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**Testosterone replacement therapy and exercise training in males with low
testosterone status and heart failure.**

Martin Stout

A Thesis Submitted in Partial Fulfilment of the Requirements of Sheffield Hallam
University for the Degree of Doctor of Philosophy.

August 2013.

Collaborating Organisations: Sheffield Teaching Hospitals NHS Foundation Trust and
University Hospital South Manchester NHS Foundation Trust.

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Acknowledgements.

I am grateful to Professors John Saxton and Kevin Channer for their continued help, support, experience and knowledge throughout the completion and write-up of this thesis.

In addition I would like to thank those who assisted during completion of the research. These include Dr Emma Scott and Anouska McConnell for the administration of trial medications and collection of blood samples. Dr Garry Tew and Dr Liam Bourke for the assistance in supervising patient exercise sessions and in blood collection. Sue Kesterton and Dr Helen Crank for assisting in supervising exercise sessions and in recruiting patients onto the study. Helen Lloyd and Alan Ruddock for their technical assistance when collecting data. These people played an important part in the smooth running and efficiency of this research.

I am also grateful to Helen Bowler, Alexis Bedford and Lance Burn at Sheffield Teaching Hospitals NHS Foundation Trust together with Teresa Coppinger, Carolyn Waywell and Diane Daniel from the Heart Failure Clinic at UHSM for their expertise and help in completing this project.

I am also indebted to Keith Pearce at the North West Heart Centre, University Hospital of South Manchester NHS Foundation Trust for allowing time to ensure this work is completed – often at short notice.

I thank all patients who agreed to participate in this research as without you it would not have been completed. I also thank their families who provided them with support and encouragement throughout the intervention period.

I would finally like to thank my wife Jess and daughter Maddie for their patience and support during the completion of this write-up. I would also like to thank my parents for their continued help and support.

Without you all, it would have been impossible to complete this work. Once again, thanks.

STATEMENT OF ORIGINALITY.

Section 1.

The acute and chronic use of testosterone replacement therapy in males with low testosterone status and chronic HF has been described in studies by members of our investigatory team (Malkin et al, 2006 and Pugh et al, 2003). More recently, researchers in Italy have conducted similar research (Caminiti et al, 2009). Although there are positive results regarding the use of testosterone in males with chronic HF, there is limited information on the physiological and biochemical consequences associated with decreased testosterone in this patient group. There are only two small correlation studies which have demonstrated moderate relationships between testosterone concentration, exercise capacity and right ventricular function in males with HF (Bocchi et al, 2008, Jankowska et al, 2009). This study will be the first direct comparison of key physiological and psychological outcomes in males with moderate to severe HF and 'low' or 'normal' levels of testosterone. For this research, the study design, regional ethical approval, research and development approval were undertaken by myself. Also 80% of the total data collection was undertaken by myself with assistance from Mr Keith Pearce and the HF specialist nurses at University Hospital South Manchester. Reproducibility of the echocardiography data was undertaken by Mr Keith Pearce and myself. Statistical analysis was performed by myself with guidance and support from Miss Sigrid Whiteside (Medical Statistician at University Hospital South Manchester).

Section 2.

The acute and chronic use of testosterone replacement therapy in males with low testosterone status and chronic HF has been described in studies by members of our investigatory team (Malkin et al, 2006 and Pugh et al, 2003). More recently, researchers in Italy have conducted similar research (Caminiti et al, 2009). However, there is no research on the combined effects of exercise training and testosterone therapy in the chronic HF population. This randomised controlled clinical trial aimed to study the effects of combined exercise training and testosterone treatment on exercise capacity, physiological function and quality of life in males with low level testosterone. During this study, 90% of all data collection and supervision of the 12 week intervention was undertaken by myself. I received assistance from Miss Helen Lloyd, Mr Alan Ruddock, Professors Kevin Channer and John Saxton during the initial baseline data collection visit. Dr Emma Scott and Miss Anouska Mc Connell provided all blinded trial medication injections. Mrs Sue Kesterton, Dr Garry Tew, Dr Liam Bourke and Dr Helen Crank all provided cover for exercise training sessions in my absence. All statistical analysis was performed by myself (blinded to group allocation) and Dr Helen Doll (Medical Statistician at the University of East Anglia) under the guidance of Professor John Saxton.

With the exception of any statements to the contrary, all the data presented in this report are the result of my own efforts. In addition, no parts of this report have been copied from other sources. I understand that any evidence of plagiarism and/or the use of unacknowledged third party data will be dealt with as a serious matter.

Signed.....

Date..... 1/3/14

Abbreviations.

17 β HSD	17 beta hydroxysteroid dehydrogenase
2D	two dimensional
3D	three dimensional
ACE	angiotensin converting enzyme
ADAM	Androgen deficiency in the ageing male questionnaire
ADP	adenosine diphosphate
AMP	adenosine monophosphate
ANCOVA	analysis of covariance
ANOVA	analysis of variance
AR	androgen receptor
A _t	anaerobic threshold
ATP	adenosine triphosphate
AVR	aortic valve replacement
BDI	Beck Depression Inventory
BMI	body mass index
BNP	brain natriuretic peptide
BP	blood pressure
Ca ²⁺	calcium ions
CABG	coronary artery bypass graft
CAD	coronary artery disease
CHAMPS Questionnaire	Community Health Activities Model Programme for Seniors Questionnaire
CHF	chronic heart failure
Cm/s	centimetres per second

CO ₂	carbon dioxide
CoA	co-enzyme A
COX-1	cyclo-oxygenase-1
CRT	cardiac resynchronisation therapy
c-Src	proto-oncogene tyrosine protein kinase
CV	cardiovascular
CVA	cerebrovascular accident
DNA	deoxyribonucleic acid
DSE	dobutamine stress echocardiography
ED	endothelial dysfunction
EDTA	ethylenediaminetetraacetic acid
eNOS	endothelial nitric oxide synthase
ERK-2	mitogen activated protein kinase-2
ET	exercise training
FADH ₂	flavin adenine dinucleotide dehydrate
Fe CO ₂	fraction of expired carbon dioxide
Fe O ₂	fraction of expired oxygen
Fi O ₂	fraction of inspired oxygen
FMD	flow mediated dilatation
FT	free testosterone
GLUT	glucose transporter
GM-CSF	granulocyte macrophage colony stimulating factor
GP	general practitioner
GTP	guanosine triphosphate
hCG	human choriogonadotropin
HF	heart failure

HPRS	Hamilton Psychiatric Rating Scale for depression
HR	heart rate
ICD	implantable cardioverter defibrillator
IGF-1	insulin like growth factor -1
IHD	ischaemic heart disease
IL	interleukin
ISWT	incremental shuttle walk test
LAEF%	left atrial active emptying fraction
LA-EI	left atrial expansion index
LA-PEF%	left atrial passive emptying fraction
LDL	low density lipoprotein
LNCaP	androgen sensitive human prostate adenocarcinoma cell line
LT	low testosterone
LV	left ventricular
LVAD	left ventricular assist device
LVEF%	left ventricular ejection fraction
m	metres
MCP-1	monocyte chemotactic protein-1
MET	metabolic equivalent
MHz	mega Hertz
MI	myocardial infarction
min	minutes
MLHFQ	Minnesota living with heart failure questionnaire
MRI	magnetic resonance imaging
MRNA	messenger ribonucleic acid
NADH	nicotinamide adenine dinucleotide hydroxide

NICE	National Institute for Clinical Excellence
NIRS	near infrared spectroscopy
nmol/L	nanomols per Litre
NT	normal testosterone
NT pro-BNP	N terminal brain natriuretic peptide
NYHA	New York Heart Association
PCG-1 α	PPAR α co-activator 1
PCWP	pulmonary capillary wedge pressure
PDH	pyruvate dehydrogenase
PDK-4	pyruvate dehydrogenase kinase isoenzyme-4
PET	positive emission tomography
PPAR	peroxisome proliferator activated receptor
PSA	prostate specific antigen
RAAS	rennin-angiotensin-aldosterone system
Raf-1	murine leukaemia viral oncogene homolog-1
RHH	Royal Hallamshire Hospital
RV	right ventricular
SAE	serious adverse event
SF-36 V2	short form 36 version 2 questionnaire
SHBG	sex hormone binding globulin
SHU	Sheffield Hallam University
sICAM	soluble intra-cellular adhesion molecule
SPECT	single photon emission computed tomography
STH	Sheffield Teaching Hospitals
St O ₂	percentage oxygen saturation
StAR	steroidogenesis activator protein

STPD	standard temperature and pressure, dry
sVCAM	soluble vascular adhesion molecule
TAPSE	tricuspid annular plane systolic excursion
TDI	tissue Doppler imaging
TEM	technical error of measurement
TNF α	tumour necrosis factor alpha
TS	testosterone supplementation
TT	total testosterone
UHSM	University Hospital South Manchester
UK	United Kingdom
VE	ventilation
V _{max}	maximum left atrial volume
V _{min}	minimum left atrial volume
VO _{2max}	maximal oxygen uptake
V _{preA}	left atrial pre-contraction volume
VSD	ventricular septal defect
α	alpha
β	beta

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Publications, presentations and conference proceedings arising from this research.

Innaugral Sports Medicine Meeting – Sheffield Teaching Hospitals NHS Foundation Trust, October 2009 – invited speaker. Testosterone supplementation in heart failure.

British Association of Sport and Exercise Science: Cardiac Rehabilitation Subgroup Meeting, Metropole Hotel, Birmingham, December 2009 – invited speaker. Testosterone and exercise training in heart failure.

European Society of Cardiology Congress, Stockholm, Sweden, May 2010 – invited speaker. The use of flow mediated dilatation: a clinical or research tool.

American College of Sports Medicine, Washington, U.S.A, October 2010 – abstract presentation. Combined exercise training and testosterone supplementation in males with low testosterone status and chronic heart failure.

European Society of Cardiology – Heart Failure Congress, Gothenburg, Sweden, May 2011 - Abstract presentation: the cardiovascular effects of testosterone and exercise training in heart failure. *European Journal of Heart Failure Supplement* (2011) (10) (suppl1): S60-S104.

Stout, M., Tew, G.A., Doll, H., Zwierska, I., Woodroffe, N., Channer, K.S. and Saxton, J.M. Testosterone therapy during exercise rehabilitation in male patients with heart failure who have low testosterone status: A double-blind randomised controlled feasibility study. *American Heart Journal* (2012), 164: 893-901.

Abstract.

Background: This cross-sectional study aimed to assess the effect of low testosterone (LT) status on functional capacity, quality of life, cardiac structure and function in males with moderate or severe stable heart failure (HF). **Methods:** 40 male patients with HF (ejection fraction (EF)% $\leq 45\%$) with low serum total testosterone (TT) ≤ 12 nmol / L (n =20, 71.9 ± 10.11 yrs) and normal range TT (n = 20, 66.9 ± 11.98 yrs) were recruited from a specialist HF clinic. The primary outcome, functional capacity, was assessed by the 6 min shuttle walk test. Health related quality of life (HRQoL) data was assessed using a licensed version of the SF36 Version 2. Additionally, Beck Depression Inventory (BDI), Minnesota Living with Heart Failure questionnaire (MLHFQ) and Androgen Deficiency in the Ageing Male (ADAM) questionnaire data was collected. Cardiac characteristics were assessed using detailed echocardiography analysis. Blood samples were taken for assessment of NT pro BNP. **Results:** Normal testosterone (NT) patients demonstrated a significantly higher 6 min walk distance (429.00 ± 126.94 m; 257.65 ± 65 m, $p < 0.01$) when compared to LT. LT patients showed significantly worse indices of quality of life using the SF36 V2 questionnaire. General health component (31.84 ± 2.85 v 59.44 ± 3.24 , $p < 0.05$), overall physical component score (41.39 ± 17.03 v 69.69 ± 19.71 , $p < 0.01$) and overall mental component score (54.88 ± 17.52 v 72.36 ± 16.58 , $p < 0.01$) higher in the NT group. There were very few cardiac changes and no differences in MLHFQ or BDI score between the groups. **Conclusion:** This is the first study to compare important prognostic outcomes in matched patients with HF and low testosterone. Additionally, this is the first paper to report significantly adverse HRQoL in male patients with low testosterone and HF when compared to normal range testosterone counterparts. LT in HF is associated with reduced functional capacity, together with attenuated general, physical and mental components of quality of life. Further research is warranted to assess the impact of testosterone supplementation on

these important prognostic outcomes in male patients with HF and low testosterone status.

Section 1: The impact of testosterone concentration in males with heart failure.

Chapter 1: General introduction.

Heart failure (HF) is a multi-organ disease, involving the musculoskeletal, respiratory and endocrine systems. It is a common, debilitating condition and a major public health and financial burden in the Western world. The incidence and prevalence of congestive HF has increased together with the resulting morbidity and mortality. Worldwide it is estimated that 15 million individuals suffer from this condition, with 400,000 new cases each year in the United States of America and a total of 4.5 million Americans effected (Erikson, 1995). In the United Kingdom (UK), it is estimated that 900,000 people have HF (Al-Mohammed et al, 2010). Surveys in North West London show prevalence rates of 0.6 per 1000 patients aged less than 65 years and 28 per 1000 patients aged 65 years and over (Parameshwar et al, 1992).

As males age, there is a gradual decline in circulating bio-available testosterone (Betocchi, 2005). There is a general consensus that testosterone levels decline about 1% per year from as early as age 30 years. Noticeable declines are common after the age of 50 years but there is great individual variability (Morales and Lunenfield, 2002).

HF also appears to be associated with decreased levels of plasma testosterone, supported by the fact that about 25% of men of all ages with HF have biochemical evidence of testosterone deficiency (Malkin et al, 2009). Low levels of testosterone have been correlated with disease progression in HF and may also be responsible for some of the features of HF, such as reduced skeletal muscle mass and function, cachexia, fatigue and depressed mood (Malkin et al, 2006). Furthermore, myocardial

cachexia, a syndrome with poor prognosis, is characterised by low levels of testosterone (Aukrust et al, 2009).

In addition to this it is well known that HF as a metabolic syndrome can adversely alter numerous endocrine, metabolic and inflammatory parameters (Jackson et al, 2000, Noutsias et al, 1999). The alterations can include changes in levels and sensitivity to insulin, growth hormone and importantly testosterone (Von Haehling et al, 2007). Reduced insulin sensitivity and the development of diabetes could be a major complicating factor of HF. It has been described that more than 40% of patients with HF have manifest disorders of glucose metabolism which appear independent of fat distribution and obesity (Aukrust et al, 2009).

Interestingly, testosterone replacement therapy at physiological doses has been shown to improve indices of physical function, cardiac function and also quality of life in HF patients (Malkin et al, 2006, Caminiti et al, 2009). These studies have shown modest improvements in cardiac output, maximal oxygen uptake, muscular strength and improved disease and health specific quality of life markers. Additionally, testosterone supplementation has been shown to improve arterial function via reduction in systemic vascular resistance (Pugh et al, 2003). Animal models have shown positive associations between testosterone supplementation and reduced levels of biochemical inflammation (Zhang et al, 2007) and also improvements in cardiac contractile performance (Scheuer et al, 1997). These studies are important because they suggest that when testosterone level is returned to the normal range there are significant improvements in important sequelae related to prognosis in HF.

Recent correlation studies have suggested that in HF, testosterone level is independently related to peak $\dot{V}O_2$, oxygen pulse and also right ventricular function (Jankowska et al, 2009 and Bocchi et al, 2008). However, there is no research that directly compares parameters of physical fitness, cardiac function, quality of life and biochemical inflammation in male HF patients with low or normal testosterone levels.

Contradictory results have been published regarding the successful administration of testosterone therapy to males with HF (Caminiti et al, 2009, Malkin et al, 2006) and no large scale trials have aimed to determine the efficacy of supplementation. Based on previous observations, the efficacy of testosterone supplementation in a male HF population with low testosterone status is uncertain. This study aims for the first time to determine the effects of testosterone concentration on exercise capacity, quality of life and cardiac function in males with low testosterone status in comparison with normal testosterone in moderate or worse HF.

2.1: Heart failure.

2.1.1 Incidence of HF.

There has been a dramatic decline in the incidence of mortality and morbidity from many cardiovascular diseases over recent decades (Sytkowski et al, 1990). Conversely, the incidence and prevalence of congestive HF, together with the resultant morbidity and mortality have shown an increase throughout this time. Worldwide it is estimated that 15 million individuals suffer from this condition, with 400,000 new cases each year in the United States of America and a total of 4.5 million Americans effected (Eriksson, 1995). In the United Kingdom (UK), surveys in North West London show prevalence rates of 0.6 per 1000 patients aged less than 65 years and 28 per 1000 patients aged 65 years and over (Parameshwar et al, 1992). This statistic is reinforced by the Framingham Study which described a doubling in the incidence of HF for every decade of life reaching 3% in those aged 85-94 years (Ho et al, 1993). Large surveys have also been conducted in the UK during the 1990's aiming to study left ventricular (LV) dysfunction using echocardiography in large populations of Glasgow and the West Midlands. In Glasgow the prevalence of significantly impaired LV systolic dysfunction in participants 25-74 years was 2.9% and in the West Midlands 1.8% in patients aged 45 years and over (Lip et al, 1997 and Mc Donagh et al, 1997). Economists have studied the costs of HF to the National Health Service in the UK. It has been found that in the year 2000 HF cost the Health Service 905 million pounds (1.91% of total UK Health Service expenditure). The most predominant factor for this cost was hospital admission (69%) followed by prescriptions for important treatment (18%). Additionally, the cost for secondary hospital admission and nursing home care for patients with HF was 750 million equating to 2% of total Health Service expenditure (Stewart et al, 2002

2.1.2: Aetiology of HF.

The aetiology of HF differs according to the population being studied. For example, in Western society, the most common causes of HF are coronary artery disease (CAD) and hypertension. In the developing countries, valvular heart disease and nutritional heart disease are most common (Lip et al, 2000). There are also a host of other conditions which can cause HF and these are listed in the summary table 1 below.

Table 1. Causes of HF adapted from Lip et al, 2000.

Causes of Heart Failure.

Coronary artery disease:

- Myocardial Infarction.
- Ischemia.

Hypertension.

Cardiomyopathy:

- Dilated (congestive).
- Hypertrophic / obstructive.
- Restrictive (e.g. amyloidosis, sarcoidosis, haemochromatosis).
- Obliterative.

Valvular and Congenital Heart Disease:

- Mitral valve disease.
- Aortic valve disease.
- Atrial septal defect / ventricular septal defect.

Arrhythmias:

- Tachycardia.
- Bradycardia (complete heart block, sick sinus syndrome).
- Loss of atrial transport (e.g. atrial fibrillation).

Alcohol and Drugs:

- Alcohol.
- Cardiac depressants (e.g. β blockers, calcium channel blockers).

'High output' cardiac failure:

- Anaemia, thyrotoxicosis, Paget's Disease, arteriovenous fistulae.

Pericardial Disease:

-
- Constrictive pericarditis.
 - Pericardial effusion.

Primary Right Heart Failure:

- Pulmonary hypertension (e.g. pulmonary embolism, cor pulmonale).
 - Tricuspid incompetence.
-

Table 2 summarises the percentages of common aetiologies of HF in the western world.

Table 2. Epidemiological studies of the aetiology of HF as percentage (adapted from Lip et al, 2000).

Aetiology.	Framingham Heart Study.			Hillingdon Study.
	Teerlink et al 1989-90.	Men.	Women.	
<i>Ischaemic.</i>	50	50	48	36
<i>Non Ischaemic:</i>				
Hypertension.	4	70	78	14
Idiopathic.	18	0	0	0
Valvular.	4	22	31	7
Other.	10	7	7	10
Unknown.	13	0	0	34

2.1.3: Pathophysiological considerations – Heart failure.

In systolic HF, there is impairment in left ventricular ejection fraction (LV EF%) resulting in a decline in cardiac output. In response to this there is activation of various neuro-hormonal compensatory mechanisms which attempt to improve the mechanical efficiency of the heart. The phenomenon of which is addressed below in more detail.

Stimulation of the renin-angiotensin-aldosterone system (RAAS) via neuro-hormonal activation and altered autonomic control increases levels of renin, plasma angiotensin II and aldosterone. Angiotensin II has been found to be a potent vasoconstrictor of the renal efferent arterioles and systemic circulation where it stimulates release of nor adrenaline from sympathetic nerve terminals, inhibits vagal tone and promotes release of aldosterone (Jackson et al, 2000). This activation results in retention of sodium and water together with increased release of potassium. Furthermore, angiotensin II has been shown to cause anorexia and weight loss in experimental models using animals (Brink et al, 1996). Additional research has also suggested an increase in the levels of endothelin (another potent vasoconstrictor peptide) in HF, constituting adverse prognosis (Tsutamoto et al, 1995). Blood plasma levels of Endothelin – 1 have been shown to be a reliable correlate to pulmonary artery capillary wedge pressure (PCWP), hospital admission and death (McMurray et al, 1992). As an early compensatory mechanism to provide inotropic support to the failing cardiac muscle, low and high pressure baroreceptors further stimulate the sympathetic nervous system to increase heart rate (HR) and hence cardiac output. Adverse consequences of prolonged elevation of adrenergic drive to the cardiac muscle involve a direct toxic effect of nor-epinephrine on cardiac myocytes (Mann et al, 2002), facilitation of the development of ventricular arrhythmias (Schwartz et al, 1994), alterations in β -adrenoceptor function (Bristow et al, 1992) and apoptosis of endothelial cells in vitro (Horiuchi et al, 1997). The alteration in

β - adrenoceptor function increases sympathetic activity and also down-regulates autonomic modulation of the sinus node leading to the notion that reduced HR variability is an important prognostic marker in patients with HF (Jackson et al, 2000).

Atrial and Brain natriuretic peptides are released by cardiac muscle in HF primarily due to volume overload and the corresponding atrial and ventricular stretch. These peptides have an antagonistic effect to those of angiotensin II on vascular tone, aldosterone secretion and renal-tubule sodium reabsorption (Jackson et al, 2000). As a result, levels of these circulating mediators in HF have become an important diagnostic marker and are also the subject of research interest in developing new medications that inhibit the enzyme that metabolises atrial natriuretic peptide (neutral endopeptidase) (Jackson et al, 2000). In untreated congestive cardiac failure, high levels of atrial and brain natriuretic peptide correlate closely with adverse prognosis and ultimately mortality.

HF also results in increased levels of circulating cytokines which include tumour necrosis factor-alpha (TNF- α), interleukins-1(IL-1), 2 (IL-2), 6 (IL-6), 8 (IL-8) and their receptors (Berry and Clarke, 2000). In more detail, research has shown that TNF- α and IL-6 levels are elevated in relation to deteriorating functional classification and in this regard should also be recognised to be associated with some of the classic hallmarks of HF including worsening LV dysfunction, pulmonary oedema and cardiac remodelling (Millar et al, 1982). In relation to this, in patients with severe HF, elevated levels of soluble TNF receptor are a stronger predictor of early mortality when compared to plasma nor adrenaline, atrial natriuretic peptide or (New York Heart Association) NYHA classification (Berry and Clarke, 2000). Physiologically in HF, there is an increased production of soluble adhesion molecules via the endothelium; these are recognised as important mediators of endothelial-leukocyte adhesion and

inflammatory response (Oppenheimer et al, 1991). This physiological process relates to the earlier notion that endothelial injury is associated with an adverse prognosis in HF. Other researchers have discovered that during hypoxia (an occurrence related to the reduced cardiac output in HF) IL-6 and the soluble adhesion molecule - 1 (sICAM-1) are secreted by injured endothelial cells (Sluiter et al, 1993) and vascular xanthine dehydrogenase enzyme is converted to xanthine oxidase. This catalyses the production of uric acid and its reactive bi-product superoxide (Yan et al, 1997). It has been hypothesised that injured vascular endothelium in HF patients may be a source of free radical production, triggering leukocyte activation and increased cytokine production (Leyva et al, 1998).

Researchers and clinicians have observed widespread endothelial cell activation followed closely by endothelial dysfunction (ED) in HF (Brutsaert, 2003). This ED has been thought to be partly responsible for the early fatigue and exercise intolerance in HF. This may be attributed to inappropriate, endothelial mediated, vasoconstrictor responses with reduced vasodilatory capacity contributing to elevated peripheral vascular resistance (Drexler et al, 1992). In relation to this, reduced gene expression of endothelial nitric oxide synthase (eNOS) and cyclo-oxygenase-1 (COX-1) has also been shown to be a factor in the early onset of ED in cardiac failure (Smith et al, 1996). Experimental research in patients with cardiac dysfunction has provided direct evidence of ED in both conductive and resistance coronary arteries (Treasure et al, 1990). In more detail, these patients displayed impaired dilatory responses to acetylcholine and bradykinin within the coronary circulation. The coronary ED has been attributed to reduced synthesis of coronary endothelial nitric oxide. The concept has emerged that coronary vascular ED could trigger coronary vasoconstriction, smooth muscle proliferation and remodelling, increased lipid deposition in the vessel wall and also

coronary thrombosis. It is evident that these processes would accelerate the development CAD, resulting in decreases in myocardial perfusion and indirectly contributing to cardiac failure.

Skeletal muscle physiology and function is abnormal in patients with HF. Muscle bulk is decreased and there are documented reductions in muscular strength and endurance (Massie et al, 1987). Early studies have found that during leg exercise, oxygen extraction to the exercising muscle and lactate efflux were increased, together with diminished total oxygen utilisation (Donald et al, 1961). This was attributed to represent the metabolic consequences of reduced blood flow to exercising muscle. To support this notion, research has suggested that skeletal muscle is histologically abnormal with a tendency towards anaerobic (type II) muscle fibres (Mancini et al, 1989). This research has also identified abnormal mitochondrial structure with reduced cristae volume and reduction in the key enzymes involved in the Krebs's cycle and the oxidative chain. This can be supported by other research which has shown more rapid declines of phosphocreatine and rises in inorganic phosphate in HF patients when compared with healthy controls. The slope of the relationship between phosphocreatine and inorganic phosphate versus muscular power output (an index of mitochondrial oxidative metabolism) was found to be steeper in HF patients suggesting impaired oxidative capacity (Wilson et al, 1985).

2.1.4: Myocardial substrate metabolism and heart failure.

In order to understand the abnormalities related to myocardial substrate metabolism in HF, it is important to review myocardial metabolism in a normal heart. In non-ischaemic conditions, the majority of adenosine triphosphate (ATP) formation in the

heart is derived from oxidative phosphorylation in the mitochondria with minor contribution from glycolysis and guanosine triphosphate (GTP) formation via the citric acid cycle (Stanley et al, 2005). The initial step in the energetic pathway is the cellular uptake of and utilisation of available substrate, their breakdown by β -oxidation and glycolysis, resulting in formation of acetyl coenzyme A (CoA) which is then fed into the Krebs cycle producing nicotinamide adenine dinucleotide hydroxide (NADH) and carbon dioxide (CO₂) (Neubauer, 2007). Under normal perfusion approximately 60-90% of CoA results from β -oxidation of free-fatty acids and 10-40% from pyruvate oxidation via glycolysis and lactate oxidation (Stanley et al, 2005).

2.1.4.1: Normal myocardial carbohydrate metabolism.

Glycolytic substrate is utilised from exogenous glucose and glycogen stores. Glucose transport into cardiac myocytes is facilitated by trans-membrane gradients and sarcolemma glucose transporters (GLUT-4 and GLUT-1) (Stanley et al, 2005). Insulin stimulation, increased cardiac demand or myocardial ischaemia result in a shift in glucose transporters from intra-cellular vesicles to the sarcolemma therefore increasing glucose transport and uptake into the cell (Stanley et al, 1997). In addition, GLUT-4 re-location into the sarcolemma may be facilitated by activation of adenosine monophosphate (AMP) -activated protein kinase, particularly during ischaemia or in response to exercise (Russell et al, 1999 and Russell et al, 2004). Intra-cellular glycogen stores may be used as an additional source of glucose 6 phosphate for uptake into the glycolytic pathway. Previous studies have suggested that glycogen concentration may be increased by an elevated supply of exogenous substrate together with, or solely by, increased levels of insulin (Kruszynska et al 1991). Glycogenolysis can therefore be activated by adrenergic stimulation, decrease in tissue adenosine triphosphate (ATP)

concentration and increase in inorganic phosphate – occurring typically with stressors such as exercise or ischaemia (Stanley et al, 1997).

Pyruvate, formed from glycolysis, may be converted to lactate, decarboxylated to CoA or carboxylated to oxaloacetate or malate (Stanley et al, 2005). The decarboxylation of pyruvate is considered to be the first irreversible step in carbohydrate oxidation, catalysed by the enzyme pyruvate dehydrogenase (PDH) (Randle, 1986). Importantly, higher levels of circulating lipids, together with increased accumulation of long chain fatty acid moieties (e.g. associated with diabetes or fasting) have been shown to increase phosphorylation inhibition of PDH and decrease oxidation of pyruvate from the glycolytic pathway and lactate oxidation (Huang et al, 2002). Furthermore, glucose and pyruvate oxidation, together with related PDH activity, are attenuated by increased rates of fatty acid oxidation; for instance when there are elevated levels of plasma free fatty acids. Conversely to this, pyruvate oxidation is increased when fatty acid oxidation is attenuated (Kruszynska et al, 1991).

2.1.4.2: Normal myocardial fatty acid metabolism.

The concentration of plasma non-esterified fatty acid levels mainly determines the rate of fatty acid uptake by the myocardium (Stanley et al, 2005). As such, metabolic stressors such as ischaemia, diabetes or starvation which increase plasma free fatty acid concentration, result in increased rate of myocardial free fatty acid uptake (Stanley et al, 2005). Regulation of plasma free fatty acid concentration is via their net release from triglycerides in adipocytes, facilitated by the action of hormone-sensitive lipase and synthesis by glycerolphosphate acyltransferase. Hormone-sensitive lipase is activated by catecholamines and inhibited by insulin. Accordingly, when insulin concentration is low and catecholamine concentration high, plasma free fatty acid concentration and

hence myocardial free fatty acid uptake and oxidation are high (Lopaschuk et al, 1994). Uptake of free fatty acids into the cardiomyocyte is promoted by passive diffusion or protein mediated transport across the sarcolemma (Stanley et al, 2005). Once within the sarcolemma, non-esterified fatty acids bind to fatty acid binding protein to be activated by esterification to fatty acyl-CoA via fatty acyl-CoA synthase (Stanley et al, 2005). Acyl-CoA transportation into the mitochondria is facilitated by a carnitine-dependent transport system, with carnitine palmitoyltransferase-I serving as the key regulator for the rate of this uptake (Lopaschuk et al, 1994). Fatty acid β -oxidation occurs within the mitochondria. This process repeatedly cleaves two carbon acetyl-CoA units in order to generate NADH and flavin adenine dinucleotide dihydroxide (FADH₂). Bing and co-workers in 1954 suggested four reactions, involving different, but specific enzymes, to facilitate fatty acid β -oxidation dependent on long, medium or short-chain fatty intermediates. Initially, catalysed by acyl-CoA dehydrogenase, followed by 2-enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase. Finally, 3-ketoacyl-CoA thiolase regenerates acyl-CoA for further β -oxidation and releases acetyl-CoA for the citric acid cycle.

2.1.4.3: Normal myocardial ketone body metabolism.

Normal arterial plasma concentration of ketone bodies is low. Fatty acid ketone body formation in the liver therefore, plays a minor role as a substrate for the myocardium (Stanley et al, 2005). It should be highlighted however, that during starvation or poorly controlled diabetes, plasma ketone bodies become elevated due to low insulin and high fatty acid levels and as such become a major substrate for myocardial metabolism (Hall et al, 1996).

2.1.4.4: Electron transport chain and oxidative phosphorylation in HF.

Research in both human and animal models of HF have shown that there is a decreased concentration of tissue ATP, increased concentration of adenosine diphosphate (ADP) and reduction in phosphorylation potential (Montgomery et al, 1992 and Shen et al, 1999). As a result, there is significant impairment of the kinetic mechanisms of ATP utilisation for myocardial cell contraction and relaxation via myosin ATPase and sarcoplasmic reticulum Ca^{2+} - ATPase (Stanley et al, 2005). It has previously been noted that HF attenuates the ability of the creatine kinase system to transfer mitochondrial ATP to the myofibril. In addition to this, impairment of the electron transport chain may be detrimental to the mitochondrial and cytosolic redox state, adversely affecting the concentration of ATP, ADP and phosphate and therefore, reducing the rate of influx through key metabolic enzymes such as PDH or phosphofructokinase (Ye et al, 2001). Abnormalities at the level of the mitochondria in HF are abundant. Previous studies in both humans and animals have suggested there is a greater incidence of mitochondrial membrane disruption and matrix depletion (Sabbah et al, 1992), a lower capacity for respiration with a variety of substrates (Sharov et al, 2000), electron transport chain defects together with decreased capacity for oxidative phosphorylation (Casademont and Miro, 2002). Although there are different theories as to the exact processes involved in electron transport chain complex dysfunction in HF, it is widely evident that there is a major disruption in oxidative metabolism at this level. To date, there is no consensus regarding the exact site of the lesions of electron transport chain dysfunction, whether the effects of this dysfunction are isolated to a group of myocytes or all cardiac myocytes as a whole or finally, whether dysfunction is localised to subsarcolemmal or intrafibrillar components of the mitochondria (Stanley et al, 2005).

2.1.5.5: Myocardial substrate metabolism in HF.

Results are conflicting regarding myocardial substrate metabolism in heart failure, particularly in human models. There is however, a consensus that HF reduces the capacity to transduce foodstuffs into ATP (Stanley et al, 2005). Initially, in the early stages of HF, fatty oxidation rate is maintained. However, Paolisso et al (1994) observed that in established HF with NYHA Class II and III, there was increased extraction and uptake of plasma free fatty acid and decreased glucose uptake. Furthermore, it has also been established that HF increases myocardial lipid oxidation by as much as 50% when compared to healthy aged matched controls (Paolisso et al, 1994). The same study also showed that in HF patients there is a significant decrease in myocardial carbohydrate oxidation when compared to controls. Increased plasma nor adrenaline concentrations corresponding to increased levels of free fatty acid were also noted in the HF population. This has been attributed to enhanced β -adrenergic stimulation and also higher levels of plasma insulin, both factors which may facilitate glucose uptake and oxidation by the myocardium (Stanley et al, 2005). Using other methods, researchers have been able to develop radiolabelled fatty acid / deoxyglucose analogues and with positive emission tomography (PET) have discovered increased uptake of fatty acid analogue and decreased uptake of deoxyglucose analogue in patients with NYHA class III HF (Taylor et al, 2001). In contradiction to this, Yazaki et al (1999) have suggested that in HF patients with idiopathic dilated cardiomyopathy, the process is reversed with increased myocardial glucose uptake and decreased fatty acid oxidation when compared to healthy controls. Davila-Roman et al (2002) have reaffirmed the findings of Yazaki and colleagues by using ^{18}F -deoxyglucose 6 phosphate infusion during PET to estimate glucose uptake. This study determined that there was increased myocardial glucose metabolism and decreased fatty acid utilisation in their population of HF patients.

Studies of patients with end-stage HF have consistently suggested that there is down regulation of myocardial fatty acid oxidative enzymes – a feature relating to the conversion of substrate metabolism from fatty acid oxidation towards glucose oxidation (Stanley et al, 2005). In more detail, explanted hearts at transplant, have demonstrated reduced messenger ribonucleic acid (mRNA) for the fatty acid oxidation enzymes long-chain acyl-CoA dehydrogenase and medium-chain acyl-CoA dehydrogenase together with significantly reduced protein levels of medium-chain acyl-CoA dehydrogenase without down-regulation of mRNA for the glycolytic enzyme glyceraldehydes-3-phosphate dehydrogenase (Sack et al, 1996). Pacing induced HF in a canine model, has also showed similar reductions in mRNA levels of key enzymes involved in the fatty acid oxidation pathway, but in addition, GLUT-1, GLUT-4, glyceraldehydes-3-phosphate dehydrogenase, PDH and pyruvate dehydrogenase kinase isoenzyme-4 (PDK-4), providing evidence that the failing heart attenuates the expression of all metabolic enzymes rather than selectively suppressing fatty acid oxidation enzyme and potentiating the carbohydrate pathway (Razeghi et al, 2001).

In HF, impaired oxidative phosphorylation is detrimental to cardiac function due to an inadequate supply of ATP to cardiac myocytes. Mitochondria in HF have been found to have abnormal structure and contribute to a substantial reduction in oxygen consumption and derangement of energy production in a failing myocardium. (Ide et al, 2001). In more detail, the action of electron-transport chain complexes and ATP synthase capacity are attenuated together with the regulation of oxidative phosphorylation by phosphate ADP, AMP and creatine (Lewandowski, 2002 and Marin-Garcia et al, 2001).

In advanced, end-stage HF, myocardial ATP levels decrease by up to 40% (Beer et al, 2002). However, it should be noted that average ATP levels remain sufficient for myosin-ATPase usage and, as such, do not contribute to pump failure (Beer et al, 2002). Phosphocreatine and total creatine levels have been shown to decline at an earlier stage and by greater values up to 70%, probably due to down-regulation of creatine-transport function (Ten-Hove et al, 2005). In relation to this, mitochondrial creatine kinase activity has been shown to reduce to 20% of normal activity and myofibrillar creatine kinase activity can decrease by as much as 50%. These processes clearly result in a drastic decline in ATP transfer, energy flux within the mitochondrial structure and a combined up to 70% reduction in energy delivery to myofibrils in a HF model (Liao et al, 1996). Increased catecholamine secretion in HF results in high workload states that promote artificial elevation in free ADP concentration to values twice that of normal human myocardium (Neubauer, 2007). Increased free ADP accumulation in the perimyofibrillar compartments together with compartments adjacent to the sarcoplasmic reticulum and sarcolemmal ion pumps serves to limit the inotropic contractile reserve of the myocardium, resulting clinically with dyspnoea during high workload states e.g. exertion (Neubauer, 2007).

³¹P-MR spectroscopy has been widely utilised to assess myocardial energetics in HF and is a robust indicator of the energetic state of the myocardium by comparing the ratio of phosphocreatine to ATP. As such, the creatine kinase reaction equilibrium favours ATP synthesis rather than phosphocreatine synthesis by a factor of 100 (Neubauer, 2007). Resultantly, if demand for ATP outweighs ATP synthesis, then phosphocreatine levels decline initially with ATP only decreasing when phosphocreatine levels are substantially reduced. In HF however, total creatine also decreases serving to reduce the phosphocreatine / ATP ratio (Hardy et al, 1991). This ratio is particularly important in

HF because it has been consistently shown to correlate well with NYHA score (Hardy et al, 1991), indexes of systolic and diastolic LV impairment (Neubauer et al, 1995, Lamb et al, 1991) and ultimately has been suggested as the strongest predictor of mortality when compared to clinical and functional characteristics (Neubauer et al, 1997).

The nuclear receptors of the peroxisome proliferator-activated receptor (PPAR) family (isoforms: PPAR α , PPAR β and PPAR γ) play an important role in cardiac lipid metabolism (Neubauer, 2007). PPAR α has been suggested to be the key determinant to cardiac lipid metabolism by controlling the expression of enzymes directly involved in fatty acid oxidation. In human models, PPAR α expression decreases in conjunction with the attenuation of fatty acid oxidation and, as such, is thought to be the principle mechanism in the switch from fatty acid substrate utilisation to glycolytic metabolism (Sack et al, 1996). PPAR γ coactivator-1 (PCG-1 α) is a nuclear receptor co-activator and plays a crucial role in mitochondrial metabolic function (Huss et al, 2004). PCG-1 α is able to activate genes responsible for fatty acid uptake and oxidation and also for oxidative phosphorylation. Primarily, these genes include PPAR α and PPAR β . PCG-1 α inhibition due to increased catecholamine levels in HF results in down-regulation of mitochondrial gene expression and therefore directly contributes to impaired oxidative phosphorylation (Garnier et al, 2003). Furthermore, development of HF is accelerated by inhibition or deficiency of PCG -1 α which suggests that this nuclear receptor co-activator may play an important cardio-protective role (Aranzy et al, 2006).

2.1.5: Diastolic heart failure.

Previously, diastolic HF was considered a more benign condition than the systolic counterpart and was also thought to affect only a relatively small cohort of patients.

More recently, perspectives have altered and the prevalence of diastolic HF has increased to 54% of all cases of HF (Owan et al, 2006). Importantly, recent evidence also suggests that diastolic HF carries a similar prognosis to that of systolic HF (Cleland et al, 2003). Pre-disposing conditions to diastolic HF include advancing age, female gender, diabetes, obesity, arterial hypertension and left ventricular hypertrophy (Fischer et al, 2003). As a result of these pre-disposing conditions and the evolution of Westernised society, diastolic HF is clearly becoming more dominant.

Historically, diastolic impairment was referred to as heart failure with a normal ejection fraction. In this sub-group of patients, evidence for diastolic impairment was provided by slow LV relaxation and increased LV stiffness. In addition, more recent arguments have suggested that although global LV systolic function may appear normal, there are significant decreases in myocardial tissue Doppler velocities and abnormalities of ventriculo-arterial coupling. However, it is now evident that diastolic dysfunction may also be present in patients with known systolic failure (Skaluba et al, 2004). Furthermore, it has also been suggested that in patients with systolic and diastolic HF, the latter correlates more closely with symptoms than the former (Skaluba et al, 2004).

As a result of the challenges faced when assessing diastolic HF and the evidence of a close relationship to systolic HF recent opinion has categorised HF of both origins into a single syndrome. This 'single syndrome' hypothesis suggests that both forms of ventricular impairment result from similar pathophysiology (i.e. increased interstitial collagen deposition and modification to extracellular matrix proteins) and therefore, there is a progression from heart failure with a normal ejection fraction to heart failure with reduced ejection fraction (Sanderson, 2007). Supporting research has established that in heart failure with a preserved ejection fraction there are increased three dimensional

(3D) LV volumes when compared to well-matched normal patients (Maurer et al, 2005). Additionally, another study has showed that in prospective follow-up of patients with heart failure and preserved ejection fraction, 20% of participants demonstrated a significant decline in LV EF below 45% during a 3 month follow-up period (Cahill et al, 2006).

Conversely, structural, functional and molecular biological theories have been suggested that categorise heart failure as a ‘two-syndrome’ condition, namely, LV systolic impairment and normal LV systolic function with features associated with diastolic LV dysfunction (Paulus et al, 2007). Related to this theory, patients with systolic HF demonstrate eccentrically dilated, remodelled ventricles with reduced pumping capacity. However, in diastolic HF, patients develop concentric hypertrophy and a high wall mass-volume ratio (Kitzman et al, 2002). In addition to this, ultra-structural studies have suggested further differences between diastolic and systolic HF. In diastolic HF cardiomyocyte diameter and myofibrillary density is increased when compared to patients with systolic HF (Van Heerebeek et al, 2006). Furthermore, there are not only structural differences at the cellular level between systolic and diastolic HF but functional cellular differences have been suggested. Cardiomyocyte resting tension is increased in diastolic HF – a factor which has been shown to significantly contribute to overall myocardial stiffness (Van Heerebeek et al, 2006). Titin, a cytoskeletal protein responsible for early diastolic recoil, demonstrates a less compliant isoform expression in diastolic HF when compared to systolic HF (Neague et al, 2002). At the functional cellular level, differences have also been observed in expression patterns of matrix metalloproteinases and tissue inhibitors of metalloproteinases (Paulus et al, 2007). In diastolic HF down-regulation of metalloproteinases combined with increased regulation of tissue inhibitors of metalloproteinases results in a decrease in matrix degradation. In

systolic HF, the opposite pattern is observed, with increased matrix degradation due to upregulation of metalloproteinases (Ahmed et al, 2006). Pharmacological intervention in diastolic HF also suggests different pathophysiological mechanisms between systolic and diastolic HF. For example, in the PEP-CHF study (Cleland et al, 2006) it was noted that perindopril treatment in diastolic HF was not as effective as when used for treating systolic HF.

2.1.6: Diagnosis, signs and symptoms – Heart failure.

The aforementioned pathophysiology of HF goes some way to explain the common clinical features and complications of this condition. Patients can present with a variety of symptoms which can often be non-specific. The main signs and symptoms of HF are listed in the table 3 below.

Table 3. Symptoms and Signs in Heart Failure.

Symptoms:

Dyspnoea.

Orthopnoea.

Paroxysmal Nocturnal Dyspnoea.

Reduced Exercise Tolerance, lethargy, fatigue.

Nocturnal cough.

Wheeze.

Ankle swelling.

Anorexia.

Signs:

Cachexia and muscle wasting.

Tachycardia.

Pulsus alternans.

Elevated jugular venous pressure.

Displaced apex beat.

Right ventricular heave.

Creptitations or wheeze.

Third heart sound.

Oedema.

Hepatomegaly (tender).

Ascites.

It should be noted that there are many non-cardiac conditions that can mimic the symptoms of HF so diagnosis by these alone is often difficult. Additionally, physical

examination has serious limitations as many patients particularly with less severe HF have very few abnormalities. The following table (4) highlights the sensitivity, specificity and predictive value of symptoms, signs and chest x-ray findings for the presence of HF.

Table 4. Sensitivity, specificity and predictive value of symptoms, signs and chest x-ray findings for presence of HF (EF <40%) in 1306 patients with CAD undergoing cardiac catheterisation (adapted from Watson et al, 2000).

Clinical Features.	Sensitivity %	Specificity %	Positive Predictive Value %
History:			
Shortness of Breath.	66	52	23
Orthopnoea.	21	81	2
Paroxysmal Nocturnal Dyspnoea.	33	76	26
History of Oedema.	23	80	22
Examination:			
Tachycardia.	7	99	6
Creptitations.	13	91	27
Oedema on examination.	10	93	3
Gallop (S3).	31	95	61
Neck vein distension.	10	97	2
Chest X-Ray Examination:			
Cardiomegaly.	62	67	32

The European Society of Cardiology (Task Force on Heart Failure of the European Society of Cardiology, 1995) has developed standardised guidelines for the diagnosis of HF. Essentially, patients must have documented symptoms of HF e.g. breathlessness, fatigue and / or ankle swelling together with objective evidence of resting cardiac dysfunction. Alternatively, if a patient responds well to treatment targeted towards HF when the diagnosis is in doubt, then this is also a non-essential diagnostic indicator.

Symptoms and exercise capacity together are used to classify the severity of HF and to also gauge the effectiveness of treatment. The most common worldwide classification system currently used to achieve these aims in clinical practise is the NYHA. This can be summarised as follows (table 5):

Table 5. NYHA Classification for Heart Failure. From the American Heart Association.

NYHA Class.

Class I: Asymptomatic.

No limitation to physical activity despite presence of heart disease confirmed by investigations. (e.g. echocardiography).

Class II: Mild.

Slight limitation in physical activity. More strenuous exercise causes shortness of breath. Most patients continue to have a normal lifestyle and employment.

Class III: Moderate.

More marked limitation of activity interfering with work and daily life. Shortness of breath when walking on the flat.

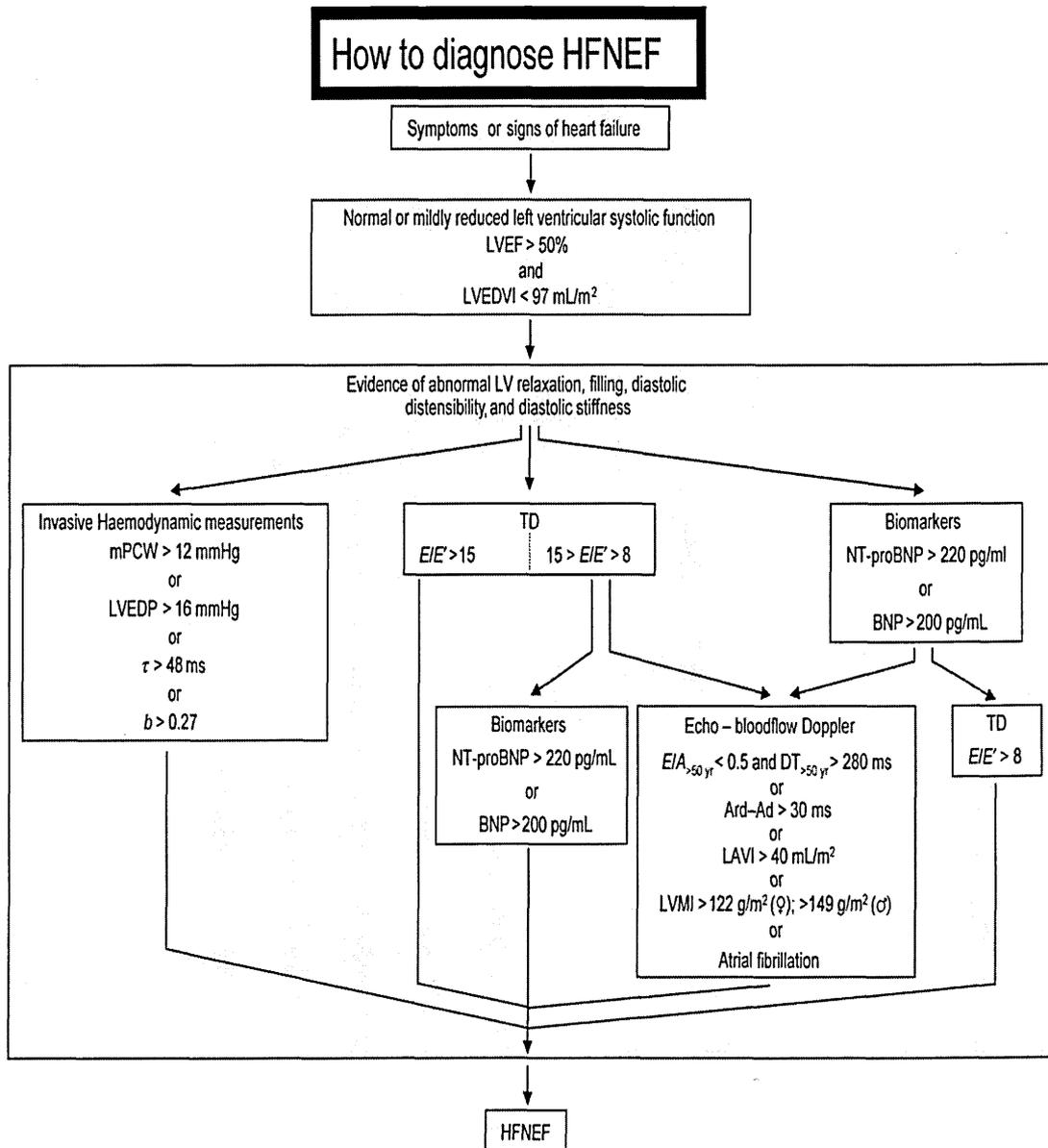
Class IV: Severe.

Unable to carry out activity without symptoms. Breathless at rest and usually housebound.

2.1.7: Diagnostic considerations for diastolic HF.

The following flowchart has been suggested for the accurate diagnosis of diastolic HF.

Figure 1. Flow chart for the suggested diagnosis of diastolic HF. (Adapted from Paulus et al, 2007).



There are three important criteria which all must be fulfilled in order to establish a diagnosis of heart failure with a normal ejection fraction. These are the presence of signs and symptoms of congestive heart failure, the presence of normal or mildly abnormal LV systolic function and finally, evidence of diastolic LV dysfunction.

In more detail, task force guidelines for the diagnosis and management of heart failure (Swedberg et al, 2005) have suggested that the primary symptoms associated with diastolic HF include lung crepitations, pulmonary oedema, ankle swelling, hepatomegaly, exertional dyspnoea and fatigue. Patients with diastolic HF often present with dyspnoea as the earliest symptom due to pulmonary congestion (Swedberg et al, 2005). In systolic HF, exercise intolerance is perhaps more applicable at the early stages due to reduced cardiac output, peripheral vasoconstriction and abnormalities at the level of skeletal muscle.

Consensus documentation suggests that an LV EF greater than 50% is considered to be a normal or mildly abnormal value for systolic function when measured using standard recommended techniques (Paulus et al, 2007). In conjunction with this however, for accurate assessment combined volumetric data of the LV should be obtained and indexed for body size (Paulus et al, 2007).

Objective evidence of diastolic dysfunction can be achieved using a number of parameters, some invasive (e.g. cardiac catheterisation) and others non-invasive (echocardiography and cardiac magnetic resonance imaging). Basic measurements of LV wall mass index may provide sufficient data to diagnose diastolic HF when tissue Doppler imaging (TDI) assessment is inconclusive or when plasma natriuretic levels are raised (Lang et al, 2006). Furthermore, blood flow Doppler parameters may be utilised during relaxation or diastole to provide further evidence of toward HF with a normal ejection fraction. Although blood flow parameters of diastolic dysfunction have received criticism, there is some evidence of the strength of these measurements. Combined use of mitral valve blood flow Doppler and pulmonary vein Doppler has resulted in 93% of patients with suspected HF with a normal ejection fraction showing

evidence of diastolic abnormalities (Badano et al, 2004). The more modern use of TDI has added further power to the assessment of diastolic parameters. Studies have suggested that E/e' greater than 15 correlates well with raised filling pressures and that values less than 8 suggest low filling pressure (Ommen et al, 2000). The close correlation of E/e' and filling pressure has also been proved in systolic HF with reduced EF below 50% (Nagueh et al, 1997). The gray zone E/e' of 8-15 values are suggested as inconclusive and must be supported by the use of other measurements to confirm or refute diastolic abnormalities. Left atrial volume measures, indexed to body surface area, are also pivotal to the assessment of diastole. Recent evidence shows that a raised left atrial volume index greater than 26 mL/m^2 can be utilised as a load independent predictor of LV filling pressures in patients with suspected HF with normal ejection fraction (Lim et al, 2006).

Assessment of natriuretic peptides, produced by atrial and ventricular myocardium has been shown to be valuable in the assessment of HF with normal ejection fraction. However, the sensitivity for this assessment is below 80% due to significant overlap between normal values and values obtained for patients with HF with normal ejection fraction. As such, current recommendations suggest the use of natriuretic peptides to exclude and not diagnose HF with normal ejection fraction, particularly when the patient presents with exertional dyspnoea. Natriuretic peptide measures must be combined with other investigations in order to gain 'stand-alone' evidence of diastolic abnormalities (Paulus et al, 2007).

2.1.8: Current treatment strategies for HF.

2.1.8.1: Ischaemic heart disease and viability.

This review has already established that in Westernised society, the most common cause of HF is ischaemic heart disease (IHD). An important facet of HF with coronary artery disease is the ability to predict the benefit of revascularisation in patients with ischaemic HF, a concept that is currently a challenge to Cardiology physicians and cardiac surgeons.

Current research evidence strongly supports the notion that LV function post myocardial infarction (MI) is an important predictor of prognosis (Buckley and Di Carli, 2011). It is important clinically to be able to differentiate between infarcted, necrosed, scarred myocardium and ischaemic myocardium that is dysfunctional but would improve following sufficient restoration of blood supply. Indeed, patients with dysfunctional but viable myocardium show substantial prognostic improvements with demonstrable attenuation of LV dysfunction following revascularisation techniques (Di Carli and Hachamovich, 2007).

There is a widely held notion that acute myocardial ischaemia rapidly results in LV contractile dysfunction that can persist for several hours after transient non-lethal ischaemia and eventually followed by restoration of myocardial contractility (Heyndrickx et al, 1975). Patients with coronary artery disease often demonstrate repeated episodes of demand ischaemia accompanied with episodes of myocardial dysfunction, cumulatively resulting in myocardial stunning and post-ischaemic LV dysfunction (Barnes et al, 2002). Historically, myocardial hibernation had been observed clinically in many patients. A cohort of patients with significantly impaired LV function who received coronary artery bypass surgery (CABG) surgery were noted to demonstrate improvements in contractility following revascularisation strategies. The underlying pathophysiology has been suggested to relate to severely reduced coronary

flow reserve and recovery of myocardial function in hibernating regions post revascularisation relates to restoration of adequate coronary flow reserve (Pagano et al, 2001). As a result of underlying pathophysiological mechanisms, clinically patients with similar reductions in LV systolic function may have significant differences in the extent of viable myocardium with even extreme wall thinned regions sometimes benefiting from revascularisation (Camici et al, 2008). The identification of viable myocardium becomes critically important prognostically when LV EF is reduced below 40% whereby 6 month mortality levels increase dramatically and decline steeply as EF decreases further (Multicentre post infarction research group, 1983).

Research has suggested that functional improvement post revascularisation relates to the amount of dysfunctional myocardium that proves to be viable (Carluccio et al, 2006). The study by Carluccio and colleagues demonstrated that patients with 6 or more dysfunctional LV regions which demonstrate viability show the greatest improvement in overall ejection fraction. Naturally, no improvement in ejection fraction is noted when there are no viable LV segments. Furthermore, significant prognostic benefit following revascularisation strategies have been shown in patients with severe ischaemic cardiomyopathy who demonstrate >20% of LV region viability when compared to patients with no viable myocardium (Afridi et al, 1998). Another study provides consistent results with the detection of myocardial viability being associated with a 79% decrease in cardiac mortality post revascularisation techniques compared to patients conservatively treated with medical therapy (Sicari et al, 2003). A comprehensive meta-analysis performed by Allman et al, 2002 evaluated the prognostic value of viability testing in 24 non-randomised studies between 1992 and 1999. This study reinforced the significant association between revascularisation and improved

survival rate in patients with LV dysfunction and evidence of myocardial viability independent of the imaging technique used for assessment.

The increased cardiovascular risk of diabetes mellitus further increases the challenge to Cardiology physicians when assessing for viability with regard to prognosis in this cohort. This is apparent in the research by Carson et al, 2002 which demonstrated significantly increased mortality rates for diabetic patients post revascularisation. Taking this into account however, there is research evidence to suggest that diabetic patients with viable myocardium have similar outcome to non-diabetic patients following revascularisation. Improvement in LV ejection fraction and attenuation of unfavourable LV remodelling was found to be similar in both diabetic and non-diabetic patients following revascularisation, together with overall prognostic outcome (Rizello et al, 2006). Furthermore, a study by Cortigiani and colleagues in 2007 showed that diabetic and non-diabetic patients with severe ischaemic cardiomyopathy had similar prognostic outcome following viability assessment and revascularisation with a 22% and 24% 4 year survival rate. Interestingly, in this study diabetes was found to be an independent predictor of mortality in patients treated conservatively with medical therapy.

Owing to the research evidence presented, both the European Society of Echocardiography and the European Association of Cardiothoracic Surgery endorsed the use of viability evaluation prior to revascularisation to assist in the management of patients with ischaemic heart failure.

The STICH trial viability sub-study, published in 2011 (Velazquez et al, 2011 and Bonow et al, 2011), demonstrated the opposite, confirming no impact of viability on

outcome of patients undergoing revascularisation when compared to medical therapy alone. Recent commentaries have however, suggested caution when interpreting the results of STICH (Cortigiani et al, 2012 and Perrone-Filardi and Pinto, 2012).

The main STICH investigation showed no significant all-cause mortality differences between patients with severe ischaemic LV impairment ($EF\% \leq 35\%$) undergoing CABG when compared to optimal medical therapy. Additionally, in the CABG group the secondary end-point of cardiovascular death was reduced by 19% with borderline significance. Composite endpoints of cardiac death and cardiovascular related hospital admission were significantly reduced by 26%. The authors concluded that the results do not definitively deny the advantage of CABG in LV dysfunction but rather provide provisional evidence favouring revascularisation in ischaemic heart failure.

The viability sub-study results were reported by Bonow and colleagues in the same journal (2011). This sub-study assessed the influence of viability on clinical outcome in patients assigned to medical therapy or revascularisation with CABG. The results, available only on half the initial STICH population, showed that survival of patients with viability was significantly longer than those patients without viability but following CABG these patients did not show any survival benefit when compared to medical therapy alone.

Invited perspectives and study commentaries (Cortigiani et al, 2012 and Perrone-Filardi and Pinto, 2012) have dissected the results of STICH and the viability sub-study. The research analysis of STICH by Bonow et al, 2011 clearly questions the guidelines and use of viability for assessment of suitability for revascularisation. Leading authority suggests such research findings are a setback, but when put in perspective the results

should not be seen as the final answer. Perrone-Filardi and Pinto, 2012 highlighted important limitations to the data collected in STICH. The limitations included a reduced final population use for data analysis (half of the main trial) with comparisons of 4 numerically unbalanced, sub-groups of patients with or without viability undergoing treatment with CABG or medical therapy – raising concerns over the statistical adequacy of the method to identify differences between subgroups. The definition of viability using single photon emission computed tomography (SPECT) was not validated in clinical trials and the power of SPECT to distinguish regional myocardial recovery was considered low. Dobutamine stress echocardiography (DSE) was used interchangeably with SPECT for viability assessment but there are well documented differences in accuracy for predicting myocardial recovery post revascularisation between the two techniques (Camici et al, 2008). In addition, Perrone-Filardi and Pinto suggest that both revascularisation and medical therapy can influence LV remodelling thus making the impact of viability on prognosis comparing CABG with medical therapy unclear; a factor which may be an explanation for the association between viability and increased survival during the study. A significant association between viability and the composite endpoints was apparent following adjustment for baseline differences, suggesting that revascularisation and optimal medical therapy may provide similar benefits to patients with viable myocardium. Commentaries conclude that although the STICH results represent a wake-up call for Cardiology, it should not close the door on myocardial viability assessment pre revascularisation but highlights the importance of new clinical trials and new diagnostic investigations (e.g. cardiac magnetic resonance imaging (MRI) or positive emission tomography (PET)) to develop improved and more refined criteria.

2.1.8.2: Pharmacological.

Until the late 1980's, the mainstay of treatment for HF relied on symptom relief of fluid congestion by the use of diuretics with or without digoxin. Since this time, newer medical therapies have been developed which impact on haemodynamics, neuro-endocrinology and inflammation. Several classes of these newer medications, unlike the earlier treatments regimes, have favourable impact on morbidity and mortality (National Clinic Guideline Centre, 2010). The morbidity and mortality rates in HF with evidence of impaired LV systolic function have declined through the cumulative effects of agents such as angiotensin converting enzyme (ACE) inhibitors, β blockers, aldosterone antagonists, arterial and venous dilators and angiotensin receptor blockers. This section of the review will briefly summarise the main breakthrough clinical trials that highlight the effective use of these classes of medications.

2.1.8.2.1: Angiotensin Converting Enzyme Inhibitors (ACE Inhibitor).

Completed systematic reviews of the use of ACE inhibitor therapy have demonstrated significant increase in life expectancy when compared to placebo in patients with HF. In addition, the observed increase appears more significant in patients with increasing severity of LV impairment and more severe symptoms. It should be noted however, that all NYHA classes show improvement in life expectancy following ACE inhibition (Flather et al, 2000). As a result of these improvements, ACE inhibition has also been demonstrated to reduce hospitalisation. The Acute Infarction Ramipril Efficacy Study (AIRE Study) with mean follow-up of 15 months after randomisation, demonstrated all-cause mortality reductions from 22.6% (placebo group) to 16.9% (treatment group). This represented an absolute mortality reduction of 5.7% and a relative risk reduction of 27% ($p=0.002$) (The AIRE Study Investigators, 1993). Further research, The Acute Infarction Ramipril Efficacy Extension Study, followed the same study population for an extra 3 years. During this study, death from all causes had occurred in 117 (38.9%)

of 301 patients randomly assigned placebo and 83 (27.5%) of 302 patients randomly assigned to ramipril. This demonstrated a relative risk reduction of 36% ($p=0.002$) and an absolute reduction in mortality of 11.4%. These figures correspond to 114 additional patients surviving for 5 years per 1000 patients treated for an average of 12.4 months (Hall et al, 1997). Further randomised controlled trials have shown that ACE inhibition can significantly improve quality of life in patients with HF (Beller et al, 1995). The effect of ACE inhibition on exercise performance has provided contrasting results. However, systematic reviews have demonstrated greater improvements in exercise performance in patients with more severe HF (Beller et al, 1995). As a result of these breakthrough clinical trials, National Institute for Clinical Excellence (NICE) Guidance for chronic heart failure (CHF) (2003) has concluded that treatment of HF with ACE inhibitors is cost effective. This is largely due to the costs saved from reduced re-hospitalisation.

2.1.8.2.2: Diuretics.

Although newer pharmacological therapies have evolved in the treatment of HF, diuretics remain an important contributor to patient treatment. As a consequence of their prolonged historical use in HF, there are currently no large scale randomised trials investigating their usage. However, there is a comprehensive systematic review of diuretic use in HF (Faris et al, 2002). This review showed that mortality rate was lower for patients treated with diuretics than for control (odds ratio for death, 0.25; 95% confidence intervals 0.07-0.84, $p = 0.03$). Furthermore, admission to hospital for worsening HF in four small trials (total patient number of 448) showed an odds ratio of 0.31 (95% CI 0.15 - 0.62, $p = 0.001$). In six small studies comparing diuretics to active control, diuretics significantly improved exercise capacity in patients with HF (odds ratio 0.37, CI 0.10-0.64, $p = 0.007$). The improvement in symptoms and exercise

capacity with diuretic use is as a result of improved fluid control and this can be achieved by using loop or potassium sparing diuretics or even a combination of both (NICE Guidance for HF, 2003). No formal economic evaluation of diuretics has been undertaken for their use in HF.

2.1.8.2.3: β -blockers.

Clinical trial data are prevalent investigating the effects of β -blockers in HF. A systematic review of 22 randomised controlled trials showed a favourable impact on mortality and hospital re-admission in HF patients receiving β -blocker therapy (Shibata et al, 2001). This review showed that death rates in patients randomised to receive β -blockers compared to controls were lower (458/5657 - 8.0% and 635/4951 - 12.8% respectively with odds ratio 0.63, 95% confidence interval of 0.55-0.72, $p < 0.00001$). Similar reductions were observed for hospital admissions for worsening HF in the β -blocker group (11.3 and 17.1%, respectively, odds ratio 0.63) and for the composite outcome of death or HF hospital admission (19.4 and 26.9%, respectively, odds ratio 0.66). The results of this systematic review show that β -blockers reduce the risk of mortality or the need for HF hospital re-admission by approximately 33%. More recently, clinical trials have been published which investigate the effects of selective and non-selective β -blockers in the treatment of HF and also the use of these agents in elderly patients. In older patients with HF, β -blockers have shown beneficial effects on all cause mortality (Deedwania et al, 2004), sudden cardiac death (Flather et al, 2005), re-hospitalisation (Deedwania et al, 2004 and Flather et al, 2005) and also quality of life as assessed by the Minnesota Living with Heart Failure questionnaire (MLHFQ) (Edes et al, 2005). Research comparing selective versus non-selective β -blockade has also been undertaken. This research has suggested that selective β -blockers are associated with significant increases in all cause mortality (39.5% versus 33.9% for non-selective,

relative risk of 1.17, confidence interval 1.06 – 1.28) and also sudden cardiac death (17.3% versus 14.4%, relative risk 1.2, confidence interval 1.01 – 1.41) (Poole-Wilson, 2003). Studies investigating quality of life with the Minnesota Heart Failure questionnaire (Sanderson et al, 1999) and adverse events (Poole Wilson, 2003) have shown no significant differences between selective and non-selective β -blockade.

2.1.8.3: Invasive procedures.

Pharmacological therapy is considered the mainstay of therapy for patients with HF. However, modern technological advances have led to the development of diagnostic and interventional invasive procedures which may provide benefit for sub-groups of patients with HF. A detailed review of these procedures and devices is beyond the scope of this review. However, there follows a short description of the major advances in this area.

2.1.8.3.1: Cardiac resynchronisation therapy.

Cardiac resynchronisation therapy (CRT) is a relatively new advance in the treatment of symptomatic HF following optimal medical therapy. Research has shown that CRT results in reduced morbidity and mortality in HF patients who demonstrate significant intra or inter-ventricular dyssynchrony (NICE Technology appraisal guidance 120, 2007).

2.1.8.3.2: Implantable Cardioverter Defibrillators.

Implantable cardioverter defibrillators (ICDs) can be utilised in HF patients who are prone to significant ventricular cardiac arrhythmias, either proven through ambulatory electrocardiograph monitoring or due to severely impaired LV performance. ICDs are

commonly combined with a CRT device. However, may also be used in isolation in selected cases (NICE Technology appraisal guidance 95, 2006).

2.1.8.8.3: Left Ventricular Assist Devices (LVADs).

Left ventricular assist devices (LVADs) are mechanical support systems for patients with end-stage HF. LVADs have been shown to improve patient survival, quality of life and functional capacity (Rose et al, 2001 and Slaughter et al, 2009). Currently in the UK, LVADs are approved as a bridge to transplantation (Clegg et al, 2005, Farrar et al, 1994). These devices have also been investigated as a bridge to decision (regarding suitability for transplantation) and destination therapy (Rose et al, 2001).

2.1.8.3.4: Cardiac Transplantation.

Ultimately, cardiac transplantation is the only long-term survival option for patients who demonstrate severe refractory symptoms of HF despite optimal medical and invasive mechanical therapy. Additionally, patients who develop refractory cardiogenic shock are also candidates for cardiac transplantation dependent on lifestyle and other co-morbidities. Details of transplant guidelines and techniques are however, beyond the scope of this review.

2.1.8.4: Rehabilitation.

Exercise rehabilitation has become commonplace in HF. A comprehensive and detailed review of exercise rehabilitation in HF is provided in section 2 of this thesis.

2.1.8.5: Importance of research into new modalities in heart failure.

There is a widely held notion within the medical community that despite technological advances in treatment of patients with HF, there is still high risk for hospitalisation and

death (O'Connor et al, 2012). The increased risk has been attributed to numerous factors which include ageing, disease progression, increase frequency of hospitalisation and high event rates following episodes of decompensated HF with up to 30% of patients experiencing adverse cardiovascular (CV) events or death following HF related hospital admission (O'Connor et al, 2005). There is no doubt that modern therapies have slowed the descent towards premature mortality with some increasing the survival trend. However, decline is inevitable and individual patients display marked variability in their prognosis and responses to treatment (Aaronson and Cowger, 2012). Untreated HF has been found to have approximately 50% survival at 12 months (Ho et al, 1993). In addition, the overall prognosis for patients with HF receiving treatment is approximately 50% at 5 years, a value that has varied little in the past 20 years (Levy et al, 2002).

Clinical trial data in HF can be misleading. Although trials do show initial improvement in symptoms, therapies do not stop but rather prolong cardiovascular related mortality. Evidence of this is available from data from numerous clinical trials. Longitudinal data from the V-Heft 2 study of 403 patients with HF being treated with ACE inhibition (Enalapril) showed that 132 of the study population had died following 2.5 years of drug administration. Of these deaths, 107 (81%) were found to be due to complications relating to congestive HF and there was physiological evidence of clinically relevant decline due to the reduced VO_{2max} values achieved in the treatment group at follow-up visits (Cohn et al, 1991). Another trial involving the use of ACE inhibition compared treatment with placebo in a population of patients with asymptomatic LV systolic impairment (Cohn et al, 1991). During this trial (SOLVD prevention study), 30% of the initially asymptomatic patients had developed symptoms of HF at 3 year follow-up. In addition, at this time-point, there were 313 deaths of which approximately 60% were

related to HF. The CONSENSUS trial (CONSENSUS trial study group, 1987) clearly showed that HF is associated with poor prognosis. Ten year follow-up data of this study was produced by Swedberg et al 1998. The initial study as reported earlier in this review, showed relative mortality reduction of 40% at 6 months and also 20% at 12 months. Ten year data show average survival of 781 days for the treatment group when compared to 521 days for the placebo group. 5 survivors only remained in the treatment group after 10 years and there was convergence of treatment and placebo curves on Kaplan-Meier plots.

Malkin and Channer (2005) reviewed many of the major trials investigating medical therapy in HF and using Kaplan-Meier plots calculated extension to survival, morbidity extension and whether the Kaplan-Meier curve was divergent, divergent-convergent or divergent parallel. Survival extension was calculated using previous work by Torp-Pederson et al (1999) by passing a line through the y axis and curves at the point of the 50th centile patient. The time difference between treatment and placebo curves on the x axis demonstrates this difference in median lifetimes (Malkin and Channer, 2005). Table 6 demonstrates the life and morbidity extension values together with Kaplan-Meier curve shape for the major clinical trials for HF medical therapy.

Table 6. Life extension and morbidity extension values together with Kaplan-Meier curve shape in the major clinical trials for HF medical treatment (adapted from Malkin and Channer, 2005).

Type of therapy.	Survival	Morbidity	Trial reference.	Type of
	extension	extension		curve
	time	time		appearance
	(months).	(months).		(Kaplan-
				Meier).
ACE inhibitor.	12	11	SAVE	Div
Post MI (no HF)			(Pfeffer et al, 1992).	
ACE inhibitor.	15.3	22	TRACE	Div/Conv
Post MI (NYHA 1-2).			(Torp-Pederson et al, 1999)	

	1	-	ISIS-4	Div
ACE inhibitor.				
Post MI (NYHA 3-4).			(4 th International Study of Infarct Survival Collaborative Group, 1995).	
ACE inhibitor. (NYHA 1-2).	0	14	SOLVD Prevention. (SOLVD Investigators, 1992).	Div
ACE inhibitor. (NYHA 2-3).	6	15	SOLVD Treatment. (SOLVD Investigators, 1992).	Div/Conv

ACE inhibitor.	9	-	CONSENSUS.	Div/Conv
(NYHA 3-4).			(Swedberg et al, 1998).	
B-blocker.	6	6	MDC.	Div/Conv
(NYHA 2-3).			(Wagstein et al, 1993)	
B-blocker.	6	9	Merit-HF.	Div/Conv
(NYHA 2-3).			(MERIT-HF	
			Research Group, 1999).	
B-blocker.	9	-	CIBIS-2.	Div
(NYHA 2-3).			(CIBIS-2	
			Investigators, 1999).	
B-blocker.	8	-	COPERNICUS.	Div
(NYHA 3-4).			(Packer et al, 2001).	

Spironolactone.	12	-	RALES.	Div
Angiotensin-2 Agonists.	0	4	(Pitt et al, 1999). Val-HeFT. (Cohn et al, 2001).	Div
Digoxin.	0	16	DIG. (Digitalis Investigation Group, 1997).	Parallel
Amiodarone.	6	-	GESICA. (Doval et al, 1994).	Div/Conv

Div (divergent), Conv (convergent).

Table 6 re-affirms the notion that despite recent medical pharmacological therapy, prognosis in HF remains poor. According to Malkin and Channer, 2007, current treatments provide an average extension to life expectancy of around 20 months. In more detail, the authors state that ACE inhibition prolongs life for around 9 months, addition of B-blocker adds approximately a further 7 months and finally adding spironolactone may confer 12 months more survival.

Of course, medical pharmacological therapy is not the only technological advance available to HF patients in order to attempt to increase life expectancy. More invasive procedures involving CRT devices + or – ICD, ventricular reconstructive surgery, LVAD implantation and cardiac transplantation are also other options for advanced severe HF patients already established on optimal medical therapy.

The CARE-HF trial (Cleland et al, 2005) randomised patients with class III and IV HF, LV dysfunction and evidence of ventricular dyssynchrony to either CRT with medical therapy or medical therapy alone. 404 patients were assigned to receive medical therapy with 409 patients randomised to CRT with medical therapy. These patients were followed up for a mean of 29 months (range 18-44 months). The composite primary end-point was death or unplanned hospital admission with a severe cardiovascular event. 159 patients reached this end-point in the CRT with medical therapy group and 224 patients reaching this in the medical therapy group. In addition, there were 222 unplanned cardiovascular related hospital admissions in the CRT plus medical therapy group when compared to 384 in the medical therapy alone group. CARE-HF clearly indicates that CRT plus medical therapy is able to reduce hospitalisation and death over the course of the follow-up period. However, there is still a substantial number of

participants in the CRT plus medical therapy group reaching composite study endpoints.

It is a widely accepted notion that only around 60-70% of patients receiving CRT devices together with medical therapy respond with clinical improvement probably resulting from lack of physiological and clinical indicators for device suitability (Hawkins et al, 2006). Current guidance suggests that CRT device implantation is warranted with electrocardiographic evidence of cardiac dyssynchrony. However, some patients do not have co-existent mechanical dyssynchrony and as such, may not benefit from device implantation (Vardas et al, 2007). As studies involving CRT therapy recruited patients with broad ≥ 120 ms QRS complex, there is no available data for patients with NYHA class III and IV HF with normal or slightly prolonged QRS duration. In addition to this data, CRT therapy is invasive and can result in potentially serious complication. For instance, during the CARE-HF trial procedure related complications included 24 patients with lead displacement, 10 patients with coronary sinus dissection, 8 patients with pacemaker pocket erosion, 6 patients with pneumothorax and 3 patients with pacemaker related infection.

Currently the only validated treatments for end-stage HF are mechanical circulatory support using LVAD's or heart transplantation. The major limitation for heart transplantation is availability of suitable organs resulting in long waiting times. At least 10% of patients actively waiting on the transplant list die each year before a suitable donor organ is discovered (Lund et al, 2010). Transplantation itself is associated with 90% survival at 1 year and 60% survival at 10 years with evidence of absence of symptoms in survivors during follow-up (Taylor et al, 2007). Unfortunately, transplantation may not be suitable due to a multitude of risk factors, contraindications

and co-morbidities. Factors which may dramatically increase the risk of transplantation include; cardio-renal syndrome namely that impaired renal function is an independent predictor of mortality post transplantation (Ganesh et al, 2004). Hyponatraemia carries a poor prognosis across all HF severities (Gheorgiade et al, 2007). Abnormal liver function defined by elevated bilirubin is a predictor of mortality in chronic HF and also post transplantation (Allen et al, 2009). Pulmonary hypertension is associated with dramatically increased risk of right heart failure post transplant (Taylor et al, 2009) and, if irreversible, is a major contraindication to transplant. Co-morbidities that increase risk, may contraindicate transplant or impact on survival post transplant include age (Taylor et al, 2009), diabetes (Mehra et al, 2006), symptomatic peripheral or cerebrovascular disease (Bravata et al, 2003), sepsis / active infection, recent pulmonary embolism, active malignancy, auto-immune disorders, substance abuse including tobacco, psychological factors associated with adherence to treatment (Banner et al, 2011). Patients who are deemed suitable physiologically for transplant must be psychologically able to commit to a lifelong programme of monitoring and drug treatment.

