METABOLIC ALTERATIONS IN PATIENTS WITH HEART DISEASES

A PhD thesis

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ABSTRACT

Despite major advances in therapies, chronic heart failure (CHF) and hypertrophic cardiomyopathy (HCM) are still associated with significant morbidity and mortality. These patients often have a significant limitation in their exercise capacity. We showed that there are widespread abnormalities of both systolic and diastolic function in HCM patients. These abnormalities contribute significantly to exercise limitation observed in these patients. Furthermore, we showed that HCM manifest a myocardial energy deficiency which was accompanied by a slowing of the energy-dependent early diastolic LV active relaxation during exercise. The present study supports the hypothesis that HCM is, at least in part, a disease of energy deficiency. Consistent with this hypothesis, we showed that metabolic modulation by perhexiline augmented myocardial energetics, and normalised diastolic ventricular filling which translated into significant subjective (improved symptoms) and objective (increased exercise capacity, peak V_{O2}) clinical improvement in HCM patients. These findings suggest that metabolic modulators, such as perhexiline, have the potential role in the management of patients with symptomatic non obstructive HCM, a condition for which there are currently limited therapeutic options. However, large scale long-term studies are still required to examine the effects of these agents on morbidity and mortality in these patients.

DECLARATION AND STATEMENTS

Declaration

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature.

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Statement 1

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STATEMENT OF CONTRIBUTION TO RESEARCH

Myself and Professor Frenneaux conceived and designed all the studies.

Execution

I performed all of the recruitment and organisation of the appointments at the three centres involved in the study. I performed metabolic exercise tests, echocardiogram, radionuclide ventriculography, cardiac MRI and cardiac MRS studies in the University of Birmingham. Metabolic exercise tests were reported by Rebekah Weaver. Perhexiline assays and LFT were performed by Dr Alan Hutchins at Llandough Hospital, Cardiff.

Analysis

All data were collated and analysed by myself. Interobserver reproducibility data were analysed by Dr Thanh Phan and Dr G Nallur-Shivu.

PUBLICATION

This PhD thesis in cardiovascular medicine is based on the following publication listed below:

Original research

- **Abozguia K,** Nallur-Shivu G, Ahmed I, Phan TT, Kalra R, McKenna W, Sanderson J, Elliott P and Frenneaux M. ***Left Ventricular Strain and Untwist in Hypertrophic Cardiomyopathy: Relation to Exercise Capacity.*** American Heart Journal. 2010 May;159(5):825-32.

- **Abozguia K**, Phan TT, Shivu GN, Maher AR, Ahmed I, Wagenmakers A, Frenneaux MP. ***Reduced in vivo skeletal muscle oxygen consumption in patients with chronic heart failure-A study using Near Infrared Spectrophotometry (NIRS***). Eur J Heart Fail. 2008 Jul;10(7):652-7.

- **Abozguia K and** Shivu GN (joint first author)**,** Phan TT, Ahmed I, Henning A, Frenneaux M**.** ***(31) P magnetic resonance spectroscopy to measure in vivo cardiac energetics in normal myocardium and hypertrophic cardiomyopathy: Experiences at 3T.*** Eur J Radiol. 2008 Dec 2.

- **Abozguia K**, Elliott P, McKenna W, Phan TT, Nallur-Shivu G, Ahmed I, Maher AR, Henning A, Ashrafian H, Watkins H and Frenneaux M. ***Metabolic Modulation***

With Perhexiline Corrects Energy Deficiency And Improves Exercise Capacity In Symptomatic Hypertrophic Cardiomyopath. (in press, Circulation).

- **Abozguia K,** Nallur-Shivu G, Phan TT, Ahmed I, Maher AR, Kalra R, McKenna W, Elliott P and Frenneaux M. ***Myocardial Energy Deficiency in Hypertrophic Cardiomyopathy: Relation to Exercise Capacity and Dynamic Diastolic Dysfunction.*** (To be submitted).

Reviews

- **Abozguia K,** Clarke K, Lee L and Frenneaux M**.** ***Modification of Myocardial Substrate Utilisation as a Therapy for Heart Failure.*** Nature (Cardiovascular) September 2006, 3(9):490-8.

- **Abozguia K,** Shivu GN, Ahmed I, Phan TT, Frenneaux MP**.** ***The heart metabolism: pathophysiological aspects in ischaemia and heart failure.*** Curr Pharm Des. 2009;15(8):827-35.

- **Abozguia K,** Nallur Shivu G, Phan TT, Maher A, Ahmed I and Frenneaux M. ***Potential of metabolic agents as adjunct therapies in heart failure.*** Future Cardiology September 2007, Vol. 3, No. 5, Pages 525-535.

- **Abozguia K,** Phan TT, Nallur Shivu G, Maher A, Ahmed I and Frenneaux M. ***Insights and Tips for Starting a Clinical Study in the United Kingdom.*** J R Soc Med 2007 Oct;100(10):469-72.

Abstracts

Oral presentations

- **Abozguia K,** Elliott P, McKenna W, Phan T, Nallur-Shivu G, Ahmed I, Maher AR, Henning A, Ashrafian H, Watkins H, Frenneaux M. ***Metabolic Modulation With Perhexiline Corrects Energy Deficiency and Improves Exercise Capacity in Symptomatic Hypertrophic Cardiomyopathy.*** American Heart Association (AHA), Orlando, Florida, Nov 14-18, 2009.

- **Abozguia K**, Nallur Shivu G, Phan TT and Frenneaux M**.** ***Non-invasive in vivo measurement of skeletal muscle oxygen consumption in chronic heart failure*****.** 13th world congress on heart disease, Vancouver, Canada, July 28-31, 2007.

- **Abozguia K,** Nallur Shivu G, Phan TT, Steeds R and Frenneaux M. ***Speckle tracking echocardiography (STE) - variability and reproducibility study.*** 13th world congress on heart disease, Vancouver, Canada, July 28-31, 2007.

Moderator poster

- **Abozguia K,** Nallur Shivu G, Ahmed I, Phan TT, McKenna W, Sanderson J, Elliott P, and Frenneaux M. ***Ventricular twist and strain in hypertrophic cardiomyopathy: relation to exercise capacity.*** British Cardiac Society (BCS) 2009 London, UK.

Posters

- **Abozguia K**, Phan TT, Ahmed I, Nallur Shivu G, Maher AR, Kalra R, McKenna W, Watkins H, Elliott PM, Frenneaux MP. ***Cardiac energetic impairment in non-obstructive hypertrophic cardiomyopathy: relation to exercise capacity and dynamic diastolic dysfunction.*** ESC Congress 2009, Barcelona, Spain.

- **Abozguia K,** Nallur Shivu G, Ahmed I, Phan TT, Maher AR, McKenna WJ, Elliott PM, Frenneaux MP - ***Reduced longitudinal and radial strain of the left ventricle in patients with hypertrophic cardiomyopathy (HCM): a study using two-dimensional speckle tracking imaging.*** ESC Congress 2008, Munich, Germany.

- **Abozguia K,** Phan TT, Nallur Shivu G, Ahmed I, Maher AR, Nassimizadeh M, Chudasama K, Neubauer S, Elliott P, Watkins H and Frenneaux M. ***Impaired myocardial energetics is associated with diastolic dysfunction at rest and during exercise in patients with symptomatic non-obstructive Hypertrophic Cardiomyopathy (HCM).*** American College of Cardiology (ACC) 2008, Chicago, USA.

- **Abozguia K,** Phan TT, Nallur Shivu G, Ahmed I, Maher AR**,** Wagenmakers A, Frenneaux M **.** ***Reduced In Vivo Oxygen Consumption is independent of oxygen saturation in skeletal muscle of Chronic Heart Failure patients.*** ESC Heart Failure Congress 2008, Milan, Italy.

- **<u>Abozguia K</u>**, Nallur-Shivu G, Ahmed I, Phan TT, Maher AR, Nassimizadeh M, Yousef Z, McKenna W, Watkins H, Elliott P, Frenneaux M. ***Reduced twisting of the left ventricle in patients with symptomatic non-obstructive Hypertrophic Cardiomyopathy (HCM): a study using two-dimensional speckle tracking imaging.*** British Cardiac Society (BCS) 2008 Manchester, UK.

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ABBREVIATIONS

2,3-DPG - 2,3 diphosphoglycerate

3T - 3Tesla

ACE - Angiotensin-converting enzyme

ADP - Adenosine diphosphate

Am - Myocardial (TDI) peak late diastole velocity

AMP - Adenosine monophosphate–activated Protein

AMPK - Adenosine monophosphate–activated protein kinase

ARB - Angiotensin receptor blockers

ASH - Asymmetric septal hypertrophy

ATP - Adenosine triphosphate

CD36 - Cluster of differentiation 36

CHF – Chronic heart failure

CK - Creatine kinase

CPT - Carnitine-palmitoyl-transferase

CoA - Coenzyme A

Dec - Deceleration

Deoxy-Hb - Deoxygenated haemoglobin concentration

DPP-IV - Dipeptidyl peptidase IV

EaI - Arterial elastance index

EDV/s - End-diastolic volumes per second

EF- Ejection fraction

E_{LV}I - LV end systolic elastance index

Em - Myocardial (TDI) peak early diastole velocity

FA - Fatty acid

FASTMAP - Fast automatic shimming technique by mapping along projections

FATP-1- Fatty acid transporter protein-1

FFA - Free fatty acid

F0 - Centre frequency

F2,6 bisphosphate - Fructose 2, 6 – bisphosphate

GLP-1 - Glucagon-like peptide-1

GLUT-1 - Glucose transporter-1

Hb-T - Total haemoglobin concentration

HCM – Hypertrophic Cardiomyopathy

H/O - History of

HV – Healthy volunteer

jMRUI – java-based magnetic resonance user interface

ICD - Implantable cardioverter defibrillator

IMM - Inner mitochondrial membrane

iNOS - Inducible nitric oxide synthase

ISIS - Image-selected *in vivo* spectroscopy

LA - Left atrium

LAVI – Left Atrium volume index

LCFA - Long chain fatty acid

LC 3-KAT - Long-chain 3-ketoacyl coenzyme A thiolase

LV- Left ventricle

LVEF – Left ventricular ejection fraction

LVOTO - Left ventricular outflow tract obstruction

MBq – Megabecquerel

MCAD - Medium chain acetyl CoA dehydrogenase

MLHF - Minnesota living with heart failure

MLHFQ - Minnesota living with heart failure questionnaire

MR - Magnetic resonance

MRI - Magnetic resonance imaging

MRS - Magnetic resonance spectroscopy

MV A – Trans mitral late diastole velocity

MV E – Trans mitral early diastole velocity

MWT - Maximal wall thickness

NIRS- Near infra red spectrophotometry

NMR - Nuclear magnetic resonance

NOE - Nuclear overhauser enhancement

NS - Non Significant

NSVT - Non-sustained ventricular tachycardia.

nTTPF - Normalised Time To Peak Filling

NYHA – New York heart association

OMM - Outer mitochondrial membrane

Oxy-Hb - Oxygenated haemoglobin concentration

PCr - Phosphocreatine

PDE - Phosphodiesters

PDH - Pyruvate dehydrogenase

Peak A - Peak late diastole

Peak E - Peak early diastole

Peak S - Peak systolic

PET - Positron emission tomography

PFK- Phosphofructokinase

Pi - Inorganic phosphates

PPAR - Peroxisome proliferators-activated receptor

pPFRK-2 - Phosphorylation of 6- phosphofructo-2-kinase

ppm - Parts per million

PW-TDI - Pulse wave tissue Doppler imaging

QOL - Quality of life

QTc- Corrected QT interval

RF - Radiofrequency

R PCT - Randomized placebo controlled trial.

