint American Thoracic Society / European Respiratory Society guidelines harmonise many of these and introduce a smoother grading scale, albeit with arbitrarily defined cut-off points. Three of these scales are summarised in Table 4-16. It should be noted that both the GOLD and ATS/ERS

scales do not define an upper boundary between mild airflow obstruction and normality. These criteria are intended primarily for obstructive lung disease which will, by definition, have an FEV_1:FVC ratio of <70% thus delineating normal from abnormal respiratory function.

Table 4-16 – Staging Criteria for Obstructive Lung Disease

	ATS / ERS (2005)	BTS / SIGN (2004)	GOLD (2003)	
Stage	**FEV_1 (%)**	**FEV_1 (%)**	**FEV_1 (%)**	**Symptoms**
I (mild)	> 70	50-80	≥ 80	±
II (moderate)	60-69	30-49	50-79	+
IIb (moderately severe)	50-59			
III (severe)	35-49	< 30	30-49	++
IV (very severe)	<35		<30	+++

Subjects with an abnormal FEV_1:FVC ratio but FEV_1 within the normal range, >80% predicted by British Thoracic Society 2004 guidelines represent an interesting subgroup. In subjects with definite obstructive airways disease an increase in morbidity and mortality is seen compared to normal subjects. In apparently healthy subjects, in whom the abnormality is detected as a part of screening, it is of uncertain significance with more detailed pulmonary function testing identifying disease in some. In others, it is thought to represent a physiological variant.

4.4.2 Results

Spirometry results were available in 82 of the 83 patients. Two classifications will be used here to describe the result. The first is a simple division into normal or abnormal respiratory function with an FEV_1:FVC ratio of <70% being the cut-off. These are then classified as to severity of airflow obstruction using the ATS/ERS criteria. Spirometry was normal in 58 patients and abnormal in 24. Of these, 20 had mild airflow obstruction and 4 had moderate. Of the 40 patients who had never smoked, 31 had normal spirometry and 9 had mild airflow obstruction.

4.5 Myocardial Perfusion

With the exception of patient 100, all patients underwent routine gated planar thallium scanning with a stress/redistribution protocol. Patient 100 underwent SPECT scanning,

also using thallium with a stress/redistribution protocol, at another local hospital less than 6 months prior to recruitment. It was not felt to be ethically justifiable to repeat the perfusion scan within this timescale for the sole purpose of the study.

4.5.1 Exercise Tolerance Test

Details of the exercise tolerance test were available for all patients other than the patient who underwent SPECT scanning. Of the remaining 82 patients, 76 underwent bicycle stress and 6 underwent vasodilator stress with dipyridamole at 0.56mg/kg. The resting ECG was normal in 61 of 83 patients. There were minor ST or T wave changes in 12 patients and a minor intraventricular conduction defect in 8 patients. The full breakdown is shown in Table 4-17.

Table 4-17 – Resting ECG types in study patients

	N	Percent
Normal	61	73.5
Resting ST/T abnormality	12	14.5
Minor IVCD	8	9.6
RBBB	1	1.2
LVH	1	1.2

Only 2 stress tests were electrically positive with planar ST depression of 1.5mm. 32 tests were negative and 48 were classified as inconclusive. This was on the basis of failure to achieve target heart rate, or ST depression which was either upsloping or less than 1mm in magnitude. The mean exercise test duration was 3 minutes 44 seconds, ranging from 2 minutes 40 seconds to 7 minutes. The mean rate-pressure product at peak stress for those undergoing bicycle stress was 24917, ranging from 8200 to 39750. This was normally distributed.

4.5.2 Thallium Scan

All scans were reported by a single operator (WM). Both stress and redistribution scans were scored for defect size and depth using the previously described 5 segments per projection model. The difference between the stress and redistribution scans, the ischaemia score, was classified on the following basis: 0-4 – no significant perfusion abnormality (NSPA), 5-8 – minor ischaemia, >8 – definite ischaemia. The differentiation

between minor and definite ischaemia is made in the basis that small, usually partially reversible, perfusion defects are often associated with angiographically normal coronary arteries. Although 46 of the 83 patients had myocardial perfusion within normal limits, 17 patients had evidence of minor ischaemia, and 20 had significant reversible ischaemia demonstrated. The highest ischaemia score was 16, representing a large area of ischaemia. The mean stress perfusion score and overall ischaemia scores are shown in Table 4-18. The stress perfusion scores are significantly higher than the ischaemia scores by virtue of the fact that the former does not take account of attenuation artefact. Attenuation artefact is particularly common in women due to breast tissue. It is usually possible to differentiate this fixed defect from infarcted myocardium by its preserved regional wall motion and typical location.

Table 4-18 – Study patient thallium myocardial perfusion results

	N	Mean Stress Score	Mean Ischaemia Score
No significant perfusion abnormality	46	6.93	1.65
Minor ischaemia	17	11.71	6.94
Definite ischaemia	20	15.75	10.75

A detailed breakdown of the myocardial perfusion scans showing definite ischaemia is given in Table 4-19. There were only 2 troponin positive acute coronary syndromes documented in this group, one occurring 2 years post scan, the other 5 years post. The first of these patients was the only patient in the entire cohort to undergo coronary revascularisation. This took place after a second acute coronary syndrome and repeat angiography which showed progression of previously non-obstructive right coronary artery disease. Perfusion scintigraphy had shown an antero-apical perfusion defect with substantial reversibility and had been attributed to left anterior descending artery disease.

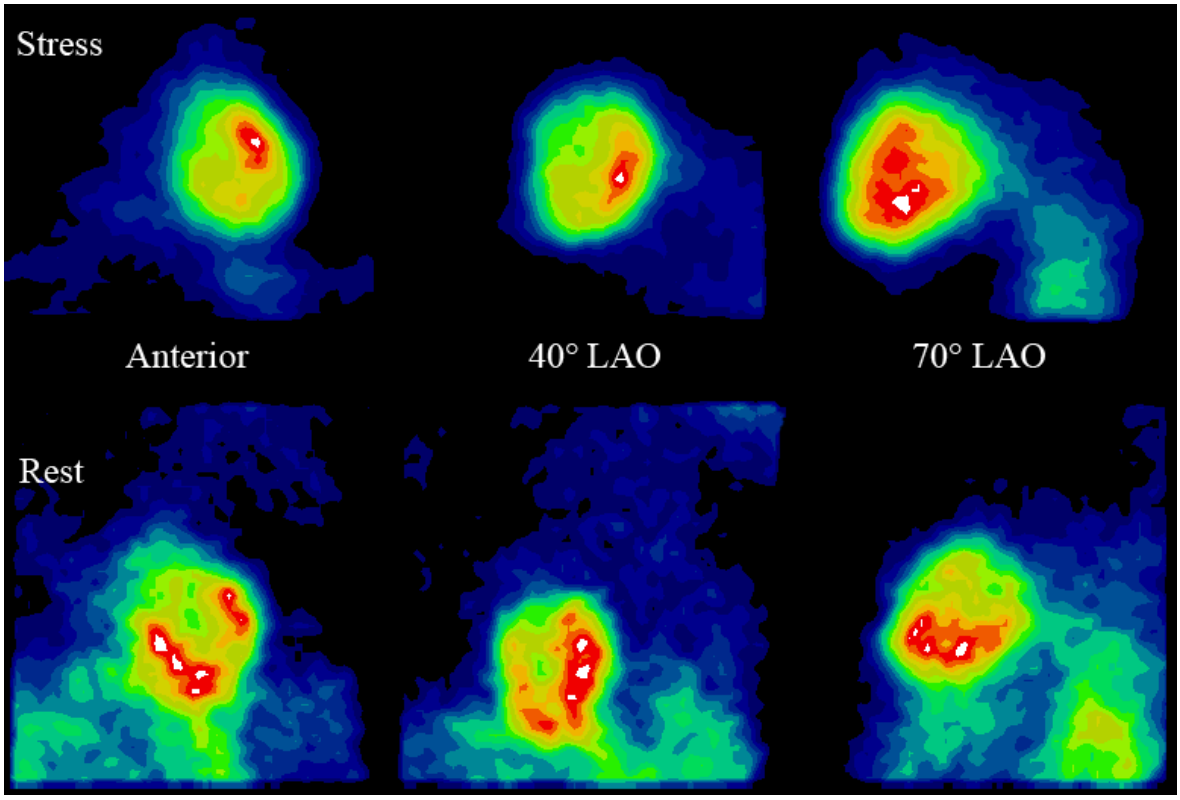
Table 4-19 – Detailed breakdown of myocardial perfusion scans showing definite ischaemia and subsequent coronary events

ID	Date Scan	Stress Score	Ischaemia Score	Predicted Territory	ACS	Angio	PCI
1	16/05/03	9	9	RCA	N	N	N
5	10/06/03	18	16	LAD	N	N	N
15	13/08/03	14	9	LAD	N	15/2/08	N
19	22/10/03	18	8	LAD	N	N	N
25	12/11/03	16	12	LAD	N	N	N
32	06/02/04	14	14	RCA	N	4/6/04	N
35	27/02/04	13	12	RCA	N	N	N
40	04/02/04	12	9	LAD	N	N	N
43	18/02/04	26	12	RCA	N	N	N
51	12/03/04	16	9	LCx	N	N	N
56	23/03/04	17	14	RCA	N	N	N
57	07/05/04	16	10	RCA	N	N	N
63	26/05/04	21	9	LAD	N	N	N
65	16/04/04	14	9	LAD	N	N	N
67	23/06/04	21	16	RCA	N	N	N
68	22/06/04	18	9	LAD	N	N	N
71	09/07/04	10	10	RCA	N	N	N
78	25/08/04	22	9	RCA	N	N	N
79	01/09/04	10	9	LAD	10/7/06	17/7/06	14/4/07 (RCA)
87	29/09/04	10	10	RCA	22/11/09	N	N

ACS = Acute Coronary Syndrome; Angio = Coronary angiography; PCI = Percutaneous Coronary Intervention

Figure 4-3 shows the stress and rest scintigraphy from patient ID number 32 with a large reversible perfusion defect in the inferior, septal and apical regions (ischaemia score 14). Reverse redistribution can be seen in the anterior wall (70° LAO projection). Coronary angiography 2 months following this scan showed only plaque disease in the first diagonal artery. No acute coronary events were documented in a 6 year follow-up period for this patient.

Figure 4-3 – Stress and rest myocardial scintigraphy from patient with large reversible inferior, septal and apical perfusion abnormalities



The results of these scans were provided to each patient's General Practitioner along with a recommendation for changes in drug therapy or referral to clinic if appropriate. Only 4 of the cohort of 83 patients underwent coronary angiography between the date of their scan and January 2010. Of these patients, 1 had possible ischaemia and 3 had definite ischaemia on thallium scanning. One of the 3 patients with definite ischaemia went on to have percutaneous revascularisation 31 months after their thallium scan. This particular patient had suffered a troponin positive acute coronary syndrome 22 months after their scan with angiography at that point showing non-obstructive disease.

A total of 4 patients suffered an acute coronary syndrome during this extended follow-up period. These events occurred at 22 months (definite ischaemia), 24 months (minor ischaemia), 44 months (no ischaemia), and 61 months (definite ischaemia).

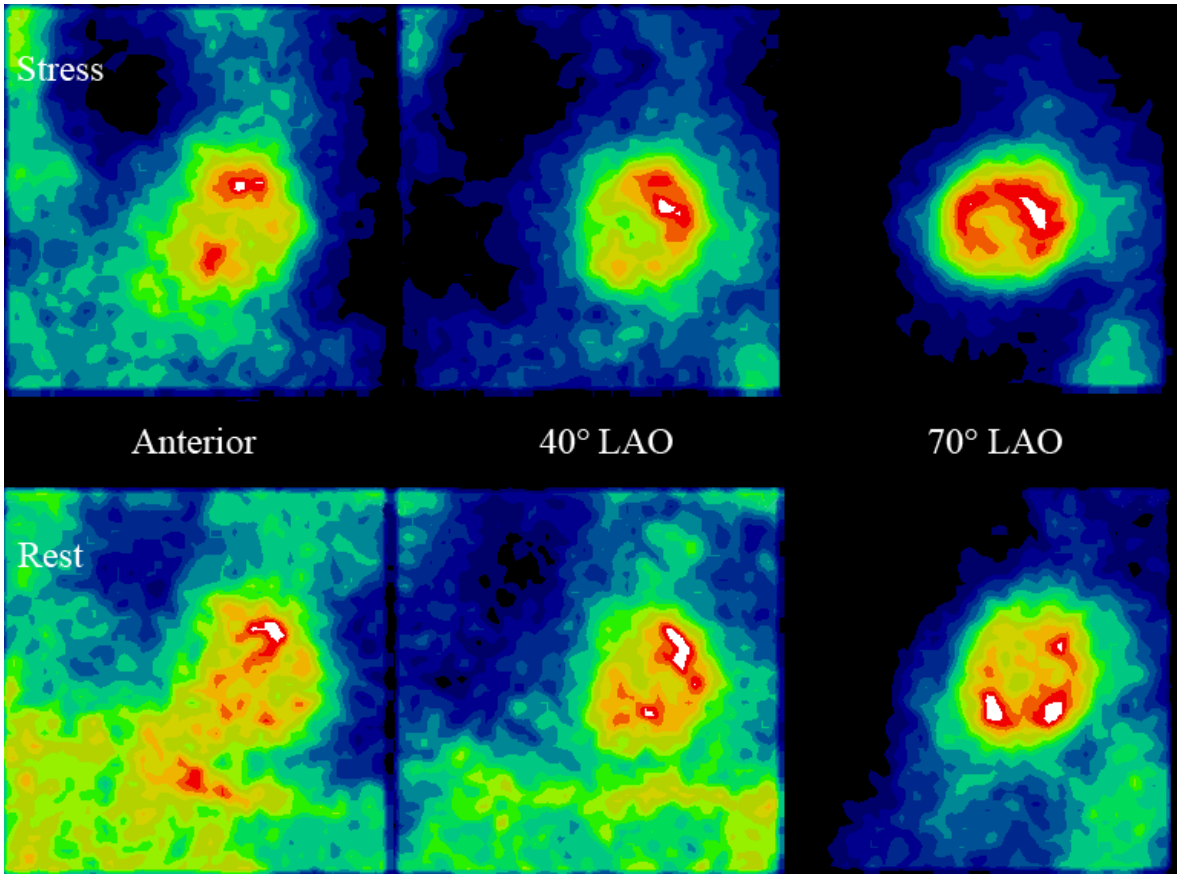
One patient was confirmed to have died, 61 months after the scan was performed. This was due to a non-cardiac cause (pancreatitis). The vital status of 5 patients could not be confirmed by searching either local hospital records or laboratory databases which included blood samples collected by local General Practitioners.

4.5.3 Lung Uptake of Thallium

Elevated lung uptake of thallium on stress has been independently associated with adverse cardiovascular prognosis in a number of situations. These include left ventricular systolic dysfunction(186), following coronary artery bypass grafting (187), and in patients with myocardial perfusion defects even in the absence of reversibility (188). Lung uptake was assessed visually by a single operator (WM) using a semi-quantitative scale: none, mild, moderate, or severely increased uptake.