Recent advances in mechanical circulatory support are providing alternatives for patients waiting for heart transplant (as a bridge to transplantation) and also for patients in who transplant is contraindicated (destination therapy). In addition, LVAD therapy may be suitable for patients in whom ventricular function is predicted to improve (bridge to recovery) (Lund et al, 2010). Outcome related to LVAD implantation is dependent upon the type of device implanted, surgical experience, device and patient characteristics (Lund et al, 2010). If patients are selected well against recognised criteria, operative mortality is approximately 5-10% (Miller et al, 2007). Other research has displayed improved survival to transplant from 33-70% due to improvements in

device function and increased knowledge regarding implantation and contraindications (Frazier et al, 2001). 1 year survival following implant is between 50 and 80% (Miller et al, 2007). Again, in comparison to transplantation, there are numerous contraindications related to LVAD implant. These include acute cardiogenic shock, non-systolic HF, co-existing illness with reduced life expectancy, severe co-morbidity (e.g. renal disease, cancer, severe liver disease, severe COPD), systemic infection or infection risk, active bleeding, reduced platelet count, right HF not secondary to left HF, severe right ventricular (RV) impairment, multi-organ failure, moderate or worse aortic insufficiency, mechanical aortic valve replacement (AVR), LV thrombus, hypertrophic cardiomyopathy, ventricular septal defect (VSD), congenital heart disease, intolerant to anti-coagulation, obesity, psychosocial limitations to adherence to device (Lund et al, 2010). Device failure in more modern devices is relatively rare due to a smaller number of moving parts. However, there are still minor risks for mechanical failure of parts, thrombus formation or battery failure – all of which could be catastrophic.

This section of the review clearly identifies technologically advanced medical pharmacological, mechanical and surgical therapy for HF treatment. However, these are not without their limitation and / or significant risk and side-effects. As a result, it is of paramount importance that further therapeutic targets are actively researched to reduce symptoms and increase life expectancy in HF.

Testosterone deficiency.

2.3: Testosterone.

2.3.1: Biochemistry and biosynthetic pathways.

In human males, testosterone is the major circulating androgen. The testes secrete approximately 6-7 mg of testosterone per day and this equates to 95% of total production of testosterone by the body (Coffey, 1988). The metabolic processes for conversion of cholesterol into androgens is facilitated within up to 500 million Leydig cells comprising a small proportion of total testicular overall volume (Rommerts, 2005). In addition to Leydig cell production testosterone can be produced within the adrenal cortex and also, in miniscule amounts, by brain cells (Baulieu, 1997).

Cholesterol may be synthesized *de novo* from acetate but it also may be synthesized from plasma lipoproteins and according to Freeman and Rommerts, 1996, for human Leydig cells the low density lipoprotein (LDL) fraction is the predominant extracellular store of cholesterol. For Leydig cells, cholesterol situated within the plasma membranes is the most widely available pool and is transported into the mitochondria by a vesicle-mediated endosomal / lysosomal network system (Rommerts, 2005). In addition, van Noort et al, 1988) have suggested that cholesterol transport within the mitochondria may be facilitated by a sterol carrier protein₂ in conjunction with the cytoskeleton and vesicular system. Although the exact mechanisms for cholesterol cell transport are uncertain, the process results in the availability of cholesterol (C₂₇) within the mitochondria to initiate the production of pregnenolone (C₂₁). This cleavage of the side chain of cholesterol and the resultant development of pregnenolone within the mitochondria, probably under the influence of luteinising hormone, marks the initiation of the steroidogenic cascade (Rommerts, 2005). In normal conditions, human pregnenolone converting enzyme systems are insufficient for the total conversion of

pregnenolone to testosterone. Therefore, intermediates of progesterone derivatives leak from Leydig cells. As a result of this, the rate-limiting step for testosterone production is at the endoplasmic reticulum, but the rate determining step for steroidogenesis sits within the mitochondria governed by cholesterol side chain cleavage activity (Rommerts, 2005, van Haren et al, 1989).

Cholesterol side chain cleavage enzyme (P450_{scc}) is located within the inner-membrane of the mitochondria and as mentioned earlier in this section, governs the initiation of the steroidogenesis cascade (Rommerts et al, 2005). Important factors regulating the generation of pregnenolone from cholesterol include cholesterol availability at the inner mitochondrial membrane, amount and/or availability of oxygen, P450_{scc} enzyme activity and the delivery of reducing equivalents to the P450_{scc} (Rommerts et al, 2005). Simply, the generation of steroids from cholesterol is mainly dependent on the supply of the substrate to the enzyme P450_{scc} and the amount of this enzyme situated within the mitochondria. It has been previously noted that P450_{scc} concentration is regulated by luteinising hormone and short-term regulation of the rate of steroidogenesis is facilitated by the intra-cellular transfer of cholesterol from the outer to inner (cholesterol deficient) mitochondrial membrane. This intra-cellular cholesterol trafficking has been shown to be dependent on a labile steroidogenesis activator protein (StAR) –not only in Leydig cells but also adrenal and ovarian cells (Stocco, 2001).

As stated earlier in this review, pregnenolone is the initial product of the cholesterol side chain process. Pregnenolone is biologically inactive and further metabolised by enzymes present in the endoplasmic reticulum (Rommerts, 2005). The conversion of C₂₁-pregnenolone to C₁₉ steroids via the enzymes 17-hydroxylase and C₁₇, 20-lyase has been shown to occur in a single protein, P450_{C17}, coded by the gene CYP17. However,

there is different expression of enzyme activities in the testes and adrenal glands, which is dependent upon the micro-environment of the enzyme in the endoplasmic reticulum (Zuber et al, 1986). Due to a high number of steroid-converting enzymes there are a number of different pathways to convert pregnenolone to testosterone. However, in the testes, most steroids are formed via the $\Delta 5$ -pathway with dehydroepiandrosterone as the initial C₁₉ intermediate (Rommerts et al, 2005). The final step of the biosynthetic pathway of testosterone is the reduction of the 17-keto-group by the 17 β -hydroxysteroid dehydrogenase (17 β HSD), represented by a number of different isoforms present in many tissues (Andersson and Moghrabi, 1997). 17 β HSD type 2 enzyme also possesses 20 α HSD activity and in the testes this type 3 isoform is present mostly in Leydig cells (Rommerts, 2005). As such, deficiency in testicular activity of 17 β HSD is accountable for the majority of testosterone deficiencies in human models (Geissler et al, 1994).

Normal testicular function is maintained by luteinising hormone and follicle stimulating hormone. In addition, Leydig cell function is primarily controlled by luteinising hormone (Rommerts, 2005). Chemes et al, 1996 suggest that although hormones regulate steroid production by controlling metabolic activities in existing cells, they are also integral to the control of the size of the Leydig cell population. In the foetus, there is a steep increase in Leydig cell population and activity. Furthermore, during the early neonate period, gonadotropins stimulate the development and activity of foetal Leydig cells resulting in similar peripheral and testicular levels of testosterone. During the rest of the first year of life, foetal Leydig cells degenerate, remaining dormant until puberty. During puberty, rising levels of plasma luteinising hormone stimulate further Leydig cell proliferation and differentiation resulting in adult levels of Leydig cells (Chemes, 1996).

Human choriogonadotropin (hCG) and luteinising hormone are both able to stimulate the luteinising hormone receptor (luteinising hormone being the natural ligand for luteinising hormone receptor). However, the binding properties of these ligands are significantly different. Wang et al, 2000 have shown that activated luteinising hormone receptors stimulate adenylyl cyclase via GTP binding proteins, resulting in the production of cyclic AMP (cAMP). cAMP has been shown to increase steroid production. However, low concentrations of luteinising hormone stimulate steroidogenesis without traceable increase in cAMP concentration possibly suggesting that cAMP is only a second messenger of the action of luteinising hormone (Rommerts, 2005). In addition to this, phospholipids, specific phospholipases, products of phospholipid metabolism, calcium ions and calmodulin are vitally important for steroidogenic activity and signal transduction (Wang et al, 2000). Research into human models of signal transduction pathways is limited. However, research with rodent models is more abundant. In these rodent models, the activation of various signal transduction pathways in Leydig cells results in activation of numerous classes of protein kinases and kinase related pathways (Richards, 2001). Of these pathways, the protein kinase-A route has assumed most importance in controlling the promoter regions of most of the genes involved in mediating the trophic effects of luteinising hormone. Additionally, trophic control of steroidogenic activities within the mitochondrial matrix and smooth endoplasmic reticulum is thought to be achieved by regulation of the biosynthesis of steroidogenic enzymes via increased levels of mRNA's (Rommerts, 2005).

Leydig cells in the testis are surrounded by cells of the seminiferous tubules (e.g. Sertoli cells) or cells in the interstitial tissue (e.g. macrophages). Numerous authors have suggested that these cells have an ability to influence the function of Leydig cells in a

paracrine manner (Saez, 1989, Saez, 1994, Gnessi et al, 1997). In more detail, follicle stimulating hormone may facilitate the production of Leydig cells, most possibly via Sertoli cell products. Additionally, conditioned media from Sertoli cells or seminiferous tubules is able to modify the steroidogenic activity of Leydig cells. Consensus exists that four criteria must be fulfilled if individual local secretion products are to be considered as potential paracrine factors. These are that the molecule should regulate at least one biological activity of the target cell, the molecule must be secreted in sufficient quantities to promote a physiological response, regulation of secretion of the molecule must be possible and changes in the local concentration of the molecule must influence *in vivo* target cell properties (Rommerts, 2005).

Androgen formation from pregnenolone occurring in the smooth endoplasmic reticulum of Leydig cells results from the interaction of membrane bound enzymes with steroids as mobile elements (Rommerts, 2005). Related to this concept, Mendel, 1989 states that movement of steroids within tissues depends mostly on diffusion. Importantly, binding proteins for androgens or other steroids play an important role in decreasing the concentration of unbound steroids outside of the cell and therefore, increase the diffusion process without effecting steroid trafficking within the cell. According to van Doorn et al, 1974, pregnenolone, progesterone and testosterone are able to pass the Leydig cell membranes and together with this are also able to equilibrate rapidly between testicular compartments. In relation to this, Maddocks and Sharpe, 1989, suggest that most of the unconjugated steroids diffuse from the interstitial space to the blood, leaving the testis via venous flow by the principles of concentration gradients and flow rates of the various fluids.

Peripherally, steroids equilibrate quickly between organs and the blood. In support of this notion, Wang et al, 1981 showed that salivary free testosterone concentration is almost identical to blood. Testosterone concentration in tissues and fluids depends largely on the availability of binding proteins such as sex hormone binding globulin (SHBG) and albumin. Mendel, 1989 iterates that binding proteins within body fluids act as a storage device for steroids which have an increased rate of metabolism during passage of blood through the liver. It should be noted however, that SHBG and albumin are not essential factors for steroid homeostasis (Mendel et al, 1989). O'Malley elaborates on this concept by stating that changes in peripheral free testosterone concentration can be sensed directly by androgen receptors if there is equilibrium between exterior and interior of androgen target cells. Furthermore, steroid receptors may become partially activated by phosphorylation and it is plausible that predisposed receptors may become further activated by binding small amounts of steroid (O'Malley et al, 1995).

Put simply, the level of biologically active steroid within the body is based on supply and demand with rate of synthesis equal to that of degradation. Supply is dependent on the rate of inward transport of active steroid whereas removal depends on outward transport and the overall rate of degradation (Rommerts, 2005). The following important factors have been shown to influence the control of steroid levels in target cells. The flow volume and rate of blood or lymph, release from binding proteins, membrane transport, connective tissue and cell layers (sometimes have the ability to inactivate steroids) are all important factors outside the target cell. Within the target cell, local activation or inactivation reactions and outwards transport (Rommerts, 2005).

Testosterone can be metabolised in many ways. Aromatisation of the Δ^4 bond of testosterone results in derivation of 17β -estradiol and 5α -dihydrotestosterone (Rommerts, 2005). In oestrogen dependent target cells, the rate of synthesis of estrogens is highly influenced by target cell aromatase activity and the supply of androgen substrate. For testosterone conversion to 5α -dihydrotestosterone, the two isoforms of 5α -reductase must be of sufficient concentration in combination with slow dihydrotestosterone metabolism (Rommerts, 2005). Furthermore, George et al, 1997 have shown that in prostate tissue, the primary oxidative enzyme 17β -hydroxysteroid dehydrogenase is able to convert testosterone to androstenedione, particularly when 5α -reductase activity is inhibited. Luke and Coffey, 1994 reported high activity of 3α HSD and low activity of 5α -reductase in order to efficiently optimise the amount of testosterone for testosterone-dependent receptor stimulation within muscle tissue. In skin and hair follicle target cells, conversion of testosterone to dihydrotestosterone is dependent on the balance of catabolism by reducing 3α or 3β -steroid dehydrogenases versus glucuronidation (Rittmaster, 1994).

Degradation of androgens in tissue is determined by the profile of those enzymes responsible for the process (Rommerts, 2005). As a result, the enzymes 5α and 5β -steroid reductases, 17β -hydroxysteroid dehydrogenase together with 3α and 3β -hydroxysteroid dehydrogenases assume importance. Steroid metabolites that are not bound to nuclear steroid receptors are still biologically active and as such, metabolism and catabolism should not be viewed as a pathway for excreting inactive steroids (Rommerts, 2005). Some androgen metabolites may be conjugated whilst others may be excreted as free steroids.

More recently, research evidence has been collected suggesting a non-genomic action of androgens away from the direct activation of deoxyribonucleic acid (DNA). Heinlein and Chang, 2002, have showed that androgens are able to activate transcription-independent signalling pathways, Gue et al, 2002 have established rapid effects of androgens on calcium fluxes and Castoria et al, 2003, have shown that androgens affect intracellular phosphorylation cascades. It should be noted that the non-genomic actions of steroids have not been as extensively studied as genomic actions. However, there is strong evidence that the nucleus is not the only direct source of action for steroids. In a typical non-genomic model, criteria, similar to those afore-mentioned in the genomic model, must be adhered to. The effects should occur within a timescale of seconds to minutes and not prolonged to several hours following steroid exposure which is typical for a genomic model (Cato et al, 1988). The response must be membrane mediated and display an action that can be induced even when the steroid is conjugated to molecules which do not directly facilitate cell entry. An interesting example of this is testosterone and the capacity for it to bind with albumin (Foradori et al, 2008). The nature of the response must be lacking transcription / translation machinery activation, hence demonstrating that the steroid response can be initiated in systems where gene transcription or protein synthesis is improbable (Foradori et al, 2008).

In research, the most consistently demonstrated non-genomic effect of androgen exposure is a rapid change in Ca^{2+} (Gue et al, 2002, Lieberherr et al, 1994, Gorczynska and Handelsman, 1995). This calcium modulation has been shown to occur rapidly within seconds to minutes and because of this, it has been suggested that the androgen must bind to a receptor on the surface of the cell. Currently, debate is underway regarding the nature of this cell. Some research suggests that the classic intra-cellular androgen receptor coupled with signal transduction pathway may be the route

(Gorczyńska and Handelsman, 1995) whereas, other authors have suggested the role of a unique protein with androgen binding capability may initiate signal transduction cascades (Steinsapir et al, 1991).

Perhaps the most pertinent physiological example to this review of a non-genomic action of androgens on Ca^{2+} are the effects on the cardiovascular system. It has been shown that administration of androgens can facilitate rapid aortic and coronary arterial vasodilatation (Yue et al, 1995). Furthermore, administration can also facilitate vasoconstriction (Masuda et al, 1991). Ceballos et al, 1999 have added to this by suggesting a non-genomic effect of testosterone, exerted at the level of the cell membrane. The authors infused testosterone into rodent coronary arteries resulting in rapid increases in vascular resistance and complete attenuation of the effects of vasodilatory agents. In addition to this, other authors have showed that testosterone is associated with a rapid Ca^{2+} increase in cardiac myocytes through the activation of a plasma membrane androgen receptor associated with the PTX-sensitive G-protein-PLC/IP3 signalling pathway (Vicencio et al, 2006).

Verbist et al, 1991, using fluorescent resonance energy transfer, have demonstrated a direct interaction between negatively charged phospholipids and membrane ATPase calcium pumps. This suggests that androgen metabolites may acquire additional charges from sulphate residues enabling them to infiltrate the lipid / protein complex of the cell membrane decreasing the membrane flexibility, modulating the actions of the enzymes responsible for ATP hydrolysis (Zylinska et al, 1999). In further support of this concept, testosterone and dihydroepiandrosterone, as hydrophobic steroids, have been shown to interact with membrane phospholipids in order to manipulate membrane fluidity (Duval et al, 1983).

Androgen receptors (AR) have demonstrated an ability to activate second messenger pathways independent of their transcriptional activity (Foradori et al, 2008). The tyrosine kinase proto-oncogene tyrosine-protein kinase (c-Src) is generally targeted to the inner surface of the plasma membrane and has been shown to interact with androgen receptors. This suggests the necessity of a membrane androgen receptor to facilitate this process (Foradori et al, 2008). To further support this notion, Migliaccio et al, 2000, have shown that the association of an androgen receptor with the SH3 domain of c-Src stimulates c-Src kinase activity in less than a minute in the androgen sensitive human prostate adenocarcinoma (LNCaP) prostate cancer cell line. In prostate cancer cells, the AR/Src/modulator of non-genomic action of oestrogen receptor association and subsequent mitogen activated protein kinase family activation has been found to be androgen dependent and also independent (Unni et al, 2004). Related to the mitogen activated protein kinase family signalling cascade, Kousteni et al, 2001, have suggested that androgen treatment may result in stimulation of two important members murine leukaemia viral oncogene homolog-1 (Raf-1) and mitogen activated protein kinase-2 (ERK-2) within a window of approximately 5 minutes. This androgen induction of c-Src/Raf/ERK signalling is impeded by c-Src kinase activity inhibition or treatment with androgen receptor antagonists (Migliaccio et al, 2000). The same authors conclude that androgens work through the known androgen receptor in order to activate a non-genomic second messenger pathway and can also function together with oestrogen receptors in order to induce c-Src kinase activity as part of a complex composed of c-Src, oestrogen receptors and the androgen receptor.

SHBG, a glycoprotein derived from the liver, is able to bind to testosterone, dehydroepiandrosterone and estradiol (Mean et al, 1977). Previous research has

suggested that serum testosterone is mainly bound to SHBG (~60%) and cell surface receptors for this glycoprotein have been identified in numerous tissues including the prostate, testis, breast and liver (Krupenko et al, 1994). The SHBG receptor is able to facilitate androgen activation of cAMP and phosphokinase (Foradori et al, 2008). As a result of this, it is feasible that androgen-SHBG stimulation of phosphokinase may alter the phosphorylation of androgen receptors and co-regulators, therefore modulating androgen receptor transcriptional activity (Foradori et al, 2008).

Research work has suggested that androgens may play a role as a membrane receptor because of the detection of specific androgen binding to plasma membranes of numerous cell types. Such cells include the endothelium (Figuroa-Valverde et al, 2002), breast and prostate cancer cells (Hatzoglou et al, 2005, Kampa et al, 2002), osteoblasts (Armen et al, 2000), macrophages (Guo et al, 2002) and T-lymphocytes (Benton et al, 1997). To this date, the assumed membrane androgen receptor has yet to be identified and it cannot be accurately determined whether the modulation of Ca²⁺ ion channel activity are mediated by a specific androgen receptor or by other signalling pathways (e.g. SHBG or c-Scr kinase-androgen receptor complex) (Foradori et al, 2008).

Androgens have been shown to have key involvement in the human reproductive system (Foradori et al, 2008). In particular, androgens are fundamental to neuro-endocrine control of the gonadotropin releasing hormone. Previous work has already shown that androgens are able to inhibit hypothalamic luteinising hormone secretion by the release of gonadotropin releasing hormone (Foradori et al, 2008). However, although androgens affect pituitary sensitivity to gonadotropin releasing hormone, research has consistently identified a neural component for the androgen regulation of

lutinising hormone secretion without a specific neural site of action (Kalra et al, 1989). Research has suggested that androgens may be affecting gonadotropin releasing hormone secretion via trans-synaptic pathways. To elaborate on this, research by Belsham and colleagues (1998) have suggested a direct action of androgens on gonadotropin releasing hormone neurons by observing the classic androgen receptor in the plasma membrane of the GT-1 hypothalamic cell line. Non-genomic actions of androgen receptors have been suggested in GT1-7 neurons and administration of dehydrotestosterone and testosterone have all resulted in significant elevation in Ca^{2+} in GT-1 cells within 200 seconds (Shakil et al, 2002). This finding is consistent with previous research which has stipulated that Ca^{2+} changes are key in the control of pulsatile gonadotropin releasing hormone secretion (Shakil et al, 2002).

2.3.2: Incidence of testosterone deficiency.

As males age, there is a gradual decline in circulating bio-available testosterone (Bettocchi, 2005). There is a general consensus that testosterone levels decline about 1% per year from as early as age 30 years. Noticeable declines are common following an age of 50 years but there is great inter-patient variability (Morales and Lunenfeld, 2002). The Hypogonadism in Males study (Mulligan et al, 2006) estimated the prevalence of hypogonadism in men aged 45 years or greater visiting primary care practises in the United States. This research identified that the prevalence of hypogonadism (i.e. testosterone level <300ng/dl) was estimated to be 37.8% - 836 patients of the 2162 sampled. Furthermore, data collected from the Centres for Disease Control and Prevention National Health Interview Survey has indicated that 74% of adult men in the United States visit a general practitioner (GP) surgery annually (Lethbridge-Cejku et al, 2004) and in 2003, 48.4 million males were aged 45 years of greater in the United States. The authors extrapolated this data and estimated that 13.8

million men aged 45 years or greater (who had visited a primary care doctor) may be hypogonadal. Additional research has also attempted to clarify the prevalence and incidence rates of androgen deficiency in a random sample of middle aged and older males (Araujo et al, 2004). This research also used three signs / symptoms of hypogonadism together with total testosterone (TT) <200 ng/dL or 200-400 ng/dL with free testosterone (FT) <8.9 ng/dL to stratify androgen deficiency. Studying initially 1691 patients and a further 1087 at follow-up, it was noted that the crude prevalence of androgen deficiency rose from 6.0% to 12.3% in males aged 40-69 years old. Furthermore, it was estimated that 2.4 million US males in this age range with androgen deficiency. The crude incidence rate of androgen deficiency was defined as 12.3 per 1000 person years with significant increases with advancing age. Finally, it was estimated that in the United States there would be 481,000 new cases of hypogonadism per year in males aged 40-69 years.

2.3.3: Aetiology of hypogonadism.

In normal healthy males, multiple neural inputs, endogenous opioids, testosterone, estradiol and other factors influence the hypothalamic secretion of gonadotropin releasing hormone. Additionally, these factors influence the ability of gonadotropin releasing hormone to facilitate secretion of luteinizing hormone (Reyes-Fuentes and Veldhuis, 1993). Release of gonadotropin releasing hormone is pulsatile and reaches the pituitary gland via the hypothalamic pituitary portal venous system which in turn is modulated by neural input (dopaminergic pathways) and neurotransmitters (e.g. galanin, neuropeptide Y and opioids). This stimulates episodic secretion of luteinizing hormone (Reyes-Fuentes and Veldhuis, 1993). The secretion of luteinizing hormone reaches the testes via the systemic circulation thus promoting tonic and episodic Leydig cell secretion of testosterone. Any unbound plasma testosterone acts upon target tissues

completing the feedback loop to inhibit gonadotropin releasing hormone and luteinizing hormone secretion (Reyes-Fuentes and Veldhuis, 1993).

Various research studies have attempted to identify the important factors affecting testosterone concentrations in healthy elderly males. There have been some reports of circannual variations in testosterone level with increases of up to 30% noted from October to December in the Western hemisphere (Smals et al, 1976). However, this research was unable to differentiate between other confounding variables such as climate and latitude. In addition to this, other research has noted peak serum testosterone levels during spring and summer and also no significant seasonal variation (Svartberg et al, 2003).

Research studies of twins have identified that genes can determine as much as 25-76% of the total variation in plasma levels of gonadotropin, testosterone, free testosterone, estradiol and estrone (Meikle et al, 1988). These studies suggested a strong genetic influence in the tissue formation and production rate of dihydrotestosterone. Some authors have identified an ethnic variation in TT and SHBG levels in African males when compared to Caucasian males (Gapstur et al, 2002). This research identified a slightly higher percentage of these levels in African males. However, following adjustment for body composition and adiposity these small differences were nullified. Other research investigating the effects of ethnicity on free testosterone in Caucasian and Asian males has found no significant differences (Lookingbill et al, 1991).

More recently, scientists have identified possible important genetic correlations affecting testosterone concentration. In more detail, this research has focussed upon AR gene polymorphism. The AR gene contains a polymorphic trinucleotide CAG repeat in exon 1. This CAG repeat encodes a physiologically relevant polyglutamine tract of variable length. The research work suggested that any CAG length greater than normal (15-31) would result in diminished AR transactivation capacity and androgen resistance (Chamberlain et al, 1994). Conversely, shorter AR CAG repeat lengths have been associated with higher prevalence of androgen sensitive diseases e.g. prostate cancer (Giovannucci et al, 1997) and with greater decline of serum and bio-available testosterone (Krithivas et al, 1999). In contrast, other research studies investigating Australian and Chinese males (Jin et al, 2000) Belgian males (Van Pottlebergh et al, 2001), German males (Zitzmann et al, 2001) and Finnish males (Harkonen et al, 2003) have failed to demonstrate a significant relationship between AR CAG repeat length and levels of androgen hormones. This suggests a lack of truly consistent findings attributing a substantive role of the AR CAG repeat in elderly male androgen deficiency.

Body mass index (BMI) has been discovered to be an important influence on testosterone concentration, probably via its effects on SHBG. Negative associations have been found between SHBG and TT (Demoor and Goosens, 1970). In addition to this, lower serum testosterone levels tend to be more pronounced when associated with higher levels of abdominal obesity (Haffner et al, 1993). Physiologically, alterations in the neuro-endocrine regulation of testosterone secretion (via reduced mean amplitude of luteinising hormone pulses) have been noted to be the foundation to this decline (Giagulli et al, 1994).

Increased levels of stress can stimulate the neuroendocrine stress-responsive corticotropic, sympatho-adrenal and somatotropic axes together with suppression of hypothalamic gonadotropin releasing hormone secretion (Bergendahl et al, 1998). In particular, inhibition of gonadotropin releasing hormone may be related to corticotropin stimulated secretion of endogenous opioids (Gambacciani et al, 1986). Various types of physical stressors (e.g. pain, injury, temperature, exercise) and also psychological stress have been implicated in inhibition of gonadal function and thus reduction in circulating testosterone concentration (Christiansen et al, 1985 and Nilsson et al, 1995).

2.3.4: Pathophysiological considerations of testosterone deficiency.

Pathophysiologically during ageing, it has been noted that although the gonadotropin releasing hormone mRNA cellular content does not change, there is a decrease in neuropeptide Y release, an excitatory peptidergic signal to gonadotropin secreting neurons (Pednekar and Mulgaonker et al, 1995). Additionally, β receptors and hypothalamic norepinephrine content has been noted to become less functional in ageing males, both of which are agonistic to gonadotropin releasing hormone secretion (Kaufman et al, 1991). Therefore, it could be stated that the reduced gonadotropin releasing hormone impulse strength may contribute to hypogonadism in ageing males. Research has also suggested that with ageing, gonadotropin releasing hormone receptor activated Ca^{2+} channels reduce their ability to mobilise Ca^{2+} for release thus negatively impacting on pituitary luteinizing hormone secretion (Miyamoto et al, 1992). Extrinsically testosterone can inhibit gonadotropin releasing hormone stimulated pituitary secretion of luteinising hormone (Miyamoto et al, 1992). This research found that dihydrotestosterone had no effect on luteinising hormone secretion in men when its

levels are decreased via a 5 alpha reductase inhibitor (finasteride). However, patients with 5 alpha reductase deficiencies exhibited significantly increased luteinising hormone pulse amplitude suggesting testosterone and its 5 alpha reductase metabolite modulate luteinising hormone secretion.

The stress of illness is also an important modulator of pituitary function. In more detail, cytokines which activate the corticotropin-adrenal axis (such as IL-1 alpha) have been suggested to impair gonadotropin secretion (Feng et al, 1991). Animal studies have shown that stress activated corticotropin releasing hormone and opiates suppress the gonadotropin releasing hormone pulse generator. For example, in ovariectomised monkeys, IL-1 alpha reduces the frequency and amplitude of luteinising hormone secretion via arginine vasopressin (Shalts et al, 1992). In humans, older men display a wider dispersion of luteinising hormone and serum testosterone levels, probably reflecting variations in health status. In relation to this, older men also exhibit suppression of serum luteinising hormone concentrations during acute illness. Cross-sectional research has reported lower testosterone levels in patients with CAD when compared with age-matched controls (Alexanderson et al, 1996). In addition to this, other research has identified that the age-related decline in testosterone level is exaggerated in males with concomitant CAD (Zmuda et al, 1997). There are however, no conclusive research studies reporting an independent association between low testosterone concentration and development of fatal or non fatal MI (Contoreggi et al, 1990). Numerous other chronic illnesses have been reported to result in lower testosterone concentration. For example, the hypercapnoea and hypoxia associated with chronic obstructive airways disease is thought to result in hypo-pituitary dysfunction and thus decreased serum testosterone levels (Semple et al, 1981). Chronic liver disease patients have been noted to present with decreased free testosterone concentrations and

increased serum concentrations of SHBG, androstenedione, and estrogens. Additionally, excess alcohol consumption in hepatic cirrhosis can have an additional effect on decreased testosterone concentration (Bannister et al, 1987). The increased plasma half life found in chronic renal impairment has been found to contribute to increased plasma gonadotropin levels. Furthermore, luteinising hormone secretion is altered due to abnormal gonadotropin hormone release and there is also evidence of impaired Leydig cell function (Handelsman and Dong, 1993).

2.3.5: Diagnosis, signs and symptoms – Hypogonadism.

Clinically, the importance of recognising and treating hypogonadism has recently become more prominent. A research study conducted in 2006 on veteran males over 40 years of age identified that hypogonadism is associated with a reduced cumulative survival level to 55% when compared to normal males (found to have a 75% survival over the 8 year study observation). Additionally, following adjustment of important confounding variables, hypogonadism was still found to be associated with increased mortality (hazard ratio of 1.88) (Laughlin et al, 2008). Importantly, further research has identified that hypogonadism (defined as a testosterone level <450 ng/dL) was significantly (45% of the study population) associated with the development of the metabolic syndrome and diabetes over an 11 year follow up period (Laaksonen et al, 2004). Moreover, a study of San Bernado males identified that those with the lowest percentile of testosterone had increased all cardiovascular mortality even when adjusted for age, smoking status, exercise levels, BMI, hip ratio and alcohol consumption (Shores et al, 2006).

Although hypogonadism has now become widely established, it is still unclear as to whether this physiological process is associated with a definitive symptom complex.

Recently published reviews and consensus panel updates have suggested that for the diagnosis of hypogonadism there must be both biochemical and functional evidence (Matsumoto, 2002). Additionally, symptom onset, age of decline of testosterone, velocity of testosterone decline and the threshold for significant testosterone decline are all uncertain factors (Martinez-Jabaloyas et al, 2007). As a consequence, together with biochemistry, a number of questionnaires have been designed and validated for use in this patient population. Perhaps the most commonly used of these questionnaires and the only one that has independent validation is the Androgen Deficiency in the Ageing Male Questionnaire (ADAM) as developed at the University of St Louis in the late 1990's (Heinmann et al, 1998). This questionnaire aims to highlight the most important functional characteristics of the age associated decline in testosterone concentration in order to aid diagnosis.

The clinical presentation of hypogonadism may be subtle but most commonly include some of the following: loss of libido, erectile dysfunction, lethargy, loss of muscle mass and / or strength, oligospermia, reduced bone density, testicular atrophy, gynecomastia and depression (Dobs, 2008). In addition to ageing, there is an expanded appreciation of the aetiologies of low testosterone in table 7 below:

Table 7. Expanded Appreciation of the Aetiologies of Low Testosterone – adapted from (Dobs, 2008).

Representative Disease Associated with Primary Testicular Failure.

Klinefelters Syndrome

Alkylating Chemotherapeutic Agents

Radiation Therapy

Auto-immune Destruction

Trauma

Viral Invasion

Ketoconazole

Representative Disease Associated with Centrally Mediated Hypogonadism.

Non-secretory Pituitary Tumours

Hyperprolactinemia

Prolactinomas

Granulomatous Invasion

Kallmans Syndrome

Opiate Use

Glucocorticoids

Representative Chronic Diseases with Both a Central and Testicular Effect.

Diabetes Mellitus

Obesity

Hyperinsulinism

Starvation

Acute Illness

Cytokine Mediated Impaired Testosterone Synthesis.

Renal Failure

Ageing

HIV

Stress

Cancer

The classic diagnosis of hypogonadism has been based on clinical presentation with laboratory confirmation. Laboratory confirmation historically was based on statistical norms (mean \pm 2 s.d.). However, with the increased appreciation of the effect of advancing age on testosterone concentration (see aforementioned Massachusetts Male Aging Study Araujo et al, 2004). it is unclear whether the normal range should become age adjusted or averaged over the lifespan (Dobs, 2008).

Importantly, testosterone levels vary significantly diurnally and also have been suggested to vary circ-annually (Smals et al, 1976). Additionally, as suggested earlier, testosterone concentration is also affected by illness and perhaps, more importantly SHBG concentration (Bhasin et al, 2006). The majority of circulating testosterone is bound to SHBG and albumin with up to approximately 3% unbound and known as 'free testosterone'. Bio available testosterone refers to the sum of testosterone bound to albumin and unbound testosterone within the circulation. Current European Endocrine Society guidelines (Bhasin et al, 2006) have proposed common values for testosterone deficiency in ageing males but have advised caution due to large patterns of variability. A cut off value of 12 nmol/L for TT has been suggested as commonplace for ageing males, whereas values as low as 10.4 nmol/L have been utilised for younger males. In addition, 0.17 nmol/L has been widely used as a cut off value for free testosterone. Bio-available testosterone refers to unbound testosterone plus the fraction loosely bound to albumin. Albumin bound testosterone is thought to be readily dissociable and is

therefore termed bio-available (Bhasin et al, 2010). Endocrinology guidelines have suggested that measurement of both free and bio-available testosterone are essential if total testosterone concentrations are borderline hypo / eugonadal or when there may be a possible alteration of SHBG. Older males, males with obesity, diabetes mellitus of chronic disease have been shown to present with increases in SHBG and thus total testosterone may not be a precise marker of androgen status in this population (Bhasin et al, 2008). Bio-available testosterone concentration ≤ 2.5 nmol/L have been associated with overt hypogonadism in younger males and levels below 4.0 nmol/L have been considered hypogonadal and suitable for testosterone supplementation (Kapoor et al, 2007 and Leifke et al, 2000).

2.3.6: Treatment of testosterone deficiency.

Testosterone therapy in young hypogonadal males has been associated with improvements in overall sexual activity scores, frequency of sexual thoughts, increased attentiveness to erotic stimuli and increase in frequency and duration of night-time erections (Alexander et al, 1997). Testosterone therapy in healthy hypogonadal males has also been found to increase fat free mass, muscular strength and also decrease overall fat mass (Wang et al, 2000, Bhasin et al, 1997 and Wang et al, 2004). In addition, testosterone therapy provides a dose-dependent increase in haemoglobin levels, which appears to be further augmented in older males (Bhasin et al, 2005). Testosterone therapy has also been noted to have a positive effect on mood, by increasing positive and decreasing negative aspects (Wang et al, 2000). Furthermore, other research has highlighted benefits of testosterone therapy on self reported energy and well-being (Rabkin et al, 1995).

There are a number of available testosterone formulations with differing clinical pharmacology. Table 8 summarises the main testosterone formulations currently in use (adapted from Bhasin et al, 2006).

Table 8. Clinical Pharmacology and Testosterone formulation. Adapted from Bhasin et al, 2006.

Formulation.	Regimen.	Pharmo-kinetic Profile.	Advantages.	Disadvantages.
T enanthate or cypionate.	100 mg / week or 200mg every other week. Intra-muscular.	After 1 injection serum T levels rise supra-normally. Gradual decline to hypogonadal levels over rest of dosing interval.	Corrects symptoms of hypogonadism. Inexpensive and self administered.	Requires IM injection. Peaks / troughs in serum T levels.
Scrotal T patch.	One scrotal patch. Delivers 6mg over 24 hours.	Normalise T levels in many but not all deficient states.	Corrects androgen deficiency symptoms.	Requires scrotal skin shaving for optimum absorption.
Nongenital transdermal system.	1 or 2 patches. Designed to deliver 5-10mg over 24 hours.	Restores levels towards normal physiological range.	Easy to apply. Corrects symptoms by mimicking the normal diurnal T variation.	For lower T levels, 2 patches may be needed daily. Often results in skin irritation.
T Gel.	5-10g gel containing 50-100mg.	Restores levels towards normal physiological range.	Corrects symptoms, provides flexibility and ease of application.	Potentially transferable to others via skin contact.

17-α-methyl T.	Avoid due to	Orally active.	Variable clinical response.
	potential hepato-toxicity.		May adversely cause hepatic toxicity.
Buccal, bio adhesive, T tablets.	30mg controlled release tablets, twice daily.	Absorbed from the buccal mucosa.	Gum related adverse events noted in 16% of treated males.
Oral T undecanoate.	40-80mg orally. 2-3 times per day with food.	In oleic acid is absorbed through the lymphatics, bypassing the portal system. High variability within and between individuals.	Not FDA approved in the USA.
Injectable long acting T undecanoate in oil.	1000mg intramuscular injection. Followed by 1000mg at 6 and 12 weeks.	In 1000mg dose – serum T levels maintained in normal range in most treated males.	Requires large volume injection (4ml).

T pellets.	4-6 200mg	T peaks at 1 month – sustained in	Corrects symptoms of	Requires surgical incision to
	pellets	normal range for 4-6 months.	androgen deficiency.	insert pellets. May
	implanted.			spontaneously extrude from
				skin.

T (testosterone), mg (milligrams), g (grams), IM (intra-muscular), FDA (Federal Drug Agency).

Bhasin et al, 2006 remark that any of the aforementioned treatment options can be considered dependent on patient preference, pharmacokinetics of testosterone action, treatment burden and also cost. The recommended achievable testosterone level should aim towards that of a normal healthy young male (dependent on local laboratory values). This holds true for older males with long-term chronic illness. However, this data was based upon males with chronic human immuno-deficiency virus (HIV) and not HF. It should be accepted that as this treatment has not been fully implemented in the HF population, these values are unavailable.

2.3.7: Testosterone as a metabolic hormone.

The metabolic syndrome has been considerably investigated in recent years, particularly due to its perceived association with common disease states for instance HF (Inglesson et al, 2006), cardiovascular disease and type II diabetes mellitus (Imam et al, 2007). The most common risk factors for the development of metabolic syndrome are obesity and physical inactivity (Ford and Li, 2006) additionally, obese individuals are more likely to develop insulin resistance when compared to non-obese. Insulin resistance may facilitate the development of additional metabolic risk factors such as elevated triglycerides, reduced high density lipoprotein (HDL) cholesterol levels, elevated fasting blood glucose concentration and hypertension (Hu et al, 2004). Geography, ethnicity, lifestyle, age and gender may all contribute to the development of the metabolic syndrome. However, research has shown that low levels of serum total testosterone and SHBG may also be considered as risk factors for its development in males (Muller et al, 2005). A recent meta-analysis supports the presence of a sex-dependent association between endogenous testosterone and the metabolic syndrome (Brand et al, 2011). This analysis identified a total of 52 studies comprising 22043

males and 7839 females, presenting relative risk estimates or hormone levels for participants with or without development of the metabolic syndrome. Males with higher serum TT displayed a lower metabolic syndrome risk (relative risk estimate = 0.38, 95% confidence interval 0.28-0.50). In addition, males with higher SHBG levels displayed a reduced risk of development of the metabolic syndrome (relative risk estimate 0.29, 95% confidence interval 0.21 – 0.41). Research conducted by Laaksonen and colleagues in 2005 has displayed the reverse to hold true. This study has indicated that male patients who have the metabolic syndrome at baseline will have an increased risk of developing testosterone deficiency (defined as total testosterone ≤ 11 nmol/L) over an extended follow-up period of 11 years.

The precise mechanisms linking testosterone deficiency with insulin resistance and type II diabetes mellitus are not fully understood. However, limited research evidence is suggestive of a role at the level of the major insulin-responsive target tissues; skeletal muscle, liver and adipose tissue. In addition, impaired insulin sensitivity in the above tissues is related to deficiencies in insulin-stimulated glucose transport activity into skeletal muscle, impaired insulin mediated inhibition of hepatic glucose production, stimulation of glycogen synthesis in the liver and an attenuation of insulin inhibited lipolysis in adipose tissue (Kelly and Jones, 2013). In response to this, dissociated fatty acid release from adipose tissue becomes surplus to the energy requirements of other tissues including skeletal muscle and the liver. Yu and Ginsberg, 2005, suggest that this lipid accumulation contributes to impaired insulin responsiveness and abnormal glucose control.

Following castration in a rodent model, it is widely accepted that there will eventually be reduced muscle levels of glycogen (Leonard, 1952). Insulin stimulated glucose uptake into skeletal muscle is mediated by the Glut4 glucose transporter isoform

(GLUT4) which is situated in membrane vesicles inside the cell during resting state (Kelly and Jones, 2013). Translocation of GLUT4 to the cell membrane, in response to insulin receptor signalling, binding of insulin substrate 1 and activation of intra-cellular signalling pathways serves to facilitate increased glucose transport into skeletal muscle (Bryant et al, 2002). This theory is supported by other research which has shown that in type II diabetics, GLUT4 expression is reduced correlating with decreased insulin responsiveness and defects at the level of the insulin receptor (Pessin and Saltiel, 2000). Cultured adipocytes and skeletal muscle cells incubated with low dose testosterone therapy have demonstrated an augmented regulation of GLUT4 and insulin receptor 1 (Chen et al, 2006). In relation to this, Sato et al, 2008 have showed that testosterone therapy in a rodent model can facilitate phosphorylation of protein kinase B and C which are key steps in the insulin receptor signalling pathways for regulation of GLUT4 translocation. The same authors have also suggested that testosterone treatment can influence the enzymes related to the glycolytic process. Increased activity of phosphofructokinase and hexokinase has been shown in cultured rat skeletal muscle cells following administration of testosterone. Furthermore, an increase in glucose oxidation has been noted in isolated sebaceous glands of castrated male mice when compared to controls in response to testosterone treatment. The observed increase in oxygenation was in parallel to an increase in glucose use in the pentose phosphate pathway and ultimate use in fatty acid synthesis (Sansone et al, 1971).

Pitteloud et al, 2005 have asserted that testosterone may beneficially influence the metabolic rate in skeletal muscle by promoting the acquisition of energy from adipose tissue in order to decrease fat mass. As such, the authors noted a correlation between expression of genes involved in skeletal muscle mitochondrial oxidative phosphorylation, impaired glucose tolerance and the presence of type II diabetes.

Furthermore, ubiquinol cytochrome c reductase binding protein, which plays a critical role in oxidative phosphorylation, was noted to have the most significant difference between healthy and diabetic muscle and also a strong correlation with testosterone and insulin resistance.

Testosterone deficiency has been found to result in significant declines in lipid oxidation rate resulting in declines in resting energy expenditure and increased adiposity in healthy males with gonadal steroid suppression (Mauras et al, 1998). In relation to this, other research has shown that two weeks of testosterone treatment resulted in stimulation of whole body fat oxidation and reduced fat mass in testosterone deficient males (Birzniece et al, 2009). Although the exact mechanism behind this phenomenon is not well known, it has been postulated that this process may be as a result of a decrease in protein oxidation (Gibney et al, 2005) or indirectly on fat oxidation by reducing glucose oxidation (Moverare-Skrtic et al, 2006). Testosterone may be able to enhance lipid oxidation in muscle, improve circulating lipid profiles, sensitise myocyte insulin signalling and glucose metabolism, therefore protecting against the detrimental myocellular consequence of deregulated lipid metabolism in type II diabetes mellitus, obesity and the metabolic syndrome (Kelly and Jones, 2013).

The liver is an important organ for the maintenance of glucose and lipid homeostasis. Decreases in testosterone have been associated with attenuated expression of GLUT4 in liver tissue, accompanied by increased blood glucose, reduced insulin and decreased glucose uptake in adipose and skeletal muscle tissues (Muthusamy et al, 2009). In rodent models, treatment with testosterone has been shown to restore liver GLUT4 expression and resultantly glucose uptake, leading the authors to hypothesize that the

aforementioned GLUT4 impairment may have resulted from castration induced deficiency in insulin (Muthusamy et al, 2009).

Obesity and the metabolic syndrome can directly impact on liver function. Free fatty acids and adipocytokines in high concentration may disturb liver metabolism and result in development of metabolic disorders. In more detail, free fatty acids decrease hepatic insulin binding and extraction, increase hepatic gluconeogenesis and increase hepatic insulin resistance (Kelly and Jones, 2013). It can be theorised that testosterone supplementation could reduce free fatty acid production and correct disorders of insulin.

In males, the distribution of adipose tissue appears to influence androgen concentration and cardiovascular risk profiles. As such, numerous research studies have suggested that reduced lean body mass and increases in central adiposity are inversely correlated with levels of testosterone (Nielsen et al, 2007, Frederiksen et al, 2012, Kapoor et al, 2007). Mechanistically, lipid breakdown for energy usage in adipose tissue, influenced by androgens, may play an important role in fat storage and obesity (Kelly and Jones, 2013). Further to this, research conducted by Hansen et al, 1980 has linked testosterone supplementation with enhanced nor-adrenaline stimulated lipolysis in isolated male rat fat cells. This is an important link because catecholamines are the main lipolysis regulating hormone in males and regulate adipocyte lipolysis through the activation of adenylate cyclase to produce cAMP. Protein kinase A activation by cAMP results in stimulation of hormone sensitive lipase and therefore facilitates accelerated lipolysis and increased breakdown of triglycerides (Arner , 2005).

Plasma concentration of bio-available testosterone has been show to inversely correlate with abdominal adipose tissue lipoprotein lipase (Ramirez et al, 1997). This enzyme has

been suggested to play a role in the pathogenesis of obesity as it resides on the extracellular surface of adipocytes and hydrolyses circulating triglyceride rich lipoproteins to fatty acids which then become esterified and stored as triglyceride (Eckel, 1989). In relation to this, Marin et al, 1995 showed that following prolonged testosterone supplementation, there was a marked decrease in lipoprotein lipase activity and hence reductions in triglyceride storage in abdominal subcutaneous adipose tissue.

Adipocytokine production is abundant from visceral fat. IL-1, IL-6 and TNF α production is increased in obesity and also stimulates production of CRP in the liver (Schuster, 2010). This production of pro-inflammatory cytokines by adipose tissue facilitates insulin resistance via direct and indirect mechanisms together with a contribution to systemic and peripheral vascular inflammation associated with cardiovascular disease (Kelly and Jones, 2013). It has already been suggested in this review that testosterone is able to decrease lipid deposition and therefore has the potential to modulate the release of harmful pro-inflammatory cytokines into the circulation. This phenomenon has been proved in numerous research studies which have shown that testosterone supplementation can beneficially effect concentrations of IL-1, IL-6, TNF α and CRP (Yang et al, 2005, Malkin et al, 2004, Kapoor et al, 2007). In more detail, retinol binding protein 4 has been found to precipitate insulin resistance by the reduction of phosphatidylinositol-3-OH-kinase signalling at the level of the muscle, which in turn increases the expression of phosphoenolpyruvate carboxykinase in the liver to inhibit tyrosine phosphorylation of IRS1 in adipose and ultimately, attenuate adipocentin and PPAR α activity which is characteristic of obesity (Yang et al, 2005).

2.3.8: HF and testosterone deficiency.

HF appears to be associated with decreased levels of plasma testosterone, supported by the fact that about 25%-30% of men with HF and a mean age of around 60 years have biochemical evidence of testosterone deficiency (Malkin et al, 2009). Some studies have actually reported that in males with HF up to 79% of patients may suffer from low testosterone levels (Guder et al, 2009). All studies do, however, report a consistently high morbidity and mortality associated with testosterone deficiency and HF.

To support this hypothesis, research has shown that in male patients with HF, anabolic hormone depletion is common and an independent marker of poor prognosis (Jankowska et al, 2006). In this study, serum levels of three important anabolic hormones were measured (TT, dehydroepiandrosterone sulphate and insulin-like growth factor 1) in 208 males with HF (median age of 63 years and median LV EF of 33%). Deficiencies in these anabolic hormones (below 10th percentile of healthy peers) were noted in all age categories of males with HF. In addition, these deficiencies were inversely correlated with NYHA Score ($p < 0.01$). More importantly, circulating TT, dehydroepiandrosterone sulphate and insulin-like growth factor 1 (IGF-1) were prognostic markers in multivariate models when adjusted for important prognostic factors ($p < 0.05$). Males with HF and normal levels of all anabolic hormones had superior 3 year survival rates (83%, 95% confidence interval 67% to 98%) when compared to deficiencies in one (74% survival rate, 95% confidence interval 65% to 84%), deficiencies in two (55% survival rate, 95% confidence interval 45% to 66%) and deficiencies in three (27% survival rate, 95% confidence interval 5% to 49%).

Low levels of testosterone have been correlated with disease progression in HF and may also be responsible for some of the common features of HF, for instance, reduced

skeletal muscle mass and function, cachexia, fatigue and depressed mood (Malkin et al, 2006). Myocardial cachexia, a syndrome with poor prognosis (defined as a non-intentional loss of 6kg lean mass in 6 months or less), has been characterised by low levels of testosterone (Aukrust et al, 2009). It is well known that HF as a metabolic syndrome can adversely alter numerous endocrine, metabolic and inflammatory parameters (Jackson et al, 2000, Noutsias et al, 1999). The alterations can include changes in levels and sensitivity to insulin, growth hormone and also testosterone (Von Haehling et al, 2007). Most patients with HF are affected by a gradual decline in muscle mass, muscular strength and also endurance performance, reflecting the pathophysiological imbalance and deficiency of anabolic hormones (Malkin et al, 2010).

The importance of the relationship between testosterone deficiency, metabolic syndrome and heart failure is clearly clinical valid. Initial reports from the Framingham Study drew attention to the potential relationship between diabetes mellitus and HF independent of age, coronary artery disease, hypertension or BMI (Kannel et al, 1979). In HF, there is derangement of the glucose-insulin axis with some studies reporting up to 43% of patients manifesting disorders of glucose metabolism (Kontoleon et al, 2003). Those patients with HF and insulin resistance display an impaired ability to promote glucose transport into the muscle and adipose tissue. Additionally, more recent research has showed that impaired insulin activity inversely correlates with severity of HF, relates to disorder of skeletal muscle physiology, facilitates reduced muscle mass and can also influence prognosis (Swan et al, 1997 and Doehner et al, 2002). Further to this, it has been shown that stress induced increased plasma glucose levels are associated with increased risk for the development of HF (Capes et al, 2000), type II diabetes mellitus can predisposes severe presentation of HF with over 33% of patients with de-

compensated HF manifesting type II diabetes mellitus (Kostis et al, 2005). Research has shown that one standard deviation increase in insulin sensitivity increased the risk of developing HF by approximately one third (Ingleson et al, 2005). Thus, there is a consensus of evidence that suggests a direct link between insulin resistance and HF, with a notion that insulin resistance may facilitate development of cardiomyopathy and also HF as a precursor to development of insulin resistance.

There are a number of theories to explain the association between altered metabolism, insulin resistance and HF. The most investigated concept relates to the aforementioned activation of the sympathetic nervous system and renin-angiotensin-aldosterone system. It has already been stated in this review that in response to sympathetic nervous system activation, there is an increase in catecholamine secretion. In addition to this however, there is also evidence of reduced cardiac catecholamine re-uptake (Eisenhofer et al, 1996). The observed increase in catecholamines can facilitate myocardial oxygen wastage by inducing marked enzyme loss due to myocardial damage (Opie et al, 1979) together with norepinephrine mediated coronary vasoconstriction and increased plasma free fatty acid levels; other factors which contribute to oxygen wastage (Kostis et al, 2005 and Paolisso et al, 1991). Increased levels of free fatty acids may also be responsible for defects in insulin signalling, potentially mediated by activation of protein kinase C- β , which phosphorylates insulin receptors and hence reduced capillary opening and myocyte glucose uptake (Wagenmakers et al, 2006). Peterson and co-workers (2004) have also shown that insulin resistance has the ability to increase cardiac free fatty acid metabolism and therefore decrease cardiac muscle contractile efficiency in their study of obese young females without other significant cardiac risk factors. At cardiac level, HF mediated increases in sympathetic nervous system activity have been shown to reduce presynaptic norepinephrine and myocardial glucose uptake

in areas of LV impairment when compared to normal myocardial ventricular segments (Ashrafian et al, 2007). This suggests that altered metabolism and insulin resistance directly relate to local cardiac sympathetic nervous system activity and are a potentially important correlation in HF. In skeletal muscle, plasma free fatty acid induced insulin resistance has been linked with increased triglyceride, increased cellular free fatty acids, increased cytoplasmic fatty acid metabolites such as diacylglycerol, and activation of protein kinase-C and nuclear factor-kappa B (Savage et al, 2005). There is a strong association between muscular triglyceride content and insulin resistance but there is controversy regarding the specific lipid species responsible for its instigation; triglyceride or intracellular free fatty acid and its metabolites (Ashrafian et al, 2007). Savage and co-workers (2005) have investigated this concept in transgenic mice. During their research, it was noted that in adiposome triglyceride lipase – null mice, despite the deletion of triglyceride, the reduced availability of lipase derived free fatty acids increased glucose utilisation and therefore facilitated a decline in cardiac insulin resistance. This research stipulates that in a transgenic rodent model, free fatty acids and their derivatives appear to be the main mediator of cardiac insulin resistance. The mechanism of this process appears to be either due to an alteration of glucose signalling or by competition with glucose signalling (Poornima et al, 2006). The accumulation of intra-muscular free fatty acid coenzyme A then limits attenuates glucose uptake by inhibiting intracellular muscle insulin signal pathways (Savage et al, 2005).

Resultantly, there is clear evidence of that testosterone deficiency and HF are interchangeable with a large body of evidence suggesting detailed relationships between the two difference clinical manifestations. It is due to this phenomenon that research studies

are warranted to address the effects of testosterone concentration on important health related outcomes in HF.

Unfortunately, currently there are only two research studies that have attempted to identify the effect of testosterone concentration on physiological function in males with HF. An initial study conducted by a Brazilian research group investigated the effects of serum testosterone concentration on cardiovascular haemodynamics and exercise capacity in males with HF (Bocchi et al, 2008). This small study recruited 15 patients with HF (EF% of $23 \pm 7\%$) and erectile dysfunction. It was noted that serum TT correlated directly with distance walked during the 6 min walk test and inversely with diastolic blood pressure (BP), right and LV EF ($p < 0.004$). The latter inverse correlation may suggest that serum testosterone could play a role in the pathophysiology of HF thereby worsening cardiac function. This could be related to the work carried out by Sullivan et al 1998, who suggested that anabolic steroids are cardiotoxic, by adversely affecting cellular and organ physiology in a similar way to cardiomyopathy. However, a contrasting hypothesis by numerous research groups has suggested that testosterone may be beneficial in HF (Malkin et al, 2006, 2009, Caminiti et al, 2009, Jankowska et al 2006 and 2009). For instance, androgen therapy has previously been shown to improve coronary blood flow and also increase fractional shortening of cardiac fibres in a rodent model (Kontoleon et al 2003). In addition, previous randomised controlled trials have identified improved walking capacity, muscular strength and endothelial function in testosterone supplemented males with chronic stable HF (Malkin et al 2006, Caminiti et al 2009).

In relation to this, another study conducted by a Polish research group (Jankowska et al 2009) has shown that circulating serum TT independently relates to exercise intolerance with further decline of testosterone indicating further reductions in exercise capacity. This study identified 205 males with HF (mean EF% of $31 \pm 8\%$) of NYHA Class I-IV. Using multivariate models, reduced peak VO_{2max} , reduced peak oxygen pulse was associated with diminished total and free serum testosterone levels ($p < 0.01$). In addition, following a further 2.4 year follow-up, additional reductions in circulating TT was associated with further reductions in peak VO_{2max} and peak oxygen pulse ($p < 0.01$). In explanation of the importance of these results, the authors accept that the role of anabolic hormones in HF patients is poorly understood. They do suggest however, that testosterone may potentially affect some of the many complex pathways associated with exercise intolerance in HF. For example, testosterone can favourably affect skeletal muscle mass and the local synthesis of important growth factors (IGF 1) and also contractile protein synthesis (Balagopal et al, 1997). It has also been suggested that testosterone may potentially positively interact with peripheral mechanisms involved in HF pathology. For example, attenuation of ED (Miller et al, 2007), improvement in lung function (Svartberg et al, 2007), normalisation of baroreflex sensitivity and autonomic imbalance (El Mas et al, 2001) and increased muscle perfusion at rest and during exercise (Jones et al, 2004).

It has also been postulated that testosterone concentration may be responsible for impaired somatic health and worsened psyche in both a general and HF population (Shores et al, 2005, Jankowska et al, 2010). Jankowska et al 2006, studied how levels of circulating anabolic hormones affected depression and outcome in males with HF. Serum TT and dehydroepiandrosterone sulphate were sampled in 163 males with stable HF (mean age 60 ± 10 years, NYHA Class I/II/III/IV) together with 316 healthy males

aged 35 - 60 years. Level of depression was assessed using the Beck Depression Inventory (BDI) and defined as a questionnaire score of ≥ 16 points. The results of this research showed that the severity of depressive symptoms was increased in men with HF when compared to healthy controls in age groups 35-49 years (Beck Score of 9.9 ± 7.3 versus 5.2 ± 4.4 points, $P < 0.001$) and 50-59 years (Beck Score of 14.3 ± 11.9 versus 9.1 ± 4.3 points, $P < 0.001$), but not among males aged 60-80 years. Furthermore, depression was found to be present in 20% of HF males without androgen deficiency, 37% of males with either testosterone or dehydroepiandrosterone sulphate deficiency and 77% of males with deficiency of both anabolic hormones ($p < 0.0001$). During regular follow-up in the outpatient clinics for a median of 28 months, there were 53% cardiovascular deaths or unplanned hospital admissions. Anabolic hormone deficiencies ($\leq 10^{\text{th}}$ percentile of serum androgen in healthy controls) and high depression score index ≥ 16 points independently predicted unfavourable outcome ($p < 0.05$).

2.4: Aims and objectives.

Research directly comparing physiological, cardiovascular and psychological function in male HF patients with low and normal range testosterone is sparse. Separate, small correlation studies have attempted to identify the relationship between testosterone concentration and exercise capacity, ventricular function and depressive status in male HF patients. This study, however will aim to directly compare detailed physiological, cardiovascular functional and quality of life parameters in male patients with low range and normal testosterone concentrations. Importantly, this study has been designed to assess in more detail than previously undertaken the functional mechanics of the heart with respect to testosterone level in HF. In addition, this study is the first to recognise the importance of free testosterone and bio-available testosterone concentration in a population whom the traditional assessment of total testosterone may be an inaccuracy due to the deleterious effects of SHBG. Recognition of salivary testosterone measurement as a direct correlate of free testosterone and also its relationship to important outcomes assessed during the study is another important facet that may, in the future, reduce the need for semi-invasive blood sampling and also allow patients to provide their own testosterone samples for analysis.

This study aims to investigate if serum total testosterone, free testosterone, bio-available and salivary testosterone concentrations influence exercise capacity, levels of NT pro-BNP and quality of life in male HF patients. Testosterone deficiency is commonplace in the male HF population with approximately 25% of this population with a mean age of around 60 years effected. Testosterone deficiency may exacerbate symptoms of HF by further attenuating the pathophysiological processes involved.

The objectives of this study are:

- To investigate whether patients with low testosterone status and HF have reduced exercise capacity, quality of life, together with an increased level of serum markers of LV impairment and attenuated indices of cardiac structure and function when compared to age-matched male HF patients with normal range testosterone.
- To gain an insight into the relationship between traditional serum measures of testosterone and salivary testosterone as a novel measure of testosterone status in the male HF population.

Chapter 3. Materials and Methods.

3.1: Study design.

This was a cross-sectional study that recruited 40 male patients from the local HF clinic at University Hospital of South Manchester (UHSM). These patients were stratified into two groups of 20 based on analysis of their serum TT level. Patients with a TT concentration ≤ 12.0 nmol/L were defined as 'testosterone deficient' and those above this value defined as 'normal range'. This cut off value is based on the range currently used by the department of clinical chemistry at University Hospital South Manchester NHS Foundation Trust and forms part of the local guideline for low testosterone at this institution. In addition, for completion, outcome measures were also assessed based on free and bio-available testosterone as recommended by European Endocrine Society practise guidelines (Bhasin et al, 2006). Consequently, a cut off of ≤ 0.17 nmol/L for free testosterone and ≤ 4.0 nmol/L for bio-available testosterone were used (Bhasin et al, 2006 and Kapoor et al, 2007). This re-allocation of the groups resulted in 17 participants with low FT and 23 participants with normal range FT and 17 with low, 23 with normal range bio-available testosterone for separate analysis. All outcome measures were assessed immediately following group allocation and independent samples t-tests were used to determine if there were significant differences based on testosterone level. Correlation studies were also performed in order to assess the relationship between testosterone status and the outcome data collected. Salivary testosterone was collected as a novel marker in the HF population and was assessed against FT (previous research in healthy participants has shown FT and salivary testosterone to correlate highly) in order to ascertain its value in assessing hypogonadal

status in a heart failure population without the need for semi-invasive blood sampling and also by way of avoiding testosterone parameters which are known to be affected by age, obesity, diabetes and chronic disease.

3.2: Patient selection and recruitment.

Following ethical approval from the local research ethics committee (appendix 1), 40 male patients with stable HF were recruited from the Specialist Heart Failure Clinic at University Hospital of South Manchester NHS Foundation Trust. Patient's were included or excluded from the study on the basis of the following grounds:

3.3: Inclusion criteria.

- Clinically stable HF prior to recruitment established on optimal medical therapy and without significant symptom change (using NYHA Score) for a period of 6 months.
- Evidence of at least moderate impairment of LV systolic function (defined by echocardiography within the last 6 months, as $EF \leq 45\%$).
- Reduced exercise tolerance (limited by fatigue or breathlessness of cardiac origin – i.e. NYHA Class II or worse).
- Over 18 years of age

3.4: Exclusion criteria.

- Unstable angina (defined by classical angina symptoms at rest).

- Severe stable angina pectoris limiting the ability of the participant to perform the 6 minute walk shuttle test.
- Recent (within the last 6 weeks) acute MI.
- Episodes of decompensated HF / clinical change in course of condition within the last 6 months (i.e. increasing symptoms or requirement of new treatment).
- Moderate or worse (defined by echocardiography) valvular heart disease.
- Uncontrolled hypertension (resting systolic BP ≥ 180 mmHg, diastolic ≥ 90 mmHg) – as per defined cut off values for performing the 6 minute walk test (Crapo et al, 2002).
- Any orthopaedic or neurological illness limiting the ability to exercise.
- Bi-ventricular pacing device / Left ventricular assist device in situ**

**bi-ventricular pacing devices and LVAD patients were excluded because of the documented ability of these devices to provide improvements in exercise capacity and quality of life, as such, potentially confounding the data collected during this study. In addition, both devices artificially alter or prevent accurate measurement of a number of the echocardiographic parameters to be collected as part of this study.

3.5: Consent procedures.

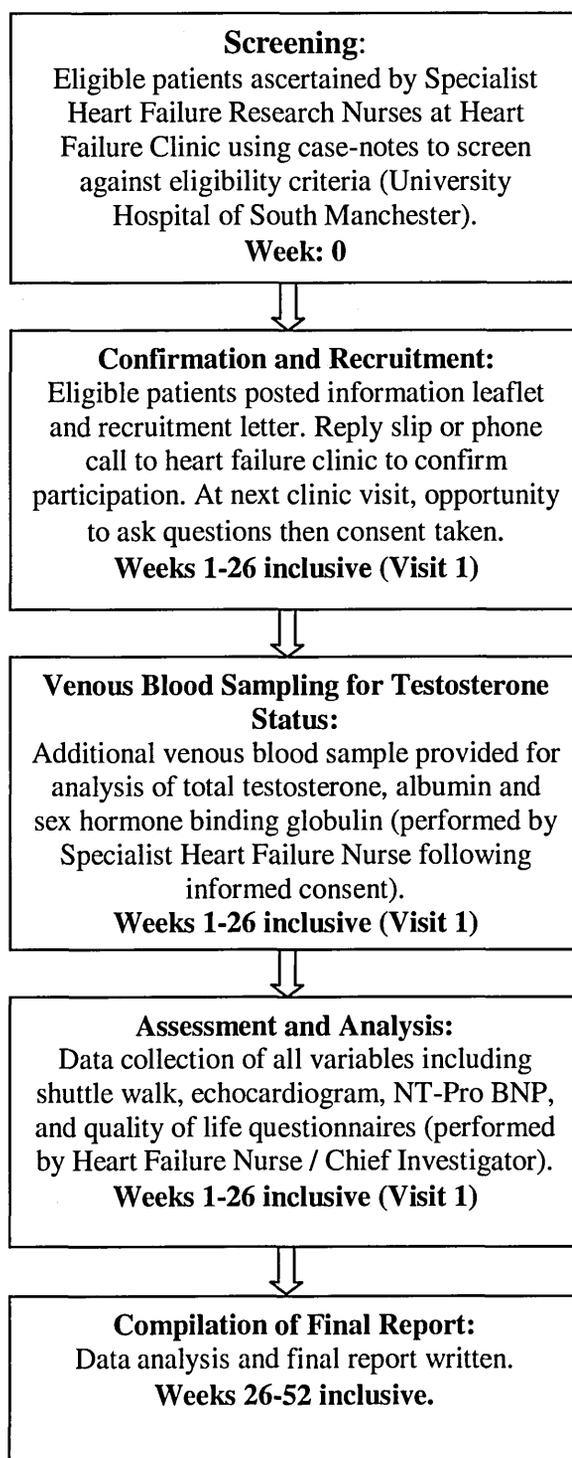
Patients who were due to attend the specialist HF clinic were identified in advance by secretarial staff. These patients were sent an official study invite which consisted of a patient information leaflet (appendix 2), invitation letter and reply slip. All interested replies were collected by the lead researcher who informed clinic staff prior to the clinic appointment. Upon arrival at clinic, interested patients were free to ask any study related questions before they were consented for study participation. Consent was obtained by trained, experienced members of the research team and copies retained in the research file, patient case notes and also by the patient themselves (appendix 3).

3.6: Withdrawal of patients.

Any patient, who at any stage of the study did not wish to continue, was able to withdraw, without their future medical care being affected. This information was made clear to each patient in the patient information sheet and throughout the study duration.

As this was a small-scale cross-sectional study without any intervention period, it was envisaged that there would be minimal risk to the patient. However, if during the course of data collection any patient became unwell or distressed, medical opinion was sought over their continuation in the study. Participants were monitored closely following data collection and, if needed, could be re-assessed by the consultant / registrar in charge prior to leaving the department. Any withdrawn patients were replaced in order to meet the required number of participants as determined by the formal power calculation.

Figure 2. Flow chart of patient journey and important time-points during the study.



3.7: Sample size.

Caminiti et al (2009) have investigated the effects of testosterone supplementation on walking ability in 31 males with low testosterone and HF. Using the 6 min walk data from this research, the authors observed a baseline walking distance of $386.6 \text{ m} \pm 121.0 \text{ m}$. Following testosterone therapy, to a physiological normal range testosterone concentration at 3 month follow-up, walking distance significantly improved to $472.8 \pm 138.4 \text{ m}$ ($p < 0.05$). The placebo group demonstrated a 6 min walk distance pre of $390.9 \text{ m} \pm 107.4 \text{ m}$ and post $428.2 \text{ m} \pm 112.0 \text{ m}$. This equates to a difference of $86.2 \text{ m} \pm 14.5 \text{ m}$ in the testosterone group (significant at $p < 0.05$) and $37.3 \text{ m} \pm 8.7 \text{ m}$ (non-significant) in the placebo group. Taking into account these data, this study will have 80% power to detect a mean difference of at least 70 m with a minimum of 7 patients in each group at the conventional 5% significance level. As this is a small number of patients, a more conventional sample size of 20 patients per group will have 80% power to detect a mean difference of at least 16 m using 12 m as an estimate of the common standard deviation and at the 5% significance level. The common SD of change (12m) was estimated from both the SD pre and post testosterone therapy from the study by Caminiti et al, 2009 as an average value (i.e. average of $\pm 14.5 \text{ m}$ and $\pm 8.7 \text{ m}$). This was considered the best option following expert medical statistical advice, as only summary data and no individual patient data from Caminiti et al (2009) were available in their published results.

3.8: Medical examination.

Before entering the study, each patient received a thorough medical examination performed by a clinician (Consultant Cardiologist or Cardiology Research Registrar),

during which details of surgical history, co-morbid conditions, risk factors and current medication details were confirmed. Patients were also screened in detail with regards to the study inclusion and exclusion criteria. Hormone profiles and concurrent medical drug use were also recorded at the beginning of the trial. Blood pressure (BP) was taken (manual sphygmomanometer) and a resting 12-lead ECG (ECG) was performed with the patient in the supine position. This formed part of the medical assessment. During this visit, initial assessments were also performed, and this was therefore classed as the patient's first assessment visit. Most patients were able to complete all data collection during the initial visit. However, on certain occasions, patients were unable to devote sufficient time for this to be achieved. As a result, a separate visit was scheduled to complete all data collection, following further patient assessment. A second blood sample for testosterone analysis was taken at the next scheduled clinic visit as a way of confirming and standardising the classification of low or normal range testosterone. Time between first and second testosterone serum collections varied depending on clinical need for patient re-assessment. Longest duration between samples was 4 months and the shortest duration 4 weeks.

3.9: Summary of known and potential risks and benefits to the patient.

The potential for risks to occur were minimised, since all patients prior to study acceptance underwent a full clinical assessment performed by a Consultant Cardiologist or Research Registrar, during which details of surgical history, co-morbid conditions, risk factors and current medication details were confirmed. However, all exercise and strength assessments were monitored by qualified medical professionals who were all at

least Intermediate Life Support Trained. In the event of a medical emergency, as this study was performed in a clinical environment, local protocol was to be followed.

There was minor potential for discomfort and distress during the exercise assessments. However, patients recovered relatively quickly and were monitored until resting values were obtained and also patients were free from symptoms before leaving the Heart Failure Clinic.

3.10: Safety assessments.

The University Hospital's formal health and safety procedure was followed. A detailed safety assessment of the study was performed as per the risk assessment documentation by the Research and Development department.

3.11: Adverse events and SAE.

All members of the research team were at least Intermediate life support (ILS) trained. The Chief Investigator and the Heart Failure Research Nurses have extensive experience in working with patients with HF (i.e. > 10 years).

All adverse events were recorded and reported, as per case report forms. The Chief Investigator was responsible for completing the appropriate paperwork at University Hospital of South Manchester. After an adverse event, each patient was to be closely monitored for at least 30 days, or longer if deemed necessary.

The following were considered Serious Adverse Events for this study:-

- Hospital admission for a cardiovascular event.
- Death during the course of the study.

All Serious Adverse Events were reported, and these followed Hospital established protocols. All Serious Adverse Events were reported to the Sponsor Research Office (University Hospital of South Manchester) within 24 hours of discovery by the research team. Dedicated report forms and reference documents justifying a Serious Adverse Event decision were also completed.

3.12: Data collection handling and record keeping.

Data were collected and retained in accordance with the Data protection Act 1998. All data were collected as per case report forms (appendix 2), validated and approved by University Hospital of South Manchester and the local research ethics committee, at the time points indicated on the flow chart.

At UHSM the Chief Investigator was responsible for the storage and quality of data collected. In relation to this, paper files of personal data were kept in a locked filing cabinet. All computer data was coded and stored on a 'trust issue – locked' memory stick. Stored patient details and data were anonymous, since patients were assigned an identification number, to ensure confidentiality.

3.13: Outcome measures.

Outcome measures were collected at the routine clinic visit only. However, if for any unforeseen reason participants needed another appointment to complete data collection, this was provided. No more than two appointments were required during data collection for the outcome measures.

3.13.1: Primary outcome measure – 6 minute walk.

The primary outcome measure was exercise capacity, assessed using the 6 minute (min) walk. The test was performed at the routine clinic consultation in accordance with normal clinical procedures and by guidelines developed by Crapo et al, 2002.

The 6 min walk is a symptom-limited exercise test designed to allow participants to achieve maximum effort tolerance. This test has been successfully utilised in a range of clinical populations but particularly pulmonary and cardiac patients. The 6 min walk has been used as a measurement of the response to a given intervention but also is utilised as a one-time measure of functional status of patients. In addition, the 6 min walk can be used as a predictor of morbidity and mortality (Crapo et al, 2002). There are numerous studies which have investigated the reproducibility and validity of the 6 min walk when compared to regular cardiopulmonary exercise testing in a HF population. Cahalin et al, 1996 showed intra-class correlation coefficients of 0.96 and moderately strong correlations with treadmill derived peak VO_{2max} . ($r = 0.64$, $p=0.001$). In addition, Morales et al, 1999 found excellent reproducibility with no significant difference between 3 separate tests ($r = 0.98$, $p = 0.33$) and also a moderate correlation with cardiopulmonary exercise testing derived VO_{2max} ($r = 0.69$, $p = 0.001$). The authors also stated that 6 min walk duration is an accurate predictor of VO_{2mzx} values below $14ml/kg/min^{-1}$. In more recent work, Guazzi et al, 2009, suggest strong correlations

between 6 minute walk distance and VO_{2max} ($r = 0.79$, $p < 0.001$) and VO_2 at anaerobic threshold ($r = 0.80$, $p < 0.001$). VE/VCO_2 slope showed a weaker but still statistically significant correlation with 6 min walk distance ($r = -0.463$, $p < 0.001$). The same authors tested day by day reproducibility of the 6 min walk in a cohort of 80 patients with good intra-class correlation coefficients of 0.78.

Prior to testing, patients were asked to rest for 10 min in a chair. During this time, BP and resting HR were recorded. Once baseline measures were taken, patients were advised as to how to accurately report their rating of perceived exertion. Following this, the timer was set and final instructions given. Participants walked back and forth along a horizontal 30 m course, marked out by two cones and completed as many shuttles as possible before the end of the 6 min period. Walking pace was determined by the patient and rest periods were also allowed during the 6 min period. Intermittently during the walking period, even tone encouragement was given as suggested by Crapo et al, 2002. The end-point (distance walked in m) was reached when the participant failed to complete the shuttle before the signalled end time. Patients were monitored by trained medical staff during and post this assessment. Heart rate, perceived exertion and BP were also collected during this period.

3.13.2: Secondary outcome measures.

3.13.2.1: Blood analysis.

Venous blood sampling was undertaken during the initial routine clinic visit and also at the next available clinical appointment in order to assess serum TT, free testosterone, bio-available testosterone, albumin and SHBG. This was in addition to routine

collection of NT-Pro Brain Natriuretic Peptide as a blood chemistry marker of HF severity for every patient in the HF clinic.

3.13.2.2: Serum testosterone, SHBG and albumin.

Serum testosterone measurements were collected before midday during each patient's routine Heart Failure clinic appointment by the Heart Failure Specialist Nurses. In order to accurately determine testosterone concentration, a separate serum sample was taken at the following routine clinic appointment to ensure consistency of results (longest duration 4 months, shortest duration 4 weeks in-between first and second samples). Unless there was noted change from hypogonadal to eugonadal status, or vice versa, on analysis, patients were allocated to groups based on their initial testosterone measurement. If a significant change of testosterone status was noted, the patient was excluded from the analysis as all samples were measured retrospective to data collection. Previously, research has showed that testosterone concentrations are at their peak during the morning period and there may also be significant variation in values obtained on different days (Bhasin et al, 2010). Therefore, practise guidelines advocate measurement of serum testosterone on two separate occasions and during the morning period (Bhasin et al, 2010). Around 30% of males with low testosterone during the afternoon period have been shown to have normal testosterone concentration during the morning hours (Brambilla et al, 2007). In addition, the same authors also suggested that in an elderly population (i.e.65-80 years of age), the circadian variation in testosterone concentration becomes significantly blunted.