RER – Respiratory exchange ratio

Rot – Rotation

Rot-r - Rotation Rate

RR- R-R interval

RV- Right Ventricle

RXR-alpha - Retinoid X receptor alpha

SC - Circumferential strain

SCD - Sudden cardiac death

SL - Longitudinal strain

Sm – Myocardial (TDI) Peak systolic velocity

SNR - Signal to noise ratio

SR - Radial strain

SrC - Circumferential strain rate

SrL - Longitudinal strain rate

SrR - Radial strain rate

STE - Speckle tracking echocardiography

STPD - Standard temperature and pressure, dry conditions

TAN - Total adenine nucleotide

Tc - Technetium

TCA - Tricarboxylic Acid

TDI - Tissue Doppler imaging

TTPF - Time to peak filling

V_{O2} - Oxygen consumption

VOI - Voxel of interest

VVC - Vasculoventricular coupling

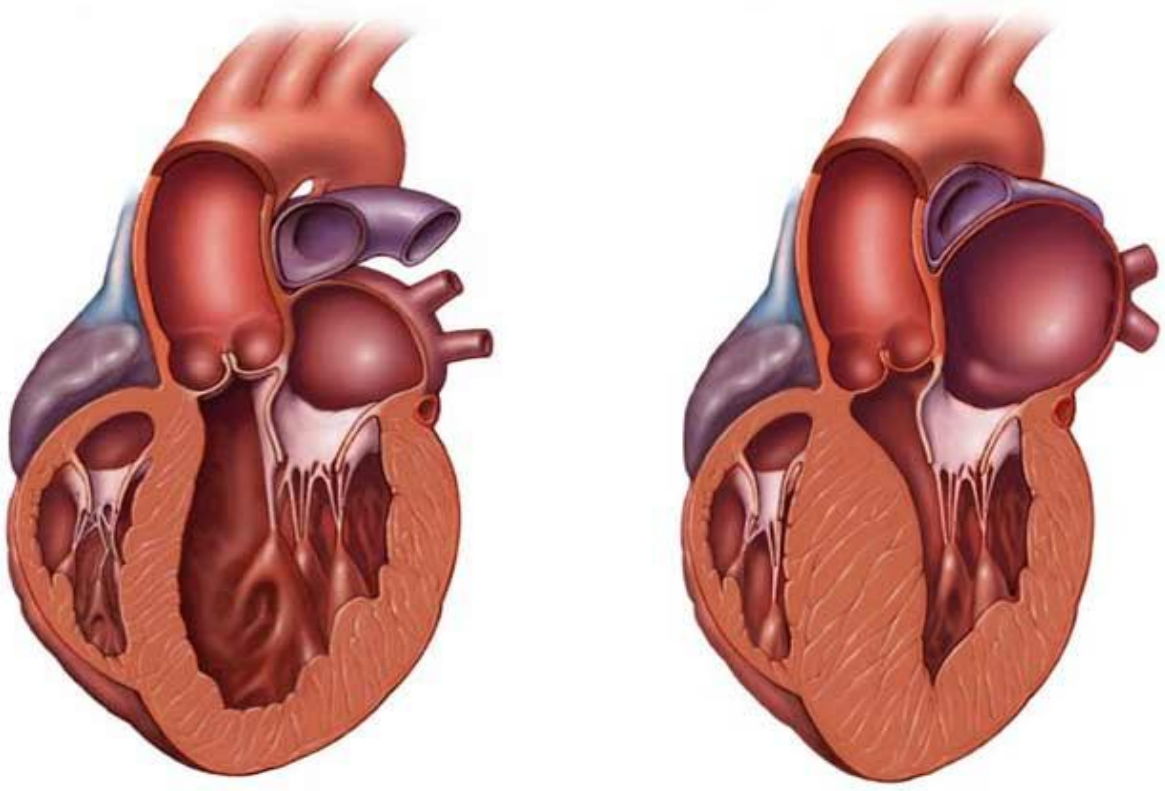
UCP - Uncoupling protein

CHAPTER 1: INTRODUCTION

Hypertrophic Cardiomyopathy

Definition

Hypertrophic cardiomyopathy (HCM) is defined as an autosomal dominant disease characterised by left ventricular hypertrophy in the absence of cardiac or systemic conditions (such as hypertension, aortic stenosis, or congenital heart defects) sufficient to explain the degree of hypertrophy (Figure 1). It is believed that myocyte and myofibrillar disarray provide the electrical substrate for malignant ventricular arrhythmias, which may be triggered by hypotension and/or ischaemia and/or a change in the autonomic milieu[1].



Normal **HCM**

Figure 1: Example of normal and HCM heart.

Courtesy of Mayo Education

Classification of HCM

There are different classifications of HCM depending on distribution of LV hypertrophy and on the presence or absence of significant LV Outflow Tract Obstruction (LVOTO) [gradient > 30 mmHg], Figure 2. Asymmetric septal hypertrophy (ASH) with and without LVOTO (Figure 2A and B respectively) is the commonest form of HCM. Other forms include concentric LVH (Figure 2C), apical HCM (Figure 2D) and mid cavity obstruction. 30% of HCM patients have obstruction at rest but this may increase with exercise and orthostatic stress. Some HCM patients may develop systolic heart failure at end stage due to left ventricular remodelling with progressive wall thinning and cavity enlargement. A minority of HCM patients may have significant restrictive physiology.

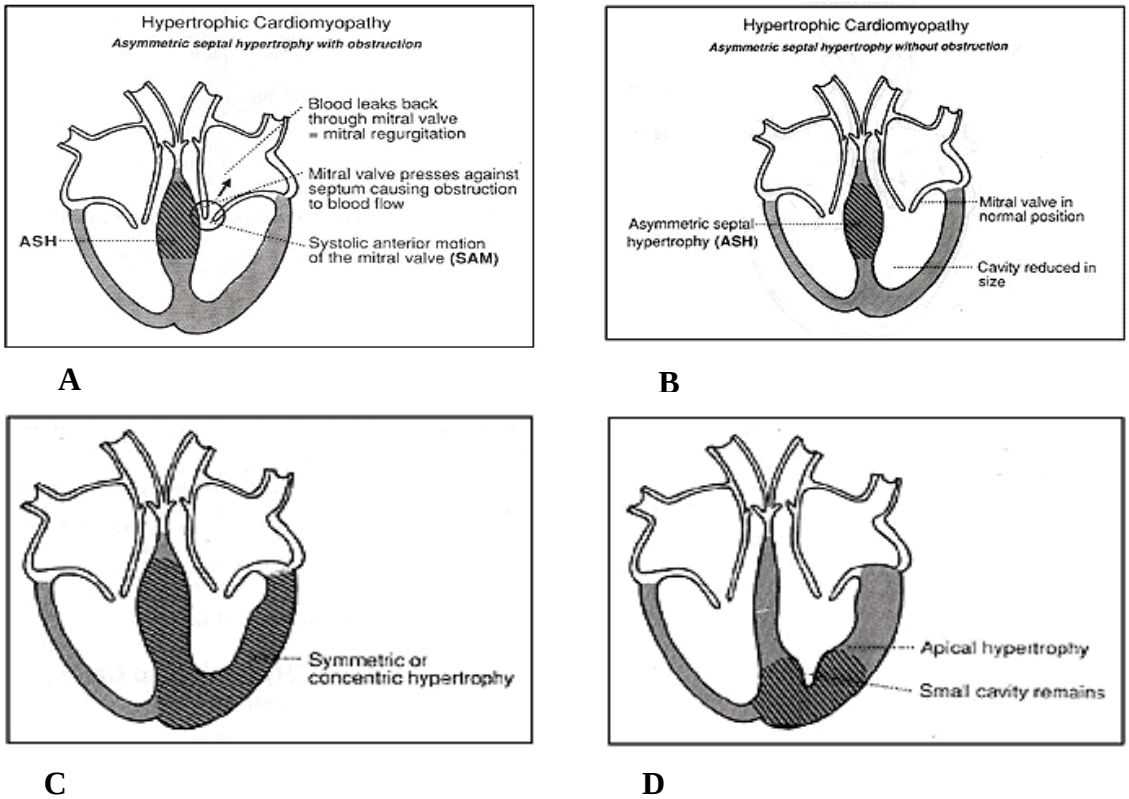


Figure 2: Anatomical classification of HCM.

Courtesy of Cardiomyopathy Association CMA

Genetics

HCM is inherited in an autosomal dominant fashion with variable penetrance. There is considerable genetic heterogeneity at both locus and allelic level, and a wide range of phenotypic expression. It is a disease of the sarcomere, with >400 mutations having been identified in approximately 10 genes encoding cardiac contractile proteins.[2] The main four genes that encode proteins of the cardiac sarcomere are: the β-myosin heavy-chain, cardiac troponin T, myosin-binding protein C genes and α-tropomyosin, Figure 3.

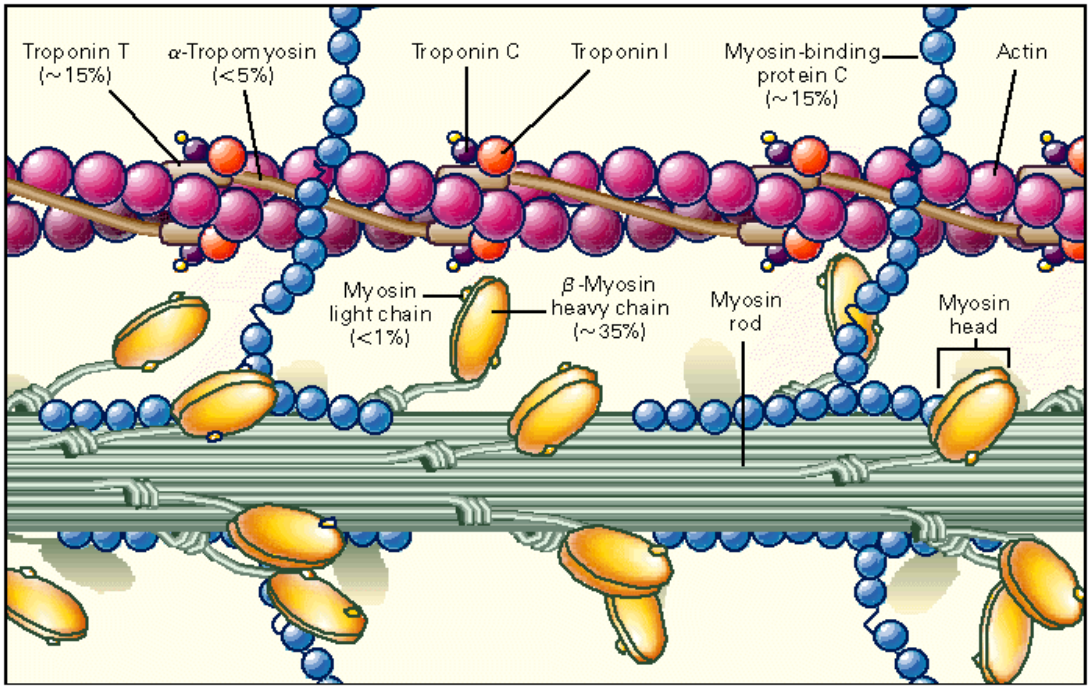


Figure 3: Component of Sarcomere in HCM.

From Spirito et al, NEJM 1997 [3]

Prevalence

HCM is the commonest inherited cardiac condition. The estimated prevalence of phenotypically expressed HCM in the adult general population is about 0.2% (1: 500) [4].

The actual prevalence is likely to be higher because patients with genotypic HCM who do not yet have hypertrophy are probably undetected clinically.

Presentation

Whilst some patients have minimal symptoms and/or a normal lifespan, others are highly symptomatic, and in others sudden cardiac death (SCD) may occur, which may or may not be preceded by symptoms of the disease. HCM is the most common cause of SCD in young people (including trained athletes) in most series [5-13]. Typical symptoms in HCM include breathlessness and/or fatigue on exertion, chest pains (which may be typically anginal, atypical, or a combination of both), palpitations (most commonly due to paroxysmal atrial fibrillation), and episodes of syncope or pre-syncope (Figure 4).

Outflow tract obstruction is present at rest in approximately one third of patients, but may be more frequent in situations associated with central hypovolaemia and or high sympathetic tone (dynamic obstruction). Outflow tract obstruction may contribute to symptoms and is usually amenable to pharmacological therapy (beta blockers and disopyramide), but in highly symptomatic medically refractory cases surgical septal myectomy or alcohol septal ablation may provide considerable symptomatic relief.

In the majority of patients however symptoms are unrelated to outflow tract obstruction. Patients with HCM typically exhibit abnormalities of diastolic function and these are thought to be important in the genesis of breathlessness. Active relaxation is slowed and filling rate diminished at rest [14].

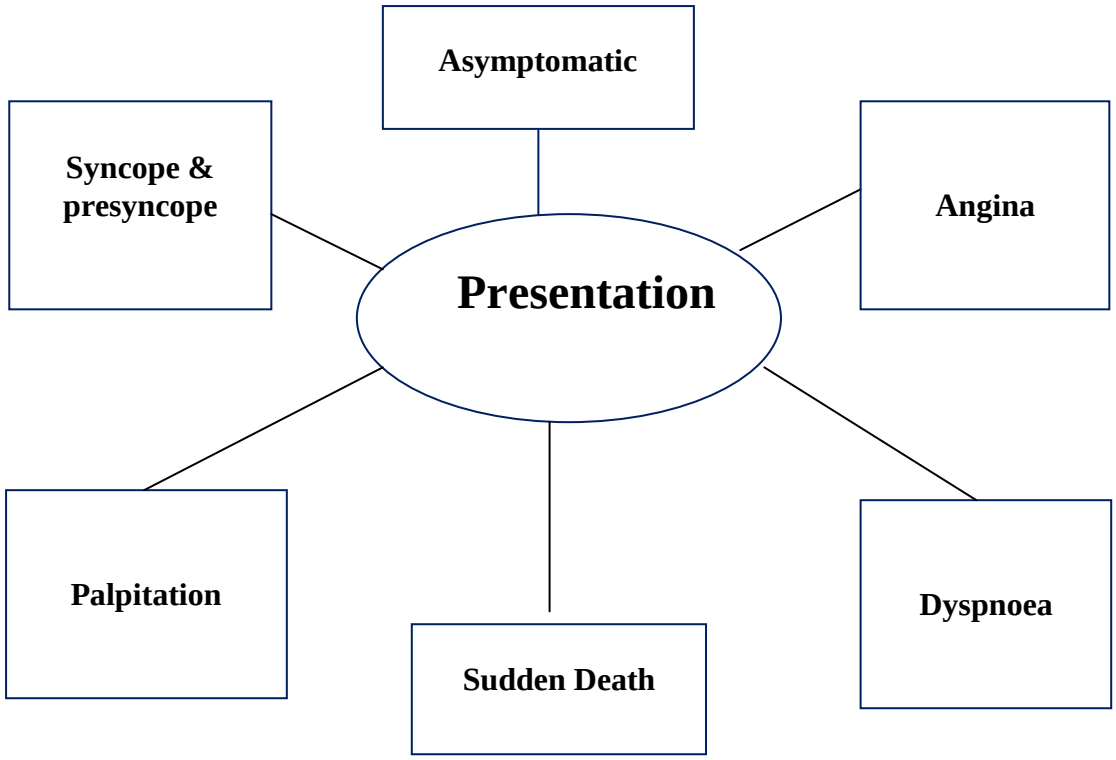


Figure 4: Broad spectrum of clinical presentations of HCM

Furthermore our group showed that, on exercise there is frequently a failure of the normal increase in rate of active relaxation on exercise, indeed in some patient's active relaxation paradoxically slowed on exertion [15]. Whilst resting measures of diastolic function were rather poorly related to exercise capacity, time to peak filling [TTPF] (an indirect measure of active relaxation) during maximal exercise was strongly related to peak oxygen consumption [15]. Izawa and colleagues subsequently confirmed these non invasive observations using invasive measures of active relaxation [16]. They demonstrated a failure of the normal shortening of active relaxation as heart rate was increased by atrial pacing and during supine exercise. Verapamil improves resting

diastolic filling parameters in the majority of patients with non obstructive HCM and improves symptoms in many, but not all [14]. The mechanisms responsible for the diastolic dysfunction are probably multifactorial. The hypertrophy, myocyte disarray and fibrosis may increase passive left ventricular stiffness. Abnormalities of active relaxation may relate to abnormal calcium handling [17], to microvascular myocardial ischaemia, to mechanical dyssynchrony [18], or to impaired myocardial energy utilisation [19].