There was no lung uptake in 38 patients, mild uptake in 33, moderate in 8 and severely increased uptake in 4 patients. Perfusion scintigraphy from one of the patients (patient 67, see also Table 4-19) with severely increased lung uptake of thallium is shown in Figure 4-4. In this patient there is a large perfusion defect on stress affecting the septum, apex and infero-septal walls which shows substantial reversibility. Across the whole group, however, lung uptake did not correlate significantly with thallium stress or ischaemia scores, left ventricular ejection fraction, left atrial diameter or volume index, NT-proBNP level, E/E' ratio (either septal or lateral), age, or smoking history. Of borderline statistical significance was a weak negative correlation with VO_2 max (Pearson correlation coefficient -0.22, p=0.06), left ventricular mass index (Pearson correlation coefficient -0.21, p=0.054), and a weak positive correlation with body mass index (Pearson correlation coefficient 0.19, p=0.09).

Figure 4-4 – Stress and rest thallium scintigraphy in a patient with severely increased lung uptake of thallium and large reversible infero-septal and apical perfusion abnormality.

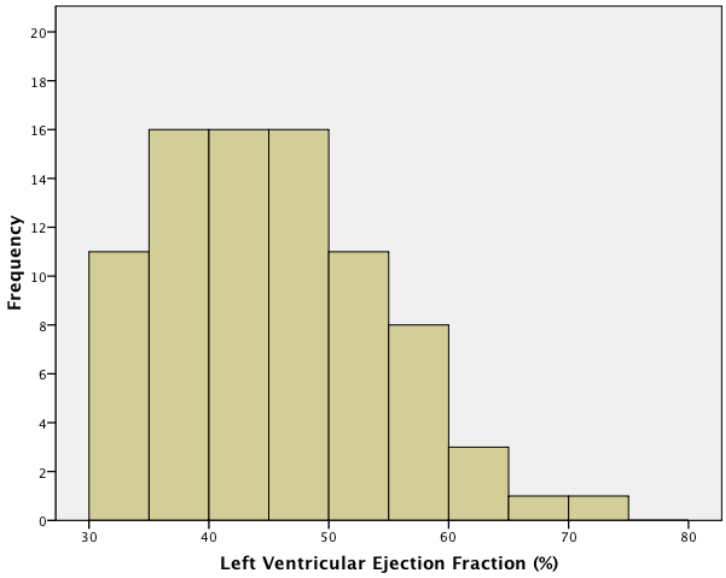


4.6 Resting Radionuclide Ventriculography

4.6.1 Left and Right Ventricular Systolic Function

Although all patients had a normal left ventricular ejection fraction as determined by visual qualitative assessment at echocardiography, 27 of the 83 patients had a radionuclide ejection fraction below the 40% lower limit of normal in routine clinical use at this institution. Of these, 16 patients had ejection fractions of between 35% and 40% indicating borderline left ventricular dysfunction. Local inter-study reproducibility has previously been estimated at ± 3% meaning 9 patients with ejection fractions of 37-40% may be classified as normal on repeat scanning. The lowest measured ejection fraction was 30%, corresponding to mild systolic dysfunction. The mean ejection fraction was 44.95%. Although the data are not normally distributed, being skewed to the right, median ejection fraction is the same at 44%. The distribution of ejection fractions is shown in Figure 4-5.

Figure 4-5 – Left ventricular ejection fraction by RNVG distribution in study patients



No significant differences were seen between the pre-defined heart rate subgroups. Although the difference in left ventricular ejection fraction between those with a resting heart rate of less than 60 and those in the 75-89 group approaches statistical significance with a p value of 0.076, this trend is not seen with the heart rate greater than 90 group. This may be because there are only 5 patients with a resting heart rate greater than 90, thus impairing the statistical power of any tests applied to this group.

Right ventricular ejection fraction has also had a lower limit of normal of 40% defined by this particular RNVG technique. Applying the same criteria as for left ventricular ejection fraction, 30 patients had a normal right ventricular ejection fraction, 43 abnormal, and 10 equivocal (37-40%). The mean right ventricular ejection fraction was 38.4%, ranging from 19.5% to 81%. If, however, the group of patients used to define the normal supine resting range for left ventricular systolic and diastolic function in Chapter 3 are used to define a normal range for the right ventricle, a lower limit of 27% is arrived at (5^{th} centile, weighted average). By this criterion, right ventricular ejection fraction was abnormal in only 11 of 83 patients. It should be borne in mind that these patients were selected on the basis of normal left ventricular function, rather than right ventricular. It should also be considered that measurement of right ventricular function by RNVG is more challenging than the left ventricle. This is due to the close proximity of the right ventricle to the liver which often interferes with background region of interest creation. Any under or overestimate of background activity secondary to this can have a substantial effect on ejection fraction.

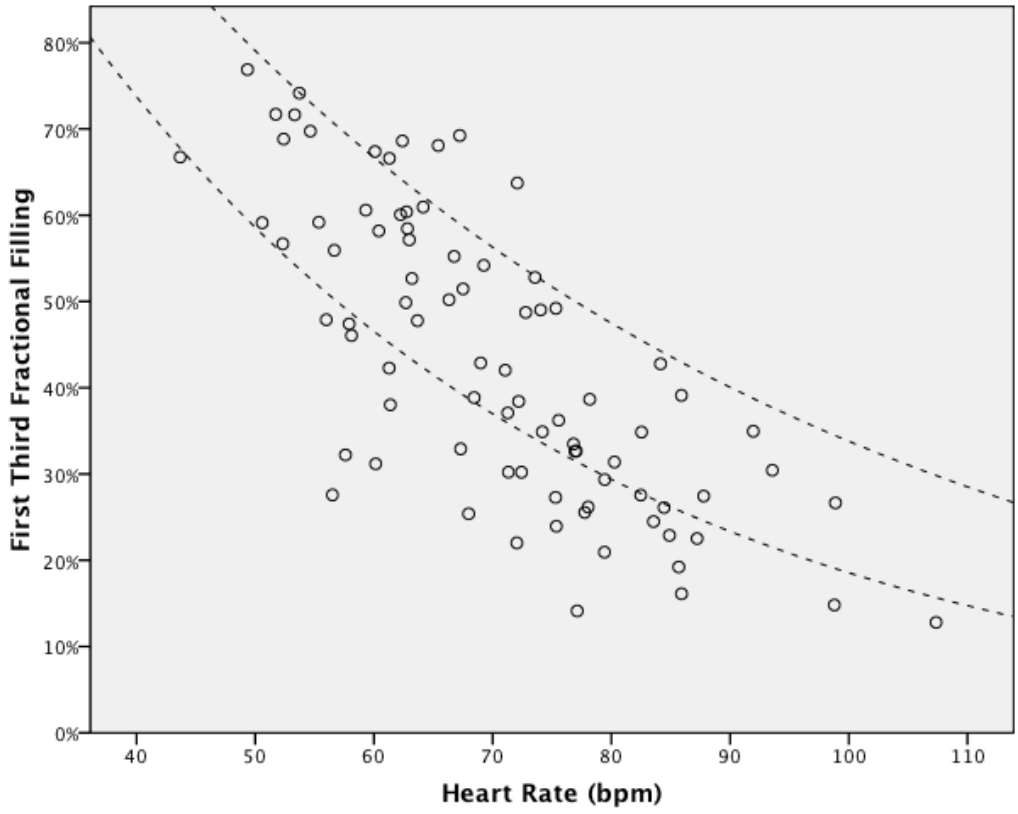
However, accepting these limitations, right ventricular ejection fraction is lower than predicted in this group as only 4 patients should have an ejection fraction below the 5th centile. This, therefore, suggests that right ventricular systolic dysfunction may be a contributing factor in the symptomatology of these patients. It is also interesting that the only significant correlation with right ventricular ejection fraction is left ventricular ejection fraction (R=0.35, p=0.001).

4.6.2 Left Ventricular Diastolic Function

4.6.2.1 First Third Fractional Filling

By applying the heart rate corrected lower limit of normal equation described in section 3.5.1.5, FTFF = 185 x $e^{-0.023xHR}$, it is possible to categorise the resting supine FTFF of each patient as normal or abnormal. By this criterion, first third fractional filling was normal in 53 of 83 patients, and abnormal in 30. The precise spread of results is shown in Figure 4-6 where the broken lines represent the upper and lower limits of normal.

Figure 4-6 – Rest supine left ventricular first third fractional filling in study patients



4.6.2.2 Peak Filling Rate

By using the heart rate subgroup normal ranges defined in section 3.5.1.5, 18 of the 83 patients had an abnormally low peak filling rate as determined by the lower limits of normal defined for each heart rate subgroup previously. As can be seen in Table 4-20, the majority of these are accounted for by the 60-69 bpm subgroup which has a substantially higher lower limit of normal than the other groups. This, however, is almost identical to the lower limit of the interquartile range when heart rate is disregarded. By using the interquartile range to define normality 32 of the 83 patients have an abnormally low peak filling rate.

Table 4-20 – Rest supine peak filling rate (normalised to RR interval). Normal versus abnormal results by heart rate subgroup

HR (bpm)	Lower Limit of Normal	Normal	Abnormal
<60	145.9	16	1
60-69	163.7	13	12
70-79	124.5	22	2
80-89	123.4	10	2
>90	128.2	4	1

4.6.2.3 Time to Peak Filling

As time to peak filling showed a strong positive correlation with heart rate, it has been analysed exclusively by heart rate subgroup. A total of 17 patients had a time to peak filling below the lower limit of normal. As with peak filling rate, the majority of these occurred within the 60-69bpm heart rate range. This may simply be coincidence as there is little overlap between the patients who had abnormal diastolic function by the two criteria. Only 4 subjects had both abnormal peak filing rate and time to peak filling.

Table 4-21 – Rest supine tine to peak filling (normalised to RR interval). Normal versus abnormal results by heart rate subgroup

HR (bpm)	Lower Limit of Normal	Normal	Abnormal
<60	0.10	16	1
60-69	0.15	11	14
70-79	0.15	22	2
80-89	0.10	12	0
>90	0.16	5	0

4.6.3 Visual Assessment of Diastolic Filling Curves

Much as it is possible to visually assess left ventricular systolic function on echocardiography, it is also possible to gain an impression of diastolic function by inspecting the activity time curve from RNVG. Although this qualitative assessment has been eschewed in favour of the quantitative first third fractional filling, it may be of use in routine practice if the two correlate well. The author undertook blinded assessment of the left ventricular activity-time curves of each of the 83 patients. These were graded as either normal or abnormal on the basis of subjectively slow early diastolic filling. This was performed a total of three times, separated by at least 1 hour to minimise recall bias, for each of the curves. The third iteration was used to resolve any discrepancy between the first two. The process was carried out with both background subtracted and non-background subtracted curves since FTFF is derived from the former and it is the latter which is displayed on screen routinely. The activity-time curves used for visual assessment were the same curves from which the supine resting first third fractional filling was calculated.

Visual assessment of ventricular filling correlated well with quantitative assessment using both types of curves, although agreement was better with non-background subtracted curves. The Chi-Square statistic for non-background subtracted curves was 30.5, $p < 0.001$, and was 7.6, $p = 0.006$ for background subtracted curves. The relative performance of the two can be seen in Table 4-22 and Table 4-23.

Table 4-22 – Visual assessment of diastolic function using non-background subtracted left ventricular activity-time curves versus first third fractional filling

			Quantitative supine resting FTFF		
			Normal	Abnormal	Total
Visual assessment	Normal	N	46	8	54
		% abnormal supine resting FTFF	86.8%	26.7%	65.1%
	Abnormal	N	7	22	29
		% abnormal supine resting FTFF	13.2%	73.3%	34.9%

Table 4-23 – Visual assessment of diastolic function using background subtracted left ventricular activity-time curves versus first third fractional filling

			Quantitative supine resting FTFF		
			Normal	Abnormal	Total
Visual assessment	Normal	N	47	19	66
		% abnormal supine resting FTFF	88.7%	63.3%	79.5%
	Abnormal	N	6	11	17
		% abnormal supine resting FTFF	13.2%	36.7%	20.5%

4.7 Stress RNVG

4.7.1 Exercise Details

72 of the entire group of patients were able to undergo stress RNVG. The mean duration and haemodynamic responses are summarised in Table 4-24. Although the initial workload was reduced below the initial workload of the thallium stress test, several patients only managed to complete 3 minutes of stress. In all cases this was due to the combination of the expired gas collection mouthpiece and close proximity to the gamma camera being too cumbersome to tolerate for any longer.

Table 4-24 – Summary of study patient stress RNVG details

	Mean	Range
Duration (seconds)	424.8	180-720
Peak Heart Rate (bpm)	119.9	80-170
Change in Heart Rate (bpm)	44.2	6-81
Peak Systolic Blood Pressure (mmHg)	190.2	135-262

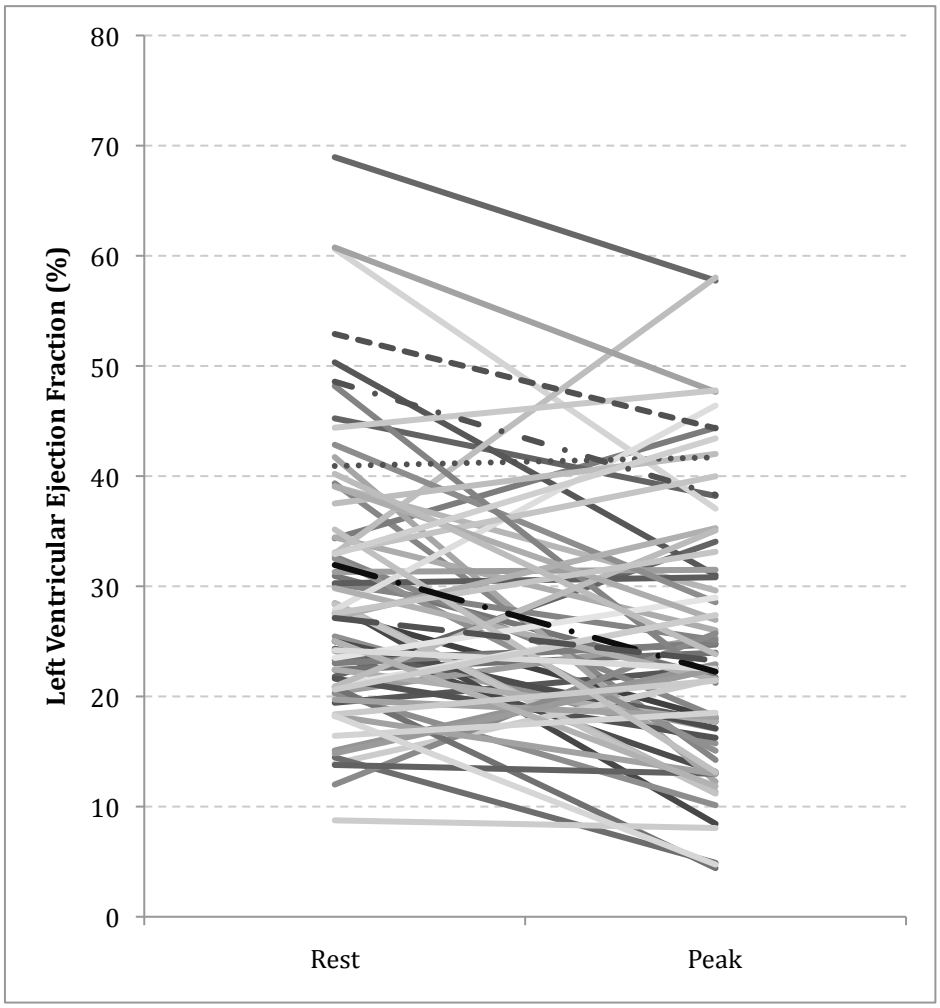
4.7.2 Left Ventricular Ejection Fraction

The mean rest erect left ventricular ejection fraction was 42.7%, ranging from 27.3% to 66.5%. On stress this rose by a mean 2.9% to 45.6%. This was statistically significant with a p value of 0.002 (paired samples T-test). The individual responses to exercise are shown in Figure 4-7. Although the majority do exhibit the expected increase in ejection fraction with stress, several show a fall. This was also seen in the control group where it was attributed to a limitation of the technique as myocardial ischaemia had already been excluded in this group by perfusion imaging. A total of 27 of 72 patients in the study group showed a fall in left ventricular ejection fraction with exercise. 16 of these occurred within the group with no significant perfusion abnormality. In the group with definite myocardial ischaemia, 10 patients showed an increase in ejection fraction whereas only one showed a fall. This is summarised in Table 4-25.