Serum total testosterone concentration represents the sum of unbound and protein bound testosterone within the circulation. The majority of circulating testosterone is bound to SHBG and albumin (Bhasin et al, 2010). A small amount of testosterone can be

described as 'free' – normally in the range of 0.5-3% of total circulating concentration (Bhasin et al, 2008). Bio-available testosterone refers to unbound testosterone plus the fraction loosely bound to albumin. Albumin bound testosterone is thought to be readily dissociable and is therefore termed bio-available (Bhasin et al, 2010). Endocrinology guidelines have suggested that measurement of both free and bio-available testosterone are essential if total testosterone concentrations are borderline hypo / eugonadal or when there may be a possible alteration of SHBG. Older males, males with obesity, diabetes mellitus or chronic disease have been shown to present with increases in SHBG and thus total testosterone may not be a precise marker of androgen status in this population (Bhasin et al, 2008). Therefore, both free and bio-available testosterone concentration were calculated during this study to increase the accuracy of overall androgen status.

Although as stated previously in the thesis, there is not a widely accepted laboratory cut-off value for testosterone deficiency. However, general agreement states that values above 12nmol/L for TT do not usually require supplementation (Wang et al, 2009). Similarly, a widely held lower limit for free testosterone has been suggested to be ≤ 0.17 nmol/L (Bhasin et al, 2006). In addition, bio-available testosterone concentration ≤ 2.5 nmol/L have been associated with overt hypogonadism in younger males and levels below 4.0 nmol/L are considered hypogonadal and are suitable for testosterone supplementation (Kapoor et al, 2007 and Leifke et al, 2000). In accordance with these pre-determined values, classification into low or normal testosterone groups was based on TT ≤ 12 nmol/L, FT concentration ≤ 0.17 nmol/L and bio-available testosterone ≤ 4.0 nmol/L.

All serum blood samples were drawn from the antecubital vein in the supine position between 0800 hrs and 1200 hrs midday. Serum aliquots were prepared for storage at -

80°C in the Clinical Chemistry Department of UHSM. Total testosterone was measured from frozen samples using liquid chromatography mass spectroscopy using a previously validated method and by accredited clinical scientists (Thienpont et al, 2008). Serum samples (200µl), standards and quality controls were extracted with methyl-tert-butyl-ether (1ml). The supernatant was then transferred and the solvent evaporated. The residue was then reconstituted with 100µl of 50:50 mobile phase (water or methanol) each containing 2mmol/L ammonium acetate and 0.1% formic acid. Extract (50µl) was directly injected onto a Synergi 4 µm Hydro-RP column (50x3mm –Phenomenex, Macclesfield, UK). Following elution from the column, each sample was directly pumped into the electrospray probe of a Quattro Micro tandem mass spectrometer (Waters, Manchester, UK). The mass spectrometer was programmed to operate in electrospray positive ionization mode. Analysis was performed isocratically with 70% mobile phase B, flow rate 0.6 ml/min for a total run time of 4.3 minutes. Primary transitions for the analytes TT 289 greater than 97 and d 2 TT 291 greater than 99 were monitored in multiple reaction monitoring quantification mode. No interfering peaks were found to be present in the extracted samples and infusion experiments indicated almost complete lack of ion suppression. The mean recovery was 93% for TT. The standard curve was linear to 50.0 nmol/L, the lower limit of quantification was 0.25nmol/L and inter / intra-assay coefficients were less than 10% for TT (range of 0.3-0.35 nmol/L).

SHBG concentrations were measured from frozen serum aliquots using competitive chemiluminescent enzyme immunoassays on an Advia Centaur device (Siemens, Eschborn, Germany). Inter assay coefficient of variation of 6.6% at the 27.1 nmol/L level, 7.6% at the 48.2 nmol/L level and 7.7% at the 52.3 nmol/L level were determined. Albumin concentration was determined using the previously validated bromcresol green

dye binding colorimetric method (Doumas et al, 1971) on a Hitachi 911 (Roche Diagnostics, Japan) analyser. Standard and specimen samples (2.5 ml, 10 μ l) were incubated for 5 minutes at 20-25°C and the increased absorbance of the binding dye complex was measured at 623nm. Within run coefficient of variation was 2.6%, run to run reproducibility was 3.1%.

Total testosterone, SHBG and albumin were used to calculate free and bio-available testosterone using an already validated formula as outlined below (Vermeulen et al, 1999).

$$\text{Bio Test (mol/L)} = \left\{ \frac{k_{at} \times [\text{albumin}] \times [FT]}{1 + k_{at} \times [FT]} \right\} + [FT]$$

$$\text{Free test (mol/L)} = \frac{-b + \sqrt{b^2 + 4a[TT]}}{2a}, \text{ in which}$$

$$a = k_{at} + k_t + (k_{at} \times k_t)([SHBG] + [\text{albumin}] - [T])$$

$$b = 1 + k_t[SHBG] + k_{at}[\text{albumin}] - (k_{at} + k_t)[T]$$

The algorithm for which can be found at <http://www.issam.ch> and is routinely used for the calculation of free and bio-available testosterone concentration in research practise. In a critical review of this technique, Vermeulen et al, 1999 have showed that this calculation represents a simple and rapid method that yields free testosterone concentrations very close, if not identical, to those obtained by equilibrium dialysis.

3.13.2.3: Salivary testosterone.

In conjunction with the National Survey of Sexual Attitudes and Lifestyles working group, the Department of Clinical Chemistry at UHSM have developed a liquid chromatography tandem mass spectroscopy assay for the evaluation of salivary

testosterone concentration. Salivary testosterone is a filtrate of plasma containing only the free fraction of testosterone and, as stated earlier in this review, free testosterone is often considered the most physiologically active form of testosterone available for metabolising in target tissue. Laboratory measurement of serum free testosterone is expensive and not a cost effective diagnostic test for most routine clinical laboratories and estimation of free testosterone from available formulae has little in the way of consensus (MacDonald et al, 2011). As a result, measurement of salivary testosterone could provide a more accurate and feasible alternative to traditional serum sampling.

Participants were asked to perform an un-stimulated passive drool technique. For this, each participant rinsed out their mouths with plain water, refrained from eating / drinking and did not brush their teeth for at least 30 minutes prior to the collection. To collect each sample, the patient was instructed to drool through a plastic straw into a collection vial. Samples were frozen at -80°C and stored in the Clinical Chemistry Department at University Hospital South Manchester. For analysis, samples were thawed, mixed and centrifuged. The clear supernatant was then utilised for the analysis. Sample preparation involved a liquid-liquid extraction requiring a $200\mu\text{l}$ sample with D₅ testosterone as internal standard and methyl-tert-butyl ether then placed at -80°C . Following 1 hour at 80°C , the organic layer was transferred and evaporated by heating and gentle N₂ gas flow with the resultant residue reconstituted with a 500ml/L methanol mobile phase before transferring to a 96-well micro-titre plate. Liquid chromatography was performed using a Waters ACQUITY Ultra Performance Liquid Chromatography system (Manchester, UK) and a C18 ACQUITY 1.8 μm HSS T₃ column (21x50mm) maintained at constant 45°C . The mass spectrometer was a Waters Quattro Premier XE (Manchester, UK) set to positive ionisation mode. Binary pump mixing of the mobile phases produced a linear gradient that increased from 50% to 90% methanol for 1.5

minutes. Overall run time was 3.5 minutes. Testosterone and D₅ testosterone co-eluted with clean, discrete and identifiable peaks at a retention time of 1.28 minutes. Intra-inter assay coefficient of variation were <15%. The mean recoveries from saliva samples at 3 concentrations were 95.6%, 100.3% and 95.8% respectively.

3.13.2.4: Serum N Terminal Pro Brain Natriuretic Peptide (NT Pro-BNP).

The notion that the heart may have an endocrine function has previously been suggested almost 55 years ago when research identified that atrial dilatation resulted in natriuresis (Henry et al, 1956). Since then, detailed research has identified that cardiac myocytes constitute the major source of BNP-related peptides in the circulation with cardiac fibroblasts recently demonstrated to produce BNP (Tsuruda et al, 2002). In more detail, cardiac myocytes produce BNP pro-hormone, pro BNP, which is encoded from the BNP gene located on chromosome 1 (Hall, 2004). Following this, the protease furin splits Pro BNP into NT-Pro BNP and BNP. NT-Pro BNP is secreted following cardiac wall stress and as HF develops there is a shift in cardiac BNP production from the atria to the ventricles. Following its secretion, BNP binds to natriuretic peptide receptors A and C, biologically resulting in diuresis, vasodilatation, inhibition of rennin and aldosterone production together with attenuation of cardiac and vascular myocyte growth (Hall, 2004).

Many studies have now suggested the routine use of NT-Pro BNP in the diagnosis of HF – as a result of LV systolic impairment (McDonagh et al, 2004). The same authors report that, in relation to other studies, the efficacy of using NT-Pro BNP as a diagnostic tool lies in its high negative predictive value. This high value makes NT-Pro BNP a useful marker to ‘exclude’ HF in a given population. It should be noted however, that elevated levels of NT-Pro BNP merely confirm an element of cardio-renal stress and not

always LV systolic dysfunction. For instance, Mc Donagh et al (2004) confirm that in their population of patients with elevated NT-Pro BNP, 10% of breathless patients had concentric LV hypertrophy without signs of systolic impairment, almost one fifth of the population had had a previous MI but no echocardiographic evidence of systolic impairment and 1.8% of the population had normal cardiac structure and function but significantly impaired renal function.

Blood samples were drawn from the antecubital vein with the patients in a supine position between 800 hrs and 1200 hrs to coincide with serum samples for testosterone analysis. Samples were collected in pre-chilled tubes containing ethylenediaminetetraacetic acid (EDTA), immediately placed on ice, and promptly centrifuged at 4°C by the research nurses in the heart failure unit. After separation, plasma was transferred to the Clinical Chemistry Department at University Hospital South Manchester and stored at -80°C. NT-pro BNP analysis was undertaken using an ELISA—a two step sandwich assay with streptavidin coated microtitre plates – a technique already validated by Karl et al, 1991. The streptavidin assay does not require sample extraction and there is no detectable cross reactivity with ANP, NT-pro ANP, BNP, or urodilatin. The inter-assay and intra-assay coefficient variances were 10% and 3% respectively. Sample recovery was between 104% and 112%.

3.13.2.5: Echocardiography.

Morphological and functional changes in cardiac structure and function were assessed using a commercially available ultrasound device (GE, Vingmed, Vivid 7, Norway) and dedicated 3.5 MHz phased array ultrasonic transducer. Each patient was assessed using guidelines produced by the American Society of Echocardiography (Gottinger, 2004).

All echocardiographic measurements were averaged over 3 cardiac cycles for patients in sinus rhythm. If patients were found to be in atrial fibrillation at the time of echocardiography, 5 cardiac cycles were recorded and subsequently analysed. No analysis was performed on or immediately prior to ectopic beats. For analysis based on two dimensional (2D) speckle tracking echocardiography, stable R-R intervals were selected in order to further increase accuracy of the analysis.

Echocardiography is one of the most commonly performed non-invasive techniques in the assessment of patients with known or suspected cardiovascular disease, providing a comprehensive evaluation of cardiovascular structure, function and hemodynamics. 2D (B mode) echocardiography displays cardiac images in real-time, allowing the operator to obtain multiple anatomic 'slices' of the heart by precise angulation of the echocardiographic transducer.

M-mode echocardiography is a continuous 1-dimensional display of structures along a single anatomic line versus time and, because of its high temporal resolution, can be used to accurately assess valve / chamber motion and measure diameter changes over time. Doppler echocardiography uses ultrasonic reflections from moving red blood cells in order to characterise flow in the central and peripheral circulation. Therefore, this technique can be used during echocardiography in order to assess systolic and diastolic flow through valves, within the cardiac chambers and also in the major cardiac vessels. The four main types of Doppler used include pulsed wave, continuous wave, tissue and Colour. Pulsed wave Doppler can detect flow in a localised area and that is moving within the physiological range of velocities. Continuous wave Doppler is useful for measuring gradients across pathological flow jets. Colour flow mapping displays information as colour coded pixels (toward and away from the echo transducer) in order

to display blood flow over a relatively large area. This technique is useful for evaluating flow through valves, assessing diastolic and systolic flow, assessing intra-cardiac shunts and measuring coronary flow.

Tissue Doppler is a relatively new technique used to detect movement of the myocardium over time. This technique has proved important in evaluating diastolic function and systolic function. In addition, during this initial study, myocardial strain was assessed using a comprehensive commercially available analysis system (EchoPac, GE, Vingmed, Norway). Longitudinal systolic strain involves assessment of the longitudinal myocardial fibres responsible for longitudinal shortening in systole. As the LV shortens in systole, thickening of the walls must also occur in order to maintain wall volume in an in-compressible structure (myocardium). Wall thickening reflects the contraction of individual muscle fibres in all directions and therefore as the outer contour shows little change during systole, the ventricle must also thicken inwards as the long axis length reduces in systole (Stoylen, 2011). As a result of this, longitudinal shortening also determines systolic wall thickening. Circumferential strain or mid-wall circumferential shortening is dependent on LV diameter only (Stoylen, 2011). Therefore, circumferential shortening is an inward movement of the circumferential line due to systolic wall thickening and as such is derived by cavity diameter, wall thickness and wall thickening.

For the echocardiographic assessment, participants lay in the left lateral position and images were obtained in concordance with the standardised minimum dataset for echocardiography in the UK (Chambers et al, 2009) by an experienced and accredited

cardiac sonographer. During the echocardiographic assessment, the following parameters were measured:

1. Detailed volumetric assessment of the left atrium was performed in both the apical 4 and apical 2 chamber imaging planes. Volumetric assessment was carried out in a similar fashion to the Biplane Method of Discs used for LV volumetric assessment. Accurate tracing of the left atrial border from the mitral valve annulus was performed at various points within the cardiac cycle and averaged according to 4 and 2 chamber values. Using this data, the following LA volumetric data could be calculated according to methods detailed by Todaro et al, 2012.

a. Left atrial pre-contraction volume (V_{preA}) corresponding to the onset of the P wave on the surface ECG.

b. Minimal left atrial volume (V_{min}), measured on mitral valve closure at end-diastole.

c. Maximal left atrial volume (V_{max}), measured immediately prior to mitral valve opening at end-systole.

Accordingly, these values were used to calculate further parameters relating to left atrial active volumetric assessment as detailed by Nikitin et al, 2003.

a. Left atrial active emptying fraction (LA-AEF%) $(V_{preA} - V_{min}) / V_{preA} \times 100$

b. Left atrial expansion index (LA-EI) $(V_{max} - V_{min}) / V_{min} \times 100$

c. Left atrial passive emptying fraction (LA-PEF%) $(V_{max} - V_{preA}) / V_{max} \times 100$

For patients who presented in atrial fibrillation at time of echocardiogram, pre contraction volumes were not calculated due to the absence of 'p' wave on the surface

ECG. As such, for these patients, LA active and passive emptying fractions were not able to be calculated.

2. Left ventricular end-diastolic diameter was measured from both parasternal long axis images and also apical 4 chamber images at end diastole. In addition, LV long axis end-diastolic length was assessed from apical 4 chamber images – measured from the level of the mitral valve annulus to the LV apical endocardial border definition. The ratio of LV short to long axis ratio was calculated (from the 4 chamber measurements) in order to obtain the Sphericity Index – an indicator of LV remodelling (Obeidat et al, 2004).

3. Left Ventricular EF was calculated using Simpsons Biplane Method of Discs in patients with clear-enough echocardiographic windows. This required accurate tracing of the endocardial border at end diastole and systole in both apical 4 and apical 2 chamber image planes using the mitral valve annulus as the base for the measurement. The percentage change in LV volume between these cycles was expressed as percentage EF and average values of 4 and 2 chamber measurements taken.

4. Sophisticated echocardiography analysis software (Q-analysis, GE Medical, Norway) was used in order to calculate longitudinal and circumferential left ventricular strain. Longitudinal strain was measured from the apical 4 and 2 chamber image planes together with the apical long axis view and averaged over at least 3 cardiac cycles with stable R-R intervals. Images were optimised for depth and sector width in order to promote accurate tracing of the myocardium with a minimum of 12 markers, allowing for detailed assessment of myocardial speckle tracking throughout the cardiac cycle. Images were acquired with frame rates between 60 and 80 per second according to pre-determined best practise criteria (Mor-Avi et al, 2011) and with the intention of avoiding loss of speckles or detriment to spatial resolution. Correlation of myocardial

movement with the marked area was checked and adjusted if necessary to ensure detailed tracking of the myocardium throughout the cardiac cycle. Longitudinal strain was measured as the observed shortening between myocardial speckles at end-systole as the aortic valve closes and also the peak systolic strain value, regardless of aortic valve closure. Timing calibration was achieved by using Doppler echocardiography of the aortic valve and the R wave on the ECG in order to accurately determine aortic valve opening and closure thereby defining systole accurately. Timing of R wave to aortic valve opening, aortic valve opening to aortic valve closure and R wave to aortic valve closure were all measured.

5. Circumferential strain was assessed in the parasternal short axis view. Accurate myocardial tracing was performed at the level of the mitral valve annulus, papillary muscle and also cardiac apex – defined as more distal to the papillary muscle structure and with none or the smallest view of the right ventricle that was achievable. The average circumferential shortening of myocardial fibres was calculated at end systole upon aortic valve closure – defined as per the timing cycles outlined earlier. In addition, circumferential strain at the mitral valve level and at the apex was used in order to calculate ‘twist’ in degrees’ using the formula of mitral valve level (basal) rotation – apical rotation. In addition to this, left ventricular twist / untwist rates together with basal and apical rotation rates and time to left ventricular untwist / peak basal and apical rotation rate were measured from the circumferential strain curves. It should be noted that the terms twist, rotation and torsion are often used interchangeably and may represent different facets of cardiac mechanics depending upon the research group. During this study, rotation was defined as rotation of sections of the myocardium in the parasternal short axis imaging plane as viewed from the apical end and also defined as the angle between radial lines connecting the centre of mass of each specific cross sectional plane to a specific point within the myocardial wall (Henson et al, 2000).

Twist, as mentioned previously was defined as the absolute base to apex difference in rotation and is most commonly expressed as the net LV twist angle (Henson et al, 2000). In this study, torsion was simply derived from net LV twist angle normalised for base to apex distance (Russell et al, 2009).

It is a widely held notion that the left ventricular base and apex rotate in opposite directions. During isovolumetric contraction the LV apex initially rotates slightly clockwise before, during LV ejection, rapidly rotating oppositely in the counter-clockwise direction (Narula et al, 2007). At the apical level, this counter clockwise twist seen during ejection is immediately followed by untwisting (clockwise rotation) during isovolumetric relaxation and the early diastolic period. Basal left ventricular rotation is observed at lower magnitude to the apex (Sengupta et al, 2008). Additionally, rotation at this level is opposite to that observed at the apex with brief counter clockwise rotation during isovolumetric contraction and clockwise rotation during the ejection phase. Naturally, there is counter clockwise rotation during isovolumetric relaxation and the early diastolic filling period (Sengupta et al, 2008). Of course, this mechanical movement of the myocardium during the cardiac cycle is closely related to myofibrillar geometry. As such, within the myocardium, myofibre geometry alters from a right handed helical structure at the sub-endocardium to a left hand structure at the level of the sub-epicardium (Taber et al, 1996). Therefore, it results that contraction of the epicardial fibres will result in apical counter clockwise rotation and basal clockwise rotation and the opposite phenomenon when the subendocardial fibres are activated. Due to the greater wrap around distance for the epicardial fibres, they become the dominant force when both types of fibres are activated simultaneously (Sengupta et al, 2008). At the onset of ventricular electromechanical activation, the natural cardiac conduction system results in initial activation of the mid-apical septal subendocardial layer and as such the activation transmits from apex to base. As the subendocardial

fibres contract, subepicardial fibres become stretched to result in the initial clockwise rotation of the LV apex (Taber et al, 1996). Electrophysiological activation from the endocardium to epicardium results in subsequent fibre shortening with the subepicardial fibres producing the highest torque due to their greater radius and therefore dominating the rotational direction to counter clockwise at the apex and clockwise towards the base. The subsequent twisting and shearing of the subendocardial fibres during the ejection period, results in the storage of potential energy which is of paramount importance for diastolic recoil (Bell et al, 2000). This torsional recoil is facilitated by lengthening-shortening gradients in the LV wall with epicardial lengthening at the base / shortening at the apex as opposed to endocardial shortening at the base / lengthening at the apex resulting in an untwisting motion opposite to that of the ejection phase (Sengupta et al, 2008). This simultaneous shortening and lengthening at the base and apex allows diastolic restoration without change to LV volume.

6. Myocardial speckle tracking techniques were used in a similar manner to that employed when assessing LV longitudinal strain for the left atrium in apical 4 chamber images. Images were optimised for depth and sector width in order to achieve frame rates in the region of 60-80 frames per second. Following this, the left atrial border was carefully marked with at least 12 points to allow accurate tracing of the atrial walls. The mitral valve annulus was used as the base for these measurements. Care was taken to avoid tracking the pulmonary veins and also to ensure atrial septal movement was accurately mimicked. All atrial strain parameters were averaged over 3 cardiac cycles in sinus rhythm and at least 5 cycles in atrial fibrillation. NB. As with the aforementioned LA mechanical volumetric measurements, patients in atrial fibrillation at the time of echocardiography did not have atrial contractile properties measured. From the ensuing left atrial strain curves, the following parameters were assessed:

a. Peak longitudinal strain of the contractile period.

- b. Peak longitudinal strain of the reservoir period.
- c. Peak longitudinal strain of the conduit period.

In more detail, myocardial shortening during the LA contractile period normally results in negative strain values. Strain values obtained for the reservoir phase (during ventricular systole) are normally positive due to the dilatation and stretch of the atria as the ventricle contracts. Left atrial conduit function is largely dependent upon LV relaxation and preload with the transfer of blood to the LV being accompanied by LA shortening giving rise to negative strain values (Todaro et al, 2012).

7. Tissue Doppler imaging was used to assess indices of both LV longitudinal systolic function and LV diastolic function together with their detailed relationship (Vinereanu et al, 2005).

- a. Longitudinal LV Systolic Function by Tissue Doppler Imaging.

The apical 4 chamber view was obtained and optimised in order to improve image quality, ensure accurate Doppler beam alignment and promote higher frame rates. Tissue Doppler Imaging was selected and the pulsed wave Doppler cursor placed over the septal (medial) and lateral positions of the mitral valve annulus. On the resultant tissue Doppler display, longitudinal systolic velocity was measured by placing a cursor upon the maximum systolic velocity (s') noted for each myocardial wall and averaged to give S' in cm/s (Ho and Solomon, 2006).

- b. LV Diastolic Function by Tissue Doppler Imaging.

In order to assess LV diastolic function using TDI, all measurements were taken in the apical 4 chamber view – optimised as previously mentioned to promote high frame rates and optimum Doppler alignment. Conventional LV inflow velocities (E and A wave)

were assessed at the tips of the mitral leaflets using pulsed wave Doppler with optimised scale settings. Average E velocity over 3 or 5 consecutive beats was recorded. In addition to this, tissue Doppler imaging was obtained as above at the septal (medial) and lateral walls. E' velocity was measured and averaged over 3 or 5 consecutive beats and also averaged over the medial and lateral walls. E/E' ratio was calculated to give an indicator of LV diastolic compliance (Nagueh et al, 1997).

In addition to left heart mechanical and functional assessment, the right heart was studied in detail for mechanical and functional properties. All measurements were undertaken on 3 separate cardiac cycles in sinus rhythm and 5 cycles when patients were in atrial fibrillation. Mechanical 2D speckle tracking and TDI parameters were measured with as constant as possible R-R interval in order to maintain consistency between measures. Using standard 2 dimensional imaging, the following parameters were assessed:

1. In the apical 4 chamber image plane, sector width and depth settings were adjusted in order to focus directly on the right atrium. When this was achieved, the image was frozen in systole when the tricuspid valve leaflets were closed. Careful border tracing of the right atrial area was undertaken using the tricuspid valve annulus as a base for the measurement.
2. In the same apical 4 chamber window, images were then optimised in order to accurately assess the right ventricle. In this view, right ventricular diastolic and systolic area was traced with reference to ECG timing of the cardiac cycle. Using the derived diastolic and systolic areas, right ventricular fractional area change % was calculated with the following formula:

$$(\text{RV end diastolic area} - \text{RV end systolic area} / \text{RV end-diastolic area})$$

3. M-mode echocardiography was used to measure Tricuspid Annular Plane Systolic Excursion (TAPSE) from apical 4 chamber images. The m-mode cursor was placed through the anterior aspect of the tricuspid valve annulus ensuring that each image was 'on axis' and in-line with the m-mode cursor. Movement of the annulus towards the apex was recorded against time and this distance measured during systole. TAPSE is a measure of right ventricular longitudinal systolic function and hence a correlate of overall right ventricular systolic function (Zeinah et al, 2009).

4. Longitudinal strain of the right ventricle was measured from the apical 4 chamber image plane and averaged over at least 3 cardiac cycles with stable R-R intervals. Images were optimised for depth and sector width in order to promote accurate tracing of the myocardium with a minimum of 12 markers, allowing for detailed assessment of myocardial speckle tracking throughout the cardiac cycle. Images were acquired with frame rates between 60 and 80 per second according to pre-determined best practise criteria (Mor-Avi et al, 2011) and with the intention of avoiding loss of speckles or detriment to spatial resolution. Correlation of myocardial movement with the marked area was checked and adjusted if necessary to ensure detailed tracking of the myocardium throughout the cardiac cycle. Longitudinal strain was measured as the observed shortening between myocardial speckles at end-systole as the pulmonary valve closes and also the peak systolic strain value, regardless of pulmonary valve closure. Timing calibration was achieved by using Doppler echocardiography of the pulmonary valve and the R wave on the ECG in order to accurately determine pulmonary valve opening and closure thereby defining right ventricular systole accurately. Timing of R wave to pulmonary valve opening, pulmonary valve opening to pulmonary valve closure and R wave to pulmonary valve closure were all measured.

3.13.2.6: Health related, disease specific and psychological quality of life.

Both HF and androgen deficiency are characterised by low mood and depression, which is improved by testosterone replacement therapy in testosterone deficient subjects. Thus, patients were instructed to complete the Minnesota Living with Heart Failure questionnaire (MLHFQ), the short form 36 version 2 (SF-36 v2) Health Survey, the Beck Depression Inventory (BDI) and the Androgen Deficiency in the Adult Male Screening Questionnaire (ADAM).

3.13.2.7: Minnesota Living with Heart Failure Questionnaire (MLHFQ).

The MLHFQ (appendix 4) has been designed as a disease-specific measure of quality of life in patients with HF (Rector et al, 1987). This questionnaire is designed to assess each participant perception of the effects of HF on the physical, socio-economic and psychological aspects of their life. There are a total of 21 questions with responses using a six-point Likert Scale (0-5). This questionnaire is simple to administer and relatively easily understood. This questionnaire has previously been shown to be valid in comparison to other health outcome scales where it has also demonstrated to differentiate between those patients with HF and more symptomatic LV impairment (Berry et al, 1999 and Gorkin et al, 1993). Test-retest techniques have suggested that initial low-scores had a tendency to increase and initial high scores tended to decrease following the second administration of the questionnaire (Bowling, 2001). Slight concerns have been raised suggesting that using the sub-scales may be less accurate in the quality of life assessment when compared to using the overall score (Sneed et al, 2001), hence in this study, total overall questionnaire score will be utilised.

3.13.2.8: SF-36 Version 2 Questionnaire.

The SF-36 Version 2 (appendix 5) is a 36 item questionnaire that measures eight multi-item dimensions of general health. These include physical functioning (10 items), social functioning (2 items), role limitations due to physical problems (4 items), role limitations due to emotional problems (3 items), mental health (5 items), energy / vitality (4 items), pain (2 items) and general health perception (5 items). For each of these dimensions, item scores are coded, summed and transformed onto a scale ranging from 0 to 100 with zero being the worst possible health state and a hundred the best possible state. In addition, two further standardised summary scores can be calculated namely, the physical component and mental health component summary. Improvements have been made in the development of the SF-36 Version 2 when compared to the older version 1. Most notably improvements to both instruction and question wording have made the questionnaire less ambiguous and have removed the contentious double negative noted in the older version (Jenkinson, 1995). More recent research by Jenkinson and co-workers (1999) has shown that the SF-36 Version 2 has good internal validity when utilising Cronbach's alpha statistic. Furthermore, the authors suggested that internal reliability statistics for the two role-functioning dimensions are higher than previously reported for the SF-36 Version 1. As claimed by the developers of the Version 2 questionnaire, fewer floor and ceiling effects were noted in the Oxford registry (1.32% of respondents scoring zero and 56.29% scoring 100 using version 2 versus 7.16% versus 75.5% respectively for version 1 when observing role-physical dimensions).

3.13.2.9: Beck Depression Inventory (BDI).

The BDI (appendix 6) is the most commonly used tool for the self-assessment of depression in clinical research (Gottlieb et al, 2004). The BDI is a short questionnaire

containing 21 items with 4 response outcomes. The questionnaire is easy to administer and has a reading level equivalent to that of the fifth grade in the USA. As such, the scale is designed to rate the severity of depression in individuals aged over 13 years. Previous assessment of the scale by Beck and colleagues (1988), has shown high levels of internal consistency both in psychiatric (0.86) and non-psychiatric participants (0.81). Furthermore, the concurrent validity of the BDI with respect to clinical ratings of depression and the Hamilton Psychiatric Rating Scale for Depression (HPRS-D) have been reported to be high (Beck et al, 1988). This research identified mean correlations of the BDI samples (in psychiatric patients) with clinical ratings and HPRS-D of 0.72 and 0.73 respectively and 0.60 and 0.74 respectively in a non-psychiatric population. Depressive status is defined as a questionnaire score of ≥ 10 .

3.13.2.10: Androgen Deficiency in the Ageing Male Questionnaire (ADAM).

The ADAM questionnaire (appendix 7) consists of 10 items with simple yes or no answers. Low testosterone status is defined as a total of 3 'yes' answers or answering 'yes' to questions 1 or 7 alone. The initial validation study of the ADAM questionnaire sampled 316 Canadian physicians aged between 40-62 years in the hope that the authors could identify a symptom complex to correlate with low testosterone levels (Morley et al, 2000). Low bio-available testosterone was noted in 25% of this population and the ADAM questionnaire was found to have 88% sensitivity and 60% specificity to low serum testosterone level. Test-retest reproducibility demonstrated a coefficient of variation of 11.5%. Furthermore, following treatment of testosterone deficiency in a subset of the same population, the ADAM questionnaire was found to demonstrate an improvement in score in 18 out of 21 patients ($p = 0.002$). Although sensitivity is high, specificity is commonly regarded as low for this questionnaire due to the recognition that depression, as well as a number of symptoms commonly seen in the older person,

will confound the attempts to successfully screen this population using a questionnaire based technique (Morley et al, 2006).

3.14: Measurement error.

A cohort of 10 patients was selected in order to assess the reproducibility of the main outcome measures. This included 6 min walk distance and important echocardiographic parameters. Reproducibility was assessed using the relative technical error of measurement (TEM) and expressed as percentage error by the formula below:

$$\%TEM = (TEM / [M1+M2] / (2) \times 100$$

Where M1 is the mean of the first series of measurements, M2 is the mean of the second series of measurements.

It should be noted that the % value obtained pertains to % change from the previous measurements and not an actual % unit difference from the last measurement (when measurements are expressed in terms of %). For example, a TEM% of 3 relates to a 3% error in the measurement in subsequent analyses and not a change from perhaps 28% to 31%.

Bland Altman plots were also used in order to assess the test-retest agreement between initial and second TT, bio-available and FT measurements and also the agreement between serum FT and salivary testosterone concentration.

3.15: Analysis of results.

Data were analysed using specialised statistical software (SPSS Version 18, Chicago, Illinois), by the Chief Investigator. Data were initially tested for normal distribution using the Shapiro Wilk statistical test due to the sample size being less than 50. Any data not normally distributed were transformed logarithmically and re-assessed for normality prior to analysis. Previously transformed data were back transformed and presented in the tables as geometric means. Independent samples T-tests were used to examine if there was a significant difference in the outcome measures between ‘low’ and ‘normal’ range testosterone. Furthermore, analysis of co-variance (ANCOVA) statistic was used to formally assess the effect of age on the primary outcome measure (6 min walk capacity) due to its role as a significant confounding variable and the noted small baseline difference in age between the groups. In addition, Pearson’s product moment correlation coefficient (r) was used to formally assess for bivariate relationships between traditional serum and salivary testosterone and pertinent outcome measures collected during this study. Significant correlations were set at the level $p < 0.05$. All data are expressed as mean \pm standard deviation unless otherwise stated. Bland Altman plots were also used in order to assess the accuracy of traditionally mathematically calculated testosterone values to newer clinical measurement techniques and to graphically represent the differences in testosterone concentration samples from different clinic visits.

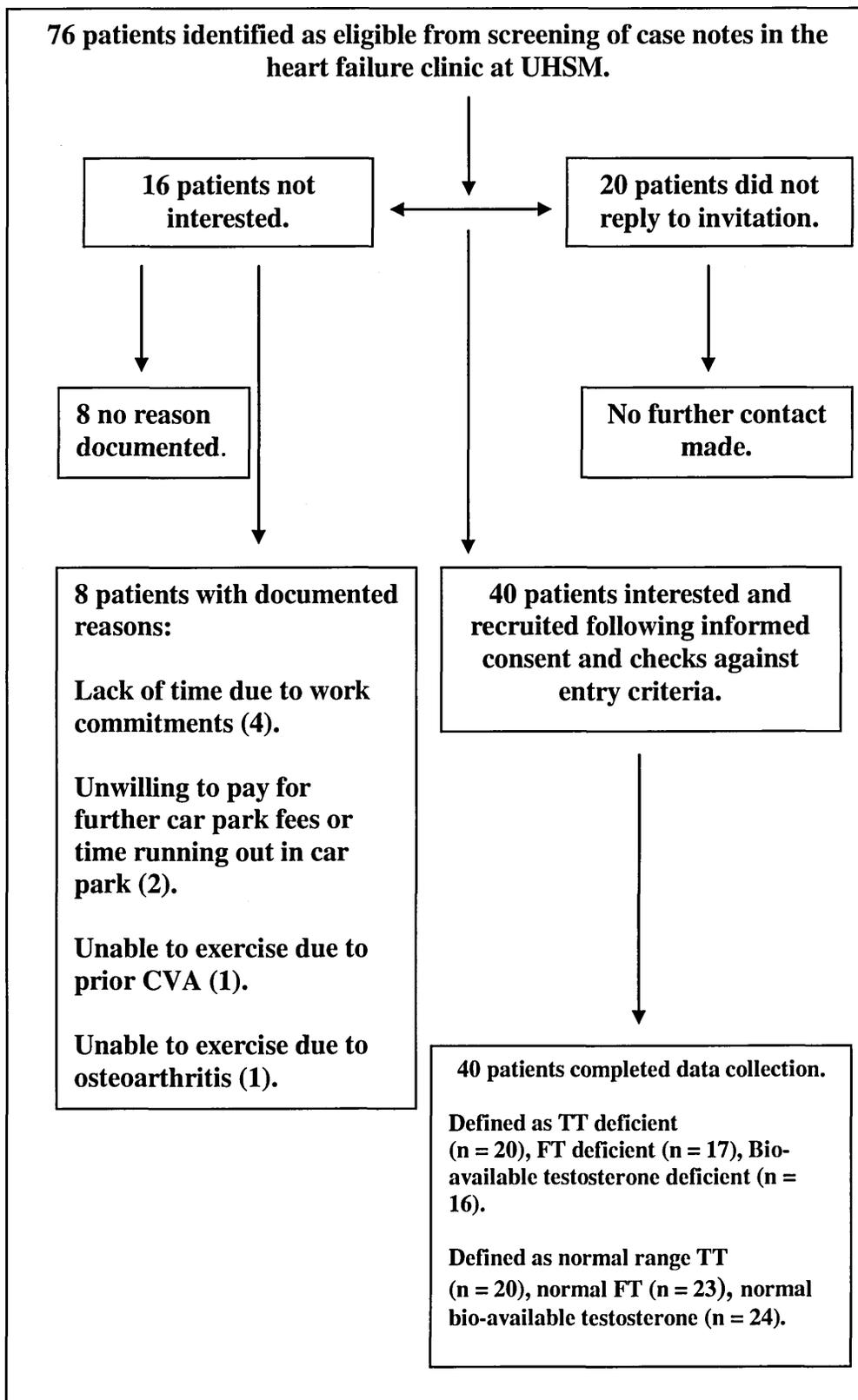
3.16: Quality control assurance and direct access to source documents.

Quality control of the study was maintained by the Chief Investigator in collaboration with the local research ethics committee and the Sponsor research and development department. All appropriate risk assessments were in place prior to commencement of the research and also reviewed throughout the study period. The research team were able to provide access to source documents for inspection by authorised outside agencies upon written request.

4.1: Demographic Data.

A total of 76 patients were identified from the screening of patient case notes in the UHSM specialist heart failure clinic. Of these, 20 patients (26%) did not reply to their initial invitation letter and 8 (11%) patients replied as 'not interested' without any documented reason. Of the remaining 8 patients (11%) who replied as 'not interested', the most common reasons were lack of time or work commitments – particularly due to the risk of over-running parking time or not wishing to pay more money for the car-park. Other, more rare, reasons included co-morbidities preventing exercise – such as previous cerebrovascular accident (CVA) (1 patient) or osteoarthritis (1 patient). As this initial study was not interventional, all outcomes were collected at this initial visit resulting in 100% compliance and no drop-out. Figure 3 below details recruitment to the study. The two groups were well-matched at baseline for key variables when grouped for TT, FT and bio-available testosterone with no significant differences observed between the groups with the exception of testosterone status ($P < 0.01$). Table 9 details the baseline demographics for the study sample based on serum total testosterone.

Figure 3. Recruitment summary.



4.2: Analyses based on total testosterone concentration.

Table 9. Baseline demographics of the study sample based on serum total testosterone.

Both groups were well matched at baseline. No significant differences were noted between the groups at baseline using independent samples T-tests with the exception of testosterone status ($P < 0.01$). Data presented as mean \pm s.d. %: percent of population ($n = 20$).

Characteristics	Low-range total testosterone (n=20)	Normal-range total testosterone (n=20)
Age (y)	71.9 \pm 10.11	66.9 \pm 11.98
Stature (m)	1.75 \pm 0.07	1.74 \pm 0.07
Body mass (kg)	84.49 \pm 16.62	80.71 \pm 13.67
Body mass index (kg/m^2)	27.62 \pm 4.88	26.38 \pm 3.55
Systolic BP (mm Hg)	125.6 \pm 21.70	118.10 \pm 12.85
Diastolic BP (mm Hg)	74.01 \pm 9.63	69.65 \pm 9.33
Resting HR (beats/min)	66.25 \pm 15.45	67.54 \pm 13.68
Total Testosterone (nmol/L)	9.21 \pm 1.84	17.82 \pm 5.31
Free Testosterone (nmol/L)	0.132 \pm 0.33	0.271 \pm 0.67

SHBG (nmol/L)	64.21 ± 9.62	59.31 ± 7.30
Albumin	39.98 ± 1.98	40.28 ± 2.01
Bio-available testosterone (nmol/L)	3.14 ± 0.98	6.97 ± 1.05
NYHA Score	2.0 ± 0.46	2.0 ± 0.32
Left ventricular end-diastolic volume (mls)	142.32 ± 29.14	144.99 ± 36.21
Left ventricular EF (%)	28.31 ± 7.07	30.72 ± 6.25
Aetiology of HF		
Ischaemic	12 (60%)	10 (50%)
Hypertensive	1 (5%)	2 (10%)
Idiopathic dilated cardiomyopathy	4 (20%)	4 (20%)
Unclear	3 (15%)	4 (20%)
Co-morbidities		
Ischaemic heart disease	12 (60%)	10 (50%)
Previously documented atrial fibrillation	8 (40%)	9 (45%)
Atrial fibrillation at data collection	3 (15%)	5 (25%)
Hypertension	15 (75%)	18 (90%)
Diabetes	8 (40%)	7 (35%)
Chronic obstructive pulmonary disease	6 (30%)	4 (20%)
Peripheral arterial disease	4 (20%)	2 (10%)
Medication		

Beta Blocker	19 (95%)	20 (100%)
ACE Inhibitor	20 (100%)	20 (100%)
Diuretic	19 (95%)	19 (95%)
Aldosterone Receptor Blocker	4 (20%)	3 (15%)
Digoxin	1 (5%)	1 (5%)
Anti-Arrhythmic	12 (60%)	10 (50%)
Anti-platelet	20 (100%)	19 (95%)
Anticoagulant	11 (22%)	8 (40%)
Statin	18 (90%)	20 (100%)

(y) years, (m) metres, (kg) kilogram's, (m²) metres squared, (mmHg millimetres of mercury, (min) minute, (nmol/L) nanomols per Litre, (NYHA Score) New York Heart Association Score, (ACE) Angiotensin converting enzyme, (EF%) ejection fraction, (SHBG) sex hormone binding globulin, (HR) heart rate, (BP) blood pressure, (%) percent of population (n = 20).

Table 10 presents the relative technical error of measurement of the outcome measures assessed in a cohort of 10 patients who underwent identical assessments on a second occasion.

Table 10. Measurement error for key outcomes.

Error expressed as percentage change between first and second measurement.

Test	TEM% (n=10)
6 min shuttle walk	4.18
EF%	8.47
Max LA volume	6.24
LA expansion index	6.60
TDI LA ventricular systole	4.65
Peak LA strain (reservoir)	4.98
LV peak systolic torsion	7.20
LV peak untwist rate	8.74
Twist	9.21
Circumferential strain	7.24
Longitudinal strain	7.65
Sphericity index	6.40
LV diastology (E/E')	4.21
LV longitudinal function (S wave)	4.87
TAPSE	3.10
RV fractional area change	5.74
RV peak systolic strain	3.98

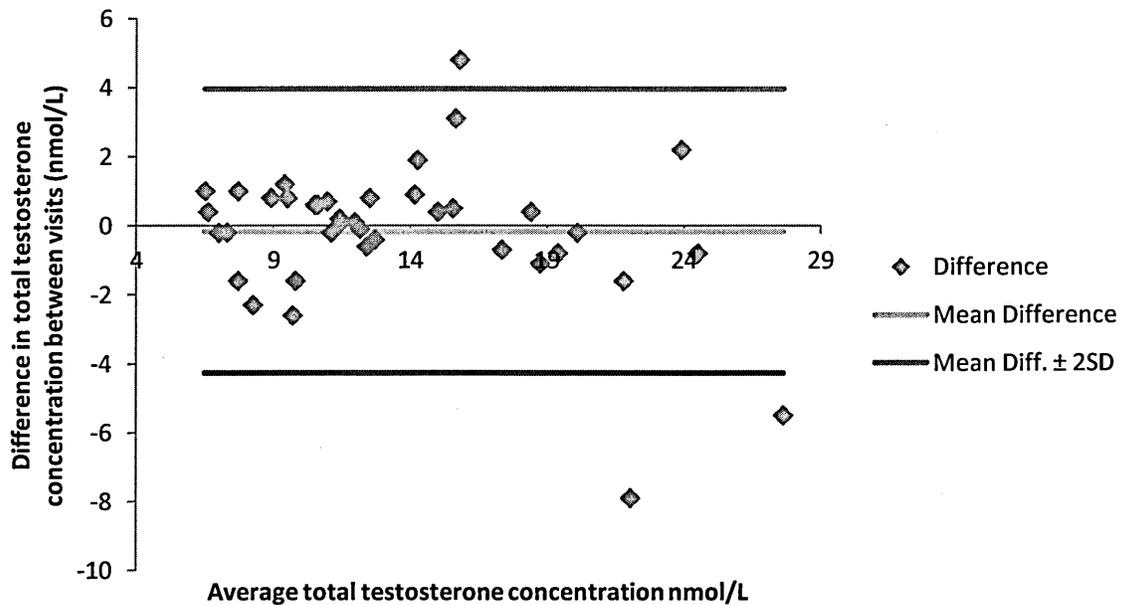
(min) minute, (EF%) ejection fraction, (max) maximum, (LA)left atrial, (TDI) tissue Doppler imaging, (LV) left ventricular, (TAPSE) tricuspid annular plane systolic excursion, (RV) right ventricular).

All outcome measures above demonstrate satisfactory technical error of measurement – all comfortably within 10% difference between repeated measurements. Left ventricular twist and untwist demonstrate the most erroneous measurement errors. However, this may be due to their reliance on other echocardiographic measures (apical and base rotation) in order to be calculated. The main outcome measure (6 min walk) demonstrates excellent reproducibility with a measurement error of 4%.

The Bland Altman plot (figure 4) shows the agreement between initial and subsequent measurement of total testosterone performed on a separate clinic visit.

Figure 4 Bland Altman plot showing the agreement between total testosterone measurements on two separate occasions.

Minimum 4 weeks between initial measurement, maximum 4 months to coincide with routine clinic visit.



The Bland Altman plot shows a mean difference between TT measurements of 0.15 nmol/L (s.d. \pm 2.05 nmol/L). Therefore, the mean difference \pm 2 s.d. is + 3.95 nmol/L and - 4.26 nmol/L. The coefficient of repeatability is 5.10 nmol/L. Greater than 95% of measurements fall acceptably within the limits of agreement of \pm 2 s.d. The outlying measurements did not represent a change of testosterone status (i.e. there was no change from hypo to eugonadal status between measurement 1 and measurement 2).

4.2.1: Primary outcome measure – 6 min walk test.

Table 11 highlights the data from the 6 min walk test for patients grouped by total testosterone.

Table 11. 6 min walk data based on total testosterone status.

Independent samples T-test data is presented as mean \pm s.d. Significance at $P < 0.05$ is highlighted with *. ** indicates $P < 0.01$.

	Low-range total testosterone (n=20)	Normal-range total testosterone (n=20)	P value
6 min walk distance (m)	257.65 \pm 106.89 _(n=20)	429.00 \pm 126.94** _(n=20)	<0.001
6 min walk HR (bpm)	97.0 \pm 18.23 _(n=20)	97.05 \pm 18.63 _(n=20)	0.993
6 min walk peak RPE	13.85 \pm 2.83 _(n=20)	12.2 \pm 3.17 _(n=20)	0.091

(m) metres, (HR) heart rate, (RPE) rating of perceived exertion, (bpm) beats per minute, (min) minute.

There was a significant difference in walking distance between normal range total testosterone when compared to low range total testosterone (257.65 \pm 106.89 m LT versus 429.00 \pm 126.94 m NT, $P < 0.001$), despite evidence of working at a similar exercise intensity (no difference noted in heart rate or perceived exertion between the groups).

4.3: Secondary outcome measures.

4.3.1: Echocardiography.

Tables 12-14 show the echocardiographic measures used to assess, in detail, cardiac structure and function. Independent samples T-test data are presented as mean \pm s.d.

Table 12. Left atrial structural and functional characteristics for normal and low range TT.

Independent samples T-test. Significance at $P < 0.05$ is highlighted with *. ** indicates $P < 0.01$. Figures in brackets indicate number of patients for each parameter measured.

	Low TT (n=20)	Normal TT (n=20)	P value
Min LA volume (mls)	62.80 ± 21.86 (n=17)	66.90 ± 22.62 (n=19)	.56
Max LA volume (mls)	74.10 ± 20.75 (n=17)	80.90 ± 20.09 (n=19)	.30
Pre-contraction LA volume (mls)	62.75 ± 17.67 (n=14)	64.60 ± 18.80 (n=14)	.78
LA expansion index	21.80 ± 11.10 (n=17)	26.15 ± 18.18 (n=19)	.26
LA passive emptying fraction	8.16 ± 3.63 (n=14)	14.05 ± 6.71 (n=14)	.005*
LA active emptying fraction	11.27 ± 5.65 (n=14)	9.56 ± 7.15 (n=19)	.47

TDI LA Sa (cm/s)	3.97 ± 1.32 (n=17)	3.98 ± 1.30 (n=19)	.97
TDI LA Ea (cm/s)	4.82 ± 1.68 (n=17)	4.74 ± 1.89 (n=19)	.91
TDI LA Aa (cm/s)	4.83 ± 1.90 (n=17)	3.61 ± 1.53 (n=19)	.06
Peak longitudinal strain reservoir (%)	21.83 ± 4.24 (n=16)	21.42 ± 3.39 (n=17)	.74
Peak longitudinal strain conduit (%)	3.87 ± 1.75 (n=16)	4.57 ± 1.66 (n=17)	.20
Peak longitudinal strain contractile (%)	-3.87 ± 1.35 (n=14)	-3.68 ± 1.02 (n=14)	.67

(min) minimum, (max) maximum, (mls) millilitres, (Sa)ventricular systole, (Ea) early diastole, (Aa)late diastole (atrial contraction), (cm/s) centimetres per second, (TDI) tissue Doppler imaging, (LA) left atrial.

There was a significant difference in left atrial passive emptying fraction between low and normal TT (P = 0.005). However, there were no other significant difference in parameters relating to left atrial structure and function between the groups based on TT.

Table 13. Left ventricular structural and functional characteristics for normal and low range TT.

Independent samples T-test. Significance at $P < 0.05$ is highlighted with *. ** indicates $P < 0.01$. Figures in brackets indicate number of patients for each parameter measured.

	Low TT (n=20)	Normal TT (n=20)	P value
LV width (end-diastole) (cm)	5.48 ± 0.74 (n = 20)	5.84 ± 1.16 (n = 20)	.24
LV end-diastolic length (cm)	8.74 ± 0.94 (n = 20)	8.78 ± 0.87 (n = 20)	.88
Biplane Simpsons EF%	28.31 ± 7.07 (n = 15)	30.72 ± 6.25 (n = 16)	.28
Sphericity Index	1.69 ± 0.51 (n = 20)	1.71 ± 0.48 (n = 20)	.86
MAPSE (mm)	10.55 ± 3.07 (n = 20)	9.82 ± 2.37 (n = 20)	.42
TDI Peak s' medial (cm/s)	3.52 ± 1.21 (n = 20)	3.37 ± 1.21 (n = 19)	.71

TDI peak s' lateral (cm/s)	4.10 ± 1.58 (n = 18)	3.85 ± 1.51 (n = 19)	.62
TDI E/e'	24.26 ± 15.31 (n = 18)	22.78 ± 16.01 (n = 19)	.60
Peak longitudinal systolic strain (%)	-6.86 ± 3.2 (n = 18)	-6.74 ± 2.29 (n = 19)	.90
Peak circumferential systolic strain (%)	-6.82 ± 2.29 (n = 15)	-7.45 ± 2.71 (n = 19)	.49
Peak systolic twist (°)	3.39 ± 2.40 (n = 16)	3.57 ± 3.11 (n = 17)	.87
Peak systolic twist rate (°/sec)	46.82 ± 11.83 (n = 17)	45.78 ± 11.29 (n = 17)	.78
Peak systolic torsion (°/cm)	0.44 ± 0.27 (n = 17)	0.41 ± 0.36 (n = 17)	.78
Peak untwisting rate (°/sec)	-46.82 ± 11.82 (n = 17)	-45.78 ± 11.29 (n = 17)	.70
Time to peak untwisting rate (% systole)	118.83 ± 4.60 (n = 17)	118.08 ± 5.89 (n = 17)	.70

Table 14. Right heart structural and functional characteristics for normal and low range TT.

Independent samples T-test. Significance at $P < 0.05$ is highlighted with *. ** indicates $P < 0.01$. Figures in brackets indicate number of patients for each parameter measured.

	Low TT (n=20)	Normal TT (n=20)	P value
RA area (systole) (cm ²)	15.34 ± 1.99 (n=20)	14.10 ± 2.49 (n=20)	.08
RV area end-diastole (cm ²)	15.55 ± 2.50 (n=20)	14.80 ± 2.46 (n=20)	.34
RV fractional area change (%)	33.20 ± 9.69 (n=20)	36.95 ± 12.20 (n=20)	.29
TAPSE	15.78 ± 3.76 (n=20)	15.23 ± 2.78 (n=20)	.69
RV peak longitudinal strain (%)	-28.97 ± 5.92 (n=15)	-29.48 ± 6.91 (n=17)	.80

(RA) right atrial, (cm²) centimetres square, (RV) right ventricular, (TAPSE) tricuspid annular plane systolic excursion (%) percent.

Based on TT and using echocardiography, there were no significant differences observed between the groups in the detailed assessment of right heart structure and function.

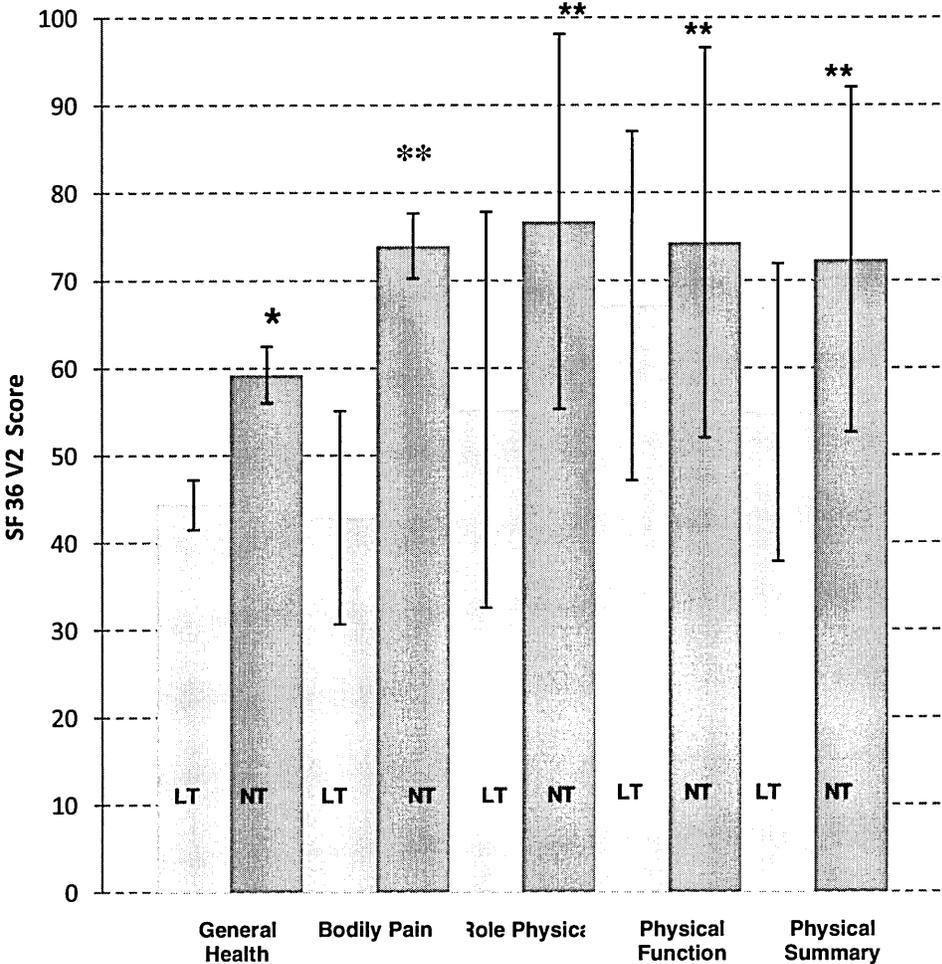
4.3.2: Quality of Life.

Figure 5 illustrates the SF36 Version 2 quality of life data for the low and normal range total testosterone groups

Figure 5. SF36 Domains based on total testosterone status.

Based on independent samples T-test data. *indicates $P < 0.05$ and ** indicates $P < 0.01$.

Error bars are representative of s.d.



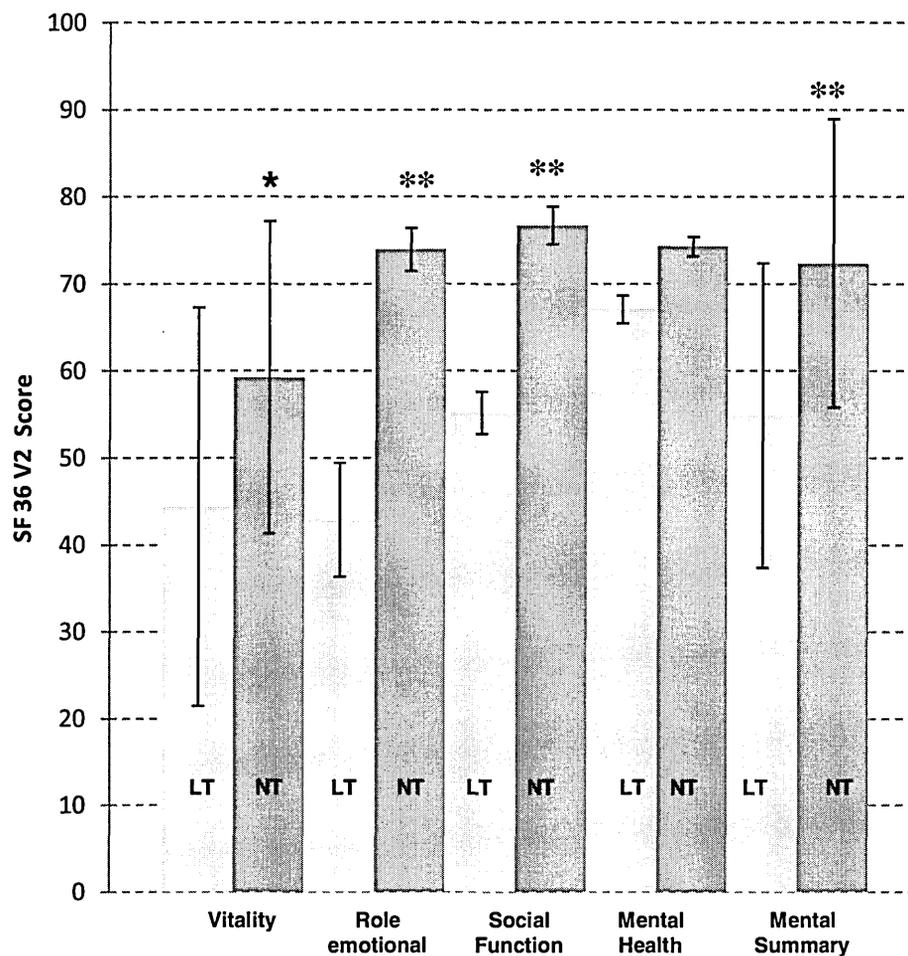


Figure 5 above illustrate numerous significant differences in SF36 domain score in the normal range testosterone group when compared to low range testosterone. There is a significantly higher score in the general health domain in the normal range testosterone group ($p < 0.05$, 36.84 ± 2.85 low versus 59.44 ± 3.24 normal). In addition, all physical components of the SF36 demonstrate significantly higher scores in the normal testosterone HF patients (bodily pain 48.86 ± 12.21 low, 64.14 ± 3.72 normal; role physical 36.25 ± 22.63 low versus 71.25 ± 21.4 normal; physical function 38.5 ± 19.94 low versus 67.5 ± 22.27 normal – all $p < 0.001$). As a result, the overall physical component summary score is significantly higher in the normal range testosterone patients (41.39 ± 17.03 in the low versus 69.69 ± 19.71 in normal, $p < 0.01$).

Similar differences are also noted in the mental domains of the SF36. Social functioning and role emotional domains are significantly higher in the normal testosterone group

with $p < 0.01$ (social functioning 55.20 ± 2.43 in low compared to 76.73 ± 2.16 in normal and role emotional 42.90 ± 6.55 in low versus 73.96 ± 2.46 in the normal). Additionally, vitality scored significantly higher in the normal testosterone group when compared to low testosterone (44.38 ± 22.95 low compared to 59.25 ± 17.96 in normal, $p < 0.05$). The mental health domain of the SF36 did not demonstrate a statistically significant difference between the two groups ($p = 0.26$). Overall mental component summary was significantly higher in the normal testosterone HF patients (54.88 ± 17.52 low when compared to 72.36 ± 16.58 in normal).

Table 15 illustrates the effect of testosterone status on disease specific quality of life using the MLHFQ, ADAM and depression status using the BDI.

Table 15. MLHFQ, ADAM and BDI score based on total testosterone status.

Independent samples T-test. Significance at P <0.05 is highlighted with *. ** indicates

P<0.01.

	Low-range testosterone (n=20)	Normal-range testosterone (n=20)	P value
MLHFQ Score	42.31 ± 20.16	32.25 ± 27.40	0.20
BDI Score	9.74 ± 6.2	6.7 ± 6.38	0.14
ADAM Score	5.41 ± 1.08	2.67 ± 0.88	0.03*

(MLHFQ) Minnesota Living with Heart Failure Questionnaire, (BDI) Beck Depression Inventory, (ADAM) Androgen deficiency in the ageing male.

There were no significant differences seen between MLHFQ score or BDI between the low and normal range testosterone HF patients. Low testosterone patients demonstrated a significantly higher ADAM score (p<0.05).

4.3.3: NT Pro BNP.

Table 16 summarises the levels of NT pro-BNP in participants with low and normal range TT.

Table 16. NT pro-BNP based on total testosterone status.

Independent samples T-test. Significance at $P < 0.05$ is highlighted with *. ** indicates $P < 0.01$.

	Low-range total testosterone (n=20)	Normal-range total testosterone (n=20)	P value
NT pro-BNP (pg/L)	129.36 ± 100.92	119.54 ± 92.21	0.45

(NT Pro-BNP) N-terminal pro brain natriuretic peptide, (pg/L) picograms per Litre

There were no observed significant differences in NT pro-BNP based on total testosterone status between the low and normal testosterone groups.

4.4: Analyses based on free testosterone.

In addition to measurements of TT, recommendations by the European Endocrine Society suggest a thorough analysis of testosterone deficiency requires calculation of FT, particularly in conditions where there may be alterations in SHBG. As a result, the following section of results summarises the difference in key outcome measures using a FT concentration of ≤ 0.17 nmol/L as a cut off value for low FT status in accordance with practise guidelines (Bhasin et al, 2006).

4.4.1: Adjustment of baseline demographics.

As a result of the change in groups, the baseline demographics for the analysis are presented in table 17. It should be highlighted that following adjustment of the low and normal groups based on FT, the group numbers change to n = 17 low free testosterone and n = 23 normal range free testosterone.

Table 17. Adjusted baseline demographics for FT.

	Low-range free testosterone (n=17)	Normal-range free testosterone (n=23)
Characteristics		
Age (y)	72.7 ± 7.1	69.96 ± 11.49
Stature (m)	1.74 ± 0.07	1.75 ± 0.07
Body mass (kg)	82.87 ± 14.35	82.4 ± 16.01
Body mass index (kg/m ²)	27.48 ± 4.57	26.64 ± 4.09
Systolic BP (mm Hg)	125.76 ± 23.16	118.96 ± 12.83
Diastolic BP (mm Hg)	68.76 ± 9.72	74.13 ± 9.08
Resting HR (beats/min)	68.21 ± 24.20	70.03 ± 18.91
Total Testosterone (nmol/L)	8.80 ± 1.67	17.0 ± 5.39
Free Testosterone (nmol/L)	0.13 ± 0.03	0.27 ± 0.07
SHBG (nmol/L)	62.34 ± 10.54	61.48 ± 8.24
Albumin	40.05 ± 2.01	40.54 ± 1.07
Bio-available testosterone (nmol/L)	2.99 ± 2.0	6.57 ± 1.99
NYHA Score	2.0 ± 0.41	2.0 ± 0.44
Left ventricular end-diastolic volume (mls)	143.78 ± 30.02	145.79 ± 23.12
Left ventricular EF (%)	28.68 ± 7.14	30.10 ± 6.45

Aetiology of HF

Ischaemic	10 (59%)	12 (52%)
Hypertensive	1 (6%)	2 (9%)
Idiopathic dilated cardiomyopathy	3 (17%)	5 (22%)
Unclear	3 (17%)	4 (17%)

Co-morbidities

Ischaemic heart disease	11 (65%)	11 (48%)
Atrial fibrillation	7 (41%)	10 (43%)
Atrial fibrillation at data collection	2 (12%)	4 (17%)
Hypertension	14 (82%)	19 (83%)
Diabetes	7 (41%)	8 (35%)
Chronic obstructive pulmonary disease	5 (29%)	5 (22%)
Peripheral arterial disease	4 (24%)	2 (9%)

Medication

Beta Blocker	17 (100%)	22 (96%)
ACE Inhibitor	17 (100%)	23 (100%)
Diuretic	16 (94%)	22 (96%)
AldosteroneReceptorBlocker	4 (24%)	3 (13%)
Digoxin	1 (6%)	1 (4%)
Anti-Arhythmic	11 (65%)	11 (48%)

Anti-platelet	17 (100%)	22 (96%)
Anticoagulant	11 (65%)	8 (35%)
Statin	15 (88%)	23 (100%)

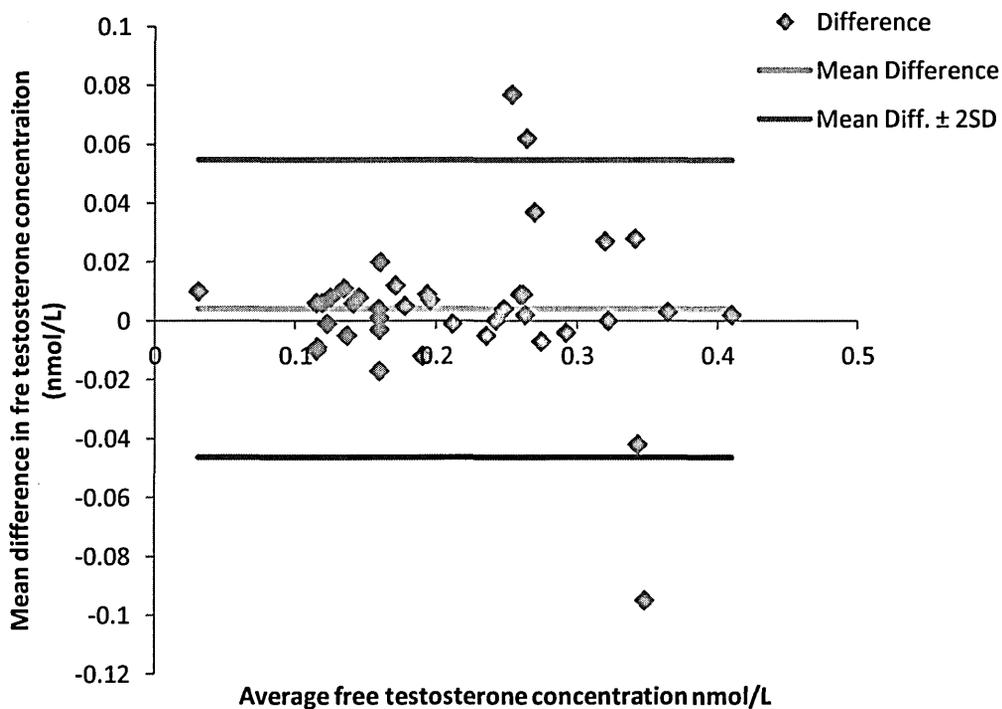
(y) years, (m) metres, (kg) kilogram's, (m²) metres squared, (mmHg millimetres of mercury, (min) minute, (nmol/L) nanomols per Litre, (NYHA Score) New York Heart Association Score, (ACE) Angiotensin converting enzyme, (EF%) ejection fraction, (SHBG) sex hormone binding globulin, (HR) heart rate, (BP) blood pressure.

Table 17 suggests that both groups are well matched at baseline. No significant differences were observed between the low and normal free testosterone groups baseline characteristics using independent samples t-tests with the exception of total testosterone and free testosterone (both P < 0.001).

The following Bland Altman plot (figure 6) shows the agreement between initial and subsequent measurement of free testosterone performed on a separate clinic visit.

Figure 6. Bland Altman plot for the agreement between free testosterone measurements on two separate occasions.

Minimum 4 weeks between initial measurement, maximum 4 months to coincide with routine clinic visit.



This Bland Altman plot shows a mean difference between FT measurements of 0.004 nmol/L (s.d. \pm 0.0025 nmol/L). Therefore, the mean difference \pm 2 s.d. is + 0.055 nmol/L and - 0.046 nmol/L. The coefficient of repeatability is 0.0050 nmol/L. Greater than 95% of measurements fall acceptably within the limits of agreement of \pm 2 s.d. The outlying measurements did not represent a change of testosterone status (i.e. there was no change from hypo to eugonadal status between measurement 1 and measurement 2).

4.4.2: Primary outcome measure – 6 min walk.

Table 18 shows 6 min walk data based on FT status. Note, again as a result of FT calculation, the group distributions change resulting in n = 17 low FT and n = 23 normal FT.

Table 18. 6 min walk data based on free testosterone status.

Independent samples T-test. Significance at P <0.05 is highlighted with *. ** indicates P <0.01.

	Low-range free testosterone (n=17)	Normal-range free testosterone (n=23)	P value
6 min walk distance (m)	249.00 ± 111.35	413.04 ± 127.09**	<0.01

(m) metres,

Patients with low FT demonstrated a significantly impaired exercise capacity when compared to those patients with normal range FT (249.00 ± 111.35 versus 413.04 ± 127.09 m, P < 0.001).

4.5: Secondary outcome measures.

4.5.1: Echocardiography.

Table 19 below illustrates differences in key echocardiographic variables of cardiac structure and function between HF patients with low FT and normal FT.

Table 19. Left atrial structural and functional characteristics for normal and low range FT.

Independent samples T-test. Significance at $P < 0.05$ is highlighted with *. ** indicates $P < 0.01$. Figures in brackets indicate number of patients for each parameter measured.

	Low FT (n = 17)	Normal FT (n=23)	P value
Min LA volume (mls)	60.82 ± 17.92 (n=17)	67.83 ± 24.63 (n = 23)	.33
Max LA volume (mls)	72.94 ± 17.36 (n=17)	80.87 ± 22.23 (n = 23)	.23
Pre-contraction LA volume (mls)	64.93 ± 16.94 (n=15)	62.43 ± 19.31 (n=19)	.70
LA expansion index	22.00 ± 10.67 (n=17)	24.52 ± 17.88 (n = 23)	.58
LA passive emptying fraction	8.12 ± 3.78 (n=15)	13.73 ± 6.60 (n=19)	.007*
LA active emptying fraction	11.12 ± 5.82 (n=15)	9.82 ± 7.00 (n=19)	.58
TDI LA Sa (cm/s)	3.62 ± 1.11 (n=17)	4.24 ± 1.37 (n = 23)	.12

TDI LA Ea (cm/s)	4.57 ± 1.67 (n=17)	4.94 ± 1.85 (n = 23)	.52
TDI LA Aa (cm/s)	4.51 ± 1.85 (n=17)	3.99 ± 1.80 (n = 23)	.43
Peak longitudinal strain reservoir (%)	21.69 ± 5.50 (n=17)	21.75 ± 3.89 (n = 23)	.82
Peak longitudinal strain conduit (%)	3.29 ± 1.57 (n=17)	4.09 ± 1.19 (n = 23)	.40
Peak longitudinal strain contractile (%)	-3.81 ± 1.98 (n = 15)	-3.84 ± 0.95 (n=19)	.88

(min) minimum, (max) maximum, (mls) millilitres, (Sa)ventricular systole, (Ea) early diastole, (Aa)late diastole (atrial contraction), (cm/s) centimetres per second, (TDI) tissue Doppler imaging, (LA) left atrial.

There was a significant difference in left atrial passive emptying fraction between low and normal range FT (8.12 ± 3.78 versus 13.73 ± 6.60, P = 0.007). No other significant differences were noted between the groups based on FT status.

Table 20. Left ventricular structural and functional characteristics for normal and low range FT.

Independent samples T-test. Significance at $P < 0.05$ is highlighted with *. ** indicates $P < 0.01$. Figures in brackets indicate number of patients for each parameter measured.

	Low FT (n = 17)	Normal FT (n=23)	P value
LV width (end-diastole) (cm)	5.43 ± 0.70 (n = 17)	5.83 ± 1.13 (n=23)	.22
LV end-diastolic length (cm)	8.69 ± 0.86 (n = 17)	8.81 ± 0.93 (n=23)	.68
Biplane Simpsons EF%	28.68 ± 7.14 (n=15)	30.10 ± 6.46 (n=19)	.53
Sphericity Index	1.62 ± 0.19 (n = 17)	1.55 ± 0.23 (n=23)	.53
MAPSE (mm)	9.84 ± 2.66 (n = 17)	10.44 ± 2.81 (n=23)	.51
TDI Peak s' medial (cm/s)	3.50 ± 1.14 (n = 17)	3.40 ± 1.26 (n=23)	.80
TDI peak s' lateral (cm/s)	3.99 ± 1.30 (n = 17)	3.97 ± 1.71 (n=23)	.96
TDI E/e'	25.62 ± 13.26 (n = 17)	21.17 ± 18.92 (n=23)	.59
Peak longitudinal systolic strain (%)	-7.08 ± 3.29 (n = 17)	-6.62 ± 2.34 (n=19)	.62

Peak circumferential systolic strain (%)	-6.67 ± 1.74 (n=17)	-7.45 ± 2.90 (n=19)	.40
Peak systolic twist (°)	4.08 ± 1.23 (n=15)	3.1 ± 3.21 (n=19)	.36
Peak systolic twist rate (°/sec)	46.69 ± 12.33 (n=15)	46.00 ± 11.00 (n=19)	.86
Peak systolic torsion (°/cm)	0.37 ± 0.37 (n=15)	0.45 ± 0.29 (n=19)	0.53
Peak untwisting rate (°/sec)	-44.58 ± 8.54 (n=15)	-41.07 ± 7.09 (n=19)	.17
Time to peak untwisting rate (% systole)	118.68 ± 5.17 (n=15)	118.28 ± 5.41 (n=19)	.82
Apical back rotation rate (°/sec)	-23.13 ± 5.76 (n=15)	-23.48 ± 5.82 (n=19)	.85
Time to peak apical back rotation rate (% systole)	114.89 ± 6.44 (n=15)	116.29 ± 3.76 (n=19)	.40
Basal back rotation rate (°/sec)	21.43 ± 6.05 (n=15)	17.59 ± 3.89 (n=19)	.02*
Time to peak basal back rotation rate (% systole)	117.34 ± 5.12 (n=15)	117.44 ± 6.10 (n=19)	.96

(LV) left ventricular, (cm) centimetres, (EF%) ejection fraction, (MAPSE) mitral annular plane systolic excursion, (mm) millimetres, (TDI) tissue Doppler imaging, (cm/s) centimetres per second, (%) percent, (°) degrees, (°/s) degrees per second.

There was a significant difference only in apical back rotation rate ($P = 0.02$) between low versus normal FT. No other significant differences were noted in echocardiographic parameters of LV structure and mechanics between the groups based on FT.

Table 21. Right heart structural and functional characteristics for normal and low range FT.

Independent samples T-test. Significance at $P < 0.05$ is highlighted with *. ** indicates $P < 0.01$. Figures in brackets indicate number of patients for each parameter measured.

	Low FT (n = 17)	Normal FT (n=23)	P value
RA area (systole) (cm ²)	14.79 ± 2.13 (n=17)	14.67 ± 2.61 (n=23)	.86
RV area end-diastole (cm ²)	26.12 ± 4.64 (n=17)	24.47 ± 6.13 (n=23)	.18
RV fractional area change (%)	35.01 ± 10.87 (n=17)	35.10 ± 11.40 (n=23)	.94
TAPSE	15.74 ± 4.0 (n=17)	15.34 ± 2.61 (n=23)	.76
RV peak longitudinal strain (%)	-29.34 ± 5.67 (n=17)	-29.13 ± 6.95 (n=23)	.92

(RA) right atrial, (cm²) centimetres square, (RV) right ventricular, (TAPSE) tricuspid annular plane systolic excursion (%) percent.

There were no significant differences in right heart structure and function noted between the groups when classified by FT.

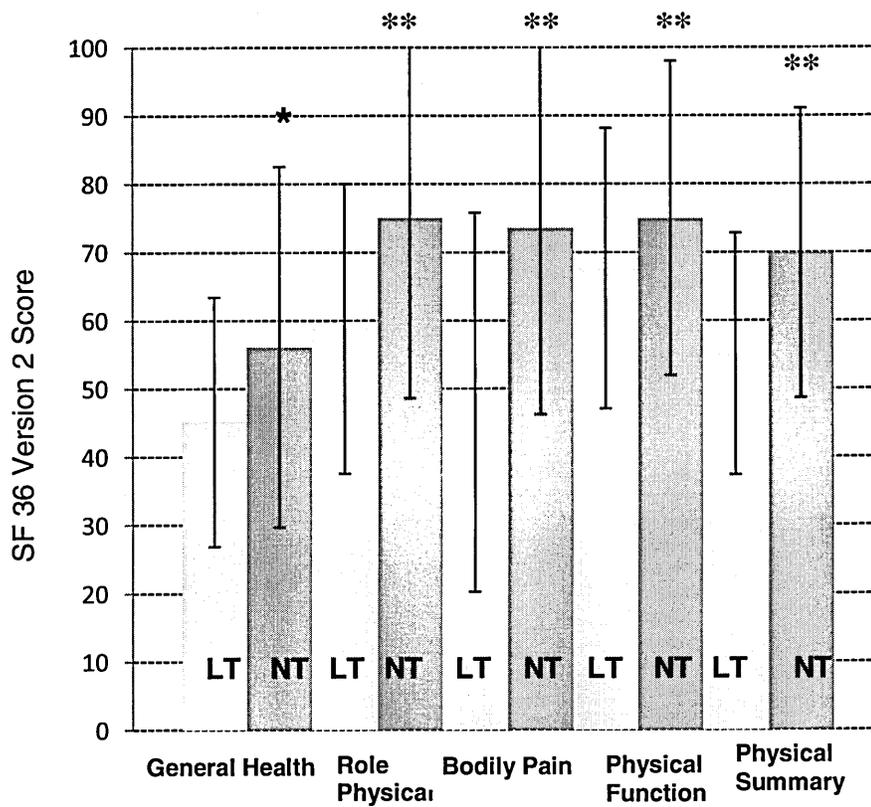
4.5.2: Quality of Life data based on FT.