Management

There are four distinct issues in the management of HCM patients. These include treatment of symptoms to improve quality of life (QOL), genetics testing, family screening and the identification of patients who are at high risk for sudden death and require aggressive therapy (Figure 5).

Pharmacological options

Conventional treatments include ß-blockers +/- disopyramide for obstructive HCM whereas calcium channel antagonists (mainly verapamil) are used for non-obstructive HCM. Beta Blockers act predominantly by lowering heart rate with a consequent prolongation of diastole and increased passive ventricular filling [20, 21]. Beta-blockers exhibit negative inotropic effects which may lessen myocardial oxygen demand and decrease the outflow gradient during exercise [20, 21]. Similarly, disopyramide may improve symptoms by reducing the outflow gradient via its inotropic effect [22].

Verapamil improves symptoms by increasing ventricular filling as a result of heart rate reduction and probably reduces myocardial ischaemia [23, 24].

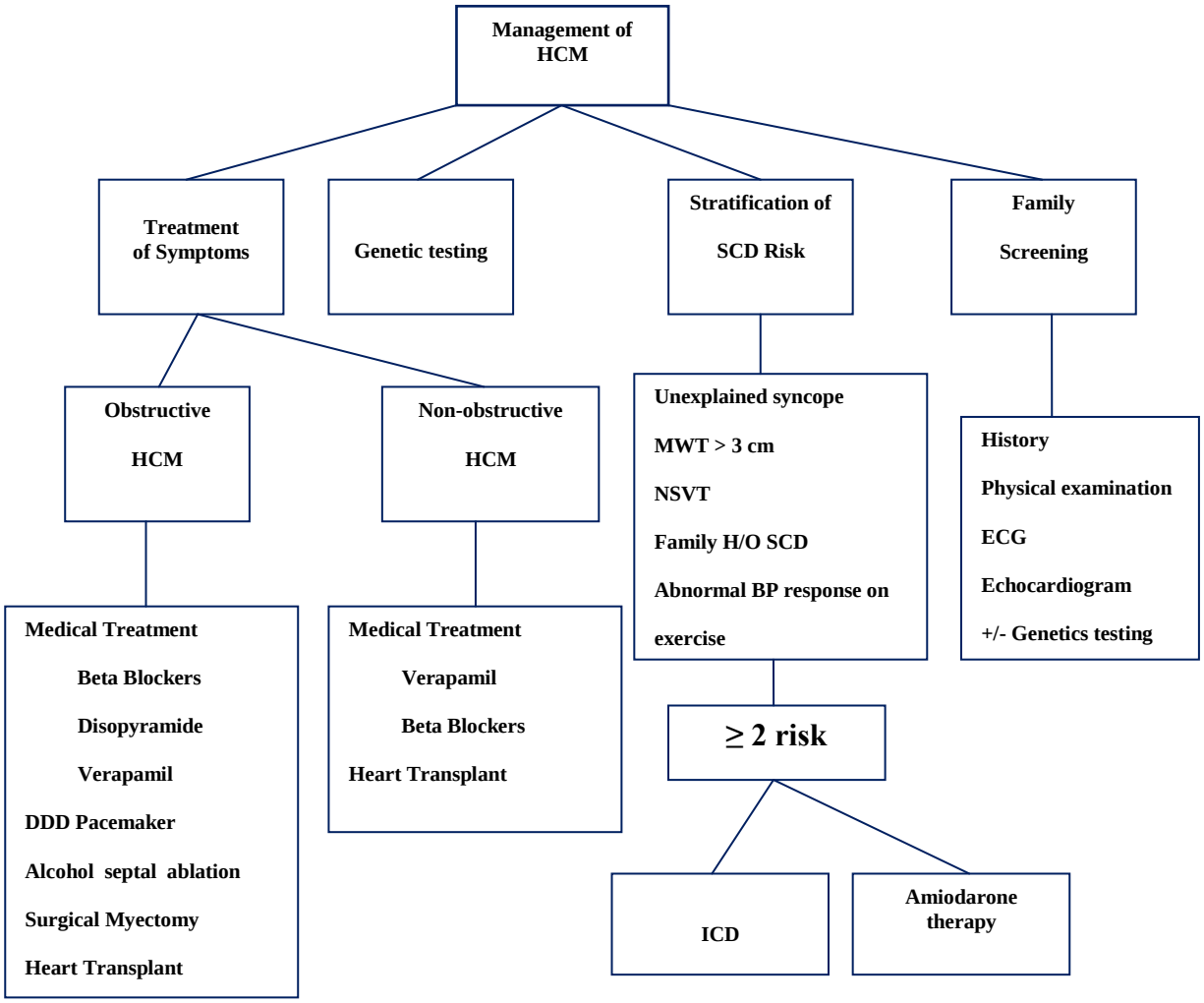


Figure 5: The management of HCM.

SCD: sudden cardiac death, H/O: history of, MWT: maximal wall thickness, ICD: Implantable Cardioverter Defibrillator, NSVT: Non-sustained ventricular tachycardia.

Dual Chamber (DDD) Pacemaker

Nonrandomized, unblinded studies reported that dual-chamber pacing is associated with both a substantial decrease in the outflow gradient and symptomatic improvement in patients with outflow obstruction [25, 26]. However, in a randomized, double-blind, crossover study, there was no significant difference in exercise capacity with and without pacing [27]. Furthermore, there was only a small decrease in the average outflow gradient during pacing and varied significantly among patients [27]. These findings suggest that the effects of DDD pacemaker to be less favourable. Additionally, there are concerns that pacing may have harmful effects on ventricular filling and cardiac output [28, 29].

Alcohol septal ablation

This is a percutaneous, intra-coronary, minimally-invasive procedure to relieve symptoms by reducing outflow gradient. 1 to 4 ml of alcohol is introduced into the target septal perforator coronary artery branch to produce myocardial infarction. This reduces basal septal thickness and enlarges the LV outflow tract.

Surgical myectomy

This is an invasive surgical operation which involves resection of a small amount of muscle (about 5 g) from the proximal septum thereby abolishing any significant obstruction to LV outflow. In established centres, it is considered as the gold standard treatment for adults with obstructive HCM and severe drug refractory symptoms.

Risk stratification for SCD

HCM is considered the most common cause of SCD in young competitive athletes [10]. Ventricular fibrillation and tachycardia are the commonest cause of sudden death in these patients. This can be a primary event related to the arrhythmogenic myocardial substrate or as a secondary occurrence triggered by myocardial ischaemia. All patients should undergo non-invasive risk factor stratification for SCD (Figure 5). Detailed history should be obtained specifically for any history of unexplained syncope, previous cardiac arrest and family history of sudden cardiac arrest. Echocardiogram is a useful tool to identify severe LV hypertrophy (MWT > 3 cm) and any significant LVOTO (gradient > 30 mmHg). NSVT on 48 hours tape is another important risk factor for SCD among these patients. Furthermore, an abnormal BP response during exercise is an important risk factor for SCD especially in young patients (less than 40 years old). This is defined as a failure to increase systolic blood pressure by > 25 mm Hg above the resting systolic blood pressure during exercise. Patients with two or more risk factors have annual sudden death rates of 3% (95% CI 2 % to 7%). These patients should be offered prophylaxis with ICD and/or (more controversially) amiodarone [5] (Figure 5).

Family screening

All first-degree relatives of the HCM patient should be offered screening. This should include detailed history, physical examination, ECG and echocardiogram. Genetic testing is only helpful if a gene had been identified in the affected person. Screening is usually performed annually from age 12-20 and every 5 years age 25 and over.

Future Treatment

While the incidence of sudden death appears to be declining, the management of symptoms remains a challenge. In particular, in non-obstructive HCM patients whose symptoms are refractory to standard drug therapy, there are no therapeutic options (unlike the options of surgical septal myectomy or alcohol septal ablation in obstructive disease), mandating a search for effective novel treatments. Small studies in animal models of HCM have shown regression of LV hypertrophy by simvastatin and losartan [30, 31]. However, these results have not been replicated in human studies. There is a strong evidence of reduced cardiac energetic in both animal and human models of HCM [32-36]. The main focus of this PhD thesis is to examine the causative role for this energy deficiency in the pathogenesis of HCM. Furthermore, the studies described assess the direct manipulation of myocardial energy utilisation with perhexiline, metabolic modulator, on subjective and objective clinical benefits in symptomatic non-obstructive HCM patients who were already on optimal medical therapy.

Metabolic alteration in heart diseases

The foetal heart uses glucose as its primary substrate, but a shift to predominant free fatty acid utilisation occurs soon after birth [37]. The healthy adult human heart is a metabolic omnivore [38], able to adapt substrate utilisation accordingly to circumstances but in general approximately 70% of energy production is derived from beta oxidation of fatty acids [39, 40] (Figure 6). During ischaemia there is a shift towards greater glucose utilisation, but free fatty acid utilisation is still typically more than 50% [40]. Changes in cardiac substrate utilisation occur in the diseased heart. In animal models of left

ventricular hypertrophy (LVH) progressing to heart failure there is a down regulation of enzymes involved in fatty acid oxidation with an increase in glucose uptake [41]. However, at least in the rat aortic banding model, there appears to be a block in the conversion of Pyruvate to Acetyl CoA. Although anaplerosis, process which replenishes the Tricarboxylic Acid (TCA) cycle, is increased this is insufficient to compensate for this block and the hypertrophied heart is energy deficient [42]**.** The spontaneous hypertensive rat is deficient in CD36 (Cluster of Differentiation 36) which is an integral membrane protein required for the uptake of long chain fatty acids into cardiac myocytes [43]. Dietary supplementation with medium chain fatty acids (that do not require CD36 to enter myocytes) prevents the development of LVH and increases resistance to adrenergic stress in this model, suggesting that the reduction in fatty acid utilisation is maladaptive in this model [44, 45]. In contrast, in the mouse banding model of LVH it appears adaptive because up regulating fatty acid utilisation by activating peroxisome proliferators-activated receptor alpha (PPAR-α) results in a loss of contractile function in this model [46]. In rapid pacing models of heart failure fatty acid utilisation appears to be preserved or even increased [47].

The mechanisms responsible for the shift to decreased fatty acid oxidation in LVH models the 'foetal phenotype' are complex. Increased Adenosine 5'-monophosphate [AMP] leads to activation of AMP-dependent protein kinase (AMPK) which acts as a 'low on fuel' warning system [48] . Increased [AMP] and AMPK activity has been reported in hypertrophied rat hearts undergoing transition to heart failure [49]. By phosphorylating several proteins, AMPK regulates free fatty acid and glucose utilisation in both cardiac and skeletal muscle to maximise ATP production. This results in an

increase in (insulin independent) GLUT 1 transporters [49] and phosphorylation of 6-phosphofructo-2-kinase (pPFRK-2) which increases synthesis of fructose 2, 6 – bisphosphate (F2,6 bisphosphate), a potent stimulator of glycolysis [50]. AMPK also regulates fatty acid metabolism. Acute increases in AMPK activity enhance fatty acid oxidation in rat skeletal muscle via a decrease in acetyl CoA carboxylase activity and a subsequent decrease in the inhibition of Carnitine palmitoyl-transferase-1 (CPT-1) by malonyl CoA [51] (Figure 6).