Table 4-25 – Response of left ventricular ejection fraction to stress depending on thallium scan result in study patients

		Ischaemia on Thallium Perfusion			
		None	**Minor**	**Definite**	**Total**
LV Ejection fraction	Rise	26	9	10	45
	Fall	16	5	6	27
	Total	42	14	16	72

Figure 4-7 – Left ventricular ejection fraction response to stress in study patients



The relative failure of ejection fraction to increase on stress does not appear to be attributable to the continued use of beta blockers in 22 of the 72 patients (Table 4-26). Although resting and peak stress heart rate is significantly lower in those patients taking beta blockers, the rise in heart rate is not. There is no significant difference in the resting, peak stress or change in ejection fraction between those beta blocked and those not (individual samples T-test).

Table 4-26 – Response of heart rate and left ventricular ejection fraction during stress RNVG depending on beta blockade

	No ß Blocker	ß Blocker	p
Resting heart rate (bpm)	80.5	65.7	<0.001
Change heart rate (bpm)	46.7	104.4	<0.001
Resting LV EF (%)	43.4	41.3	0.26
Change LV EF (%)	3.1	2.4	0.67

The most likely explanation for this ejection fraction behaviour is the combination of upright exercise and the manual single region of interest technique used to define the left ventricle here. In contrast to supine exercise, left ventricular end diastolic volume increases with erect exercise. The manual single ROI technique underestimates ejection fraction by underestimating background counts contributed by mediastinal structures. As end-diastolic volume increases with exercise, the background ROI moves further away from the mediastinum, further underestimating background activity and reducing the apparent ejection fraction further. By this mechanism, the expected rise in ejection fraction with exercise may be attenuated or even reversed.

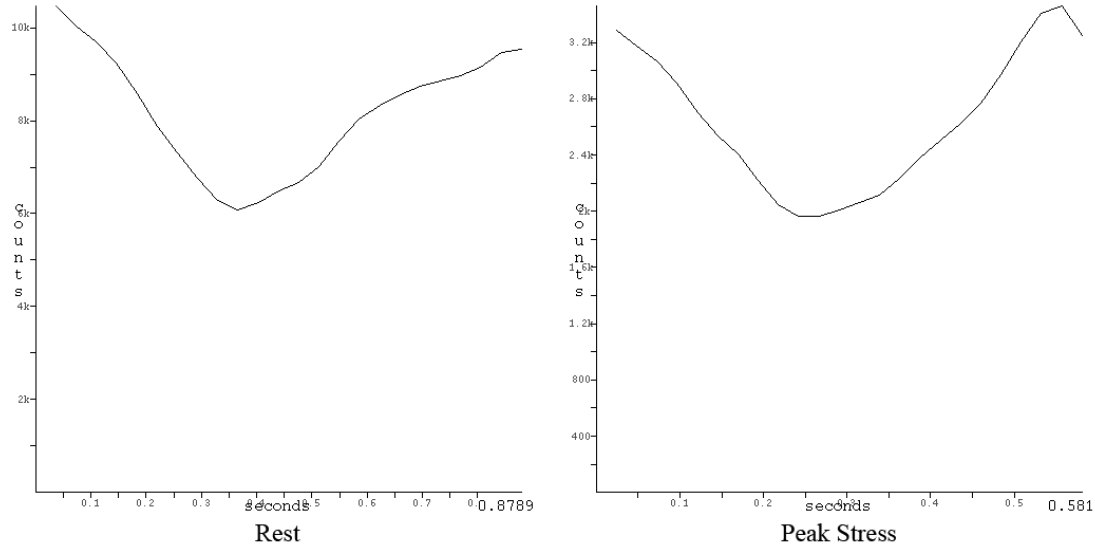
4.7.3 Right Ventricular Ejection Fraction

Mean right ventricular ejection fraction was 33.2% at rest which rose by 3.8% to 37% at peak stress. This was also statistically significant with a p value of 0.004.

4.7.4 First Third Fractional Filling

By applying the lower limit of normal equation derived for the control stress patients in section 4.2.3.3 ($FTFF = 52.8 \times e^{-0.008 \times HR}$) it is possible to categorise the observed FTFF at stress and rest in each patient as normal or abnormal. At rest 38 of the 72 patients (53%) undergoing stress RNVG had abnormal erect first third fractional filling. This is greater than the proportion who had abnormal resting supine FTFF (36%). At peak stress, only 25 patients (35%) were categorised as abnormal. 22 patients who were abnormal at rest fell back within the normal range at peak exercise. 9 patients who were normal at rest became abnormal on stress. An example of this is shown in Figure 4-8 where there is visible attenuation of the rate of ventricular filling in early to mid diastole. Of those who showed no change in status, 16 remained abnormal and 25 remained within the normal range.

Figure 4-8 – Background subtracted left ventricular activity-time curves at rest and peak stress from patient with normal first third fractional filling at rest which becomes abnormal on stress



4.7.5 Peak Filling Rate

Peak filling rate at both rest and stress fell within the normal range for the majority of study subjects. By using the interquartile range for erect resting, 7 patients fell outwith the normal range. The same number were defined as abnormal using the heart rate subgroups (Table 4-27).

Table 4-27 – Rest erect peak filling rate in study patients by heart rate subgroup

HR (bpm)	Lower Limit of Normal	Normal	Abnormal
<70	74.5	24	0
70-79	90.4	14	2
80-89	66.4	17	0
>90	164.7	7	5

At peak stress only 1 patient fell outwith the normal range using the heart rate subgroups (Table 4-28). Using the 5th centile as a cutpoint for the whole group only 3 patients were abnormal. The apparent disparity in results between first third fractional filling and peak filling rate is likely to be secondary to the wide normal ranges for peak filling rate reducing its sensitivity below a useful level.

Table 4-28 – Peak erect stress peak filling rate in study patients by heart rate subgroup

HR (bpm)	Lower Limit of Normal	Normal	Abnormal
<120	106.8	35	0
120-140	117.3	23	1
>140	148.3	13	0

4.7.6 Time to Peak Filling

Similar numbers of patients are found to be abnormal by time to peak filling at rest (Table 4-29) and peak stress (Table 4-30) as was the case with peak filling rate. Again, this very low yield of patients with abnormal results likely reflects the weakness identified in this parameter by virtue of its very wide normal range.

Table 4-29 – Rest erect time to peak filling in study patients by heart rate subgroup

HR (bpm)	Lower Limit of Normal	Normal	Abnormal
<70	0.162	22	5
70-79	0.047	16	0
80-89	0.053	17	0
>90	0.072	11	1

Table 4-30 – Peak erect stress time to peak filling in study patients by heart rate subgroup

HR (bpm)	Lower Limit of Normal	Normal	Abnormal
<120	0.037	35	0
120-140	0.154	24	0
>140	0.155	12	1

4.8 VO_2 Assessment

Of the 72 patients who underwent stress RNVG, it was possible to perform VO_2 max testing on 69 of these. Only 6 of these patients did not reach anaerobic threshold during exercise. The level of peak oxygen consumption achieved was, on the whole, low. The spread of results is shown in Figure 4-9. However, maximal oxygen consumption is age

and gender dependent. The lower limit of normal for the average female of age 70-79 years old is 20ml/kg/min which adds a degree of perspective to the results. The mean VO_2 max achieved was 9.6ml/kg/min. This ranged from 4.7 to 21ml/kg/min.

What is more useful is to examine is the achieved percentage of the predicted VO_2 max for each patient's age and gender (Figure 4-10). The group achieved an average of 53.8% of their predicted VO_2 max, ranging from 30.3% to 92.3%.

Figure 4-9 – Distribution of VO_2 max results in study patients

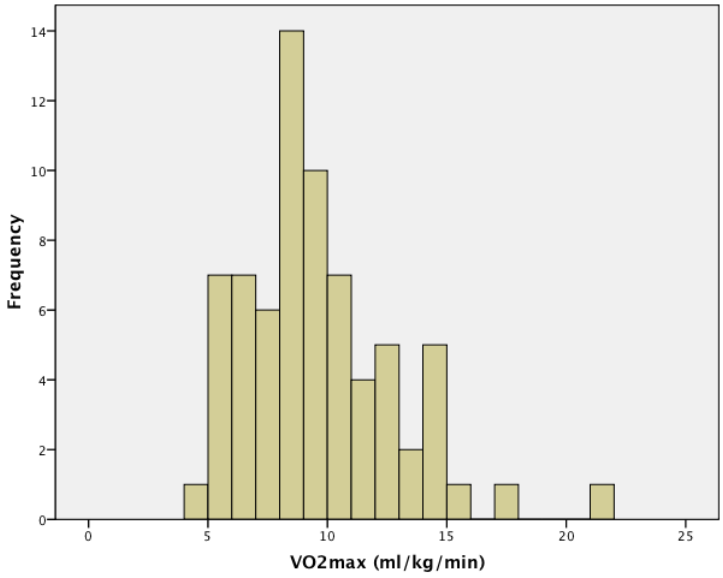
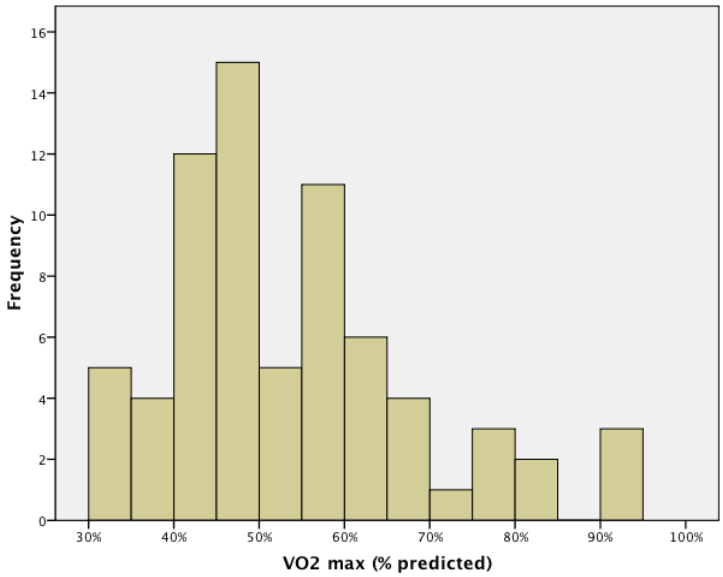


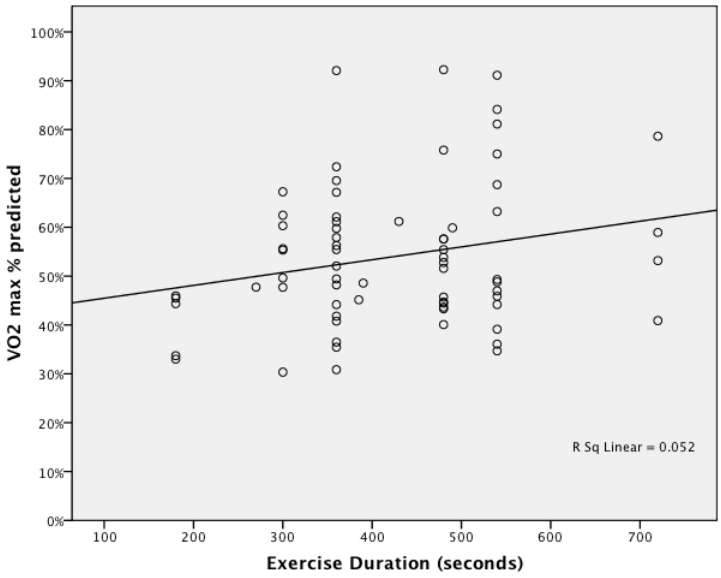
Figure 4-10 – Distribution of VO_2 max results as percentage of predicted



4.8.1 Correlates of VO_2 Max

There was only a weak positive correlation between exercise duration and percentage of predicted VO_2 max achieved (correlation coefficient 0.23, p=0.059). This is shown in Figure 4-11.

Figure 4-11 – Correlation between exercise duration and percentage of predicted VO_2 max achieved

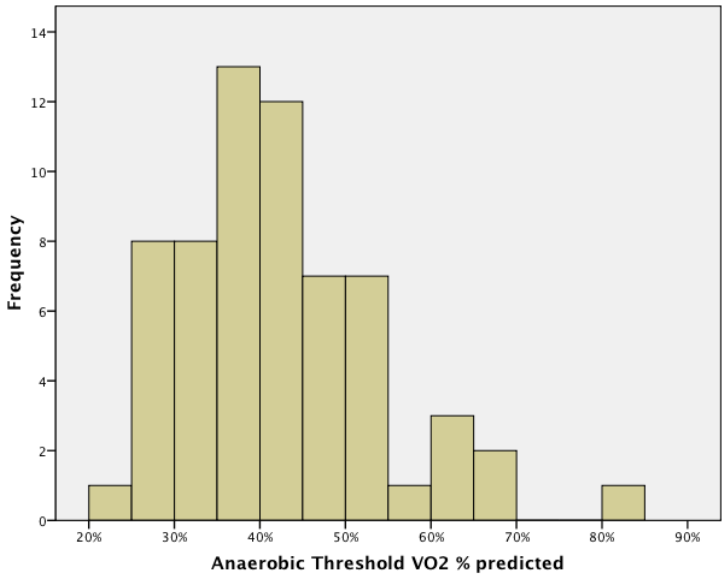


There was no significant correlation between percentage VO2 max achieved and the other measured indices of respiratory function, FEV_1 and FEV_1/FVC. In addition, there was no correlation in this patient group with left ventricular ejection fraction, the E/E' ratio, the E/A ratio, thallium ischaemia score or body mass index.

4.8.2 Anaerobic Threshold

VO_2 at anaerobic threshold was assessed in the same manner as VO_2 max, that is expressed as a percentage of the age and gender specific normal achieved. The mean percentage achieved was 42.2%, ranging from 24% to 81%. The spread of results (Figure 4-12) is similar to that seen with VO_2 max. As with VO_2 max there is no significant correlation with left ventricular ejection fraction, the E/E' ratio, the E/A ratio, thallium ischaemia score, body mass index, or other indices of respiratory function.

Figure 4-12 – Distribution of VO_2 at anaerobic threshold results as percentage of predicted



4.9 Resting First Third Fractional Filling as a Reference Standard

4.9.1 Rationale

The lack of a single, universally accepted, gold standard for diastolic function means that it is necessary to adopt a reference standard from the parameters assessed here. The other parameters may then be compared to this. Its performance may, in part, assessed by the prevalence of conditions such as left ventricular hypertrophy and hypertension where diastolic dysfunction might be expected. The resting supine left ventricular first third filling fraction has been chosen as this reference standard as it is this that is most widely available. First third fractional filling at peak stress will be considered separately to assess whether this performs better. Due to the heart rate dependency of FTFF, it has been dichotomised as normal or abnormal as described in section 4.6.2.1.