Figure 7 illustrates the differences in quality of life between HF patients based on low and normal FT.

Figure 7. SF 36 V2 Domains based on free testosterone.

Graphical data based on independent samples T-test. Significance at $P < 0.05$ is

highlighted with *. ** indicates $P < 0.01$. Error bars representative of standard deviation.



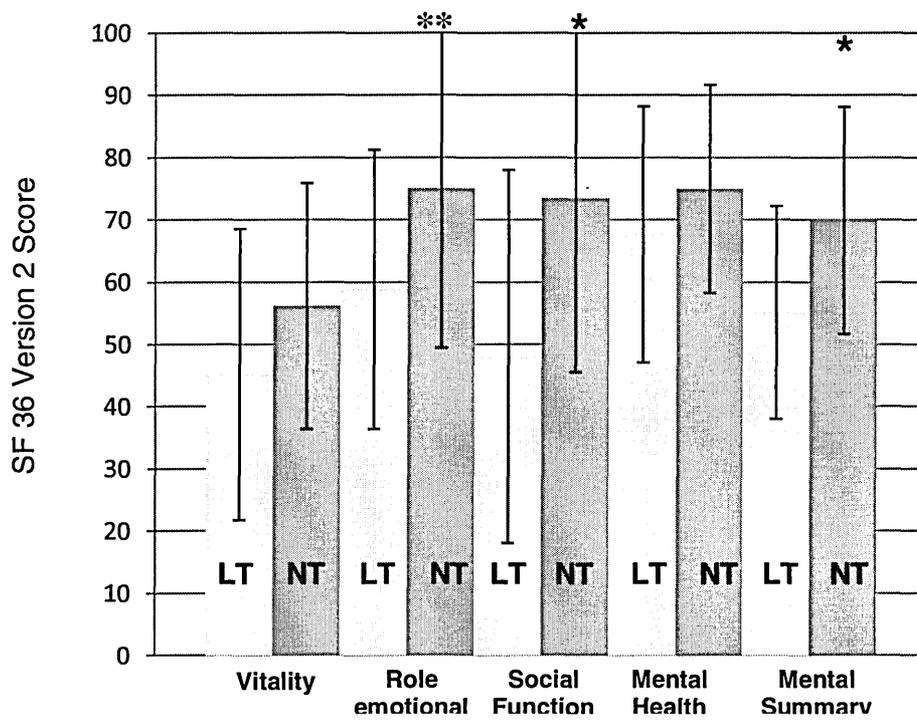


Figure 7 above illustrate that using calculated free testosterone values as a marker of low versus normal testosterone status, resulted in similar differences in quality of life between the groups as was observed when adjusted for total testosterone. In exception to the aforementioned results, no significant difference was noted in the vitality domain of the SF36 when groups were stratified by FT.

Table 22 illustrates how the differences between low and normal free testosterone status impacts upon disease specific quality of life using the MLHFQ, ADAM and depression status using the BDI.

Table 22. MLHFQ, ADAM and BDI Score based on Free Testosterone status.

Independent samples T-test. Significance at P <0.05 is highlighted with *. ** indicates

P <0.01.

	Low-range FT (n=17)	Normal-range FT (n=23)	P value
MLHFQ Score	41.19 ± 20.60	34.35 ± 27.78	0.40
BDI Score	9.31 ± 6.26	7.39 ± 6.50	0.36
ADAM Score	6.50 ± 2.34	5.43 ± 2.97	0.24

(MLHFQ) Minnesota Living with Heart Failure Questionnaire, (BDI) Beck Depression Inventory, (ADAM) Androgen deficiency in the ageing male.

There were no significant differences seen between MLHFQ score or BDI between the low and normal range free testosterone HF patients. In contradiction to previous results, there was no significant difference in ADAM score between the low and normal FT groups.

4.5.3: NT pro BNP based on FT.

Table 23 summarises the levels of NT pro-BNP in participants with low and normal range FT.

Table 23. NT pro-BNP based on free testosterone status.

Independent samples T-test. Significance at P <0.05 is highlighted with *. ** indicates

P <0.01.

	Low-range FT (n=17)	Normal-range FT (n=23)	P value
NT pro-BNP (pg/L)	129.69 ± 101.17	117.42 ± 92.01	0.51

(NT pro BNP) N terminal brain natriuretic peptide, (pg/L) picograms per Litre.

There were no observed significant differences in NT pro-BNP based on free testosterone status between the low and normal testosterone groups.

4.6: Analyses based on bio-available testosterone.

This section displays the results based on determination of bio-available testosterone and a cut off value of $\leq 4.0\text{nmol/L}$ for testosterone deficiency as per the guidance stated previously.

4.6.1: Adjusted baseline demographics.

Adjustment of data based on bio-available testosterone results in 17 patients with low bio-available testosterone and 23 patients with normal range bio-available testosterone.

Table 24 shows the adjusted baseline demographics for this sample.

Table. 24. Adjusted baseline demographics for Bio-available testosterone.

Characteristics	Low-range bio-available testosterone (n=17)	Normal-range bio-available testosterone (n=23)
Age (y)	71.13 ± 12.08	69.25 ± 8.80
Stature (m)	1.74 ± 0.76	1.75 ± 0.72
Body mass (kg)	83.74 ± 16.30	84.42 ± 12.17
Body mass index (kg/m ²)	27.50 ± 4.83	27.47 ± 2.05
Systolic BP (mm Hg)	124.38 ± 20.43	120.58 ± 12.62
Diastolic BP (mm Hg)	70.92 ± 10.61	73.50 ± 8.54
Resting HR (beats/min)	67.50 ± 10.87	69.33 ± 11.74
Total Testosterone (nmol/L)	9.45 ± 2.08	20.38 ± 5.33
Free Testosterone (nmol/L)	0.16 ± 0.06	0.30 ± 0.07
SHBG (nmol/L)	52.29 ± 28.77	56.25 ± 23.73

Albumin	39.98 ± 2.29	40.01 ± 3.52
Bio-available testosterone (nmol/L)	3.04 ± 0.91	6.62 ± 1.15
NYHA Score	2.0 ± 0.42	2.0 ± 0.42
Left ventricular end-diastolic volume (mls)	142.18 ± 31.12	143.90 ± 22.19
Left ventricular EF (%)	29.47 ± 7.11	28.50 ± 6.62
Aetiology of HF		
Ischaemic	11 (65%)	11 (48%)
Hypertensive	1 (6%)	2 (9%)
Idiopathic dilated cardiomyopathy	3 (17%)	5 (22%)
Unclear	3 (17%)	4 (17%)
Co-morbidities		
Ischaemic heart disease	11 (65%)	11 (48%)
Atrial fibrillation	7 (41%)	10 (43%)
Atrial fibrillation at data collection	2 (12%)	4 (17%)
Hypertension	14 (82%)	19 (83%)
Diabetes	7 (41%)	8 (35%)
Chronic obstructive pulmonary disease	7 (41%)	4 (17%)
Peripheral arterial disease	2 (12%)	4 (17%)

Medication

Beta Blocker	17 (100%)	22 (96%)
ACE Inhibitor	17 (100%)	23 (100%)
Diuretic	16 (94%)	22 (96%)
Aldosterone Receptor Blocker	4 (24%)	3 (13%)
Digoxin	1 (6%)	1 (4%)
Anti-Arrhythmic	11 (65%)	11 (48%)
Anti-platelet	17 (100%)	22 (96%)
Anticoagulant	11 (65%)	8 (35%)
Statin	15 (88%)	23 (100%)

(y) years, (m) metres, (kg) kilogram's, (m²) metres squared, (mmHg millimetres of mercury, (min) minute, (nmol/L) nanomols per Litre,

(NYHA Score) New York Heart Association Score, (ACE) Angiotensin converting enzyme, (EF%) ejection fraction, (SHBG) sex hormone

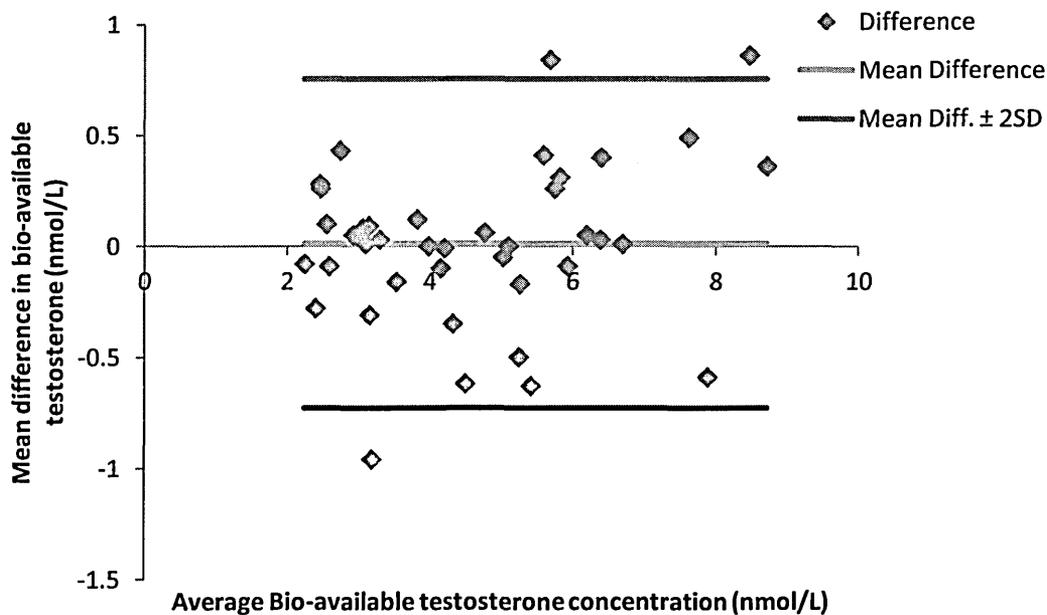
binding globulin, (HR) heart rate, (BP) blood pressure

Table 24 above suggests that both groups are well matched at baseline. No significant differences were observed between low and normal testosterone group baseline characteristics using independent samples t-tests with the exception of total testosterone, bio-available and free testosterone (all $p < 0.001$).

Figure 8, Bland Altman plot shows the agreement between initial and subsequent measurement of bio-available testosterone performed on a separate clinic visit.

Figure 8. Bland Altman plot for the agreement between bio-available testosterone measurements on two separate occasions.

Minimum 4 weeks between initial measurement, maximum 4 months to coincide with routine clinic visit.



The Bland Altman plot shows a mean difference between bio-available testosterone measurements of 0.014 nmol/L (s.d. \pm 0.38 nmol/L). Therefore, the mean difference \pm 2 s.d. is + 0.76 nmol/L and – 0.73 nmol/L. The coefficient of repeatability is 0.76 nmol/L. Greater than 95% of measurements fall acceptably within the limits of agreement of \pm 2 s.d. The outlying measurements did not represent a change of testosterone status (i.e. there was no change from hypo to eugonadal status between measurement 1 and measurement 2).

4.6.2: Primary outcome measure – 6 minute walk.

Table 25 shows 6 min walk data based on bio-available testosterone status.

Table 25. 6 min walk data based on bio-available testosterone status.

Independent samples T-test. Significance at P <0.05 is highlighted with *. ** indicates P <0.01.

	Low-range bio-available testosterone (n=17)	Normal-range bio-available testosterone (n=23)	P value
6 min walk distance (m)	291.62 \pm 110.44	420.88 \pm 158.30**	<0.01

(min) minute, (m) metres

Patients with low bio-available testosterone demonstrated a significantly impaired exercise capacity when compared to those patients with normal range bio-available testosterone (291.62 ± 110.44 versus 420.88 ± 158.30 m, $p < 0.01$).

4.7: Secondary outcome measures.

4.7.1: Echocardiography based on bio-available testosterone.

Tables 26-28 below illustrate differences in key echocardiographic variables of cardiac structure and function between HF patients with low and normal bio-available testosterone.

Table 26. Left atrial structural and functional characteristics for normal and low range Bio-available testosterone.

Independent samples T-test. Significance at $P < 0.05$ is highlighted with *. ** indicates $P < 0.01$. Figures in brackets indicate number of patients for each parameter measured.

	Low bio-available testosterone (n = 17)	Normal bio-available testosterone (n=23)	P value
Min LA volume (mls)	62.75 ± 16.19 (n=17)	65.27 ± 21.53 (n = 23)	.72
Max LA volume (mls)	73.46 ± 19.30 (n=17)	83.56 ± 21.26 (n = 23)	.14
Pre-contraction LA volume (mls)	63.88 ± 17.91 (n=14)	63.03 ± 17.31 (n=19)	.78
LA expansion index	8.67 ± 3.62 (n=17)	15.27 ± 7.33 (n = 23)	.02**
LA passive emptying fraction	11.41 ± 5.36 (n=14)	8.68 ± 7.87 (n=19)	.26

LA active emptying fraction	35.96 ± 11.80 (n=14)	33.75 ± 10.00 (n=19)	.58
TDI LA Sa (cm/s)	4.89 ± 1.86 (n=17)	4.62 ± 1.66 (n=23)	.65
TDI LA Ea (cm/s)	4.57 ± 1.88 (n=17)	3.63 ± 1.59 (n=23)	.17
TDI LA Aa (cm/s)	4.86 ± 1.59 (n=17)	4.91 ± 1.79 (n=23)	.72
Peak longitudinal strain reservoir (%)	21.45 ± 4.50 (n=17)	21.75 ± 3.34 (n=23)	.82
Peak longitudinal strain conduit (%)	3.95 ± 1.92 (n=17)	4.41 ± 1.57 (n=23)	.40
Peak longitudinal strain contractile (%)	-3.81 ± 1.59 (n=14)	-3.74 ± 0.99 (n=19)	.88

(min) minimum, (max) maximum, (mls) millilitres, (Sa)ventricular systole, (Ea) early diastole, (Aa)late diastole (atrial contraction), (cm/s) centimetres per second, (TDI) tissue Doppler imaging, (LA) left atrial.

There was a significant difference in left atrial expansion index between low and normal range bio-available testosterone (8.67 ± 3.62 versus 15.27 ± 7.33 , $P = 0.02$). No other significant differences were noted between the groups based on bio-available testosterone status.

Table 27. Left ventricular structural and functional characteristics for normal and low range bio-available testosterone.

Independent samples T-test. Significance at $P < 0.05$ is highlighted with *. ** indicates $P < 0.01$. Figures in brackets indicate number of patients for each parameter measured.

	Low bio-available testosterone (n = 17)	Normal bio-available testosterone (n=23)	P value
LV width (end-diastole) (cm)	5.38 ± 0.72 (n = 17)	5.80 ± 1.03 (n=23)	.20
LV end-diastolic length (cm)	8.71 ± 0.88(n=17)	8.87 ± 0.91 (n=23)	.66
Biplane Simpsons EF %	29.47 ± 7.11 (n=14)	28.50 ± 6.62 (n=17)	.78
Sphericity Index	1.64 ± 0.21 (n=17)	1.57 ± 0.26 (n=23)	.55
MAPSE (mm)	9.84 ± 2.54 (n=17)	10.48± 2.80 (n=23)	.52
TDI Peak s' medial (cm/s)	3.70 ± 1.18 (n=17)	3.60 ± 1.29 (n=23)	.60

TDI peak s' lateral (cm/s)	3.79 ± 1.41 (n=17)	3.77 ± 1.77 (n=23)	.96
TDI E/e'	20.99 ± 12.00 (n=17)	21.19 ± 18.82 (n=23)	.60
Peak longitudinal systolic strain (%)	-7.01 ± 3.33 (n=16)	-6.80 ± 2.39 (n=19)	.69
Peak circumferential systolic strain (%)	-6.67 ± 1.90 (n=17)	-7.45 ± 2.09 (n=19)	.40
Peak systolic twist (°)	3.68 ± 1.09 (n=15)	3.29 ± 3.00 (n=19)	.46
Peak systolic twist rate (°/sec)	46.82 ± 12.88 (n=15)	46.09 ± 15.03 (n=19)	.89
Peak systolic torsion (°/cm)	0.39 ± 0.37 (n=15)	0.45 ± 0.30 (n=19)	0.48
Peak untwisting rate (°/sec)	-40.55 ± 8.96 (n=15)	-41.99 ± 7.99 (n=19)	.37
Time to peak untwisting rate (% systole)	121.69 ± 5.18 (n=15)	119.99 ± 5.44 (n=19)	.80

Apical back rotation rate (°/sec)	-23.88 ± 5.86 (n=15)	-23.08 ± 5.88 (n=19)	.88
Time to peak apical back rotation rate (% systole)	115.85 ± 6.54 (n=15)	116.55 ± 3.56 (n=19)	.40
Basal back rotation rate (°/sec)	21.93 ± 6.00 (n=15)	17.09 ± 3.04 (n=19)	.02*
Time to peak basal back rotation rate (% systole)	119.34 ± 5.74 (n=15)	118.47 ± 6.17 (n=19)	.92

(LV) left ventricular, (cm) centimetres, (EF%) ejection fraction, (MAPSE) mitral annular plane systolic excursion, (mm) millimetres, (TDI) tissue Doppler imaging, (cm/s) centimetres per second, (%%) percent, (°) degrees, (°/s) degrees per second.

There was a significant difference only in apical back rotation rate (21.93 ± 6.00 versus 17.09 ± 3.04, P = 0.02) between low versus normal bio-available testosterone. No other significant differences were noted in echocardiographic parameters of LV structure and mechanics between the groups based on bio-available testosterone. These results mirror those obtained when grouped for free testosterone.

Table 28. Right heart structural and functional characteristics for normal and low range bio-available testosterone.

Independent samples T-test. Significance at $P < 0.05$ is highlighted with *. ** indicates $P < 0.01$. Figures in brackets indicate number of patients for each parameter measured.

	Low bio-available testosterone (n = 17)	Normal bio-available testosterone (n=23)	P value
RA area (systole) (cm²)	15.67 ± 2.73 (n=17)	14.43 ± 1.86 (n=23)	.09
RV area end-diastole (cm²)	30.39 ± 5.98 (n=17)	27.47 ± 6.70 (n=23)	.16
RV fractional area change (%)	35.01 ± 10.87 (n=17)	35.10 ± 11.40 (n=23)	.22
TAPSE	15.04 ± 4.21 (n=17)	15.14 ± 2.88 (n=23)	.90
RV peak longitudinal strain (%)	-28.38 ± 5.87 (n=17)	-29.88 ± 6.88 (n=23)	.92

(RA) right atrial, (cm²) centimetres square, (RV) right ventricular, (TAPSE) tricuspid annular plane systolic excursion (%) percent.

There were no significant differences in right heart structure and function noted between the groups when classified by bio-available testosterone. This mirrors the findings of both total and free testosterone group allocations.

4.7.2: Quality of life parameters based on bio-available testosterone.

Table 29 below summarises the main quality of life outcomes based on low and normal bio-available testosterone.

Table 29 . Quality of Life outcome measures based on normal versus low bio-available testosterone concentration.

Independent samples T-test. Significance at $P < 0.05$ is highlighted with *. ** indicates $P < 0.01$.

Quality of Life Measure.	Low bio-available testosterone (n = 17)	Normal bio-available testosterone (n=23)	P value
SF 36 General Health	47.97 ± 23.33	55.62 ± 26.64	.34
SF 36 Role Physical	44.01 ± 25.90	68.35 ± 25.26**	.006
SF 36 Bodily Pain	57.81 ± 30.13	74.22 ± 30.00**	.008
SF 36 Physical Function	44.17 ± 23.00	66.25 ± 23.91**	.007
SF 36 Physical Summary	48.49 ± 21.32	66.11 ± 22.31*	.02

SF 36 Vitality	46.61 ± 21.65	59.61 ± 19.99	.06
SF 36 Social Function	61.46 ± 23.58	73.43 ± 17.84*	.04
SF 36 Role emotional	55.54 ± 30.86	73.43 ± 29.38	.07
SF 36 Mental Health	70.83 ± 19.32	73.40 ± 17.84	.67
SF 36 Mental Component Summary	58.61 ± 18.43	71.14 ± 17.89*	.04
MLHFQ	38.96 ± 21.52	32.13 ± 29.23	.43
BDI	8.33 ± 6.23	7.43 ± 6.93	.68
ADAM	6.08 ± 3.00	5.19 ± 2.97	.35

(SF-36) short form 36 version 2 questionnaire, (MLHFQ) Minnesota Living with Heart Failure Questionnaire, (BDI) Beck depression inventory, (ADAM) Androgen deficiency in the ageing male questionnaire.

Table 29 shows significant differences in several of the physical and mental domains of the SF-36 questionnaire. Role physical, physical functioning and bodily pain are all significantly higher in the normal bio-available testosterone group (44.01 ± 25.90 versus 68.35 ± 25.26 , 44.17 ± 23.00 versus 66.25 ± 23.91 and 57.81 ± 30.13 versus 74.22 ± 30.00 respectively. All $P < 0.01$). This results in a significant difference in overall physical component score in favour of the normal bio-available testosterone group (48.49 ± 21.32 versus 66.11 ± 22.31 , $P = 0.02$). For the mental components of the SF-36, there is a significant difference in social functioning (61.46 ± 23.58 versus 73.43 ± 17.84 , $P = 0.04$) and a significant difference in overall mental component summary (58.61 ± 18.43 versus 71.14 ± 17.89 , $P = 0.04$) in favour of the normal bio-available testosterone group. There were no significant differences in MLHFQ, BDI or ADAM scores between low and normal bio-available testosterone.

4.7.3: NT pro BNP based on bio-available testosterone.

Table 30 documents NT pro-BNP values based on bio-available testosterone concentration.

Table 30. NT pro-BNP based on bio-available testosterone status.

Independent samples T-test. Significance at P <0.05 is highlighted with *. ** indicates

P <0.01.

	Low-range Bio-available testosterone (n=17)	Normal-range Bio-available testosterone (n=23)	P value
NT pro-BNP (pg/L)	112.37 ± 84.42	143.63 ± 76.56	0.23

(NT pro BNP) N terminal brain natriuretic peptide, (pg/L) picograms per Litre.

There were no observed significant differences in NT pro-BNP based on bio-available testosterone status between the LT and NT groups.

4.8: Correlation studies.

Tables 31-33 highlight the relationship observed between total, free and bio-available serum testosterone concentration and the main important outcome measures assessed during the course of the study.

Table 31. Summary of main outcome measure univariate Pearson's correlation (r)with total testosterone. * indicates $P < 0.05$, ** $P < 0.01$

Variable	TT r value	TT P value
6 min walk distance (m)	.504**	<0.001
SF36 General Health	.154	0.33
SF36 Bodily Pain	.196	0.23
SF36 Role Physical	.467**	<0.01
SF36 Physical Function	.354*	0.02
SF36 Physical Summary	.343*	0.03
SF36 Vitality	-.003	0.96
SF36 Role emotion	.274	0.09
SF36 Social functioning	.052	0.75
SF36 Mental health	-.050	0.76
SF36 Mental Summary	.117	0.47
EF%	-.024	0.89
LA max volume	.049	0.88
LA strain conduit	.024	0.91
LA strain reservoir	.019	0.78
LA strain contractile	.079	0.49
LV twist	.087	0.74
LV untwist	.054	0.65
LV torsion	.079	0.55
RV area change %	.120	0.29

TAPSE (mm)	.016	0.93
Peak S wave velocity (cm/s)	.029	0.86
Age (yrs)	-.155	0.34
NYHA Score	-.011	0.95

(min) minute, (m) metres, (SF-36) short form 36 version 2 questionnaire, (EF%) ejection fraction, (%) percentage, (LA) left atrium, (LV) left ventricle, (RV) right ventricle, (TAPSE) tricuspid annular plane systolic excursion, (mm) millimetres, (cm/s) centimetres per second, (yrs) years, (NYHA) New York Heart Association.

Table 32. Summary of main outcome measure univariate Pearson's correlation (r)with free testosterone. * indicates $P < 0.05$, ** $P < 0.01$

Variable	FT r value	FT P value
6 min walk distance (m)	.589**	<0.001
SF36 General Health	.240	0.14
SF36 Bodily Pain	.090	0.88
SF36 Role Physical	.540**	<0.001
SF36 Physical Function	0.451**	0.003
SF36 Physical Summary	0.441**	0.004
SF36 Vitality	.068	0.68
SF36 Role emotion	.361*	0.02
SF36 Social functioning	.143	0.38
SF36 Mental health	.026	0.14
SF36 Mental Summary	.220	0.17
EF %	.011	0.95
LA max volume	.044	0.87
LA strain conduit	.029	0.90
LA strain reservoir	.019	0.79
LA strain contractile	.088	0.55
LV twist	.090	0.81
LV untwist	.050	0.75
LV torsion	.088	0.60
RV area change %	.201	0.19

TAPSE (mm)	.020	0.92
Peak S wave velocity (cm/s)	-.002	0.99
Age (yrs)	.017	0.92
NYHA Score	.006	0.97

(min) minute, (m) metres, (SF-36) short form 36 version 2 questionnaire, (EF%) ejection fraction, (%) percentage, (LA) left atrium, (LV) left ventricle, (RV) right ventricle, (TAPSE) tricuspid annular plane systolic excursion, (mm) millimetres, (cm/s) centimetres per second, (yrs) years, (NYHA) New York Heart Association.

Table 33 Summary of main outcome measure univariate Pearson's correlation (r)with bio-available testosterone. * indicates $P < 0.05$, ** $P < 0.01$

Variable	BioT r value	BioT P value
6 min walk distance (m)	.579**	<0.001
SF36 General Health	.244	0.19
SF36 Bodily Pain	.78	0.74
SF36 Role Physical	.598**	<0.01
SF36 Physical Function	.499**	0.003
SF36 Physical Summary	.487**	0.002
SF36 Vitality	.070	0.28
SF36 Role emotion	.387*	0.03
SF36 Social functioning	.097	0.44
SF36 Mental health	.036	0.54
SF36 Mental Summary	.297	0.30
EF%	.021	0.90
LA max volume	.052	0.81
LA strain conduit	.087	0.90
LA strain reservoir	.081	0.70
LA strain contractile	.080	0.50
LV twist	.074	0.60
LV untwist	.041	0.71
LV torsion	.088	0.71
RV area change %	.201	0.22

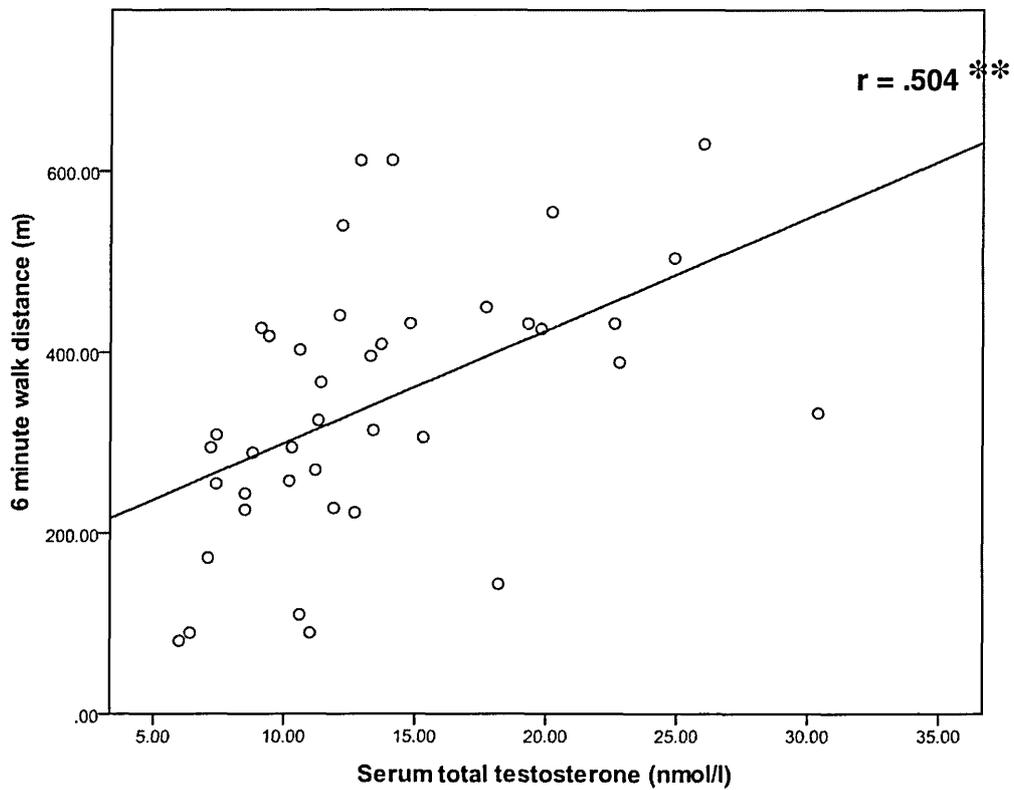
TAPSE (mm)	.100	0.82
Peak S wave velocity (cm/s)	.004	0.79
Age (yrs)	.011	0.45
NYHA Score	.001	0.92

(min) minute, (m) metres, (SF-36) short form 36 version 2 questionnaire, (EF%) ejection fraction, (%) percentage, (LA) left atrium, (LV) left ventricle, (RV) right ventricle), (TAPSE) tricuspid annular plane systolic excursion, (mm) millimetres, (cm/s) centimetres per second, (yrs) years, (NYHA) New York Heart Association.

These tables (31-33) indicate that there is a strong positive relationship between total, free and bio-available testosterone concentration and 6 minute walking distance ($p < 0.01$). In addition, a strong positive relationship exists between total, free and bio-available testosterone concentration and the role physical, physical function and physical component summary domains of the SF36 ($p < 0.05$ for TT and $p < 0.001$ for FT and Bio-available testosterone).

Figure 9 shows the linear relationship between total testosterone and the main study outcome (6 minute walk).

Figure 9. Scatter-plot of serum total testosterone and 6 min walk distance. Linear regression lines included to demonstrate the relationship between the indices. * indicates $P < 0.05$ and ** indicates $P < 0.01$.

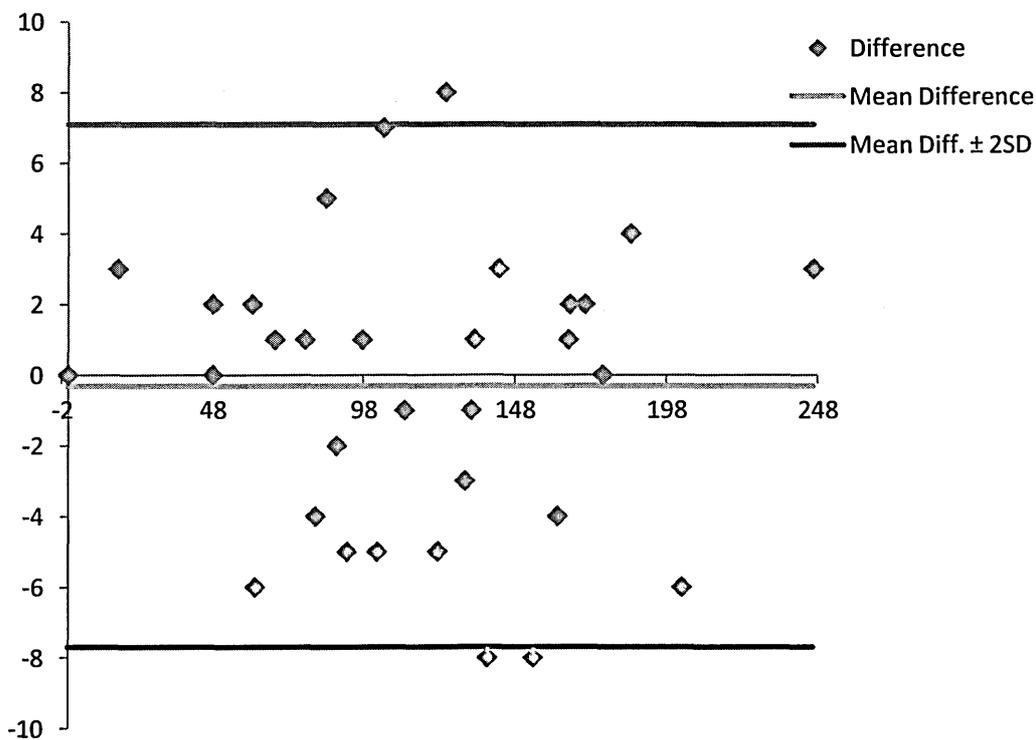


4.9: Salivary testosterone.

As a novel marker of testosterone concentration in a male HF population, salivary testosterone was measured. The following Bland Altman plot shows the agreement between initial and subsequent measurement of salivary testosterone performed on a separate clinic visit.

Figure 10. Bland Altman plot showing agreement between salivary testosterone measurements on 2 separate visits.

Minimum 4 weeks between initial measurement, maximum 4 months to coincide with routine clinic visit.



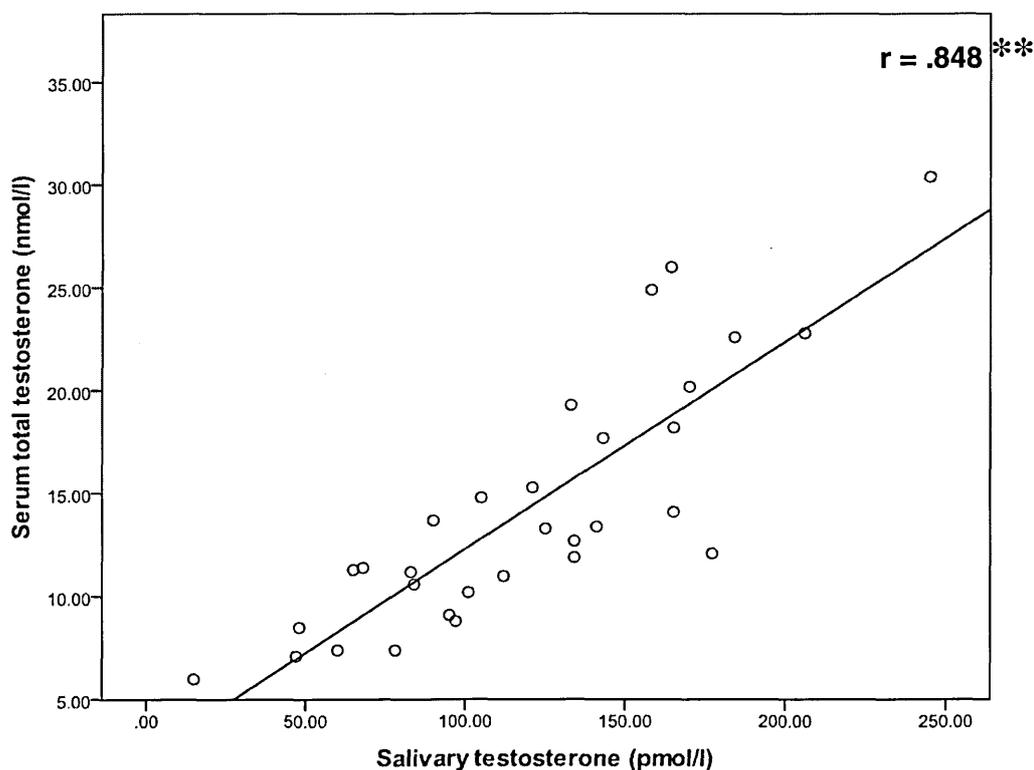
The Bland Altman plot shows a mean difference between salivary testosterone measurements of -0.31 nmol/L (s.d. \pm 3.70 nmol/L). Therefore, the mean difference \pm 2 s.d. is + 7.09 nmol/L and -7.70 nmol/L. The coefficient of repeatability is 7.14 nmol/L.

Greater than 95% of measurements fall acceptably within the limits of agreement of ± 2 s.d.

The scatterplot in figure 11 shows the relationship between salivary and total testosterone concentrations. Linear regression lines included to demonstrate the relationship between the indices. * indicates $P < 0.05$ and ** indicates $P < 0.01$.

Figure 11. Scatter plot of total testosterone and salivary testosterone concentration.

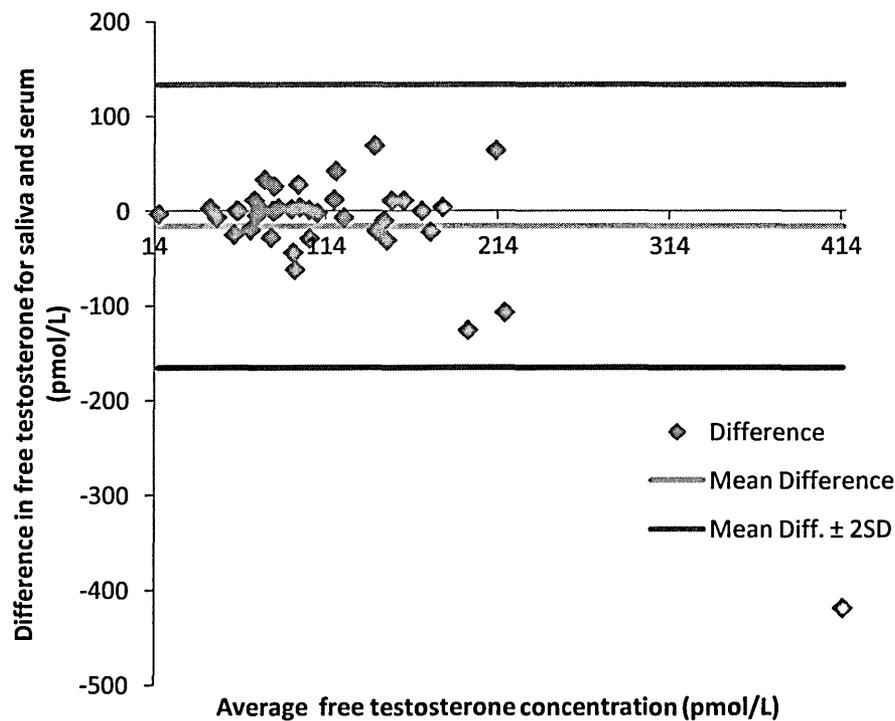
Linear regression lines included to demonstrate the relationship between the indices. * indicates $P < 0.05$ and ** indicates $P < 0.01$.



The scatter-plot illustrates a strong positive correlation between salivary testosterone, total testosterone ($r = .848$, $p < 0.001$) and free testosterone ($r = .865$, $p < 0.001$).

Salivary testosterone is a filtrate of plasma containing only the free fraction of testosterone and, as stated earlier in this review, free testosterone is often considered the most physiologically active form of testosterone available for metabolising in target tissue. As such, a Bland Altman plot is appropriate to assess the agreement between traditional serum sampling for measurement of FT and the use of simple salivary collection as a novel technique in a heart failure population.

Figure 12 Bland Altman plot for the agreement between salivary and serum free testosterone measurements.



This plot shows good agreement between two different methods for the analysis of free testosterone concentration. >95% of values fall within ± 2 s.d with one clear outlying value. The data show a mean difference of -16.05 pmol/L with s.d. of 74.57 pmol/L.

This results in + 2 s.d of 133.09 pmol/L and – 2 s.d. of 165.19 pmol/L. The calculated coefficient of variation is 149.14 pmol/L.

In addition, table 34 below details the observed relationship between salivary testosterone concentration and the main outcome measures summarised previously in this result section.

Table 34. Summary of main outcome measure univariate Pearson's correlation (r) with salivary testosterone as a marker of serum free testosterone status.

* indicates $P < 0.05$, ** $P < 0.01$

Variable	Salivary testosterone r value	Salivary testosterone P value
6 min walk distance (m)	.592**	<0.001
SF36 General Health	.242	0.18
SF36 Bodily Pain	.096	0.89
SF36 Role Physical	.518**	<0.001
SF36 Physical Function	.481**	0.004
SF36 Physical Summary	.451**	0.004
SF36 Vitality	.061	0.61
SF36 Role emotion	.385*	0.02
SF36 Social functioning	.149	0.42

SF36 Mental health	.031	0.22
SF36 Mental Summary	.229	0.19
EF%	.009	0.92
LA max volume	.044	0.87
LA strain conduit	.022	0.87
LA strain reservoir	.017	0.77
LA strain contractile	.089	0.54
LV twist	.097	0.83
LV untwist	.055	0.78
LV torsion	.088	0.60
RV area change %	.200	0.18
TAPSE (mm)	.027	0.90
Peak S wave velocity (cm/s)	-.011	0.88
Age (yrs)	.011	0.93
NYHA Score	.004	0.95

(min) minute, (m) metres, (SF-36) short form 36 version 2 questionnaire, (EF%) ejection fraction, (%) percentage, (LA) left atrium, (LV) left ventricle, (RV) right ventricle, (TAPSE) tricuspid annular plane systolic excursion, (mm) millimetres, (cm/s) centimetres per second, (yrs) years, (NYHA) New York Heart Association.

Given the close relationship observed between serum free testosterone and salivary testosterone concentration, there are similar correlations observed based on salivary testosterone.

There is a strong positive relationship between salivary testosterone concentration and walking distance ($p < 0.01$). In addition, a strong positive relationship exists between salivary testosterone concentration and the role physical, physical function and physical component summary domains of the SF36 ($p < 0.001$).

4.10: Additional statistics performed based on observations of initial data.

Although not statistically significant using independent samples t-tests, it is evident from the baseline characteristics that low testosterone participants are, on average, slightly older than the normal testosterone counterparts. It is possible that age could influence the results of the primary outcome measure hence resulting in a significant between group differences in walking ability. In order to adjust the comparison taking into account age as a covariate, ANCOVA was undertaken to assess further the effect of age in the comparison. The results for TT and FT are presented in the table 35 below.

Table 35. ANCOVA statistic comparing 6 minute walk capacity between low and normal testosterone with age added as a covariate. * P <0.05, ** P<0.01

	6 minute walk distance (m).	6 min walk p value.	P value for age as a covariate.
Low-range TT	257.65 ± 106.87	<0.001	0.07
Normal-range TT	429.00 ± 126.94		
Low-range FT	249.00 ± 111.35	<0.001	0.06
Normal range FT	413.04 ± 144.71		

TT (total testosterone), FT (free testosterone)

The table for ANCOVA indicates that age does not significantly affect walking distance when added as a covariate to the between group analysis. However, it does demonstrate a trend towards effecting walking distance of both low and normal range TT and FT.

Chapter 5. Discussion.

This is the only study to date to compare two well-matched groups of male patients with HF and biochemical and functional evidence of low or normal total, free and bio-available testosterone concentration. Participants recruited to the low TT group were found to have an average TT concentration of 9.21 ± 1.84 nmol/L compared to a mean TT concentration of 17.82 ± 5.31 nmol/L in those with normal range TT. A value of 9.21 nmol/L for TT is parallel with previously published European Endocrinology guidance that suggests a total testosterone concentration of ≤ 10.4 nmol/L as a marker of testosterone deficiency (Bhasin et al, 2006) and also further evidence suggesting that values of ≥ 12.4 nmol/L generally do not require supplementation (Wang et al, 2009). Nevertheless, these recommendations also state that functional evidence of testosterone deficiency should be present, and as such, all patients in the low TT group demonstrated a positive androgen deprivation score of ≥ 3 using the ADAM questionnaire. Although, as stated later in this discussion, our data suggests that there should be caution when applying the ADAM questionnaire in this patient population.

More rigorous analysis of testosterone concentration is now widely recommended in patients who have borderline hypogonadal TT concentration or in patients who may have possible alterations of SHBG. More elderly males, males with obesity, diabetes mellitus or chronic diseases (such as cancer, lung disease, heart failure and HIV) have been shown to present with increases in SHBG and thus total testosterone may not be a precise marker of androgen status in this population (Bhasin et al, 2008). In response to this, a novel feature of this research was the comparison of groups based upon calculation of bio-available and free testosterone concentration. Calculated FT values for the low testosterone group were 0.132 ± 0.33 nmol/L and 0.271 ± 0.67 nmol/L for the normal testosterone group and bio-available concentrations were 3.04 ± 0.91 nmol/L

and 6.62 ± 1.15 nmol/L for low and normal testosterone groups respectively. There is much less published evidence available regarding specific cut off values for low FT and bio-available testosterone. However, suggestions from the European Endocrine Society are that FT values ≤ 0.17 nmol/L represent hypogonadism and usually require supplementation (Bhasin et al, 2006). In addition, Leifke and colleagues (2000) have shown that bio-available testosterone concentration in an older population ≤ 4 nmol/L is consistent with hypogonadism and these patients would benefit from testosterone supplementation.

There are wide variations in laboratory 'cut-off' values for low total testosterone varying from 8.0 nmol/L to 12.0 nmol/L in healthy populations (Bhasin et al, 2006). Endocrinology guidelines are clear that variations in laboratory serological techniques and the use of questionnaires can further impact on the diagnostic accuracy of measurement of testosterone concentration. Total testosterone is widely reported to be at its lowest in the early hours of the day before 10:00 am (Diver et al, 2003). Many of the studies assessing diurnal testosterone variation were performed in a younger population with diminished numbers of older participants. In elderly patients, although the diurnal variation in testosterone concentration persists, it may be substantially blunted (Crawford et al, 2007). Additionally it has been suggested that testosterone levels vary markedly throughout a 24 hour period and measuring testosterone levels in the same individual in a 12 hour period cannot be relied upon to make accurate conclusions over androgen status (Spratt et al, 1988). Furthermore, around 30% of males with low testosterone sampled during the afternoon period have been shown to have normal testosterone concentration when sampled during the morning hours (Brambilla et al, 2007). In this study, all patients recruited were sampled for testosterone status on two separate occasions prior to midday. All second samples were

taken between a period of 4 weeks to 4 months following the initial visit to coincide with routine clinical appointments. Bland Altman analysis of the repeated measures of testosterone did not, on the whole, show any marked deviation from initial measurements (mean difference between TT measurements of 0.15 nmol/L, s.d. \pm 2.05 nmol/L, mean difference \pm 2 s.d. + 3.95 nmol/L and - 4.26 nmol/L., coefficient of repeatability 5.10 nmol/L, mean difference for FT of 0.004 nmol/L, s.d. \pm 0.0025 nmol/L, mean difference \pm 2 s.d. + 0.055 nmol/L and - 0.046 nmol/L. coefficient of repeatability 0.0050 nmol/L and bio-available testosterone mean difference 0.014 nmol/L, s.d. \pm 0.38 nmol/L, mean difference \pm 2 s.d. + 0.76 nmol/L and - 0.73 nmol/L. with a coefficient of repeatability is 0.76 nmol/L). More than 95% of repeated measurements were within \pm 2 s.d. of the mean difference for all of the constituents of testosterone concentration. There were a minor amount of outlying values of which none resulted in a shift from eugonadal to hypogonadal status or vice-versa. All samples were batch analysed using the same serological technique and additional parameters calculated using well-recognised formulae by an accredited biochemistry laboratory and clinical scientist. Initial samples only were used for the group stratification and subsequent analysis. Secondary samples were used merely to provide clarity to the initial measurement and to provide a gauge to the reproducibility of measurements in a variable known to display significant variation.

Recruitment to the study was uncomplicated with target numbers according to the power calculation achieved within the set timescales. Furthermore, all participants who entered the study completed all data collection on the same day as their regular outpatient clinic appointment. As such, there was a full data-set for each of the participants. Some echocardiographic parameters were not measured in all patients due to the limitation of ultrasound in patients with increased BMI and a minority of patients

with atrial fibrillation at the time of analysis. The two groups were well matched at baseline using total serum testosterone, free testosterone and bio-available testosterone as the grouping variable. Potential confounding variables to total testosterone concentration were those factors which are known to alter SHBG. For this study these variables were age, weight, co-morbidities and BMI. Although the low testosterone group is slightly more elderly (71.9 ± 10 yrs compared to 66.9 ± 11.98 yrs) there was no significant difference at baseline using independent samples t-tests ($p = 0.162$). Further assessment of the groups using ANCOVA and age as a covariate in the between groups analysis resulted in no significant effect of age on walking distance when stratified by either TT or FT ($p = 0.06$ and 0.07 respectively). Clearly this indicates a strong trend towards age influencing the results and hence future studies with larger sample sizes should be undertaken to fully assess the effect of age on walking distance in this population.

Other potential confounding variables to 6 min walk performance in HF (i.e. NYHA class, EF, NT pro-BNP, age and BMI) were not statistically different, using independent samples t-tests, between the two groups at baseline. The only significant differences observed in baseline parameters were total, bio-available and free testosterone concentration ($p < 0.001$). The baseline characteristics of the study population were in-keeping with other trials investigating the impact of low testosterone in the male HF population and typically a more elderly population (Malkin et al, 2006, Malkin et al, 2004, Caminiti et al, 2009, Jankowska et al, 2009).

This initial study shows that HF patients with low testosterone have significantly diminished exercise capacity (as assessed by a 6 min shuttle walk) when compared to a well-matched group of HF patients with normal range testosterone. This study reported

a mean increase of 171.35 ± 37.10 m in HF patients with normal total testosterone concentration when compared to those patients with low total testosterone ($p < 0.001$). These differences were observed despite objective evidence of the participants exercising to a similar intensity (i.e. no significant difference in maximal HR or RPE between the groups) and were also apparent when data was stratified according to free and bio-available testosterone. Other studies using a different research design have also indicated that total testosterone and other anabolic hormone deficiency can detrimentally impact on exercise performance in HF. These studies associated reduced levels of TT to significant reductions in peak $\dot{V}O_2$ (Jankowska et al, 2009, Caminiti et al, 2009) and 6 min walk distance (Bocchi et al, 2008). Furthermore, the work of Jankowska et al, 2009 showed similar positive correlations between exercise capacity and testosterone concentration as observed in our study. Such findings have also proved consistent with associations observed in elderly males without clinical evidence of HF (Haydar et al, 2000). No other research has sought to investigate parameters related to endurance performance in a similar population based on free and bio-available testosterone.

Previous work has suggested that improved exercise capacity in HF associated with higher levels of TT may be due to improved indices of cardiac function (Bocchi et al, 2008 and Jankowska et al, 2009). This hypothesis has been formulated on the basis that TT has been shown to inversely correlate with both LV and RV EF% (Bocchi et al 2008) and testosterone therapy has been shown to improve indices of cardiac structure and function in animal and human models (Yan-Zhou et al, 2007 and Malkin et al, 2006). However, this study generally provides contradictory results. Largely, there were no significant differences in any of the detailed indices of cardiac structure, function and mechanics based upon testosterone concentration as assessed by echocardiography

during the course of this study. Only significant differences in left atrial passive emptying fraction and left ventricular basal back rotation rate were observed for total and free testosterone with the addition of left atrial expansion index when groups were based on bio-available testosterone.

No other study has extended to measuring cardiac functional characteristics in such detail in this population and it is plausible that these significant differences may reflect a subtle improvement in diastolic cardiac parameters based on testosterone concentration. Left atrial passive emptying fraction is a representative marker of left atrial conduit function and as such, early left ventricular diastolic filling (Nikitin et al, 2003). Left atrial expansion index is a marker of atrial reservoir function. The left atrium acts as a reservoir by collecting blood from the pulmonary veins during ventricular systole and as such, this has been shown to be an important determinate of cardiac output (Suga, 1974). The rate at which the left ventricle untwists has previously been proposed to represent left ventricular relaxation (Dong et al, 2001). Diastolic untwist represents elastic recoil due to the release of restoring forces generated during the preceding systole (Nagueh et al, 2009). Untwisting rate or recoil rate plays an important role in diastolic filling due to suction generation and attenuation of this phenomenon has been assumed to contribute to diastolic impairment in diseased hearts (Fuchs et al, 2004). More recently however, it has been shown that untwisting rates are retained with diastolic abnormalities and normal ejection fraction but impaired in patients with significant systolic dysfunction (Wang et al, 2007). Furthermore, time to left ventricular untwist and untwist velocities have both been shown to be reduced in patients with systolic dysfunction and increased filling pressures (Sengupta et al, 2008). In this study, patients with low and normal testosterone concentration all demonstrated significant LV systolic impairment (e.g. for total testosterone an EF% of 28.31 ± 7.07

for low versus 30.72 ± 6.25 for normal range testosterone). Thus, the observed difference in twist mechanics together with subtle changes in left atrial function may be an indicator of higher filling pressure in patients with lower levels of testosterone. In contradiction to this theory, many other important echocardiographic parameters assessed during the course of this study that relate to diastolic filling show no significant difference between groups based on testosterone concentration.

There are however, limitations to the echocardiographic parameters used during this study for the assessment of both LV systolic and diastolic function. For LV systolic function, clinical trial echocardiographic guidance suggests that measurement of LV ejection fraction based on LV volumes obtained by the method of discs should be performed (Gottdiener et al, 2004). This technique minimises but not eliminates the mathematical assumptions evident in other methods and also allows for corrections based on LV geometry. However, limitations arise when the apex is fore-shortened, the endocardium is inadequately viewed and there is limitation by reliance on only two LV planes. The literature quotes Biplane Simpsons EF reproducibility of $\pm 7\%$ (Himmelman et al, 1988) and test-re-test reliability of $\pm 5\%$ (Gottdiener et al, 1995). In this current study, we quoted a 8.5% measurement error in EF by the same operator on two different occasions. This error and the documented limitations in the literature may be a factor in the lack of statistical change noted in our results. Longitudinal velocity assessment using PW Doppler can also be limited by a number of factors. PW Doppler assessment of tissue movement is only able to provide information regarding a specific point of the myocardium determined by sample volume positioning, components perpendicular to the ultrasound beam remain unknown and there is significant angle dependency. TDI velocities may also be influenced by global heart motion, movement of adjacent structures and also blood flow (Mor-Avi et al, 2011). During this study we

demonstrated good reproducibility of the TDI peak s' velocities when measured on separate occasions (measurement error around 5%) and many of the limitations of TDI can be overcome using 2D speckle tracking echocardiography. Furthermore, our TDI velocities were measured at end-expiration in order to reduce global heart motion. 2D speckle tracking echocardiography also demonstrates some technical limitations. In more obese populations with limited echocardiographic images, it is possible that endocardial border tracking may be inaccurate. 2D speckle tracking echocardiography is also limited in patients with acoustic shadowing or reverberations (Mor-Avi et al, 2011). Tracking software algorithms use a priori knowledge of 'normal' LV function and as such, there may be errors when assessing regional abnormalities or when assessing neighbouring segments (Mor-Avi et al, 2011). It is important to recognise the afore-mentioned limitations of the techniques when evaluating these results. The use of contrast agents to effectively assess LV endocardial borders can be undertaken to enhance the quality of data and utilising other techniques previously shown to provide more accurate assessment of LV volume and EF such as 3D echocardiography or cardiac MR could be performed in newer studies.

In the present study, LV diastology was assessed in detail. However, in order to provide an improved assessment of LV diastolic function, more parameters could be assessed. Although guidelines for the assessment of diastolic function in clinical trials suggest using E/e' as this parameter seems the least subject to inter/intra-observer variability (Gottdiener et al, 2004), more recent recommendations have suggested the use of additional parameters to increase the accuracy of results (Nagueh et al, 2009). For instance, more detailed assessment of MV inflow properties (e.g. isovolumetric relaxation time, E/A ratio, deceleration time as a minimum), assessment of pulmonary venous flow and analysis of flow propagation velocities. When assessing clinical trial

participants with significantly impaired LV systolic function (EF% <40%), MV E:A ratio, isovolumetric relaxation time and MV deceleration time have been shown to provide an excellent indication of filling pressures (Gottdiener et al 2004). Assessment of diastolic parameters in this study is further compromised by a small proportion of patients in atrial fibrillation at time of echocardiographic examination (15% normal TT and 25% low TT). Atrial fibrillation may cause artificial increases in LA volumes together with artificial remodelling of the LA independent of filling pressures, loss of atrial contractile properties and significant beat to beat variability in measurements obtained. In order to counteract this last point, all measurements in patients with atrial fibrillation were repeated over at least 5 cardiac cycles but overall diastolic assessment in atrial fibrillation is limited and this should be remembered when interpreting the results of this study.

Correlation analyses incorporating total, free and bio-available testosterone did not reveal any significant relationships between testosterone concentration and cardiac structure, function and mechanics. It is feasible that this study was under-powered to detect many changes in cardiac structure and function however, the lack of trends to differences in the majority of measures and the weak correlations observed raises doubt over this hypothesis. Echocardiography cannot exclude changes at the bio-chemical and cellular myocardial level, and as such, future research should aim to measure these outcomes for a more in-depth cardiac assessment. Perhaps future research should aim to involve more detailed cardiac investigation, for instance cardiac MRI. In relation to cardiac function, there was no significant difference in NT pro-BNP concentration between the groups. As NT pro-BNP is a marker of severity of HF this finding lends further support to the lack of observed differences in aspects of cardiac function.

Testosterone may act more directly at the muscular or vascular level in order to promote increases in walking performance. Testosterone can favourably affect skeletal muscle mass and the local synthesis of growth factors (IGF 1) and acontractile protein synthesis (Balagopal et al, 1997). Testosterone may also positively interact with peripheral mechanisms involved in HF pathology. For example, attenuate ED (Miller et al, 2007), improve lung function (Svartberg et al, 2007), normalise baroreflex sensitivity and autonomic imbalance (El Mas et al, 2001), reduce the levels of circulating inflammatory cytokines found to impede LV contractility and muscular performance (Yan Zhou et al, 2009) and increase muscle perfusion at rest and during exercise (Jones et al, 2004). Future research should aim to investigate these concepts in more detail. These studies should aim to directly measure endothelial function and perform detailed muscle strength and endurance assessment in order to elucidate the role of testosterone concentration on both vascular reactivity and muscular function. In addition, future research should collect information as to the levels of important inflammatory cytokines which may promote cardiac and skeletal muscle dysfunction.

Importantly, in HF, there are other factors which may influence endurance capacity, overall muscular strength and cardiac function. For instance in HF there is derangement of the glucose-insulin axis with some studies reporting up to 43% of patients manifesting disorders of glucose metabolism (Kontoleon et al, 2003). It has been shown that low levels of serum total testosterone and SHBG are risk factors for development of the metabolic syndrome in males (Muller et al, 2005). Patients with HF and insulin resistance have an impaired ability to promote glucose transport into skeletal muscle cells and adipose tissue. Moreover, research has showed that impaired insulin activity inversely correlates with severity of HF, relates to disorder of skeletal muscle physiology, facilitates reduced muscle mass and can also influence prognosis (Swan et

al, 1997 and Doehner et al, 2002). Further reports have also suggested that insulin resistance may deteriorate cardiac performance in HF, due to cellular disruption of cardiac metabolism (Chang et al, 2005). Testosterone supplementation towards the physiological range has been shown to significantly improve fasting blood glucose and insulin levels, together with significant improvements in cholesterol level and waist to hip ratio (Kapoor et al, 2007). Mechanistically, cultured adipocytes and skeletal muscle cells incubated with low dose testosterone therapy have demonstrated improved regulation of GLUT4 and insulin receptor 1 (Chen et al, 2006). Furthermore, Sato et al, 2008 have showed that testosterone therapy in a rodent model can facilitate phosphorylation of protein kinase B and C, key pathways in insulin receptor signalling pathways for the regulation of GLUT4 translocation. The same authors have also suggested that testosterone treatment can influence enzymes related to the glycolytic process. Increased activity of phosphofructokinase and hexokinase has been discovered in cultured rat skeletal muscle cells following administration of testosterone. Hence normal levels of free and bio-available testosterone may therefore indirectly correct the aforementioned abnormalities at skeletal muscle level in testosterone deficient participants to promote increases in walking performance. Previously, bio-available testosterone has been shown to inversely correlate with abdominal adipose tissue lipoprotein lipase (Ramirez et al, 1997), an enzyme suggested to play a role in the pathogenesis of obesity as it resides on the extra-cellular surface of adipocytes and hydrolyses circulating triglyceride rich lipoproteins to fatty acids which then become esterified and stored as triglyceride (Eckel, 1989). Marin et al, 1995 showed that following prolonged testosterone supplementation towards a normal range, there was a marked decrease in lipoprotein lipase activity and hence significant reductions in triglyceride storage in abdominal subcutaneous adipose tissue. It is plausible that in our study, disorders of glucose metabolism and insulin resistance may have contributed to

the differences seen in exercise capacity and indirectly quality of life between low and normal testosterone groups. In particular, gross malfunction of skeletal muscle energetics and attenuated ability to utilise energy for muscular work would directly result in the observed difference in 6 minute walk distance. This study showed no differences in body mass, BMI or resting BP between the groups, but blood samples were not taken for fasting insulin or glucose assessment. As a result, the impact of insulin resistance on exercise tolerance in our cohort of patients with low testosterone status remains unknown. This clearly warrants investigation in future research which should aim to collect data regarding components of the metabolic syndrome together with detailed aspects of skeletal muscle contractile function and energetic in a male heart failure population.

There were significant improvements in many domains of the SF36 version 2 in both the physical and mental components in normal testosterone when compared to low testosterone. These improvements resulted in highly significant improvements in overall physical component summary ($p < 0.001$) and significant improvements in mental health component summary ($p < 0.05$). In addition, significant correlations were seen between testosterone concentration and physical function, role physical and physical component summary domains of the SF36. No other research has compared quality of life variables based on testosterone concentration in HF, particularly with patients also grouped by free and bio-available facets. However, in testosterone deficient, healthy, elderly males, testosterone therapy has been shown to significantly improve overall quality of life as assessed using the SF-12 questionnaire following 48 weeks of regular supplementation when compared to placebo (Tong et al, 2010). Logically, the significant improvements noted in exercise ability in this study should translate to improved overall perception of

physical and mental quality of life and the aforementioned physiological theories to improved 6 minute walk performance could be indirectly proposed as precursors to these improvements in quality of life. However, Malkin et al, 2006 observed no improvement in general health score following testosterone therapy in their elderly HF patients, despite significant improvements in exercise capacity. Interestingly, during this study no significant changes were seen in BDI or MLHFQ score between groups based on testosterone concentration. This is in contradiction to other research which has shown that low levels of circulating anabolic hormones resulted in increased depressive symptoms in younger males with HF (Jankowska et al, 2006). The same authors in an older population however, were unable to replicate this improvement in depressive symptoms, a finding similar to this study. During this study, no change was noted in NYHA score between the two groups. In contradiction to this, Malkin et al, 2006 observed improvements in NYHA score in testosterone treated HF males when compared to placebo. Similarly to the current study, no changes were noted in relation to depression or HF related quality of life.

An interesting outcome in this study was that there were significant differences in many generic SF-36 health outcomes but no improvements noted in disease specific quality of life using the MLHFQ. Although there is some overlap of inquiry between the two questionnaires – particularly with regard to physical fitness, the MLHFQ is shorter with less emphasis on these parameters. The MLHFQ also questions the clinical consequences of HF by enquiring about hospital admission, the financial impact and side effects related to medical treatment. It is probable that for this study, the MLHFQ was insufficiently focussed around the physical and mental parameters to allow for significant differences to be achieved when compared to the more detailed SF-36 Version 2. Additionally, as this study was short duration and the MLHFQ enquires

regarding only recent (last month) changes in HF status, this questionnaire may have been limited by the strict inclusion and exclusion criteria to our study. Patients were clinically stable for a period of 6 months without any change to medication or NYHA status and as such, these facets may decrease the power of disease specific questionnaires to detect significant differences between the groups in this study.

As stated above, in the current study, all participants were clinically stable and also actively taking a full complement of anti-failure medications for a period of at least 6 months. This may, in some part, explain the lack of change in HF specific quality of life (NYHA and MLHFQ). ADAM questionnaire score was significantly higher in the low total testosterone participants but not when the groups were based on free or bio-available testosterone. This data reinforces the use of the ADAM questionnaire as a valuable addition to serological assessment of total testosterone deficiency but does not support its use in patients who have low free or bio-available testosterone concentration. Importantly, many facets examined within the ADAM questionnaire overlap significantly with the clinical symptoms of HF, (e.g. reduced libido, lack of energy, decreased strength and / or endurance, depressive symptoms, tiredness and lack of concentration). This may partially explain the lack of observed difference in ADAM score when groups were based on free and bio-available testosterone. Future research in larger clinical populations should aim to identify if using a hypogonadal screening questionnaire such as the ADAM is feasible in patients with established HF.

There were no significant differences noted between NT pro-BNP concentrations in low testosterone when compared to normal testosterone. To date, no research has compared this outcome in this patient group. As NT pro-BNP represents a marker of severity of

HF, the lack of significant differences implies that HF is not responsible for the differences between low and normal testosterone patients in this study. To lend further support to this theory, no differences were seen in NYHA score and MLHFQ score between the groups – however, the limitations of the MLHFQ have been previously mentioned. Other researchers have also demonstrated no improvement in NT pro-BNP concentration despite testosterone supplementation in deficient HF males (Malkin et al, 2006). In concurrence with this study, the authors observed no change in NT pro-BNP, but a significant improvement in exercise capacity suggestive of a role other than cardiac for the mechanical improvements noted in exercise ability based on testosterone concentration. There is evidence however, that testosterone supplementation can improve serum markers of inflammation and cardiac dysfunction. Yan-Zhou et al (2007) demonstrated in rodent models that testosterone supplementation reduces the imbalance between IL-10 and TNF- α thereby reducing LV remodelling and improving LV systolic function. This study did not measure serum markers of inflammation and as such, further research is implied to assess for this more closely in this patient population.

A novel feature of this study is the additional measurement of salivary testosterone in the HF population. At present, there is no consensus cut off value for low levels of salivary testosterone due to inconsistencies in analysis techniques and lack of available data in different clinical populations. In normal healthy populations, salivary testosterone is a filtrate of plasma containing only the free fraction of testosterone and, as stated earlier in this review, free testosterone is often considered the most physiologically active form of testosterone available for metabolising in target tissue (MacDonald et al, 2011). In addition, following exogenous administration of testosterone, salivary testosterone increased in parallel with total testosterone without significant change in SHBG binding capacity in serum (Wang et al, 1981). This further

clarifies the suggestion that salivary testosterone represents closely serum unbound or free testosterone (Nieschlag et al, 2006).

This study has demonstrated for the first time that salivary testosterone measurement is valuable in the HF population. Salivary testosterone concentration correlated strongly and significantly ($p < 0.001$) with total testosterone in this study. In addition, Bland Altman plot analysis showed that repeated measurements of salivary testosterone are accurate (mean difference of -0.31 nmol/L, s.d. ± 3.70 nmol/L, mean difference ± 2 s.d. $+ 7.09$ nmol/L and -7.70 nmol/L) and also confirms the close agreement between traditional serum FT measurements in a HF population (mean difference -16.05 pmol/L, s.d. of 74.57 pmol/L, $+ 2$ s.d of 133.09 pmol/L and $- 2$ s.d. of 165.19 pmol/L). This perhaps may reduce the need for semi-invasive blood sampling or allowing patients to provide their own sample without need for medical assistance in the future.

Undoubtedly, the aforementioned close relationships between salivary testosterone and free testosterone during this study resulted in similar relationships seen against important outcome measures when using Pearson product moment correlation. Larger scale studies should aim to accurately address the true cut-off value for salivary testosterone which predicts true testosterone deficiency in a HF population. This study had an insufficient sample size for this to be achieved. However, it is predictable that such values will correlate closely to those of the free fraction of testosterone given the closeness of the observed relationship in this study.

The results of this study suggest a possible role for testosterone replacement therapy in this patient population to augment both exercise capacity and quality of life. Although work has already been undertaken in this area, results are contradictory and there is

scope for adequately powered studies to investigate more detailed aspects of physiological function. Studies should also aim to identify if testosterone supplementation can be combined with other therapeutic interventions such as exercise training to augment any improvements that may be seen. Detailed physiological, biochemical and cardiovascular assessments should be undertaken in any future research to ascertain the mechanisms behind any observed improvements. In particular, future research should aim to measure aspects of skeletal muscle function – including strength and endurance, fasting blood glucose, insulin and vascular endothelial function in order to bring further clarity to the mechanisms involved any improvements seen.

Chapter 6. Summary and Future Research.

This study was designed to investigate the physiological and psychological impact of low testosterone status on functional capacity, cardiac function and quality of life in males with stable HF. Currently, limited data is available regarding the effects of low testosterone on the aforementioned outcomes in males with HF. Initial work by Jankowska et al, 2009 and Bocchi et al, 2008 have showed that testosterone levels are correlated to VO_{2max} and RV function in HF. However, no direct comparison between low and normal range testosterone has been undertaken and there is currently no research investigating the effects of low testosterone on general health related quality of life and advanced aspects of cardiac structure and function in HF. Importantly, this study has been designed to assess in more detail than previously undertaken the functional mechanics of the heart with respect to testosterone level in HF. In addition, this study is the first to recognise the importance of free testosterone and bio-available testosterone concentration in a population whom the traditional assessment of total testosterone may be an inaccurate due to the deleterious effects of SHBG. Recognition of salivary testosterone measurement as a direct correlate of free testosterone and also its relationship to important outcomes assessed during the study is another important facet that may, in the future, reduce the need for semi-invasive blood sampling and also allow patients to provide their own testosterone samples from home for analysis.

The principle finding of this study is that male patients with low serum and salivary testosterone together with moderate or worse systolic HF have significantly impaired exercise capacity as assessed by the 6 min walk when compared to males with normal range testosterone. Furthermore, several SF36 domains of quality of life in both the mental and physical domains are significantly worse in HF patients with low levels of testosterone. These differences seem independent of age, NYHA score, BMI, NT-Pro

BNP and EF%. Detailed cardiac assessment largely revealed that there was no significant difference in cardiac function based on testosterone concentration. However, there were minor interesting differences seen in some parameters relating to left ventricular diastole.

Future research should aim to assess adequately the safety and efficacy of testosterone supplementation in male patients with stable HF. In addition, future studies should aim to address, in more detail, the physiological mechanisms responsible for poorer exercise capacity in this patient population. Important outcomes should assess detailed, structural skeletal muscle parameters which related to improvements in strength and endurance, vascular adaptation with a focus on endothelial function, inflammatory markers which may affect detailed indices of cardiac and skeletal muscle cellular function, glucose and insulin levels with other components of the Metabolic Syndrome and perhaps use more detailed techniques to investigate cardiac function (e.g. 3D echocardiography or cardiac MRI).

To date, there are only small-scale studies that have addressed the effects of testosterone therapy on important prognostic outcomes in HF (Malkin et al, 2004 and 2006, Caminiti et al, 2009). Any future studies should be designed in order to inform a larger, perhaps multi-centre trial investigating the effects of testosterone supplementation in HF. This should be achieved by not only focussing on the changes in important health variables but by also providing detailed feasibility data. Earlier in this study it was highlighted that although treatments for HF have improved prognosis, there is still substantial morbidity and mortality associated with this condition. It is important that future research explores strategies that could augment the physiological benefits of

testosterone supplementation in order to provide improved prognostic outcome in patients with HF to reduce the burden of HF related costs on health services.

Section 2. Testosterone therapy during exercise rehabilitation in males with heart failure who are testosterone deficient.

Abstract.

Background: The efficacy of exercise therapy for evoking improvements in key health outcomes in elderly male CHF patients with low testosterone status ($\leq 15\text{mmol/l}$) is unknown. The primary aim of this study was to assess the feasibility of a 12-week exercise program, with and without testosterone supplementation, in this patient subgroup. Secondly, to collect preliminary data on changes in key health outcomes evoked by the two interventions. **Methods:** Forty male CHF patients (67.1 ± 6.3 years with low testosterone $\leq 15\text{mmol/l}$) were randomly allocated to exercise training plus testosterone supplementation or exercise training plus placebo. Intramuscular injections of testosterone/placebo were administered at baseline and then every other week during the intervention period. Compliance to the intervention, shuttle walk distance, peak oxygen uptake, muscular strength, muscle oxygen saturation, central and peripheral cardiovascular function, biochemical profile and health and disease related quality of life were assessed at baseline and immediately after the 12-week intervention. **Results:** 28 patients completed the study. Peak oxygen uptake, time-to-minimum tissue oxygenation, and incremental shuttle walk test performance were improved in both groups ($p < 0.05$), with no significant group-by-time interaction. Body mass index was decreased in the exercise plus testosterone group only ($p < 0.05$). Leg strength and biochemical status did not change in either group ($p > 0.05$). **Conclusion:** An exercise and testosterone intervention in hypogonadal males with CHF is safe and feasible. Experience recruiting these patients and the attrition data produced from this study suggest that future exercise and testosterone interventions in this clinical group should be conducted as multi-centre clinical trials.

Chapter 1. General introduction.

Testosterone deficiency is positively correlated with cardiac output (Kontoleon et al, 2003) and exercise capacity (Malkin et al, 2006) in patients with HF. Significant improvement in both these parameters has been observed following testosterone replacement therapy (Parameshwar et al, 1992, Pugh et al, 2002 and Pugh et al, 2003). Although the mechanisms behind this process are poorly understood, improvement in exercise capacity has been shown to be positively correlated with increase in serum testosterone level (Malkin et al, 2006). Additionally, this was accompanied by a small increase in internal LV length in a recent study (Malkin et al, 2006). Evidence from animal studies suggests that anabolic androgens can attenuate skeletal muscle fatigue in response to exercise (Tamaki et al, 2001). Testosterone replacement therapy has also been shown to reduce circulating levels of inflammatory mediators, such as TNF- α and IL-1 β as well as total cholesterol in patients with established CAD and testosterone deficiency (Malkin et al, 2004, Malkin et al, 2004). Circulating levels of inflammatory mediators are elevated in HF and may be related to ED and clinical deterioration in these patients.

Interestingly, chronic administration of physiologic replacement doses of testosterone can delay the time to ischemic threshold during treadmill walking in elderly males with established CAD (Malkin et al, 2004). A rapid mode of action is indicated, as improvements in ischemic threshold have been observed after as little as one month of intramuscular testosterone replacement compared with placebo (Malkin et al, 2004). As testosterone is a vasodilator, this could explain its anti-ischemic effects on cardiac function during exercise. However, it is currently unknown whether the vasodilatory

effects of testosterone can influence the fatigability of skeletal muscle in a similar fashion.

It is now widely accepted that exercise training (ET) can safely increase exercise capacity in stable HF patients. An improvement in skeletal muscle strength and endurance has been reported after resistance training regimens (Gordon et al, 1996 and Pu et al, 2001). More recent studies have also reported improvements in dynamic quadriceps and hamstrings strength and endurance following combined aerobic and resistance training programmes (Senden et al, 2005) and provided evidence that this combined training approach is superior to aerobic training alone for improvement of LV function in patients with HF (Delagardelle et al, 2002). There is also evidence that short-term programmes of ET lasting for 12 weeks can reduce the circulating levels of inflammatory mediators such as TNF- α , soluble intracellular adhesion molecule (sICAM-1) and soluble vascular adhesion molecule (sVCAM-1), which are elevated in HF (Adamopoulos et al, 2001). However, a need for more clinical trials aimed at evaluating the long-term effects of physical training on functional status, morbidity and mortality in these patients has been highlighted (Freimark et al, 2007).

Considering the low functional capacity of male HF patients (Malkin et al, 2006), an investigation of strategies that have the potential to augment the response to exercise rehabilitation in order to improve prognosis is clearly warranted in this patient group. To date, the efficacy of exercise therapy for evoking improvements in key health outcomes in elderly male patients with HF and low testosterone status have not been studied. Neither is it known whether the effects of exercise training in HF can be augmented by testosterone supplementation.

Chapter 2. Literature review.

2.1: Exercise training and HF.

2.1.1: Exercise intolerance.

The inability to perform exercise without discomfort is often one of the primary symptoms exhibited by patients with HF and is often the sole reason for seeking medical attention. As discussed earlier in this review, the main pathological consideration in systolic HF is impairment in LV EF resulting in a decline in cardiac output and in up to 50% of patients may be related to HF with normal ejection fraction but impairment in filling parameters. It is therefore expected that there is a strong correlation between resting ventricular function and exercise capacity. Previous reports however, contrast this theory and have shown poor correlations between resting EF and exercise tolerance (Franciosa et al, 1981). This has prompted numerous investigators to attempt to elucidate the important factors involved in exercise intolerance and the use of therapeutic measures to improve exercise tolerance in patients with HF. This section of this literature review will aim to understand the main pathological factors involved in exercise intolerance in HF and will review the effect of ET in attempts to improve physiological correlates and symptom onset.

A reduced ability to perform aerobic exercise is commonplace in HF. This has been attributed to inadequate blood flow to active skeletal muscle due to reduced cardiac output (Sullivan and Cobb, 1992). Patients with HF typically achieve less than 50% of the maximum cardiac output achieved by healthy individuals at peak exercise. Stroke volume is decreased at rest and only rises to approximately 50-65ml compared to 100ml in healthy participants. This is in addition to a significant reduction in exercising maximal HR in HF patients (Franciosa et al, 1981). Dilated left ventricles with

significantly reduced EF also demonstrate poor increases in stroke volume during exercise, primarily because of a blunted ability to increase preload and EF (Hakki et al, 1984). Simply, an already dilated left ventricle operates close to its maximal volume having already utilised most of its preload reserve. Failure to increase systolic emptying by increasing EF derives from impaired intrinsic contractility, reduced α -adrenergic responsiveness, elevated systemic vascular resistance (see earlier discussion surrounding the renin-angiotensin system) and a blunted peripheral arterial vasodilator response to exercise (Pina et al, 2003). Consequently, the only way patients with HF can increase cardiac output is by increase in HR. Previous studies have shown only slightly reduced maximal HR in HF patients with almost identical slope of increase when compared to normal participants. However, many patients with HF have elevated resting HR and therefore reduced HR reserve and also achieve lower HR at maximal exercise (Sullivan and Cobb, 1992). Although a strong correlation has been observed between the degree of aerobic capacity impairment and the reduction in maximal cardiac output (Sullivan and Cobb, 1992), other research has demonstrated weak correlations between peak exercise $\dot{V}O_2$ and EF in patients with HF (Wilson et al, 1995). In relation to this, it has also been discovered that peak $\dot{V}O_2$ does not correlate closely with NYHA score or subjective quality of life questionnaire data (Wilson et al, 1995). This data suggests that although reduced EF and hence cardiac output is an important factor in exercise intolerance in patients with HF, there are other detrimental physiological processes that contribute to this phenomenon.