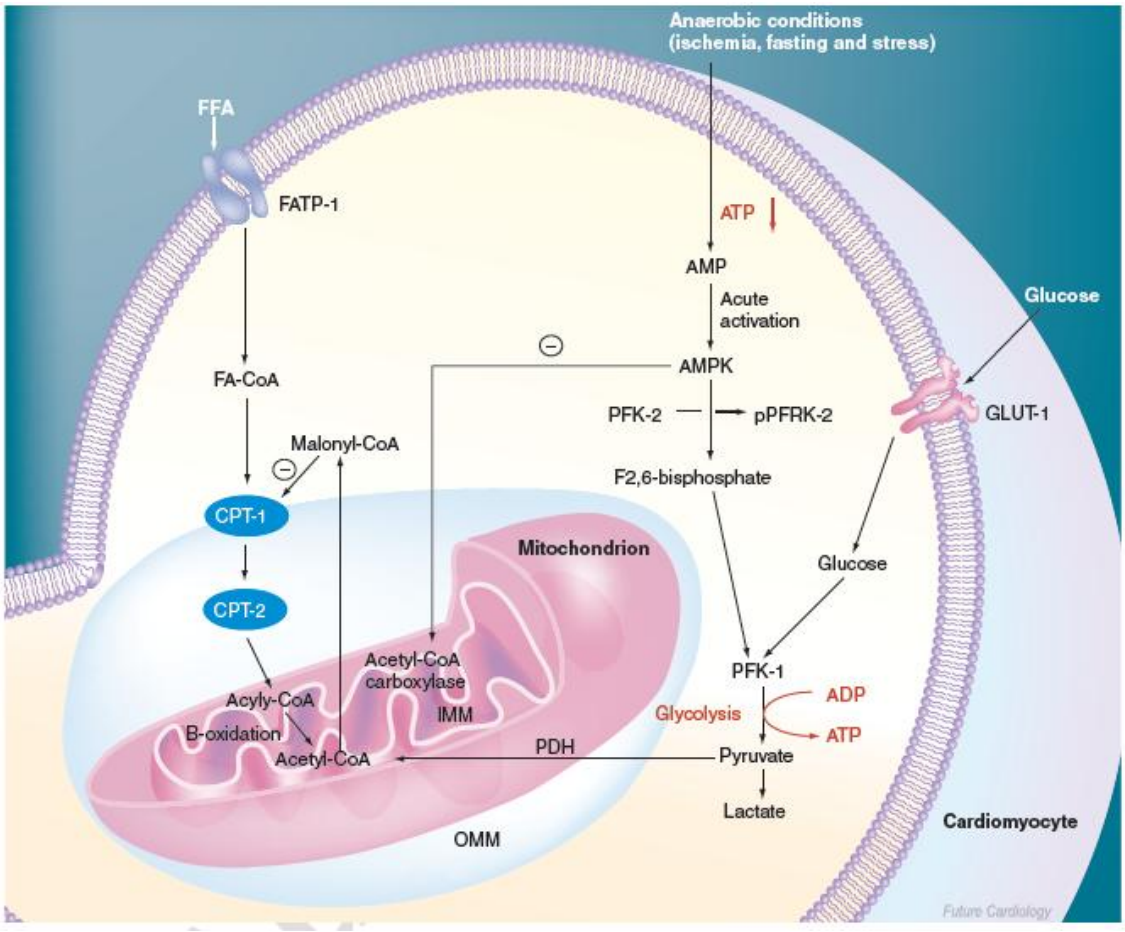


Figure 6: Myocardial metabolism.

With permission from Future cardiology [52]

In contrast chronic AMPK activation by pressure overload was associated with decreased fatty acid utilisation via marked reductions in the expression of CPT-1 and medium chain acetyl CoA dehydrogenase (MCAD), the rate limiting steps for this pathway [49]. Increased AMPK activity also increases expression of the nuclear transcription factor peroxisome proliferators-activated receptor gamma co-activator (PPAR-γ). This promotes mitochondrial biogenesis and oxidative phosphorylation [53, 54].

In contrast to LVH models, most studies of rapid pacing models have shown increased dependency on FA metabolism [47]. However, one study in the rapid pacing canine heart failure model showed a marked reduction in fatty acid utilisation and an increase in glucose utilisation and that was attributed to reductions in the expression and activity of Retinoid X receptor alpha (RXR-alpha), a nuclear receptor known to stimulate expression of enzymes involved in free fatty acid oxidation, and of the enzyme MCAD. [55]. There was no change in expression of PPAR alpha. The mechanisms responsible for altered expression and activity were not delineated. In contrast to the rapid pacing model, in pressure overload hypertrophy there is also down regulation of PPAR alpha expression [56].

There remains some controversy as to whether the shift towards glucose utilisation in animal models of Left Ventricular Hypertrophy (LVH) and heart failure is adaptive or maladaptive. Human genetic disorders of fatty acid beta oxidation may be associated with the development of heart failure [57]. The spontaneously hypertensive rat has an impaired ability to withstand adrenergic stress [58]. This appears to be because the reduced ability to utilise long chain fatty acids is not fully compensated under

conditions of stress by increased glucose utilisation, increased breakdown of intracellular triglycerides and increased flux through the anaplerotic reaction. In this model dietary supplementation with medium chain fatty acids, which do not enter the myocyte via the defective Long Chain Fatty Acid (LCFA) transporter (CD36), prevents the development of left ventricular hypertrophy [59] and restores the ability to withstand acute adrenergic stress [58] . These observations suggest that the shift to glucose utilisation may be maladaptive. On the other hand, cardiac specific over expression of the GLUT 1 transporter prevents the transition from left ventricular hypertrophy to heart failure in mice subjected to pressure overload [60] , whereas activation of the PPAR alpha receptor (which increases fatty acid utilisation) in the mouse aortic banding model, causes contractile dysfunction [46].

It appears that the direction of the metabolic substrate shifts varies with aetiology and severity of ventricular dysfunction [61]. Therefore, the severity of heart failure and temporal differences during the progressive remodelling that characterised the transition to heart failure may play important role in the mechanism of metabolic substrate shift. Unlike the above animal experimental models that undergo transition from LVH to heart failure and in which there is a shift to a 'foetal' pattern of substrate use, the available evidence suggests that human heart failure may not be associated with a shift towards predominant glucose utilization, indeed the converse may be true [62, 63]. Using Positron emission tomography (PET) technique, Taylor et al demonstrated increased myocardial FFA uptake and reduced glucose uptake in human heart failure. There are several potential explanations for this. Firstly, insulin resistance is commonly present in CHF [64]. Nikolaidis *et al* demonstrated a progressive increase in cardiac insulin

resistance during disease progression in the canine rapid pacing heart failure model [65]. There was reduced cardiac basal and insulin stimulated glucose uptake (associated with reduced insulin-stimulated GLUT-4 translocation) [65]. Secondly, increased plasma FFAs are characteristically found in CHF due to sympathetic activation and contribute to insulin resistance.

Despite the uncertainty of whether the shift towards glucose metabolism in experimental models of heart failure is adaptive or maladaptive in nature, observations with perhexiline (8 weeks) [66] and those with trimetazidine (up to 18 months) [67] suggest that pharmacologically induced inhibition of fatty acid metabolism has beneficial effects on cardiac performance, exercise capacity and symptoms in patients with chronic heart failure.

Substrate Utilisation and Myocardial Oxygen Utilisation

Simple stochiometry suggests that utilisation of fatty acids should cost approximately 12% more oxygen per unit of ATP generated than glucose. In practice the increased oxygen requirement when plasma FFAs are increased compared with dominant glucose utilisation appears to be substantially greater (up to 40 %) [68-70], implying the presence of an 'oxygen wasting' phenomenon with FFA usage. In the isolated perfused rat heart, increasing the palmitate and octanoate in the perfusate disproportionately increased oxygen utilisation and this was only partially abolished by administration of an inhibitor of fatty acid beta oxidation, suggesting that a component of the oxygen 'wasting effect' of increased FFAs is not related to mitochondrial long chain fatty acid oxidation [71]. It is

possible that it may be due to the up-regulation of uncoupling protein expression in the mitochondria via FFA activation of peroxisome proliferators-activated receptor alpha (PPAR $\propto$) [72]. Normally the electron transport chain results in the generation of a net proton gradient across the inner mitochondrial membrane (IMM) and very little proton leak occurs and all the energy built up in the respiratory chain can be used to phosphorylate ADP to generate ATP. When mitochondria are 'uncoupled' there is proton leakage and the electrochemical gradient is dissipated as heat [73]. Emerging evidence suggests that the primary role of uncoupling proteins (UCP) is the export of lipid peroxides and long chain fatty acids out from the mitochondrion. This protects mitochondrial DNA from oxidation damage. Uncoupling occurs when fatty acid delivery exceeds oxidative capacity and when there is an accumulation lipid peroxides [73]. The activity of UCPs is also increased by lipid peroxides. Heart failure is associated with elevated free fatty acid concentrations which might increase uncoupling. Consistent with this concept, Murray et al showed that mitochondrial uncoupling protein expression in human cardiac muscle correlated positively with plasma FFA concentration [74]. Furthermore, high levels of FFA may trigger intracellular futile metabolic cycles such as intramyocardial lipolysis and resterification [69, 75, 76].

Myocardial energetics in Heart Diseases

Heart Failure

Heart failure continues to have a significant morbidity and mortality despite the several advances in treatment such as additional neurohumoral blockades and cardiac resynchronisation therapy. There is compelling evidence that an impairment of cardiac

energetic status may play an important role in the pathophysiology of heart failure even in the absence of coronary artery disease. Studies of human biopsy specimens and others using *in vivo* ^{31}P NMR spectroscopy suggest that [ATP] is approximately 25-30% lower in the failing human heart. Studies in animal models have shown that there is a global loss of the total adenine nucleotide (TAN) pool [77, 78]. It is not clear whether this is a consequence of reduced (or inadequate) *De Novo* purine synthesis or increased breakdown to diffusible purine nucleosides. The energetic abnormality may in turn exacerbate the loss of TAN in that increased [AMP] activates cytosolic 5'AMP – specific 5'nucleotidase, the primary enzyme responsible for the conversion of AMP to adenosine in muscle cells. Shen and colleagues investigated the changes in energetic status occurring during the evolution of heart failure in the canine rapid pacing model. There was a progressive monotonic decay in total adenine nucleotides and creatine from the onset of pacing. In other words, the onset of the decline in energetic status preceded objective evidence of cardiac contractile dysfunction [77].

Phosphocreatine (PCr) is an important energy buffer molecule that maintains a high phosphorylation potential under conditions of increased energy demand such as exercise. The movement of a phosphoryl group from PCr to ADP by the enzyme Creatine kinase (CK) generates ATP approximately 10 times faster than the maximum rate of ATP generation via oxidative phosphorylation [79]. Cardiac creatine content is determined in large part by the activity of the creatine transporter which is responsible for creatine uptake into the myocyte against a concentration gradient [80, 81]. Studies in animal models of LVH / heart failure and in human heart failure have shown that there is a progressive reduction in the creatine pool as high as 60% and the level of reduction

is related to heart failure severity [82-84]. In the healthy heart approximately two thirds of the total creatine pool is phosphorylated via the CK reaction to form PCr. The reduction of total creatine pool in condition such as heart failure results in PCr depletion and also a reduction in PCr/ATP ratios as measured by ^{31}P NMR spectroscopy [48, 85]. The magnitude of the reduction of this ratio predicts mortality [86]. The reduction in PCr in heart failure appears to be partly due to a decreased number of creatine transporters [81] and a reduction in CK isoenzyme expression activity, particularly mitochondrial CK leading to a reduced [PCr]/ [Total Creatine] [87]. The reduced mitochondrial CK activity may in turn be partly mediated by the effect of inducible nitric oxide synthase (iNOS)-derived Nitric Oxide [88]. Creatine supplements are frequently taken by competitive athletes, despite the fact that the myocyte levels are primarily limited by the transporter. Short term oral creatine supplementation in chronic heart failure (CHF) has been shown to increase skeletal muscle function, but no effect was observed on left ventricular ejection fraction (LVEF) [89, 90] . In summary, there is clear evidence that even in the absence of coronary artery disease (or of myocardial hypoxia) there is reduced energy reserve in the myocardium in heart failure, which although related to heart failure severity, may begin before the development of overt heart failure.

HCM

Biochemical and biophysical analyses of the mutant sarcomeric proteins that cause HCM led Watkins and colleagues to propose that energy depletion may underlie HCM [91-93]. Consistent with this, unexplained left ventricular hypertrophy is a feature of disorders associated with a variety of metabolic genes that lead to defects in energy

production in the heart [91]. In a recent study using *in vivo* cardiac MR spectroscopy, resting PCr/ATP ratio was diminished in patients with sarcomeric HCM, indicating reduced energy availability [34]. Importantly, patients with genotypic HCM who do not yet have hypertrophy have a similar degree of impairment of cardiac PCr/ATP ratio as do patients with marked hypertrophy, implying that the disturbance may be an early feature of the disease and is not simply due to the hypertrophy [32]. Consistent with this concept is the recent observation that although LVEF is normal or supra-normal in the majority of HCM patients, there is nevertheless usually impairment in long axis systolic function which is apparent even in young patients before hypertrophy develops [94]. Tagged MRI studies have shown that radial contractile function may also be impaired even though EF is supra-normal [95].

Anginal chest pains in HCM patients may be due to increased myocardial oxygen demand, a reduced transcoronary perfusion gradient, microvascular disease and potentially to the disturbed energy utilisation described above. Reduced skeletal muscle maximal oxidative capacity has also been reported in HCM patients with Beta Myosin mutations (expressed in skeletal muscle) but not in those with Troponin mutations [96]. Whether this relates to a similar primary energetic disorder, to an impairment of skeletal muscle nutritive flow, or to skeletal myopathy is unknown [97].