4.9.2 Correlation With Predictors of Diastolic Dysfunction

4.9.2.1 Sex

Although diastolic dysfunction, or heart failure with preserved ejection fraction, is predominantly a condition affecting females, this may be due to a higher prevalence of

ischaemic heart disease and systolic dysfunction in men. In this group there was no association between abnormal FTFF and female sex (p = 0.4, X^2 test).

4.9.2.2 Hypertension

A history of hypertension was present in 23 of the 30 patients with an abnormal resting FTFF (76.7%), and in 37 of the 53 patients (69.8%) with a normal FTFF. There was no evidence of a significant correlation (p = 0.34, Fisher's Exact Test). In those with a systolic blood pressure of 160mmHg or greater at rest, irrespective of an established history of hypertension, the findings were similar with 13 of 30 (43.3%) of hypertensive patients having diastolic dysfunction and 20 of 53 (37.7%) having normal diastolic dysfunction. Again, although there is a slightly higher prevalence of hypertension in patients with abnormal resting FTFF, this did not achieve statistical significance (p = 0.39, Fisher's Exact Test).

Table 4-31 – Correlation between abnormal resting supine left ventricular first third fractional filling and either history of hypertension or presence of systolic blood pressure ≥ 160mmHg

		Supine Resting FTFF		
		Normal	Abnormal	Total
History of hypertension	No	16	7	23
	Yes	37	23	60
Uncontrolled BP	No	33	17	50
	Yes	20	13	33

4.9.2.3 Obesity

Although there is an association between obesity and diastolic dysfunction(189, 190), this is not seen with resting first third fractional filling (Table 4-32). The difference between those with normal and abnormal FTFF is not significant over the spectrum of obesity seen (p = 0.52, Chi-square test).

Table 4-32 – Correlation between abnormal resting supine left ventricular first third fractional filling and obesity (body mass index)

		Supine Resting FTFF		
		Normal	Abnormal	Total
BMI	<25	7	3	10
	25-30	12	10	22
	30-35	25	10	35
	>35	9	7	16

4.9.2.4 Left Atrial Size

Left atrial size, dichotomised as normal or dilated at an antero-posterior diameter of 40mm, shows no significant correlation with abnormal resting first third fractional filling ($p = 0.59$, Fisher's Exact Test). The proportion of patients with dilated atria and normal or abnormal FTFF are almost identical at 20.8% and 20% respectively (Table 4-33).

Table 4-33 – Correlation between abnormal resting supine left ventricular first third fractional filling and left atrial dilatation by antero-posterior diameter

		Supine Resting FTFF		
		Normal	Abnormal	Total
Left atrial size	Normal	42	24	66
	Dilated	11	6	17

Left atrial volume index does show some promise in that the left atrium is dilated in 5 of 11 patients (45.5%) with abnormal FTFF and only 3 of 16 patients (18.8%) with normal FTFF. However, this does not achieve statistical significance ($p = 0.14$, Fisher's Exact Test). This may be due to left atrial volume index only being available in 27 of the 83 study patients.

4.9.2.5 Left Ventricular Hypertrophy

Left ventricular hypertrophy as defined by a left ventricular mass index above the sex corrected upper limit of normal is slightly more prevalent in those with abnormal resting FTFF. Left ventricular hypertrophy is present in 23 of 29 (79.3%) of patients with

diastolic dysfunction and in 34 of 53 (64.1%) of those without. The correlation is, however, not statistically significant (p = 0.12, Fisher's Exact Test)

Table 4-34 – Correlation between abnormal resting supine left ventricular first third fractional filling and left ventricular hypertrophy by left ventricular mass index

		Supine Resting FTFF		
		Normal	Abnormal	Total
LVH by LV Mass Index	No LVH	19	6	25
	LVH	34	23	57

4.9.2.6 Left Ventricular Ejection Fraction

It has already been shown in section 4.1.4.1 that there is no relationship between left ventricular ejection fraction and first third fractional filling uncorrected for heart rate. This is also the case once first third fractional filling has been corrected for heart rate. Left ventricular ejection fraction was abnormal in 6 of 30 patients (20%) with abnormal FTFF, and in 12 of 53 (22.6%) patients with normal FTFF. This is not statistically significant (p = 0.59, Chi-square test).

Table 4-35 – Correlation between abnormal resting supine left ventricular first third fractional filling and normal, borderline or abnormal left ventricular ejection fraction

		Supine Resting FTFF		
		Normal	Abnormal	Total
LV Ejection Fraction	Normal	34	22	56
	Borderline (37-40%)	7	2	9
	Abnormal	12	6	18

4.9.2.7 E/E' Ratio

Neither the septal nor lateral E/E' ratio show a significant relationship with an abnormal resting first third fractional filling (p = 0.47 and p = 0.09 respectively, Chi-square test). There is almost no difference in the percentage of patients with an elevated septal E/E' ratio and normal (44.2%) or abnormal FTFF (43.3%) (Table 4-36). There is actually a preponderance of patients with normal FTFF and elevated E/E' lateral ratio compared to abnormal FTFF (Table 4-37).

Table 4-36 – Correlation between abnormal resting supine left ventricular first third fractional filling and normal, equivocal or elevated septal E/E' ratio

		Supine Resting FTFF		
		Normal	Abnormal	Total
E/E' septal	Normal	10	9	19
	Equivocal	19	8	27
	Elevated	23	13	36

Table 4-37 – Correlation between abnormal resting supine left ventricular first third fractional filling and normal or elevated lateral E/E' ratio

		Supine Resting FTFF		
		Normal	Abnormal	Total
E/E' lateral	Normal	19	16	35
	Elevated	34	14	48

4.9.2.8 E/A Ratio

There is a statistically significant association between an abnormal resting FTFF and an E/A ratio outwith the normal range (p = 0.02, Chi-Square test). No patients with a normal FTFF have an abnormal E/A ratio and only 3 (10%) of patients with an abnormal FTFF have a normal E/A ratio (Table 4-38).

Table 4-38 – Correlation between abnormal resting supine left ventricular first third fractional filling and normal, equivocal or abnormal E/A ratio

		Supine Resting FTFF		
		Normal	Abnormal	Total
Abnormal E/A ratio	Normal	17	3	20
	Equivocal	36	25	61
	Abnormal	0	2	2

4.9.2.9 NT-proBNP

NT-proBNP does not show any statistically significant association with abnormal first third fractional filling (p = 0.32, Fisher's Exact Test). The breakdown of patients is shown in Table 4-39.

Table 4-39 – Correlation between abnormal resting supine left ventricular first third fractional filling and normal or elevated NT-proBNP

		Supine Resting FTFF		
		Normal	Abnormal	Total
NT-proBNP	Elevated	15	6	21
	Normal	38	23	61

4.9.2.10 Myocardial Ischaemia

Although a relationship has previously been observed between the presence of inducible ischaemia on myocardial perfusion imaging and impaired first third fractional filling (191), an inverse relationship is seen in this group. The trend for higher levels of diastolic dysfunction in the absence of inducible ischaemia seen in Table 4-40 is statistically significant ($p = 0.046$, Chi-Square test).

Table 4-40 – Correlation between abnormal resting supine left ventricular first third fractional filling and extent of inducible myocardial ischaemia on thallium scintigraphy

	Supine Resting FTFF		
	Normal	Abnormal	Total
Normal	24	22	46
Minor ischaemia	13	4	17
Definite ischaemia	16	4	20

4.9.2.11 Failure to Reach Anaerobic Threshold

The failure to reach anaerobic threshold on exercise suggests a cardiac, rather than respiratory, limitation to exercise. In the absence of other causes such as valvular heart disease, systolic dysfunction or ischaemia this may indicate diastolic dysfunction. In this patient group there was no significant difference in the proportions with those with abnormal FTFF (2 of 23, 8.7%) and those with normal FTFF (6 of 48, 12.5%) failing to reach anaerobic threshold ($p = 0.49$, Fisher's Exact test).

4.9.3 Summary

Of the indices one might expect to correlate with diastolic dysfunction and abnormal resting first third fractional filling, only the E/A ratio behaves as expected and is statistically significant. The lack of correlation with other indices and the counterintuitive relationship with myocardial ischaemia leads to the conclusion that first third fractional filling, at least at rest, is no better than other indces of diastolic function in this patient group. There is overall a poor correlation between these indices.

4.10 Peak Stress First Third Fractional Filling as a Reference Standard

4.10.1 Correlation With Predictors of Diastolic Dysfunction

4.10.1.1 Sex

As with resting FTFF there is no significant association between female sex and abnormal peak stress first third fractional filling ($p = 0.51$, Fisher's Exact test). The proportions of females and males with abnormal peak stress FTFF are 35.6% and 30.8% respectively.

4.10.1.2 Hypertension

Neither a history of hypertension nor uncontrolled hypertension at baseline showed a significant association with abnormal peak stress FTFF ($p = 0.12$ and $p = 0.47$ respectively, Fisher's Exact test). The proportion of patients with abnormal peak stress FTFF and a history of hypertension was 80% compared to 63.8% of patients who had normal FTFF. Of those with uncontrolled hypertension these proportions were 40% and 36.2% respectively.

4.10.1.3 Obesity

There is no significant association between increasing body mass index and abnormal peak stress first third fractional filling ($p = 0.3$, Chi-Square test).

4.10.1.4 Left Atrial Size

Although there is a higher proportion of patients with a dilated left atrium by antero-posterior diameter with abnormal peak stress FTFF (28% vs 14.9% with normal FTFF), this difference is not statistically significant (p = 0.15, Fisher's Exact test).

4.10.1.5 Left Ventricular Hypertrophy

Left ventricular hypertrophy, defined by left ventricular mass index, is also slightly more common in patients with abnormal peak stress FTFF (76% vs 60.9% with normal FTFF). As with left atrial size, the difference is not statistically significant (p = 0.15, Fisher's Exact test).

4.10.1.6 Left Ventricular Ejection Fraction

Just as myocardial ischaemia showed the opposite relationship to first third fractional filling at rest, left ventricular ejection shows a similarly unexpected relationship to peak stress FTFF (Table 4-41). Patients with a depressed left ventricular ejection fraction are more likely to have normal peak stress first third fractional filling than abnormal FTFF. This is statistically significant (p = 0.02, Chi-Square test) and is particularly unexpected given the lack of any relationship with resting FTFF, even in the larger control group (section 4.1.4.1).

Table 4-41 – Correlation between abnormal peak exercise supine left ventricular first third fractional filling and left ventricular ejection fraction

		Peak stress FTFF		
		Normal	**Abnormal**	**Total**
LV Ejection Fraction	**Normal**	26	22	48
	Borderline (37-40%)	7	1	8
	Abnormal	14	2	16

4.10.1.7 E/E' Ratio

Both the septal and lateral E/E' ratios show no significant relationship to abnormal peak stress FTFF (p = 0.86 and p = 0.96 respectively, Chi-Square test). This can be seen in

Table 4-42 and Table 4-43 where the proportion of subjects with a normal or elevated E/E' ratio shows almost no difference between the normal or abnormal stress FTFF groups.

Table 4-42 – Correlation between abnormal peak stress left ventricular first third fractional filling and normal, equivocal or elevated septal E/E' ratio

		Peak Stress FTFF		
		Normal	Abnormal	Total
E/E' septal	Normal	12	5	17
	Equivocal	15	9	24
	Elevated	20	10	30

Table 4-43 – Correlation between abnormal peak stress left ventricular first third fractional filling and normal or elevated lateral E/E' ratio

		Peak Stress FTFF		
		Normal	Abnormal	Total
E/E' lateral	Normal	21	11	32
	Elevated	26	14	40

4.10.1.8 E/A Ratio

Whilst the E/A ratio showed a significant correlation with resting first third fractional filling, this relationship disappears with peak stress FTFF ($p = 0.13$, Chi-Square test).

4.10.1.9 NT-proBNP

As with resting first third fractional filling, abnormal peak stress FTFF has no significant relationship with an elevated NT-pro BNP level ($p = 0.15$, Chi-Square test). Of those with normal peak stress FTFF 15 of 47 patients (31.9%) had an elevated NT-proBNP compared with 4 of 25 patients (16%) with abnormal FTFF.

4.10.1.10 Myocardial Ischaemia

The trend for first third fractional filling to correlate inversely with myocardial ischaemia on thallium scintigraphy, significant at rest, is also seen with peak stress FTFF (Table 4-44). It is, however, not statistically significant at peak stress ($p = 0.3$, Chi-Square test).

This suggests that the significant result at rest may be a statistical aberration due to the relatively small patient numbers.

Table 4-44 – Correlation between abnormal peak exercise supine left ventricular first third fractional filling and extent of inducible myocardial ischaemia on thallium scintigraphy

	Peak stress FTFF		
	Normal	**Abnormal**	**Total**
Normal	25	17	42
Minor ischaemia	9	5	14
Definite ischaemia	13	3	16

4.10.1.11 Failure to Reach Anaerobic Threshold

Unlike its resting equivalent, peak stress first third fractional filling shows a significant association with failure to reach anaerobic threshold ($p = 0.01$, Fisher's Exact test). Of the 23 patients with abnormal peak stress FTFF 5 (21.7%) failed to reach anaerobic threshold compared to 1 of 46 (2.2%) with normal FTFF (Table 4-45).

Table 4-45 – Correlation between abnormal peak exercise supine left ventricular first third fractional filling and failure to reach anaerobic threshold

		Peak stress FTFF		
		Normal	**Abnormal**	**Total**
Anaerobic threshold reached	**No**	1	5	6
	Yes	45	18	63

4.10.2 Summary

Like resting first third fractional filling, peak stress FTFF shows a generally poor level of correlation with predictors of and other parameters of diastolic dysfunction. However, a statistically significant correlation which behaves as expected is with failure to reach anaerobic threshold on exercise. This is potentially useful as it shows correlation between an objective measure of diastolic dysfunction and reduced exercise capacity of a potentially cardiac cause.

Like the unexpected inverse correlation between myocardial ischaemia and resting FTFF, the association between impaired left ventricular systolic function and normal FTFF on stress is the opposite one would expect. This does raise doubts as to the reliability of first third fractional filling as an index of diastolic function. It is clear that, with the exception of the correlation with anaerobic threshold, assessment of FTFF on stress is no more reliable than at rest, at least in this patient group. This is a heterogeneous patient group and although they could be labelled as having diastolic dysfunction on the basis of symptoms and signs, many of them may not actually have this condition. This limitation is not exclusive to this work, the lack of a reliable gold standard means it is inherent in all the major trial work published to date. This may explain the disappointing results and lack of firm evidence base in diastolic heart failure. A drug can only be proven effective where the correct pathology has been identified.

4.11 Conclusions

4.11.1 RNVG in the Diagnosis of Diastolic Dysfunction

The prevalence of diastolic dysfunction as defined by abnormal first third fractional filling was 60% (53 of 83 patients) at rest and 35% (25 of 72 patients) on stress. The reason for this large change with exercise is unclear. At rest RNVG performs poorly against the better regarded markers including NT-proBNP, tissue Doppler echocardiography and left atrial dimensions. The addition of assessment during stress does little to improve these correlations with the exception of providing a link between reduced exercise capacity and the failure to reach anaerobic threshold with an objective measurement of diastolic function. It is therefore difficult to argue that the observed change in abnormal FTFF with exercise is due to an improvement in accuracy with stress assessment. The poor correlation with other markers of diastolic function may reflect weakness in these other markers and the lack of a gold standard.