Many of the pathophysiological characteristics of HF as mentioned previously in this thesis would have an adverse effect on exercise tolerance. For example, stimulation of the RAAS (Vinereanu et al, 2005) would preclude inadequate blood flow to exercising

muscles via an inability to decrease vascular resistance by vasodilatation. This theory has been supported by research which discovered reduced fore-arm blood flow at rest and during exercise in patients with HF when compared to healthy controls (Zelis et al, 1974) and also failure of exercising leg vascular resistance to decrease normally during exercise in HF potentially as a result of excessive sympathetic stimulation (Zelis et al, 1988).

The afore-mentioned widespread endothelial cell activation and dysfunction (Brutsaert, 2003) would negatively impact on vasodilatory capacity during exercise in HF. This notion has been supported by studies demonstrating that endothelial-dependent dilatation of the forearm vasculature is impaired in HF via a reduction in the release of nitric oxide (Kubo et al, 1991). The peripheral vasculature endothelium secretes the lipid soluble gas nitric oxide in response to increased flow through the target vessel lumen via its enzyme endothelial nitric oxide synthase (eNOS) in a similar manner to coronary resistance vessels mentioned previously. This release of nitric oxide via shear increased pulse pressure and pulsatility in the resistance vessels promotes vasodilatation in normal animal models in order to normalise shear stress (Green et al, 2004). Abnormalities of this response can cause reduced peripheral vasodilatation and hence tissue perfusion in patients with HF. Additionally, it has been shown that degree of impairment in endothelial-dependent dilatation is correlated with exercise tolerance and NYHA score (Nakamura et al, 1994).

Perhaps closely related to these concepts is the capacity of the vasculature to redistribute cardiac output to the working skeletal muscle during exercise. There is some research evidence that suggests that the reduction in muscle blood flow correlates closely with reductions in cardiac output (Yamabe et al, 1995). However, other research

suggests that this reduction is somewhat out of proportion to maximal cardiac output. Researchers at Duke University in the U.S.A have identified that the percentage of cardiac output distributed to both legs during exercise was attenuated in patients with HF (51%) when compared to healthy controls (76%) (Sullivan and Cobb,1992). Therefore, as a result of abnormal vascular resistance in exercising muscle and further abnormalities in redistribution of flow (e.g. maintained flow to non-exercising tissue) there may be considerable hypo-perfusion and fatigue in exercising muscle.

The pathophysiological characteristics of skeletal muscle in HF described earlier in this review can also contribute adversely to exercise tolerance in this patient group. For example, increased oxygen extraction and lactate efflux together with diminished total muscular oxygen utilisation (Donald et al, 1961), identified changes in mitochondrial structure and function together with increased type II muscle fibres (Mancini et al, 1989) and declines in phosphocreatine with rises in inorganic phosphate (Wilson et al, 1985) suggests that skeletal muscle alterations may contribute to abnormal oxygen extraction, substrate utilisation and consequently exercise tolerance in HF.

Researchers in London, U.K. have described a newer 'muscle hypothesis' that may contribute to HF related exercise intolerance (Piepoli et al, 1996). The metabolic stasis of the muscle is monitored by the activation of ergoreceptors whose fibres increase ventilation and sympathetic outflow. Reduced LV function leads to skeletal muscle wasting together with associated abnormalities in muscle metabolism and function. In response to this, exaggerated ergoreflex activation occurs that leads to excessive sympathetic vasoconstriction of non-exercising beds and an excessive ventilatory response to exercise. Consequently this increases peripheral resistance and reduces

muscle perfusion. The authors also state that this process combined with a catabolic state (see earlier) may eventually lead to worsening LV function and remodelling thus progressing severity of HF.

In HF, there is derangement of the glucose-insulin axis with some studies reporting up to 43% of patients manifesting disorders of glucose metabolism (Kontoleon et al, 2003). Initial reports from the Framingham Study drew attention to the potential relationship between diabetes mellitus and HF independent of age, coronary artery disease, hypertension or BMI (Kannel et al, 1979). Patients with HF and insulin resistance display an impaired ability to promote glucose transport into the muscle and adipose tissue – factors which may clearly affect physiological function of exercising muscle. Additionally, more recent research has showed that impaired insulin activity inversely correlates with severity of HF, relates to disorder of skeletal muscle physiology, facilitates reduced muscle mass and can also influence prognosis (Swan et al, 1997 and Doehner et al, 2002).

2.2: Benefits of ET in HF.

2.2.1: Exercise capacity.

Numerous studies have identified positive benefit effects of ET relating to exercise capacity considering both exercise duration and peak $\dot{V}O_2$. Study design has included randomised controlled trials, parallel or cross-over training with a marked variance in participant numbers ranging from 6 to 99. Exercise intervention duration has ranged from 3 weeks up to 24 weeks with periods of maintenance extending up to 1 year. The ET programme has varied by numerous factors including setting (home versus gym

supervised), type of exercise modality (cycling, treadmill, Pole Striding, outdoor exercise) and exercise intensity from low level to moderate – high intensity training. Another study has included both aerobic conditioning elements combined with strength training (Smart et al, 2007). Improvements in peak oxygen uptake have ranged from 6% in a study of Pole Striding for 30 minutes three times per week (Collins et al, 2004) to 31% in a study simply using outdoor walking for 10 minutes six times per day, twice a week (Hambrecht et al, 1995). A fairly recent meta-analysis has suggested that the greatest mean increase in peak $\dot{V}O_2$ was identified in the research studies involving either continuous or intermittent aerobic exercise or combined aerobic and strength training (Smart et al, 2004). The authors reported increases in $\dot{V}O_2$ of 16.5% and 15% compared with increases of 9% in studies involving only strength training. This analysis also asserted that most improvement in oxygen uptake seems to occur early in the intervention (by week 3) but can continue up to 6 months dependent upon patient compliance to the ET regimen. Overall, average increase in $\dot{V}O_2$ in the same meta-analysis of 81 studies with a total of 2387 patients was identified as 17% in the 57 studies that incorporated direct measurement of oxygen consumption.

A study conducted by the European Heart Failure Training Group (1998) reviewed the progress of 134 stable HF patients studied in randomised controlled trials of physical training. All patients underwent training on a cycle ergometer for 20 minutes 4-5 days per week. Patients were instructed to exercise at 70-80% of a pre-determined peak HR with calisthenics added in 40% of the patient population. Exercise training duration ranged from 6 weeks to a maximum of 16 weeks with the majority (69.4%) of patients training at home. The study concluded that the positive effects of exercise in stable HF are confirmed and independent of age and gender. A significant training effect of 13%

increase in peak $\dot{V}O_2$ and 17% increase in exercise duration was found to be associated with improved autonomic indices such as resting catecholamines and HR variability. Interestingly, in this study, improvement in oxygen consumption was higher following 16 weeks of training when compared to 6 weeks – contradictory to other research (Smart et al, 2004).

More recent research has used high intensity exercise training up to 90-95% of peak $\dot{V}O_2$ for 4 minute intervals (Wisloff et al, 2007). This study recruited 27 patients with stable HF post MI all receiving optimal medical therapy. These patients were elderly with a mean age of 75.11 ± 11.1 years and had severely impaired LV systolic function (mean EF% of 29%). Patients were randomised to receive high intensity ET or moderate continuous ET for a total of three sessions per week (2 supervised and 1 unsupervised home based). High intensity ET as above was interpolated with 3 minute active recovery pauses for a total exercise time including warm and cool-down of 38 minutes. Moderate continuous ET involved walking for a total of 47 minutes at 70-75% of peak $\dot{V}O_2$. Results showed that aerobic interval ET was superior to moderate continuous ET by evoking significant improvement in $\dot{V}O_2$ (46% versus 14%, $p < 0.001$). In addition to this, improvements in peak $\dot{V}O_2$ were associated with decreased LV volumes in systole and diastole, increased EF by approximately 35% and also decreased pro-BNP by 40%. Improvements were also identified in FMD of the brachial artery and vastus lateralis muscle mitochondrial function in the aerobic interval ET group only. This study supports the hypothesis that high intensity aerobic interval ET is safe in elderly patients with HF. Additionally, this type of training can produce superior effects to traditional moderate continuous training. The authors suggest that a combination of LV reverse remodelling, improved LV contractility, increased

endothelial function and mitochondrial function all result in superior exercise capacity as assessed by peak $\dot{V}O_2$ in post-infarct HF patients.

Mechanistically, the integration of several factors seems to be responsible for the increase in peak oxygen uptake following ET in patients with HF. Central myocardial function appears to be improved documented by increases in peak cardiac output response. A study performed by researchers in the Duke University and Durham Veterans Medical Centre in the U.S.A identified slightly decreased or unchanged cardiac output during rest or sub maximal exercise but increased maximal exercise cardiac output from 8.9 ± 2.9 L/min to 9.9 ± 3.2 L/min following ET (Sullivan et al, 1988). However, it is suggested that this increase is not solely responsible for the increases in peak $\dot{V}O_2$ following training (Working Group on Cardiac Rehabilitation and Exercise Physiology, 2001). Reduced vasoconstriction of nutritive arterioles within the active skeletal muscles in combination with improved oxygen extraction and metabolic function at the active skeletal muscle are also important contributing factors. Research has identified significant systemic vasodilatation at rest, during sub maximal and peak exercise following ET intervention in HF patients (Coats et al, 1992 and Sullivan et al, 1988). This work highlighted increased blood flow to the exercising leg during peak exercise (from 2.5 ± 0.7 L/min to 3.0 ± 0.8 L/min) together with a strong tendency for decreased leg vascular resistance at peak exercise (from 48 ± 14 mmHg /L/min to 42 ± 12 mmHg/L/min). Consequently this research also identified that time course to ventilator derived anaerobic threshold and oxygen consumption at this point are also increased due to ET (+ 1.8 minutes at $\dot{V}O_2$ of 13.7 ± 0.7 ml/kg/min – compared to a $\dot{V}O_2$ of 11.9 ± 0.8 ml/kg/min prior to exercise training). This suggests delayed

reliance on inefficient anaerobic pathways for energy production during exercise training.

2.2.2: Exercise training and cardiac systolic function.

There are a number of inconsistent results focussing on myocardial adaptations to ET in the HF population. Much work has suggested that any improvement in exercise capacity is due to peripheral adaptations. However, there is research evidence that suggests ET has a favourable effect on myocardial and coronary vessel adaptation. A recent meta-analysis has identified a number of research studies that have investigated the effects of ET on LV systolic function and LV volumes (Haykowsky et al, 2007).

Studies indicate that aerobic training can reduce adverse LV remodelling in patients with HF. These changes are supplementary to any benefits that occur due to pharmacological intervention. In more detail, studies indicate that aerobic training can provide consistent benefits to EF. Haykowsky et al (2007) examined nine trials utilising aerobic training and measuring its effect on EF. They observed a mean weighted difference in EF of +2.59% to +3.21%. Strength training in isolation was found to be inconclusive when assessing benefits to EF. However, this is based solely on one trial of strength training alone in HF. Trials have used a combination of aerobic and strength training interventions in patients with HF. These studies have also proved inconclusive for their effects on EF with a weighted mean difference of 0.37% to 2.97% (Haykowsky et al, 2007). Conversely, other research studies that have investigated the effects of aerobic training on myocardial function in HF patients have not documented any significant changes in resting or exercising EF (Belardinelli et al, 1995 and 1998).

There appears to be more convincing evidence within the literature in favour of the beneficial effect of ET on LV volume. Aerobic training has predominantly been utilised in this HF population. However, there are also studies combining aerobic and strength training. Using weighted mean difference, ET has been found to be associated with a significant decline in end diastolic volume (-9.75 ml) and also end systolic volume (-12.31 ml) (Haykowsky et al, 2007). These findings are in contrast to early observations by Judgutt and colleagues (Judgutt et al, 1988) who found that ET early post MI was associated with increased myocardial thinning, remodelling and worsening EF. This early observation served to fuel concerns over the effectiveness of ET in a post MI population. However, more recent observations have confirmed that ET not only reduced LV volumes (Haykowsky et al, 2007), has showed no detrimental effects to LV wall thickness and thinned infarcted segments (Dubach et al, 1997), but has been shown to attenuate unfavourable LV remodelling (using long-term exercise training) in post MI patients with significant LV dysfunction (Gianuzzi et al, 1997).

The mechanisms behind improvements in central cardiac function are not well known. Theories have however, been postulated by various investigators. Studies have found that ET can result in decreased levels of the important patho-physiological vasoconstrictive hormones common to HF. An important study by Braith and co-workers has suggested that ET favourably affects levels of aldosterone, angiotensin II, vasopressin, atrial natriuretic peptide and brain natriuretic peptide, epinephrine and norepinephrine levels (Braith et al, 1999). The importance of these hormones in HF has been discussed earlier in this review. Reduced levels of these hormones serve to promote peripheral vasodilatation. This reduction in cardiac afterload reduces pumping

strain on an already compromised ventricle and hence attenuates the process of ventricular remodelling. In relation to this point, other research has shown that ET can also decrease sympathetic tone and increase vagal activity in patients with HF (Coats et al, 1992). Such improvement in sympatho-vagal activity can further contribute to reduced vascular load and attenuation of LV remodelling. The work carried out by Hambrecht and colleagues (Hambrecht et al 1995) and Bellardinelli and co-workers (1995, 1996, 1999) has shown that beneficial changes in LV volume and peak exercise stroke volume as a result of aerobic ET were correlated to the decline in both resting and exercising systemic vascular resistance. Furthermore, it was discovered that this type of training can facilitate improvements in myocardial contractility and diastolic filling in patients with markedly impaired LV systolic function. Therefore, the finding of improved EF in a recent meta-analysis of clinical trials (Haykowsky et al, 2007) may be explained thus; peripheral vasodilatation results in decreased cardiac afterload – attenuating LV remodelling. Improvements in venous return due to peripheral vasodilatation results in increased diastolic filling and improved stretching of cardiac myocytes as the ventricle swells to an increased end diastolic volume (increased cardiac preload). Myocyte stretching increases the sarcomere length resulting in an increase in force generation via additional increases in troponin-c calcium sensitivity and the related increased rate of cross bridge attachment and detachment (amount of tension developed by individual muscle fibres). This mechanism enables the cardiac muscle to eject the additional venous return and improve cardiac stroke volume.

2.2.3: Exercise training and cardiac diastolic function.

The effects of ET on LV diastolic function are less studied when compared to systolic functional parameters. However, the relevance of diastolic function in determining

prognosis and survival in HF has emerged as clinically important. Research studies have identified that ventricular filling abnormalities, together with systolic dysfunction, not only increase severity of patient symptoms (Rihal et al, 1994), but are also a powerful predictor of future cardiac events and consequently mortality (Pinamonti et al, 1993). To reinforce this concept, previous research has identified that exercise intolerance in HF is more closely related to left atrial pressure than to LV systolic EF (Bellardinelli et al, 1996). As a result, abnormalities of relaxation and filling during exercise can potentially be involved in reducing aerobic exercise capacity in this patient group. Within the literature, there is conflicting data with regard to the effects of ET on parameters of diastolic function. In studies of patients with diastolic dysfunction alone, there are similar improvements to exercise capacity and quality of life as seen with those patients who have systolic HF (Freimark et al, 2005). There are however, contradictory results regarding the effects of ET on central cardiac aspects of diastology.

There are contradictory results regarding the effects of ET on diastolic function in HF patients. Studies have however, noted significant improvements in some diastolic parameters in response to ET when compared to controls (Bellardinelli et al, 1996, Stolen et al, 2003, Cheuk-Man et al, 2004, Malfatto et al, 2009). In particular, the improvement in diastolic parameters has also been found to correlate well with improvements in peak $\dot{V}O_2$ suggesting an important role of diastole in the increased endurance performance noted as a result of ET in HF patients (Bellardinelli et al, 1996). It has been hypothesised that an altered loading state of the LV as a response to ET could be an important mechanism in the reduction of LV stiffness. No specific mechanism has been suggested to preclude this phenomenon. Together with the

aforementioned peripheral adaptations which may contribute to an improvement in systolic contractility and also diastolic filling, there are also possible coronary vascular and myocardial adaptations that may contribute to enhanced diastolic function following exercise training. For example, animal studies have suggested that the uptake of calcium by the myocardial sarcoplasmic reticulum can be enhanced by ET in HF patients, inducing both functional and structural coronary vascular adaptation (Laughlin and Mc Allistair, 1992). ET has also been found to attenuate the vasoconstrictor response to norepinephrine via decreased receptor signal transduction rate in α -adrenergic receptors of coronary vascular smooth muscle (Carrier et al, 1978). ET can also improve bradykinin induced coronary endothelial secretion of relaxing factors, thus improving coronary artery sensitivity (Muller et al, 1991). Together these factors can drastically improve myocardial perfusion which may play an important role in enhancing early diastolic filling (Bellardinelli et al, 1996). It should be noted that all of the echocardiographic indexes of diastolic function used in the studies above are directly load-dependent. Therefore, as previously discussed, it is sensible to suggest that any therapeutic intervention that affects chamber volume and peripheral resistance can have positive effects on diastolic function. Improved peripheral arterial function via the positive effects of ET on the detrimental pathological hormones secreted in HF (Braith et al, 1999) serves to improve venous return, thus enhancing diastolic filling. The significant reduction in LV elastance following physical training noted in one of the studies above (Malfatto et al, 2009) could be related to positive ventricular remodelling – particularly, because in the same study, there was a significant improvement in a correlate of remodelling namely LV mass index (Colucci et al, 2007).

2.2.4: Exercise training and muscle function.

As previously highlighted in this review, peripheral muscle abnormalities can be regarded as one of the principle reasons for poor exercise tolerance in patients with HF. Reduced muscle mass and structural mal-adaptation occurs frequently and is linked to malnutrition, de-conditioning and the toxicity of circulating inflammatory cytokines. Researchers have investigated the effects of ET on patients with HF and have determined that the biochemical and histologic changes in skeletal muscle have been found to correlate closely with those of classical de-conditioning (Holloszy and Coyle, 1984). Early studies investigating the effects of ET on skeletal muscle structure and function showed that regular physical training delays anaerobic metabolism also improving aerobic exercise capacity via increased oxidative capacity of the working skeletal muscle (Hambrecht et al, 1995). This initial study showed significant increases in the oxidative capacity of skeletal muscle as suggested by changes in volume density of cytochrome oxidase positive mitochondria which were also found to be significantly correlated with changes in functional capacity and peak oxygen uptake.

Similar improvements in oxidative metabolism have been suggested by other researchers (Minotti et al, 1990). Single arm ET for 28 days yielded a two-threefold increase in endurance associated with a slower increase in inorganic phosphate and a decline in phosphocreatine ratio versus work load slope – together indicating an improvement in oxidative metabolism. Needle biopsies of the vastus lateralis muscle following aerobic ET in patients with HF has derived that ultra-structural abnormalities in muscle fibre distribution of HF patients can be corrected and that any increases in aerobic capacity are closely linked to changes in mitochondrial ultrastructure (Hambrecht et al, 1997). In this study, surface density of mitochondrial inner border membrane increased by 92% and surface density of mitochondria cristae by 41%. The more pronounced increase in inner border membranes suggests a higher increase in

cytochrome c-oxidase activity (a rate limiting enzyme) at inner border membranes of mitochondria when compared to mitochondrial cristae in muscle cells.

HF patients are known to have an increase in type II muscle fibres with low aerobic potential (Mancini et al, 1989). An increased quantity of these muscle fibres therefore promotes reduced muscle performance during exercise as slowly contracting, high endurance fibres are required to perform low intensity exercise such as walking or climbing stairs. Hambrecht and colleagues (Hambrecht et al, 1997) documented that physical exercise enhances oxidative capacity across both muscle fibre types and hence a correction towards improved oxidative capacity. In addition, the increase in aerobic enzyme content was found to be an important contributing factor to the reduced levels of lactate accumulation in exercising muscle at sub-maximal workloads. More recent research by Norwegian investigators (Larsen et al, 2002) has suggested that aerobic ET promotes an increased area and thickness of type IIB muscle fibres. Together with this, they found no significant correlation between these muscular adaptations and increased endurance performance. The authors suggest that any increases in maximal aerobic capacity is partly due to a shift toward aerobic metabolism with less lactic acidosis, increased respiratory muscle strength and a further shift to a normalised ergo-reflex response to exercise thus reducing pathological hyperventilation.

Researchers in the UK used ^{31}P nuclear magnetic resonance spectroscopy to measure phosphocreatine depletion, muscle acidification and adenosine diphosphate increase during plantar flexion exercise in the calf muscle of HF patients before and following 8 weeks of physical training (Adamopoulos et al, 1993). Initially, all variables were significantly increased in HF patients when compared to controls, but following ET,

phosphocreatine depletion and the increase in adenosine diphosphate during exercise were significantly reduced. Additionally, phosphocreatine recovery half time was also significantly reduced. Together, these findings indicate a marked correction of the impaired oxidative capacity of skeletal muscle in HF achieved by exercise training. Skeletal muscle mitochondrial adenosine triphosphate production rate has been postulated as an integrated measure of muscle oxidative capacity (Williams et al, 2007). During this research, resistance training was found to be significantly associated with several indices of muscle oxidative capacity. These included citrate synthase activity (an oxidative enzyme) and intact mitochondrial adenosine triphosphate production rate. With these changes being strongly related to increases in peak $\dot{V}O_2$ following a period of circuit resistance training. Interestingly, the substrate combination with the most significant response (pyruvate and malate) stimulates the effect of carbohydrate metabolism. This is appropriate because during the intervention for this study patients exercised at high intensity (>85% of peak). As a result, much of the increased demand for adenosine triphosphate resynthesis via oxidative mechanisms would be met by carbohydrate metabolism. Another finding of this study was the increase in the lactate threshold as a result of ET when compared to controls. Intra-cellular acidosis inhibits adenosine triphosphate supply from oxidative phosphorylation in skeletal muscle (Jubrais et al, 2003) thus limiting oxidative flux to levels below mitochondrial capacity. The delayed onset of lactic acidosis during exercise serves to maintain oxidative flux for longer periods during incremental exercise.

Improvements in major aerobic enzymes such as cytochrome c-oxidase has also been related to improvements in local and systemic levels of some of the detrimental inflammatory cytokines found in HF as mentioned previously in this review.

Collaborative work between German and Swiss researchers has studied the influence of regular physical exercise on skeletal muscle cytokine and inducible nitric oxide synthase expression (a known inhibitor of cytochrome c-oxidase production) (Gielen et al, 2003). Baseline analysis during this study showed that patients with HF demonstrate increased expression of TNF- α , IL-1 α and IL-6 in biopsies from skeletal muscle indicating a local intramyocyte production of inflammatory cytokines. Following a 6 month programme of physical exercise there was a significant reduction in local expression of these inflammatory cytokines in the skeletal muscle of patients with HF with virtually unchanged levels of serum cytokine levels. Additionally, expression of inducible nitric oxide synthase was reduced by over half following the 6 months of exercise training. It has been postulated that in HF, the combination of a low cardiac output and ED, may promote repetitive skeletal muscle ischemia (Paulus, 1999). In ischemia-reperfusion situations, reactive oxygen species are rapidly generated and have been suggested to play a pro-inflammatory role at muscular level in HF (Gielen et al, 2003).

It has already been established that regular physical exercise beneficially effects anti-oxidative enzyme expression (Powers et al, 1994) and this could serve to decrease local pro-inflammatory cytokine production. Similarly, oxidative stress has been shown to play an important role in inducible nitric oxide synthase production (Punzalan et al, 1999). The inducible nitric oxide synthase promoter region has been found to contain an anti-oxidant responsive element whereby reactive oxygen species increase inducible nitric oxide synthase expression. Therefore, it is plausible that the previously mentioned anti-oxidant effects of ET may play a direct role in the attenuation of inducible nitric oxide synthase expression. Niebauer and colleagues (Niebauer et al, 1998) have noted

an association between low serum levels of insulin like growth factor-I and reduction in lean muscle mass of patients with HF.

Other research has identified that local insulin like growth factor-I expression is significantly attenuated in HF patients when assessed using skeletal muscle biopsy (Schulze et al, 2000). These studies promote the hypothesis that decreased levels of systemic and local insulin like growth factor-I occur with advancing stages of HF. In the latter of these two studies, it was noted that the local reduction in insulin like growth factor-I levels was independent of serum concentrations which remained virtually normal thus suggesting an independent local mechanism for insulin like growth factor-I production. ET is a natural form of muscular stretch exposure and has been found to significantly increase skeletal muscle insulin like growth factor-I protein levels without significant change in serum concentrations in animal studies (Eliakim et al, 1997). Consequently, a research study investigating the effects of ET on insulin like growth factor-I expression in patients with HF has resulted in a twofold increase in insulin like growth factor-I levels post intervention (Hambrecht et al, 2000). This seminal research suggests that with adequate aerobic training the catabolic state of skeletal muscle in HF can be attenuated or even reversed as a result of exercise training.

2.2.5: Endothelial function.

As previously stated in this review, vasodilatory capacity during exercise is severely blunted in patients with HF. The reduction in arterial response during exercise due to alterations in endothelial and flow-dependent vasodilatation of resistance arteries seems

to be a key factor in the pathophysiology of reduced exercise tolerance. Research work has however, proposed that HF patients who have undergone ET show marked improvements in endothelium dependent relaxation.

Endothelial cell dysfunction is commonplace in HF and other cardiovascular disease states (Kubo et al, 1991, Green et al, 2004, Nakamura et al, 1994) and can detrimentally reduce peripheral vasodilatation during exercise thus resulting in poor exercise performance. The importance of ET in these conditions has become more relevant because decreased endothelial function (as characterised by decreased nitric oxide vasodilator activity) has been shown to be an important early marker of vascular disease (Green et al, 2004). Exercise training itself promotes repeated exposure to significant alterations in cardiovascular haemodynamics. The onset of physical activity and the related mechanical contraction of exercising muscles results in an immediate increase in blood flow to the working muscles which can rise to levels up to 100-fold (Anderson et al, 1985). In order to facilitate this increased flow, vaso-active metabolites are released from the muscle into the interstitial fluid to act directly on terminal arterioles.

It has been suggested that flow changes associated with metabolic vasodilatation are not enough to account for the significant augmentation of flow to the periphery during ET (Maiorana et al, 2003). The same authors have considered an additional hypothesis to explain the co-ordinated dilatation of smaller and larger vessels to improve peripheral blood flow. The 'ascending vasodilatation' hypothesis theorises that vasodilatation begins in the micro-vessels and serially progresses more proximally along the arterial tree to larger size vessels. Physiologically, it has been proposed that during exercise training, target vessels are bathed in interstitial fluid containing by-products of

metabolism which encourages peripheral dilatation. In normal subjects, this dilatation encourages increased flow velocity in more proximal vessels and hence increases shear stress, promoting endothelial release of nitric oxide and more proximal vasodilatation. Further research has indirectly supported this concept by concluding that flow mediated dilatation of conduit arteries in response to muscle ischemia is in the majority dependent upon an increase in nitric oxide bio-availability in larger arteries (Joannides et al, 1995).

The effects of ET on endothelial function have been thoroughly investigated. Peripheral abnormalities in HF have been previously determined in this review and their relationship to peak oxygen uptake and therefore prognosis warrant this level of investigation. Two early studies in the late 1990's have found significant improvements in nitric oxide related endothelial dependent function following 4 and 8 weeks of handgrip ET in patients with HF. This significant change was not associated with any improvement in endothelial independent dilatation (Hornig et al, 1996 and Katz et al, 1997). The earlier of these studies demonstrated no significant improvement in nitric oxide related endothelial function in the untrained contra-lateral limb which strongly supports the argument that training adaptations to handgrip exercise result from local adaptations (Hornig et al, 1996).

A further study conducted by Hambrecht and colleagues (Hambrecht et al, 2000) noted that combined supplementation with oral L-arginine and handgrip ET resulted in superior blood flow responses to acetyl choline when compared to either intervention alone. Thus suggesting that increased substrate availability compliments shear stress induced up-regulation of endothelial nitric oxide synthase. It should be noted that other research has provided contradictory results to those mentioned above. Bank and

colleagues observed no significant improvement in acetyl choline response to handgrip ET in their HF cohort when compared to healthy controls who had significant improvements in both endothelial dependent vasodilatation and peak hyperaemic blood flow (Bank et al, 1998).

More research has been undertaken assessing the effects of whole body exercise on vascular function. 6 months of cycle training resulted in localised improvements in femoral artery basal and stimulated nitric oxide related dilatation (Hambrecht et al, 1998). This occurred without change in smooth muscle response to glyceryl trinitrate. Perhaps more importantly, this type of ET was associated with significant improvements in exercise capacity, reduced LV end diastolic diameter and reduced total peripheral resistance. Furthermore, the reduced total peripheral resistance correlated well with acetyl choline enhanced flow in the lower limb. This improvement could explain the previously mentioned role of the peripheral circulation in reducing afterload, increasing preload and therefore improving cardiac systolic and diastolic parameters (Bellardinelli et al, 1996).

Other research has used combined aerobic and resistance ET in the form of circuit sessions for an intervention period of 8 weeks (Maiorana et al, 2000). This type of training was found to significantly improve forearm resistance vessel function in patients with HF when compared to controls. Furthermore, this study specifically avoided repetitive forearm muscle training during the circuit training intervention thus suggesting a systemic effect of training. This type of training also suggested slight improvement to endothelial dependent vasodilatation which is consistent with concomitant changes to vascular structure and smooth muscle function. Further

clarification of a systemic effect of ET on nitric oxide related endothelial function was provided by Linke and co-workers (Linke et al, 2001). This group studied the effects of cycle training over a period of 4 weeks on endothelial function in patients with HF when compared to controls. Training of the legs resulted in improved acetyl choline mediated and flow mediated dilatation responses in the radial artery with no concurrent change in vascular smooth muscle function.

To conclude, in patients with HF, it appears that ET of high enough intensity and that activates sufficient muscle mass to produce increases in pulse wave dynamics and shear stress can improve localised and systemic arterial nitric oxide related ED without significant changes in vascular smooth muscle function.

Exercise training in patients with CAD has also been shown to bring about similar improvements to those with individuals HF. ET large muscle groups predominate this research and the studied effects range from the periphery to the coronary arterial tree. Hambrecht and colleagues (Hambrecht et al, 2000) used coronary arterial flow wires during cardiac catheterisation to assess the effects of cycle training for 4 weeks on coronary endothelial function. The intervention resulted in significant improvements in endothelial dependent vasodilatation in epicardial coronary and resistance vessels without significant change in independent endothelial function.

Research focussing upon ET and peripheral arterial function in CAD has yielded positive results. Gocke and co-workers (Gocke et al, 2002) described improved flow mediated dilatation in the tibial artery following 10 weeks of leg ET without any change

in response to glyceryl trinitrate. Disappointingly, although there was a 1.9% increase in brachial artery flow mediated dilatation this was not statistically significant. Another study focussing on circuit training, which again avoided forearm exercise, resulted in significant improvements in brachial artery flow mediated dilatation without any change in vascular smooth muscle function (Walsh et al, 2003). This is further satisfactory evidence suggesting that ET of sufficient calibre and intensity can improve systemic nitric oxide mediated endothelial function in patients with CAD. Mechanistically, endothelial nitric oxide synthase mRNA and vascular protein expression have been found to be higher in trained individuals with CAD when compared to controls (Hambrecht et al, 2003). Additionally, increased endothelial nitric oxide synthase phosphorylation was found to be significantly correlated with the changes in acetyl choline induced peak flow velocity following exercise training, supporting the theory that increased quantity of endothelial nitric oxide synthase protein may promote an improvement in endothelial function via shear stress induced phosphorylation.

It appears that ET can improve endothelial function in patients with known CAD. Clearly, any improvements in coronary artery function may improve myocardial perfusion and hence cardiac function together with reductions in the incidence and severity of myocardial ischemia (Schuler et al, 1992).

2.2.6: Biochemical and inflammatory markers.

The increased activation of several neuro-hormonal systems and increased production of peptides such as nor-epinephrene, angiotensin II, aldosterone, endothelin-1 and pro-

inflammatory cytokines are well documented pathophysiological hallmarks of HF and have been previously described in this review.

Cytokines are small molecular weight protein molecules secreted by cells in response to a variety of stimuli. Cytokines may act in an autocrine, juxtacrine or paracrine manner and are suggested to be secreted by neighbouring 'producer' cells (Bozkurt, 2009). They are able to influence the biological behaviour of other neighbouring 'target' cells. Overproduction of cytokines results in over-spill into the peripheral circulation. Once freely available within the peripheral circulation cytokines are able to exert endocrine-like effects (Bozkurt, 2009). Additionally, cytokines can be produced by a number of different cell types of numerous tissue origins. In HF, two major classes of cytokine have been identified. These are vasoconstrictor cytokines (e.g. endothelin) and vasodepressor cytokines such as TNF α and IL-6.

Early reports from the Framingham study (2003) showed the inflammatory cytokines IL-6 and TNF α were able to identify asymptomatic older participants at future risk of developing HF. Initial research studies have suggested that the pro-inflammatory cytokines TNF α , IL-1, 6 and 18 are produced by nucleated cells within the myocardium (Anker and von Haehling 2004). The cytokine hypothesis of HF describes the process of over-secretion of inflammatory cytokines in response to a particular trigger (e.g. ischaemia or cardiac injury) Braunwald (2009). Furthermore, this hypothesis suggests that TNF α , IL-1, 6 and 18 are produced by the damaged myocardium, a factor which is augmented by stimulation of the sympathetic nervous system. This process results skeletal and cardiac muscle hypoperfusion due to reductions in cardiac output and allows the production of monocytes. These monocytes, in turn, produce further

inflammatory cytokines which are able to exert their biological effect on the myocardium resulting in cell apoptosis and necrosis (Anker and von Haehling, 2004). IL-6 levels have been shown to increase in humans following acute myocardial infarction (Finkel et al, 1992). This glycoprotein consists of two functional chains, as a binding protein (IL6R) and also as a docking protein able to transmit the intracellular signal (Hirano, 1991).

Further research has suggested that IL-6 is able to induce a hypertrophic response in cardiac myocytes via activation of the common receptor gp130 (Yoshida et al, 1996) and TNF α results in left ventricular dilatation via the activation of matrix metalloproteinases (Seta et al, 1996) and is able to induce immediate and delayed negative inotropic effects to impair myocardial contractility (Bozkurt, 2009). The immediate pathway has been shown to be mediated by activation of the neuromyelinase pathway (Oral et al, 1997) and the delayed pathway mediated by nitric oxide derived blunting of β adrenergic signalling (Balligand et al, 1993). In fact, when expressed at sufficiently high concentration, cytokines are able to mimic many important aspects of the HF phenotype such as pulmonary oedema, LV remodelling, foetal gene expression and cardiomyopathy (Bozkurt, 2009).

In particular, TNF is able to bind to the adult cardiac monocyte via the receptors TNFR1 and TNFR2 (Torre-Amione et al, 1996). This research also showed that adult human cardiac myocytes express both types of TNF receptors with TNFR1 responsible for mediating negative inotropic effects. In addition, when both receptors are proteolytically cleaved from cell membranes they are able to subsist in the circulation as soluble receptors sTNFR1 and sTNFR2 (Bozkurt et al, 1996). These receptors are

able to bind to their ligand as well as inhibit the cardiotoxic effects of TNF and as such are capable of rapidly neutralising the aforementioned cardiotoxic effects (Bozkurt et al, 2009).

Inflammatory cytokines such as TNF α , IL-1 and IL-6 have been shown to increase the expression of oxygen free radicals, inducible nitric oxide synthase and stimulate apoptosis in endothelial cells via oxidative stress, thus facilitating widespread ED (Adamopoulos et al, 2001). Increased activation of pro-inflammatory cytokines has also been associated with increased expression of inducible nitric oxide synthase and decreased expression of insulin like growth factor-1 in the skeletal muscle of patients with HF. As mentioned above, this creates potential for decreased skeletal muscle performance by attenuating mitochondrial efficiency and also promoting skeletal muscle apoptosis (Hambrecht et al, 2000).

Cell adhesion molecules are a diverse system of proteins and adhesive receptors which have the ability to orchestrate biological actions such as embryogenesis, cell growth and differentiation as well as inflammatory processes (Mulvihill et al, 2002). These adhesion molecules have been shown to be important in the mediation of the pathological processes involved in the progression of atherosclerosis and acute coronary syndromes. The most abundant family of adhesion molecules within the human body are the immunoglobulin superfamily (Mulvihill et al, 2002). This family, which accounts for up to 50% of leukocyte membrane glycoproteins, are able to serve as

receptors for growth factors, receptors for the Fc region of immunoglobulins and also as cellular counter-receptors for integrins (Mulvihill et al, 2002).

Increased release of cytokines and oxygen free radicals in HF has been shown to induce the expression of endothelial adhesion molecules (intercellular adhesion molecule-1, vascular cell adhesion molecule-1). These adhesion molecules enter the circulation via activation of endothelial cells (measured as soluble adhesion molecules sICAM -1 and sVCAM-1) and may play an important role in deterioration of endothelial function in this patient group (Noutsias et al, 1999). The exact biological role of soluble cell adhesion molecules is fairly unclear. These soluble forms are released into the circulation from the cell surface via proteolytic cleavage of the extra-cellular protein (Gearing et al, 1993). This process has been suggested as a means to controlling cell adhesion (Mulhivill et al, 2002). Importantly, much research has suggested a strong link between serum levels of soluble adhesion molecules and cardiovascular events. For instance, research has shown that sICAM-1 levels, in patients enrolled into the Physicians Health Study (Ridker et al, 1998), are elevated in 'healthy' males who are at increased risk for future cardio-vascular events. A similar finding has been observed in the female population. In the Womens Health study, sICAM-1 levels were found to correlate well with future risk of cardiovascular events in apparently healthy females (Ridker et al, 2000).

Similarly in patients with established coronary heart disease and in acute coronary syndromes, disruptions to sICAM-1 and sVCAM-1 levels in serum can be observed. In patients with angiographically diagnosed coronary artery disease, increased levels of sICAM-1 and sVCAM-1 were predictive of future cardiovascular events (Blankenberg

et al, 2001). Additionally, Shyu et al, 1996 reported increased levels of sICAM-1 in patients with acute coronary syndrome. sICAM-1 was been established as the most powerful predictor of future cardiovascular events in an asymptomatic population and sVCAM-1 has been shown to be more powerful when predicting future events in patients with established atherosclerotic disease (Mulvihill et al, 2002). Therefore, there is a commonly held belief that serum soluble adhesion molecules represent the vascular inflammatory component of the atherosclerotic process.

In further detail, ICAM-1 is expressed at low levels by numerous cell types to include leukocytes and endothelial cells. As a result, Ridker et al, 1997) suggest that elevated levels of sICAM-1 in healthy individuals who later develop cardiovascular events probably represents a low level inflammatory process analogous to elevated levels of C-reactive protein. Expression of VCAM-1 has been shown to occur only on activated endothelial and vascular smooth muscle cells. This may suggest that sVCAM-1 can serve as a potential marker of overall plaque burden and has already shown its predictive value in patients with diabetes and established atherosclerotic disease (Jager et al, 2000 and Blankenberg et al, 2001), groups who are known to have an activated endothelium.

Table 36 summarises the known pathophysiological effects of inflammatory cytokines.

Table 36. Adverse Pathophysiological Effects of Inflammatory Cytokines in HF

(adapted from (Adamopoulos et al, 2001).

1. Cardiac.

Promotion of LV remodelling.

Attenuated cardiac contractility.

Cardiomyocyte hypertrophy.

Cardiomyocyte apoptosis.

Cardiac Fibrosis.

2. Vascular.

Progression of atherosclerotic process.

Oxidative stress.

Vasoconstriction.

Endothelial cell apoptosis.

Adverse vascular remodelling.

3. Skeletal Muscle.

Reduced skeletal muscle blood flow.

Anabolic / catabolic metabolism imbalance.

Inhibition of protein synthesis.

Muscle cell apoptosis.

BNP is a cardiac neurohormone secreted from the ventricles in response to volume expansion, pressure overload and hence myocyte stress. As previously mentioned in this review (and comparable to the above table), the most important adverse effects of increased levels of BNP include reduced skeletal muscle blood flow and cachexia, promotion of LV remodelling, reduced cardiac contractility, progression of atherosclerosis, increased oxidative stress, nitric oxide impairment, vasoconstriction, endothelial cell apoptosis and adverse vascular remodelling (Anker et al, 2004).

Intense physical exercise has been shown to influence inflammatory reactions within the blood as denoted by delayed increase of acute phase proteins such as C-Reactive Protein and an attenuated acute phase reaction following regular physical exercise. Taken together this suggests an anti-inflammatory role of regular ET (Mattusch et al, 2002). A number of studies have investigated the effects of regular physical ET on markers of inflammation in patients with heart disease. Aerobic ET can be utilised as an immunomodulatory treatment in HF. Early research has shown significantly reduced plasma TNF- α levels together with increased skeletal muscle capillary density, which resulted in improved flow reserve of exercising skeletal muscle in HF patients (Larsen et al, 2001). Similar ET programmes have been shown to positively intervene in the inflammatory and apoptotic cascade in patients with HF. There can be potential significant reductions in the major potent cytokines TNF- α and IL-6 (Larsen et al, 2002, Adamopoulos et al, 2001, Adamopoulos et al, 2002, Le Maitre et al, 2004), but also the soluble receptors of these cytokines which are the products of a monocyte / endothelial cell interaction and biological determinants of circulating inflammatory cytokine

function (Adamopoulos et al, 2002). Additionally, representative apoptosis mediators such as soluble Fas and soluble apoptosis inducer Fas ligand (which are known to be elevated in HF), can also be mediated by aerobic ET. It has been suggested that this attenuation may play an important beneficial role in the peripheral immune response, improve patient symptoms and improve prognosis in patients with HF (Adamopoulos et al, 2002). Soluble apoptosis inducer Fas ligand is a TNF- α related cytokine which is synthesized as a membrane bound protein. This can be changed via proteolytic cleavage into soluble form to promote intercellular Ca^{++} , homeostatic alterations, caspase activation, apoptotic gene transcription and apoptotic cell death following cross-linkage with receptor Fas (Adamopoulos et al, 2003). The significant correlations noted between improved exercise capacity and reduced levels of TNF- α and sFasL plasma levels in response to a programme of aerobic ET suggests that this type of intervention can, at least partially, suppress pro-inflammatory cytokine activation and therefore attenuate the clinical deterioration noted in HF (Adamopoulos et al, 2002).

Other research from the same group in Greece has demonstrated that aerobic ET can reduce certain peripheral inflammatory markers (Adamopoulos et al, 2001) representative of macrophage-endothelial cell adhesive interaction and basal parts of the complex inflammatory cytokine network. This study utilised home-based aerobic training to evaluate the levels of inflammatory cell growth factor granulocyte macrophage colony-stimulating factor (GM-CSF), monocyte chemotactic protein cytokine (MCP-1) and the soluble adhesion molecules sICAM-1 and sVCAM-1. The significant improvement noted in all of these inflammatory markers was closely correlated with improvements in endothelial function and exercise capacity. This suggests that regular aerobic ET can, by virtue of its anti-inflammatory and anti-apoptotic roles, beneficially affect peripheral immune responses perhaps by reversing

the aforementioned deleterious effects of inflammation on endothelial structure and function.

2.2.7: Exercise training and the Metabolic Syndrome.

The metabolic syndrome is a public health problem worldwide with significant evidence, already published, suggesting that levels of fitness and increasing physical activity are essential in regression of this condition. The earlier section of this thesis has already highlighted the possible relationship between components of the metabolic syndrome and heart failure, particularly with respect to insulin resistance. Therefore, the close relationship between the metabolic syndrome and cardiovascular disease and the potential beneficial effects warrants discussion as part of this thesis.

Lakka et al, 2003 showed important correlations between cardiorespiratory fitness, amount of leisure time physical activity with development and progression of the metabolic syndrome in over a thousand males with no evidence of type II diabetes, CVD or cancer. This study unequivocally demonstrated that males engaging in less than one hour physical activity per week were 60% more likely to develop or already have the metabolic syndrome. Additionally, La Monte et al, 2005 demonstrated similar correlations between cardiorespiratory fitness and incidence of the metabolic syndrome in over ten thousand participants (1491 of these female). Furthermore, adjustment for lifestyle factors including dietary choices has been shown not to alter the association between overall cardiorespiratory fitness and the metabolic syndrome (Finley et al, 2006). Not only overall cardiorespiratory fitness has been shown to be an important predictor or marker for development of the metabolic syndrome. Muscular strength and

lean body mass are also related to this condition. In the large Aerobics Centre Longitudinal Study, Jurca et al (2004) demonstrated that each of the five metabolic syndrome components were inversely related to muscular strength as determined by a one repetition maximum bench press and leg press. In addition, the effects of muscular strength on the metabolic syndrome components were independent to cardiorespiratory fitness and also BMI. This data provides significant evidence that both aerobic fitness and muscular strength provide preventative effects on metabolic syndrome risk. Overall mortality data demonstrates strongly the public health burden of the metabolic syndrome. In a large study, Katzmarzyk et al (2004) investigated the effects of cardiorespiratory fitness and mortality on almost twenty thousand males separated into healthy and those with metabolic syndrome components. Accruing 196,466 man years of follow-up, the authors showed relative risks of all cause and CVD mortality of 1.29 and 1.89 for men with the metabolic syndrome when compared to healthy counterparts. Additionally, overall mortality risk was 5.18% in the healthy unfit group compared to 1.95% in the healthy fit group. In the metabolic syndrome, overall mortality risk was 5.15% in the unfit group and 2.69% in the fit group indicating that unfit healthy males show higher all-cause and CVD mortality rates per 10,000 man years of follow up than fit males with the metabolic syndrome. There was also a significant dose-response relationship between cardiorespiratory fitness and mortality in males with the metabolic syndrome reinforcing the aforementioned concept that cardiorespiratory fitness may be as powerful predictor of mortality as the entire metabolic syndrome components combined and should be included as a feature of the syndrome.

There are relatively few studies of sufficient detail that have investigated fully the effects of exercise training on the metabolic syndrome in entirety, rather than individual components of the syndrome. Perhaps the two most detailed and landmark trials are the

Studies of a Targeted Risk Reduction Intervention through Defined Exercise Trial (STRRIDE) (Johnson et al, 2007) and the study of Katzmarzyk et al 2003, which assessed the effects of ET in the metabolic syndrome on the population of the HERITAGE Family Study. The STRRIDE study showed that a low amount of moderate intensity exercise (e.g. around 19km of walking per week) and a high amount of vigorous activity (e.g. around 32km of jogging per week) improved the metabolic syndrome when compared to controls. The high amount, vigorous intensity group only reported decreases in BMI. In the HERITAGE study, the presence of metabolic syndrome and component risk factors were determined prior to and following 20 weeks of supervised ET (3 days per week at 55% VO_{2max} for a duration of around 30 minutes, progressing up to 75% VO_{2max} for 50 minutes duration). This study reported that following ET, 31% of participants diagnosed with the metabolic syndrome using the ATP III criteria were no longer classified following ET.

Insulin resistance has been suggested as the leading connection between physical inactivity and the metabolic syndrome (Roberts et al, 2013). As discussed previously in this review, aerobic training has been shown to increase insulin action in the skeletal muscle of healthy individuals and research evidence has shown that ET can increase insulin sensitivity from 25-50% in numerous age and population groups to include normal healthy males (Dela et al, 1992), healthy females (Evans et al, 2001), obese populations (De Fronzo et al, 1987), young participants (Yfanti et al, 2011), older participants (Di Pietro et al, 2001), first degree relatives of known diabetics (Barwell et al, 2008), type II diabetics (Dela et al, 1995) and sedentary adolescents (van der Heijden et al, 2009).

Studies investigating aerobic training versus control with respect to insulin sensitivity have identified differences in glycogen storage in response to insulin (Ebeling et al, 1993), increases in insulin signalling proteins (glycogen synthase, GLUT4, GLUT4 vesicle associated protein) (Ebeling et al, 1993) and decreased insulin receptor substrate 1 /2, insulin receptor with no difference in protein kinase B (Yu et al, 2001). Research conducted by Dela and colleagues 1993 and 1995 has identified increases in non-oxidative glucose disposal which was associated with increased glycogen synthase mRNA and also increases in GLUT4 protein concentration (26%) which matched their observed increases in maximum insulin stimulated leg glucose uptake (25%), a strong positive correlation noted between the two variables. Other research has also shown significant improvements in GLUT4 uptake in response to exercise training with values ranging from 22% up to 60% (Christ-Roberts et al, 2004, Houmard et al, 1996 and Dela et al, 1994). In addition, Christ-Roberts et al, 2004 have shown that ET resulted in increased insulin sensitivity, increased glycogen synthase fractional activity together with increased phosphoinositide-3 kinase activity. No changes were seen in insulin receptor substrate-1 associated phosphoinositide-3 kinase activity, in agreement with other research investigating short-term exercise training in the metabolic syndrome (Tanner et al, 2002).

In addition to traditional continuous aerobic training, research has attempted to identify the benefits of more modern high intensity interval training in the metabolic syndrome. It has already been established earlier in this review that intermittent high intensity interval training can result in greater improvement in VO_{2max} when compared to traditional training methods (Wisloff et al, 2007). Furthermore, research by Tremblay et al, 1994 has shown that 20 weeks of interval training resulted in greater reduction in percent body fat when compared to continuous aerobic training despite over 50% less

total energy consumption during the interval training regimen. This, for the first time, demonstrated that improvements in fat mass are not related to actual energy expended during exercise but related to exercise intensity. Little et al (2010 and 2011) has shown that interval training can increase insulin receptor phosphorylation to a greater extent than continuous training and also noted significant improvements (119% increase) in GLUT4. It has been hypothesized that glycogen depletion as a result of high intensity intervals may induce the improvements in insulin sensitivity.

2.2.8: Exercise training and quality of life.

Many reports have assessed the effects of different types and intensities of ET on quality of life and health status in patients with HF, either as a primary or secondary outcome measure. Studies have used numerous, validated questionnaires to assess varying aspects of health status including general health, mental health, physical fitness anxiety and depression and disease specific quality of life indices.

Earlier studies performed in the late 1990's have used a variety of questionnaire models to assess quality of life in HF patients. Exercise training resulted in significant improvements in the Feelings of Being Disabled subscale of the Heart Patients Psychological Questionnaire and also highly significant improvements in the Self Assessment on General Wellbeing Questionnaire (Wielenga et al, 1998). The same authors did not discover any significant improvements in the Sickness Impact Profile which was attributed to the fact that this is a generic questionnaire and not specifically tailored to HF patients. Conversely, other research conducted around the same time has shown significant improvements in the Sickness Impact Profile (Tyni-Lenne et al,

1998). It was noted that the documented significant improvements in functional capacity during this study could explain the perceived reduction of disability. Another study (Bellardinelli et al, 1999) utilised the popular Minnesota Living with Heart Failure Questionnaire. This study again, documented significant improvements in Quality of Life which closely followed the course of improved peak oxygen uptake. The same improvements have also been noted by other research groups (Miche et al, 2009). However, other research using the Minnesota Questionnaire has not found significant improvements in health related quality of life (Gottlieb et al, 1999). The same authors also did not find any significant improvement in other measures of quality of life, namely the Functional Assessment Questionnaire and the Centre for Epidemiological Studies Depression Questionnaire. It was suggested that either there was a poor correlation between peak exercise performance and the ability to perform routine daily activities or the tests utilised were not sensitive enough to detect significant improvements.

More recent research conducted since the turn of the century has again produced contradictory results. Of those studies that utilised the Minnesota Living with Heart Failure Questionnaire (Tyni-Lenne et al, 1998, Smart et al, 2007, Janowska et al, 2008) significant improvements in health related quality of life were noted in all patients with systolic dysfunction and even in a group of patients with diastolic dysfunction following supervised exercise training. Simply, these authors have suggested that by physical training all the significant major muscle groups involved in daily activities it is possible to reduce the burden of HF on everyday tasks and hence improve health related quality of life. The documented physiological improvement in physical function in many of these studies serves to support this theory.

Two more recent studies have used the generic SF36 questionnaire to look at quality of life in more detail (Smart et al, 2007, Miche et al, 2009). Both of these studies demonstrated statistically significant improvements in quality of life score. One study showed significant improvements in all domains of the SF36 following supervised ET in a group of patient with LV systolic dysfunction (Smart et al, 2007) whilst the other research study documented changes in the physical sum scale only Miche et al, 2009). Additionally, in patients with diastolic dysfunction, some improvements in SF36 domains were also noted following ET (Smart et al, 2007).

Two studies utilised specific questionnaires assessing anxiety and depression before and following ET in HF patients (Smart et al, 2007, Miche et al, 2009). The results were contradictory. The initial study conducted by an Australian research group (Smart et al, 2007) suggested significant improvements in the Harc-Davis Cardiac Depression Scale. However, more recent research has shown no significant improvements in the Hospital Anxiety and Depression Scale in their sample of HF patients (Miche et al, 2009). This discrepancy could be explained thus; patients who exhibit improved functional capacity and improvement in their ability to perform daily activities can display an improved outlook due to their improved sense of wellbeing. However, as the majority of patients studied with HF are of the older generation, these patients tend to feel more content with their surroundings which makes it more difficult to observe significant improvements following an exercise intervention. This is reinforced by Fahrenberg and co-workers (Fahrenberg et al, 2000) who suggest that as cardiovascular patients age their reported levels of contentment improve to levels of 'extremely high'. Another suggested reason for the lack of significant change in the older generation has been the

'disability paradox'. This implies that patients who have a poor physical condition report a good sense of physical and emotional health (Paul et al, 2007).

The timescale of the improvements in quality of life noted in HF patients as a response to ET has been highlighted by the work in the HF Action Trial (Flynn et al, 2009). This large, randomised controlled trial showed that health status improved more during the initial 3 months of the intervention for the exercise group when compared to the controls. Additionally, this improvement persisted throughout the follow-up period and included important subscales of the Kansas City Cardiomyopathy Questionnaire namely; physical limitations, quality of life and social limitations. This highlights that short interventions of ET in the HF population (<3 months) is sufficient to exert changes in domains of quality of life and also that these changes can be maintained over a long period of follow-up (up to 3 years).

2.3: Testosterone supplementation in HF.

2.3.1: Testosterone replacement therapy and functional capacity.

Currently there are only a small number of randomised trials that have assessed the use of testosterone replacement therapy in patients with HF and low testosterone. Larger, multicentre trials are needed in order to ascertain the complete importance of this therapeutic aid in this population. However, some important factors must be considered prior to initiation of these trials.

High doses of testosterone can be cardiotoxic and may also adversely affect the progression of CAD (Malkin et al, 2009). It should be noted however, that all studies to date have utilised low physiological replacement doses of testosterone in patients with known hypogonadal levels. It has to date not been established if testosterone therapy can be safely employed in patients with HF and normal testosterone levels.

It has been recently discovered that in males with hypogonadism and HF, the level of circulating testosterone is independently related to peak oxygen consumption and peak oxygen pulse (Jankowska et al, 2009). A small number of randomised studies have assessed the effect of physiological testosterone replacement therapy on exercise capacity and endurance performance in the HF population. The following table (37) illustrates the main studies that have addressed this.

Table 37. The Effects of Testosterone Supplementation on Exercise Capacity in HF.

Study.	Sample.	Testosterone.	Duration.	Measures of Exercise Capacity.	Outcomes.
Pugh et al (2004)	Treatment group n=10, placebo n=10. Combined age median 62yrs (range 44-81), EF% 35±8 with reduced exercise tolerance.	Intramuscular injection of testosterone (Sustanon 100 – 1ml in 0.9% saline) given every 2 weeks or placebo (normal saline injection).	12 weeks.	Incremental Shuttle walk Test.	Significant increase in distance walked in testosterone group only – 91m versus 26m. Represents a treatment effect of 65±24m and absolute increase of 32.9% (9.1) versus 10% (4.8).
Malkin et al (2006)	Active group n=37 (63.1±10.7yrs). EF% of 33.8±10.4, NYHA II,III,IV.	Adhesive skin patch of testosterone (Androderm). 5mg patch applied every	12 months.	Incremental Shuttle Walk Test.	Significant increase in distance walked in treatment group when compared to controls (p =

	Placebo group n=39 (64.9±9.3yrs). EF% of 33.1±11.8, NYHA II,III,IV. All stable HF for >6months.	night throughout intervention or placebo patch. Avoid bony prominences and rotate site.		0.006 ANOVA).
			Mean change at 12 months was +25±15m corresponding to a 15±11% improvement.	
Caminiti et al (2009)	Treatment group n=35 (71yrs, 67-76 range). NYHA II and III, EF% 31.5±9.9. Placebo group n=35 (69yrs, 66-74 range). NYHA II, III. EF% 33.8±6.5.	Long acting intramuscular administration of testosterone undecanoate (1000mg Nebido) at baseline, 6 and 12 weeks or saline injection of same dose.	12 weeks 6 min walk test and cardiopulmonary exercise test.	Significant improvement of peak $\dot{V}O_2$, maximal voluntary muscle contraction and 6 min walk distance in the treatment group only (within and between group $p<0.05$).
	Both idiopathic and dilated cardiomyopathy.			

Although there are currently few studies available investigating the effects of testosterone supplementation on exercise tolerance in HF, there is conclusive evidence that testosterone therapy can improve exercise tolerance. This occurs in short and long term follow-up and with testosterone administration of different sources (i.e. injection fortnightly, long acting injection or adhesive skin patch). Not only is there a documented increased walking distance on shuttle tests but also physiologically documented increases in key determinants of endurance performance such as peak $\dot{V}O_2$. Explanation of the improvement in exercise tolerance remains unclear. However, an Italian research group have identified that changes in muscle performance may provide some insight (Caminiti et al, 2009). It has been stated previously in this review that there are documented changes in morphology and function of skeletal muscles in patients with HF – independent to reduced peripheral flow (Hare et al, 1999, Senden et al, 2005, Janowska et al, 2008, Kida et al, 2008, Williams et al, 2007). Maladaptive muscle fibre atrophy, increased abundance of type II glycolytic fibres and the resultant reduced oxidative metabolic capacity have been shown to correlate with poor exercise tolerance and increased VE/VCO₂ slope in HF (abnormal enhanced muscle ergoreflex) (Hambrecht et al, 1995). The work undertaken in Italy documented significantly increased muscle strength and work output following testosterone supplementation. The authors hypothesised that this representative improvement in overall muscle function may decrease the abnormal muscle metaboreflex contribution to the ventilatory response to exercise, reducing the VE/VCO₂ slope and therefore improving exercise tolerance.

To further reinforce this hypothesis, other work (in non-HF animal models) has shown that anabolic supplementation at physiological doses is capable of accelerating fast to

slow twitch muscle fibre type conversion and also increase the number of type I oxidative fibres, co-existent in improving the relative oxidative capacity of skeletal muscle (Ustunel et al, 2003). Although explained in less detail, the work undertaken by Pugh and colleagues (Pugh et al, 2004) has also suggested that microscopic changes in muscle metabolism and structure not detected by their muscle analysis techniques may explain the increase in exercise tolerance noted in their study. However, it should be noted that there was not a significant increase in muscle strength in this small-scale pilot study.

2.3.2: Testosterone replacement therapy and cardiac function.

Data regarding the effect of testosterone supplementation on cardiac structure and function in HF patients is sparse. It has previously been determined that in HF patients with erectile dysfunction there is a significant inverse correlation between resting right and LV EF and testosterone level (Bocchi et al, 2008). This suggests testosterone levels may exert an influence on cardiac structure and function. However, research studies so far in human subjects have showed little or no effect of testosterone supplementation in physiological doses. Two of the studies in the table above measured indices of cardiac structure and function (Malkin et al, 2006, Caminiti et al, 2009). The earlier study conducted by Malkin et al demonstrated a minor but significant increase in LV length – but no serial change in other aspects of cardiac function. The later study by Caminiti et al does not show any significant effect of testosterone supplementation on cardiac structure or function as measured by echocardiography. This is disappointing as animal models appear to demonstrate a more pronounced cardiac response to testosterone supplementation. For example, an early study has shown that following gonadectomy, both male and female rats demonstrated loss of weight and reduced cardiac contractile

function. Furthermore, supplementation with testosterone was shown to improve cardiac contractile function in male rats and also (but to a lesser extent) in female rats with concomitant testosterone and estrogen supplementation (Scheuer et al, 1997). Physiologically, gonadectomy was found to be associated by decreases in cardiac myosin ATPase activity and a shift in heavy chain myosin isoenzymes from pattern V₁ to V₃. The administration of testosterone in males and estrogen and testosterone in females seemed to reverse this pathological process. Additionally, gonadectomised male rats were found to have an increased duration of cardiac relaxation in perfused hearts (with moderate afterload) when compared to their intact counterparts. Administration of testosterone was successful in resolving this process to normal, probably via alterations in biochemical control of diastole such as by the sarcoplasmic reticulum (Scheuer et al, 1997). This early study does suggest that sex hormones can have an important modulating role on cardiac systolic and diastolic function. More recently, research in rats has suggested that administration of testosterone can attenuate unfavourable cardiac remodelling and improve contractile performance probably mediated by decreasing levels of TNF- α (Zhang et al, 2007). As discussed previously, TNF- α is one of the primary cytokines involved in the catabolic processes in HF. It is known to result in LV dysfunction, remodelling, pulmonary oedema, muscle wasting and cardiomyopathy in human subjects when overexpressed (Adamopoulos et al, 2001). It was identified that one important factor in the cardiac remodelling process in HF is extra-cellular matrix fibrosis or over-degradation (Zhang et al, 2007). Matrix metalloproteinases have been shown to be the most prominent system in this process with TNF- α being the main stimulatory mechanism (Miyamoto et al, 2005). Testosterone therapy has been shown to significantly decrease the myocardial expression of matrix metalloproteinases and also reduce levels of myocardial hydroxyproline (elevated in HF and reflect the information of deposited collagen proteins) hence, attenuating excessive proliferation of collagen

proteins to prevent ventricular remodelling. These beneficial effects of testosterone were attributed to TNF- α suppression or down regulation of matrix metalloproteinase directly (Zhang et al, 2007). It should be noted however, anabolic androgen abuse such as that traditionally noted in high level competitive athletes has been shown to drastically alter myocardial function. Increases in cardiac mass due to severe LV hypertrophy can result in severe diastolic dysfunction – independently of the effects of strength training (Urhausen et al, 2004). As a result of this, testosterone dose level compared to body weight may be an important factor in any resultant central cardiac response. It is important however, to maintain safe levels of supplementation to avoid adverse physiological effects and there is little or no published research on the safety of intermediate levels of testosterone supplementation on cardiac function in any patient group.

2.3.3: Testosterone replacement therapy and arterial function.

To date, there is only one study available that has assessed the effects of testosterone replacement therapy on arterial function in males with HF. This small-scale study utilised a balloon flotation catheter inserted into the pulmonary circulation in order to measure systemic vascular resistance, PCWP and pulmonary artery pressure in 12 males who received 60mg of testosterone via the buccal route (Pugh et al, 2003). Haemodynamic parameters were assessed at baseline and at increments up to 360 minutes post treatment. Acute administration of testosterone was noted to significantly increase cardiac output when compared to placebo ($10.3 \pm 4.6\%$ increase from baseline) accompanied by a reduction in systemic vascular resistance ($-17.4 \pm 9\%$ from baseline, $p < 0.0001$) in the treatment group only. Importantly, the relative decrease in systemic vascular resistance suggests that the increased cardiac output is due to arteriolar

vasodilatation and therefore reduction of cardiac afterload. Moreover, it is previously well documented that testosterone acts as an acute vasodilator in coronary, mesenteric, subcutaneous and pulmonary vascular beds (Jones et al, 2004). Detailed laboratory investigation has suggested that testosterone acts as an L-type calcium channel blocker at the smooth muscle level via a non-genomic pathway (English et al, 2002). Indeed, early studies investigating calcium channel blockers has shown that they too can acutely decrease systemic vascular resistance and increase cardiac output in a similar fashion to acute testosterone supplementation (Vetrovec et al, 1993).

Testosterone supplementation has also been shown to be particularly beneficial to arterial function in patients with CAD and hypogonadism. This becomes relevant to this review because ischaemic heart disease is well established as the most common cause of HF (Malkin et al, 2010). Furthermore, as coronary arterial disease may co-exist with HF the increased myocardial oxygen demand resulting from poor cardiac output and significant coronary artery stenosis may impact more prominently on quality of life.

A study conducted by researchers in the UK assessed the effects of high dose and physiological dose intravenous testosterone on brachial artery reactivity in 22 males with angiographically significant ($\geq 70\%$) coronary artery stenosis (Ong et al, 2000). Patients were randomly assigned to receive testosterone or placebo and crossed over the following week to receive the physiological dose versus placebo. Brachial artery studies were then performed 1 hour following drug infusion. This was the first randomised study that demonstrated a significantly increased flow mediated response to testosterone supplementation in males with CAD. High dose intravenous testosterone more than doubled the flow mediated dilatation (FMD) response in males with CAD (placebo

FMD% 3.16 ± 1.9 versus high dose testosterone FMD% of 6.86 ± 3.72). Physiological dose of testosterone also demonstrated a trend to increased flow mediated response but this was non-significant (p 0.59). It was suggested that testosterone administration enhances flow mediated endothelium-dependent mechanisms rather than receptor mediated responses. The authors conclude that testosterone may act in a similar role to anti-oxidants by prolonging the vascular effect of nitric oxide.

In support of this, other researchers have assessed brachial artery response to long-term oral supplementation of testosterone in patients with significant CAD (Kang et al, 2002). This study recruited 35 males who were randomised to placebo and treatment groups. The treatment group received oral testosterone undecanoate 160mg daily for 4 weeks followed by 80mg testosterone undecanoate for 8 weeks in addition to all concurrent medication. Brachial artery reactivity was assessed in response to flow mediated dilatation and also nitro-glycerin mediated dilatation. There was a significant increase in brachial artery flow and nitroglycerin mediated dilatation in the testosterone group only when compared to baseline (FMD% 3.2 ± 3.0 increase to 5.6 ± 4.0 and GTN 4.7 ± 4.1 increase to 9.7 ± 5.0 both $p < 0.05$). The authors concluded in a similar fashion to previous reports that testosterone supplementation improves endothelial dependent and in this report endothelial independent dilatation probably via testosterone prolonging the vascular effects of nitric oxide. The only study to date assessing the effects of testosterone replacement therapy in hypogonadal males with CAD was performed by researchers in Sheffield, UK (Malkin et al, 2004). This randomised, single blind, crossover study recruited 12 males with angiographically significant coronary artery stenosis ($\geq 70\%$) and biochemical evidence of hypogonadism. Participants were randomly assigned to receive testosterone (fortnightly intra-muscular injection of 1ml – 100mg) or placebo for 1 month. Exercise treadmill tests were performed at baseline and

immediately following cessation of intervention. Following 1 month participants were crossed over to the other arm of the trial. The results of this study indicated that TS significantly increased time taken to reach ischemic threshold during exercise tolerance testing when compared to placebo (81 second (48) average increase in time taken to reach -1mm ST depression in the treatment group – 7.2 seconds (32) in controls). It was suggested that TS in this form has a rapid mode of action as these changes were seen following only 1 month of treatment. In addition, the vasodilatory properties of testosterone were attributed to the improved anti-ischemic action.

2.3.4: Testosterone replacement therapy and muscle function.

Currently there are few studies that have investigated the effects of testosterone supplementation on muscular strength, muscular endurance and detailed physiological aspects of muscle function in patients with HF. Of course, it is widely acknowledged that androgen supplementation can increase lean mass, voluntary muscular strength and reduce fat mass in healthy populations (Isidori et al, 2005). Additionally, research has investigated the effects of testosterone supplementation in patients with morbid conditions such as HIV, metabolic syndrome, advanced malignancy, lung disease and chronic kidney failure. These trials have again, showed consistent improvements in body composition and physical strength (Johns et al, 2005 and Johansen et al, 1999).

Perhaps the most detailed assessment of testosterone administration and muscular strength in HF patients has been recently undertaken by Caminiti and colleagues in Italy (Caminiti et al, 2009). This double-blind randomised controlled trial, as previously mentioned, investigated the effects of testosterone supplementation in 70 males with HF

on various indices of physical, biochemical and social function. Assessment of leg muscle strength was performed using isokinetic and isometric dynamometry of the quadriceps muscle group of the dominant leg. The results of the study indicated that supplementation can significantly increase maximum voluntary contraction (from 116.7 ± 26.3 to 135.6 ± 21.2 Newton m) when compared to placebo who did not demonstrate any significant change. Furthermore, it was also noted that the treatment group could produce a significantly higher peak torque from baseline (74.0 ± 17.4 to 83.4 ± 16.3 Newton m) when compared to placebo. This study also calculated quadriceps muscle fatigue index using an in-built computer package within the dynamometer. There was a significantly improved fatigue index from baseline in the treatment group only ($-42.4 \pm 13\%$, $p = 0.03$) when compared to the placebo group. This research group postulated that the improvements in leg muscle strength and work output may be due to similar mechanisms as to the afore-mentioned improvements in endurance capacity.

The predominant shift toward type II glycolytic muscle fibres in HF; the 'muscle-hypothesis' results in an abnormal metabo-reflex during exercise which results in detrimental neuro-hormonal activation, abnormal haemodynamic, autonomic and ventilatory response to exercise (Janowska et al, 2008 and Hambrecht et al, 1995). As stated previously, possible reductions in the abnormal muscle ergoreflex contribution to ventilatory response to exercise would improve VE/VCO_2 slope therefore improving ventilatory efficiency. Importantly, other research groups can support this notion. Anabolic administration at physiological dosage has been shown to accelerate fast to slow twitch muscle fibre conversion and also significantly increase the abundance of type I oxidative fibres – both of which are pertinent in the pathophysiology of HF (Hambrecht et al, 1997 and Ustunel et al, 2003).

The earlier research work of Malkin and colleagues highlighted in the table above (2006) also assessed a crude marker of muscular strength. Dominant handgrip strength was assessed both pre and post treatment with testosterone or placebo. It was noted that the testosterone group only showed significant improvements in handgrip strength (treatment effect of $+1.6 \pm 1.1$, $p = 0.04$ testosterone versus placebo at 12 months). No physiological mechanism other than improved anabolic function was discussed as the precursor of this change. However, it is plausible that the physiological mechanism as proposed by Caminiti et al (2009) could contribute to this improvement.

2.3.5: Testosterone replacement therapy and inflammatory markers.

As previously highlighted in detail during this review, HF is characterised by significant sub-clinical inflammation. Inflammatory cytokines such as tumour necrosis factor alpha, IL-1 and 6 and soluble adhesion molecules are elevated and known to contribute to insulin resistance, cachexia and poor prognosis in HF. Anticytokine biological drugs such as etanercept and infliximab have been developed and also shown to reduce the levels of circulating cytokines but have also been documented to increase all-cause mortality (Anker et al, 2002).

In animal models testosterone supplementation has been shown to favourably impact on cytokine levels and also improve LV remodelling in HF (Yan-Zhou Zhang et al, 2007). This research, conducted in China, investigated the effects of testosterone supplementation in 85 Sprague-Dawley rats with induced MI and HF. Following this surgery, rats were randomly assigned to receive 12 weeks supplementation with

testosterone or placebo. Echocardiography, blood sampling and tissue analysis was performed in order to determine cardiac function, biochemical levels of inflammatory cytokines and cardiac tissue levels of inflammation. The results of the study showed that rats supplemented with testosterone (5mg/kg every 2 weeks for 12 weeks) documented higher LV EF ($41.9 \pm 10.5\%$ versus $33.2 \pm 7.5\%$) when compared to placebo. Additionally, the treatment group also demonstrated higher levels of serum IL-10 (an anti-inflammatory cytokine which induces the production of specific cytokine inhibitors such as IL-1 receptor antagonist, thus serving as a counter mechanism to TNF- α production) and lower levels of TNF- α (both $p < 0.05$) when compared to the placebo. Cardiac tissue levels of TNF- α , mRNA and matrix metalloproteinase mRNA was also significantly lower in the testosterone group when compared to placebo. Physiologically, the imbalance of inflammation and anti-inflammation may result in decreased myocardial contractility, ventricular dilatation, myocardial remodelling, increased cardiac myocyte apoptosis and cardiac cachexia (Ceconi et al, 2001). The authors hypothesise that this imbalance in a HF model is likely due to the presence of LV dysfunction. Testosterone supplementation serves to partially preserve LV function and this is associated with a concomitant modestly prevented inflammation / anti-inflammation imbalance. Therefore, the testosterone associated increase in IL-10 and decrease in TNF- α has been proposed to be due to suppression of macrophage production of TNF- α with an increase in CD⁴⁺T lymphocyte production of IL-10 (D'Agostino et al, 1999 and Liva and Voskhul, 2001).

Human studies investigating the effects of testosterone supplementation in HF on inflammation have however, produced disappointing results. The only available data investigating this was conducted by Pugh et al (2004). This research investigated the effects of testosterone administered via three different routes (i.e. 2, 30mg buccal tablets

over a period of 6 hours, fortnightly 100mg intra-muscular injection for 3 months or 5mg transdermal daily for 3 months) on serum levels of TNF- α in 94 patients with hypogonadism and HF. The results of this investigation showed that although whole blood incubation with testosterone reduced significantly lipo-polysaccharide induction of TNF production, there was no significant change in serum levels of TNF- α pre and post TS in any of the three routes of administration. In conclusion, the authors suggest that the clinical improvements noted in trials of testosterone therapy and HF are likely due to other factors rather than reduced serum levels of inflammation.

Research in other clinical populations has however, suggested a beneficial role for the effect of testosterone supplementation on inflammatory cytokines in males with low testosterone. A study conducted by Malkin and colleagues (2004) investigated the effects of testosterone supplementation on inflammatory cytokines in hypogonadal males. Many of the participants included in the study were diagnosed with primary testicular failure. Participants received standard fortnightly intra-muscular injections of testosterone. Serum samples were collected for the assessment of TNF- α , IL-1, 6 and 10 pre and post the intervention period of 1 month. The results showed significant declines in TNF α and IL-1 when compared to baseline (TNF α -3.1 ± 8.3 pg/ml and IL-1 -1.32 ± 5.2 pg/ml, $P = 0.012$). Although no exact mechanism for this process was discussed, the authors declared that this trial is one of very few investigating the in-vivo effects of testosterone supplementation on cytokine levels and stated a benefit to patients presenting with testosterone deficiency and established vascular disease.

Perhaps leading on from, but contradicting, this initial research, Kapoor et al, 2007 investigated the effects of testosterone supplementation on adipocytokines in

hypogonadal males with type II diabetes mellitus. This double-blind placebo controlled randomised controlled trial recruited patients to testosterone supplementation versus placebo using a similar supplementation methodology to above. The authors did not observe significant decreases in TNF- α or IL-1 levels as a result of testosterone replacement therapy during this trial. However, at baseline there were significant correlations between low levels of testosterone and an elevated inflammatory profile.

2.3.6: Testosterone replacement therapy and quality of life.

Data regarding the effects of testosterone supplementation on quality of life is sparse. This research has already discussed the impact of low testosterone status on quality of life in both healthy and chronically diseased population (see section 1). One of the seminal studies investigating the effects of testosterone replacement therapy on ischaemic threshold and quality of life in HF males with low testosterone (Malkin et al, 2004) reported significant improvements from baseline in Beck Depression Inventory (BDI), general health screen questionnaire and Androgen deficiency in ageing male (ADAM) scores (all Wilcoxon matched pairs - $p < 0.05$) in the treatment arm of the trial only. This study supplemented a small number of males ($n = 10$) intra-muscularly with Sustanon 100 fortnightly for a total of 12 weeks. When analysed for significant differences between the treatment and placebo groups, both BDI and ADAM demonstrated significant between group improvements. No discussion of the mechanisms behind this improvement was postulated by the authors. However, their findings of improved time to ischaemic threshold and reduced levels of circulating inflammatory markers such as TNF- α can potentially facilitate improvement to quality of life by reducing patient symptoms and increasing their exercise ability. Naturally, due to the increased serum concentration of testosterone towards normal range in the

treatment group, it is plausible that improved ADAM scores are related to increased serum TT concentration.

A further study conducted by Malkin and co-workers (2006) investigated the effects of a skin patch preparation of testosterone supplementation in males with HF and low testosterone when compared with placebo. This 12 month trial demonstrated no significant improvement in BDI or Minnesota living with heart failure questionnaire (MLHFQ) score following the intervention period. This was despite significant improvement in functional capacity as assessed by the incremental shuttle walk test (ISWT) and heart failure symptoms as assessed by NYHA score. The authors suggest that route of testosterone administration may play a role in the non-significant quality of life scores obtained in the study. The skin patch preparation was poorly tolerated and also resulted in lower mean total testosterone levels in the treatment group when compared to previous studies. However, this contradicts the observed improvement in functional capacity. Simply, this small-scale study may have been under-powered to detect significant changes in questionnaire data.

2.4: Aims.

To date the efficacy of exercise therapy for evoking improvements in key health outcomes in males with HF and low testosterone status has not been studied. Neither is it known whether the effects of exercise training can be augmented by testosterone supplementation in this patient group. The literature provides compelling evidence of benefit for both exercise training and testosterone replacement therapy on numerous physiological and psychological outcomes in HF patients.