In summary there is clear evidence of an energetic impairment in both cardiac and skeletal muscle in HCM, whether due to ischaemia or abnormal energy utilisation. Exercise limitation in HCM patients appears to be due to a dynamic impairment of active relaxation during exercise, probably related to this energetic impairment. From

the above it will be clear that an agent which increased the efficiency of myocardial energy production (i.e. increased production without increased oxygen cost) would have the potential to improve symptoms of breathlessness and angina in patients with HCM.

Metabolic Agents

In a study in the late 1990's intracoronary pyruvate was shown to acutely increase stroke volume and reduce pulmonary capillary wedge pressure in heart failure patients, implying acute beneficial effects of a shift away from fatty acid metabolism [98]. This renewed interest in the potential beneficial effects of modifying substrate utilisation in chronic heart failure. A number of pharmacological agents have been shown to inhibit FFA utilisation in the heart, either by inhibiting FFA uptake into the mitochondrion or by inhibiting beta oxidation (Table 1 and Figure 7).

FFA uptake inhibitors	Perhexiline Oxfenicine Etmoxir Beta-Blockers
FFA β-Oxidation inhibitors	Trimetazidine
Others	Ranolazine* D-Ribose Proprionyl-L-Carnitine Glucagon-like Peptide-1

Table 1: Classification of metabolic agents.

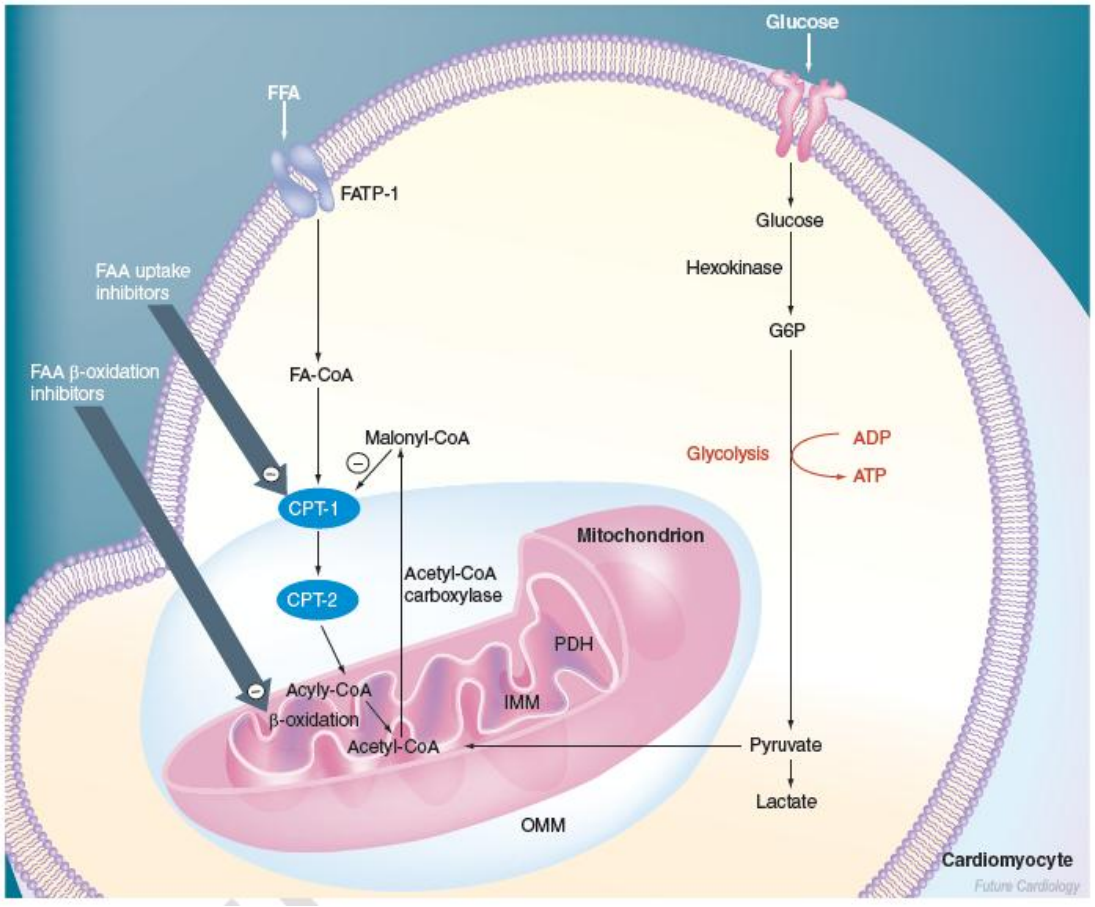


Figure 7: Effects of metabolic agents on myocardial metabolism.

With permission from Future cardiology [52]

Some of these agents have been used as antianginal agents because of their 'oxygen sparing' actions. Summary of clinical trials of metabolic agents are shown in Table 2.

FFA Uptake Inhibitors

Perhexiline

Perhexiline is a metabolic modulator which causes a shift in cardiac substrate use from fatty acids towards carbohydrates. It is a reversible inhibitor of both CPT1 and CPT2 which are key enzymes involved in the transport of free fatty acids into the

mitochondria[99, 100]. It is also effective as an adjunct to other anti-anginal therapies such as beta-blockades and calcium channel blockades. Its use however declined dramatically in the late 1970s and early 1980s following reports of hepatotoxic effects and peripheral neuropathy [101-103].

Metabolic Agents	Study (Ref)	Study design	No of Patients	Patient Characteristics	Study duration	Outcome
Perhexiline	Lee *et al* 2005 [66]	R PCT* Double blind	56	EF≤ 45 Non-ischemic & Ischemic CHF NYHA II-IV	2 months	Improvement in: LVEF Peak V_{o2} QOL
Trimetazidine	Vitale C *et al* 2004 [104]	R PCT	47	EF< 50% Chronic stable angina NYHA I-III	6 months	Improvement in: LVEF QOL
Trimetazidine	Di Napoli et al 2005 [67]	Open label	61	LVEF <40% Ischemic LV dysfunction NYHA II-IV	18 months	Improvement in: LVEF NYHA
Trimetazidine	Fragasso G *et al* 2006 [105]	Open label	55	LVEF<45% Ischemic and Non-ischemic CHF NYHA II-III	13 months	Improvement in: LVEF NYHA
Etomoxir	Schmidt-Schweda *et al* 2000 [106]	Open label	10	LVEF<40% Dilated Cardiomyopathy NYHAII-III	3 months	Improvement in LVEF Increased stroke volume during exercise
Etomoxir	Holubarsch CJ *et al* 2007 [107]	R PCT	350	LVEF<40% Ischemic and Non-ischemic moderate CHF NYHA II-III	6 months	Study stopped prematurely because of hepatotoxicity.
D-Ribose	Omran *et al* 2003 [108]	R PCT Cross over	15	Ischemic Cardiomyopathy NYHA II-III	3 weeks	Improved functional capacity and diastolic function
L-Carnitine	Anand *et al* 1998 [109]	R PCT Single blind	30	LVEF<40% NYHA II-III Ischemic and non-ischemic cardiomyopathy	4 weeks	Improvement in: Peak V_{O2} but no change in LVEF
L-Carnitine	Eur Heart Journal 1999 [110]	R PCT Double Blind	537	LVEF <40% NYHA II-III Ischemic and non-ischemic cardiomyopathy	6 months	No increase in exercise capacity
Glucagon-like Peptide 1	Sokos *et al* 2006 [111]	Open label	12	NYHA III-IV	5 weeks	Improvement in: LVEF QOL

Table 2: Summary of human clinical trials of metabolic agents for treatments of chronic heart failure.

These effects were later demonstrated to occur in patients, in whom hydroxylation was slow because they processed a genetic variant of the cytochrome P450 2D6 enzyme, which metabolizes the drug, resulting in drug accumulation and, consequently, accumulation of phospholipids in the liver and nerves [112]. The risk of toxic effects is virtually eliminated by maintaining plasma concentrations at between 150 and 600 ng/ml, at this level the drug also remains efficacious [113, 114]. As a result, in some countries particularly Australia, the perhexiline use has been rekindled as an adjunctive therapy for refractory angina, with good results.

In the therapeutic range, perhexiline has no effects on systemic vascular resistance and is not negatively inotropic [115-117]. Horowitz and colleagues showed that perhexiline improve mechanical efficiency but without alteration in FFA uptake [118]. In a double-blind, randomized, placebo-controlled trial, our group have shown short-term beneficial effects of perhexiline in patients with ischaemic or non-ischaemic heart failure [66]. Patients taking optimum conventional medical therapy were randomized to perhexiline or placebo for 8 weeks, with dummy dose adjustment in the placebo group. We noted a large increase in the combined primary endpoint of peak oxygen uptake (≈ 3 ml/kg/min) and LVEF (≈ 10 percentage points), and improvement in symptoms as assessed by the Minnesota Living with Heart Failure (MLHF) Questionnaire. The study design involved separate randomization of the ischaemic and non ischaemic groups, permitting separate analysis of the primary endpoint in each group. Maximum oxygen uptake was significantly increased in both the ischaemic and non-ischaemic groups; therefore suggesting the mechanism of benefit is not primarily anti-ischaemic. The study was however of short duration and therefore, further work is required to show that the

clinical benefits of perhexiline are prolonged and that it reduces hospitalization and mortality.

Oxfenicine

Oxfenicine is an irreversible inhibitor of carnitine palmitoyltransferase I (CPT1). Oxfenicine has been shown to delay development of terminal heart failure, attenuate adverse hemodynamic changes, prevent wall thinning, prevent the activation of matrix metalloproteinases, and result in the transcriptional down regulation of CPT1 and of key enzymes involved in cardiac energy metabolism in animal models of heart failure [119]. However a dose related increase in cardiac weight due to uniform myocardial fiber hypertrophy of all cardiac chambers was noted in animal studies [120]. There was an increase in liver and kidney weights as well [121]. No human studies have been reported with use of oxfenicine in heart failure.

Etomoxir

Etomoxir an irreversible inhibitor of CPT1 was initially introduced as a therapy for diabetes due to its hypoglycemic properties [122, 123]. It has been shown to favorably modify ventricular mass, geometry and function in pressure-overloaded rats [124], and reduce the occurrence of ventricular failure in diabetic rat hearts [125]. In humans, a 3-month period, open-label, uncontrolled trial in 10 patients with NYHA class II–III heart failure, etomoxir treatment in addition to standard therapy significantly improved LVEF, cardiac output at peak exercise, and clinical status [106]. However, recently a large randomized placebo controlled study was terminated prematurely because of the

development of significant hepatotoxicity in four subjects [107]. Therefore etomoxir may not be a suitable metabolic modulator for use in heart failure.

Beta Blockers

β-adrenoreceptor blockade improves left ventricular function and prognosis in patients with ischaemic or nonischaemic cardiomyopathy [126, 127]. Interestingly, Wallhaus et al. demonstrated carvedilol in patients with heart failure caused a 57% reduction in myocardial free fatty acid uptake [128]. However, neither mean myocardial uptake of labeled glucose tracers nor the rates of glucose use were significantly increased. This could be because of a type II error, since a marked fall in the ratio of myocardial free fatty acid to glucose use does suggest a so-called metabolic shift. This effect may be due to CPT1 inhibition. In another study, Al-Hesayen and colleagues showed that after 4 months of carvedilol therapy, myocardial lactate consumption was increased and myocardial uptake of free fatty acids in patients with CHF was reduced [129]. This suggests that carvedilol therapy may cause a significant shift in myocardial substrate use from free fatty acids to glucose.

FFA β-Oxidation inhibitors

Trimetazidine

There remain controversies regarding the exact mechanism of action of trimetazidine. Kantor and colleagues showed that trimetazidine reduces the oxidation of free fatty acids via inhibition of the enzyme long-chain 3-ketoacyl coenzyme A thiolase (LC 3-KAT), which is crucial in the β-oxidation pathway [130]. However, recent data failed to replicate these finding. In fact, MacInnes and colleagues showed that trimetazidine

exhibits anti-anginal effects without any significant inhibition of LC 3-KAT or any component of ß-oxidation [131]. It is an effective anti-anginal agent with no significant vasodilator properties at rest or during dynamic exercise [132]. In a double-blind, placebo-controlled trial involving 47 patients with coronary artery disease and a reduced left ventricular function, limited by angina but not by heart failure, trimetazidine therapy improved left ventricular systolic and diastolic function and QOL [104].

A number of other studies also demonstrated benefits with trimetazidine. Di Napoli et al demonstrated in an 18 month, open-label study a significant improvement in left ventricular function in patients with ischaemic cardiomyopathy with reduced LVEF [67]. Patients were excluded if they had experienced acute myocardial infarction less than 3 months previously, or had acute heart failure. Many patients however were taking suboptimum conventional therapy. Rosano and colleagues demonstrated improvements in LVEF among patients with diabetes and coronary heart disease and left ventricular systolic dysfunction, but without frank heart failure, following 6 months of trimetazidine therapy [133].

Recently, Fragasso et al demonstrated in patients with both ischaemic and non-ischaemic cardiomyopathy increase in LVEF and NYHA class with the use of trimetazidine [105]. However, in another study, which assessed the effects of trimetazidine in patients with heart failure who were diabetic, revealed no significant improvement in exercise capacity and only minor effects on left ventricular systolic function in both resting and exercise states [134].