There are a number of reasons which RNVG and in particular stress RNVG has not performed as well as hoped for. First and foremost amongst these is the small, heterogeneous patient group studied. The presence of confounding diagnoses was almost universal despite attempting to screen out these patients at the recruitment stage. This coupled with the lack of a gold standard indicator of diastolic dysfunction means that the proportion of the patients recruited who actually had diastolic dysfunction may have been

relatively small. This will have substantially reduced the power of the study to demonstrate the utility of assessing diastolic function on exercise by RNVG.

The difficulty in defining a normal range for parameters of diastolic function by RNVG, particularly those that are highly heart rate dependent, is another source of weakness. A novel approach has been taken here in the correction of first third fractional filling for heart rate. Whilst data from a large control group has been used to generate the resting formal range for FTFF, data from control subjects on stress is more limited. The wide confidence intervals seen with all of the parameters of diastolic function examined greatly increase the probability of a false negative diagnosis.

Stress RNVG is no longer in routine use for the diagnosis of ischaemic heart disease, having been surpassed by myocardial perfusion scintigraphy. As a tool for assessing diastolic function, stress RNVG is unlikely to enter routine use but there has been further research interest in this field recently. At present, however, stress RNVG, whilst attractive in concept, has not been shown here to be a useful tool for the assessment of diastolic function. The hypothesis that stress RNVG is more sensitive and correlates better with other indices of diastolic function than assessment at rest has, therefore, not been proven.

4.11.2 Diagnoses Other Than Diastolic Dysfunction

The very high prevalence of confounding diagnoses other than diastolic dysfunction is the most striking aspect of this study. In only one patient of the 83 was none of these prespecified alternative diagnoses found. The virtual ubiquity of these diagnoses illustrates that, even if diastolic dysfunction was to be disregarded as a potential diagnosis, significant pathology is present and requires appropriate investigation to uncover.

In 56 of the 83 patients evidence of at least one cardiac source of dyspnoea, either abnormal systolic function by RNVG, ischaemia or an elevated NT-proBNP was found. Left ventricular systolic dysfunction not detected on echocardiography was present in 18 of 83 patients and was borderline abnormal in a further 9. The ability of echocardiography to accurately assess ventricular function is dependent both on operator experience and on image quality. The patients in this cohort were assessed by two experienced operators, reducing the impact of this factor. However, image quality can be significantly degraded by obesity and respiratory disease, both of which were common here. Right ventricular systolic dysfunction, which is difficult to assess by echocardiography, was present in 43

patients and was borderline in 10 patients. There was, therefore evidence of LV systolic dysfunction in nearly one third of patients and RV systolic dysfunction in nearly two thirds.

Previously undiagnosed ischaemic heart disease was also common. 37 of the 83 patients had some evidence of inducible myocardial ischaemia. 20 of these had significant, unequivocal evidence of ischaemia. Of the 25 patients with diastolic dysfunction demonstrated on exercise only 8 had myocardial ischaemia demonstrated. Although ischaemia was common, with a follow-up time of between 6 and 7 years, this patient group has proven itself to be at relatively low cardiovascular risk. Only 4 patients suffered an acute coronary syndrome, an annual incidence of 0.7%, and one patient died of non-cardiovascular causes

Obesity was a common finding. Only 10 patients had a normal body mass index of 25 or less. 22 patients were overweight with BMIs of 25-30, 35 patients were obese with a BMI of 30-35, and 16 patients had a BMI of greater than 35.

Of the 82 patients with spirometry results available, 58 had normal spirometry, 20 had mild airflow obstruction and 4 had moderate airflow obstruction.

4.11.3 Agreement Between Assessments of Diastolic Function

First third fractional filling at rest supine correlated strongly with rest erect FTFF, but not with peak stress FTFF. Similarly it correlated well with resting values of peak filling rate, but not stress values. There was also a modest correlation with an abnormal E/A ratio (R=-0.3, p=0.007). However, it only correlated with the E/E' septal ratio when the rest erect value was used.

A similar pattern was seen between the other indices examined. Where statistically significant correlations were seen, these were generally weak and inconsistent between supine, erect and stress measurements.

NT-proBNP level did not correlate with any of the measured indices of diastolic function either at rest or at peak stress.

With the proviso that this is a small group, albeit representative of usual clinical practice, the parameters of diastolic function measured either by echocardiography, rest or stress radionuclide ventriculography, or biochemical marker such as NT-proBNP show generally poor correlation with each other. By measuring a wide number of indices, the majority of patients can be shown to have at least 1 abnormal parameter of diastolic function. Partly due to the heterogeneity of the group, possible alternative diagnosis are almost universal.

5. Discussion – Multimodality Imaging of Suspected Diastolic Dysfunction

5.1 Scope of this Study

This thesis set out to look in detail at a common clinical problem, the assessment of patients referred from primary care with suspected heart failure but normal systolic function on echocardiography. Specifically, this thesis has examined the role of radionuclide ventriculography in the assessment of diastolic function and whether the use of stress RNVG improves this assessment. In this respect, stress RNVG has been found wanting. However, this should not be regarded as the end of the road for RNVG and stress RNVG. The biggest limitation of this study is that many of the recruited patients, in all likelihood, did not have diastolic heart failure. Only 21 of the 83 patients had an NT-proBNP level above the reference range suggesting that stress RNVG only had an opportunity to show its worth in a much smaller patient group than anticipated. If a population with a higher probability of a positive finding, for example those with an elevated NT-proBNP level and a recent acute hospital admission with heart failure, were to be studied, the finding may have been significantly different.

The most significant findings of this study were the very high frequency of alternative diagnoses such as ischaemic heart disease and respiratory disease, and the relatively poor performance of echocardiography.

5.2 Identification of Confounding Diagnoses

It has been demonstrated, both in this work and previously, that diagnoses other that diastolic dysfunction are frequently present in patients with dyspnoea without echocardiographic evidence of systolic dysfunction. What is also clear from this work is that there is often not a single diagnosis in each patient. Many of the diagnoses identified may not only cause dyspnoea and clinical signs of heart failure in their own right, but are also potential causes of diastolic dysfunction. In the investigation of the breathless patient, it should no longer be sufficient to simply document normal left ventricular systolic function on echocardiography and not investigate further. Where there is sufficient clinical suspicion that there is pathology to account for symptoms beyond physical deconditioning

or obesity, these should be actively sought. This work has demonstrated that a significant number of new diagnoses of ischaemic heart disease and chronic obstructive pulmonary disease can be made in this patient group. This, therefore, allows inappropriate medication to be discontinued and more appropriate management strategies pursued.

Although some patients will ultimately undergo as comprehensive an assessment as undertaken here, a more discriminating approach should normally be taken. Where a patient has a definite history of ischaemic heart disease, the initial focus, after echocardiography, should be on identifying myocardial ischaemia. The most effective means of doing this has been the subject of much debate with each modality championed by enthusiasts. It is well recognised that the treadmill test is often unhelpful. Notwithstanding the limited sensitivity of treadmill testing, tests may be inconclusive on the basis of poor exercise capacity. Dyspnoea as a limiting symptom in exercise tolerance testing may simply reflect physical deconditioning but may also be a symptom of cardiac ischaemia that the ECG is too insensitive to show. Myocardial perfusion imaging with either thallium or technetium based tracers is more sensitive and offers the possibility of pharmacological stress. There are two downsides to nuclear imaging. First, its specificity is imperfect and small perfusion defects may be false positive results, necessitating a degree of subjectivity in interpretation. Second, it involves exposure to ionising radiation. Whilst this is not a feature of stress echocardiography, image quality can be significantly impaired by obesity and respiratory disease even with the use of echocardiographic contrast agents. Whilst the avoidance of radiation exposure is desirable, the correct investigation must be selected for the correct patient.

Where the main issue is dyspnoea rather than clinical signs of heart failure, the most appropriate initial investigation may not be echocardiography. If the likelihood of respiratory disease is high, a 12 lead ECG may be an appropriate initial screening tool given the low probability of left ventricular systolic dysfunction with a normal ECG. Ultimately, most patients will have an echocardiogram. This may be to exclude right heart pathology in respiratory disease, assess valvular pathology, or simply to provide reassurance and confirm the result of more basic tests.

Perhaps the most important distinction to make is between cardiac and non-cardiac pathology. A wrong supposition at an early stage may lead to a delay in the correct

diagnosis and treatment as each specialty pursues its own set of diagnoses and battery of tests.

The cardiac causes of heart failure-like symptoms which should be considered are:

- Left ventricular systolic dysfunction
- Valvular heart disease
- Ischaemic heart disease and the anginal equivalent in the absence of fixed ventricular dysfunction
- Arrhythmias, including atrial fibrillation which may be symptomatic even with a controlled ventricular response, or show an exaggerated heart rate response to even minimal exertion.
- Diastolic dysfunction, or normal ejection fraction heart failure

Diastolic dysfunction is intentionally placed at the bottom of this list, partly as it should be considered a diagnosis of exclusion. It is also outranked by the other cardiac diagnoses as, in contrast to these, it does not have a solid evidence base of treatment.

The non-cardiac diagnoses which should be considered are:

- Respiratory pathology including chronic obstructive pulmonary disease and interstitial lung disease
- Obesity
- Physical deconditioning – this may include patients with unrealistic expectations where their symptoms reflect the normal aging process rather than pathology.
- Anaemia
- Hypothyroidism
- Depression – this may have prominent symptoms of fatigue, exercise intolerance and dyspnoea. There may be a degree of overlap with chronic fatigue syndrome, although this remains a controversial diagnosis.

5.3 The Diagnostic Accuracy of Open Access Echocardiography

The concept of echocardiography services being directly available to general practitioners has been with us for more than a decade now. But does the service achieve what it set out

to do, or is the yield of pathology insufficient to justify the quantity of resources devoted to it? Another question that must be addressed when evaluating the service is whether or not the results are sufficiently robust given this may be the patient's only contact with secondary care. There is no reason to suspect that the patient group here is different to that presenting to any other open access echocardiography service. Yet, despite assessment by two experienced operators, mild left ventricular systolic dysfunction was not detected in up to 1 in 3 patients.

5.3.1 Origins and Scope of the Service

Whilst some pilot schemes existed prior to 1995 (192), a major expansion began in the field of open access echocardiography in this year. A telephone survey of 100 hospitals of greater than 300 beds in 1996 (193) found that 30 of these offered a service of some description and of these, 21 had opened the service within the past year. Such a rate of expansion has not been maintained – a survey of western European primary care physicians in 2000 found that only 22% of respondents had direct access to echocardiography from the community. This is partly due to some schemes which had been funded by pharmaceutical companies closing once this funding was withdrawn (194).

The impetus for open access echocardiography comes from observations that heart failure was being neither adequately investigated nor optimally treated(195). A task force set up by the European Society of Cardiology reiterated in 1995 that evidence of cardiac structural abnormality was essential for the accurate diagnosis of heart failure, with echocardiography being the key investigation (16). This conclusion had already been reached in 1994 by Dargie and M^cMurray(196) who suggested that the provision of readily available direct access echocardiography services to general practitioners was crucial in addressing this.

With the background of a desire to improve the diagnosis and management of heart failure in the community, some early services focussed solely on this to the exclusion of other structural cardiac disease. The service in Edinburgh (152) invited referrals solely for suspected heart failure, whether treated or not, and of patients at risk of having asymptomatic left ventricular dysfunction. This was not a universally held opinion however. The open access echocardiography service launched in Brighton in 1994 (192) was also intended to assess murmurs. Of the 106 patients referred in the first ten months of

the service, 46 were referred solely for the assessment of murmurs. 25 of these were found to have no abnormality. A negative finding in this context is as beneficial as a positive one as is will have avoided an unnecessary referral to a cardiologist. Further to this, unless patients could be guaranteed echocardiography on the same day as their first clinic visit, patients being evaluated by a non direct access route would have at least one additional hospital visit and additional delay in definitive diagnosis.

5.3.2 The Missed Diagnosis

Almost one third, 27 of 83, of the patients in this study had a radionuclide ejection fraction below the reference range of 40%. Whilst 16 of these had only borderline impairment and the remainder mild systolic dysfunction, this highlights the potential weakness of echocardiography in this population. This is an area where evidence based treatment is available and represents a missed opportunity to influence prognosis in a positive manner. Whether or not this degree of systolic dysfunction is sufficient to account for the symptoms present does not affect its prognostic significance. The SOLVD(197) and SAVE(198) trials examined the use of the ACE inhibitors captopril and enalapril in asymptomatic left ventricular systolic dysfunction. Both showed significant reductions in morbidity and mortality, with a reduction in hospitalisations due to heart failure of up to one third. Even in a population with no history of cardiovascular disease asymptomatic left ventricular dysfunction carries an adverse prognosis(199).

In this patient group the primary echocardiographic parameter of left ventricular systolic function was the qualitative visual assessment. Whilst this is limited by its subjective nature, it better reflects the real world and usual clinical practice than the Simpson's biplane ejection fraction. This was only possible in a modest subset of the patients here. Previous studies from a similar population locally managed to measure ejection fraction by this method in approximately 90% of patients(200). The reason for the discrepancy is not clear but many patients in this study were rejected for biplane ejection fraction measurement on the basis of suboptimal image quality. The rationale in this case was that a qualitative assessment was preferable to the potentially inaccurate ejection fraction due to 'guessing' the contours of the endocardial border. Had ejection fraction been measurable with confidence in more patients, the number of missed diagnoses of left ventricular systolic dysfunction may have been fewer.

There are two sources of inaccuracy in the echocardiographic assessment of suspected left ventricular dysfunction. These are patient related factors, and operator related factors with a degree of interplay between the two.

The patient related factors are well recognised and impact upon image quality. Obesity, an increasingly common problem, particularly in this patient population, may significantly reduce image quality. The greater depth the ultrasound has to penetrate to results in a lower image frame rate, making the assessment of subtle wall motion abnormalities more challenging. Lower ultrasound frequencies may also be necessary in order to achieve adequate tissue penetration. This results in poorer spatial resolution, again reducing diagnostic confidence in less obvious abnormalities. This has been a more significant problem historically than at present due to the advent of harmonic imaging. Harmonic ultrasound combines the advantages of low frequency transmitted pulses to achieve tissue penetration with the use of secondary harmonics reflected back from the tissue. These secondary harmonics are typically at double the transmitted frequency and have far superior spatial resolution. In comparison, traditional fundamental imaging transmits and receives at the same frequency resulting in poor image quality in patients requiring lower frequencies.

Respiratory disease also has a significant impact on the performance of echocardiography. Ultrasound is not efficiently transmitted through air and, as air trapping and hyperinflation of the lungs may be a feature of chronic respiratory disease, image quality is degraded. Hyperinflation of the lungs may also render standard transthoracic echocardiography windows useless. An experienced operator may be able to salvage the study with a comprehensive subcostal examination, but Doppler measurements may be inaccurate due to the beam not being parallel to flow from this angle.

Operator experience is a critical factor in echocardiography. Ultrasound, unlike other imaging techniques such as computed tomography, is reliant on a comprehensive set of images being acquired at the time of the study. If a particular view or Doppler measurement is not present, it may necessitate a repeat study. An experienced operator is more likely to acquire a comprehensive set of images, and to optimise these well, compensating for any adverse patient factors. Experience is also important in the interpretation of echocardiography. The most common method of assessing left ventricular systolic function is the qualitative visual assessment. As this is a subjective assessment, a

degree of experience is required to differentiate the sluggish ventricle of a heavily beta blocked patient from genuine dysfunction. Experience is also required to integrate mitral regurgitation assessment and the effect this has on flattering an impaired left ventricle by offering a low pressure escape route.