Therefore the aims of this study are:

- To assess the feasibility of a combined 12 week exercise rehabilitation and adjunctive testosterone therapy intervention in males with HF and low testosterone status in terms of recruitment, compliance to the intervention, reproducibility of outcome measures and attrition.
- To obtain preliminary data on the impact of the exercise and testosterone intervention on key health outcomes assessing both physiological and psychological function in this patient group.

Chapter 3. Materials and Methods.

3.1: Trial Design.

This feasibility study was designed as a two-arm, double-blind, placebo-controlled trial in order to provide sufficient detail to inform a larger scale phase III clinical trial. After completion of the baseline assessments, treatment groups were allocated according to a pre-determined randomisation schedule. The clinical trials pharmacist prepared a blocked randomisation schedule using a table of random numbers. Medication numbers were allocated in ascending sequential order to ET plus testosterone supplementation (TS) or ET plus placebo. Outcome measures were assessed at baseline and 12 weeks.

Patients randomised to testosterone therapy were administered Sustanon injection (Sustanon 100, Organon Laboratories, Cambridge, UK), and by form of comparator, those patients randomised to placebo therapy received saline (18mg in 2ml, 0.9% w/v NaCl, PL01502/0006R, Hameln Pharmaceuticals Ltd, Gloucester, UK). Testosterone and placebo therapy were administered by intramuscular injection every two weeks for 12 weeks.

3.2: Randomisation.

Patients were randomly allocated to one of the two study groups. Treatment groups were allocated according to a pre-determined randomisation schedule. The pharmacy department at Sheffield Teaching Hospitals NHS Foundation Trust prepared a blocked randomisation schedule using a table of random numbers. Medication numbers were allocated in ascending sequential order. The blocked randomisation protocol was designed to ensure equal treatment numbers at fixed points throughout the course of the

study. During this study, permuted blocks were used and randomisation into these blocks was achieved using a table of random numbers. A limitation of using blocked randomisation, particularly small blocks, is the possibility of researchers predicting the next treatment. However, this is certainly less possible in a double-blind trial design. In order to reduce selection bias, the investigators were unaware of block size. All randomisation and allocation was performed by a clinical trials pharmacist who was not involved in the research study and had significant experience in administration medication for clinical trials. All randomisation procedures and treatment allocations were double-checked by other members of the clinical trials pharmacy team also not involved in the study.

3.3: Blinding.

Members of the research team responsible for the assessment of the primary and secondary outcomes and those who supervised the exercise training sessions were blinded to group allocation. These team members were also unaware as to treatment allocation for each participant until study closure. This ensured that both groups were identically encouraged and motivated to undertake exercise training, in order to minimise bias.

The duration and sequence of all study visits and procedures to be undertaken, was as per the flow chart below (figure 13).

The end of the trial was classed as the date when the last patient attended and performed their last post-intervention assessment. Following this visit, and once the data was analysed and inputted, the database was locked.

3.4: Patient selection and recruitment.

Following ethical approval from the local research ethics committee (appendix 8), 41 ambulant male patients, over 18 years, with stable HF (longer than 6 months) and initially with a blood testosterone level of less than 12 nmol/L and symptoms of hypogonadism were recruited from Cardiology Clinics at the Royal Hallamshire Hospital, Sheffield between September 2007 and October 2009. Due to recruitment problems and time constraints for study completion, the total testosterone cut off value was increased to 15 nmol/L based on guidance from the Clinical Chemistry Department at Sheffield Teaching Hospitals NHS Foundation Trust and discussion with the lead investigator who had significant experience in working with patients with low testosterone and heart failure. It should be noted however, that during this recruitment phase (cut of ≤ 15 nmol/L) only one participant (total testosterone 12.9 nmol/L) was over the previous cut-off value and all other participants recruited (n=5 displayed testosterone concentration below the initial cut-off value of ≤ 12 nmol/L). Patients were included and excluded from study participation on the following grounds.

3.5: Inclusion Criteria.

- Symptoms of hypogonadism – defined by positive androgen deprivation score.
- Serum TT concentration up to 12 nmol/L initially, extended to 15 nmol/L latterly.

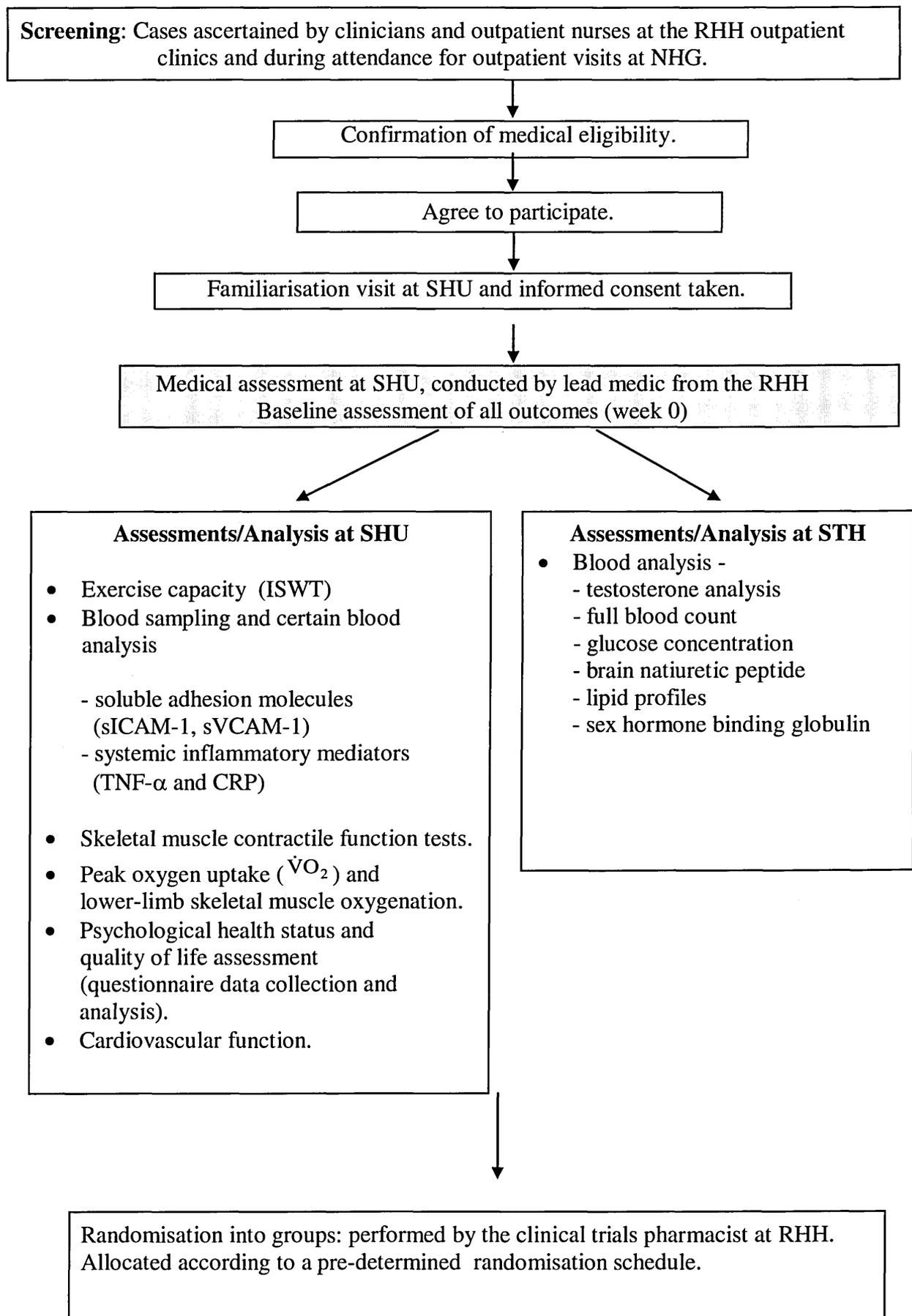
- Clinically stable HF prior to recruitment established on optimal medical therapy and without significant symptom change (using NYHA Score) for a period of 6 months.
- Evidence of severe or worse impairment of LV systolic function (defined by echocardiography as EF less than 35%).
- Reduced exercise tolerance (limited by fatigue or breathlessness of cardiac origin, i.e. NYHA Class II or worse).
- Over 18 years of age.

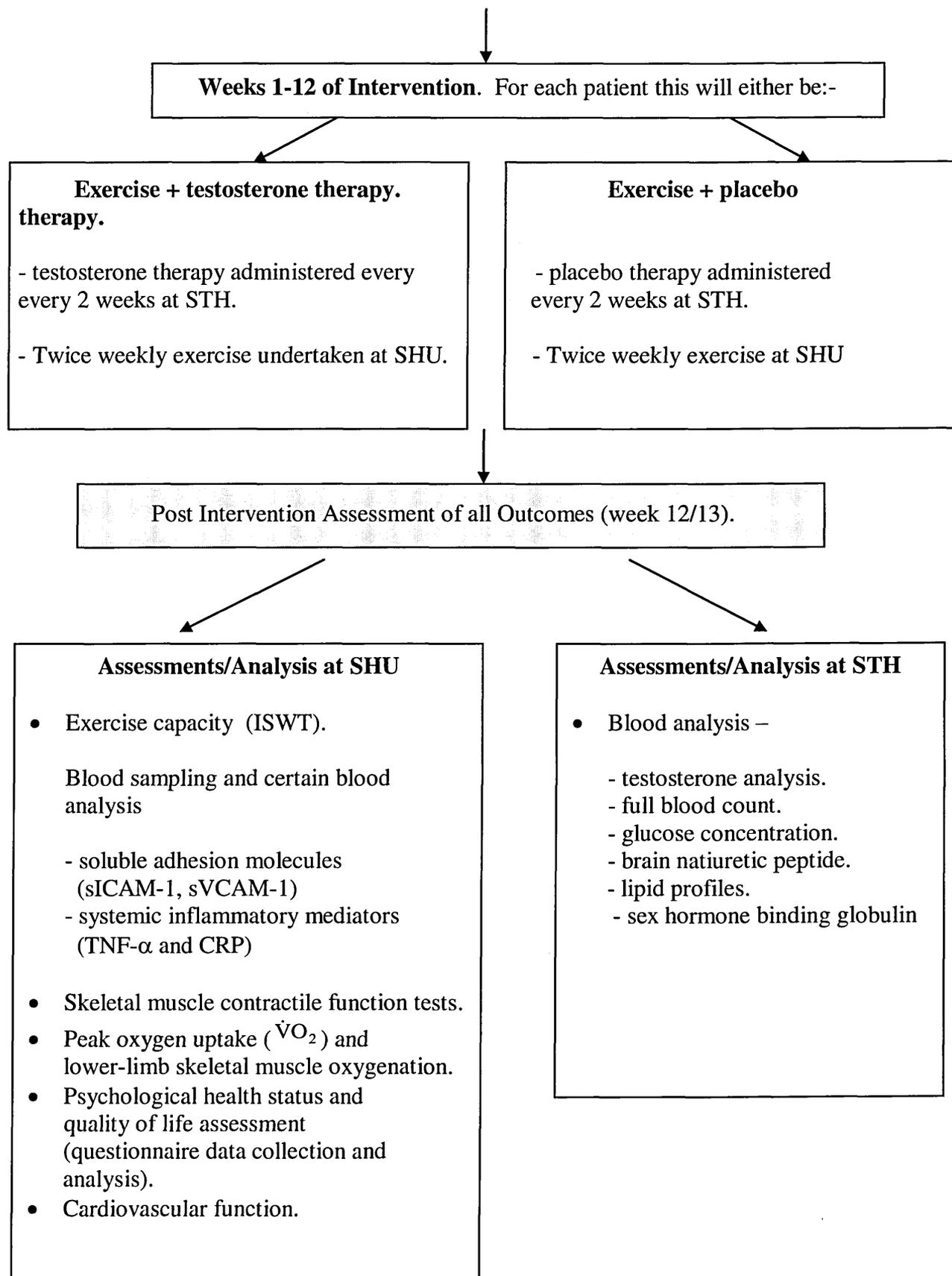
3.6: Exclusion Criteria.

- Unstable angina (defined by classical angina symptoms at rest).
- Recent (within the last 6 weeks) acute MI.
- Episodes of decompensated HF / clinical change in course of condition within the last 6 months (i.e. increasing symptoms or requirement of new treatment).
- Moderate or worse (defined by echocardiography) valvular heart disease.
- Uncontrolled hypertension (resting systolic BP ≥ 180 mmHg, diastolic ≥ 100 mmHg) – as per defined cut off values for performing exercise tolerance testing (British Cardiac Society / Society for Cardiological Science and Technology Joint Guidelines for Physiologist Led exercise testing, 2008).
- Renal insufficiency (serum creatinine > 200umol/l).
- Urologic disorders.
- Any orthopaedic or neurologic illness limiting the ability to exercise.
- Patients with prostate specific antigen (PSA) level above the age adjusted normal range.

- Patients already prescribed androgen replacement therapy.
- Patients with a peanut/soya allergy – due to the nature of the testosterone replacement therapy formulation.

Figure 13. Flow chart demonstrating participant journey and important time-points during the study.





Eligible patients were identified based on strict adherence to the aforementioned inclusion and exclusion criteria both by trained research nurses and the investigator. Eligibility was confirmed through screening patient notes of all males who attended the Cardiology outpatient clinics at the Royal Hallamshire Hospital. In addition, weekly visits by the research nurse to the Northern General Hospital (part of Sheffield Teaching Hospital NHS Foundation Trust) were undertaken in order to follow the same process for the Cardiology clinics over at that site.

3.7: Consent procedures.

In addition to the initial letter sent out to patients with the patient information sheet (appendix 9), a tear-off slip was also attached, which patients returned to the primary investigators, to register interest in study participation. Patients had approximately 10-14 days to decide whether or not to take part in the research. If any patients did not initially return the tear-off slip attached to the patient information sheet, they were sent a follow-up letter reminding them of the study. Patients who did not respond to this letter were not contacted further, since it was assumed that these patients did not wish to participate in the study.

Informed consent was obtained from those patients who were interested in study participation. This was obtained at the initial consultation session, following prior familiarisation with all equipment and the study protocol, and once all patient's questions and concerns have been answered and addressed. Informed consent was obtained in the presence of a witness, by one of the primary investigators who had considerable work experience with cardiac patients (> 5 years).

3.8: Withdrawal of patients.

Any patient, who at any stage of the study did not wish to continue, could withdraw, without their future medical care being affected. This information was made clear to each patient in the patient information sheet and throughout the duration of the study.

Throughout the study each patient was carefully monitored, in terms of health and fitness status, and also in terms of blood testosterone levels. Patients were withdrawn from the study immediately, if they developed undue discomfort or distress, resulting from the exercise sessions, if it was deemed to be negative to their health. Equally, patients were withdrawn if they developed chest pain, indicated by an abnormal ECG to be cardiac related, or if testosterone or prostate specific antigen (PSA) status was altered. Data obtained up to the time of withdrawal from each patient, was utilised in the overall analysis of the study data, on the basis of intention to treat analysis. However, once withdrawn, then no further data was collected from these patients. For safety reasons these patients were followed up for at least 30 days after the last administration of testosterone / placebo, as applicable.

3.9: Sample size.

A formal power calculation was not undertaken, as the primary aim of this study was to assess the feasibility of a 12-week program of exercise rehabilitation (with and without TS) in male HF patients with low testosterone status, in terms of recruitment, willingness to be randomized, compliance and attrition (Arain et al, 2010). A second aim of the study was to obtain preliminary data and report on outcome standard deviations to inform sample size estimates for a larger scale randomized controlled trial.

3.10: Medical examination.

Before entering the trial, each patient received a thorough medical examination performed by a clinician (Consultant Cardiologist or Research Registrar), during which details of surgical history, co-morbid conditions, risk factors and current medication details were confirmed. Hormone profiles and drug use were also recorded at the beginning of the trial. Blood pressure was taken (manual sphygmomanometer) and a resting 12-lead ECG performed with the patient in the supine position.

3.11: Testosterone replacement therapy.

It was imperative that prior to patients undertaking the study they had not previously received testosterone replacement therapy. During this study patients were administered testosterone therapy or placebo in an identical fashion and also blinded to which treatment they were receiving. For ease of administration (since the preparation was readily available, cheaper than adhesive skin patches and provided a direct comparison with placebo), a commercially available testosterone replacement therapy (*Sustanon 100, Organon Laboratories, Cambridge, UK*) or placebo therapy (*0.9% saline, Hameln Pharmaceuticals Ltd Gloucester, UK*) was given by a trained researcher, not in any way involved in the study, via intramuscular injection of 1ml every two weeks for 12 weeks, as previously found successful in a similar patient population (Malkin et al, 2006).

Study medication was stored and dispensed by the trial sites' pharmacy department – clinical trial section in accordance with good clinical and manufacturing practice. Drug

accountability was monitored as per the attached case report forms. Professor Kevin Channer (Consultant Cardiologist, Royal Hallamshire Hospital (RHH)) was responsible for overseeing the administration of the respective drug, and completion of the appropriate paperwork. For patients recruited onto the trial, study medications were stored at the Sheffield Hallam University Centre for Sport and Exercise Science site in a locked cupboard within a temperature controlled room with restricted access.

3.12: Description of the packaging and labelling on the investigational medicinal product (IMP).

For the placebo arm of the study, sodium chloride (0.9% 2ml glass ampoules) was purchased. Due to the fact that saline ampoules cannot be provided to match Sustanon directly, the pharmacy department at the RHH, under the supervision of Helen Bowler (Pharmacy Clinical Trials Manager) packed the ampoules in identical boxes labelled so as to blind the contents. As per the protocol and to maintain blinding, only 1ml of saline was drawn, and injected into the patient. The remaining amount in each ampoule was discarded.

The identity of the ampoules was only revealed once the box was opened, thus the doctor / research assistant administering the injections was un-blinded to the randomisation schedule. Therefore, this individual was not responsible for the recruitment, selection, assessment or training of patients during the study period. In order to maintain patient blinding, all injections were drawn and prepared (with packages discarded) prior to patients entering the clinical room for administration.

Figure 14. An example of the labelling on the IMP.

Keep out of reach of children

Store in a dry place, below 26 degrees C, protect from light

1 Ampoule for injection containing either:

SODIUM CHLORIDE B.P 0.9% w/v

or **SUSTANON 100 INJECTION**

Inject 1ml by intramuscular injection

in accordance with study protocol STH14394

Discard any remainder immediately

For single use only. Use only if clear and particle free

FOR CLINICAL TRIAL USE ONLY

RHH Dept. of Cardiology

Investigator Prof. K. Channer, Sponsor STH NHS Foundation Trust

Patient name. _____ Date: _____.

Medication No _____ Visit No _____

Pharmacy Clinical Trials, RHH, Sheffield. S10 2JF.

3.13: Potential interactions with testosterone.

For study safety, all known potential, negative interactions with testosterone replacement therapy (Sustanon 100) were studied and patients were checked for these concomitant interactions prior to enrolment onto the study.

3.13.1: Anticoagulants.

Increased anticoagulant effects and bleeding have been observed in patients who were taking a number of oral anticoagulants when prescribed concomitantly with anabolic steroids or other related therapies (e.g. danazol, testosterone). Bleeding may occur if the

anticoagulant dosage is not reduced appropriately. The principle author's previous research studies using *Sustanon* in patients taking warfarin have not found any significant interaction or side effects. However, as a result, during this study patients who were taking warfarin had an additional INR measurement at 5-7 days after starting drug therapy (active and placebo) so that warfarin dose adjustments could be made if deemed necessary.

3.13.2: Insulin.

Testosterone can enhance the blood sugar reducing effects of insulin. The concurrent use was monitored, and a reduction in the dosage of the anti-diabetic agent was undertaken if needed.

3.13.3: Ciclosporin.

Theoretically, concomitant administration of ciclosporin and anabolic steroids may result in increased ciclosporin blood levels and toxicity. Patients receiving concomitant therapy were advised to attend for additional blood tests to ensure safe levels of ciclosporin.

3.13.4: Paclitaxel.

Testosterone, a known inhibitor of the isoenzyme CYP2C8, inhibits the metabolism of paclitaxel to its primary metabolite 6-alpha-hydroxypaclitaxel, *in vitro*. The pharmacokinetics of paclitaxel *may* also be altered *in vivo* in the presence of a CYP2C8 inhibitor. Therefore, patients receiving paclitaxel therapy were not recruited for study participation.

3.13.5: Peanut / Soya allergy.

Sustanon 100 contains Arachis oil (peanut oil) and should not be taken / applied by patients known to be allergic to peanut. As there is a possible relationship between allergy to peanut and allergy to Soya, patients with Soya allergy were not recruited to the study.

3.14: Monitoring of patient compliance.

A record was maintained to monitor patient attendance and compliance to the study. Patients unable to attend a pre-arranged appointment time were offered an alternative appointment. Those patients absent without prior notification were contacted on the day, and a further suitable appointment will be offered.

3.15: Adverse Events and Serious Adverse Events (SAE's).

All members of the research team were at least Intermediate Life Support trained. The Chief Investigator (Prof Kevin S. Channer) also had extensive experience in running and administering testosterone therapy trials (Pugh et al, 2004, Malkin et al, 2006, Pugh et al, 2004, Malkin et al, 2004).

All adverse events were recorded and reported, as per the case report forms. At each visit, each patient was questioned regarding AE's, either to the exercise regimen or to the drug therapy. The author and chief investigator were responsible for completing the appropriate paperwork at Sheffield Hallam University and at the Royal Hallamshire

Hospital, respectively. After an AE each patient was closely monitored for at least 30 days, or longer if deemed necessary.

During the research study, the following adverse events were documented:

1. Patient unable to exercise due to gout – related to cardiac medication.
2. Patient unable to continue exercise due to increase in severity of peripheral vascular disease.
3. Recurrence of paroxysmal atrial fibrillation previously documented in patient notes. Patient was unwilling to continue.
4. Complications with regard to ICD and anti-arrhythmic medication. This patient also contracted swine flu during the intervention and therefore was excluded due to a long interruption in exercise sessions.
5. One patient was severely injured in a road traffic accident following the initial study consultation but prior to baseline assessments.

None of these adverse events were deemed study related by the lead physician and hence no further action was taken to alter study protocol. All adverse events were classified using a combination of Good Clinical Practise guidance and the clinical knowledge of the research team.

The following were considered SAE's for this study:-

1. Hospital admission for a cardiovascular event.

2. Diagnosis of prostate cancer after 30 days of entry on to the trial.
3. Death during the course of the study.

All SAE's were to be reported to the research office at Sheffield Teaching Hospitals NHS Foundation Trust within 24 hours of discovery by the research team. Dedicated report forms and reference documents justifying a SAE decision were completed. However, no SAE were noted for this research study.

3.16: Safety assessments.

The University's formal health and safety procedures were strictly followed. A detailed safety assessment of the study was performed as per risk assessment documentation. This was designed to identify the likelihood and consequences of an untoward event occurring.

3.17: Data collection handling and record keeping.

Data was collected and retained in accordance with the Data protection Act 1998. All data was collected as per the case report forms (appendix 9), validated and approved by STH, at the time points indicated on the flow chart which clearly outlines which data was collected at SHU (the Centre for Sport and Exercise Science) and which at STH (Sheffield Teaching Hospitals - Royal Hallamshire Hospital).

At the Centre for Sport and Exercise Science, the author was responsible for the data collection, recording and quality of data at this site. The chief investigator had the

overall responsibility for data collected and recorded at the Royal Hallamshire Hospital site.

At both sites, any paper files of personal data were kept in a locked filing cabinet. Stored patient details and data were anonymous, following randomisation, since patients were assigned an identification number, to ensure confidentiality. Any computer generated data was coded and stored on 'locked' memory sticks. All data is to be stored for a maximum of 2 years, in order to ensure that all data analysis has been performed.

3.18: Exercise training.

Cycle ergometry constituted the main component of the aerobic ET regimen. Exercise intensity was set at 50% of the maximum short-term exercise performance, determined by a steep ramp test every 4 weeks (3 minutes of unloaded pedalling, then work rate increments of 12.5 W every 10 seconds at a crank rate of 60 rpm) (Senden et al, 2005). An interval training regimen was used, incorporating 30 seconds of exercise with one minute interpolated rest periods for a total exercise time of 15 minutes (i.e. 10 repeats). Each aerobic exercise session was followed by two to three sets of low to moderate intensity resistance exercises for five main muscle groups: quadriceps, hamstrings, pectorals, latissimus-dorsi and deltoids. Each supervised exercise session lasted for approximately 45-50 minutes in total and patients attended 2 sessions per week. The venue for supervised exercise was the Exercise Science Laboratory at Sheffield Hallam University.

3.19: Outcome measures.

3.19.1: Incremental shuttle walk test.

Unless otherwise stated, outcome measures were assessed at baseline and 12 weeks in all patients. The primary outcome measure was exercise capacity, assessed using the incremental shuttle walk test (ISWT), as described in previously published work (Malkin et al, 2006). Two tests were performed prior to starting treatment, one at the screening visit following informed consent and the second before randomisation during collection of all baseline outcomes in order to allow for a learning effect (as per ISWT instruction documents – available from Singh, S. Dept Respiratory Medicine, Glenfield Hospital – initial reference study - Singh et al, 1992). The best ISWT result was recorded and this was consistently the second ISWT in all patients.

The ISWT is a symptom-limited exercise test with a progressive increase in workload designed to allow subjects to achieve maximum effort tolerance. The test was originally developed to assess patients with chronic obstructive pulmonary disease (COPD) and it is in this cohort, that the original correlations with peak $\dot{V}O_{2max}$ were observed (Singh et al, 1992). More recently, Lewis et al (2001) described successful reproducibility of this test in patients with severe HF awaiting cardiac transplantation. The authors suggested a close correlation between ISWT distance and peak $\dot{V}O_2$ which is represented by the regression equation $\dot{V}O_2 = 0.022 \times \text{distance} + 6.4$ (where $\dot{V}O_2 = \text{peak } \dot{V}O_2$ and distance = distance walked during ISWT) with $r = 0.73$, $p = 0.0001$. In addition, the ISWT was found to be highly reproducible with mean walking distances of $400 \pm 146\text{m}$ (walk 1) versus $401.3 \pm 129\text{m}$ (walk 2), $r = 0.90$, $p = 0.0001$.

Prior to commencement of the ISWT, patients rested for 10 minutes. During this period, resting BP and HR were recorded. In addition, a thorough and detailed explanation of the Borg rating scale of Perceived Exertion was undertaken. Participants were instructed on how to safely perform the test and to also detail any symptoms observed both during and following testing. As this test is maximal, trained intermediate life support staff and resuscitation equipment were on hand to ensure the safety of each patient. During the ISWT, subjects walked back and forth along a horizontal 10 m course, marked out by two cones aiming to complete the shuttle before a pre-recorded signal from a cassette player, which shortened incrementally after each shuttle. The end-point (distance walked in metres) was reached when the subject failed to complete the shuttle before the signal. Immediately on completion of the ISWT, BP, HR and RPE were recorded. Each participant was then allowed to recover in the sitting position for a length of time that allowed all symptoms, BP and HR measurements to return to pre-testing values.

3.19.2: Blood analysis.

Serum blood samples for assessment of IL-6, TNF α , sVCAM-1 and sICAM-1 were collected at the time of the initial data collection visit at Sheffield Hallam University Centre for Sport and Exercise Science. All serum blood samples were drawn from the antecubital vein in the supine position. Serum aliquots were prepared for storage at -80°C in Centre for Sport and Exercise Science at Sheffield Hallam University. Samples were also collected at the end of the study during the post intervention assessment. A batch analysis of pre and post intervention samples was performed, on study completion, by an accredited laboratory scientist as part of the research team in the Biomedical Research Centre at Sheffield Hallam University.

Total testosterone, glucose, insulin, SHBG, albumin, full blood count, lipid profiles (triglyceride, high and low density lipoprotein (HDL) and (LDL)), C-reactive protein (CRP) and NT pro-BNP were collected during the initial screening visit to the Royal Hallamshire Hospital prior to official study enrolment. Two samples were collected for total testosterone and SHBG assessment on separate hospital visits with the initial total testosterone value obtained used for acceptance onto and analysis during the study. Patients were advised to attend the blood sampling clinic as early as possible during the morning hours. However, no official times of attendance were logged during the study.

Testosterone and SHBG analyses were performed on the day of patient attendance at the Royal Hallamshire Hospital Clinical Chemistry Department for the initial screening visit in order to ascertain total testosterone values against the inclusion criteria for the study. During post intervention assessments at Sheffield Hallam University, blood samples were taken for the later assessment of testosterone, SHBG and albumin at the Royal Hallamshire Hospital. These samples were prepared and stored at -80°C at the Centre for Sport and Exercise Science for batch analysis at study completion. All sample analysis at the Royal Hallamshire Hospital Clinical Chemistry department was undertaken by accredited laboratory technicians. Laboratory techniques for the analysis of serum testosterone, SHBG, albumin and NT pro-BNP were documented in detail in the previous section of this thesis, together with the equations used in order to calculate free and bio-available testosterone.

All Quantikine HS ELISA human immuno-assays were manufactured by R and D Systems Inc, Minneapolis, USA. IL-6, TNF α , sICAM-1 and sVCAM-1 were analysed employing a quantitative sandwich enzyme immuno-assay technique with mono-clonal

specific antibodies pre-coated onto microplates by an accredited laboratory scientist as part of the research team in the Biomedical Research Centre at Sheffield Hallam University.

For the assessment of IL-6, a serum separator tube was used and samples were allowed to clot for 30 minutes before centrifugation at 1000 x g for a total of 15 minutes. Aliquots were prepared and stored at -80°C for batch analysis to be performed at the end of the study. Reagent preparation involved reconstitution of the lyophilized substrate with 6.0mL of substrate diluent, 10 minutes prior to use. Amplifier solution was reconstituted with the lyophilized amplifier with 6.0mL of amplifier diluents, again at least 10 minutes prior to usage. IL-6 standard was reconstituted with 5.0mL of the calibrator diluent (RD6-11) resulting in a stock solution of 10 pg/mL. The standard was allowed to sit for 15 minutes with gentle agitation prior to making the stock solutions. 100µL of assay diluent RD1-75 was added to each well, followed by 100µL of standard or sample. This was covered with an adhesive strip and incubated for 2 hours at room temperature on a horizontal orbital microplate shaker set at 500 rpm. Wells were washed using wash buffer and 200µL of IL-6 conjugate added. This was covered with an adhesive strip and further incubated for 2 hours at room temperature on the shaker. Following further washing, 50µL of substrate solution was added to each well and incubated for 60 minutes at room temperature. Then, 50µL of amplifier solution was added to each well and incubated for a further 30 minutes at room temperature following being covered with an adhesive strip. 50µL of stop solution was then added to the wells. A microplate reader as used to determine the optical density of each well at 490nm. Wavelengths were corrected to 650nm prior to calculation of results. Average duplicate readings were obtained for each standard, control and sample and the average zero standard optical density subtracted from this value. Standard curves were

produced for each set of samples analysed. Intra-assay precision was calculated on three samples of known concentration and tested 20 times. Co-efficient of variation were 6.9%, 7.8% and 7.4% respectively. Inter-assay precision was determined on 3 samples of known concentration. Co-efficient of variation were 9.6%, 7.2% and 6.5%. Average recovery was 94% (range 87-99%).

For sICAM analysis, serum aliquots were prepared in the same way as above. Reagent preparation involved dilution of 23mL of wash buffer concentrate into de-ionized water to prepare 500mL of wash buffer. The substrate solution was prepared by mixing the colour reagents A and B and protecting from direct light. ICAM-1 standard was reconstituted with 1.0mL of de-ionized water to produce a stock solution of 250 ng/mL, mixed and allowed to sit for 15 minutes with gentle agitation. 800 μ L of calibrator diluents was pipetted into 50ng/mL tubes and 500 μ L of calibrator diluents into the remaining tubes. The stock solution was used to produce a dilution series. 100 μ L of sICAM-1 conjugate was added to each well and also 100 μ L of standard, control and sample to each well before covering and incubation at room temperature for 90 minutes on a horizontal microplate shaker. Following the washing procedure, 200 μ L of substrate solution was added to each well and incubated on the bench for 30 minutes at room temperature. Then 50 μ L of stop solution was added to each well prior to determining the optical density of each well within a 30 minute period. Wavelengths were corrected to 570nm and standard curves created from average duplicate readings of standards, control and sample. Intra-assay co-efficient of variation for 3 samples were 3.7%, 5.2% and 5.0% respectively. Inter-assay co-efficient were 6.7%, 5.4% and 4.4%. Mean recovery was 104% (range 90-109%).

sVCAM-1 serum aliquots were prepared in a similar manner to previous analyses. Substrate solution, wash buffer, calibrator diluent and sVCAM standard were all prepared in a similar manner to that for sICAM-1. 500 μ L of calibrator diluents (RD5P) was pipetted into tubes and the stock solution used to make a dilution series. 100 μ L of sVCAM-1 conjugate was added to each well followed by 100 μ L of standard, control or sample. This was covered with an adhesive strip and allowed to incubate for 90 minutes at room temperature. Following washing with the pre-prepared buffer solution, 100 μ L of substrate solution was added to each well immediately, protected from light and incubated for a further 20 minutes. 50 μ L of stop solution was added to each well and colour change observed. The optical density of each well was determined within 30 minutes and wavelength corrected to 570nm. As previously, average duplicate readings were made for each standard, sample and control. Intra-assay co-efficient of 2.3%, 3.5% and 3.6% were noted. Inter-assay co-efficients were 7.8%, 7.7% and 5.5%. Mean recovery for serum samples was 96% (range 89-99%).

TNF α serum aliquots were prepared in the same way as those above. Wash buffer was prepared by diluting 100 mL of wash buffer concentrate into de-ionized water to prepare 1000 mL. Lyophilized substrate was reconstituted with 6.0mL of substrate diluent 10 minutes prior to use. The lyophilized amplifier solution was reconstituted with 6.0mL of amplifier diluent and allowed to stand for 10 minutes before use. TNF α standard was reconstituted with the volume of calibrator diluent (RD6-13) to produce a stock solution of 32 pg/ml. This was allowed to sit for at least 15 minutes before gently agitating prior to making the dilutions. Poly propylene tubes were used to make a dilution series. 50 μ L of assay diluents (RD1F) was added to each well and mixed well prior to use. 200 μ L of standard, sample or control were also added per well. This was then incubated at room temperature for 3 hours. Following washing, 200 μ L of TNF α

HS conjugate was added to each well (allowed to incubate for 2 hours at room temperature) and after further washing, 50 μ L of substrate solution added to each well. This was further incubated for 1 hour at room temperature before adding 50 μ L of amplifier solution to each well and incubating for a further 30 minutes. Finally, 50 μ L of stop solution was added to each well and determination of optical density of each well performed within 30 minutes using a microplate reader set to 490nm. Wavelength correction was set to 690nm. Average duplicate readings were calculated for each standard, control or sample and standard curves developed. Intra-assay co-efficient were 8.7%, 4.3% and 3.1% and inter-assay co-efficient were 10.4%, 7.2% and 7.4% respectively. Mean recovery was 93% for serum samples (range 85-98%).

CRP serum aliquots were prepared in the same way as those previously mentioned. 20mL of wash buffer was diluted into de-ionized water to make 500mL of wash buffer. 20 mL of calibrator diluent (RD5P) was diluted into 80mL of de-ionized water. Colour reagents A and B were mixed together with equal volume within 15 minutes of use. These were protected from light. The CRP standard was prepared by pipetting 200 μ L of calibrator diluent into 6 polypropylene tubes. 200 μ L of the standard was added to the 25ng/ml tube in order to create the dilution series. 100 μ L of assay diluent was added to each well and 50 μ L of standard, control or sample also added per well. This was covered with the provided adhesive strips and allowed to incubate at room temperature for 2 hours. Following a detailed washing procedure, 200 μ L of CRP conjugate was added to each well and further incubated for 2 hours at room temperature. Following a final washing procedure, 200 μ L of substrate solution was added to each well, protected from light and incubated on the bench at room temperature for 30 minutes. Finally, 50 μ L of stop solution was added and colour changes observed. Optical density was assessed within 30 minutes with a microplate reader set at 450nm. Average duplicate

readings were calculated for each standard, sample or control and subtracted from the average zero standard optical density. Standard curves were produced. Intra-assay co-efficient were 4.4%, 3.8% and 8.3%. Inter-assay co-efficient were 6.0%, 7.0% and 6.6%. The mean recovery was 100% (range 92-11%).

3.19.3: Skeletal muscle contractile function.

Dynamic strength and endurance of the dominant leg quadriceps and hamstrings was measured using an isokinetic dynamometer (Biodex System 3 dynamometer, Biodex Medical, NY, USA). The test protocol comprised a 5-minute warm-up on a cycle ergometer, 3 repetitions at an angular velocity of $1.0 \text{ rad}\cdot\text{s}^{-1}$, 5 repetitions at $3.0 \text{ rad}\cdot\text{s}^{-1}$ and 20 repetitions at $5.3 \text{ rad}\cdot\text{s}^{-1}$, followed by a cool-down on a cycle ergometer. A 2-minute rest period was applied between each set of repetitions and the main outcome measures were peak torque and total amount of muscular work (endurance) performed at each angular velocity. This protocol was recently shown to be safe for measuring skeletal muscle strength and endurance in HF patients (Senden et al, 2005). This protocol took approximately 15 -20 minutes to complete. Isometric strength of the forearm muscles was also assessed using a hand-grip dynamometer.

3.19.4: Peak oxygen uptake ($\dot{V}O_2$) and lower-limb skeletal muscle oxygenation.

Peak $\dot{V}O_2$ was assessed using incremental cycle ergometry (Excalibur, Lode, Groningen, The Netherlands). The test began at a power output of 25 W and increased by increments of 25 W every 3 minutes to maximal exercise tolerance. The test was terminated when the patient became restricted by clinical symptoms or the crank rate of 60 rpm could not be maintained. Blood pressure, HR, perceived exertion (Borg scale

range 6-20) and 12 lead ECG recordings (Marquette CaSE 15, Wisconsin, U.S.A.) were made after 10 minutes of supine rest and within the last 30 seconds of each work-rate increment. Pulmonary gas exchange variables were also measured breath-by-breath (CaSE EX670 PulmoLab, Kent, U.K.) for assessment of peak $\dot{V}O_2$ and anaerobic threshold (A_T). The system oxygen and carbon dioxide analysers were calibrated before each test using gases of known concentrations. Inspired and expired volumes were also calibrated using a 3-L syringe. A_T is used to detect the beginning of excess CO_2 production resulting from the buffering of H^+ arising from lactic acid production and represents the shift towards anaerobic metabolism seen towards the end of intense exercise. Anaerobic threshold was derived using the ventilatory slope (VE/VCO_2) method. Peak oxygen uptake was derived as the highest 20 second average. Patients were monitored closely during recovery until all physiological variables and any reported symptoms returned to baseline values. As the test was maximal, medical supervision was on hand together with emergency equipment and appropriately trained staff.

Ventilatory gas exchange data obtained during exercise testing aims to provide a more precise measurement of total body workload together with a significant increase in the information gained regarding cardio-pulmonary function when compared to exercise time alone (Sullivan et al, 1989). Such techniques are remarkably valuable in patients with HF whereby the main symptom is exercise intolerance together with exertional dyspnoea, partly related to ventilation-perfusion mismatch at the level of the lung (Creager et al, 1982). In order to accurately determine oxygen uptake, it is imperative to accurately measure a number of variables. In a basic model, determination needs only 3 components, the fraction of oxygen in the expired air, the fraction of carbon dioxide in

the expired air and the volume of inspired or expired air. In this model, oxygen uptake can be simply equated to the product of ventilation (VE) in a known interval together with the fraction of oxygen in the same ventilation which has been utilised by working muscle (Myers, 1997). However, more accurate and correct measures of oxygen uptake require the input of more detailed components. The previous statement assumes that expired air is dry and also that inspired / expired volumes are equal. Initially, the fraction of inspired oxygen (FiO₂) is affected by ambient temperature, barometric pressure and humidity. These variables must therefore be assessed and FiO₂ adjusted as a consequence. Furthermore, as oxygen uptake is defined by the difference between the fraction of oxygen in inspired and expired ventilation, both inspired and expired ventilation values must be known. Inspired volume can be measured from the expired volume and the relative concentrations of carbon dioxide and oxygen, utilising the difference of between the fraction of inert gases (which do not affect physiological gas exchange in humans) in the expired air and the fraction of inert gases in the atmosphere (Myers, 1997). As a result of these detailed inter-variable relationships, oxygen uptake becomes:

$$\dot{V}O_2 \text{ l/min STPD} = (1 - F_{eO_2} - F_{eCO_2}) / 0.7904 \times (F_{iO_2} \times F_{eCO_2}) \times VE \text{ L/minSTPD}$$

Where: STPD is standard temperature and pressure, dry, F_{eO₂} is the fraction of expired oxygen, F_{eCO₂} is the fraction of expired CO₂, F_{iO₂} is the fraction of inspired oxygen, VE is expired volume and 0.7904 is the concentration of nitrogen in the inspired air.

Peak $\dot{V}O_2$ and A_T have become widely utilised measurements in the assessment of patients with HF (Kleber et al, 2004). Low levels of peak $\dot{V}O_2$ are well known to identify patients at increased mortality risk (Francis et al, 2000). Relating to this,

VE/VCO₂ slope enhancement in HF, which may be due to increased physiological / anatomical dead space, ventilation-perfusion mismatch, abnormal pulmonary vascular haemodynamics and disordered ventilatory reflex control have been suggested to yield similar prognostic power to peak $\dot{V}O_2$. It is for this reason, that A_T has been suggested as a routine component of the analysis of cardiopulmonary exercise testing in the HF population (Francis et al, 2000).

Local muscle haemoglobin saturation was monitored at rest, during exercise and recovery using near infrared spectroscopy (NIRS; NIRO 300, Hamamatsu, Welwyn Garden, UK). This is a non-invasive technique, in which a light emitting/detecting probe is placed on the skin over the vastus lateralis muscle. NIRS is an optical technique that can be used for the non-invasive measurement of tissue oxygenation and haemodynamics. It is based on the relative tissue transparency for light in the near-infrared region and on the oxygen-dependent absorption changes of haemoglobin and myoglobin. Haemoglobin, myoglobin, and to a lesser extent cytochrome oxidase, are the most important chromophores absorbing near-infrared light in skeletal muscle tissue. Haemoglobin is the main component of erythrocytes and the oxygen carrier of the blood. Myoglobin is present within the skeletal muscle cells and facilitates intracellular oxygen transport. Due to identical spectral characteristics, it is not possible with NIRS to distinguish between haemoglobin and myoglobin. However, the contribution of myoglobin to the overall signal has previously been described as minimal (~10% Mancini et al, 1994). Therefore, the degree of light absorption is thought to be primarily dependent on the amount of oxygen attached to haemoglobin in the small arterioles, venules and capillaries (Boushel et al, 2001). Cytochrome oxidase is the terminal enzyme of the mitochondrial respiratory chain reaction transferring the

electrons to molecular oxygen. Because the amount of cytochrome oxidase in muscle is relatively low as compared with haemoglobin and myoglobin, changes in cytochrome oxidase are lost within the noise of the signal. Therefore, the contribution of cytochrome oxidase in the studies of this thesis has been omitted.

The continuous-wave NIRS employed in this study generates light at four wavelengths (775, 810, 850 and 910 nm), which is transported to the tissue by means of an optical fibre bundle called an optode. A second optode transports the light to the detector and is placed parallel to the light source, directly on the skin over the muscle or other tissue of interest. The light penetrates the skin, sub-cutaneous fat layer and muscle, and is either scattered or absorbed within the tissue. The light scattering originating from the source occurs in any direction, but the light detected by the second optode is thought to describe a banana shape (Boushel et al, 2001). Back-scattered light is returned as an optical signal and analysed using a procedure called spatially resolved spectroscopy to produce a ratio of oxygenated haemoglobin / myoglobin to total haemoglobin / myoglobin, expressed as percentage saturation (StO_2). Muscle StO_2 changes during exercise reflect the balance between oxygen delivery and tissue oxygen utilisation; a mismatch being reflected by a drop in StO_2 relative to baseline. Resting StO_2 , StO_2 peak drop during exercise, and StO_2 at the end of exercise were assessed during the exercise protocol.

3.19.5: Echocardiography.

Morphological and functional changes in cardiac structure and function were assessed using a commercially available ultrasound device (Terason T3000, Terason Ultrasound, Teratech, Burlington, U.S.A.) and dedicated 3.5 MHz phased array ultrasonic transducer. Each patient was assessed using guidelines produced by the American Society of Echocardiography (Gottinger, 2004). A detailed description of the use of echocardiography and the methods employed in this study are given in section 1, but due to functional restrictions of the ultrasonic device, not all the previous parameters could be assessed in this study population. The following echocardiographic parameters were assessed during this study (please see section 1 for added detail about each method).

1. LA volumes were measured at the following points:

c. Left atrial pre-contraction volume (V_{preA}) corresponding to the onset of the P wave on the surface ECG.

d. Minimal left atrial volume (V_{min}), measured on mitral valve closure at end-diastole.

e. Maximal left atrial volume (V_{max}), measured immediately prior to mitral valve opening at end-systole.

These values were used to calculate further parameters relating to left atrial active volumetric assessment

a. Left atrial active emptying fraction (LA-AEF%) $(V_{preA} - V_{min}) / V_{preA} \times 100$

b. Left atrial expansion index (LA-EI) $(V_{max} - V_{min}) / V_{min} \times 100$

c. Left atrial passive emptying fraction (LA-PEF%) $(V_{max} - V_{preA}) / V_{max} \times 100$

For patients who presented in atrial fibrillation at time of echocardiogram, pre contraction volumes were not calculated due to the absence of 'p' wave on the surface ECG. As such, for these patients, LA active and passive emptying fractions were not able to be calculated.

8. Left ventricular end-diastolic diameter was measured from both parasternal long axis images and also apical 4 chamber images at end diastole. LV long axis end-diastolic length was assessed from apical 4 chamber images – measured from the level of the mitral valve annulus to the LV apical endocardial border definition. The ratio of LV short to long axis ratio was calculated (from the 4 chamber measurements) in order to obtain the Sphericity Index.

9. Left Ventricular EF was calculated using Simpsons Biplane Method of Discs in patients with clear-enough echocardiographic windows.

10. Tissue Doppler imaging was used to assess indices of both LV longitudinal systolic function and LV diastolic function together with their detailed relationship

11. Careful border tracing of right atrial area was undertaken using the tricuspid valve annulus as a base for the measurement.

12. Right ventricular diastolic and systolic area was traced with reference to ECG timing of the cardiac cycle. Using the derived diastolic and systolic areas, right ventricular fractional area change % was calculated with the following formula:

$$(\text{RV end diastolic area} - \text{RV end systolic area} / \text{RV end-diastolic area})$$

13. M-mode echocardiography was used to measure Tricuspid Annular Systolic Plane Excursion (TAPSE) from apical 4 chamber images.

3.19.6: Assessment of arterial reactivity using Flow Mediated Dilatation.

Endothelial dependent flow mediated dilatation of the femoral artery was assessed using a commercially available ultrasound device (Terason T3000, Terason Ultrasound, Teratech, Burlington, U.S.A.) and a compatible 7-12 MHz linear array vascular probe. The primary goal of this assessment was to create a shear stress stimulus that produces a nitric oxide dependent response in order that flow mediated dilatation can be used as a direct marker of nitric oxide availability and hence endothelial function. A common technique originally applied by Celermajer and co workers (Gottinger, 2004) was used in order to achieve this. Baseline femoral arterial diameter was measured in the longitudinal plane by a qualified ultra-sonographer together with pulsed wave Doppler measurements of arterial flow. A BP cuff was placed immediately above the knee joint and inflated rapidly to supra-systolic pressure for a period of 3 minutes. The occlusion of blood flow to the lower limb serves to produce an ischemia induced reactive hyperaemia. Following the 3 minute period of continued arterial imaging, the BP cuff was rapidly deflated resulting in increased shear stress to return blood flow to the ischemic region. A further 2 minutes of arterial scanning was performed in order to allow adequate time for sufficient arterial response. Images were recorded and edited for analysis of arterial diameter to be undertaken by a dedicated computer software package (Brachial Tools, MIA, IA, U.S.A) previously validated in a number of research studies (Stout, 2009). The computer software was calibrated to accurately reflect arterial diameter over time and changes in vessel diameter were logged graphically in frames per second ratio. Maximum percentage change in arterial diameter following cuff deflation was noted when compared to an averaged baseline trace using a graphically represented diagram of arterial diameter change over time.

3.19.7: Psychological health status and quality of life.

Both HF and androgen deficiency are characterised by low mood and depression, which is improved by testosterone replacement therapy in testosterone deficient subjects. Thus, at baseline and 12 weeks, patients were asked to complete the MLHF questionnaire, the SF-36 v2 Health Survey, the BDI and the ADAM screening Questionnaire. A detailed explanation of the MLHF, SF-36 version 2, BDI and ADAM questionnaires can be found in section 1 of this thesis.

3.19.7.1: Community Healthy Activities Model Program for Seniors Questionnaire (CHAMPS) Physical Activity Questionnaire.

Physical activity behaviour in everyday life, outside of the exercise intervention, was measured at baseline and 12 weeks by the Community Healthy Activities Model Program for Seniors Questionnaire (CHAMPS) Physical Activity Questionnaire for older adults. The CHAMPS questionnaire has shown a 6 month stability range from 0.58 to 0.67 using intra-class correlation coefficients and significant sensitivity to change ($p < 0.001$) Stewart et al (2001). See appendix 11.

In order to adequately address the issue of measuring physical activity levels in older adults as outcomes of interventions, several issues need to be considered. The most important of these concepts have been described as; assessing appropriate types and amounts of the activities assessed, designing questions and methods to allow accurate reporting, minimising socially desirable responding and enhancing sensitivity to change (Stewart et al, 2001). As a consequence, Stewart and co-workers (2001) have designed a self report questionnaire which can provide several physical activity measures in the

older generation. The questionnaire houses 41 items assessing a number of physical activities both light and more vigorous that is applicable to an older generation. The initial response to each item is a simple 'yes' or 'no' to participation in that activity in the last 4 weeks. Following this, the participant must decide how many times a week the activity was usually performed. A space is provided to write this answer. Finally, for each activity performed, the participant must circle how many total hours (in a typical week) the activity was undertaken.

From the questionnaire responses, Stewart and colleagues (2001) have provided coded algorithms so that researchers are able to derive important physical activity measures from the data obtained; namely frequency per week and estimated caloric expenditure per week in physical activity. In order to achieve this, Stewart et al (2001) made adjustments to the originally devised metabolic equivalent values (MET values), of Ainsworth et al (1993) to values that correspond with older adults performing the same activities. As part of the coding process, researchers must create a new duration variable for each activity. The new duration variable is then multiplied with the corresponding 'adjusted' MET value to create a new weighted duration variable. The caloric expenditure per week variable can then be calculated for each activity by multiplying the weighted duration variable by 3.5 (ml/kg/min which is equal to 1 MET) and then 60 to give METS / hour and by weight in kilograms (kg) divided by 200. This formula must be applied across all activities to give total caloric expenditure per week. During this study, total METS/hour over all activities were calculated as a comparator between treatment groups. This is summarised by the formula below:

$$\text{METS/hour} = \text{New coded duration variable} \times \text{adjusted 'older' person MET value for activity} \times 3.5 \times 60$$

This formula was used for each activity performed and added together for the total MET/hour score, where $3.5 \text{ (ml/kg/min)} = 1 \text{ metabolic equivalent}$. Therefore $3.5 \times \text{MET}$ value will give actual ml/kg/min of oxygen uptake for each exercise undertaken (from ACSM Guidelines for Exercise Testing and Prescription, 7th Edition, 2006).

3.19.8: Qualitative assessment of testosterone therapy and exercise intervention

(Focus Groups).

Focus groups were utilized as the method of qualitative inquiry because they facilitate group interaction and, in turn, may generate more abundant data than single or group interviews (Kitzinger, 1995). Participants are therefore, encouraged to talk to each other, ask questions, exchange anecdotes and comment regarding their own experiences and points of view. Focus groups are particularly useful for exploring the knowledge and experiences of study participants and can be used to examine not only patients thoughts, but expanding on this to generate data on how and why each patient thinks the way they do (Kitzinger, 1994).

In addition to this, the focus group method can facilitate exploration and clarification of participant opinion using means that would be less accessible in one to one interviews (Kitzinger, 1995). Focus group discussion appears particularly appropriate when the facilitator provides a series of open ended questions and aims to encourage their participants to explore issues of importance to them, using their own vocabulary, perhaps even generating their own questions and pursuing topics that are important to them (Basch, 1987).

Importantly, focus groups allow researchers to utilize different forms of communication that people use in day to day interaction, including jokes, anecdotes, teasing, and arguing. Therefore, participant responses become more varied and may even be less formal than responses obtained during an interview situation (Kitzinger, 1995).

Four groups of 6 participants ($n = 24$) were randomly chosen from those patients who were consented for and completed the intervention study. Patients were randomly selected using a fishbowl technique based on each participants allocated study number. As part of this technique, a researcher not related to study data collection randomly picked out 24 patient numbers from a container. These participants were then invited to partake in the focus group sessions. There was no other specific inclusion criterion. Patients were of a mixed age and consisted of those who received testosterone therapy and those who received placebo. At the time of the discussions, patients and researchers were unaware of the group allocation. However, these participants consisted of 12 patients from testosterone and exercise group and 12 patients from testosterone and placebo group. Some of the patients involved already attended local health clubs regularly. Each focus group meeting was undertaken in a private meeting room at the Centre for Sport and Exercise Science, Sheffield Hallam University, lasted for approximately 30-45 minutes and were all performed one week following completion of the final participants post intervention assessment. A topic schedule was created in order to achieve the aforementioned aims of the sessions (table 38). However, groups were encouraged to elaborate and raise their own issues. Each focus group was chaired by the chief investigator who has substantial experience (>15 years) of working with patients with cardiovascular disease but lesser experience when conducting focus group sessions. Resultingly, advice was sought from experienced qualitative researchers regarding the effective management and scheduling of each session. Each focus group

session was digitally recorded and subsequently transcribed (appendix 13 shows example of transcription). The facilitator together with two other (non-study related researchers) coded all the transcripts with the computer package (QSR NVivo Version 8. QSR International 1999-2009). The resulting data were analysed using the principle of constant comparison in order to identify the most important themes and categories of response.

Table 38. Topic schedule for the focus group session.

<i>Process evaluation of the lifestyle intervention.</i>	<i>Outcomes from the lifestyle intervention.</i>
Reasons for participation.	Perceived benefits of the lifestyle intervention.
Frequency, intensity and duration of exercise sessions.	Perceived problems with the lifestyle intervention.
Contact with exercise specialists.	Disease specific quality of life.
Format of the exercise training sessions.	Relationship with health professionals.
Reasons for continuation with programme.	Future continuation with similar training regimen.
Influences of structured programme on current methods of exercise training.	Advice to other patients with heart failure.
Opinions of other medical professionals involved with patient outside of study.	
Barriers to exercising.	
Support received.	
Role of different medical professionals involved in the programme.	
Comparison to current commercial gym environment and staffing.	
Negative factors associated with the intervention.	
Recommendation for future exercise intervention design and implementation.	

3.19.9: Focus group data analysis.

Qualitative research is becoming increasingly more popular in health service research and can be used to study novel interventions designed to improve patient care (Dy et al, 2005). No singularly appropriate way to conduct qualitative data analysis has been proposed. However, it is well regarded that the process begins during collection of the data and continues throughout the course of the study (Bradley et al, 2007). For accuracy and consistency in qualitative data analysis, the following steps have been suggested to ensure a systematic approach allowing for open discovery of emergent concepts (Bradley et al, 2007).

Initially, reading of the data facilitates deeper understanding of its meaning, together with the identification of emergent themes without losing connections between concepts and context. Following this initial phase, the data can now become coded. These codes should be applied to paragraphs, sentences or even words within the data to catalogue key concepts without losing their context. Using a 'grounded theory' approach, data is reviewed line by line and as concepts are selected, codes are assigned. During progression of data analysis, specifications of the codes can be developed and refined to fit the data. In this approach, the researcher can identify text that represents similar data to that already coded to ascertain if they reflect the same concept. The constant comparison method, initially developed by Glaser and Strauss, 1967, involves the refinement of existing codes and the development of new codes to fit with the context of the data being analysed. In this way, the codes structure evolves inductively, reflecting the 'ground' or direct experiences of the participants (Bradley et al, 2007).

3.20: Statistical analyses.

On the whole, data were normally distributed, with the exceptions of the NYHA questionnaire, ISWT perceived exertion, inflammatory markers and NT pro-BNP, which were normalised using logarithmic transformation before analysis. Analysis of Covariance (ANCOVA) was used to assess differences between the groups, with the baseline value used as a covariate (Vickers et al, 2001). Analysis of Variance (ANOVA) was used to assess differences in the baseline characteristics of the groups and paired t-tests were used to assess changes from baseline within the groups. Only data for patients who completed the study were included in the analyses, and no adjustments were made for multiple comparisons. Bland Altman plots were formulated in order to assess the accuracy or repeated measurements of testosterone and Pearson product moment correlation studies were performed on all baseline data (including participants who did not complete the programme) to assess for relationships between testosterone and outcome measures assessed during the study. Statistical significance was set at $P \leq 0.05$, and results are expressed as means \pm SD.

3.21: Measurement error.

A cohort of 10 patients was selected in order to assess the reproducibility of the main outcome measures. This included 6 min walk distance, $\dot{V}O_2$, endothelial function using flow mediated dilatation and important echocardiographic parameters. Reproducibility was assessed using the relative technical error of measurement (TEM) and expressed as percentage error by the formula below:

$$\%TEM = (TEM / [M1+M2] / (2) \times 100$$

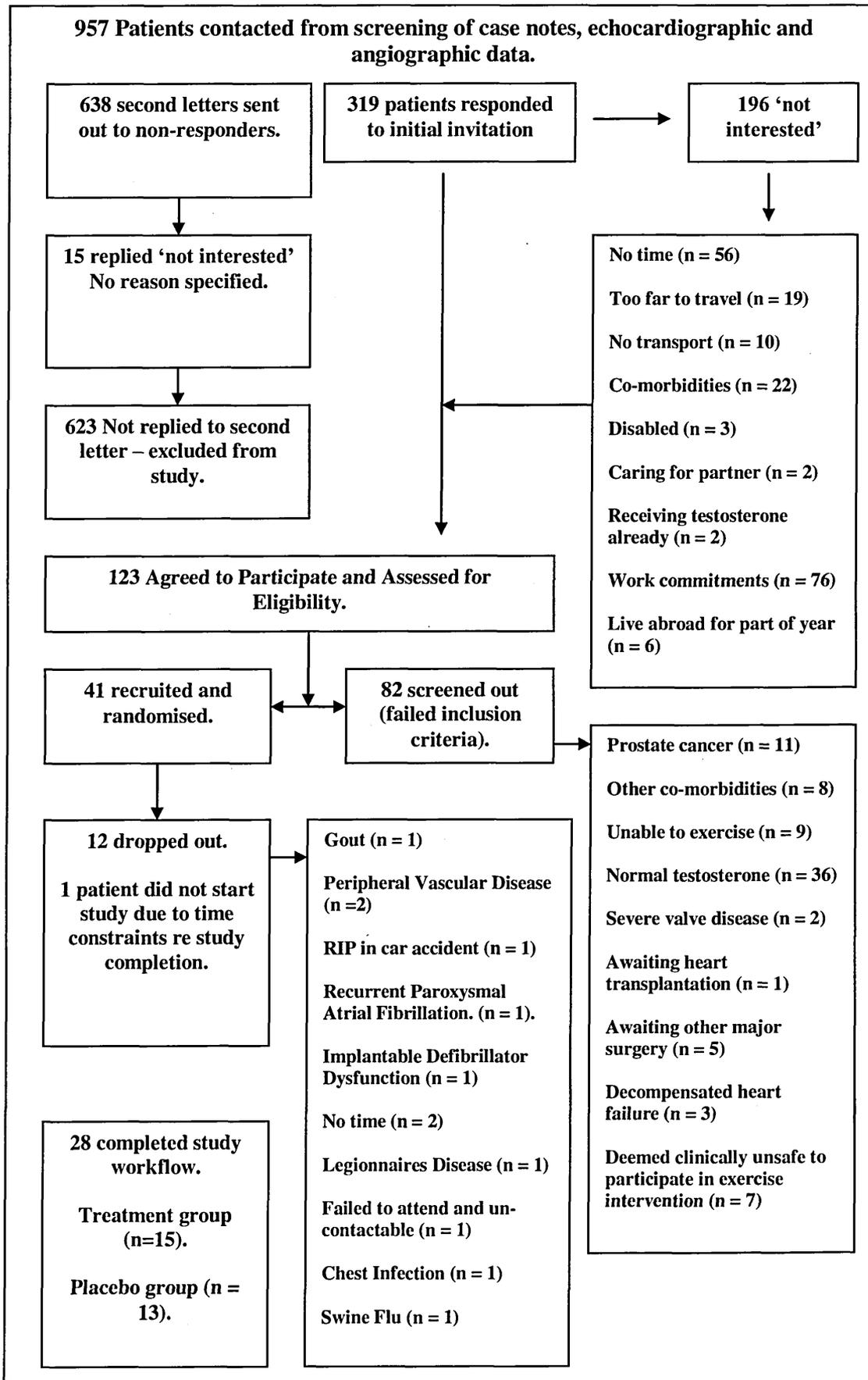
Where M1 is the mean of the first series of measurements, M2 is the mean of the second series of measurements.

Chapter 4. Results.

4.1: Demographic data.

A total of 957 patients were identified from the screening of patient notes, echocardiographic and angiographic data (figure 14). These were sent an official letter inviting them to participate in the study. Of these, 638 patients (67%) did not reply and were sent another invitation letter. Following this, 15 replied as 'not interested' without any documented reason and 623 did not respond to the second invitation letter. Of the 196 patients (20%) who replied as 'not interested' to the original invitation letter, the most common reasons for not wanting to participate were lack of time or work commitments. Other reasons included co-morbidities that the participant felt would prevent them from exercising, a lack of transport and/or too great a distance to travel to the research centre. Two patients were unable to participate because they were already receiving testosterone therapy. Of the remaining 123 patients who were assessed for entry into the study, 82 were screened out for not fulfilling the eligibility criteria and the remaining 41 patients (4% of those initially contacted) were successfully recruited and randomized. 1 of these patients did not start the study intervention due to time constraints re study completion. Attrition over the 12-week period of the exercise intervention was high. Five patients from the testosterone group (25%) and seven patients in the placebo group (35%) were lost to follow-up (overall 30%). Two patients dropped out due to time constraints but all others were due to co-morbidities. None of the patients dropped out due to study-related adverse events. Hence, 28 patients (3% of those contacted initially) completed the study protocol (n=15 testosterone and n = 13 placebo; figure 15).

Figure 15. Recruitment summary.



As this was a preliminary/feasibility trial, data analysis was based on the 28 patients that completed the study. We successfully implemented the exercise intervention and injections in all these patients. Additionally, compliance to the twice-weekly exercise sessions was excellent, with all 24 supervised exercise sessions being attended (100%) and patients in both groups receiving all six fortnightly injections (either testosterone or placebo). The groups were particularly well matched at baseline. There was a strong trend to an increased walking distance at baseline in the placebo group ($p = 0.05$). No other differences were noted. Baseline demographics of the cohort are presented in table 39.

Table 39. Baseline characteristics of the study sample.

	Testosterone (N=15)	Placebo (N=13)
Characteristics		
Age (y)	68.3 ± 5.3	65.9 ± 8.8
Stature (m)	178.1 ± 4.5	176.4 ± 3.1
Body mass (kg)	90.2 ± 7.4	93.6 ± 11.1
Body mass index (kg/m ²)	28.7 ± 1.8	30.1 ± 3.5
Systolic BP (mm Hg)	137 ± 11	139 ± 18
Diastolic BP (mm Hg)	80 ± 5	84 ± 9
Resting HR (beats/min)	76 ± 13	71 ± 9
Total Testosterone (nmol/L)	10.4 ± 2.7	11.2 ± 2.6
Free Testosterone (nmol/L)	0.230 ± 0.081	0.230 ± 0.070
Bio-available testosterone (nmol/L)	3.98 ± 1.07	3.75 ± 1.48
SHBG (nmol/L)	31.4 ± 9.7	34.7 ± 11.6
Albumin (g/L)	39.87 ± 2.98	40.24 ± 1.74
NYHA Score	2.5 ± 0.5	2.5 ± 0.5
Left ventricular end-diastolic volume (mls)	118.04 ± 36.12	131.64 ± 32.67
Left ventricular EF (%)	21.3 ± 9.7	28.0 ± 6.0
Aetiology of HF		
Ischaemic	13 (87%)	7 (54%)
Hypertensive	0	3 (23%)
Idiopathic dilated cardiomyopathy	1 (7%)	2 (15%)
Unclear	1 (7%)	1 (8%)
Co-morbidities		
Ischaemic heart disease	12 (80%)	6 (46%)
History of previous Atrial fibrillation	5 (33%)	5 (38%)
Atrial fibrillation at data collection	2 (13%)	2 (15%)
Hypertension	5 (33%)	4 (31%)
Diabetes	5 (33%)	4 (31%)
Chronic obstructive pulmonary disease	4 (27%)	5 (38%)

Peripheral arterial disease	1 (7%)	0
Medication		
Beta Blocker	12 (80%)	10 (77%)
ACE Inhibitor	15 (100%)	11 (85%)
Calcium Channel Blocker	2 (13%)	1 (8%)
Diuretic	6 (40%)	7 (54%)
Aldosterone Receptor Blocker	2 (13%)	2 (15%)
Digoxin	1 (7%)	1 (8%)
Anti-Arrhythmic	4 (27%)	2 (15%)
Anti-platelet	7 (47%)	9 (69%)
Anticoagulant	5 (33%)	5 (38%)
Statin	12 (80%)	10 (77%)

(y) years, (m) metres, (kg) kilogrammes, (m²) metres squared, (mmHg) millimetres of mercury, (min) minute, (nmol/L) nanomols per Litre, (NYHA Score) New York Heart Association Score, (ACE) Angiotensin converting enzyme, (EF%) ejection fraction, (SHBG) sex hormone binding globulin, (HR) heart rate, (BP) blood pressure

4.1.1: Measurement error of data collection techniques.

Table 40 presents the relative technical error of measurement of the outcome measures assessed in a cohort of 10 patients who underwent identical assessments on a second occasion. Error is expressed as percentage change between initial and second measurement on a separate occasion.

Table 40: Technical Error of Measurement.

Test	TEM%
ISWT	3.58
$\dot{V}O_2$	4.10
Handgrip strength	3.70
FMD%	21.63
Muscular strength and endurance	4.10
NIRS time to minimum TOI	19.30
Sphericity index	7.21
LV diastology (E/E')	4.66
LV longitudinal function (S wave)	5.25
LV EF%	8.94
LA max volume (mls)	5.12
LA min volume (mls)	5.10
RV area diastole (cm²)	5.10
RA area systole (cm²)	4.98
LA expansion index	6.98
LA passive emptying fraction	6.41
LA active emptying fraction	7.14

(ISWT) incremental shuttle walk test, (VO_2) maximal oxygen uptake,(FMD) flow mediated dilatation, (%) percentage, (NIRS) near infrared spectroscopy, (TOI) tissue oxygenation, (LV) left ventricular, (LA) left atrial, (RV) right ventricular, (mls) millilitres, (cm²) centimetres square.

With the exception of NIRS time to minimum TOI (19.3%) and FMD (21.6%), other tests demonstrate good reproducibility with values under 10%. It has been recognised that one of the major limitations of FMD assessment is its poor reproducibility, even when performed by skilled operators (Stout, 2009). Time to minimum TOI data collection was hindered during testing by the inability to completely block out all light to the sensing electrodes. This was particularly difficult to achieve especially when the participant was performing intensive dynamic leg exercise during the recording period. The vigorous leg movement also resulted in oxygenation curves which were, at times, difficult to interpret.

4.2: Endurance performance.

All patients underwent detailed physiological assessment of endurance and strength performance. Table 41 illustrates the data obtained to assess aerobic and strength capability pre and post the 12 week intervention period.

Table 41. Endurance performance, muscular strength and muscular oxygenation of the treatment and placebo groups.

Data is reported as mean \pm SD. P value for ANCOVA is shown in the far right column. * indicates significance at $P < 0.05$, ** at $P < 0.01$ for changes from baseline within each group (using paired samples t-test).

	Testosterone (N=15)		Placebo (N=13)		P value
	Baseline	End-point	Baseline	End-point	
Shuttle walk distance (m)	418.7 \pm 153.7	492.7 \pm 215.3*	556.2 \pm 112.1	661.5 \pm 158.8*	.68
Incremental cycle ergometer test					
<i>Peak $\dot{V}O_2$ (ml/kg/min)</i>	15.0 \pm 4.4	18.2 \pm 4.8**	17.4 \pm 3.6	18.8 \pm 3.4	.42
<i>Peak work rate (W)</i>	88.3 \pm 20.8	103.3 \pm 31.1*	94.2 \pm 23.2	105.8 \pm 20.8*	.79
<i>Time to minimum tissue oxygenation (s)</i>	550.8 \pm 170.5	718.5 \pm 325.8*	581.8 \pm 232.1	804.6 \pm 164.4**	.85
Peak quadriceps torque production (Nm)					
<i>Knee Extension 60°/s (1 rad/s)</i>	122.2 \pm 51.7	134.0 \pm 44.7*	141.6 \pm 42.1	145.0 \pm 40.9	.63
<i>Knee Extension 180°/s (3 rad/s)</i>	78.5 \pm 30.5	83.5 \pm 31.4	89.0 \pm 25.2	96.0 \pm 33.0	.64
<i>Knee Extension 300°/s (5.3 rad/s)</i>	67.5 \pm 24.9	70.7 \pm 23.2	73.3 \pm 22.6	80.1 \pm 24.2	.42
<i>Knee Flexion 60°/s (1 rad/s)</i>	50.3 \pm 21.5	62.9 \pm 22.0*	64.2 \pm 23.8	62.9 \pm 19.0	.22
<i>Knee Flexion 180°/s (3 rad/s)</i>	46.5 \pm 20.8	55.3 \pm 24.6*	49.8 \pm 18.6	56.4 \pm 13.8	.86
<i>Knee Flexion 300°/s (5.3 rad/s)</i>	53.1 \pm 25.6	56.2 \pm 28.9	52.3 \pm 17.0	57.8 \pm 12.9	.81

Handgrip Strength (kg) 38.6 ± 8.6 40.7 ± 7.1 44.5 ± 7.6 47.0 ± 9.0* .50

(*m*) metres, (*VO₂*) maximal oxygen uptake, (*ml/kg/min*) millilitres per kilogram per minute, (*W*) watts, (*s*) seconds, (*Nm*) newton metres, (*°/s*) degrees per second, (*1rad/s*) radians per second, (*kg*) kilogrammes.

No significant differences were observed in markers of endurance performance between the groups. Numerous significant within group improvements were however noted. Maximum shuttle walk distance (418.7 ± 153.7 m increased to 492.7 ± 215.3 m in the treatment group and 556.2 ± 112.1 m versus 661.5 ± 158.8 m in placebo, $p < 0.05$), peak $\dot{V}O_2$ (15.0 ± 4.4 ml/kg/min increasing to 18.2 ± 4.8 ml/kg/min in the treatment arm ($p < 0.001$) with no significant difference in placebo) and maximum power output obtained during cycle ergometry (88.3 ± 20.8 W to 103.3 ± 31.1 W in treatment group and 94.2 ± 23.2 W increasing to 105.8 ± 20.8 W, $p < 0.05$ in the exercise only group) all increased in relation to baseline. In addition, the time to minimum tissue oxygenation during cycle ergometry significantly increased in both groups ($p < 0.01$ exercise only and $p < 0.05$ in the treatment group) suggesting delayed onset of muscular fatigue.

In addition to the endurance parameters, there were significant within group improvements in muscular strength confined to the testosterone treated group only. Between group differences were insufficient to become statistically significant. Knee extension and flexion at $60^\circ/s$ demonstrated increased torque in the treatment arm only (122.2 ± 51.7 Nm compared to 134.0 ± 44.7 Nm, $p < 0.05$ and 50.3 ± 21.5 Nm increasing to 62.9 ± 22.0 Nm, $p < 0.05$ respectively). There was also a significant increase in knee flexion at $180^\circ/s$ in the treatment arm only (46.5 ± 20.8 Nm to 55.3 ± 24.6 Nm, $p < 0.05$).

Handgrip strength demonstrated a significant within group improvement in the placebo group (44.5 ± 7.6 kg to 47.0 ± 9.0 kg, $p < 0.05$). No significant within group differences were seen.

4.3: Echocardiography parameters of cardiac structure and function.

Echocardiographic parameters of cardiac structure and function are presented in the table 42.

Table 42. Echocardiography outcomes pre and post intervention.

All data is expressed as mean \pm SD. Within group significant differences are highlighted in bold. * indicates significance at the $P < 0.05$ level and ** indicates significance at the $P < 0.001$ level. Between group differences are highlighted in the final column. Significant ANCOVA p values are highlighted in bold in the right hand column. Subscript in parenthesis shows number of patients measured for each parameter.