Others

Ranolazine

Ranolazine has been shown to be a partial inhibitor of fatty acid β oxidation *in vitro* [135, 136], but this has not been reliably replicated *in vivo*. There is emerging evidence that ranolazine blocks the late cardiac sodium current [137]. Ranolazine reduces sodium entry into ischaemic myocardial cells and, therefore, is thought to indirectly reduce calcium uptake via the sodium–calcium exchanger, to preserve ionic homeostasis, and to reverse ischaemia-induced contractile dysfunction [138].

The drug has been shown to be an effective anti-anginal drug [139, 140]. In a study of a canine microembolization model of heart failure, intravenous ranolazine increased myocardial work without increasing myocardial oxygen use, which implies an increase in cardiac efficiency [141]. Acute intravenous administration of ranolazine has also improved left ventricular systolic function in dogs with heart failure [142]. So far there have been no studies looking at the effects of ranolazine in humans with chronic heart failure.

There are reports that the drug slightly prolongs the QT interval on electrocardiograms. This effect raises concerns about possible complications such as torsade de pointes polymorphic ventricular tachycardia and SCD [143]. Therefore, the long-term safety of ranolazine needs further investigations.

Glucagon-Like Peptide 1

Glucagon-like peptide-1 (GLP-1) is a naturally occurring incretin with both insulinotropic and insulinomimetic properties with resultant increases in myocardial glucose uptake. In a dog model of heart failure, GLP-1 improved LV function and systemic hemodynamic [65]. In a small open label study, chronic infusion of GLP-1 significantly improved left ventricular function, functional status, and QOL in patients with severe heart failure [111]. GLP-1 is administrated subcutaneously because of breakdown in the gut. GLP-1 is currently unsuitable for oral or IV injection due to the rapid enzymatic degradation of the molecule by dipeptidyl peptidase IV (DPP-IV) enzyme [144]. Inhibitors of this enzyme are recently launched for treatment of diabetes [145]. Coadminstration might allow oral administration of GLP-1.

D-Ribose

D-Ribose is a pentose sugar which enhances ATP production by entering the pentose phosphate pathway and bypassing rate limiting steps of glycolysis. Omran et al studied the effect of ribose supplementation on cardiac hemodynamics and QOL in patients with ischaemic cardiomyopathy. This was a prospective, double blind, randomised, cross over design study which showed improved QOL and improved diastolic function with ribose supplementation [108]. However this has not been confirmed with large scale studies.

Proprionyl-L-Carnitine

Previous studies have demonstrated carnitine deficiency in heart failure patients [146-148]. Carnitine is an important co-factor in intermediary metabolism of the myocardium

which improves utilisation of pyruvate in the Krebs cycle. Studies with carnitine supplementation have shown mixed results. A small study by Anand et al showed improvement in peak V_{O2} but no change in EF [109]. However a larger study in 1999 with 537 patients showed no improvement in exercise capacity [110].

CHAPTER 2: STUDY OBJECTIVES

Study Objectives

First aim:

To measure peripheral metabolic rate in CHF patients at rest and to compare it with that in age-matched healthy volunteers (HVs).

Hypothesis: we hypothesize that resting skeletal muscle oxygen consumption is reduced in CHF patients as compared to age-matched HVs.

Second aim:

To assess the roles of left ventricular strain and untwist in limiting exercise capacity in HCM patients.

Hypothesis: we hypothesize that despite hyperdynamic LV systolic function, patients with HCM exhibit abnormalities of left ventricular strain and untwist as compared to age and gender matched controls. These abnormalities may contribute significantly to exercise limitation observed in these patients.

Third aim:

To evaluate the reproducibility and feasibility of performing cardiac ^{31}P magnetic resonance spectroscopy (MRS) to measure high energy phosphate myocardial kinetics using 3 Tesla Philips MR scanner.

Hypothesis: higher field strength of 3T offers better spatial resolution and signal to noise ratio (SNR) than conventional 1.5T. We hypothesize that ^{31}P magnetic

resonance spectroscopy of the myocardium at 3T is feasible and reproducible in the measurement of high energy phosphate kinetics. This non-invasive *in-vivo* technique will allow the assessment of metabolic modulation effects of perhexiline on cardiac energetic in HCM patients.

Fourth aim:

To assess the relationship between cardiac energetics and diastolic function at rest and during exercise in HCM patients.

Hypothesis: we hypothesized that impaired cardiac energetics may lead to diastolic dysfunction in symptomatic HCM patients.

Fifth aim:

To assess the effect of a metabolic agent, perhexiline, on cardiac energetics in HCM patients.

Hypothesis: we hypothesized that the metabolic modulator, perhexiline would ameliorate myocardial energy deficiency in symptomatic HCM patients.

Sixth aim:

To assess the effects of a metabolic agent, perhexiline, on symptoms and exercise capacity in HCM patients.

Hypothesis: we hypothesized that the metabolic modulator, perhexiline would ameliorate myocardial energy deficiency and thereby improve symptoms and exercise capacity in symptomatic HCM patients.

Seventh aim:

To assess the effects of a metabolic agent, perhexiline, on resting and dynamic diastolic dysfunction in HCM patients.

Hypothesis: we hypothesized that the metabolic modulator, perhexiline would ameliorate myocardial energy deficiency and thereby improve resting and dynamic dysfunction in symptomatic HCM patients.

CHAPTER 3: METHODS

Transthoracic Echocardiography

Echocardiography was performed with participants in the left lateral decubitus position with a GE Vivid 7 echocardiographic machine [Horten, Norway] and a 2.5-MHz transducer. Measurements were averaged for 3 beats and were stored digitally and analyzed off-line. Resting scans were acquired in standard long and short parasternal basal level (at mitral valve), papillary muscle level, and apical level (distal LV cavity where papillary muscle is not visible) as previously described [149] as well as apical 4-chamber and apical 2-chamber axis.

LV volumes were obtained by biplane echocardiography, and LVEF was derived from a modified Simpson's formula [150]. LA volumes were measured by area length method from apical 2 and 4 chambers as previously described [150] and indexed to body surface area to derive LA volume index (LAVI). LV mass was measured by area length method and indexed to body surface area to derive LV mass index as previously described [151]. The greatest thickness measured in the LV wall at any short axis parasternal view was considered to represent maximal wall thickness (MWT) in HCM patients [13].

A pulse wave Doppler sample volume was placed at the mitral valve tips to record 3 cardiac cycles. Mitral annulus velocities (pulse wave tissue Doppler imaging [PW-TDI]) were recorded from basal anterolateral and basal inferoseptal segment in apical 4-chamber view. Subjects with poor echocardiographic windows were excluded from analysis.

Speckle Tracking Echocardiography (STE)

STE is a non-invasive tool which allows measurement of strain, strain rate and rotation from grey echocardiographic images. It simply tracks characteristic speckle patterns created by interference of ultrasound beams in the myocardium which is based on greyscale B-mode images, independently of both cardiac translation and the insonation angle [152]. STE was measured using Echopac workstation (version 4.2.0.) [GE Medical System, Horten, Norway].

In this speckle tacking method [153-155], the displacement of speckles of myocardium in each spot were analyzed and tracked from frame to frame. We selected the best-quality digital two-dimensional image cardiac cycle and the left ventricle (LV) endocardium was traced at end-systole [156]. The region of interest width was adjusted as required to fit the wall thickness. The software package then automatically tracked the motion through the rest of the cardiac cycle. Adequate tracking was verified in real time. A tracking score of ≤ 2.5 was accepted with frame rate between 60-100 Hz. LV peak Strain and Strain Rate in each view were calculated with the use of the entire length of the LV myocardium. The basal and apical LV global rotation (rot) and LV rotation rate (rot-r) STE data were measured from basal and apical short axis view respectively and were then exported to a spreadsheet program (Excel 2003, Microsoft Corp, Seattle, Washington) and then exported to DPlot Graph Software (2001-2008 by HydeSoft Computing, Inc, Vicksburg, Mississippi, USA) to calculate LV twisting and untwisting rate [157] (Figure 8).

At apical level, the LV rotates counter clockwise as viewed from apex and results in a positive value during systole, whereas the base rotates clockwise and results in a negative value, as in this representative case. To adjust for heart rate, R-R interval was converted to 100 %. Left ventricular twist was calculated as the simultaneous net difference between apical and basal rotation. Left ventricular twist rate was calculated as a differential curve of the twist curve.

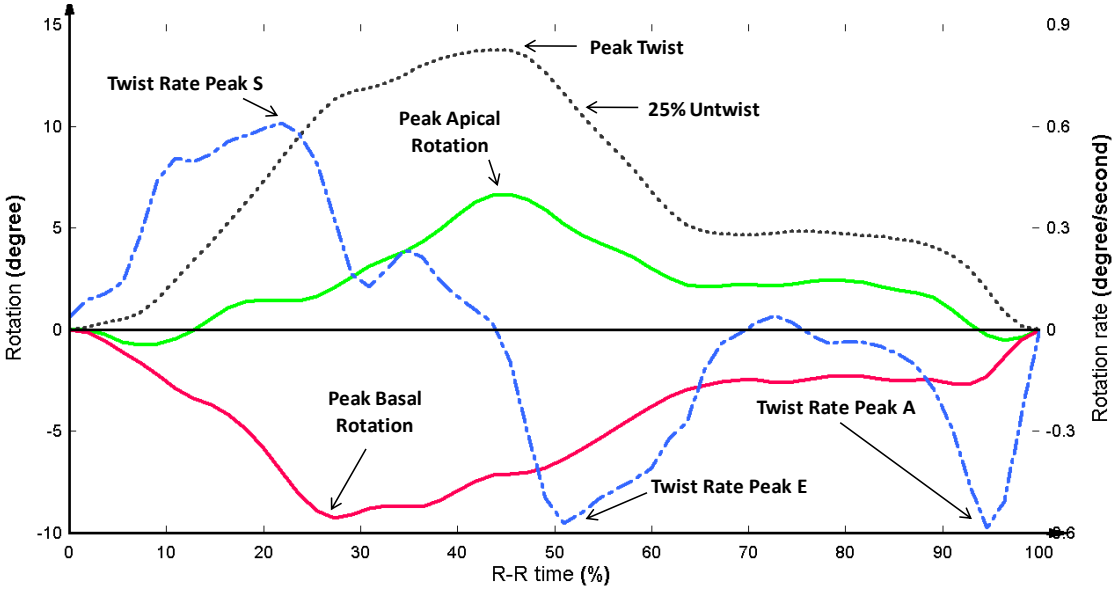


Figure 8: Illustration of STE measurement of rotation and twist.

Green line represents global apical rotation whereas red line represents global basal rotation. Black dotted line represents left ventricular twist which was calculated as the simultaneous net difference between apical and basal rotation. Blue dotted line represents the left ventricular twist rate which was calculated as a differential curve of the twist curve.

Cardiopulmonary exercise test

This was performed using a Schiller CS-200 Ergo-Spiro exercise machine, [Baar, Switzerland] (Figure 9) which was calibrated before every study. Subjects underwent spirometry and this was followed by symptom-limited erect treadmill exercise testing using a standard ramp protocol with simultaneous respiratory gas analysis [158, 159]. Samplings of expired gases were performed continuously, and data were expressed as 30-second means. Minute ventilation, oxygen consumption, carbon dioxide production, and respiratory exchange ratio (RER) were obtained. Peak oxygen consumption (peak V_{O2}) was defined as the highest V_{O2} achieved during exercise and was expressed in ml/min/kg.

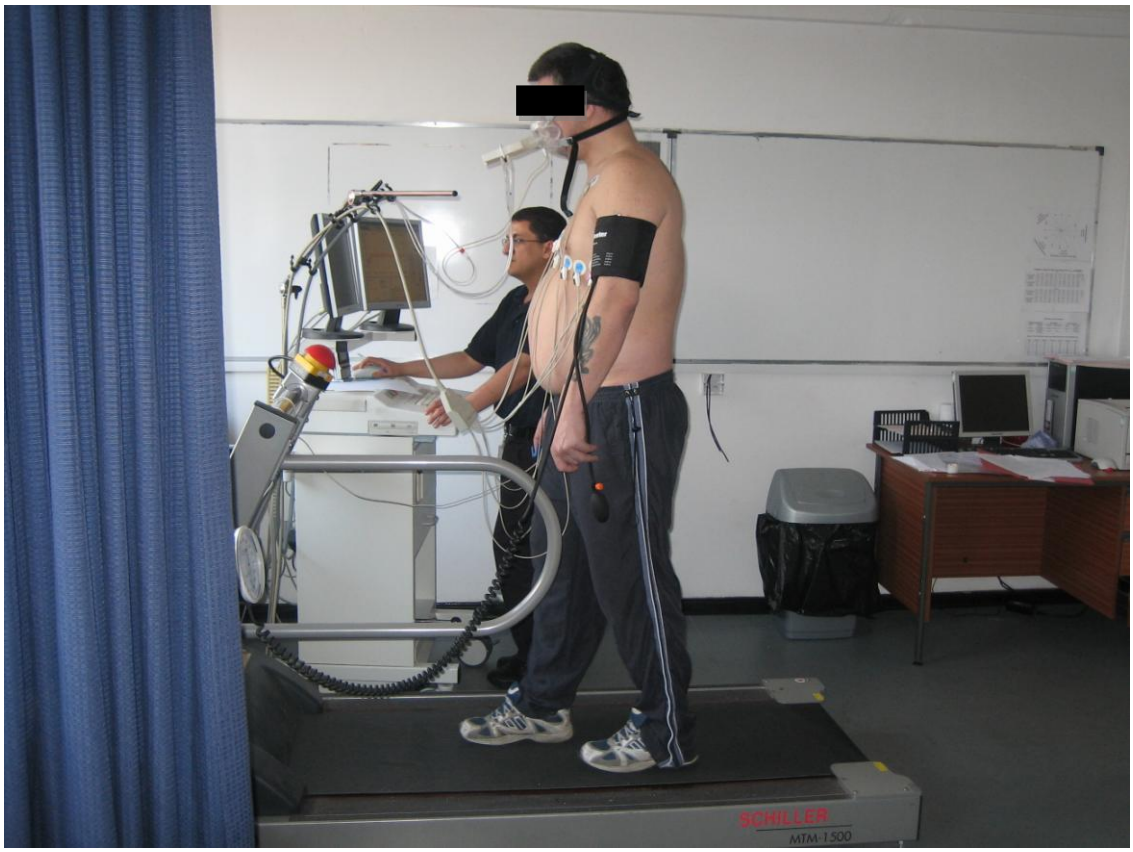


Figure 9: Schiller CS-200 Ergo-Spiro exercise machine

Blood pressure and ECG were monitored throughout. Participants were encouraged to exercise to exhaustion with a minimal requirement of RER > 1.