The problem with the often subjective nature of left ventricular function assessment may, in part, be overcome by the use of more objective quantitative methods. Foremost amongst these is the calculation of ejection fraction by means of the Simpson's biplane method. This is less reliant on geometrical assumptions than the Teicholtz method but is still subject to errors. It is very dependent on image quality on two fronts. As the technique involves tracing the endocardial border at end systole and end diastole, poor endocardial border definition can result in inaccurate volume estimation. Although this can be partly overcome with the use of left ventricular contrast, this is not commonly used outwith specialist settings such as stress echocardiography. Its administration requires venous access and the presence of a physician, making its use in routine, technician delivered, echocardiography unlikely to expand significantly. The use of left ventricular contrast agents for all studies would also be prohibitively expensive and expose patients to a small (1 in 10,000) risk of serious adverse reaction.

The second image quality related issue, that of image foreshortening, cannot be solved with left ventricular contrast. Often it is necessary to move the ultrasound probe one rib space higher than the true apex due to a poor acoustic window. This has the effect of reducing the left ventricular volumes measured and usually results in an elevation in measured ejection fraction. The apical 2 chamber view is particularly affected as the probe lies obliquely across the ribs. Matrix probes which allow the acquisition of three dimensional or tri-plane images may overcome this problem in some patients but the large probe footprint results in acoustic access problems in more challenging patients.

5.3.3 The Appropriateness of Echocardiography in Suspected Heart Failure

The yield of left ventricular systolic dysfunction in those referred with suspected heart failure varies from 1 in 4 to 1 in 5(152, 201). This modest yield partly reflects the difficulties inherent in the accurate diagnosis of heart failure in the community. Given this, and the high rates of mild left ventricular dysfunction not appreciated on

echocardiography, its usefulness in this setting must be questioned. With the use of screening investigations it should be possible to improve the proportion of those found to have impaired left ventricular function by creating a more targeted lower volume, higher quality service.

5.3.3.1 ECG as a screening tool?

Although data for diastolic dysfunction are lacking, the 12 lead ECG has shown promise as an effective screening tool for the detection of left ventricular systolic dysfunction. However, different series have yielded significantly different results. Davie et al (202) found that a 'normal' ECG had a 98% negative predictive value for left ventricular dysfunction. 534 patients referred with suspected heart failure to an open access echocardiography service were reviewed, of whom 96 (18%) had left ventricular systolic dysfunction, a level broadly in keeping with other similar series. Of these, 90 had what was regarded as a major ECG abnormality (atrial fibrillation, left ventricular hypertrophy, bundle branch block, left axis deviation, or previous myocardial infarction). The remaining 6 had minor ECG abnormalities – none had an entirely normal ECG. Of the 438 patients with normal ventricular function, 269 had a 'normal' ECG and 169 had a major ECG abnormality. Therefore, although it would appear that 6.25% (6/96) of LVSD would be missed by ECG screening, only 2.2% (6/275) of those with a normal ECG had LVSD. Therefore, a normal ECG is highly sensitive for normal LV systolic function; an abnormal ECG is, however, rather non-specific with 65% (169/259) of these having normal LV function.

The sensitivity and specificity of the ECG is, of course, dependent on the patient population. In a population with a significantly higher prevalence of left ventricular systolic dysfunction, the ECG performed less well(203). Of the 200 patients in this series only 35 had normal LV function. Perhaps because of the clearly different population being seen in this series, a normal ECG was a poor predictor of normal LV function – 53% of those with a normal ECG had LVSD. It should be noted that in the whole series only 34 ECGs were regarded as normal. There was also a difference in how the ECGs were interpreted. Two of the three interpreters were general practitioners who had to make a binary decision of normal or abnormal. This, therefore, raises the possibility of misinterpretation – either overreporting of minor abnormalities, or more subtle abnormalities being missed.

The question then arises as to whether the automated analysis software found in most modern ECG machines should be relied on instead. Many general practitioners rely on these automated reports and so this is not unreasonable. An attempt was made to answer this question which was unfortunately flawed by the failure to distinguish between valvular disease and left ventricular dysfunction in the analysis of the sensitivity of the ECG as a screening tool (204). UnforECG has not been proposed as a screening tool for valvular heart disease – that is the role of the stethoscope.

Whether entrusting ECG analysis and potentially the decision on echocardiography to such a system is wise is a matter of concern. Whilst electronic ECG analysis improved the speed and accuracy of interpretation by cardiologists without increasing false positive diagnoses (205), primary care physicians tended to be misled by incorrect ECG analysis and could potentially miss important diagnoses missed by the computer (206). This is presumably a matter of training, clinical experience and an awareness of the potential limitations of automated analysis. Indeed the American College of Cardiology have published guidelines suggesting that following an initial training process of 500 supervised ECG interpretations and formal assessment of competency, a minimum of 100 ECGs should be interpreted each year to maintain competency (207).

To suggest that a patient should be denied echocardiography on the basis of a normal ECG is clearly wrong. This is not to say that it cannot be used as a valuable screening tool. In a case where heart failure is considered as a possible diagnosis, a normal ECG should be used to cast significant doubt on the diagnosis and alternative diagnoses actively considered and investigated. An echocardiogram ordered where an alternative diagnosis is unlikely or excluded, would represent the most appropriate and effective use of resources.

5.3.3.2 B-type Natriuretic Peptides

In asymptomatic populations, screening for left ventricular systolic dysfunction by means of either plasma(208) or combined urinary and plasma NT-proBNP(209) followed by echocardiography in those with a positive result has been shown to be cost effective and accurate. In a symptomatic population this should allow more appropriate targeting of echocardiography and earlier identification of the correct investigation and treatment pathways.

NT-proBNP did not perform as well in the identification of left ventricular hypertrophy, a possible cause of diastolic dysfunction. It should also be remembered that other conditions such as atrial fibrillation and renal dysfunction will confound this and result in elevated BNP levels in the absence of left ventricular systolic dysfunction.

BNP is not yet universally available either in primary or secondary care. Where it is, however, it is able to function as an excellent screening tool, at least for systolic heart failure. The diagnostic confidence it provides beyond simple tools such as clinical examination and the ECG should streamline the process of differentiating heart failure from non-cardiac pathology.

5.3.3.3 The Use of Non-cardiac Investigations

When presented with a patient with suspected heart failure one must keep an open mind to other potential diagnoses and the use of screening tests to identify these. In terms of simple tests which do not require specialist knowledge to obtain or interpret, and are universally available, blood tests such as a full blood count should be regarded as a standard baseline investigation. Significant anaemia may result in dyspnoea in the absence of any other pathology. It may also exacerbate the symptoms of left ventricular dysfunction, valvular disease, pulmonary disease or obesity and is therefore an essential test even when other pathology is proven or strongly suspected.

Although more common in a hospital setting than in the community, hypoalbuminaemia may result in significant peripheral oedema which may be mistaken for heart failure. Also, given the insidious nature of hypothyroidism which may mimic many of the features of heart failure, including myxoedema, this should also be actively considered.

Although the chest x-ray is relatively insensitive for the detection of chronic obstructive pulmonary disease, it may nonetheless provide useful information. Increased lung volumes secondary to emphysema may be seen, or evidence of interstitial lung disease. A normal cardiothoracic ratio and no radiographic evidence of pulmonary venous congestion may provide evidence against a diagnosis of heart failure. Depending on local policy, open access echocardiography may not be available, in which case chest radiography might be an acceptable prelude to more definitive investigation. Even where open access echocardiography is available, a chest x-ray can usually be obtained far more rapidly.

5.4 Comprehensive Assessment of the Dyspnoeic Patient

The sequence of investigations employed here will identify any significant cardiorespiratory disease but is clearly not suitable for all patients. It is resource intensive, particularly if cardiopulmonary exercise testing is employed, and may represent over-investigation of patients whose initial tests suggest there is no pathology to be found.

5.4.1 Sequence of Investigations

Prior to referral to secondary care, simple readily available tests should be performed which would allow triage to the most appropriate specialty. A full blood count, ECG and chest x-ray would allow the identification of anaemia and significant cardiorespiratory disease. Depending on local policy it might be desirable to require some of these prior to referral to secondary care.

Once presented with the dyspnoeic patient in secondary care, a tiered approach to assessment should be adopted. The simplest, broadest and least invasive investigations are performed on the first tier, more advanced and focussed tests on the second tier, and the most specialised investigations on a third tier. This should be reserved for patients with objective evidence of pathology but inconclusive results from investigations on a lower tier.

Tier 1
Exercise tolerance test (treadmill); spirometry; BNP

Tier 2
Echocardiography; myocardial perfusion scintigraphy, radionuclide ventriculography

Tier 3
Cardiopulmonary exercise testing

A number of patients may progress beyond the scope of the investigations untaken in this thesis. Cardiac catheterisation may be appropriate for those in whom significant ischaemia has been detected, although cardiac CT now offers a non-invasive alternative with excellent negative predictive accuracy. Cardiac catheterisation may also be necessary to

formally document intracardiac pressures, of particular relevance in pericardial constriction where non-invasive measures may be inconclusive.

5.4.2 The Undiagnosable Patient

Ultimately, a proportion of patients will have no pathology identified to account for their symptoms. This is a situation encountered not infrequently in clinical practice and may result in frustration on the part of the patient. Where no abnormality has been discovered there are three possible explanations: the patient has been insufficiently investigated; the investigations used failed to identify an abnormality that was present; or there is no abnormality to be found. Inappropriate expectations on the part of the patient should be considered when there is no objective evidence of heart failure and investigations do not reveal any cardiorespiratory disease. There is a normal age related decline in cardiac and respiratory function and it may be difficult for some patients, particularly those who have led active lives, to accept this.

If one were to accept the argument that measurement of diastolic function is unnecessary by virtue of the fact that some evidence of abnormality can usually be found, these patients would all be labelled with diastolic heart failure. Desirable as it is to give a patient a diagnosis, no diagnosis is preferable to an incorrect diagnosis given the unnecessary treatment, worry and insurance implications this may have.

It is important to identify this category of patient efficiently in order to minimise the sequence of ultimately fruitless investigations. This will also minimise the impact on out patient clinics in secondary care. Unfortunately for primary care, the burden of long term follow up of these patients must fall to them. There must be a good relationship between primary and secondary care with regards these patients to them being repeatedly passed back and forth for reassessment.

5.5 Future Directions

Despite the theoretical promise of the assessment of diastolic function during stress, the radionuclide parameters have failed to produce conclusive results. There are several factors in this. The heterogeneity of the patient group, many of whom may not have diastolic dysfunction, is prime amongst these. The wide confidence intervals seen in the

normal ranges for most of the radionuclide parameters of diastolic function, even within the relatively large group used to define normal resting ranges (section 3.5.1), is a serious limitation to their use. Although the radiation dose from a radionuclide ventriculogram is modest, there is a drive towards minimising dose and substituting investigations that do not involve ionising radiation. Stress echocardiography, therefore, may be an avenue for future research for the assessment of diastolic function with either physiological or pharmacological stress.

Current tissue Doppler based indices of left ventricular filling pressures show only moderate degrees of correlation with invasive measurements, particularly where systolic function is preserved. They are based upon focal measurements of mitral annular velocity as a surrogate for what is a global measurement. Tissue Doppler is also limited by inaccuracies introduced by off-axis images. Using the advancing field of speckle tracking to derive the same parameters as tissue Doppler may improve the correlation with invasive measurements. More advanced techniques such as myocardial strain and strain rate imaging, particularly combined with stress, may be of use.

Although radionuclide ventriculography provides a good estimate of left ventricular volume, it is a planar technique. Activity from the posterior aspect of the heart is underrepresented due to attenuation artefact as it passes through the heart itself. It is possible to calculate left ventricular volumes and ejection fraction from myocardial perfusion SPECT images. As this is a tomographic technique, it should provide a more accurate estimate of left ventricular volume by delineating the endocardial border for the whole circumference of the ventricle. However, this technique is reliant on adequate myocardial perfusion to define this endocardial border. A sufficiently deep perfusion defect reduces the accuracy of border definition and, therefore, volume estimation. In principle, it is possible to acquire blood pool ventriculography data using SPECT imaging. This has the advantage of not being susceptible to myocardial perfusion defects but loses one of the key benefits of planar RNVG, its lack of reliance on spatial resolution. It is potentially more affected by problems with low count statistics and the necessary trade-off with temporal resolution than its planar counterpart. However, the advent of solid state detectors and resolution recovery algorithms mean that many of these weaknesses are mitigate. SPECT RNVG, therefore has the potential to be a useful research tool and the use of stress assessment can be explored further.

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Appendix 1 – The Reproducibility of Systolic and Diastolic Function by RNVG – additional data

1.1 Ejection Fraction

Table Ap1-1 – LV ejection fraction Intra-study/Intra-observer Reproducibility – 5-S sub-acquisition comparison (paired samples T test)

		Paired Differences – Sub-acquisitions 5 and S				
		Mean	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
				Lower	Upper	
24L	AMC	-0.11	2.39	-0.70	0.48	0.71
	AR	-0.23	2.50	-0.85	0.38	0.45
32L	AMC	0.06	2.39	-0.53	0.65	0.84
	AR	0.004	2.60	-0.64	0.64	0.99
24M	AMC	0.05	2.38	-0.54	0.64	0.87
	AR	-0.03	2.46	-0.64	0.57	0.92

Table Ap1-2 – LV ejection fraction Intra-study/Inter-observer reproducibility – 24 frame variable width (paired samples T-test)

		Paired Differences – 24 Frame List Mode				
		Mean	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
				Lower	Upper	
2	AMC-AR	-1.70	3.36	-2.53	-0.88	<0.001
3	AMC-AR	-1.61	3.28	-2.42	-0.81	<0.001
5	AMC-AR	-1.64	3.21	-2.43	-0.86	<0.001
S	AMC-AR	-1.77	3.20	-2.56	-0.98	<0.001
A	AMC-AR	-1.68	3.21	-2.47	-0.89	<0.001
2R	AMC-AR	-1.67	3.42	-2.52	-0.83	<0.001
3R	AMC-AR	-1.79	3.45	-2.64	-0.94	<0.001
5R	AMC-AR	-1.92	3.41	-2.76	-1.09	<0.001

Table Ap1-3 – Intra-observer/Inter-study reproducibility of LV ejection fraction (24 frame variable width) (paired samples T test)

		Paired Differences – 24 Frame List Mode				
		Mean	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
				Lower	Upper	
AMC	2-2R	-0.87	5.18	-2.14	0.40	0.176
	3-3R	-0.66	5.19	-1.93	0.62	0.308
	5-5R	-0.27	4.71	-1.42	0.89	0.649
AR	2-2R	-0.84	5.35	-2.16	0.47	0.206
	3-3R	-0.83	5.50	-2.18	0.52	0.225
	5-5R	-0.55	4.98	-1.77	0.68	0.377

Table Ap1-4 – Inter-observer/inter-study reproducibility of LV ejection fraction (paired samples T-test)

		Paired Differences				
		Mean	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
				Lower	Upper	
24L	AMC-AR – 2-2R	-2.55	6.06	-4.04	-1.06	0.001
	AMC-AR – 3-3R	-2.44	6.47	-4.03	-0.85	0.003
	AMC-AR – 5-5R	-2.19	5.89	-3.64	-0.74	0.004
32L	AMC-AR – 2-2R	-2.46	5.94	-3.92	-1.0	0.001
	AMC-AR – 3-3R	-2.30	6.13	-3.81	-0.79	0.003
	AMC-AR – 5-5R	-2.19	5.93	-3.65	-0.73	0.004
24M	AMC-AR – 2-2R	-2.32	5.84	-3.75	-0.88	0.002
	AMC-AR – 3-3R	-2.19	6.34	-3.75	-0.63	0.007
	AMC-AR – 5-5R	-2.12	5.78	-3.54	-0.70	0.004