	Testosterone (N=11)		Placebo (N=10)		<i>P value</i>
	Baseline	End-point	Baseline	End-point	
Left atrial diameter (cm)	3.8 \pm 0.4 ₍₁₁₎	3.8 \pm 0.4 ₍₁₁₎	4.0 \pm 0.5 ₍₁₀₎	4.1 \pm 0.4 ₍₁₀₎	.60
Left atrial maximum volume (mls)	72.18 \pm 14.12 ₍₁₁₎	71.96 \pm 10.98 ₍₁₁₎	69.59 \pm 18.74 ₍₁₀₎	71.01 \pm 15.95 ₍₁₀₎	.72
Left atrial minimum volume (mls)	60.24 \pm 12.21 ₍₁₁₎	59.88 \pm 12.98 ₍₁₁₎	58.98 \pm 9.97 ₍₁₀₎	59.64 \pm 12.10 ₍₁₀₎	.77
Left atrial pre contraction volume (mls)	61.78 \pm 7.12 ₍₉₎	63.48 \pm 9.16 ₍₉₎	59.99 \pm 12.74 ₍₈₎	63.24 \pm 9.64 ₍₈₎	.70
Left atrial expansion index	19.82 \pm 5.14 ₍₁₁₎	20.17 \pm 4.87 ₍₁₁₎	17.99 \pm 4.41 ₍₁₀₎	19.06 \pm 4.98 ₍₁₀₎	.74
Left atrial passive emptying fraction	14.41 \pm 3.27 ₍₉₎	11.78 \pm 4.11 ₍₉₎	13.80 \pm 4.10 ₍₈₎	10.94 \pm 3.47 ₍₈₎	.64

Left atrial active emptying fraction	2.49 ± 1.54 ⁽⁹⁾	5.67 ± 1.88* ⁽⁹⁾	1.68 ± 1.04 ⁽⁸⁾	5.69 ± 2.11* ⁽⁸⁾	.68
LV end-diastolic diameter (cm)	4.9 ± 0.6 ⁽¹¹⁾	5.1 ± 0.5 ⁽¹¹⁾	5.2 ± 0.6 ⁽¹⁰⁾	5.1 ± 0.5 ⁽¹⁰⁾	.36
LV long axis length (cm)	8.5 ± 1.4 ⁽¹¹⁾	8.5 ± 1.5 ⁽¹¹⁾	8.7 ± 1.6 ⁽¹⁰⁾	8.5 ± 1.6 ⁽¹⁰⁾	.10
Biplane Simpsons LV EF(%)	21.3 ± 9.7 ⁽⁸⁾	23.84 ± 8.4 ⁽⁸⁾	28.0 ± 6.0 ⁽⁹⁾	24.87 ± 10.21 ⁽⁷⁾	.64
Sphericity Index (ratio)	1.72 ± 0.15 ⁽¹¹⁾	1.68 ± 0.19 ⁽¹¹⁾	1.67 ± 0.21 ⁽¹⁰⁾	1.65 ± 0.22 ⁽¹⁰⁾	.78
RA systolic area (cm²)	13.98 ± 2.24 ⁽¹⁰⁾	14.01 ± 1.65 ⁽¹¹⁾	14.56 ± 2.64 ⁽⁹⁾	13.97 ± 2.45 ⁽¹⁰⁾	.58
RV end diastolic area (cm²)	19.64 ± 3.98 ⁽⁶⁾	18.77 ± 3.24 ⁽⁸⁾	21.15 ± 4.19 ⁽⁸⁾	21.50 ± 6.21 ⁽⁸⁾	.40
RV fractional area change (%)	33.01 ± 7.48 ⁽⁶⁾	32.48 ± 8.97 ⁽⁸⁾	30.98 ± 6.87 ⁽⁸⁾	31.01 ± 8.41 ⁽⁸⁾	.92
TAPSE (mm)	19.19 ± 2.24 ⁽¹¹⁾	19.58 ± 2.34 ⁽¹¹⁾	18.73 ± 3.58 ⁽¹⁰⁾	18.54 ± 3.88 ⁽¹⁰⁾	.50
Longitudinal LV systolic function peak s' velocity(cm/s)	1.2 ± 0.10 ⁽¹⁰⁾	1.3 ± 0.21 ⁽¹⁰⁾	1.2 ± 0.22 ⁽¹⁰⁾	2.1 ± 0.27 ⁽¹⁰⁾	.35
LV diastolic function E/e'(ratio)	11.19 ± 3.31 ⁽⁸⁾	9.49 ± 2.41 ⁽¹⁰⁾	8.86 ± 2.90 ⁽¹⁰⁾	8.48 ± 2.47 ⁽¹⁰⁾	.96

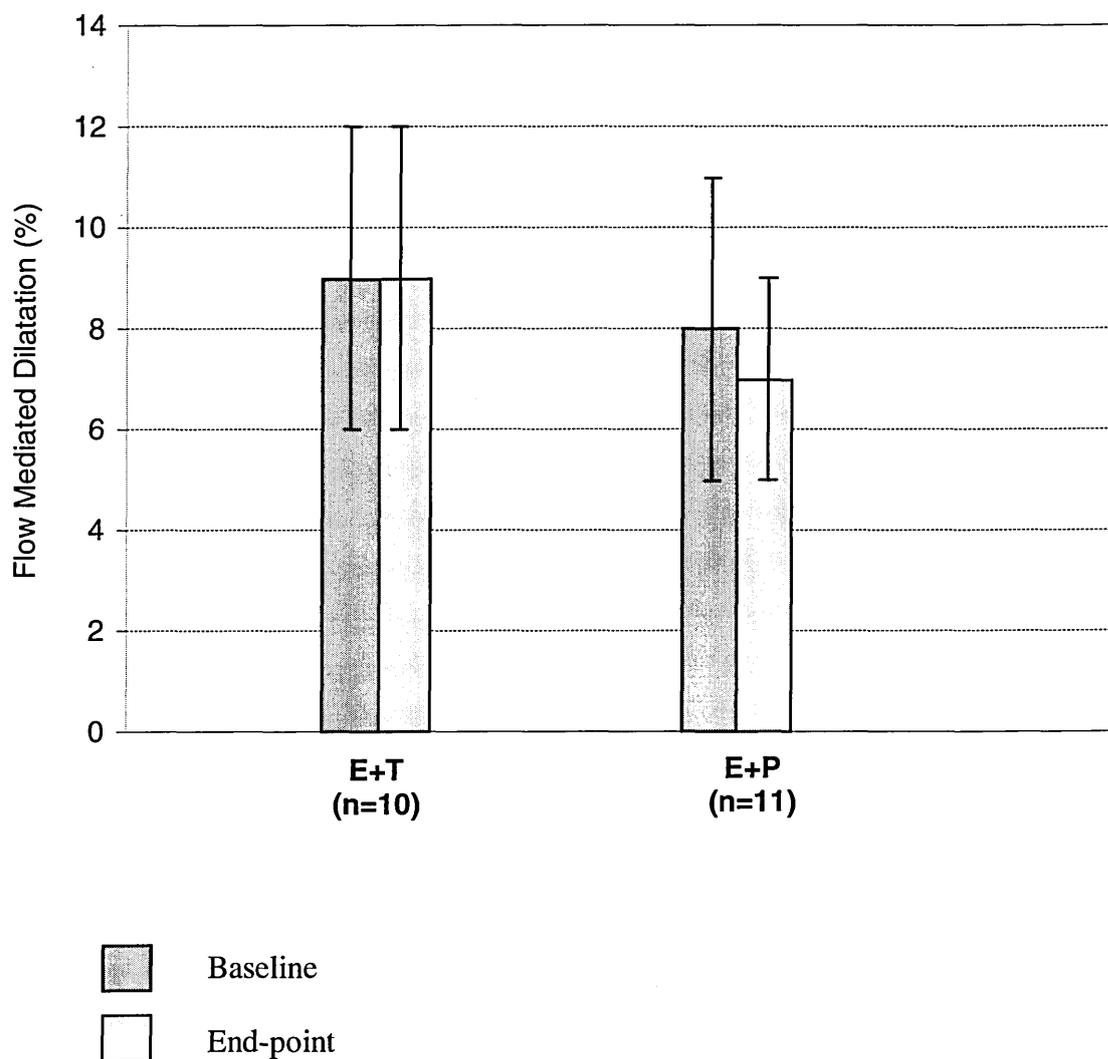
(cm) centimetres, (LV) left ventricular, (EF%) ejection fraction, (RA) right atrial, (RV) right ventricular, (cm²) centimetres square, (TAPSE) tricuspid annular plane systolic excursion, (mm) millimetres, (cm/s) centimetres per second.

No significant changes in cardiac structure and function were observed between the two groups. There was a significant ($p < 0.05$) increase in left atrial emptying fraction within both groups (2.49 ± 1.54 versus 5.67 ± 1.88 for exercise and testosterone and 1.68 ± 1.04 versus 5.69 ± 2.11 for exercise and placebo).

Figure 16 details the observed change in FMD % from baseline in the testosterone treated and placebo groups.

Figure. 16. Changes in flow mediated dilatation of the femoral artery in both groups.

Data are presented as means with error bars representing SD. E+T: testosterone supplementation (ET); E+P: placebo (EP).



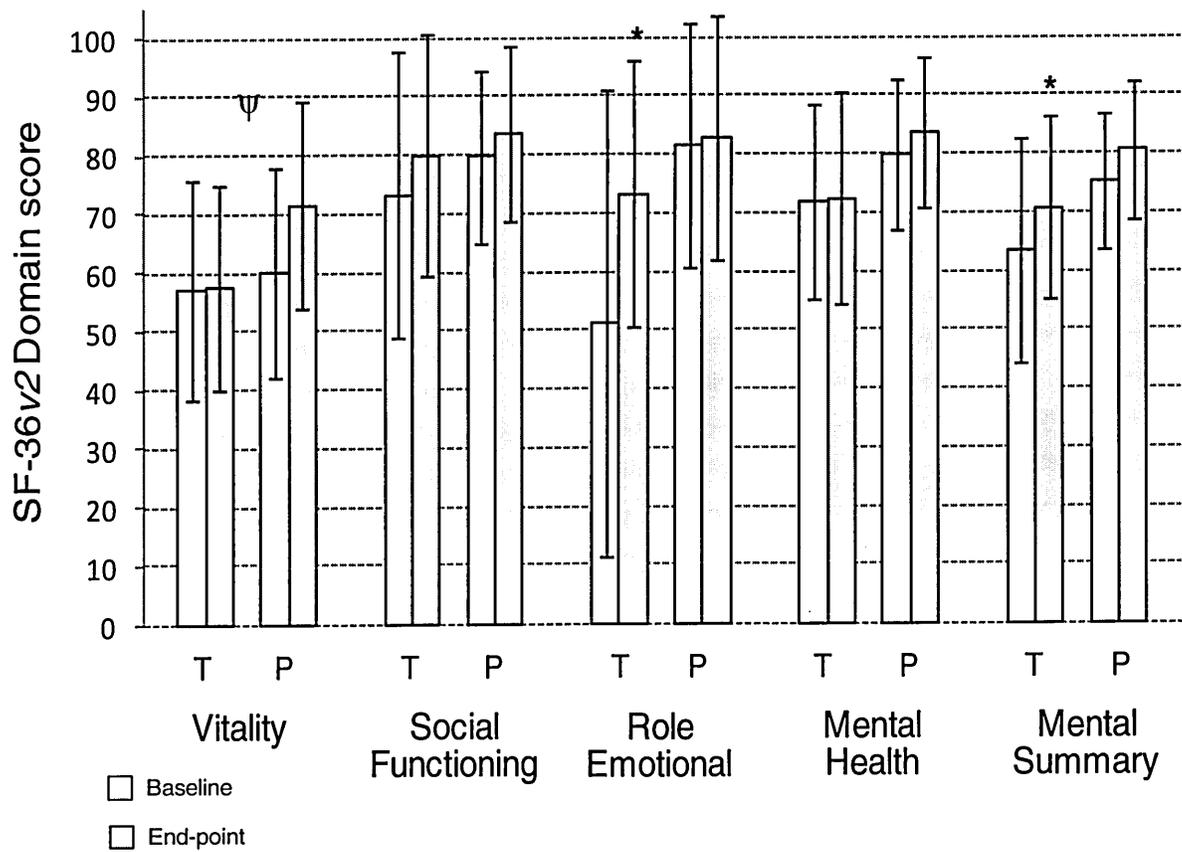
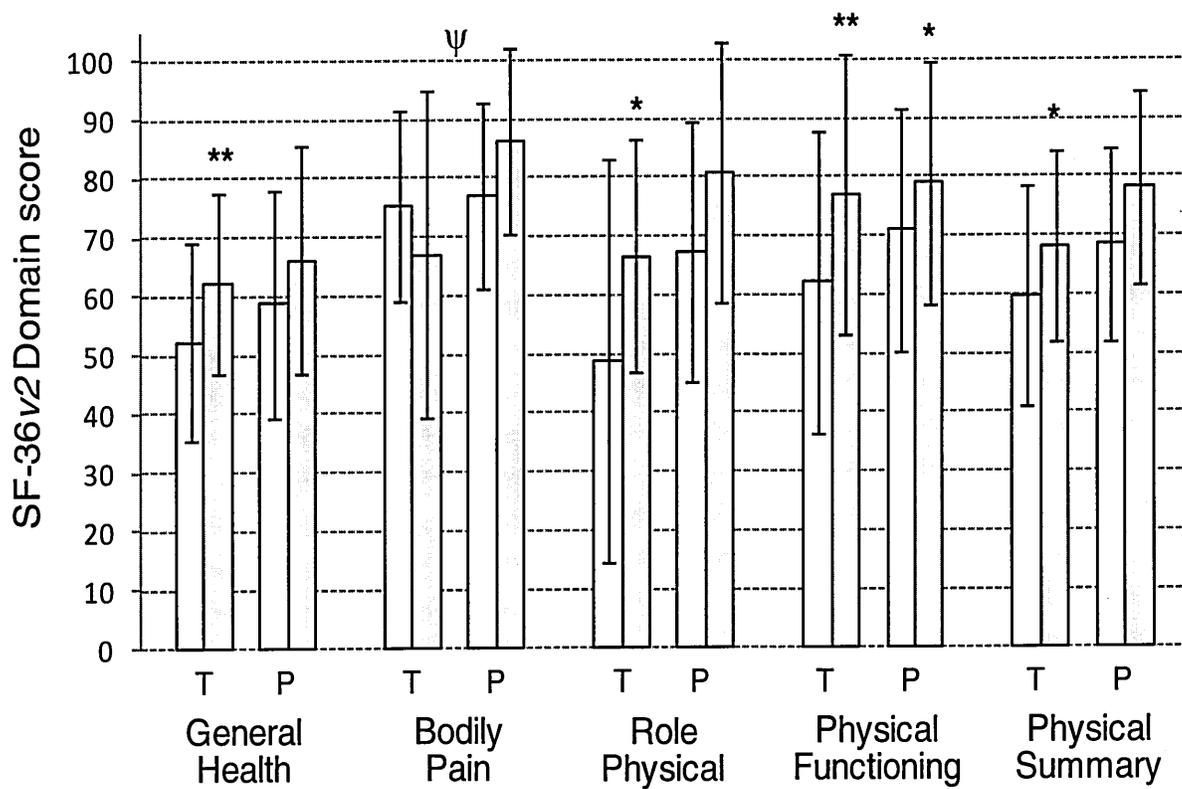
No significant change in flow mediated dilatation of the femoral artery was noted in either group in response to treatment or placebo when compared to baseline.

4.4: Indices of quality of life.

Questionnaire data pre and post intervention are presented in the figures 17 and table 42.

Figure 17. SF-36v2 quality of life domains at baseline and end-point.

Data are presented as means with error bars representing SD. T: testosterone supplementation P: placebo. *P<0.05; **P<0.01 indicates significance of group difference from baseline (paired t-test). [‡]P<0.05 significant ANCOVA.



There was a significant improvement in the general health ($p < 0.001$), role physical ($p < 0.05$), physical summary ($p < 0.05$), role emotional ($p < 0.05$) and mental health summary ($p < 0.05$) domains of the SF36 questionnaire in the treatment group only following the intervention period. ANCOVA results were only significant for the SF-36v2 domains of bodily pain ($P = 0.037$) and vitality ($P = 0.046$), favouring the EP group. There was also a significant improvement in physical functioning in the EP group ($p < 0.05$).

Table 43. Changes in Aspects of Health and Disease Related Quality of Life from Baseline in both Groups.

Data is reported as mean \pm SD. P value for ANCOVA is shown in the far right column. * indicates significance at $P < 0.05$, ** at $P < 0.01$ for changes from baseline within each group (using paired samples t-test).

	Testosterone (N=15)		Placebo (N=13)		P value
	Baseline	End-point	Baseline	End-point	
NYHA Class	2.5 \pm 0.5	1.8 \pm 0.7**	2.5 \pm 0.5	1.8 \pm 0.4**	.81
ADAM	6.2 \pm 1.7	4.6 \pm 2.5*	4.1 \pm 2.3	4.0 \pm 3.1	.24
MLHFQ	21.6 \pm 13.6	17.5 \pm 10.9	15.0 \pm 15.2	13.4 \pm 10.0	.48
BDI	10.4 \pm 8.7	6.6 \pm 3.8*	7.1 \pm 5.2	4.6 \pm 3.4	.39
CHAMPS (met hours/week)	45.5 \pm 34.1	56.7 \pm 22.0	58.4 \pm 49.2	72.6 \pm 41.7	.29

(NYHA) New York Heart Association, (ADAM) Androgen deficiency in the ageing male, (MLHFQ) Minnesota living with heart failure questionnaire, (BDI) Beck depression inventory, (CHAMPS) Community healthy activities model programme in seniors questionnaire.

There was no significant between group difference in New York Heart Association Score in the treatment and placebo groups. There was however, significant within group differences in NYHA Score pre and post intervention (testosterone group 2.5 ± 0.5 to 1.8 ± 0.7 , $p < 0.001$ and placebo 2.5 ± 0.5 to 1.8 ± 0.4 , $p < 0.001$).

Non-significant increases in self-reported physical activity levels from baseline were observed in both groups (45.5 ± 34.1 to 56.7 ± 22.0 MET-hours/week and 58.4 ± 49.2 to 72.6 ± 41.7 MET-hours/week for EaT versus EP, respectively; ANCOVA $p = 0.291$).

There was a significant improvement in Beck Depression Questionnaire Score in the testosterone group (10.4 ± 8.7 to 6.6 ± 3.8 , $p < 0.05$) following the intervention period. No such differences were observed in the placebo group. ANCOVA however, was not significant ($p = 0.39$).

There is a significant reduction in ADAM Questionnaire Score (and hence improved androgen status) in the testosterone and exercise group (6.2 ± 1.7 to 4.6 ± 2.5), there is no significant change in the EP group. No significant ANCOVA statistic between groups is noted ($p = 0.24$).

Slight trends towards improved Minnesota living with HF score were noted in both groups. However, these were not statistically significant.

4.5: Blood chemistry.

Blood chemistry variables pre and post intervention are presented in the table 44.

Table 44. NT pro-BNP and inflammatory markers at baseline and end-point.

Data is expressed as mean \pm SD. * indicates significance at the P<0.05 level and ** indicates significance at the P<0.001 level. Between group P values are highlighted in the final column.

	Testosterone (N=15)		Placebo (N=13)		P value
	Baseline	End-point	Baseline	End-point	
NT pro-BNP (pg/mL)	625 \pm 1205	486 \pm 587	678 \pm 1304	660 \pm 1353	.41
TNF- α (pg/mL)	1.44 \pm 0.77	1.32 \pm 0.53	1.95 \pm 1.97	1.69 \pm 1.67*	.81
IL-6 (pg/mL)	6.57 \pm 9.56	5.63 \pm 7.52	3.48 \pm 2.72	3.45 \pm 2.09	.88
hs-CRP (mg/L)	3.46 \pm 5.67	2.51 \pm 2.70	2.04 \pm 2.27	2.60 \pm 2.63	.43
sICAM (ng/mL)	337 \pm 82	345 \pm 81	330 \pm 105	356 \pm 107	.61
sVCAM (ng/mL)	1139 \pm 323	1177 \pm 457	1080 \pm 506	1108 \pm 484	.99

(NT pro BNP)N terminal brain natriuretic peptide, (pg/mL) pictograms per millilitre, (TNF α)tumour necrosis factor alpha,

(IL)interleukin,(hs-CRP) high sensitivity C-reactive protein, (mg/L) milligrams per litre,(sICAM)soluble intracellular adhesion molecule,

(sVCAM)soluble vascular adhesion molecule, (ng/mL) nanograms per millilitre.

There were no differences between the groups in circulating levels of NT pro-BNP or inflammatory markers. However, a decrease in TNF- α from baseline is noted in the EP group only ($p < 0.05$). This decrease is not statistically significant using between group analysis.

5.0 Correlation and Bland Altman analyses.

In order to assess for detailed relationships between important variables involved in the analyses, Pearson product moment correlation were performed on important health outcomes assessed during this study. These analyses were based on total testosterone, free testosterone and bio-available testosterone in the same format as study 1. Tables 45-48 detail the correlations observed. Additionally, Bland Altman plot was used to show the agreement between initial and second measurement of total testosterone. For completeness, these plots are also presented for free and bio-available testosterone. Figures 18-21 represent Bland Altman limits of agreement plots for total testosterone, free testosterone, bio-available testosterone and SHBG.

Table 45. Pearson correlation coefficient for strength and endurance parameters based on total, free and bio-available testosterone.

* indicates $P < 0.05$, ** $P < 0.01$

Parameter.	TT r value	TT p value	FT r value	FT p value	BioT r value	BioT p value
ISWT distance (m)	.198	0.21	.202	0.20	.20	0.21
Peak VO₂ (ml/kg/min)	.145	0.37	.164	0.40	.171	0.42
Peak work (Watts)	.100	0.51	.098	0.54	.099	0.54
Time to minimum tissue oxygenation (s)	.014	0.93	.090	0.95	.010	0.94
Knee extension 180°/S	.011	0.95	.009	0.92	.010	0.94
Knee flexion 180°/S	.100	0.51	.092	0.57	.088	0.61

(ISWT) incremental shuttle walk test, (m) metres, (VO₂) peak oxygen uptake, (ml/kg/min) millilitres per kilogram per minute, (s) seconds, (°/s) degrees per second. (TT) total testosterone, (FT) free testosterone, (BioT) bio available testosterone.

There were no significant correlations between markers of endurance performance and muscular strength when based on total, free or bio-available testosterone concentration.

Table 46 details the Pearson correlation coefficient for important quality of life parameters based on total, free and bio-available testosterone.

* indicates $P < 0.05$, ** $P < 0.01$

Parameter.	TT r value	TT p value	FT r value	FT p value	BioT r value	BioT p value
SF36 General Health	.202	0.21	.198	0.21	.184	0.25
SF36 Bodily Pain	.011	0.95	.009	0.97	.008	0.98
SF36 Role Physical	.191	0.23	.180	0.26	.177	0.27
SF36 Physical Function	.205	0.20	.200	0.21	.199	0.21
SF36 Physical Summary	.099	0.54	.084	0.60	.079	0.62
SF36 Vitality	.004	0.98	.010	0.96	.009	0.97

SF36 Role emotion	.007	0.97	.004	0.98	.004	0.98
SF36 Social functioning	.080	0.59	.047	0.77	.054	0.73
SF36 Mental health	.100	0.53	.094	0.56	.090	0.58
SF36 Mental Summary	.007	0.97	.007	0.97	.005	0.96
MLHFQ	.088	0.58	.087	0.59	.071	0.66
BDI	.004	0.98	.003	0.99	.003	0.99

(SF36) short form 36 version 2, (MLHFQ) Minnesota living with heart failure questionnaire, (BDI) Beck depression inventory. (TT) total testosterone, (FT) free testosterone, (BioT) bio available testosterone.

There were no significant correlations between markers of general health related or disease specific quality of life when based on total, free or bio-available testosterone concentration.

Table 47 details Pearson correlation coefficient for echocardiographic parameters based on total, free and bio-available testosterone.

* indicates $P < 0.05$, ** $P < 0.01$

Parameter.	TT r value	TT p value	FT r value	FT p value	BioT r value	BioT p value
Left atrial expansion index	.025	0.88	.031	0.85	.029	0.86
Left atrial passive emptying fraction	.140	0.38	.099	0.54	.109	0.50
Left atrial active emptying fraction	.084	0.60	.075	0.64	.077	0.63
LV long axis length (cm)	.101	0.53	.111	0.49	.099	0.54
Biplane Simpsons LV EF (%)	-.005	0.96	-.002	0.98	-.004	0.97
RV fractional area change (%)	.062	0.70	.054	0.74	.066	0.68
TAPSE (mm)	.041	0.80	.044	0.78	.044	0.78

Longitudinal LV systolic function peak s' velocity(cm/s)	.034	0.83	.045	0.77	.044	0.78
LV diastolic function E/e'(ratio)	.003	0.98	.004	0.97	.002	0.99

(LV) left ventricular, (cm) centimetres, (EF%) ejection fraction, (RV) right ventricular, (TAPSE) tricuspid annular plane systolic excursion, (cm/s) centimetres per second. (TT) total testosterone, (FT) free testosterone, (BioT) bio available testosterone.

There were no significant correlations between markers cardiac structure and function when based on total, free or bio-available testosterone concentration.

Table 48 details Pearson correlation coefficient for NT pro-BNP, soluble adhesion molecules and inflammatory mediators based on total, free and bio-available testosterone.

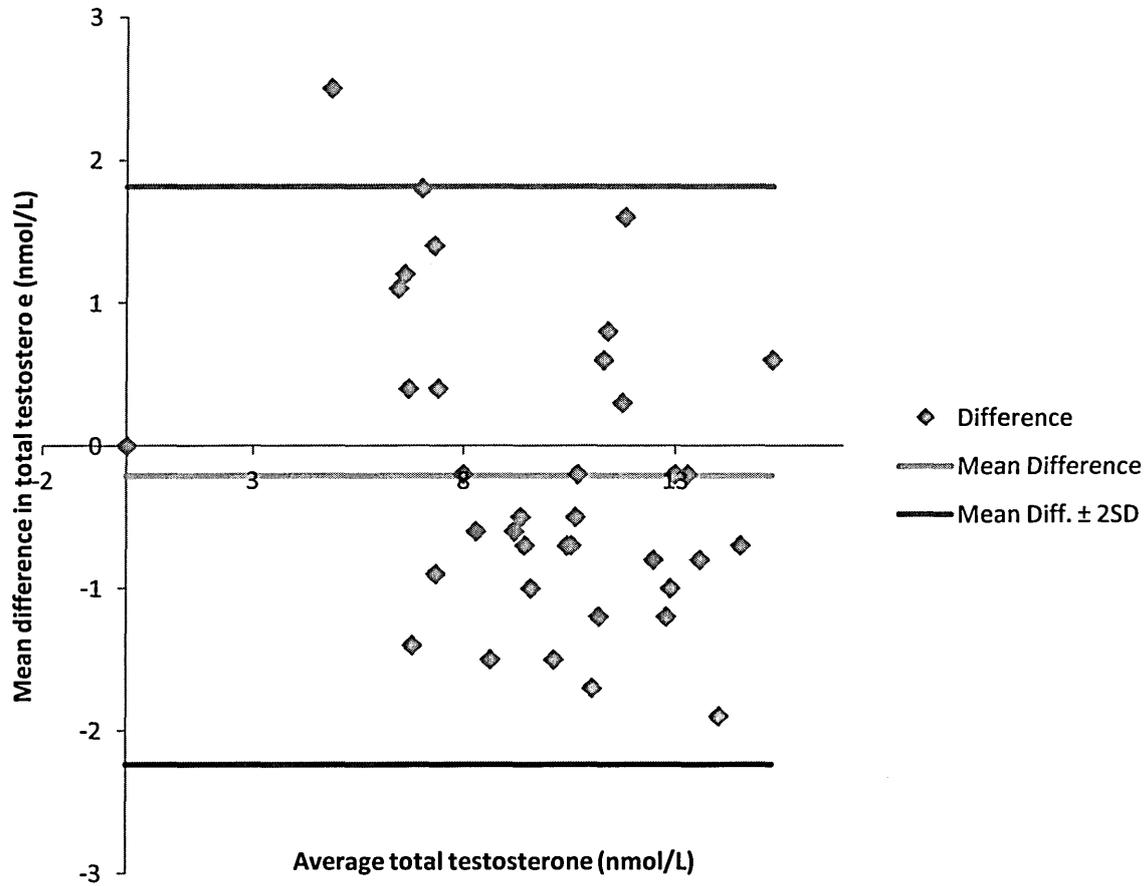
* indicates $P < 0.05$, ** $P < 0.01$

Parameter.	TT r value	TT p value	FT r value	FT p value	BioT r value	BioT p value
NT Pro-BNP (pg/mL)	.068	0.67	.071	0.66	.077	0.63
TNFα (pg/mL)	.009	0.96	.010	0.95	.011	0.94
IL-6 (pg/mL)	.007	0.97	.004	0.98	.006	0.97
sICAM-1 (ng/mL)	.109	0.50	.111	0.48	.098	0.54
sVCAM-1 (ng/mL)	.099	0.54	.098	0.54	.091	0.57
CRP (mg/L)	.004	0.98	.004	0.98	.008	0.97

(NT pro BNP) N terminal brain natriuretic peptide, (TNF α) tumour necrosis factor alpha, (IL-6) interleukin-6, (sICAM) soluble intracellular adhesion molecule, (sVCAM) soluble vascular adhesion molecule, (CRP) C reactive protein, (pg/mL) picogrammes per millilitre, (ng/mL) nanogramms per millilitre, (mg/L) milligramms per litre, (TT) total testosterone, (FT) free testosterone, (BioT) bio available testosterone.

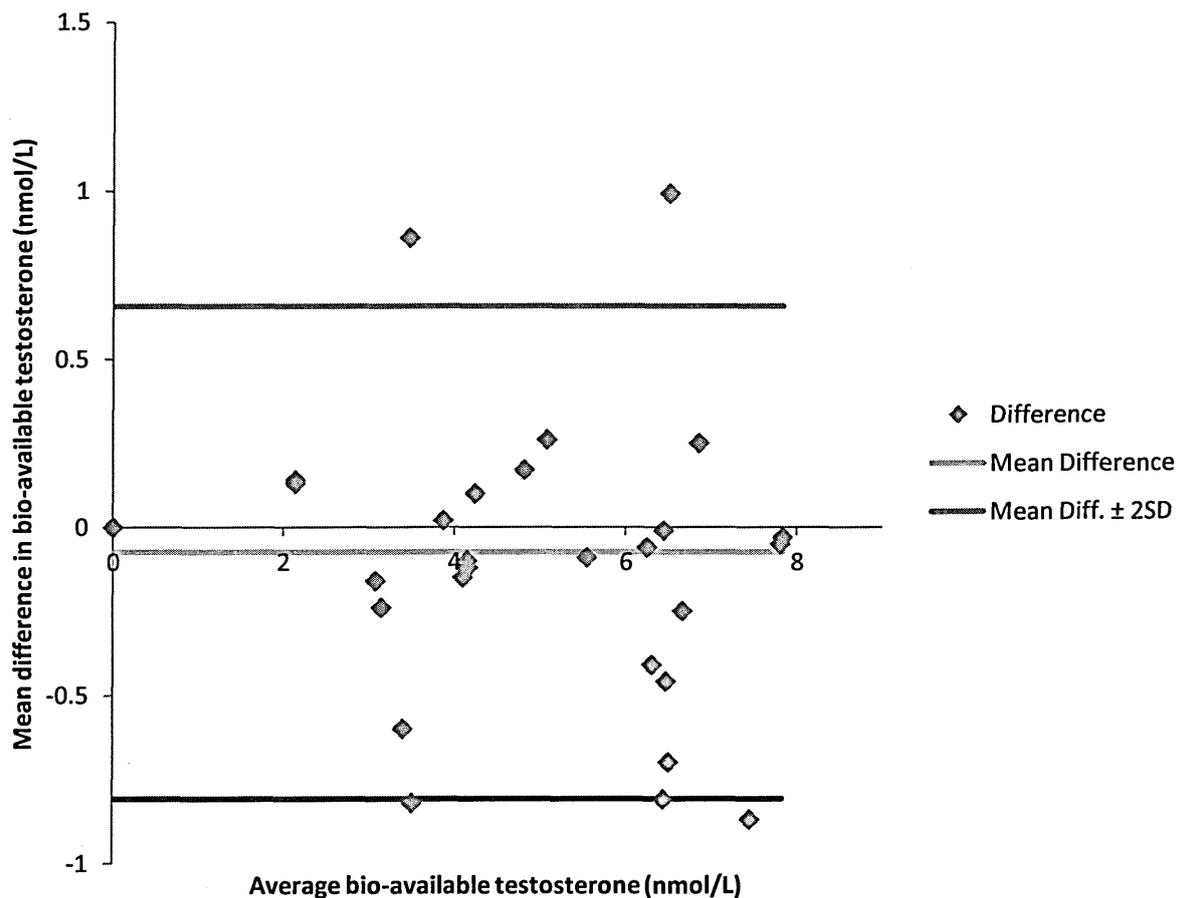
There were no significant correlations noted between NT pro-BNP, adhesion molecules or inflammatory cytokines when based on total, free or bio-available testosterone.

Figure 18. Bland Altman Plot showing the agreement between initial and subsequent total testosterone analysis.



There is good agreement between sequential measures of total testosterone on two separate visits. Mean difference of -0.21 nmol/L , s.d. 1.01 nmol/L. Therefore, mean difference $+2$ s.d. is 1.82 nmol/L and -2 s.d. is -2.24 nmol/L. Coefficient of reproducibility is 2.02 . One value out lies the ± 2 s.d.

Figure 20. Bland Altman Plot showing the agreement between initial and subsequent bio-available testosterone analysis.



The Bland Altman plot displays reasonable agreement between subsequent calculations of bio-available testosterone based on total testosterone, albumin and SHBG. The mean difference is -0.08 nmol/L with s.d. of 0.37 nmol/L. Therefore, +2 s.d. is 0.67 nmol/L and -2 s.d. is -0.81 nmol/L. The coefficient of reproducibility is 0.74.

6.0 Focus group evaluation of the intervention.

There were seven important themes and categories of response that emerged from the focus group discussions. These are presented below. Participant quotes have been included from different focus groups to provide a detailed portrait of the types of responses for each theme.

Confidence to continue exercising in the future

Patients recognised that continuation with ET was important for maintaining the perceived benefits gained from study participation. However, it was also recognised that without the support of the study team this would be a difficult undertaking. Participants were concerned that without having strict appointment times to attend for sessions and a supervised ET session with others in the same situation they would discontinue ET and be reluctant to attend other gymnasiums. In contrast however, it was noted that the experience of ET taught the participants how to perform different types of exercise safely in their own homes or in local health clubs. Although many participants did not regularly exercise previously due to concerns about their condition, they felt that they now have more idea about the level they can push themselves to during ET and daily activities. This has given them more confidence to perform ET. However, patients were in agreement that once the exercise sessions were completed it was unlikely that many of them would continue with ET due to the lack of experienced instructors in health clubs in whom they felt confident during ET.

“What you have to do when you finish is to keep the momentum going.

Otherwise you start settling back into old ways”.

“You just need someone to push you into it. You know, you’ve made an

appointment and so you come – don’t you?”

“It needs to be that we go somewhere with your mates so that you can push

each other. If you are doing it at home then you just say - Oh, I don’t feel

like doing it today”.

“It is up to us to look after our own health and this programme has

reminded me that I should be doing this myself. We can do a lot of the

exercises at home and that is important”.

“There is nothing to take you any further at the end”.

“For people like us in order to take the burden off the NHS you really need

something in place at the end”.

Role in giving advice to patient’s with similar conditions

Patients indicated that they themselves could undertake an important role in giving advice to other patients with the same condition. It was stated that the experience of performing ET and the benefits gained from the sessions should be promoted to other patients with testosterone deficiency and HF. Participants appeared confident

in being able to discuss the benefits of the intervention and how these benefits translate to everyday activities and to improvements in quality of life. In addition, it was generally felt that large organisations such as the National Health Service were failing in this respect and were wasting money and resources in other ‘less important’ areas.

“They must do exercise. It is important and gets you much fitter”.

“It makes daily life easier”.

“It is up to people like us to promote this – we should advertise these sort of programmes and more people will benefit”.

“The NHS is not doing a good enough job and wasting money on other things that are pointless”.

Perceptions about the exercise intervention

Patients felt that they could have been pushed harder by increasing the intensity of the ET. Although, it was thought that initially the programme was strenuous enough, participants felt that later during the trial they could have tolerated a more strenuous programme. It was also felt that there could have been more variety during the ET, perhaps by the use of a wider range of gym equipment. It was suggested that more variety could have helped participants in extending themselves while allowing them to learn how to use equipment found in local health clubs. It was noted that to reduce

the burden on the National Health Service, there should be more community based specialist exercise facilities especially since a minority of patients found travel to and from the exercise centre difficult. Some believed they would have benefited from more formal educational advice surrounding ET and diet to promote more effective weight loss. One member of the group thought that the ET sessions were a little rushed and they could be improved by allowing more time in the gymnasium.

“I could probably have been pushed a little harder and maybe got even more fit”.

“I felt as though it could be a little more intensive. For instance I would have liked to have done a warm up on the treadmill or perhaps the main training on a treadmill rather than a bike. That way, you can get your whole body working rather than just your legs”.

“I would have preferred more time walking on the treadmill because that’s what I do all the time. It helps me improve that specific area. I never cycle anywhere”.

“Maybe dietary and educational advice should also be included because weight is a massive problem for lots of people”.

Perceived roles of medical professionals

There were varied responses regarding the role of medical professionals in promoting and understanding ET and testosterone therapy in HF. Patients felt more positive when the general practitioner and consultant cardiologists were interested in the research and the format of the ET sessions. In contrast, there were a number of negative comments related to medical professionals. It was felt that many nurses still hold the opinion that patients with HF are unable to exercise and must dramatically reduce the level of physical activity within their daily lives. It was felt that general practitioners did not do enough to promote ET, or know about appropriate health clubs or instructors qualified to supervise ET for HF patients. Annual check-ups or meetings with heart specialists were suggested as an important factor for both maintaining health and preventing disease progression. Some of the participants had not seen a medical professional for a couple of years and thought that screening and education for those patients with features of HF at an early stage would be an acceptable way to prevent significant disease progression and could ultimately save National Health Service resources. Of those patients who had joined health clubs, there was concern that instructors could not safely supervise HF patients and would be unable to recommend adequate lifestyle changes or beneficial exercises to perform. This resulted in participants not renewing gym memberships for fear of becoming unwell during exercise and with the perception that instructors were not qualified to deal with adverse health effects. Finally, it was perceived that physicians do not have enough time to adequately explain the condition of HF and the range of therapeutic options available to the patients. Contact times were too short and seen as quick 'in and out' appointments.

“Doctors and nurses are interested in the course [trial] and also the outcome of it”.

“My doctor is keen to see the results and I have asked if he will prescribe me testosterone”.

“They have different opinions, some say don’t exercise. That’s rubbish because I know I can”.

“I never see the consultant anymore. I have been discharged and they don’t follow me up to check anything. They don’t force you to exercise”.

“If they spent more they may save money by sorting out problems in the earlier stages where it doesn’t cost as much”.

“The [health-club] instructors seem clueless and unable to help especially if you tell them you’ve got heart problems”.

“I asked my GP to send me to an exercise class but he didn’t know about any or where to go. That’s useless. They should be promoting these things to heart patients not making it more difficult to get there”.

“They know what things can help us. They just don’t push it enough because they are too busy and want you in and out quickly”.

Motivation during the trial

Motivational factors identified by patients included the perception of significant improvement in exercise capacity and strength through participation. Patients felt that motivation was enhanced by the use of group sessions where competition and team behaviour proved beneficial. In particular, patients appreciated making new contacts with others who had similar health complaints. Some participants described significant weight loss as another important factor in wanting to attend the sessions. Other important motivational factors included social support, interest in the results of the trial and also the fact that they perceived that this provision was the only safe option available for HF patients. The two groups who were felt to have provided the most support to participants during the intervention were family and fellow participants. Family members encouraged patients to attend the sessions and were able to motivate them to attend. Many participants stated that they continued in the trial because of the opportunity to meet with others who had similar health issues. The support provided by health professionals, physicians and researchers involved in the programme was seen as positive.

“My motivation was that I wanted to get fitter and also find out if it was the exercise or testosterone or whatever made you feel better”.

“I could see myself getting fitter and losing weight”.

“People will be a lot more motivated when you get to know the results. The

encouragement will come from the results”.

“The best thing for me was coming and seeing the other guys in my group every week. We could chat about things and still get on with the exercises. It made the sessions seem more fun”.

“I prefer to come here with the people who have similar problems”.

“We all got on together so that was an important part of the programme”.

“I’m grateful to all the people here who have helped me”.

“My family also motivated me to come. They wanted what was best for me so made it easier for me to get here”.

“Family was very important in getting me out of the house and attending”.

“We need more places like this with experienced staff that you have confidence in when you are exercising”.

Negative effects

Minor physical effects of the ET were discussed, and included buttock soreness from exercising on the cycle ergometer and local muscular pain from strength training

exercises. Some patients described delayed onset muscular soreness following the ET sessions and reported general tiredness towards the end of the week – especially those patients who were also still in full time employment.

“Tired at first but the more I did I felt better”.

“Sometimes sore in the morning but that soon wore off”.

“Night and day after were difficult as I used to ache quite a bit. I thought I might get use to it but I never did. Maybe I was pushing too hard at times”.

“Sore back after cycling and when doing sit ups”.

“Muscle aches when doing some of the strength exercises”.

Perceived health benefits

Most participants felt that the main benefit of the intervention was a sense of increased fitness. Participants were encouraged by the increases in endurance capacity, muscular strength and improvements in health related quality of life. They described a sense of improved confidence in performing daily tasks and, for some of them, more confidence in attending health clubs for the first time. The significant weight loss associated with the ET was also a source of increased confidence and

wellbeing. Participants valued the professionalism of the fitness instructors and researchers and the excellent facilities provided within the research centre – particularly the exercise and fitness assessment equipment.

“For me it’s been an excellent course and I’m grateful to all the people here who have helped me. I have noticed it very much because I do a physical job and my breathing has improved very much recently”

“Just fitness alone and the confidence definitely makes you improve”.

“I could walk further and get up hills without getting as short of breath”.

“Training has made me more confident and fitter”.

“Exercise and injections made me feel great during the day”.

“Yes the strength is coming back more and more”.

“The professionalism was important together with meeting friends in the gym. I really enjoyed the training”.

“I have lost weight as a result of doing this”.

“I had to buy another pair of trousers last week because my suit is too big. That must be very beneficial to us heart patients – losing weight”.

Chapter 5: Discussion.

This is the first study to evaluate the feasibility of a 12-week program of exercise rehabilitation, with and without testosterone replacement therapy, in a more elderly (mean age of 68.3 ± 5.3 years for the treatment group and 65.9 ± 8.8 years for the placebo group respectively) male HF population with bio-chemical evidence of low testosterone. This study was designed as a feasibility study because the efficacy of ET in patients with HF and testosterone deficiency had not yet been studied and, in addition, neither was it known whether testosterone treatment was able to augment the effects of exercise in this population. Thus by collecting this preliminary data, the impact of this intervention on pertinent health outcomes was studied with the aim of planning a future larger scale trial and detail issues which may impact on the successful management of future research studies.

Patients recruited to this trial had average total and free testosterone levels of 10.9 nmol/L (314 ng/dl) and 0.229 nmol/L (6.6 ng/dl), respectively. These values are similar to those reported by Vermeulen et al, 2001 for the lowest 1% of healthy non-obese males aged 20-40 y (11.0 nmol/L and 0.225 nmol/L, respectively) and are widely considered to represent the threshold for androgen deficiency (Vermeulen et al, 2001). Using these cut-off values, 61% of the men in our cohort were classified as androgen deficient on the basis of testosterone status, although all but two of them (95%) with testosterone levels of 11.9 nmol/L (343 ng/dl) and 12.9 nmol/L (371 ng/dl) were experiencing symptoms of hypogonadism (as evidenced a score of ≥ 3 on the ADAM questionnaire). Previous clinical experiences have demonstrated that the official diagnosis of hypogonadism is challenging. Guidelines have reported wide

variations in laboratory 'cut-off' values for low testosterone varying from 8.0nmol/L up to 12.0nmol/L in healthy populations (Bhasin et al, 2006). In addition, the same guidelines are clear that variations in laboratory serological techniques and the additive use of questionnaires can further impact on the diagnostic accuracy of testosterone deficiency. Using the upper cut off value of 12 nmol/L as suggested by Bhasin et al, 2006, then all but one of our participants would be classified as testosterone deficient based on total testosterone concentration. Due to challenges faced in recruitment to the trial, a decision was made to increase the acceptable total testosterone concentration for inclusion into the study to ≤ 15 nmol/L. Clearly, this increase would have resulted in recruitment of participants with 'threshold' or even lower end of normal range testosterone onto the trial and as such, affected the results. During the time of increasing the cut-off range, only one participant (with a testosterone concentration of 12.9 nmol/L) was recruited with a value above the originally defined range of ≤ 12 nmol/L.

Previous work has reported that in order to fully assess testosterone status, sampling of both bio-available and free testosterone concentration is paramount. Elderly males, males with obesity, diabetes mellitus or chronic diseases (such as cancer, lung disease, heart failure and HIV) have been shown to present with abnormal increases in SHBG and thus due to its detailed binding relationship with testosterone, total testosterone may not be a precise marker of androgen status in this population (Bhasin et al, 2008). The mean free testosterone concentration observed during this study (0.229 nmol/L) is higher than the suggested cut-off range of 0.17 nmol/L for low free testosterone suggested by Bhasin et al, 2006. Therefore, as this population is more elderly, a proportion of patients were obese, with diabetes mellitus and

taking into account the history of chronic disease (HF), then it is plausible that inclusion into the study based solely on total testosterone may be unsuitable and a combination of free and bio-available concentrations should also be used. It could therefore, be argued that based on free testosterone concentration, this sample was not truly testosterone deficient. However, in support of our research design, all other research investigating testosterone deficiency in a cardiac population have used cut-off values based solely on total testosterone (Caminiti et al, 2009, Malkin et al, 2006, Malkin et al, 2004).

Testosterone levels are widely reported to be at their lowest in the early hours of the day before 10:00am (Diver et al, 2003). However, many of the studies assessing diurnal testosterone variation were performed in a younger population with a minority of older participants. In more elderly populations, the diurnal variation in testosterone concentration persists, but is substantially blunted (Crawford et al, 2007). It has been reported that testosterone levels vary markedly throughout a 24 hour period and measuring testosterone levels in the same individual in a 12 hour period cannot be relied upon to make accurate conclusions over androgen status (Spratt et al, 1988). Around 30% of males with low testosterone sampled during the afternoon period have been shown to have normal range testosterone concentration when sampled during the morning hours (Brambilla et al, 2007). Each participant in the current study was advised to attend testosterone screening as early as possible in the morning and were sampled over two visits on separate days with the initial value being used for study inclusion. No patients switched from hypo to eugonadal status following second testosterone measurement and these values were consequently used to advise as to the accuracy of repeated measurements in this population whilst

confirming the initial diagnosis of testosterone deficiency. Bland Altman limits of agreement plots confirm that over the two visits to the clinic there was good agreement between respective measures of testosterone. An important limitation of the sampling technique was that participants were screened at a busy out-patient hospital clinic and this ensured that definitive times of blood collection could not be confirmed.

Exercise intervention duration in HF populations have ranged from 3 weeks up to 24 weeks with periods of maintenance extending up to 1 year, incorporating sessions 2 or 3 times per week. Our ET programme lasted for a period of 12 weeks and incorporated sessions twice weekly. Previous ET programmes in HF have varied by setting (home versus gym supervised), type of exercise modality (cycling, treadmill, Pole Striding, outdoor exercise) and exercise intensity from low level to moderate – high intensity training. In our study, the ET regime involved high intensity cycling interval training followed by endurance related resistance training of the major muscle groups used in everyday activities. The incorporation of high intensity exercise resulted from recent work by Wisloff et al, 2007 who demonstrated greater improvements in aerobic conditioning, endothelial function and levels of inflammation following such a regime in HF patients when compared to more traditional moderate continuous training when performed thrice weekly over a period of 3 months. Although overall duration of exercise programme is the same in our study, recruitment challenges related to time commitments of patients and the amount of travel required to and from the research centre resulted in reducing our planned number of exercise sessions from three to two per week. Most other research has used exercise sessions at least thrice weekly in their interventions in HF patients and as such, it is plausible that greater improvements may have been noted in our

study with an increased number of exercise sessions per week. Combining our reduced number of exercise training sessions per week and the borderline testosterone deficient status when using free and bio-available testosterone, it is possible that greater within and between group interactions may have been noted if patients were defined as hypogonadal with all measures of testosterone and also if there were greater than two exercise sessions per week. As such, the design of future research should aim to increase the frequency of exercise sessions and also be more stringent on the bio-chemical definition of hypogonadism in order to provide an increased opportunity for health outcome improvement in response to the intervention. Any future design however, should be mindful of the challenges presented in this study regarding recruitment and attrition.

The recruitment rate for this study was very low (4%) but similar to that reported for a recent randomized controlled trial of similar community-living elderly people (Forster et al, 2010). Despite this, the characteristics of our patient cohort, including age, BMI, baseline aerobic fitness and quality of life, were comparable to those previously reported for HF patients recruited to exercise trials (Nilsson et al, 2008, Wisloff et al, 2007, Sabelis et al, 2004, Kemps et al, 2008). The observed difficulties experienced in recruitment to the trial may be due to a number of factors.

Patients were initially contacted by post by gathering details from Cardiology clinic patient lists and assessment of eligibility through access to patient notes. It could be suggested that approaching participants via post is a less reliable method of recruitment when compared to other more pro-active methods. Research conducted by Treweek et al, 2009 has assessed the effectiveness of techniques used to recruit

participants to clinical trials. This showed that better recruitment techniques include providing educational material together with study invitations, including video material documenting the health benefits involved in trial participation, providing telephone contact from patients with similar health conditions to encourage recruitment and also providing significant financial remuneration for trial participation. Although this study did not have sufficient funding for the development of video aids, booklets or to provide significant financial remuneration (although travel and parking subsidies were provided), recruitment may have been improved with increased presence by research staff at specialist HF clinics, by using telephone reminders in conjunction with invite letters in a similar manner to Nystuen et al, 2004 who found this method significantly improved participation, or by improved promotion of the research by consulting physicians.

Attrition was also high in our patient cohort (30%), but within the range (0-39%) of that reported in a meta-analysis of physical activity interventions in cardiac patients (Conn et al, 2009). The main reasons for patient drop-out were age-related co-morbidities and infectious disease, consistent with previous studies of elderly HF patients (Lloyd-Williams et al, 2003). A proportion of the participants without significant co-morbidities preventing continued participation dropped out due to time constraints. This was also a major factor involved in the initial recruitment process. The study design involved significant travel to and from the recruiting centre, together with occasional visits, (on top of already scheduled standard care clinic visits) to the sponsoring institution. This significant time-burden was sufficient to deter many participants from entering onto the study, particularly when still in full-time employment. This has also been documented by other researchers who have

showed that tiredness on top of a working week can be a main determinant of drop-out in exercise based interventions in HF patients (Wisloff et al, 2007). During this study, the researchers did provide scheduled exercise training sessions outside of normal working hours but the extra pressure of these on top of a working week was considered too great in some cases.

In order to improve attrition in future trials, it may be beneficial to reduce the commitment of travel to other centres by employing a part home based exercise regime similar to that performed by participants in the BRUM-CHF Study (Jolly et al, 2009). However, although this study significantly reduced drop-out when compared to other exercise trials in HF, there were no significant improvement in health related outcomes from baseline. This may, in part, be due to the reduction in supervision and therefore decreases in training intensity and lack of compliance with individual exercise prescription. Other methods which have been shown to improve attrition in exercise based trials in HF, and may have improved attrition in this study, have included cognitive behavioural strategies and especially motivational interviewing. These strategies encompass goal setting, problem solving, positive feedback, reinforcement and group interaction. Importantly, such strategies were found beneficial in the short-term only (6 months) and ineffective when used in the longer duration (Tierney et al, 2012). In addition, studies have shown that self-efficacy when undertaking exercise to be an important determinant of future adherence (Anderson et al, 2006). These strategies employ peer support, observation methods of other patients with similar conditions performing training and support from family and friends in order to overcome barriers to exercise.

Although attrition was low, compliance to the exercise sessions and injections was excellent (100%) in the 28 patients who completed all assessments. It is likely that the latter was strongly influenced by our flexible approach to organising exercise sessions around patient commitments and contributing to their travel expenses. There were no adverse events resulting from the exercise sessions or injections. Minor local reaction (redness) was noted in the injection site on a small number of occasions. In addition, patients taking Warfarin experienced slightly increased bleeding from the injection site when compared to the other participants. Due to the effects of testosterone therapy on anti-coagulation and blood glucose concentration, there was a possibility that Warfarin and / or anti-diabetic agent dosage may have been altered as part of patients' regular clinic regimen. All patients who were taking Warfarin attended for an additional INR measurement at around 5-7 days following their first injection, independent of their treatment allocation and, as these potential side effects to the therapy were explained to each participant prior to recruitment and were also detailed in the study information leaflet, it is feasible that changes made to their regular medical therapy may have un-blinded patients to their treatment allocation during the trial. At the initial exercise training session of each new week, patients were screened in detail regarding their symptoms, side-effects and for any alterations to their medication. It should be noted that no changes were needed to either Warfarin or anti-diabetic medication dosage as part of this trial for patients receiving either testosterone therapy or placebo. Patients in both the treatment and placebo groups attended for extra blood analysis at 5-7 days, therefore there was no discrimination based on group allocation during the study and hence no un-blinding at this stage.

Testosterone is a widely used steroid and has received media coverage for its use in the sporting arena as a successful doping method to increase muscular strength, endurance and aggression. As such, many participants who took part in the trial were able to use their own knowledge of the suspected effects of the treatment, together with the information provided in the study leaflet, to speculate as to the nature of their allocation during the trial. This treatment effect has been previously reported by Schultz and Grimes, 2002 who suggest that treatment side-effects or significant clinical improvements are both potential factors that can un-blind both participants and researchers to group allocation. During our trial, many participants at some point commented that they were probably receiving testosterone and not placebo. However, the same participants at other times were not convinced re their group allocation. There were no significant side-effects related to testosterone therapy during the trial and as such, these could not be used to suggest a group allocation. In addition, similar improvements in most outcomes were seen in both treatment and placebo groups suggesting that the addition of exercise therapy to testosterone treatment may have masked the effects of testosterone treatment.

Although caution needs to be applied when interpreting preliminary outcome data from feasibility trials, improvements in peak power output in the treatment and placebo groups (17% v 12%, respectively) were accompanied by improvements in shuttle walk performance (18% v 19%). Furthermore, the treatment group showed improvements from baseline in peak oxygen uptake (21%), time to minimum tissue oxygenation and leg strength, which were not apparent in placebo. The observed improvement in aerobic exercise capacity was similar to that reported previously for

HF patients following programs of aerobic exercise lasting 4-24 weeks (Bellardinelli et al, 1995, Dubach et al, 1997, Jette et al, 1991) but greater than that reported for younger HF patients after a similar 26-week program of combined aerobic interval exercise and resistance training (Senden et al, 2005, Sabelis et al, 2004) Considered together, our results show that male patients with low testosterone status achieved similar exercise-induced improvements in aerobic exercise tolerance as other patient cohorts who were not classified as androgen deficient.

The observed greater improvements in aerobic exercise tolerance, muscle oxygenation and leg strength in the treatment group when compared to placebo were non-significant using ANCOVA for between group comparisons. This might simply reflect the small sample size, but we cannot exclude the possibility that synergy between exercise and testosterone treatment is short-lived. Indeed, although supplementation at similar doses has been shown to acutely improve muscular strength, aerobic capacity and time to ischemic threshold in men with HF (Malkin et al, 2006, Malkin et al, 2004) and CAD (Pugh et al, 2004) supra-physiologic levels of testosterone shortly after administration are often followed by sub-physiologic levels towards the end of the dosage cycle (Nieschlag et al, 2004). Our study supports this, as the testosterone levels assessed two weeks after the final injection were similar to those measured at baseline in the treatment group. While higher dosage testosterone treatment might be needed to markedly augment the effects of exercise and testosterone therapy in this patient group, higher dosage testosterone has been associated with elevated risk of cardiovascular events in a recent study of frail elderly males with mobility limitations (Basaria et al, 2010). This finding however, is not consistent with previous meta-analyses (Calof et al, 2006, Haddard et al, 2007,

Fernandez-Balsells et al, 2010) and might be a reflection of the small sample size and characteristics of the study population. Further trials with larger numbers of participants are needed to establish whether higher dosage supplementation when administered in the clinical setting is safe and can augment the improvements in aerobic exercise capacity and muscular strength observed in this study. In order to alleviate the pulsatile nature of testosterone injections, other preparations are available that may provide a 'long-acting' effect. Testosterone dermal patches have traditionally provided a response that mimicks the natural diurnal variation of testosterone over a 24 hour period (Bhasin et al, 2006). These patches can be applied daily or can even be long acting therefore reducing the need for daily application. Perhaps the major implication of the use of such patches is the previously observed high rate of skin reactions and therefore intolerance to the treatment (Malkin et al, 2004).

Physiological mechanisms for the noted improvements in aerobic capacity, tissue oxygenation and muscular strength and endurance, although non-significant, have been suggested in the literature. In HF, there is derangement of the glucose-insulin axis with studies reporting up to 43% of patients manifesting disorders of glucose metabolism (Kontoleon et al, 2003). Research has shown that low levels of serum total testosterone and SHBG are risk factors for development of the metabolic syndrome in males (Muller et al, 2005). Patients with HF and insulin resistance have an impaired ability to promote glucose transport into skeletal muscle cells and adipose tissue. Moreover, research has showed that impaired insulin activity inversely correlates with severity of HF, relates to disorder of skeletal muscle physiology, facilitates reduced muscle mass and can also influence prognosis (Swan

et al, 1997 and Doehner et al, 2002). Further reports have also suggested that insulin resistance may further deteriorate cardiac performance in HF, due to cellular disruption of cardiac metabolism (Chang et al, 2005). Testosterone supplementation towards the physiological range has been shown to significantly improve fasting blood glucose and insulin levels, together with significant improvements in cholesterol level and waist to hip ratio (Kapoor et al, 2007). Mechanistically, cultured adipocytes and skeletal muscle cells incubated with low dose testosterone therapy have demonstrated improved regulation of GLUT4 and insulin receptor 1 (Chen et al, 2006). Furthermore, Sato et al, 2008 have showed that testosterone therapy in a rodent model can facilitate phosphorylation of protein kinase B and C, key pathways in insulin receptor signalling pathways for the regulation of GLUT4 translocation. The same authors have also suggested that testosterone treatment can influence enzymes related to the glycolytic process. Increased activity of phosphofructokinase and hexokinase has been discovered in cultured rat skeletal muscle cells following administration of testosterone. Physiological replacement of testosterone may therefore indirectly correct the aforementioned abnormalities at skeletal muscle ultrastructural and cellular level to promote increases in walking performance.

Other research has also identified that changes in muscle performance may explain improvements in exercise capacity in HF patients receiving testosterone replacement therapy (Caminiti et al, 2009). Maladaptive muscle fibre atrophy, increased abundance of type II glycolytic fibres and reduced oxidative metabolic capacity have been shown to correlate with poor exercise tolerance and increased VE/VCO₂ slope

in HF (abnormal enhanced muscle ergoreflex) (Hambrecht et al, 1995). It has been hypothesised that a representative improvement in overall muscle function may decrease the abnormal muscle metaboreflex contribution to the ventilatory response to exercise, reducing the VE/VCO_2 slope and therefore improving exercise tolerance (Caminiti et al, 2009). To further reinforce this hypothesis, work in non-HF animal models has shown that anabolic supplementation at physiological doses is capable of accelerating fast to slow twitch muscle fibre type conversion and also increase the number of type I oxidative fibres, co-existent in improving the relative oxidative capacity of skeletal muscle (Ustunel et al, 2003). In relation to this, muscle oxygenation assessment in our study, using NIRS, demonstrated improved time to minimum oxygen saturation reinforcing the aforementioned concepts that there is an improved ability of the skeletal muscle to uptake and utilise oxygen to power muscular work. Furthermore, Pugh et al, 2004 have also suggested that microscopic changes in muscle metabolism may explain the increase in exercise tolerance noted in their earlier study.

During this study, post intervention blood samples were not taken for fasting insulin or glucose assessment. Hence, the impact of insulin resistance on exercise tolerance in our cohort of patients with low testosterone status is unknown. Additionally, detailed microscopic analysis of muscle fibre types or invasive muscle biopsy was not performed as part of our study and therefore no information regarding a switch of fibre types or increase in key energy related enzymes or molecules could be elucidated from our data. These facets clearly warrant further investigation and other studies should attempt to provide a more holistic approach to skeletal muscle structure and function. In addition, NIRS assessment of muscle oxygenation in our

study demonstrated poor reproducibility (up to 19% error between repeated measurements) mainly due to the difficulties experienced in completely blocking artificial light sources and the mechanics of cycle ergometry with a probe placed on the thigh muscle. Our patients were generally overweight (mean 90.2 ± 7.4 kg for treatment and 93.6 ± 11.1 kg for placebo with BMI of 28.7 ± 1.8 versus 30.1 ± 3.5 respectively) and subcutaneous fat has been shown to have a large impact on NIRS signal (Mc Cully and Hamaoka et al 2000). These authors suggest that increase in adipose tissue results in poor absorption, higher returning signals and thus less signal change in experimental conditions. Mc Cully et al, 1997 have argued that for clinically reliable measurements, in their cohort of elderly patients with vascular disease, BMI measurements would need to be certainly less than 32, a value close to those observed in our present study. The lack of reproducibility and difficulties observed in measurements suggest that perhaps other techniques should be utilised to provide a more accurate assessment of muscle oxygenation in future studies.

Small reductions in body mass and BMI were observed in the treatment and placebo groups. Previously, Malkin et al, 2006 reported no change in BMI in hypogonadal HF patients receiving chronic testosterone supplementation or placebo control, whereas other studies have reported increases in body mass and BMI in HF patients and other clinical groups receiving testosterone treatment (Caminiti et al, 2009, Johns et al, 2004, Johansen et al, 1999). Hansen et al, 1980 has linked testosterone supplementation with enhanced nor-adrenaline stimulated lipolysis. This is an important link because catecholamines are the main lipolysis regulating hormone in males and regulate adipocyte lipolysis through the activation of adenylate cyclase to produce cAMP. Protein kinase A activation by cAMP results in stimulation of

hormone sensitive lipase and therefore facilitates accelerated lipolysis and increased breakdown of triglycerides to reduce subcutaneous fat (Arner , 2005). Additionally, plasma concentration of bio-available testosterone inversely correlates with abdominal adipose tissue lipoprotein lipase (Ramirez et al, 1997). This enzyme has been suggested to play a role in the pathogenesis of obesity as it resides on the extra-cellular surface of adipocytes and hydrolyses circulating triglyceride rich lipoproteins to fatty acids which then become esterified and stored as triglyceride (Eckel, 1989). Marin et al, 1995 showed that following prolonged testosterone supplementation, there was a marked decrease in lipoprotein lipase activity and hence significant reductions in triglyceride storage in abdominal subcutaneous adipose tissue. Additionally, it is widely appreciated that ET facilitates overall weight loss and it is possible that an increase in total energy expenditure during the study (i.e. two 60 minute, supervised exercise sessions per week) could facilitate enough calorific deficit to promote weight loss. Hence, the reductions in body mass and BMI that we observed may have resulted from significant alteration in the amount and function of the proteins / enzymes responsible for lipolysis or fatty acid breakdown or more simply by a decrease in body fat percentage associated with increased energy expenditure attributable to the supervised exercise sessions or free living physical activity (CHAMPS questionnaire data) but in the absence of body fat measures or biochemical analysis of subcutaneous adipose, this could not be verified.

No changes in circulating levels of NT pro-BNP or the inflammatory markers were observed in either group, except for a small decrease in TNF- α from baseline in the placebo group. Interestingly mean NT pro-BNP concentrations were elevated in both

groups pre and post intervention. All patients entering onto this trial were clinically stable HF patients with no recent change in medication or increase in severity of disease classified by change in NYHA score. However, all patients in this study demonstrated significantly impaired LV EF% (mean $21.3 \pm 9.7\%$ for the treatment group and $28.0 \pm 6.0\%$ for the placebo group), there was overall evidence of increased left ventricular end-diastolic volume (118.04 ± 36.12 treatment versus 131.64 ± 32.67 placebo), and evidence of increased left atrial volume (72.18 ± 14.12 mls treatment versus 69.59 ± 18.74 mls placebo), diastolic E/e' was borderline or normal and there was evidence of reduced longitudinal systolic velocities measured by TDI peak s' wave. It is feasible that some of these parameters could contribute to an increase in NT pro-BNP. Importantly, Maeder et al, 2010 have noted that lower EF%, lower peak longitudinal s' velocities and increased LV end-systolic wall stress are all important determinants of increased levels of NT pro-BNP. Furthermore, the same authors state that LV contractile dysfunction per-se is not representative of a mechanical load that could contribute to BNP gene expression and it is probable that LV end systolic wall stress is a more likely regulator of this. LV end systolic wall stress could not be determined accurately from the measurements obtained during this study as it requires more invasive cardiac assessment. However, reduced longitudinal velocities could play a role in the elevated values obtained. S' is dependent on afterload and as such may represent structural abnormalities of the myocardium such as fibrosis (Borlaug et al, 2007). In particular, s', in addition to NT pro-BNP, has been shown to independently predict mortality in community based studies (Mogelvang et al, 2009). The paracrine effects of BNP have been shown to inhibit myocardial fibrosis in a rodent model (Walther et al, 2003). Therefore a plausible theory for increased BNP expression in this study may be a

physiological attempt to impair the fibrotic process in the presence of a reduced s' velocity. As no direct markers of cardiac fibrosis were measured during this study, this theory can only be speculative and warrants further investigation.

Kim and Januzzi (2011) suggest applying caution to elevated NT pro-BNP levels. Although elevated levels are certainly important prognostic indicators of acute and /or deteriorating HF, other factors may contribute to increases in concentration. Pulmonary hypertension, valvular disease, atrial fibrillation, advancing age and renal dysfunction may all contribute to increasing NT pro-BNP levels. (Kim and Januzzi, 2011). Pulmonary artery pressure and renal function were not measured during the course of this study so could not be commented upon. However, patients were excluded from analysis if there was presence of significant valve disease and only a minority of patients had documented persistent atrial fibrillation. It has been mentioned previously however, that this was a more elderly study population which may, in part, explain the elevation to NT pro-BNP. Other researchers have observed considerable (up to 30% co-efficient of variation) difference in NT pro-BNP in stable HF patients at yearly follow-up (Schou et al, 2007). This is further reinforced by Bruins et al, 2004 who noted significant within day, day to day and week to week coefficient of variability in NT pro-BNP measurements of 9, 20 and 35% respectively. As only pre and post samples were analysed, we are unable to comment on variation to levels as part of this study.

The lack of observed change in NT pro-BNP in the present study contradicts the findings of other studies investigating ET in HF. Previous work has demonstrated significant reductions in NT pro-BNP as a result of aerobic ET when compared to no

training in a HF population (Giallauria et al, 2008, Passino et al, 2008). Previous studies have also provided evidence that aerobic ET can attenuate and/or improve adverse LV remodelling in HF (remodelling which can occur as a result of increased inflammatory markers such as TNF- α) (Giannuzzi et al, 1997, Haykowski et al, 2007) whereas the evidence for changes following testosterone therapy is equivocal (Malkin et al, 2006, Caminiti et al, 2009). Aerobic ET has also been reported to reduce levels of TNF- α in HF patients (Larson et al, 2001, Adamopoulos et al, 2001) and there is evidence that testosterone therapy favourably affects serum levels of inflammatory markers in patients with established HF and hypogonadism (Malkin et al, 2004, Malkin et al, 2004). While the exercise and testosterone stimulus in the treatment group may have been insufficient to evoke changes in cardiac parameters and inflammatory markers, it is also likely that the study was underpowered for detecting changes in these outcomes.

In relation to no significant differences in inflammatory markers or NT pro-BNP in this study, there were no significant differences noted with respect to cardiac structure or function. In the current literature there are equivocal results regarding the effects of ET on central cardiac structure and function. Some studies have shown marked improvements in EF (Myers et al, 2002, Gianuzzi et al, 2003). Conversely, others have demonstrated no improvement and perhaps detriment to overall EF (Bellardinelli et al, 1995 and 1996, Klocek et al, 2005). ET has been shown to improve diastology via an increase in early filling rates and more favourable E:A ratio using mitral valve Doppler (Stolen et al, 2003, Cheuk-Man et al, 2004). In addition, testosterone supplementation has been shown to increase overall LV length (Malkin et al, 2006) but overall cardiac assessment in testosterone studies has been

fairly limited. There is evidence in rodent models that testosterone supplementation can improve EF due to the marked reduction of circulating inflammatory markers responsible for cardiac apoptosis (Zhang et al, 2007), a finding not yet observed in human models or in those of the present study. The lack of significant change noted in this study may simply reflect an absence of synergy between testosterone therapy and cardiac parameters. However, the lack of change may also be reflected by a small sample size (under-powered) or perhaps an inadequate assessment of cardiac functional parameters in this study.

There are several limitations to the echocardiographic parameters used during this study for the assessment of LV systolic and diastolic function. For LV systolic function, clinical trial echocardiographic guidance suggests that measurement of LV ejection fraction based on LV volumes obtained by the method of discs should be performed (Gottdiener et al, 2004). This technique minimises but not eliminates the mathematical assumptions evident in other methods and also allows for corrections based on LV geometry. However, limitations arise when the apex is fore-shortened, the endocardium is inadequately viewed and there is limitation by reliance on only two LV planes. The literature quotes Biplane Simpsons EF reproducibility of $\pm 7\%$ (Himmelman et al, 1988) and test-re-test reliability of $\pm 5\%$ (Gottdiener et al, 1995). In this current study, we quoted a 9% measurement error in EF by the same operator on two different occasions. This error and the documented limitations in the literature may be a factor in the lack of statistical change noted in our results. Longitudinal velocity assessment using PW Doppler can also be limited by a number of factors. PW Doppler assessment of tissue movement is only able to provide information regarding a specific point of the myocardium determined by sample

volume positioning, components perpendicular to the ultrasound beam remain unknown and there is significant angle dependency. TDI velocities may also be influenced by global heart motion, movement of adjacent structures and also blood flow (Mor-Avi et al, 2011). During this study we demonstrated good reproducibility of the TDI peak s' velocities when measured on separate occasions (measurement error around 5%). Our TDI velocities were measured at end-expiration in order to reduce global heart motion. It is important to recognise the afore-mentioned limitations of the technique when evaluating these results. In order to improve accuracy and reliability in future studies more advanced 2D echocardiographic techniques can be utilised. For instance, more detailed information regarding cardiac deformation using speckle tracking echocardiography (unable to be performed in the present study due to limitations of equipment) and use of contrast agents to effectively assess LV endocardial borders can be undertaken to enhance the quality of data. Additionally, other techniques have been shown to provide more accurate assessment of LV volume and EF such as 3D echocardiography or cardiac MR. Future studies should attempt to use these techniques in sufficiently powered samples in order to more accurately address the issue of systolic function in this population.

In the present study, LV diastology was assessed using LA diameter, LA volume and TDI E/e'. In order to provide an improved assessment of LV diastolic function, more parameters can be assessed in any future work. Although guidelines for the assessment of diastolic function in clinical trials suggest using E/e' as this parameter seems the least subject to inter/intra-observer variability (Gottdiener et al, 2004), more recent recommendations have suggested the use of additional parameters to

increase the accuracy of results (Nagueh et al, 2009). For instance, more detailed assessment of LA function throughout the cardiac cycle due to its role in modulating ventricular filling through the reservoir, conduit and contractile properties, assessment of pulmonary artery systolic and diastolic pressures, more detailed assessment of MV inflow properties (e.g. isovolumetric relaxation time, E/A ratio, deceleration time as a minimum), assessment of pulmonary venous flow, analysis of flow propagation velocities and detailed deformation measurements of the LV - particularly the untwisting phase may have yielded further important information regarding diastolic function. Furthermore, in clinical trial participants with significantly impaired LV systolic function (EF% <40%), MV E:A ratio, isovolumetric relaxation time and MV deceleration time have been shown to provide an excellent indication of filling pressures (Gottdiener et al 2004). Assessment of diastolic parameters in this study is further compromised by a small proportion of patients in atrial fibrillation at time of echocardiographic examination (13% treatment and 15% placebo groups). Atrial fibrillation may cause artificial increases in LA volumes together with artificial remodelling of the LA independent of filling pressures, loss of atrial contractile properties and significant beat to beat variability in measurements obtained. In order to counteract this last point, all measurements in patients with atrial fibrillation were repeated over at least 5 cardiac cycles. Studies are needed on sufficiently modern echocardiographic devices to address these imaging limitations in order to provide more accurate and comprehensive assessment of diastole in this clinical population.

Early work conducted by Malkin et al (2006) has shown that testosterone acutely dilates resistance vessels in patients with HF, supported by the earlier research of

Pugh et al, 2003. There was no change noted in FMD as an indirect marker of endothelial function in either group following the intervention in our study. Pugh et al, 2003 observed that testosterone therapy acutely reduces peripheral vascular resistance in males with stable HF. Additionally, ET has also been shown to improve the vasodilatory properties of resistance vessels by evoking changes in endothelial function together with LV pre and afterload (Gokce et al, 2002, Walsh et al, 2003, Hambrecht et al, 2003). Research investigating the effects of ET in HF has also shown beneficial improvements in flow mediated dilatation response (Gokce et al, 2002, Walsh et al, 2003) particularly in the arterial territory of frequently exercised muscles. FMD assessment using ultrasonography during this study demonstrated poor reproducibility and this together with a likely underpowered population may explain the lack of significant change in parameters of cardiovascular structure and function.

Atrial fibrillation has been shown to impair vascular responsiveness to vascular shear stress and the loss of cyclic stretch of atrial endocardial cells (the atria being an important endocrine organ in the modulation of vascular function) may result in attenuated expression of nitric oxide synthase by the increased atrial production of superoxide and resultant oxidative injury from disorganised atrial cell contraction (Guazzi et al, 2006). In fact, restoration of sinus rhythm has been shown to improve endothelial function in patients with lone AF and also partially in patients with AF and other co-morbidities such as hypertension and diabetes (Geneveley and Pfeffer, 1988). A minority of patients in the present study had atrial fibrillation at time of FMD assessment. However, a greater number of patients in both groups had a significant past history of paroxysmal AF and this coupled with evidence of

increased left atrial volumes may contribute to the lack of differences observed both within and between groups.

Improvements from baseline were observed in several SF-36v2 domains for treatment group together with reductions in BDI and ADAM scores. However, significant between group effects were only observed for the SF-36v2 domains of bodily pain and vitality in favour of the placebo group. In placebo, there was also an increase from baseline in the physical functioning domain of the SF-36v2 but no change in BDI or ADAM scores. Importantly, many facets examined within the ADAM questionnaire overlap significantly with the clinical symptoms of HF, (e.g. reduced libido, lack of energy, decreased strength and / or endurance, depressive symptoms, tiredness and lack of concentration). This may partially explain the lack of observed difference in ADAM score between groups, and as such, suggests that this questionnaire may not be as effaceable in the setting of testosterone deficiency and HF. Aerobic exercise has previously been shown to evoke improvements in total SF-36 score (Smart et al, 2007, Miche et al, 2009) and Minnesota Living with Heart Failure Questionnaire score (Smart et al, 2007, Tyni-Lynn et al, 1998, Jankowska et al, 2008) in male HF patients, though superior changes have been reported after high-intensity interval exercise (Nilsson et al, 2008, Wisloff et al, 2007). Evidence for the effect of exercise training on depression scores is equivocal, with improvements in younger patients (Smart et al, 2007) and no change in older patients (Miche et al, 2009) being previously reported. Furthermore, testosterone therapy has been shown to improve New York Heart Association HF classification, without changing depression index or HF symptom score (Malkin et al, 2006). Our results

suggest that testosterone supplementation might augment the health benefits of exercise in terms of depression and androgen deficiency symptoms in male HF patients with low testosterone status. However, the treatment group had greater perceived health impediments (as evidenced by the MLHFQ scores) and higher depression scores at baseline. As the effects of exercise on these health outcomes might be more pronounced in patients with lower initial perceived health status and there is considerable overlap of ADAM and MLHFQ symptoms, the true impact of testosterone therapy during exercise rehabilitation needs to be confirmed in larger-scale randomized controlled trials.

This is the first study to provide a qualitative evaluation of exercise training in elderly male HF patients with low testosterone status. Seven emergent themes emerged from the qualitative data analysis, namely confidence to continue exercising in the future, role in giving advice to patient's with similar conditions, perceptions about the exercise intervention, perceived roles of medical professionals, motivation during the trial, negative effects and perceived health benefits.

Confidence to continue exercising following the intervention was an emergent theme with both positive and negative considerations. Exercise that can be undertaken within the patients' own home was considered important. Participants were less keen to attend public exercise facilities and the knowledge gained on how to exercise safely and intensely in their own environment was important to them. In relation to this point, other participants suggested that without the motivation of a 'strict' appointment time as part of a supervised research study, they may soon return back

to their old lifestyle despite the noticeable improvements brought about by ET. Previous research has identified significant improvements in physiological function and quality of life with home-based exercise programmes in cardiac patients (Chien et al, 2008, De Mello-Franco et al, 2006). However, scientific literature has shown that adherence to home based ET is not comparable to supervised programmes, with reported 60-70% adherence initially, declining consistently throughout the intervention period (Hambrecht et al, 2000). Reduced adherence associated with home-based ET may result in smaller training effects and hence, less noticeable improvements in quality of life when compared to supervised ET (Chien et al, 2008).

Patients indicated that they could play an important role in health promotion by encouraging the use of exercise training to patients with HF. They were keen to suggest that the health service could utilise their knowledge to help educate other patients on the benefits of ET in HF. Research using HF patients as a source of education to peers is sparse. However, one study has suggested that group education by peers can produce a positive effect on coping, confidence, outlook and spousal relationships in a HF population (Stewart et al, 2001). The main drivers to improvement were described as patient input, co-facilitation, similarity of group members and the provision of information and support.

Perceptions of the exercise intervention were mainly centred on how it could be improved by increasing the variety of exercise equipment, the addition of lifestyle advice (education sessions), increasing the intensity of the sessions and progression onto other ET programmes at the end of the intervention period. Patients exercised at pre-determined intensities as part of an aerobic interval training programme (Wisloff

et al, 2007) and had limited choice of exercise intensity or modality. Lifestyle advice has become an integral feature of ET in cardiac rehabilitation (Blue et al, 2005) and has been shown to improve patient outcome in HF patients (Mc Allister et al, 2004). Hence, a more challenging exercise programme, incorporating lifestyle advice, a broader range of exercise training modalities, and a clear plan for self-directed exercise at the end of the period of supervision would have been more attuned to the specific needs of our patient cohort.

Patients observed that other medical professionals were vital in educating patients about the benefits of ET and testosterone therapy. However, there were varied opinions regarding the ability of medical professionals to perform this role. Specialist HF nurses are now employed in most major cardiac centres and their role is to co-ordinate a multi-disciplinary team of medical professionals to care for patients in and outside of the hospital setting. The role of HF nurses is to evaluate the learning needs of patients, assess how best to provide the information and under what timescales. In addition, HF nurses have the skills and motivation to provide individualised, evidence-based education to HF patients (Blue et al, 2005). Additional educational resources such as books, leaflets, videos, web-pages, computer packages have also been used successfully in patient education, particularly when combined with tele-monitoring of patient progress (Louis et al 2003). Despite this, qualitative perceptions from our cohort of elderly males with low testosterone status suggest that more could be done to promote the benefits of exercise and provide structures to facilitate engagement.

Key motivational factors for adherence to the programme were the perceived increases in strength and endurance, together with a description of significant weight loss. Scientific literature supports the use of ET and testosterone therapy for increasing muscular strength and endurance in HF patients (Pugh et al, 2004, Caminiti et al 2009, Jankowska et al 2008, Malkin et al 2006), and our results suggest that reinforcing these health benefits to patients could be important for continued participation. Social support was also important to programme adherence. The main support network comprised family members, other participants and health professionals involved in the research study. This support network has been described in the scientific literature. It has been shown previously that quantity of contact and social support from researchers, health professionals and fellow exercise participants during an intervention influences the impact that the intervention has on functional and psychological quality of life dimensions and adherence (Burke et al, 2006). Furthermore, intention to engage in physical activity, self-efficacy for physical activity and magnitude of the health effects experienced from engraining in a physically active lifestyle are all associated with the level of support from important others and family (Carron et al, 1996).

Many of the patients had not previously undertaken regular physical activity. Negative physical side effects were an emergent theme, however, no side effects were considered serious adverse events and most were related to muscle soreness following strength training. Tiredness was also a factor in patients that were combining exercise sessions with a full working week. Previous research in an elderly HF population supports our finding that high intensity aerobic interval training (Wisloff et al 2007) and supervised strength training (Radzewitz et al, 2002)

can be safely implemented in HF patients without observing serious adverse events. However, HF symptoms such as tiredness can be a main determinant of drop-out from ET training interventions (Piepoli et al, 2011). Future research should be mindful of the potential for negative health effects and include strategies to address them.