Cardiac ^{31}P MRS

Magnetic resonance spectroscopy (MRS) is a non-invasive technique which allows *in-vivo* assessment of cardiac energetics. ^{31}P MRS measures the high energy phosphates PCr, three phosphate components of adenosine triphosphate (ATP), 2,3 Diphosphoglycerate (2, 3-DPG) and phosphodiester compounds (PDE), (Figure 10).

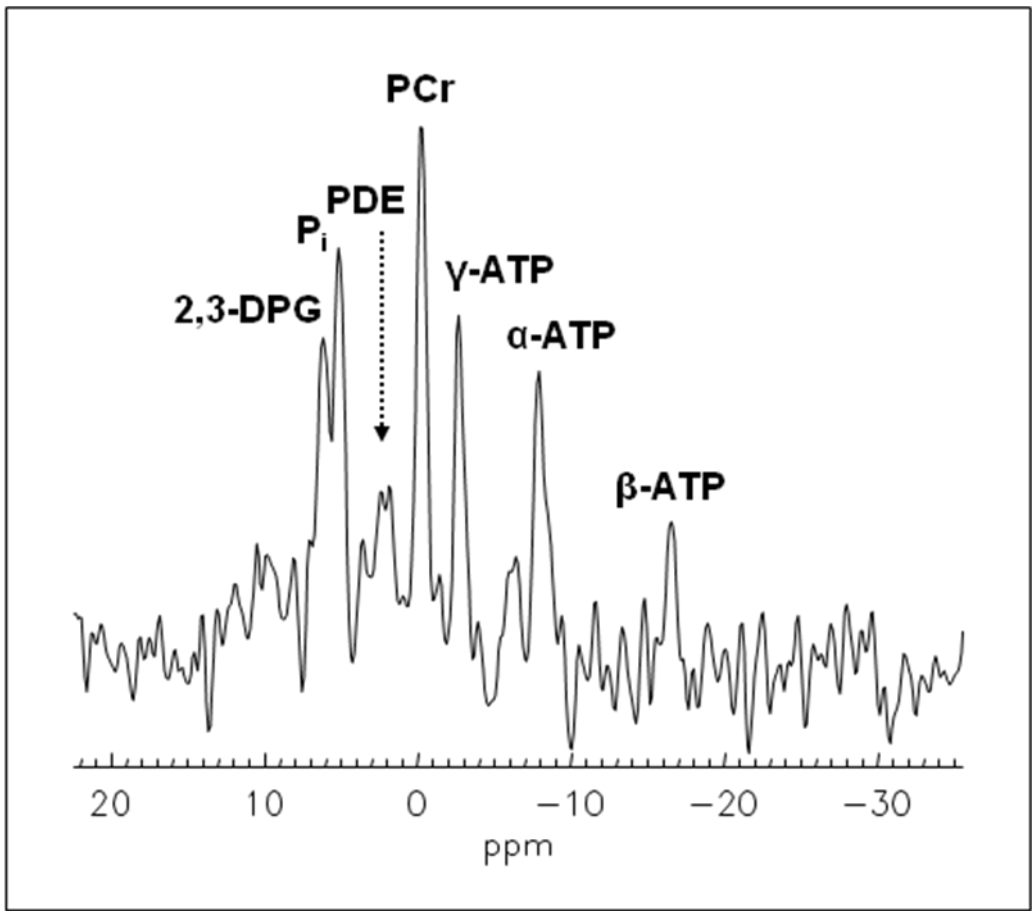


Figure 10: An example of a typical ^{31} P cardiac spectra.

PCr is an important energy buffer molecule that maintains a high phosphorylation potential under conditions of increased energy demand such as exercise (Figure 11). The movement of a phosphoryl group from PCr to ADP by the enzyme Creatine kinase (CK) generates ATP approximately 10 times faster than the maximum rate of ATP generation via oxidative phosphorylation [79]. PCr/ATP ratio reflects the energetic state of the myocardium. The magnitude of the reduction of this ratio predicts mortality in heart disease [86].

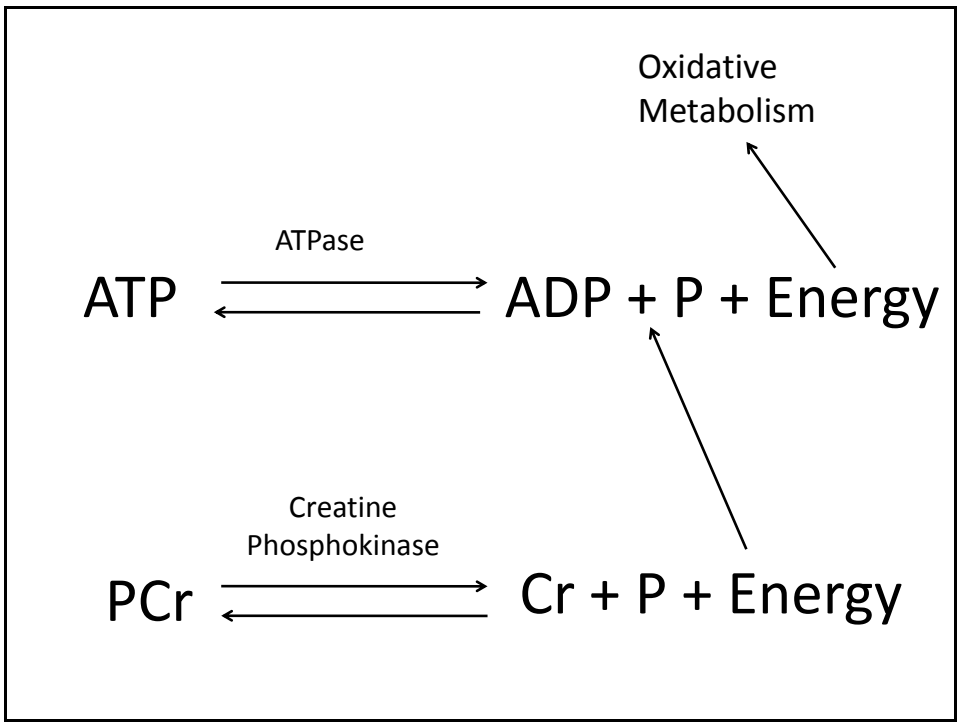


Figure 11: Creatine Phosphokinase reaction.

^{31}P cardiac magnetic resonance spectroscopy was performed using a Phillips Achieva 3T scanner (Figure 13) and a linearly polarized transmitter and receiver ^{31}P coil with a diameter of 14 cm (Figure 14). Localization was achieved by ISIS (image-selected *in vivo* spectroscopy) [160] volume selection. The participants were positioned supine with

the coil directly over the precordium. The coil was secured in place by straps wrapped around the upper body and coil. The participants were then positioned inside the magnet with the centre of the coil at the isocentre of the magnet. Survey images were obtained to check the position of the coil (Figure 12). The subjects and/or the coil were repositioned if required to ensure that the distance between coil and septum and apex of the heart was minimized.

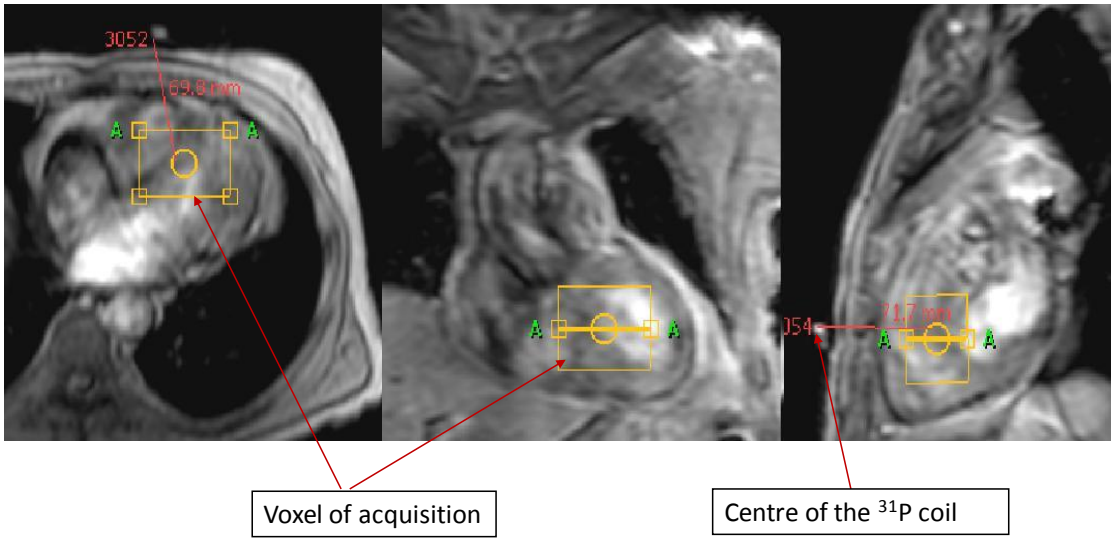


Figure 12: Position of voxel of acquisition.

Survey images showing the position of voxel of interest (VOI) and centre of the ^{31}P coil.

The standard phosphorus spectroscopy sequence provided by the manufacturer was used. It was based on hyperbolic secant pulses for slice selective inversion and adiabatic half passage RF pulse for non-selective excitation. In contrast to the standard procedure manual

fine adjustment of centre frequency (F0) was performed if the automatic F0 determination was not correct in order to ensure the correct voxel position. In contrast to the default iterative or FASTMAP (Fast Automatic Shimming Technique by Mapping Along Projections) based shimming algorithm, which was based on the selected spectroscopy VOI, an image guided shim volume was selected that included the entire myocardium.

Figure 13: 3 T Phillips Achieva MRI scanner.

A short axis cine scan was acquired to calculate the trigger delay for ECG triggering and check quality of shimming and F0 determination. The trigger delay was calculated such that the spectra were acquired in the diastolic period. The 3-D voxel of acquisition

was planned to include most of the septum and apex of the heart. Care was taken to minimize blood contamination from the right ventricle (RV) as much as possible. The voxel size was kept constant at 89.54 ml (44 x 55 x 37 mm^3) so that comparisons could be made between different subjects and scans.

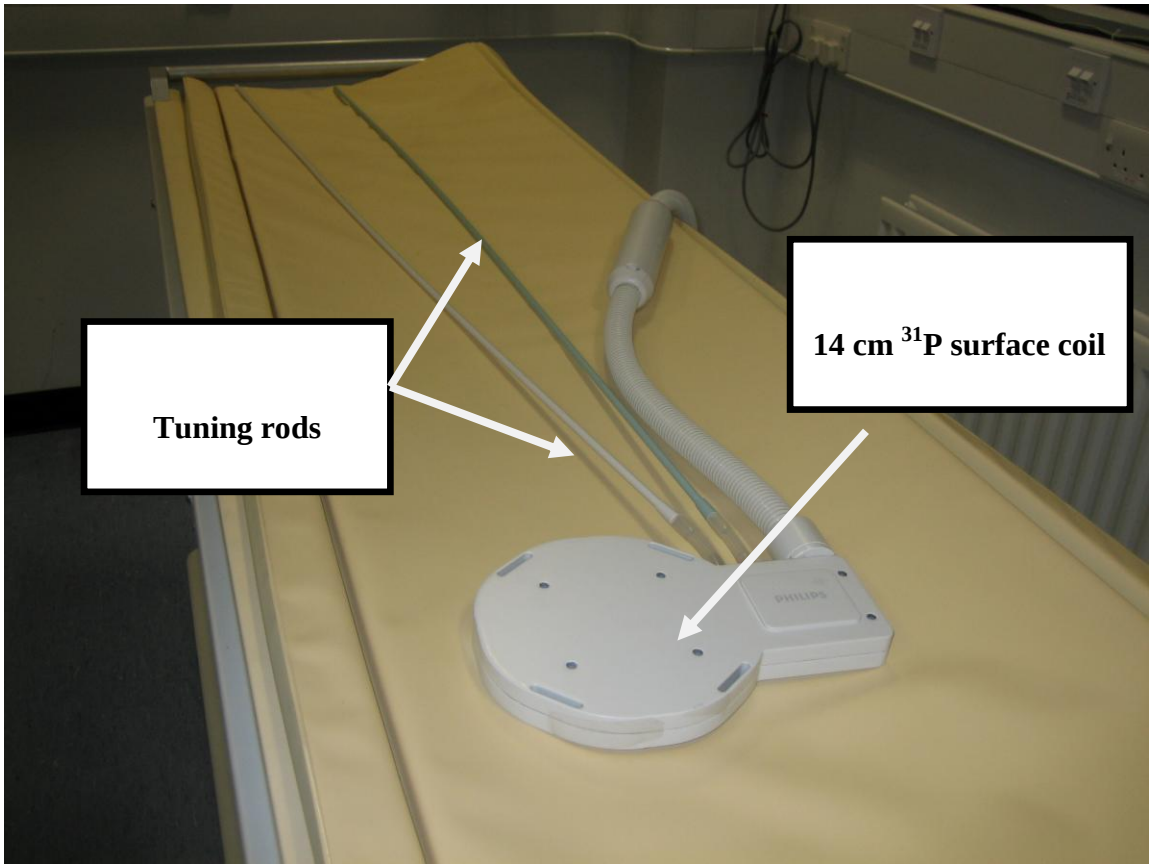


Figure 14: Transmitter and receiver ^{31}P surface coil.