Table Ap1-5 – Inter-observer/inter-study reproducibility of LV ejection fraction (Pearson correlation coefficient)

		24L	32L	24M
AMC-AR	2-2R	0.95	0.95	0.95
	3-3R	0.94	0.95	0.94
	5-5R	0.95	0.95	0.95

Table Ap1-6 – Effect of reducing acquisition time to 2 or 3 minutes versus 10 minutes on left ventricular ejection fraction (paired samples T-test)

		Paired Differences				
		Mean	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
				Lower	Upper	
24L	AMC – 2-A	0.31	1.94	-0.17	0.79	0.197
	AMC – 3-A	0.21	1.36	-0.12	0.55	0.208
	AR – 2-A	0.33	2.01	-0.16	0.83	0.183
	AR – 3-A	0.14	1.43	-0.21	0.50	0.418
32L	AMC – 2-A	1.06	2.12	0.54	1.58	<0.001
	AMC – 3-A	0.63	1.62	0.23	1.03	0.002
	AMC – 5-A	0.42	1.12	0.14	0.69	0.004
	AR – 2-A	1.07	2.11	0.55	1.59	<0.001
	AR – 3-A	0.57	1.60	0.18	0.97	0.005
	AR – 5-A	0.38	1.14	0.10	0.65	0.009
24M	AMC – 2-A	0.56	2.23	0.02	1.11	0.044
	AMC – 3-A	0.26	1.59	-0.13	0.65	0.194
	AR – 2-A	0.50	2.20	-0.04	1.04	0.069
	AR – 3-A	0.20	1.46	-0.16	0.56	0.278

1.2 First Third Fractional Filling

Table Ap1-7 – Left ventricular first third fractional filling Intra-study/Intra-observer reproducibility (paired samples T test)

		Paired Differences – Sub-acquisitions 5 and S				
		Mean	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
				Lower	Upper	
24L	AMC	-1.26	13.38	-4.55	2.03	0.447
	AR	0.17	11.76	-2.72	3.07	0.904
32L	AMC	-2.03	15.28	-5.79	1.72	0.284
	AR	-2.05	13.15	-5.28	1.19	0.211
24M	AMC	0.09	13.53	-3.24	3.41	0.958
	AR	0.67	14.45	-2.88	4.22	0.708

Table Ap1-8 – Left ventricular first third fractional filling intra-study/inter-observer reproducibility – Pearson correlation coefficient

		24L	32L	24M
2	AMC-AR	0.934	0.944	0.960
3	AMC-AR	0.968	0.940	0.916
5	AMC-AR	0.967	0.956	0.980
S	AMC-AR	0.983	0.961	0.928
A	AMC-AR	0.956	0.981	0.984
2R	AMC-AR	0.757	0.754	0.943
3R	AMC-AR	0.966	0.944	0.964
5R	AMC-AR	0.955	0.934	0.959

Table Ap1-9 – Intra-study/Inter-observer reproducibility of left ventricular first third fractional filling (24 frame variable width) – Paired samples T-test

		Paired Differences – 24 Frame List Mode				
		Mean	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
				Lower	Upper	
2	AMC-AR	-0.34	6.27	-1.89	1.20	0.657
3	AMC-AR	-0.24	4.47	-1.34	0.86	0.664
5	AMC-AR	-1.09	4.56	-2.21	0.03	0.056
S	AMC-AR	0.34	3.29	-0.47	1.15	0.399
A	AMC-AR	-0.02	5.16	-1.29	1.24	0.970
2R	AMC-AR	-0.06	11.20	-2.81	2.70	0.966
3R	AMC-AR	0.84	4.14	-0.18	1.86	0.104
5R	AMC-AR	-0.23	5.09	-1.48	1.02	0.716

Table Ap1-10 – Intra-study/Inter-observer reproducibility of left ventricular first third fractional filling (32 frame variable width) – Paired samples T-test

		Paired Differences – 32 Frame List Mode				
		Mean	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
				Lower	Upper	
2	AMC-AR	1.24	5.97	-0.22	2.71	0.096
3	AMC-AR	-0.20	5.82	-1.63	1.24	0.786
5	AMC-AR	-0.006	5.27	-1.30	1.29	0.992
S	AMC-AR	-0.02	5.27	-1.32	1.28	0.975
A	AMC-AR	0.25	3.58	-0.63	1.13	0.572
2R	AMC-AR	-0.71	12.48	-3.78	2.35	0.644
3R	AMC-AR	-0.02	5.87	-1.46	1.42	0.976
5R	AMC-AR	0.22	6.19	-1.30	1.74	0.774

Table Ap1-11 – Intra-study/Inter-observer reproducibility of left ventricular first third fractional filling (24 frame fixed width) – Paired samples T-test

		Paired Differences – 24 Frame Fixed Width				
		Mean	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
				Lower	Upper	
2	AMC-AR	-0.53	5.72	-1.94	0.87	0.452
3	AMC-AR	0.27	7.48	-1.57	2.10	0.774
5	AMC-AR	-0.49	3.58	-1.37	0.39	0.271
S	AMC-AR	0.09	6.91	-1.60	1.79	0.912
A	AMC-AR	0.26	3.05	-0.49	1.01	0.497
2R	AMC-AR	-0.63	5.73	-2.04	0.78	0.374
3R	AMC-AR	0.63	4.52	-0.48	1.74	0.260
5R	AMC-AR	0.40	4.83	-0.78	1.59	0.499

Table Ap1-12 – Intra-observer/Inter-study reproducibility of left ventricular first third fractional filling (24 frame variable width) – Pearson correlation coefficient

		24L	32L	24M
AMC	2-2R	0.55	0.44	0.61
	3-3R	0.65	0.57	0.70
	5-5R	0.67	0.58	0.62
AR	2-2R	0.42	0.31	0.53
	3-3R	0.59	0.50	0.70
	5-5R	0.67	0.57	0.64

Table Ap1-13 – Intra-observer/Inter-study reproducibility of left ventricular first third fractional filling (24 frame variable width) (paired samples T test)

		Paired Differences – 24 Frame List Mode				
				95% Confidence Interval of the Difference		Sig. (2-tailed)
		Mean	Std. Deviation	Lower	Upper	
AMC	2-2R	-2.28	16.15	-6.25	1.69	0.255
	3-3R	-1.21	13.70	-4.58	2.15	0.474
	5-5R	-0.07	14.04	-3.52	3.38	0.968
	S-5R	1.19	12.21	-1.81	4.19	0.431
AR	2-2R	-2.00	17.57	-6.32	2.32	0.359
	3-3R	-0.13	15.35	-3.91	3.64	0.944
	5-5R	0.79	14.29	-2.72	4.30	0.654
	S-5R	0.62	12.83	-2.54	3.77	0.697

Table Ap1-14 – Intra-observer/Inter-study reproducibility of left ventricular first third fractional filling (24 frame fixed width) (paired samples t test)

		Paired Differences – 24 Frame Fixed Width Mode				
				95% Confidence Interval of the Difference		Sig. (2-tailed)
		Mean	Std. Deviation	Lower	Upper	
AMC	2-2R	0.53	16.63	-3.56	4.62	0.796
	3-3R	0.16	13.62	-3.19	3.51	0.923
	5-5R	-0.74	15.05	-4.44	2.96	0.690
	S-5R	-0.83	14.01	-4.27	2.61	0.632
AR	2-2R	0.43	18.15	-4.03	4.90	0.847
	3-3R	0.53	13.89	-2.89	3.94	0.759
	5-5R	0.15	14.79	-3.49	3.79	0.935
	S-5R	-0.52	14.14	-4.00	2.96	0.766

Table Ap1-15 – Intra-observer/Inter-study reproducibility of left ventricular first third fractional filling (32 frame variable width) (paired samples T test)

		Paired Differences – 32 Frame List Mode				
				95% Confidence Interval of the Difference		Sig. (2-tailed)
		Mean	Std. Deviation	Lower	Upper	
AMC	2-2R	1.49	19.20	-3.23	6.21	0.530
	3-3R	-2.39	15.32	-6.16	1.38	0.210
	5-5R	-0.24	15.28	-3.99	3.52	0.901
	S-5R	1.80	15.49	-2.01	5.60	0.349
AR	2-2R	-0.46	20.55	-5.51	4.59	0.856
	3-3R	-2.22	17.35	-6.48	2.05	0.303
	5-5R	-0.008	16.25	-4.00	3.99	0.996
	S-5R	2.04	15.71	-1.82	5.90	0.296

Table Ap1-16 – Effect of reducing acquisition time to 2 or 3 minutes versus 10 minutes on left ventricular first third fractional filling (paired samples T-test)

		Paired Differences – 2 or 3 versus 10 minutes				
		Mean	Std. Deviation	95% Confidence Interval of the Difference		Sig (2-tailed)
				Lower	Upper	
24L	AMC – 2-A	-0.23	10.84	-2.89	2.44	0.866
	AMC – 3-A	-0.19	9.93	-2.63	2.25	0.876
	AR – 2-A	0.09	12.92	-3.08	3.27	0.953
	AR – 3-A	0.02	9.72	-2.37	2.42	0.983
32L	AMC – 2-A	2.36	12.67	-0.75	5.47	0.135
	AMC – 3-A	-0.24	11.06	-2.96	2.48	0.861
	AR – 2-A	1.37	12.01	-1.58	4.32	0.358
	AR – 3-A	0.21	10.17	-2.29	2.71	0.869
24M	AMC – 2-A	0.45	11.33	-2.33	3.24	0.747
	AMC – 3-A	-0.73	11.07	-3.45	1.99	0.595
	AR – 2-A	1.24	12.07	-1.73	4.21	0.407
	AR – 3-A	-0.74	10.76	-3.38	1.91	0.580

Table Ap1-17 – Effect of reducing acquisition time to 2 or 3 minutes versus 10 minutes on left ventricular first third fractional filling – Pearson correlation coefficient

		24L	32L	24M
AMC	2-A	0.81	0.75	0.83
	3-A	0.83	0.80	0.80
AR	2-A	0.72	0.78	0.80
	3-A	0.85	0.83	0.82

1.3 Peak Filling Rate (3 point regression)

Table Ap1-18 – Left Ventricular Peak Filling Rate Intra-study/Intra-observer Reproducibility – 5 and S sub-acquisition comparison (paired samples T test)

		Paired Differences – Sub-acquisitions 5 and S				
		Mean	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
				Lower	Upper	
24L	AMC	-4.01	26.67	-10.56	2.55	0.226
	AR	-3.96	28.83	-11.05	3.12	0.268
32L	AMC	-8.07	41.26	-18.22	2.07	0.117
	AR	-8.38	44.60	-19.35	2.58	0.132
24M	AMC	-4.63	26.30	-11.10	1.83	0.157
	AR	-4.98	29.22	-12.16	2.21	0.171

Figure Ap1-1 – Bland-Altman analysis of left ventricular Peak Filling Rate intra-study/intra-observer reproducibility (24 frame list mode AMC 5-S acquisitions)

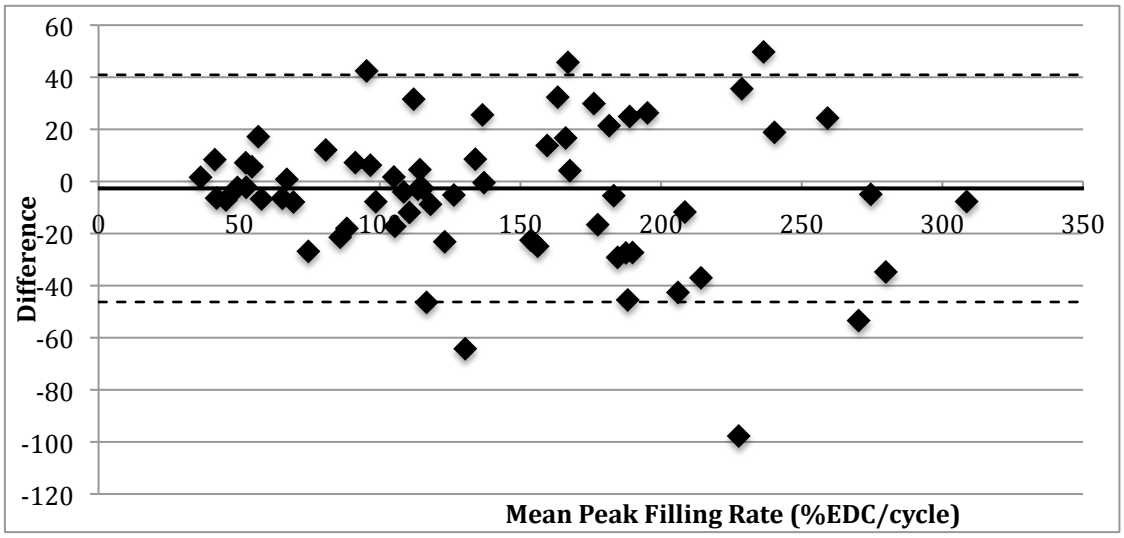

Table Ap1-19 – LV Peak Filling Rate (3 point regression) Intra-study/Inter-observer Reproducibility – 24 frame variable width (paired samples T-test)

		Paired Differences – 24 Frame Variable Width				
		Mean	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
				Lower	Upper	
2	AMC-AR	-5.59	13.90	-9.01	-2.17	0.002
3	AMC-AR	-5.20	12.62	-8.30	-2.10	0.001
5	AMC-AR	-5.92	12.99	-9.11	-2.73	<0.001
S	AMC-AR	-5.88	16.02	-9.81	-1.94	0.004
A	AMC-AR	-5.70	13.17	-8.94	-2.46	0.001
2R	AMC-AR	-7.95	16.85	-12.09	-3.81	<0.001
3R	AMC-AR	-8.03	15.83	-11.92	-4.14	<0.001
5R	AMC-AR	-7.14	14.96	-10.82	-3.46	<0.001

Figure Ap1-2 – Bland-Altman plot of Peak Filling Rate (3 point regression) – AMC-AR 24 frame variable width 10 minute acquisition

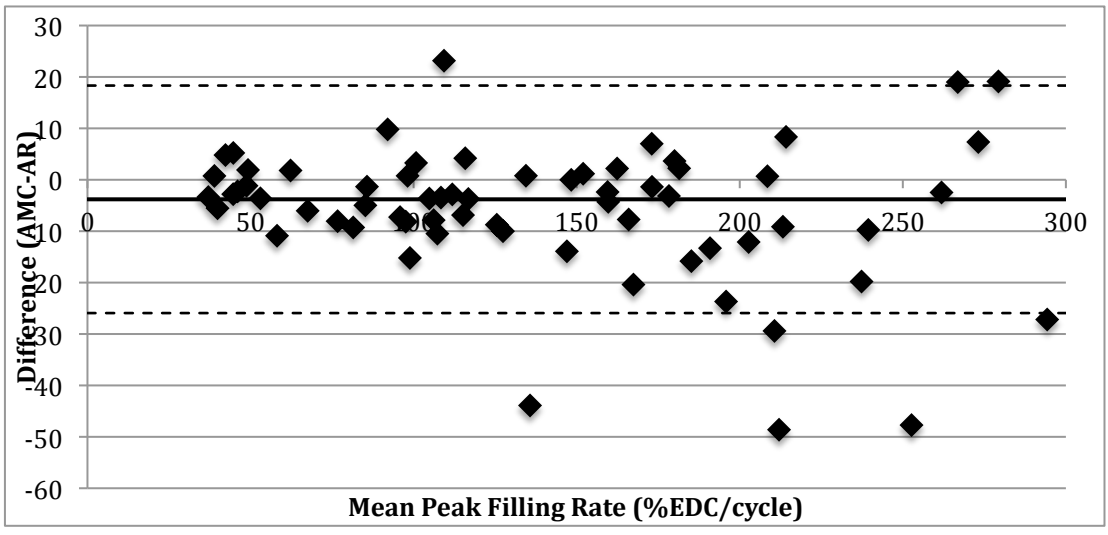

Table Ap1-20 – Intra-observer/Inter-study LV Peak Filling Rate (3 point regression) reproducibility – Pearson correlation coefficient