Patients experienced health benefits from their engagement with the intervention, consistent with previous studies (Pugh et al, 2004, Caminiti et al 2009, Jankowska et al 2008, Malkin et al 2006). Perceived improvements in endurance capacity, muscular strength, weight loss and overall quality of life were important, as they enabled patients to increase their daily activities and consider health club membership. Many studies have shown that regular ET in HF results in significant gains in aerobic exercise capacity (Flynn et al, 2009, McKelvie et al, 2002, Hambrecht et al, 2000, Wisloff et al, 2007), local muscular performance (Smart et al, 2007, Flynn et al 2009) and quality of life (Jankowska et al, 2008, Larsen et al, 2001, Wisloff et al, 2007, Radzewitz et al, 2002). Our results show that ET is a valuable therapeutic tool to increase confidence, physical activity levels and important health outcomes in HF patients with low testosterone status. Furthermore, patient perceptions of improved health status appear to be an important motivational factor for continued participation in ET

Analysis of the focus groups should be interpreted in the context of some limitations. The views expressed are from 24 males with HF and testosterone deficiency who consented to be randomised into a clinical trial. Therefore, these views may not be representative of all males with HF and low testosterone status and cannot be

extrapolated to female HF patients. As low testosterone status is prevalent in up to 25% of elderly male HF patients, more research aimed at investigating adherence, attrition, motivational characteristics and changes key health outcomes resulting from programmes of ET is warranted. Finally, 12 weeks may be considered a relatively short duration for a therapeutic interventions and more valuable insights may be gained from interventions of longer duration.

In summary, this randomized controlled double-blind feasibility trial showed that testosterone therapy during a program of exercise rehabilitation is feasible in elderly HF patients with low testosterone status. We also showed that elderly male HF patients with low testosterone status can tolerate high-intensity aerobic interval training and our data show that these patients can experience physiological benefits from participation. Additionally, testosterone supplementation induced some improvements in aerobic fitness, leg strength, depression and SF-36v2 quality of life domains that were not observed in the placebo group. While being mindful of the challenges encountered during recruitment and the observed level of attrition, some of our preliminary results provide a solid foundation for a larger-scale, multi-centred randomized controlled trial.

Qualitatively, ET is well tolerated and induces important perceived health benefits in elderly male HF patients with low testosterone status. Perceived health benefits were associated with the social experience of exercising with other participants and noticeable improvements in functional capacity and/or weight loss. Social support and the health benefits gained were important motivating factors for adherence to the ET programme. Knowledge gained on the safety of exercising with HF enabled

some patients to become more physically active in their home environment. However, there were some concerns that adherence to ET may decline in the future due to a lack of provision following the intervention. Future interventions for this patient group should be designed to optimise adherence and long-term participation in ET, giving consideration to the variety of exercise modalities available, the addition of lifestyle advice and education materials and the provision of similar ET programmes in the community supported by qualified exercise instructors.

Chapter 6: Feasibility and power calculation for phase II efficacy trial.

For scientific rigour and ethical considerations, the sample size for a phase II efficacy or phase III trial needs to be carefully considered, striking a balance between both clinical and statistical factors. Phase II efficacy and III RCTs should be based on clinically important changes in key health outcomes, whilst being mindful of patient attrition data that has been observed in a previously executed phase II trial (Altman et al, 2001). Key prognostic outcomes for HF patients with low testosterone status are exercise capacity as assessed by the ISWT and peak oxygen uptake ($\dot{V}O_2$) (Malkin et al, 2006, Caminiti et al, 2009, Jankowska et al, 2009). Based on the outcomes for ISWT and peak $\dot{V}O_2$ in this study, the following can be calculated:

From the 12 weeks intervention, the data distribution for changes in shuttle walk performance was skewed. As a result, natural logarithm transformation was performed. Following transformation, change ratios were obtained. The log transformed change ratio for each group was 0.152 for testosterone treated and 0.166 for placebo corresponding to percentage increases of 16 and 18% from pre to post. The common log transformed SD for change was 0.197. Consequently, the required phase III RCT sample size is estimated at 1890 participants per group. Given a 30% dropout rate in the present study over the course of the intervention, this would therefore require a cohort of 4914 (2457 in each group) patients in order to detect significance at α level of 0.05 with 80% power.

The changes noted following the intervention in peak $\dot{V}O_2$ were 3.19 for treatment and 1.79 for placebo, a difference of 1.4. The common standard deviation for change

was 3.45. Therefore, the required phase III RCT sample size is estimated at 97 participants per group. Given a 30% dropout rate in the present study over the course of the intervention, this would therefore require a cohort of 252 (126 in each group) patients in order to detect significance at α level of 0.05 with 80% power.

These sample size calculations are based on clinically important changes in the key health outcomes (Malkin et al, 2006, Caminiti et al, 2009 and Jankowska et al, 2009) taken from data presented in the current study. Given that the primary outcome measure (ISWT) calculation suggests a sample of 4914, a multi-centre phase III trial approach is perhaps the best way to ensure adequate patient recruitment for such a trial. Given that recruitment is difficult to such a trial and also the requirement of a large cohort it is advisable that any future trials should be powered to use peak $\dot{V}O_2$ as a primary outcome measure in replacement of ISWT. It could also be argued that utilising peak $\dot{V}O_2$ as a primary outcome measure is potentially suitable for a single-centre trial dependent upon the size of the individual heart failure service.

Chapter 7: Summary and future research.

Study 2 was designed to build upon previous research that has demonstrated reduced exercise capacity and worse prognosis in HF with testosterone deficiency and also research suggesting the positive effects of ET (Jette et al, 1991, Coats et al, 1992, Smart et al, 2007) and testosterone supplementation (Malkin et al, 2004 and 2006) in HF. The aim of the study was to assess the feasibility of a combined ET and testosterone therapy intervention for the first time in males with low testosterone and stable HF. In addition, the rationale for the study is further underpinned by study 1 which details for the first time significant differences in exercise capacity and quality of life in males with HF and low versus normal testosterone values.

For feasibility research, the principle findings must relate to recruitment and study design as well as any observed changes in the outcome measures assessed. The major findings of the study indicated that recruitment to such trials is difficult. This is probably due to the nature of the population sampled rather than related to the intervention itself. Patients with HF often have numerous co-morbidities which may prevent them from exercising, together with the psychological notion that HF may not allow sufficient tolerance to exercise interventions. As testosterone levels decline with age, it follows that many of the eligible participants are elderly. As such, it also follows that an elderly population will have numerous co-morbidities that prevent them from exercising adequately. Commitment to prolonged interventions is also an issue with older participants unwilling to travel to the research centre and younger participants unable to attend due to work commitments. Although recruitment was difficult, all patients who completed the intervention attended every ET session and

there was 100% compliance to the IM testosterone injections. This demonstrates that a flexible approach to such an intervention by the investigators is key to ensuring that patients maintain compliance throughout such an intervention.

Testosterone therapy together with ET resulted in significant improvements in peak $\dot{V}O_2$ and leg strength following the intervention when compared to baseline values. These changes were not observed in the EP group. In addition, testosterone treatment also resulted in improvements in many SF36 domains, reduced depression scores and reduced androgen deprivation scores which were not observed in the EP group. Both groups demonstrated similar improvements to peak power output during cycle ergometry, ISWT distance and reductions to BMI.

It can be concluded that ET and testosterone replacement therapy in HF patients is feasible and safe. The observed improvements in the treatment group not apparent in the EP group provide a solid foundation for future research. The planning of future trials should take into account the difficulties in recruitment, patient perception of this intervention and also the required power to obtain statistically meaningful results.

Further research should aim to clarify if ET and testosterone replacement therapy can provide significant benefits over placebo in the same population and this can be achieved from the foundation work of this feasibility trial. Furthermore, these studies should aim to address the physiological basis behind observed improvements in

exercise capacity and muscular strength using more detailed physiological testing which demonstrates good reproducibility in this population. Longer duration studies using a variety of testosterone preparations should also be undertaken in order to fully comprehend the best mode of administration and to determine the safety of long-term physiological testosterone replacement.

Chapter 8: Overall thesis summary and conclusions.

This chapter provides a summary and conclusions for the thesis as a whole and, for the purposes of clarity, is sub-divided into the following sections: overview of findings and contribution to knowledge; testosterone and HF / combined ET and testosterone therapy - take-home messages; and, implications for clinical practice.

8.1: Overview and contribution to knowledge.

Section 1 of this thesis was designed to ascertain for the first time if there are significant differences in exercise capacity, quality of life and detailed indices of cardiac structure and function in male HF patients with low and normal range testosterone. Previously, two small studies have used correlation and regression models to identify relationships between peak $\dot{V}O_2$, right and left ventricular function and total testosterone level (Bocchi et al, 2008 and Jankowska et al, 2009). This study aimed to build upon this initial research with a more robust study design and integration of more detailed aspects of quality of life and cardiac structure and function, together with a more accurate definition of hypogonadism using free and bio-available testosterone. The results of this study indicated that indeed there was significantly improved exercise capacity together with significantly improved physical and mental quality of life scores in males with HF and normal testosterone when compared to males with HF and low testosterone status. No change was noted in detailed echocardiographic assessment of cardiac function, which may be due to an under-powered design, but may also suggest that the mechanisms for improved exercise capacity in patients with normal testosterone concentration and HF may be

at the levels of the vasculature and / or skeletal muscle. Future research should build upon this notion and provide a more detailed insight into these mechanisms.

Section 2 of this thesis was designed to build upon the notions that testosterone deficiency is an important determinant of poorer outcome in HF and also the previous contradictory research investigating the effects of testosterone supplementation in males with testosterone deficiency and HF. The purpose of this study was to assess the feasibility of a combined exercise training and testosterone supplementation intervention on more detailed physiological and psychological outcomes than had previously been published in males with low testosterone and HF. This feasibility study was designed as a double-blind placebo controlled RCT with participants randomised to treatment or placebo. Importantly, high intensity interval ET and combined testosterone therapy was found to be safe in this patient group with no serious adverse events documented. Recruitment to the trial was challenging and together with a high attrition rate (although concordant to other research in a similar population) this must be considered when planning further research in this area. Testosterone therapy together with ET resulted in significant improvements in peak $\dot{V}O_2$ and leg strength following the intervention when compared to baseline values. These changes were not observed in the placebo group but were not statistically significant between the groups. In addition, supplementation together with exercise also resulted in improvements in many SF36 domains, reduced depression scores and reduced androgen deprivation scores which were not observed in the placebo group. Both groups demonstrated similar improvements to peak power output during cycle ergometry, ISWT distance and reductions to BMI. The main outcome data was used in a power calculation to inform future phase II or III RCT's and focus groups were performed in order to provide a detailed patient focussed insight into the future

design of such trials. As a result of this, any future trials should be ideally be multi-centre or have access to a large heart failure patient cohort and also bear in mind the difficulties experienced during recruitment.

8.2: Testosterone status and HF – take home message.

Testosterone deficiency in HF appears to result in reduced exercise capacity as assessed by the 6 min walk test together with worse indices of quality of life both physical and mental as assessed by the SF36 Version 2 questionnaire. These reductions are independent of other important confounding variables such as age, NYHA score, NT-pro BNP, EF and BMI. Ultimately, as diminished exercise capacity and poor quality of life in HF are markers of poor prognosis, strategies to improve these should be investigated. The value of serological testosterone screening in HF needs to be established using economic evaluations and using data from large RCT's of supplementation in HF in order to ascertain if testosterone screening and physiological replacement has a role in routine clinical care. This topic will become more pertinent with a continually ageing population, increased treatment costs and the scarcity of transplanted organs.

8.3: Combined ET and TS – take home message.

Combined testosterone therapy and ET is safe and feasible in males with HF and low testosterone. However, recruitment to such trials is challenging due to co-morbidities associated with both HF and an ageing population. Testosterone treatment and exercise training result in improvements to important prognostic markers in HF

which are not apparent in the ET and placebo group. In addition, high intensity interval training in HF can be deemed safe, resulting in a shift from traditional viewpoints. Qualitative focus group data suggests that patients benefit from such interventions and encourage their promotion in a clinical environment. However, power calculation and attrition data suggest that future phase III RCT's must be multi-centre in order to achieve the required data in order to inform meaningful clinical decisions. Further trials are mandatory if testosterone sampling and physiological replacement are to become part of the clinical treatment regimen in eligible male HF patients.

8.4: Implications for clinical practise.

Clinicians must realise that testosterone deficiency, particularly in elderly HF male patients, but also in the younger population, can significantly augment the pathological processes of HF. This includes further reductions in exercise capacity and significantly attenuated general health related quality of life indices. As a result of this, there is an important clinical question to ask. Do clinicians need to routinely sample for testosterone deficiency in their HF patients? I believe that this question remains partly unanswered. Most evidence now points to worse outcome in testosterone deficient patients and of the small number of studies performed there is some observed benefits to physiological testosterone supplementation. However, with regard to screening cost and implications arising when testosterone deficiency is detected, it is prudent that much larger research trials assessing the effect of supplementation must be performed. Only when sufficiently powered and suitable length trials are performed can the safety, physiological and psychological benefits

together with wise economic analysis of supplementation be sufficiently explored to inform future therapeutic treatment with testosterone. The research performed as part of this thesis reinforces the clinical concepts that exercise training is safe in HF and should be promoted as part of routine clinical care. I believe this information is now widely accepted in current clinical practise. However, traditionally exercise training has been performed at lower intensities in HF. It seems that higher intensity interval training can provide added physiological and psychological benefits and can be deemed safe in moderate to severe HF. As treatment costs rise in the NHS and the burden of HF and testosterone deficiency increases in line with with an ageing population, combined treatment strategies as promoted by this thesis should be investigated in order to provide augmentation to currently accepted treatment methods.

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National Research Ethics Service

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4 March 2011

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Private & Confidential

Mr Martin Stout, Advanced Specialist Cardiac Physiologist
Wythenshawe Hospital
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Dear Mr Stout

Study Title: The Effect of Testosterone Status on Exercise Capacity,
Muscular Strength and Quality of Life in Males with
Chronic Heart Failure.

REC reference number: 11/NW/0058

The Research Ethics Committee reviewed the above application at the meeting held on 22 February 2011. Thank you for attending the meeting with Mr Pearce to discuss the study.

Discussion

The Committee commented that this was a very good study with few ethical issues.

It was pointed out that question A6-2 does not list the summary of ethical issues but concentrates more on the design of the study, and you took note of this.

You confirmed that you have changed your plan for the way participants will be identified and receive information about the study. You clarified that you will identify which male patients are due to come to the clinic; the lists are done 2 weeks in advance. The information sheet will be mail-shot to suitable candidates and if they are interested they can express interest by means the self addressed envelope provided. If they are not interested, the patient will not be approached further. Consent will be obtained at the clinic visit and this will be undertaken by the nurses.

You clarified that the participants will be tested before consent is obtained. You also explained that the intention now is to recruit more than 50 patients because you hope to achieve 25 patients in each group; the original figure of 50 does not allow for people dropping out and you therefore intend to recruit up to 80 participants.

It was asked what will happen with patients who are not followed up and recruited into the study. You commented that they will be made aware of the situation. You commented that the participants most likely to be turned away are those whose test results are normal, so they will have no need to worry about their results. You also confirmed that there is no set cut-off for high and normal testosterone levels.

It was agreed that the GP letter was not needed and you agreed to remove this.

You clarified that the questionnaires used in this study will be standard validated tools. The Committee advised that these should be submitted and kept on record.

The payment of expenses was queried. You advised that patients will not be paid expenses in respect of routine visits but expenses can be paid for participating in the study. It was pointed out that this should be stated in the information sheet and you agreed to do so. ²

It was asked whether you are proposing to give all the results to the participants. You said that you will give them a summary of the results and a lay summary of the overall findings. These will be fed back through the standard clinic visit.

Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, **subject to** the conditions specified below.

If you have any queries concerning the decision, please contact the Committee Co-ordinator (elaine.hutchings@northwest.nhs.uk) in the first instance.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study:

Standard condition

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

For NHS research sites only, management permission for research ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at <http://www.rdforum.nhs.uk>.

Where the only involvement of the NHS organisation is as a Participant Identification Centre (PIC), management permission for research is not required but the R&D office should be notified of the study and agree to the organisation's involvement. Guidance on procedures for PICs is available in IRAS. Further advice should be sought from the R&D office where necessary.

Sponsors are not required to notify the Committee of approvals from host organisations.

Additional conditions

- a. The following revisions are required to the Information Sheet:
 - i. Under the heading 'Visit (approximately 60 minutes duration)', at points 3 & 4 reference has been made to 'your recovery', this should be replaced with 'whilst resting' e.g. 'This test will last no more than 15 minutes in total and you will be monitored whilst resting.' ✓
Point 4 should begin with 'Whilst resting you will be asked to complete....' ✓
 - ii. Under the heading 'What are the possible results from measurements taken?', at point 4, insert the word 'are' between 'inflammation' and 'caused' so that it reads '...level of inflammation are caused by your heart failure.' ✓
 - iii.

Membership of the Committee

The members of the Ethics Committee present at the meeting are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Service website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

11/NW/0058

Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Yours sincerely



Mr Francis Chan
Chair

Email: elaine.hutchings@northwest.nhs.uk

Enclosures: List of names and professions of members present at the meeting.
"After ethical review – guidance for researchers"

Copy to: Andrew Maines, R&D, University Hospital South Manchester.



National Research Ethics Service

North West 8 Research Ethics Committee – Greater Manchester East
3rd Floor, Barlow House
4 Minshull Street
Manchester
M1 3DZ

Telephone: 0161 625 7820

29 March 2011

Mr M Stout
Advanced Specialist Cardiac Physiologist
University Hospital South Manchester
Wythenshawe Hospital
Southmoor Road
Manchester
M23 9LT

Dear Mr Stout

Full title of study: The Effect of Testosterone Status on Exercise Capacity, Muscular Strength and Quality of Life in Males with Chronic Heart Failure.

REC reference number: 11/NW/0058

Thank you for your letter of 10 March 2011. I can confirm the REC has received the documents listed below as evidence of compliance with the approval conditions detailed in our letter dated 22 February 2011. Please note these documents are for information only and have not been reviewed by the committee.

Documents received

The documents received were as follows:

<i>Document</i>	<i>Version</i>	<i>Date</i>
Covering Letter		
Questionnaire: SF-36 v2		
Participant Information Sheet	2	07 March 2011
Participant Consent Form	2	07 March 2011
Questionnaire: Beck Depression Inventory		
Questionnaire: Minnesota Living with Heart Failure		
Questionnaire: ADAM Questionnaire		

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor's responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

Research & Development Directorate
Ground Floor, Education & Research Centre
Tel: 0161 291 5768 Fax: 0161 291 5771
jennifer.boyle@manchester.ac.uk

Wythenshawe Hospital
Southmoor Road
Wythenshawe
Manchester
M23 9LT
Tel: 0161 998 7070

Mr Martin Stout
Advanced Specialist Cardiac Physiologist
Cardiology Department
Wythenshawe Hospital
Southmoor Road
Manchester
M23 9LT

6th May 2011

Dear Mr Stout,

Study Title: The effect of testosterone status on exercise capacity, muscular strength and quality of life in males with chronic heart failure.

R&D Ref: 2011CD001
REC Ref: 11/NW/0058

Thank you for providing us with all of the documentation for the above mentioned research project.

This research project has now been given R&D Management Approval.

I have enclosed some labels for you to affix to any Trust hospital case notes used in your research project.

This is a requirement of the Trust Case Note Destruction Policy. These labels will ensure that case notes used for research projects, which involve the treatment of patients, are kept for the required number of years. You will need to define the 'do not destroy before' date, according to the minimum term defined in the ethical approval. Please note this will vary for different types of studies.

It is your responsibility to ensure that these labels are affixed on the **BACK INSIDE COVER** of each case note. We will provide you with additional labels; please contact us when you require them.

You are also required to document patient participation in a clinical trial on the alert page at the front of the patient case notes, and to place any supporting documentation behind this page.



Chairman - Felicity Goodey, CBE, DL
Chief Executive - Julian Hartley, BA, MBA



Please note it is a requirement of the approval given by the Trust that the research project is being conducted in line with the guidance given within the Research Governance Framework as issued by the DH: (from the R&D website www.researchdirectorates.org.uk click on the link 'Carrying out research').

May I also draw to your attention the need to comply with the Health & Safety at Work Act, the Data Protection Act and the Human Tissue Act 2004.

I would also be grateful if you could supply us with copies of any substantial amendments submitted to the REC throughout the course of the study, along with a copy of the REC end of study report when it has been completed.

Thank you for your assistance. If you require any further information please do not hesitate to contact us on the above numbers.

Yours sincerely

A handwritten signature in cursive script, appearing to read 'J Boyle', written in black ink.

Jennifer Boyle
Research Governance Co-ordinator
University Hospital of South Manchester NHS Foundation Trust

PARTICIPANT INFORMATION SHEET

Study name: The Effects of Testosterone Status on Exercise Capacity, Muscular Strength and Quality of Life in Male Patients with Chronic Heart Failure.

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Please ask us if there is anything that is not clear, or if you would like more information.

In Brief:

This study will build on work already completed as part of another project and will involve the collection of a blood sample to determine testosterone level. This study will collect data on male heart failure patients with normal and low testosterone and assess their walking ability, strength and quality of life. This data will then be compared to see if there are any significant differences between the two groups.

What is the purpose of the study?

The main purpose of this study is to see if males with heart failure and low testosterone level have lower levels of functional capacity, muscular strength and quality of life when compared to heart failure patients with normal testosterone.

Previous research work has already been completed which has suggested positive effects of testosterone replacement therapy in males with heart failure and low testosterone levels. This work has showed improved exercise performance, strength parameters, quality of life and in some cases, improved heart function. To date, there is no conclusive research that has shown that in male heart failure patients low testosterone level adversely effects these measurements.

Why have I been chosen?

You have been chosen to take part in this research because you have been diagnosed with heart failure and are male.

Do I have to take part?

It is up to you to decide whether or not to take part, although the study is designed to cause minimal inconvenience to you. If you decide to take part, you are free to withdraw consent at any time without giving a reason. This would not affect the standard of care you receive. If you decide that you no longer wish to continue with the study, we would like to retain any data already obtained about you, unless you request otherwise.

What will happen to me if I agree to take part?

In this study you will take part in one research visit whereby we will collect information about your physical fitness, strength, quality of life (using questionnaires) and level of inflammation within the body (using a blood sample).

Will my travel expenses be paid?

We will be unable to make payments regarding travel expenses as this study aims to complete data whilst you are here for your regular clinic appointment. However, if extra visits are required or contributions need to be made toward parking expenses in extended visits, extra expenses incurred will be made available.

Visit (approximately 60 minutes duration)

We will make an appointment for you at the Heart Failure Clinic.

If you are eligible and willing to participate in the study:

1. You will be assessed by your Consultant's medical team as part of the normal work-up of your clinic appointment. During this assessment an additional tube of blood and a saliva sample will be collected and sent for analysis of testosterone level.
2. If you are asked to take part (based on your testosterone level) we will perform an ultrasound scan of your heart (an "echocardiogram" or "echo"). This is a safe and painless procedure that takes approximately 20-30 minutes. You will be asked to lie on a couch on your left side. A "probe" is placed on your chest and lubricating jelly is used to make good contact with the skin. Ultrasound waves then create images of your heart on the scanner monitor and we are able to look at the overall structure and function of your heart, including the valves.
3. Following this we will ask you to complete a shuttle walking test. As part of this test you will be asked to walk back and forth over a 10 m course for 6 mins, as fast as is physically possible. We will record your maximum walking distance together with your monitored heart rates and perceived effort. This test will last no more than 15 minutes in total and you will be monitored whilst resting following test completion.
4. Whilst resting you will be asked to complete some questionnaires that assess your quality of life and the effect of heart failure on your ability to complete daily activities. This should take approximately 10 minutes.
5. Finally, you will be asked to complete a short test of fore-arm strength by gripping a hand strength test machine tightly. You will be asked to do 3 tests on your left and right hands. This will last for about 5 minutes in total.

What are the possible results from measurements taken?

The results of this study will give us information regarding the following health parameters:

1. Details of how well your heart is pumping. We will use modern techniques to assess (in-depth) the function of your heart during the echocardiogram.
2. We will gain information as to your endurance capacity and an approximation of how strong you are. This information is important in heart failure as these levels are usually reduced and impact on quality of life.
3. The questionnaires will help us find out about your general health, how well you can cope with different activities in your daily life and if you have increased levels of depression.
4. Blood tests will help us determine if your testosterone level and level of inflammation are caused by your heart failure.

Are there any possible risks from taking part?

Possible risks include discomfort due to the blood and walking tests. Tiredness is also common after completing the walking test and you will be closely monitored during the clinic visit and also will be re-assessed before you are asked to leave the department. Ultrasound is painless and has been shown to be a safe procedure with no side effects known.

What are the possible benefits?

The major benefit of this study is that your participation may help highlight that testosterone level is an important factor in male patients with heart failure. It is possible that lower levels of testosterone result in increased symptoms and therefore poorer quality of life. Research has already shown some positive effects of testosterone replacement therapy in male heart failure patients and this study will contribute to that data. Ultimately, this research may help initiate the use of testosterone supplementation as a therapeutic treatment in the male heart failure population.

What happens when the research study stops?

When this study is completed it will be written up and used as part of a PhD thesis. Additionally, it will be published in respected medical journals and presented at important meetings. It is hoped that this study will help raise awareness of the importance of testosterone status in heart failure.

Will my taking part in the study be kept confidential?

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. No personal data will be published at any time and your confidentiality is of the utmost importance to us. Data will be held in secure databases to which only authorised people will have access.

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

PART 2**What will happen if I don't want to carry on with the study?**

You are free to withdraw from the study at any time. If you wish, we can then just make use of the information we already have about you. Alternatively, we can ensure if your samples and information are used for future research it is entirely anonymously, or we can destroy any identifiable samples or information we hold about you.

What if something goes wrong or I have a complaint?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions (contact Martin Stout on 0161 291 4632). If you wish to complain formally, you can do this through the NHS Complaints Procedure. In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against South Manchester Universities NHS Trust, but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (if appropriate). Compensation for any injury caused by taking part in this study will be in accordance with NHS indemnity.

What will happen to the data collected in this study?

If you take part in the study, this will be indicated on your medical records. Some parts of your medical records and the data collected from the study would be looked at by authorised persons to check that the study is being carried out correctly. They may also be looked at by authorised

persons from the NHS Trust. All investigators have a duty of confidentiality to you as a research participant and nothing that could reveal your identity would be disclosed outside the research sites.

Participation in future research

If you consent to be approached for involvement in future research, we will store your contact details separately from research data you have provided. Both your details and data will carry the same ID which can "link up" your details to your data. In this way we can identify research relevant to your particular healthcare status, and approach you appropriately. You can withdraw your consent to be contacted in the future at any time.

What will happen to the results of the research study?

We anticipate that the results will be published in a scientific journal for the benefit of the wider medical community. However, individual patients will not be identified in any publication and your personal and clinical details will remain strictly confidential. Any scientific publications arising from the study will be available on request to all participants. You will have no legal right to a share of any profits that may arise from the research.

Who is organising and funding the research?

This study will be led by Martin Stout under the supervision of Professor John Saxton and Professor Kevin Channer who are the Chief Investigator's supervisory team for his PhD research. Additionally, expert support will be received from Dr Simon Williams (Consultant Cardiologist), Dr Steve Shaw (Cardiology Registrar), Keith Pearce (Principal Cardiac Physiologist) and the Heart Failure Research Nurses that you visit during your clinic appointments.

Who has reviewed the study?

The study has been reviewed by North West 8 Research Ethics Committee - Greater Manchester East.

Where can I find independent information about taking part in research?

You can contact local branches of the NHS Patient Advisory Liaison Service (PALS). Here is their website: <http://www.pals.nhs.uk/> Contact phone number is 0161 291 5600 or email: pls@uhsm.nhs.uk

Further information and contact details

Martin Stout.
Advanced Specialist Cardiac Physiologist.
Northwest Heart Centre.
Wythenshawe Hospital.
Southmoor Road.
Manchester.
M23 9LT.
Tel: 0161-2914632.

If you are concerned about any aspect of the study and feel the need for independent advice you are advised to approach your GP

Appendix 3. Case report forms study 1.

The Effects of Testosterone Status on Exercise Capacity, Muscular Strength and Quality of Life in Males with Chronic Heart Failure.

Baseline Data Collection Sheet.

Participant number

Date

Age	Height	Weight	BMI	NYHA Score	Systolic BP	Diastolic BP	HR

Current Medication and Dosage:

Serum Free Testosterone (mmol/l)	Serum Total Testosterone (mmol/l)	Salivary Testosterone (mmol/l)	NT Pro BNP (pg/dl)

Completed by: _____ Date: _____

**The Effects of Testosterone Status on Exercise Capacity, Muscular Strength
 and Quality of Life in Males with Chronic Heart Failure.**

Primary Outcome Measure: 6 minute walk test.

Participant Number

Date

Resting HR

Rest BP

Shuttle Number	Shuttle Distance (metres)	Max HR (bpm)	Max RPE	Recovery HR (bpm) 1 (3mins)	Recovery HR (bpm) 2 (6mins)	Recovery HR (bpm) 3 (9 mins)

Collected by: _____

Date: _____

The Effects of Testosterone Status on Exercise Capacity, Muscular Strength and Quality of Life in Males with Chronic Heart Failure.

Secondary Outcome Measures:

Handgrip Strength (kg), Echocardiography and Quality of Life.

Participant Number

Date

jection raction %	TDI E/E'	TDI Peak S wave velocity (cm/s)	LV length (cm)	LV diameter (cm)	Sphericity Index	TAPSE (mm)	Handgrip Strength (kg).	SF 36 completed	Beck Depression Completed	Minnesota Questionn Comple

Data collected by: _____

Date:

Appendix 4. MLHFQ.

MINNESOTA LIVING WITH HEART FAILURE® QUESTIONNAIRE

The following questions ask how much your heart failure (heart condition) affected your life during the past month (4 weeks). After each question, circle the 0, 1, 2, 3, 4 or 5 to show how much your life was affected. If a question does not apply to you, circle the 0 after that question.

Did your heart failure prevent you from living as you wanted during the past month (4 weeks) by -	No	Very Little			Very Much	
		0	1	2	3	4
1. causing swelling in your ankles or legs?	0	1	2	3	4	5
2. making you sit or lie down to rest during the day?	0	1	2	3	4	5
3. making your walking about or climbing stairs difficult?	0	1	2	3	4	5
4. making your working around the house or yard difficult?	0	1	2	3	4	5
5. making your going places away from home difficult?	0	1	2	3	4	5
6. making your sleeping well at night difficult?	0	1	2	3	4	5
7. making your relating to or doing things with your friends or family difficult?	0	1	2	3	4	5
8. making your working to earn a living difficult?	0	1	2	3	4	5
9. making your recreational pastimes, sports or hobbies difficult?	0	1	2	3	4	5
10. making your sexual activities difficult?	0	1	2	3	4	5
11. making you eat less of the foods you like?	0	1	2	3	4	5
12. making you short of breath?	0	1	2	3	4	5
13. making you tired, fatigued, or low on energy?	0	1	2	3	4	5
14. making you stay in a hospital?	0	1	2	3	4	5
15. costing you money for medical care?	0	1	2	3	4	5
16. giving you side effects from treatments?	0	1	2	3	4	5
17. making you feel you are a burden to your family or friends?	0	1	2	3	4	5
18. making you feel a loss of self-control in your life?	0	1	2	3	4	5
19. making you worry?	0	1	2	3	4	5
20. making it difficult for you to concentrate or remember things?	0	1	2	3	4	5
21. making you feel depressed?	0	1	2	3	4	5

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11/10/04

Appendix 5. Example of informed consent form.

The Effects of Testosterone Status on Exercise Capacity, Muscular Strength and Quality of Life in Male Patients with Chronic Heart Failure.

Participant CONSENT FORM (To be completed at study visit)

Patient Identification Number for this study:

Name of Lead Researcher: Martin Stout.

Please initial all the relevant boxes

1. I confirm that I have read and understood the information sheet (version 2) dated 07/03/2011 for the above study and have had the opportunity to ask questions.
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.
3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by relevant regulatory authorities including responsible members of UHSM Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.
4. I agree to gift my blood and saliva samples for future research
5. I agree to take part in the above study.
6. I agree to being approached in future for possible participation in Ethics approved research relevant to me.
7. I understand that information held by the NHS and records maintained by The NHS Information Centre and the NHS Central Register may be used to help contact me and provide information about my health status.

Name of Patient

Date

Signature

Person Taking Consent
(if different from Researcher)

Date

Signature

Researcher

Date

Signature

Appendix 6. SF 36 Version 2 Questionnaire.

SF-36 v2™ Health Survey

(SF-36 v2 Standard, US Version 2.0)

To be completed by the PATIENT

Name (Last, First, Middle Initial)
Identification Number
Event

Directions: Answer every question by filling in the correct circle or writing in the information. If you need to change an answer, completely erase the incorrect mark and fill in the correct circle. If you are unsure about how to answer a question, please give the best answer you can. Mark only one answer for each question unless instructed otherwise.

Today's Date (MM/DD/YY)

		/			/		
--	--	---	--	--	---	--	--

Shade circles like this: ●
Not like this: ⊗ ⊕

Mark only one answer for each question.
Please do not mark outside the circles or make stray marks on the questionnaire.

01. In general, would you say your health is:

- Excellent ○ Very Good ○ Good ○ Fair ○ Poor

02. Compared to one year ago, how would you rate your health in general now?

- Much better ○ Somewhat better ○ About the same ○ Somewhat worse ○ Much worse

The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
03. Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	○	○	○
04. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	○	○	○
05. Lifting or carrying groceries	○	○	○
06. Climbing several flights of stairs	○	○	○
07. Climbing one flight of stairs	○	○	○
08. Bending, kneeling, or stooping	○	○	○
09. Walking more than a mile	○	○	○
10. Walking several hundred yards	○	○	○
11. Walking one hundred yards	○	○	○
12. Bathing or dressing yourself	○	○	○

During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
13. Cut down on the amount of time you spent on work or other activities	○	○	○	○	○
14. Accomplished less than you would like	○	○	○	○	○
15. Were limited in the kind of work or other activities	○	○	○	○	○
16. Had difficulty performing the work or other activities (for example, it took extra effort)	○	○	○	○	○

Please continue on next page

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52852



Patient Last Name: _____

During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
17. Cut down the amount of time you spent on work or other activities	<input type="radio"/>				
18. Accomplished less than you would like	<input type="radio"/>				
19. Did work or activities less carefully than usual	<input type="radio"/>				

20. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

- Not at all Slightly Moderately Quite a bit Extremely

21. How much bodily pain have you had during the past 4 weeks?

- None Very mild Mild Moderate Severe Very severe

22. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

- Not at all A little bit Moderately Quite a bit Extremely

These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks...

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
23. Did you feel full of life?	<input type="radio"/>				
24. Have you been very nervous?	<input type="radio"/>				
25. Have you felt so down in the dumps that nothing could cheer you up?	<input type="radio"/>				
26. Have you felt calm and peaceful?	<input type="radio"/>				
27. Did you have a lot of energy?	<input type="radio"/>				
28. Have you felt downhearted and depressed?	<input type="radio"/>				
29. Did you feel worn out?	<input type="radio"/>				
30. Have you been happy?	<input type="radio"/>				
31. Did you feel tired?	<input type="radio"/>				
32. During the <u>past 4 weeks</u> , how much of the time has your <u>physical health or emotional problems</u> interfered with your social activities (like visiting friends, relatives, etc.)?	<input type="radio"/>				

How TRUE or FALSE is each of the following statements for you?

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
33. I seem to get sick a little easier than other people	<input type="radio"/>				
34. I am as healthy as anybody I know	<input type="radio"/>				
35. I expect my health to get worse	<input type="radio"/>				
36. My health is excellent	<input type="radio"/>				

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Appendix 7. BDI.

Beck Depression Inventory

Please circle the appropriate answer for each question. Please circle only 1 answer.

1.

0 I do not feel sad.

1 I feel sad

2 I am sad all the time and I can't snap out of it.

3 I am so sad and unhappy that I can't stand it.

2.

0 I am not particularly discouraged about the future.

1 I feel discouraged about the future.

2 I feel I have nothing to look forward to.

3 I feel the future is hopeless and that things cannot improve.

3.

0 I do not feel like a failure.

1 I feel I have failed more than the average person.

2 As I look back on my life, all I can see is a lot of failures.

3 I feel I am a complete failure as a person.

4.

0 I get as much satisfaction out of things as I used to.

1 I don't enjoy things the way I used to.

2 I don't get real satisfaction out of anything anymore.

3 I am dissatisfied or bored with everything.

5.

0 I don't feel particularly guilty

1 I feel guilty a good part of the time.

2 I feel quite guilty most of the time.

3 I feel guilty all of the time.

6.

0 I don't feel I am being punished.

1 I feel I may be punished.

2 I expect to be punished.

3 I feel I am being punished.

7.

0 I don't feel disappointed in myself.

1 I am disappointed in myself.

2 I am disgusted with myself.

3 I hate myself.

8.

0 I don't feel I am any worse than anybody else.

1 I am critical of myself for my weaknesses or mistakes.

2 I blame myself all the time for my faults.

3 I blame myself for everything bad that happens.

9.

0 I don't have any thoughts of killing myself.

1 I have thoughts of killing myself, but I would not carry them out.

2 I would like to kill myself.

3 I would kill myself if I had the chance.

10.

0 I don't cry any more than usual.

1 I cry more now than I used to.

2 I cry all the time now.

3 I used to be able to cry, but now I can't cry even though I want to.

11.

0 I am no more irritated by things than I ever was.

1 I am slightly more irritated now than usual.

2 I am quite annoyed or irritated a good deal of the time.

3 I feel irritated all the time.

12.

0 I have not lost interest in other people.

1 I am less interested in other people than I used to be.

2 I have lost most of my interest in other people. 3 I have lost all of my interest in other people.

13.

0 I make decisions about as well as I ever could.

1 I put off making decisions more than I used to.

2 I have greater difficulty in making decisions more than I used to.

3 I can't make decisions at all anymore.

14.

0 I don't feel that I look any worse than I used to.

1 I am worried that I am looking old or unattractive.

2 I feel there are permanent changes in my appearance that make me look unattractive

3 I believe that I look ugly.

15.

0 I can work about as well as before.

1 It takes an extra effort to get started at doing something.

2 I have to push myself very hard to do anything.

3 I can't do any work at all.

16.

0 I can sleep as well as usual.

1 I don't sleep as well as I used to.

2 I wake up 1-2 hours earlier than usual and find it hard to get back to sleep.

3 I wake up several hours earlier than I used to and cannot get back to sleep.

17.

- 0 I don't get more tired than usual.
- 1 I get tired more easily than I used to.
- 2 I get tired from doing almost anything.
- 3 I am too tired to do anything.

18.

- 0 My appetite is no worse than usual.
- 1 My appetite is not as good as it used to be.
- 2 My appetite is much worse now.
- 3 I have no appetite at all anymore.

19.

- 0 I haven't lost much weight, if any, lately.
- 1 I have lost more than five pounds.
- 2 I have lost more than ten pounds.
- 3 I have lost more than fifteen pounds.

20.

- 0 I am no more worried about my health than usual.
- 1 I am worried about physical problems like aches, pains, upset stomach, or constipation.
- 2 I am very worried about physical problems and it's hard to think of much else.
- 3 I am so worried about my physical problems that I cannot think of anything else.

21.

- 0 I have not noticed any recent change in my interest in sex.
- 1 I am less interested in sex than I used to be.
- 2 I have almost no interest in sex.
- 3 I have lost interest in sex completely.

Appendix 8. ADAM Questionnaire.

ADAM questionnaire about symptoms of low testosterone - (Androgen Deficiency in the Aging Male).

This basic questionnaire can be very useful for men to describe the kind and severity of their low testosterone symptoms.

1. Do you have a decrease in libido (sex drive)?
Yes No
2. Do you have a lack of energy?
Yes No
3. Do you have a decrease in strength and/or endurance?
Yes No
4. Have you lost height?
Yes No
5. Have you noticed a decreased "enjoyment of life"
Yes No
6. Are you sad and/or grumpy?
Yes No
7. Are your erections less strong?
Yes No
8. Have you noticed a recent deterioration in your ability to play sports?
Yes No
9. Are you falling asleep after dinner?
Yes No
10. Has there been a recent deterioration in your work performance?
Yes No

If you answer **Yes** to number **1** or **7** or if you answer **Yes** to more than **3** questions, you may have low Testosterone.

Appendix 9. Ethics form for study 2.



North Sheffield Ethics Office

1st Floor Vickers Corridor

Direct Line: 0114 271 4894 or 271 4011

Fax: 0114 256 2469

Email: sue.rose@sth.nhs.uk

Northern General Hospital

Herries Road

Sheffield

S5 7AU

Saxton Study

02 June 2006

Professor Kevin S Channer
Consultant Cardiologist
Royal Hallamshire Hospital
Glossop Road
Sheffield
S10 2JF

Dear Professor Channer

Full title of study: Testosterone therapy as an adjunct to exercise rehabilitation: effects on exercise capacity, inflammatory markers and quality of life in hypogonadal males with chronic heart failure.

REC reference number: 06/Q2308/73

Protocol number: 4

EudraCT number: 2006-001090-23

Thank you for your letter of 31 May 2006, responding to the Committee's request for further information on the above research [and submitting revised documentation].

The further information has been considered on behalf of the Committee by the Chairman.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation [as revised].

Ethical review of research sites

The favourable opinion applies to the research sites listed on the attached form.

The Site Specific Assessment for the Hallam University Site will be assessed by a Sub-Committee of the Research Ethics Committee.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

An advisory committee to South Yorkshire Strategic Health Authority

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

<i>Document</i>	<i>Version</i>	<i>Date</i>
Application		13 April 2006
Investigator CV		
Protocol	4	11 May 2006
Summary/Synopsis	1	
Questionnaires (CHAMPS/Health and Wellbeing/Minesota living with Heart Failure/ADAM)		
Letter of invitation to participant	3	11 May 2006
GP/Consultant Information Sheets	3	11 May 2006
Participant Information Sheet: Main Study	4	31 May 2006
Participant Information Sheet: Sub study	3	11 May 2006
Participant Consent Form: Main study	2	14 December 2005
Participant Consent Form: MRI sub study	2	14 December 2005
Response to Request for Further Information		11 May 2006
Response to Request for Further Information		31 May 2006
Request for authorisation of CTIMP to the MHRA		04 April 2006

Research governance approval

The study should not commence at any NHS site until the local Principal Investigator has obtained final research governance approval from the R&D Department for the relevant NHS care organisation.

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

North Sheffield Local Research Ethics Committee
LIST OF SITES WITH A FAVOURABLE ETHICAL OPINION

For all studies requiring site-specific assessment, this form is issued by the main REC to the Chief Investigator and sponsor with the favourable opinion letter and following subsequent notifications from site assessors. For Issue 2 onwards, all sites with a favourable opinion are listed, adding the new sites approved.

REC reference number:	06/Q2308/73	Issue number:	1	Date of Issue:	02 June 2006
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Chief Investigator: Professor Kevin S Channer

Full title of study: Testosterone therapy as an adjunct to exercise rehabilitation: effects on exercise capacity, inflammatory markers and quality of life in hypogonadal males with chronic heart failure.

This study was given a favourable ethical opinion by North Sheffield Local Research Ethics Committee on 02 June 2006. The favourable opinion is extended to each of the sites listed below. The research may commence at each NHS site when management approval from the relevant NHS care organisation has been confirmed.

Principal Investigator	Post	Research site	Site assessor	Date of favourable opinion for this site	Notes (*)
Professor Kevin S Channer	Consultant Cardiologist	Sheffield Teaching Hospitals NHS Foundation Trust	North Sheffield Local Research Ethics Committee	02/06/2006	

Approved by the Chair on behalf of the REC:


 (Signature of Chair/Administrator)
 (delete as applicable)
 S. Channer (Name)

(*) The notes column may be used by the main REC to record the early closure or withdrawal of a site (where notified by the Chief Investigator or sponsor), the suspension of termination of the favourable opinion for an individual site, or any other relevant development. The date should be recorded.

Appendix 10. Case report forms for study 2

Study Number STH

1	4	3	9	4
---	---	---	---	---

Patients Initials

--	--	--

Patient ID No.

--	--

Date

--	--	--	--	--	--

Visit No.

--	--

**Primary Outcome Measure –
a) Incremental Shuttle Walk Test.**

Time (mins)	Shuttle Number	Max HR (bpm)	Max RPE

b) Recovery measures

Recovery time (mins)	HR (bpm)	BP (mmHg)

Secondary Outcome Measures –

a) Contractile Function and Isometric Strength

<p>Contractile Function (Nm) (highest peak torque)</p>	<table style="width: 100%; border: none;"> <tr> <td style="border: none;"><input type="checkbox"/></td> </tr> <tr> <td style="border: none;">1.0 rad.s⁻¹</td> <td style="border: none;"></td> <td style="border: none;">3.0 rad.s⁻¹</td> <td style="border: none;"></td> <td style="border: none;">5.3 rad.s⁻¹</td> <td style="border: none;"></td> </tr> </table>	<input type="checkbox"/>	1.0 rad.s ⁻¹		3.0 rad.s ⁻¹		5.3 rad.s ⁻¹						
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>								
1.0 rad.s ⁻¹		3.0 rad.s ⁻¹		5.3 rad.s ⁻¹									
<p>Isometric Strength (N) (using a handgrip dynamometer)</p>	<table style="border: none;"> <tr> <td style="border: none;"><input type="checkbox"/></td> <td style="border: none;"><input type="checkbox"/></td> <td style="border: none;"><input type="checkbox"/></td> </tr> </table>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>									
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>											

b) Peak Oxygen Uptake and Lower-Limb Skeletal Oxygenation

Workrate (W)	Rest	25	50	75	100	125	150	175	200
Time (mins)		0	3	6	9	12	15	18	21
Heart Rate (beats.min ⁻¹)									
Blood Pressure (mmHg)									
RPE (Borg 6-20)									
VO ₂ (l.min ⁻¹)									
VCO ₂ (l.min ⁻¹)									
RER (VCO ₂ /VO ₂)									
VE (l.min ⁻¹)									
Vt (l)									
Bf (br.min ⁻¹)									
Deoxygenation HHb (NIRS)									

Test duration = _____

Signature of person completing this form: _____

Study Number STH

1	4	3	9	4
---	---	---	---	---

Patients Initials

--	--

Patient ID No.

--	--	--

Date

--	--	--	--	--	--

Visit No.

--	--

Secondary Outcome Measures – Recovery Following Exercise

Start Time

--	--	--	--

Stop Time

--	--	--	--

Time after exercise (mins)	0	5	10	15	20	25	30
Heart Rate (beats.min ⁻¹)							
Blood Pressure (mmHg)							
Deoxygenation HHb (NIRS)							

STH 14394 ECHOCARDIOGRAPHY OUTCOME MEASURE

2D measurements:

LV wall thickness (cm)	IVS
	LVPW
LV dimension (cm)	LVEDD
	LVESD
Left atrial diameter (cm)	
Aortic dimension (cm)	Annulus
Right atrium (cm)	
Right ventricle (cm)	

LV systolic / diastolic function.

Left ventricular ejection fraction (%)	
Left ventricular peak systolic excursion (TDI)	
Left ventricular diastology E/E"	
	E/A ratio

Doppler.

Mitral valve	E velocity (cm/s)
	A velocity (cm/s)
	E decel time (cm/s)
Aortic valve	Peak systolic velocity (cm/s)
	Peak systolic gradient (mmHg)
Pulmonary valve	Peak systolic velocity (cm/s)
	Peak systolic gradient (mmHg)
Tricuspid valve	Peak regurgitant velocity (cm/s)
	Peak RA pressure (mmHg)
	Peak PA pressure

Report / Comments.

Sonographer.....

Date.....

Reported by.....

Appendix 11. CHAMPS Questionnaire.

CHAMPS Activities Questionnaire for Older Adults

CHAMPS: Community Healthy Activities Model Program for Seniors
Institute for Health & Aging, University of California San Francisco
Stanford Center for Research in Disease Prevention, Stanford University
(11/06/00) © Copyright 1998
Do not reproduce without permission of the CHAMPS staff
Contact: Anita L. Stewart, Ph.D., UCSF, anitast@itsa.ucsf.edu

Date: _____

Name or

This questionnaire is about activities that you may have done in the past 4 weeks. The questions on the following pages are similar to the example shown below.

INSTRUCTIONS

If you **DID** the activity in the past 4 weeks:

Step #1 Check the **YES** box.

Step #2 Think about how many TIMES a week you usually did it, and write your response in the space provided.

Step #3 Circle how many **TOTAL HOURS in a typical week** you did the activity.

Here is an example of how Mrs. Jones would answer question #1: Mrs. Jones usually visits her friends Maria and Olga twice a week. She usually spends one hour on Monday with Maria and two hours on Wednesday with Olga. Therefore, the total hours a week that she visits with friends is 3 hours a week.

<p>In a typical week during the past 4 weeks, did you...</p>	
<p>1. Visit with friends or family (other than those you live with)?</p> <p><input checked="" type="checkbox"/> YES How many TIMES a week? <u>2</u> →</p> <p><input type="checkbox"/> NO</p>	<p>How many TOTAL <u>hours a week</u> did you usually do it? →</p> <p>Less than 1 hour 1-2½ hours 3-4½ hours 5-6½ hours 7-8½ hours 9 or more hours</p>

If you DID NOT do the activity:

- **Check the NO box and move to the next question**

In a typical week during the past 4 weeks, did you ...

1. Visit with friends or family (other than those you live with)? YES How many TIMES a week? _____ NO

How many TOTAL hours a week did you usually do it? Less than 1 hour 1-2½ hours 3-4½ hours 5-6½ hours 7-8½ hours 9 or more hours

2. Go to the senior center? YES How many TIMES a week? _____ NO

How many TOTAL hours a week did you usually do it? Less than 1 hour 1-2½ hours 3-4½ hours 5-6½ hours 7-8½ hours 9 or more hours

3. Do volunteer work? YES How many TIMES a week? _____ NO

How many TOTAL hours a week did you usually do it? Less than 1 hour 1-2½ hours 3-4½ hours 5-6½ hours 7-8½ hours 9 or more hours

4. Attend church or take part in church activities? YES How many TIMES a week? _____ NO

How many TOTAL hours a week did you usually do it? Less than 1 hour 1-2½ hours 3-4½ hours 5-6½ hours 7-8½ hours 9 or more hours

5. Attend other club or group meetings? YES How many TIMES a week? _____ NO

How many TOTAL hours a week did you usually do it? Less than 1 hour 1-2½ hours 3-4½ hours 5-6½ hours 7-8½ hours 9 or more hours

6. Use a computer? YES How many TIMES a week? _____ NO

How many TOTAL hours a week did you usually do it? Less than 1 hour 1-2½ hours 3-4½ hours 5-6½ hours 7-8½ hours 9 or more hours

In a typical week during the past 4 weeks, did you ...

7. Dance (such as square, folk, line, ballroom) (do not count aerobic dance here)?
 YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →
 Less than 1 hour
 1-2½ hours
 3-4½ hours
 5-6½ hours
 7-8½ hours
 9 or more hours

8. Do woodworking, needlework, drawing, or other arts or crafts?
 YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →
 Less than 1 hour
 1-2½ hours
 3-4½ hours
 5-6½ hours
 7-8½ hours
 9 or more hours

9. Play golf, carrying or pulling your equipment (count walking time only)?
 YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →
 Less than 1 hour
 1-2½ hours
 3-4½ hours
 5-6½ hours
 7-8½ hours
 9 or more hours

10. Play golf, riding a cart (count walking time only)?
 YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →
 Less than 1 hour
 1-2½ hours
 3-4½ hours
 5-6½ hours
 7-8½ hours
 9 or more hours

11. Attend a concert, movie, lecture, or sport event?
 YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →
 Less than 1 hour
 1-2½ hours
 3-4½ hours
 5-6½ hours
 7-8½ hours
 9 or more hours

In a typical week during the past 4 weeks, did you ...

12. Play cards, bingo, or board games with other people?

- YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →

Less than 1 hour
 1-2½ hours
 3-4½ hours
 5-6½ hours
 7-8½ hours
 9 or more hours

13. Shoot pool or billiards?

- YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →

Less than 1 hour
 1-2½ hours
 3-4½ hours
 5-6½ hours
 7-8½ hours
 9 or more hours

14. Play singles tennis (do not count doubles)?

- YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →

Less than 1 hour
 1-2½ hours
 3-4½ hours
 5-6½ hours
 7-8½ hours
 9 or more hours

15. Play doubles tennis (do not count singles)?

- YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →

Less than 1 hour
 1-2½ hours
 3-4½ hours
 5-6½ hours
 7-8½ hours
 9 or more hours

16. Skate (ice, roller, in-line)?

- YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →

Less than 1 hour
 1-2½ hours
 3-4½ hours
 5-6½ hours
 7-8½ hours
 9 or more hours

17. Play a musical instrument?

- YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →

Less than 1 hour
 1-2½ hours
 3-4½ hours
 5-6½ hours
 7-8½ hours
 9 or more hours

In a typical week during the past 4 weeks, did you ...

18. Read?

- YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →

Less than 1 hour
 1-2½ hours
 3-4½ hours
 5-6½ hours
 7-8½ hours
 9 or more hours

19. Do heavy work around the house (such as washing windows, cleaning gutters)?

- YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →

Less than 1 hour
 1-2½ hours
 3-4½ hours
 5-6½ hours
 7-8½ hours
 9 or more hours

20. Do light work around the house (such as sweeping or vacuuming)?

- YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →

Less than 1 hour
 1-2½ hours
 3-4½ hours
 5-6½ hours
 7-8½ hours
 9 or more hours

21. Do heavy gardening (such as spading, raking)?

- YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →

Less than 1 hour
 1-2½ hours
 3-4½ hours
 5-6½ hours
 7-8½ hours
 9 or more hours

22. Do light gardening (such as watering plants)?

- YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →

Less than 1 hour
 1-2½ hours
 3-4½ hours
 5-6½ hours
 7-8½ hours
 9 or more hours

In a typical week during the past 4 weeks, did you ...

23. Work on your car, truck, lawn mower, or other machinery?
 YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →
 Less than 1 hour
 1-2½ hours
 3-4½ hours
 5-6½ hours
 7-8½ hours
 9 or more hours

****Please note: For the following questions about running and walking, include use of a treadmill.**

24. Jog or run?
 YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →
 Less than 1 hour
 1-2½ hours
 3-4½ hours
 5-6½ hours
 7-8½ hours
 9 or more hours

25. Walk uphill or hike uphill (count only uphill part)?
 YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →
 Less than 1 hour
 1-2½ hours
 3-4½ hours
 5-6½ hours
 7-8½ hours
 9 or more hours

26. Walk fast or briskly for exercise (do not count walking leisurely or uphill)?
 YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →
 Less than 1 hour
 1-2½ hours
 3-4½ hours
 5-6½ hours
 7-8½ hours
 9 or more hours

In a typical week during the past 4 weeks, did you ...

27. Walk to do errands (such as to/from a store or to take children to school (count walk time only))? **→**

- YES How many TIMES a week? _____ **→**
 NO

How many TOTAL hours a week did you usually do it? **→**

Less than 1 hour 1-2½ hours 3-4½ hours 5-6½ hours 7-8½ hours 9 or more hours

28. Walk leisurely for exercise or pleasure? **→**

- YES How many TIMES a week? _____ **→**
 NO

How many TOTAL hours a week did you usually do it? **→**

Less than 1 hour 1-2½ hours 3-4½ hours 5-6½ hours 7-8½ hours 9 or more hours

29. Ride a bicycle or stationary cycle? **→**

- YES How many TIMES a week? _____ **→**
 NO

How many TOTAL hours a week did you usually do it? **→**

Less than 1 hour 1-2½ hours 3-4½ hours 5-6½ hours 7-8½ hours 9 or more hours

30. Do other aerobic machines such as rowing, or step machines (do not count treadmill or stationary cycle)? **→**

- YES How many TIMES a week? _____ **→**
 NO

How many TOTAL hours a week did you usually do it? **→**

Less than 1 hour 1-2½ hours 3-4½ hours 5-6½ hours 7-8½ hours 9 or more hours

31. Do water exercises (do not count other swimming)? **→**

- YES How many TIMES a week? _____ **→**
 NO

How many TOTAL hours a week did you usually do it? **→**

Less than 1 hour 1-2½ hours 3-4½ hours 5-6½ hours 7-8½ hours 9 or more hours

In a typical week during the past 4 weeks, did you ...

32. Swim moderately or fast?

- YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →

Less than 1 hour 1-2½ hours 3-4½ hours 5-6½ hours 7-8½ hours 9 or more hours

33. Swim gently?

- YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →

Less than 1 hour 1-2½ hours 3-4½ hours 5-6½ hours 7-8½ hours 9 or more hours

34. Do stretching or flexibility exercises (do not count yoga or Tai-chi)?

- YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →

Less than 1 hour 1-2½ hours 3-4½ hours 5-6½ hours 7-8½ hours 9 or more hours

35. Do yoga or Tai-chi?

- YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →

Less than 1 hour 1-2½ hours 3-4½ hours 5-6½ hours 7-8½ hours 9 or more hours

36. Do aerobics or aerobic dancing?

- YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →

Less than 1 hour 1-2½ hours 3-4½ hours 5-6½ hours 7-8½ hours 9 or more hours

In a typical week during the past 4 weeks, did you ...

37. Do moderate to heavy strength training (such as hand-held weights of more than 5 lbs., weight machines, or push-ups)?
 YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →

Less than 1 hour
 1-2½ hours
 3-4½ hours
 5-6½ hours
 7-8½ hours
 9 or more hours

38. Do light strength training (such as hand-held weights of 5 lbs. or less or elastic bands)?
 YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →

Less than 1 hour
 1-2½ hours
 3-4½ hours
 5-6½ hours
 7-8½ hours
 9 or more hours

39. Do general conditioning exercises, such as light calisthenics or chair exercises (do not count strength training)?
 YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →

Less than 1 hour
 1-2½ hours
 3-4½ hours
 5-6½ hours
 7-8½ hours
 9 or more hours

40. Play basketball, soccer, or racquetball (do not count time on sidelines)?
 YES How many TIMES a week? _____ →
 NO

How many TOTAL hours a week did you usually do it? →

Less than 1 hour
 1-2½ hours
 3-4½ hours
 5-6½ hours
 7-8½ hours
 9 or more hours

In a typical week during the past 4 weeks, did you ...

41. Do other types of physical activity not previously mentioned (please specify)?

YES How many TIMES a week? _____ →

NO

How many TOTAL hours a week did you usually do it? →

Less than 1 hour

1-2½ hours

3-4½ hours

5-6½ hours

7-8½ hours

9 or more hours

Thank You

Sheffield Teaching Hospitals



NHS Foundation Trust



Sheffield Hallam University

EXERCISE AND TESTOSTERONE REPLACEMENT THERAPY IN MEN WITH HEART FAILURE

Introduction

Testosterone deficiency is a common occurrence in men with chronic heart failure (CHF). Previous studies have shown that exercise rehabilitation can safely increase exercise capacity in stable patients with CHF. This is a pilot study, which will assess whether testosterone replacement therapy in addition to a 12-week programme of exercise rehabilitation, can improve the exercise capacity of men with CHF who also have low circulating testosterone levels. Patients will be randomly allocated into one of two groups. You will either receive testosterone or placebo therapy. Both groups will receive exercise rehabilitation, which will involve a combined programme of moderate intensity aerobic exercise and resistance training.

Exercise capacity will be assessed using an incremental shuttle walk test. We will also assess your cardiac and skeletal muscle function and take blood tests, and assess whether there are changes in your quality of life as a result of undertaking this study. The data from this pilot study will provide the basis for a further clinical trial, which will be aimed at developing more effective strategies for the treatment of patients with CHF by evaluating the long-term effects of physical training on functional status, morbidity and mortality in stable CHF patients.

Please find attached the patient information sheet for this study, which answers the most frequently asked questions. This is your copy to retain.

PATIENT INFORMATION SHEET

Q: What is the main purpose of this study?

A: In men with chronic heart failure testosterone deficiency is a common occurrence. Replacing the level of testosterone improves symptoms and exercise capability, as does a programme of exercise. The main purpose of this study is to investigate whether testosterone therapy given during a programme of exercises can provide additional benefit in comparison to exercise training alone.

Q: Why has my doctor asked me to take part in this study?

A: You have been selected as being a suitable patient from your medical history.

Q: What will I have to do?

A: All patients entering the study will have a medical examination and will be randomly allocated to one of two groups. Before you can be given any testosterone replacement therapy it is essential to ensure that you do not have prostate cancer. There is no evidence that testosterone replacement will cause cancer, but it may make small pre-existing cancers grow more quickly. We will therefore need to check a special blood test called prostate specific antigen and perform a prostate digital examination. This involves the doctor placing his gloved finger inside your rectum. If the blood test or digital examination, are abnormal this does not necessarily mean that you have prostate cancer but we will arrange for you to be seen by one of our Consultant Urologists who will do further tests to be sure and provide any treatment that may be necessary. If the prostate tests are abnormal you will not be able to continue to take testosterone replacement therapy.

Both groups will undertake a supervised 12-week exercise training programme at the **Centre for Sport and Exercise Science**, Sheffield Hallam University, Collegiate Crescent Campus (off Ecclesall Road). During the 12-week period one group will receive testosterone replacement, and the other will receive placebo or dummy testosterone treatment. Both testosterone replacement and the placebo will be administered via intramuscular injection. You will not know which group you were in until the end of the study. The placebo group is required so that direct comparisons can be made to see whether testosterone therapy provides additional improvement, compared to only exercise rehabilitation. Both groups are equally important for the success of this study.

Q: What will the assessment visits entail?

A: Unless otherwise stated all assessments will be performed at the start and end of the study period. During the assessment visits we will:-

1. Assess the distance that you can walk, using an incremental shuttle-walk test. The test will involve walking back and forth along a horizontal 10 m course.
2. Assess your heart function using special scanning techniques. This may be by ultra-sound (an echocardiogram which you will have had before) or by magnetic resonance imaging which is a painless technique which does not use radiation.
3. Measure skeletal muscle strength and endurance of your leg muscles. This procedure is safe and will take approximately 15-20 minutes to complete.
4. Measure lower-limb arterial function using ultrasound. During this procedure, a cuff will be placed around your lower thigh and inflated to occlude the circulation for 5 minutes. We will need to do this twice for each leg. You are likely to experience a degree of discomfort in the leg whilst the cuff is blown up. This will resolve when the cuff is let down.
5. Measure your oxygen consumption, blood pressure, heart rate and perceived exertion whilst you are undertaking cycle exercise. You will also have a small piece of apparatus strapped to your calf, whilst we perform this test. This technique is completely painless and harmless.
6. Take a blood sample from your arm every 4 weeks during the study.
7. Ask you to complete four brief questionnaires at the start and end of the study. We will assess your physical activity status every 4 weeks during the study.

Q: How long will the study last?

A: The programme will last for a total of 12 weeks. We will monitor everyone's progress throughout, with assessments at the start and at the end of the programme period using a standard walking test. We will also do some blood tests looking at the effects of exercise and testosterone on markers of inflammation in the blood. We want to know what effects the two treatments have on heart function so we will also do heart scans.

Q: How long will I have to exercise for?

A: The main component of the exercise training programme is stationary cycling, which will be followed by some moderate

weight lifting exercises. Each exercise session will last for approximately 45-50 minutes in total and patients will be required to attend 2 sessions per week for all 12 weeks. All exercise will be carefully **supervised** and you will be shown how to use the equipment.

Q: Will my travel expenses be reimbursed?

A: Your travel expenses to the Centre for Sport and Exercise Science will be reimbursed at the end of the 12-week study period. A £5 reimbursement per visit will be made.

Q: What are the alternatives for diagnosis or treatment?

A: If you are diagnosed with testosterone deficiency, your doctor or Consultant might discuss testosterone replacement therapy with you. In stable CHF patients your GP might also encourage exercise rehabilitation.

Q: What are the possible benefits of taking part in this study?

A: We cannot promise that the study will help you, but the information which we obtain might help improve the treatment of patients with heart failure.

Q: Are there side-effects of any treatment received when taking part?

A: We expect that exercise will make you feel tired, but as you do it more regularly you will feel increasingly better. Testosterone will be administered via intramuscular injection. Possible side effects might include pain and redness around the injection site. Testosterone, should not be used in men who are trying to start a family, as it can temporarily reduce sperm counts. Other rare side effects of testosterone include headaches, slight fluid retention causing swelling of the ankles, and a small increase in the number of red blood cells. Some men notice an increased libido after testosterone treatment. There is no evidence that this replacement dose of testosterone will cause problems with the prostate gland, but this will be monitored regularly throughout the study, using blood tests.

Q: What are the possible disadvantages and risks of taking part?

A: The potential for risks to occur will be minimised, since all patients prior to study acceptance will have a full clinical assessment performed by the Consultant Cardiologist or

Research Registrar. The likelihood of anything untoward happening during the exercise will be minimal.

Q: What about the risks of intramuscular injections for patients on warfarin?

A: The risk of local bleeding complications could be increased when patients on warfarin are given intramuscular testosterone injections. However, the risks will be minimised by extra blood tests (known as INR tests), which will be undertaken before your first injection, within 5-7 days after your first injection. These extra blood tests will be taken to see if your warfarin dosage needs to be adjusted whilst you are participating in the study. In addition, pressure will be applied to the injection site for five minutes and you will be advised to keep a check on the injection site for up to two-hours to see if any serious bruising occurs. Any concerns you have about the injection site should be discussed with a clinical member of the research team.

Q: If I decide to participate, will my GP be notified?

A: With your consent, we will write and inform your GP about your decision to take part.

Q: What if I do not wish to take part?

A: This will not affect your treatment at all.

Q: What if I change my mind during the study?

A: You are free to withdraw from the study at any time without affecting your treatment.

Q: What will happen to the information from the study?

A: The overall conclusions of the study will be available to you, however it will not be possible to produce an individualised report of your performance.

Q: Will my taking part in this study be kept confidential?

A: The confidentiality of our patients and the data, which this study will generate, is of utmost importance. All data from this study will be anonymised. In brief, you will be allocated a number during the study. We will need to obtain your permission to allow access for the research team to your medical records, and to information collected during the study. This is one of the clauses, which you will sign in agreement on the official consent form. Our procedures for handling, processing and storage of and destruction of data are compliant with the Data Protection Act 1998.

Q: Who is organising and funding the research?

A: The research is funded by Heart Research UK. The study is organised and sponsored by the Sheffield Teaching Hospital.

Q: Who has reviewed this study?

A: The North Sheffield Research Ethics Committee has reviewed this study.

Q: What if I have further questions?

A: If you have any further questions with regards to this study you may phone:-

IF YOUR QUERY IS REGARDING ...	CONTACT	AT THE	TELEPHONE NUMBER
Participating in this study or the arrangements for the training and assessment sessions at the:- CENTRE FOR SPORT & EXERCISE SCIENCE	Mr MARTIN STOUT	CENTRE FOR SPORT & EXERCISE SCIENCE	<u>0114 225 5690</u> (DIRECT LINE)
	Or Dr JOHN SAXTON	(SHEFFIELD HALLAM UNIVERSITY)	<u>0114 225 4414</u> (DIRECT LINE)
Your health (i.e. medically related)	PROF KEVIN CHANNER	CARDIOLOGY DEPT, ROYAL HALLAMSHIRE HOSPITAL	<u>0114 271 3473</u>

Q: What if I wish to complain about the way this study has been conducted?

A: If you have any cause to complain about any aspect of the way in which you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you and are not compromised in any way because you have taken part in a research study. The normal hospital complaints procedure does apply, and you should contact the following person:

Name: Professor Chris Welch (Medical Director) Tel: 0114 271 1900

You can also complain to any individual of the research team, contact details as above.

Q: What if I am Harmed?

A: In the event that something does go wrong and you are harmed during the research study, there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for legal action for compensation, but you may have to pay your legal costs.

Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms will still be available to you.

Version 6 13th September 2007

Appendix 13. Focus group transcript example – unrelated discussion

excluded.

How do you feel overall following the 12 week intervention period?

P1. Yes I felt a lot fitter – definitely. But, I've been thinking about it recently, I could probably have been pushed a little harder and maybe got even more fit. I thought after perhaps 2-3 weeks I could have been definitely pushed harder.

P2. I thought it was alright. But what you have to do when you finish is to keep the momentum going. Otherwise you start settling back into old ways. I mean I felt great straight after the exercise sessions but some mornings when I came in I felt a bit sore. Afterwards though, I felt great.

P3. I felt as though it could be a little more intensive. For instance I would have liked to have done a warm up on the treadmill or perhaps the main training on a treadmill rather than a bike. That way, you can get your whole body working rather than just your legs.

P4. For me it's been an excellent course and I'm grateful to all the people here who have helped me. I have noticed it very much because I do a physical job and my breathing has improved very much recently.

P5. I agree that the course too could have been a little more physical. I feel my fitness has improved but at the end there is nothing to take you any further at the end. For people like us in order to take the burden off the NHS you really need

something in place at the end. Just fitness alone and the confidence definitely makes you improve.

P4. It definitely gives you confidence because the nurses tend to want to put you in bed, the confidence gives you value to go to the gymnasium.

P2. You just need to be careful that you know who is best suited to the exercise because you don't want someone collapsing at the university.

P5. I agree too. That's what puts you off going to a regular gym because the instructors there may not be qualified. As those here apparently are (laughter).

P1. Yeah that's right. But I still would like to know exactly how far I can actually go.

P6. You just need someone to push you into it. You know, you've made an appointment and so you come – don't you? If you were left at home, I mean I've got a bit of a gym there but I don't use it.

Is there anything that the researchers could have done to make you continue with exercise following the programme?

P1. I don't think we could have done these exercises at home. It needs to be that we go somewhere with your mates so that you can push each other. If you are doing it at home then you just say "Oh, I don't feel like doing it today".

P3. I would have been happy coming for more than 12 weeks – whether it be 14 or 16 or more.

P2. Yes, I would have liked maybe more sessions in the week. I was motivated so could have come every day.

P5. Difficult if you're working though.

P1. Twice was enough for me. It gives you time to do other things.

Is there anything that you particularly disliked about the exercise sessions?

P2. No.

P1. Perhaps not being pushed and a bit more variety. Because if you want to carry on after you want to know how far you can push yourself.

P2. Sometimes you see other people exercising on other equipment and it makes you want to have a go on that too.

P4. I thought the sessions were well structured although it got a bit monotonous towards the end doing the cycling all the time.

What was it that made you want to keep on coming?

P1. The exercise. You've got someone else there doing it with you. It makes you want to do it and you have got good facilities.

P2. Yes. Having others here who are all in the same boat helped a lot. I've made some friends.

P4. The wife and kids encouraged me to go. It means I had a purpose and was getting motivated to get fit. They helped me a lot.

P1. Martin, I think you helped too. You and the other instructors were encouraging and made us feel safe and comfortable in what we were doing.

So, does there being a group of you make it easier to come?

P5. I don't know because I often came on my own. My motivation was that I wanted to get fitter and also find out if it was the exercise or testosterone or whatever made you feel better.

P2. It definitely does. Motivation like.

P3. I always wanted to beat.... during the exercises. We pushed ourselves hard to win.

P1. You also can chat to each other – it makes the session not feel so difficult even though you are working hard.

P3. Good for when you've got problems – everyone has the same condition so you can ask questions and get re-assurance from each other about things.

P4. Like my gout!

What about family and friends – do they give you support?

P3. Yes family have supported me. Doctors and nurses are interested in the course and also the outcome of it.

P2. Yes I had some support.

P3. I would have come anyway I think.

What has been your doctors opinion?

P3. They have different opinions, some say don't exercise. Thats rubbish because I know I can.

P4. Family are very protective. You sometimes have to hide it from them.

P1. Yes they are. It would help if you knew how far you can push yourself and what a safe limit is. You think wow I can do that and how far can I go.

P3. That's up to yourself and you shouldn't put so much pressure on those people here. You still have to be careful not to push too hard. The last thing they want to do here is call an ambulance.

How do you feel about coming to the University to exercise?

P5. There is no alternative. There's no option to have one nearer to where I live. It was an incentive to get up and get going.

P2. It gets you up and out of bed.

P4. It's okay but when you are working or busy you need alternatives like.

P6. Good point. Why isn't there more stuff like this near to our homes? I mean, there is a gym around the corner from me but I wouldn't want to go there with all the meat heads. We need something tailored to our condition and someone who knows what we can do safely.

P2. That's why coming here was the best thing. And we are giving something back through research.

If you had to design a programme such as this – would you do anything different?

P1. Yeah. There would be more variety.

P5. I would put the rowing machine in.

P2. Variety, definitely.

P2. The cross-walker too.

P6. Sometimes it was very rushed. I was often trying my best to get through things quickly.

P3. For me it has been a lesson. I can now go to the gym with my wife again with the confidence that I know what I am doing. I think I would be okay.

Sometimes the traffic is bad and it takes time to get here but the gym is on my doorstep.

How would you feel if we opened up these sessions to family?

P3. That would be excellent and I think it would make more people want to come.

P2. I think it would be okay but then you have a work situation with people not being able to find the time to get here together.

P3. What we have got is a situation where we have had 30 patients and you get a picture of people who have been satisfied and benefited you should use them as an advert for others to attend.

P6. Excuse me. We have all come here because we have a problem, an illness. We've come here to go on a test to see if it benefits us. What you're talking about is a free gym. You will have no problem getting people there. It should be strictly for those who are ill and can benefit from the programmes. It's a medical situation – not a free gym.

P2. Some people would not come regularly. I liked coming with your mates who are doing the same thing. Not your family.

P1. We have all got similar problems and it's good to chat and compare with each other. You can't do that with just your family.

What advice would you give to other heart patients?

P1. It has got to be encouraged.

P2. Definitely.

P6. People will be a lot more motivated when you get to know the results. The encouragement will come from the results.

P5. We should promote this because it clearly has worked. Some of us have lost weight and we all fit a lot more fit.

P2. We are examples that exercise does work and is safe.

P3. It's not just us though. The doctors and nurses should know we can safely do this – why aren't they encouraging us more?

Do you think if this research is published in medical journals that will influence doctors?

P1. You have got to exercise otherwise you go backwards and they should know that.

P5. The GP's should promote this and should know about it. They should use us as examples that it works.

P1. I never see the consultant anymore. I have been discharged and they don't follow me up to check anything. They don't force you to exercise.

P6. Did they not tell you to exercise?

P1. Yes they did but they don't check you up.

P3. Sometimes you have to moan. Otherwise, you don't get anything. Then it gets too late.

P1. National Health is not bothered unless you are ill. But some don't even go then. There is no annual check up unless you go yourself. I think every year they should get in touch with you.

P6. Cost wise that is unrealistic. They cannot afford to do that.

P1. Other countries have annual physical check-ups.

P6. You will have to name those – most countries you have got to pay for healthcare.

P1. If they spent more they may save money by sorting out problems in the earlier stages where it doesn't cost as much.

P1 and P4 we would pay for annual screening if it was on offer.

How did you benefit as a result of the intervention?

P5. Yes I could walk further and get up hills without getting as short of breath.

P3. Most of us in here definitely get short of breath. It feels harder to get blood and oxygen to the muscles.

P2. Lost loads of weight and feel better gardening.

P4. It gives you more confidence.

P5. Them questionnaires we filled in must have proved that we are better than before. I was depressed and not doing very much. Now I know I can it changes a lot of my answers this last time.

P6. Even things like walking upstairs to the toilet. I'm not as short of breath when I get there now.

P3. I have improved – no doubt.

Any negative effects of the exercise?

P1. Tired at first but the more I did I felt better.

P3. Not many aches no.

P2. Sometimes sore in the morning but that soon wore off.

P1. Injections made you bleed a bit.

P6. At first, I was aching all the time afterwards but after a couple of weeks I was okay. That's what made me want to try and do some more to push myself harder.

P2. That seat on the bike. It hurt after about ten minutes. That's why I started bringing my cushion!

Has what we have done here influenced how you exercise at the gym?

P5. Yes I do more cardio now.

P4. I'm joining up again I think.

P3. I'm going more with my wife now I know what I'm doing.

P2. I can't afford a gym – but you can do most of the stuff at home; walking, jogging and some of the exercises too.

Any other comments.

P2. If you have any exercise kit at home you should try and use it. Put some music on and away you go.

P6. Stop us putting weight on.

P2. Weight is a big issue and hinders you.

P4. It is difficult to lose weight and if you're fat then you are at risk.

P1. Maybe dietary and educational advice should also be included because weight is a massive problem for lots of people.

P2. Just knowing what foods you can eat or should eat then that's a good start.

P6. I once joined weight watchers and that worked because I got into the habit of getting weighed. Here is the same, you come every week and it keeps working. You need to keep at it.

P5. Weight loss and exercise go hand in hand.

P1. We could all do with losing a little bit.

P2. I hope I don't settle back into my old ways because this has stopped. I'm going to look into some cardiac classes from the council or hospital.

P4. Let us know if you find any – I'd be interested in that.

P5. Can we not pay and keep attending here?

P2. You need to think of more research so we can keep coming doing exercises – it's popular there were lots of people with other illness attending at the same time as me. I was interested when talking to them about their problems too.

P6. My wife kept wanting to come in and exercise with me – she watched a few sessions though. Something about insurance cover so she couldn't use the equipment.