		24L	32L	24M
AMC	2-2R	0.817	0.722	0.850
	3-3R	0.870	0.796	0.827
	5-5R	0.875	0.807	0.880
AR	2-2R	0.834	0.795	0.868
	3-3R	0.885	0.796	0.838
	5-5R	0.891	0.803	0.891

Table Ap1-21 – LV Peak Filling Rate (3 point regression) Intra-observer/Inter-study Reproducibility – 24 frame variable width (paired samples T-test)

		Paired Differences – 24 Frame Variable Width				
		Mean	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
				Lower	Upper	
AMC	2-2R	-1.95	41.03	-12.03	8.14	0.701
	3-3R	3.24	34.73	-5.30	11.77	0.452
	5-5R	1.10	33.15	-7.05	9.25	0.788
AR	2-2R	-4.30	42.09	-14.65	6.04	0.409
	3-3R	0.41	34.54	-8.08	8.90	0.924
	5-5R	-0.12	32.82	-8.19	7.95	0.977

Table Ap1-22 – Effect of reducing acquisition time to 2 or 3 minutes versus 10 minutes on LV Peak Filling Rate (3 point regression) – Pearson correlation coefficient

		24L	32L	24M
AMC	2-A	0.93	0.87	0.93
	3-A	0.96	0.91	0.95
AR	2-A	0.94	0.89	0.93
	3-A	0.96	0.90	0.95

Table Ap1-23 – Effect of reducing acquisition time to 2 or 3 minutes versus 10 minutes on LV Peak Filling Rate (3 point regression) (paired samples T-test)

		Paired Differences – 2 or 3 minutes vs 10				
		Mean	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
				Lower	Upper	
24L	AMC – 2-A	8.77	26.10	2.36	15.19	0.008
	AMC – 3-A	5.76	19.53	0.96	10.56	0.020
	AR – 2-A	8.66	25.51	2.39	14.93	0.008
	AR – 3-A	5.25	19.83	0.38	10.13	0.035
32L	AMC – 2-A	15.51	36.57	6.52	24.50	0.001
	AMC – 3-A	10.46	31.00	2.84	18.08	0.008
	AR – 2-A	15.88	36.26	6.97	24.79	0.001
	AR – 3-A	11.34	34.44	2.87	19.81	0.009
24M	AMC – 2-A	13.24	30.94	5.64	20.85	0.001
	AMC – 3-A	4.38	22.26	-1.09	9.86	0.114
	AR – 2-A	14.41	33.37	6.21	22.61	0.001
	AR – 3-A	4.32	23.52	-1.46	10.11	0.140

1.4 Time to Peak Filling

Table Ap1-24 – LV Time to Peak Filling (RR) Intra-study/Intra-observer Reproducibility (paired samples T-test)

		Paired Differences – 5 and S sub-acquisitions				
		Mean	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
				Lower	Upper	
24L	AMC	0.005	0.10	-0.012	0.029	0.702
	AR	0.005	0.09	-0.018	0.027	0.689
32L	AMC	0.024	0.19	-0.023	0.071	0.313
	AR	0.025	0.18	-0.019	0.069	0.257
24M	AMC	0.003	0.13	-0.029	0.036	0.839
	AR	-0.0004	0.14	-0.034	0.033	0.978

Table Ap1-25 – LV Time to Peak Filling (RR) Intra-study/Inter-observer Pearson correlation coefficient

		24L	32L	24M
2	AMC-AR	0.94	0.84	0.95
3	AMC-AR	0.93	0.95	0.96
5	AMC-AR	0.95	0.86	0.93
S	AMC-AR	0.97	0.43	0.31
A	AMC-AR	0.79	0.94	0.88
2R	AMC-AR	0.87	0.81	0.87
3R	AMC-AR	0.83	0.80	0.83
5R	AMC-AR	0.86	0.90	0.87

Table Ap1-26 – LV Time to Peak Filling (RR) Intra-study/Inter-observer Reproducibility - 24 frame variable width (paired samples T-test)

		Paired Differences -24 Frame Variable Width				
		Mean	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
				Lower	Upper	
2	AMC-AR	-0.003	0.037	-0.012	0.006	0.537
3	AMC-AR	0.0009	0.036	-0.008	0.010	0.833
5	AMC-AR	0.004	0.030	-0.003	0.012	0.228
S	AMC-AR	0.004	0.028	-0.003	0.011	0.213
A	AMC-AR	0.004	0.059	-0.010	0.019	0.592
2R	AMC-AR	-0.008	0.062	-0.023	0.008	0.326
3R	AMC-AR	-0.020	0.068	-0.036	-0.003	0.019
5R	AMC-AR	-0.005	0.064	-0.021	0.011	0.551

Table Ap1-27 – Intra-observer/Inter-study LV Time to Peak Filling (RR) reproducibility - Pearson correlation coefficient

		24L	32L	24M
AMC	2-2R	0.35	0.28	0.43
	3-3R	0.42	0.39	0.36
	5-5R	0.42	0.39	0.25
AR	2-2R	0.24	0.14	0.31
	3-3R	0.34	0.34	0.46
	5-5R	0.54	0.53	0.43

Table Ap1-28 – LV Time to Peak Filling (RR) Intra-observer/Inter-study Reproducibility - 24 frame variable width (paired samples T-test)

		Paired Differences – 24 Frame Variable Width				
		Mean	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
				Lower	Upper	
AMC	2-2R	0.017	0.12	-0.012	0.046	0.254
	3-3R	0.008	0.11	-0.020	0.035	0.581
	5-5R	-0.010	0.11	-0.038	0.018	0.468
AR	2-3R	0.012	0.14	-0.023	0.048	0.498
	3-3R	-0.014	0.13	-0.045	0.018	0.390
	5-5R	-0.019	0.11	-0.046	0.008	0.155

Table Ap1-29 – Effect of reducing acquisition time to 2 or 3 minutes versus 10 minutes on LV Time to Peak Filling (RR) (paired samples T-test)

		Paired Differences – 2 or 3 minutes versus 10				
		Mean	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
				Lower	Upper	
24L	AMC – 2-A	0.013	0.10	-0.012	0.038	0.312
	AMC – 3-A	0.002	0.09	-0.021	0.024	0.888
	AR – 2-A	0.020	0.10	-0.005	0.044	0.112
	AR – 3-A	0.005	0.09	-0.018	0.028	0.693
32L	AMC – 2-A	-0.008	0.13	-0.040	0.024	0.618
	AMC – 3-A	0.001	0.15	-0.037	0.039	0.955
	AR – 2-A	-0.014	0.13	-0.047	0.018	0.383
	AR – 3-A	0.010	0.15	-0.026	0.047	0.574
24M	AMC – 2-A	0.017	0.12	-0.012	0.046	0.251
	AMC – 3-A	0.020	0.12	-0.010	0.048	0.173
	AR – 2-A	0.010	0.14	-0.023	0.044	0.542
	AR – 3-A	0.010	0.12	-0.020	0.040	0.502

Table Ap1-30 – Effect of reducing acquisition time to 2 or 3 minutes versus 10 minutes on LV Time to Peak Filling (RR) – Pearson correlation coefficient

		24L	32L	24M
AMC	2-A	0.46	0.55	0.45
	3-A	0.54	0.39	0.51
AR	2-A	0.51	0.50	0.37
	3-A	0.48	0.48	0.51

1.5 Peak Emptying Rate (RR)

Table Ap1-31 – LV Peak Emptying Rate (RR) Intra-study/Intra-observer Reproducibility (paired samples T-test)

		Paired Differences – 5 and S sub-acquisitions				
		Mean	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
				Lower	Upper	
24L	AMC	-3.14	21.63	-8.46	2.17	0.242
	AR	-2.79	23.46	-8.55	2.98	0.338
32L	AMC	-5.36	29.22	-12.55	1.82	0.141
	AR	-5.82	33.66	-14.10	2.45	0.165
24M	AMC	-0.23	19.24	-4.96	4.50	0.924
	AR	-0.34	20.38	-5.35	4.67	0.893

Table Ap1-32 – LV Peak Emptying Rate (RR) Intra-study/Inter-observer Pearson correlation coefficient

		24L	32L	24M
2	AMC-AR	0.978	0.971	0.981
3	AMC-AR	0.977	0.976	0.978
5	AMC-AR	0.980	0.977	0.980
S	AMC-AR	0.976	0.970	0.977
A	AMC-AR	0.980	0.976	0.980
2R	AMC-AR	0.975	0.976	0.978
3R	AMC-AR	0.975	0.975	0.978
5R	AMC-AR	0.980	0.980	0.981

Table Ap1-33 – LV Peak Emptying Rate (RR) Intra-study/Inter-observer Reproducibility - 24 frame variable width (paired samples T-test)

		Paired Differences – 24 Frame Variable Width				
		Mean	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
				Lower	Upper	
2	AMC-AR	-9.01	18.20	-13.48	-4.53	<0.001
3	AMC-AR	-9.13	18.39	-13.66	-4.61	<0.001
5	AMC-AR	-9.41	16.88	-13.56	-5.26	<0.001
S	AMC-AR	-9.05	17.82	-13.43	-4.67	<0.001
A	AMC-AR	-9.63	16.38	-13.65	-5.60	<0.001
2R	AMC-AR	-9.47	21.19	-14.68	-4.26	0.001
3R	AMC-AR	-10.03	21.07	-15.21	-4.85	<0.001
5R	AMC-AR	-10.13	19.22	-14.85	-5.40	<0.001

Table Ap1-34 – Intra-observer/Inter-study LV Peak Emptying Rate (RR) reproducibility - Pearson correlation coefficient

		24L	32L	24M
AMC	2-2R	0.87	0.84	0.88
	3-3R	0.87	0.84	0.89
	5-5R	0.92	0.88	0.91
AR	2-2R	0.87	0.86	0.88
	3-3R	0.87	0.84	0.90
	5-5R	0.92	0.88	0.91

Table Ap1-35 – LV Peak Emptying Rate (RR) Intra-observer/Inter-study Reproducibility - 24 frame variable width (paired samples T-test)

		Paired Differences – 24 Frame Variable Width				
		Mean	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
				Lower	Upper	
AMC	2-2R	-11.34	41.00	-21.42	-1.27	0.028
	3-3R	-11.77	41.71	-22.02	-1.51	0.025
	5-5R	-8.25	33.56	-16.51	-0.004	0.050
AR	2-2R	-11.81	44.84	-22.83	-0.79	0.036
	3-3R	-12.66	46.12	-24.00	-1.32	0.029
	5-5R	-8.97	36.66	-17.98	0.04	0.051

Table Ap1-36 – LV Peak Emptying Rate (RR) Intra-observer/Inter-study Reproducibility - 24 frame fixed width (paired samples T-test)

		Paired Differences – 24 Frame Fixed Width				
		Mean	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
				Lower	Upper	
AMC	2-2R	-9.98	40.19	-19.86	-0.10	0.048
	3-3R	-11.12	38.17	-20.50	-1.73	0.021
	5-5R	-9.19	34.43	-17.65	-0.72	0.034
AR	2-3R	-11.56	43.76	-22.31	-0.80	0.036
	3-3R	-12.21	39.35	-21.88	-2.54	0.014
	5-5R	-11.18	37.17	-20.32	-2.04	0.017

Table Ap1-37 – LV Peak Emptying Rate (RR) Intra-observer/Inter-study Reproducibility - 32 frame variable width (paired samples T-test)

		Paired Differences – 32 Frame Variable Width				
		Mean	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
				Lower	Upper	
AMC	2-2R	-22.76	52.87	-35.75	-9.76	0.001
	3-3R	-22.78	53.49	-35.93	-9.63	0.001
	5-5R	-16.25	44.23	-27.12	-5.37	0.004
AR	2-2R	-23.43	54.56	-36.85	-10.02	0.001
	2-2R	-23.78	57.04	-37.80	-9.75	0.001
	3-3R	-17.85	48.43	-29.75	-5.94	0.004

Table Ap1-38 – Effect of reducing acquisition time to 2 or 3 minutes versus 10 minutes on LV Peak Emptying Rate (RR) – Pearson correlation coefficient

		24L	32L	24M
AMC	2-A	0.97	0.96	0.97
	3-A	0.98	0.97	0.98
AR	2-A	0.97	0.96	0.97
	3-A	0.98	0.96	0.98

Table Ap1-39 – Effect of reducing acquisition time to 2 or 3 minutes versus 10 minutes on LV Peak Emptying Rate (RR) (paired samples T-test)

		Paired Differences – 2 or 3 versus 10 minutes				
		Mean	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
				Lower	Upper	
24L	AMC – 2-A	9.22	17.07	5.02	13.41	<0.001
	AMC – 3-A	5.36	13.90	1.94	8.78	0.003
	AR – 2-A	8.60	18.27	4.11	13.09	<0.001
	AR – 3-A	4.87	16.07	0.92	8.82	0.016
32L	AMC – 2-A	21.30	24.07	15.38	27.22	<0.001
	AMC – 3-A	13.06	20.06	8.13	17.99	<0.001
	AR – 2-A	21.47	26.02	15.07	27.86	<0.001
	AR – 3-A	12.77	26.67	6.22	19.33	<0.001
24M	AMC – 2-A	11.46	19.61	6.64	16.28	<0.001
	AMC – 3-A	6.30	14.47	2.74	9.86	0.001
	AR – 2-A	10.76	20.94	5.61	15.91	<0.001
	AR – 3-A	6.10	16.25	2.11	10.10	0.003

Table Ap1-40 – Effect of reducing acquisition time to 2 or 3 minutes versus 5 minutes on LV Peak Emptying Rate (RR) (paired samples T-test)

		Paired Differences – 2 or 3 versus 5 minutes				
		Mean	Std. Deviation	95% Confidence Interval of the Difference		Sig. (2-tailed)
				Lower	Upper	
24L	AMC – 2-5	6.72	18.82	2.10	11.35	0.005
	AMC – 3-5	2.87	12.81	-0.28	6.02	0.074
	AR – 2-5	6.32	19.87	1.43	11.20	0.012
	AR – 3-5	2.59	13.62	-0.76	5.94	0.127
32L	AMC – 2-5	15.26	27.60	8.47	22.04	<0.001
	AMC – 3-5	7.01	19.02	2.34	11.69	0.004
	AR – 2-5	16.04	28.15	9.12	22.96	<0.001
	AR – 3-5	7.35	22.28	1.87	12.83	0.009
24M	AMC – 2-5	8.32	20.31	3.33	13.31	0.001
	AMC – 3-5	3.16	14.10	-0.30	6.63	0.073
	AR – 2-5	7.80	21.96	2.40	13.20	0.005
	AR – 3-5	3.14	14.67	-0.46	6.75	0.087