Initially, ^{1}H spectra were acquired from the same voxel without water suppression and repetition time of 2000 ms (total scan time of 16 sec). This helped to ensure adequate shim quality and correct F0 determination. F0 could be manually adjusted if necessary. Following this the ^{31} P spectra were acquired with a repetition time of 10000 ms, 136 averages and 512 samples. A repetition time of 10000 ms was found to be optimal to

adequately reduce saturation effects without increasing the scan time greatly. The spectral acquisition was ECG gated and the trigger delay was set to acquire spectra mainly in diastole. The trigger delay was measured by subtracting 250-300 from the total length of the cardiac cycle which allowed 250-300 msec of the cardiac cycle left for spectral acquisition (acquisition time is 170 msec). The total scan time was 23 minutes.

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

MRS Analysis

The spectra were analysed and quantified on jMRUI (java-based Magnetic Resonance User Interface) software using AMARES a time domain fitting program [161]. Post-processing was performed with 15Hz Gaussian line broadening and Fourier transformation. Phase correction was performed with PCr peak as the reference peak. Quantification was performed with AMARES using a prior knowledge file to preselect the peaks. The concentrations of PCr, ATP and 2, 3-Diphosphoglycerate (2, 3-DPG)

were calculated as the area under the peaks. Cramer Rao lower bounds [162] were then calculated. PCr/ATP ratio was determined after correcting the ATP peak for blood contamination as described previously [163].

Radionuclide Ventriculography

Left ventricular EF and diastolic filling were assessed by radionuclide ventriculography using a gamma camera (Olivetti Modulo-M-200ESL, Figure 15) at rest and during graded semi erect exercise on a cycle ergometer (Lode B.V medical technology, Groningen, Netherlands, Figure 15), as previously described by our group [15].

Ten minutes after an intravenous injection of 0.03 mg/kg stannous pyrophosphate, 5 mL of blood was drawn into a heparinised syringe and incubated for 10 minutes with 925 MBq (25 mCi) of 99mTc pertechnetate before reinjection. Studies were acquired on a small-field-of-view gamma camera fitted with a low-energy, general-purpose, parallel-hole collimator and interfaced to a dedicated minicomputer. With the patient on the cycle ergo meter, the detector was adjusted for the left anterior oblique view with the best ventricular separation and 10° to 15° of caudal tilt. A 20% tolerance window was set about the patient's heart rate, and each RR interval was divided into 28 equal frames throughout. A constant number of frames per RR interval ensure constant temporal resolution during diastole at all heart rates.

Data from each beat were acquired into a memory buffer in a 64 x 64 "word" matrix and if accepted, were reformatted with two-thirds forward, one-third backward gating.

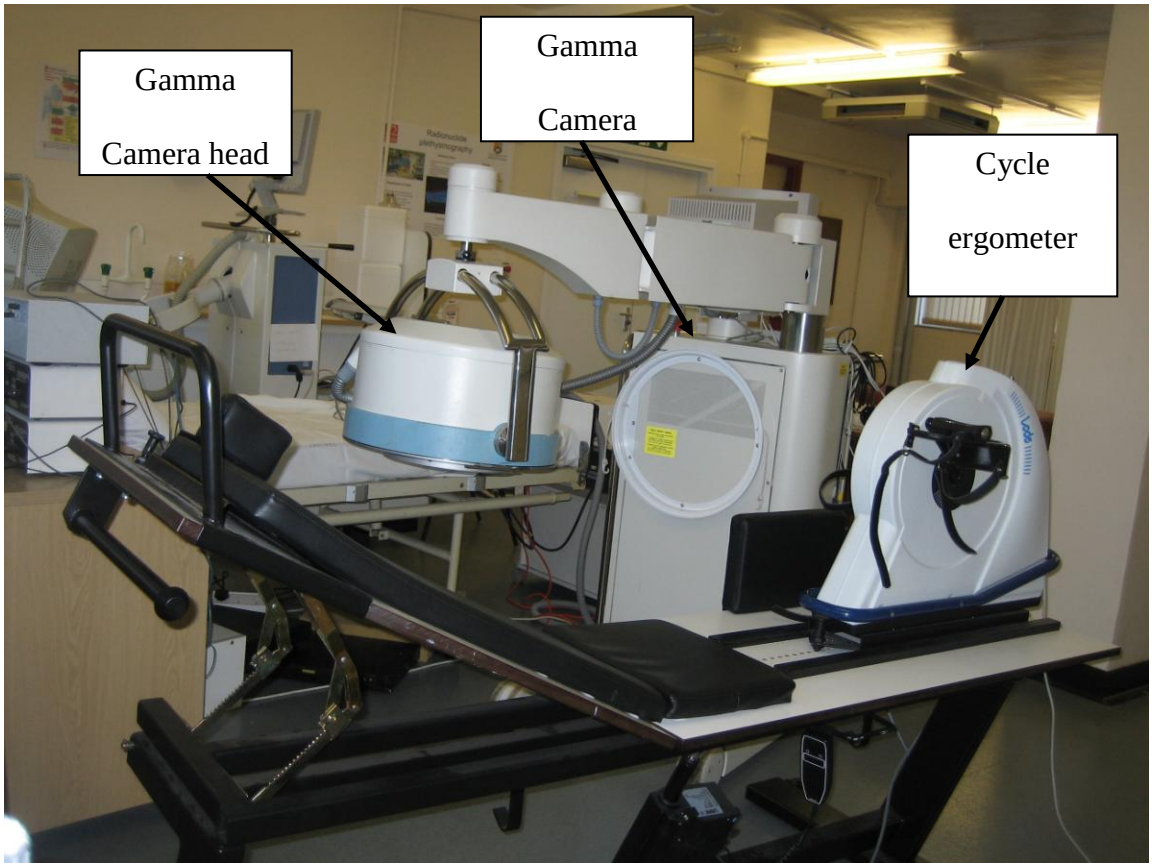


Figure 15: Radionuclide Ventriculography equipment.

Gamma camera (Olivetti Modulo-M-200ESL) machine and cycle ergometer (Lode B.V medical technology, Groningen, Netherlands).

Three minutes of data were acquired at rest and at each level of exercise after a 1 minute period for stabilisation of heart rate at the commencement of each stage. Exercise was performed at 20%, 35% and 50% workloads of heart rate reserve. The composite cycle derived from each stage was spatially and temporally filtered. Left ventricular counts in each frame were determined by a semi automated edge-detection algorithm. Data were analysed using LinkMedical MAPS software, Sun Microsystems. Peak left ventricular

filling rate in terms of end-diastolic volumes per second (EDV/s) and TTPF in milliseconds after end systole were calculated from the second derivative of the diastolic activity-time curve (Figure 16). The validity of these radionuclide measures of diastolic filling at high heart rates has been established previously (21; 22).

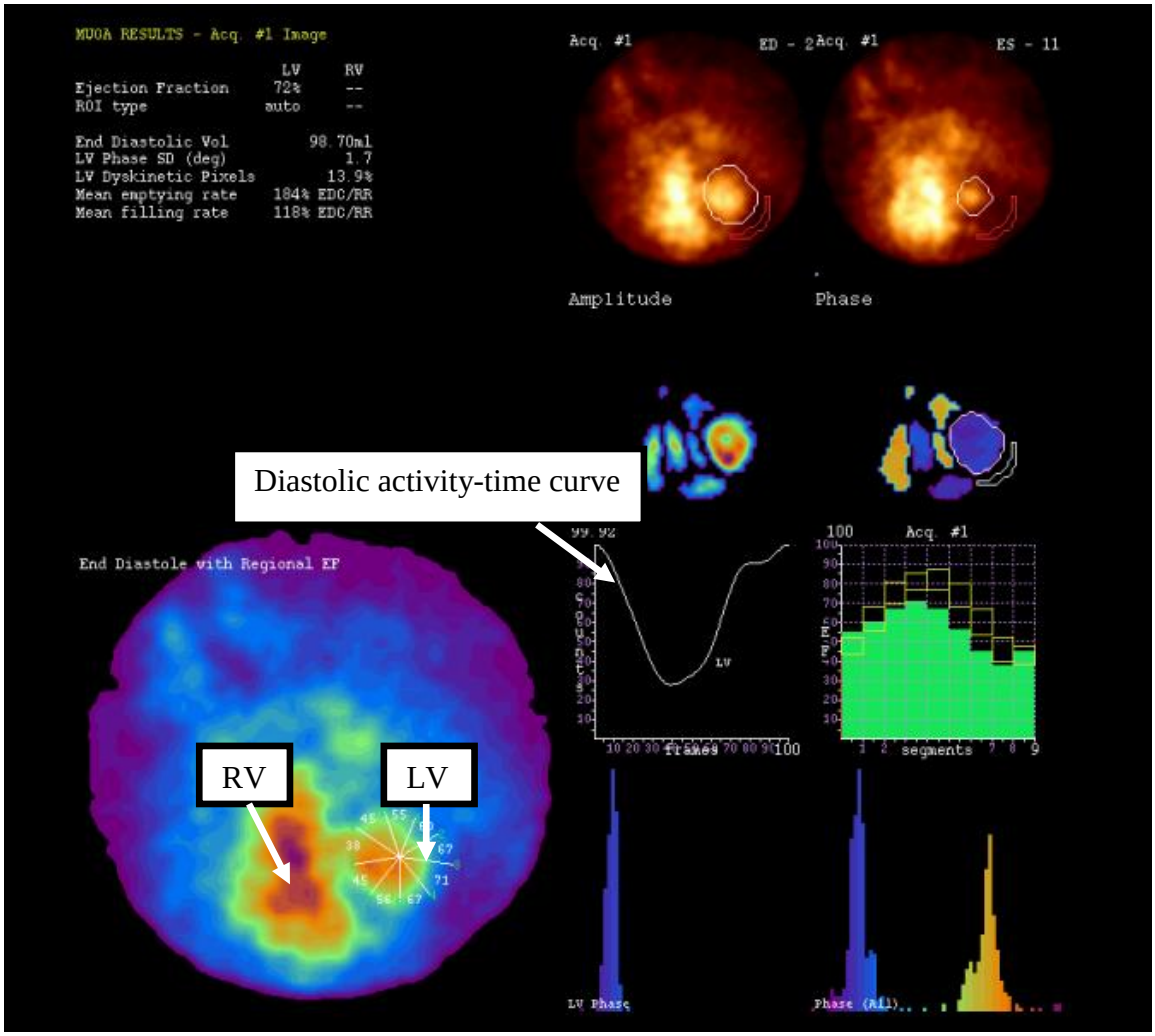


Figure 16: Example of diastolic activity-time curve from radionuclide ventriculography.

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

Near Infra Red Spectrophotometry (NIRS)

NIRS is a non-invasive optical technique that is increasingly used to assess changes in tissue oxygenation in skeletal muscle [164]. This technique is based upon the principle that NIRS light easily penetrates skeletal muscle, where it is absorbed by the iron or copper content of haemoglobin and myoglobin [165]. The majority of NIRS light absorption is due to the presence of haemoglobin in the small arterioles, capillaries, and venules of the microcirculation [164]. These features have led to growing interest in the use of NIRS as a non-invasive technique to measure changes in muscle oxygenation and blood flow at rest and during both submaximal and maximal exercise.

Subjects were investigated in the overnight fasted state. The hair was removed from the forearm of participants using a disposable shaver and the skin thickness was measured using skin callipers. Skeletal muscle oxygenated haemoglobin concentration [Oxy-Hb],

deoxygenated haemoglobin concentration [Deoxy-Hb] and total haemoglobin concentration [HbT] were measured with the OxiplexTS near infrared tissue oximeter (ISS Inc., Champaign, IL, USA), Figure 17 .

This is a frequency domain multi-distance NIRS using 4 laser-diode light sources at two wavelengths (692 and 834 nm) and one detector. The NIRS probe used had source-detector distances of 3.0 - 4.4.cm to limit the contribution of skin and subcutaneous non-muscle tissue and was placed over the brachioradialis muscle. The absorbances measured represent the sum of haemoglobin in the microvasculature and myoglobin in the myocytes.

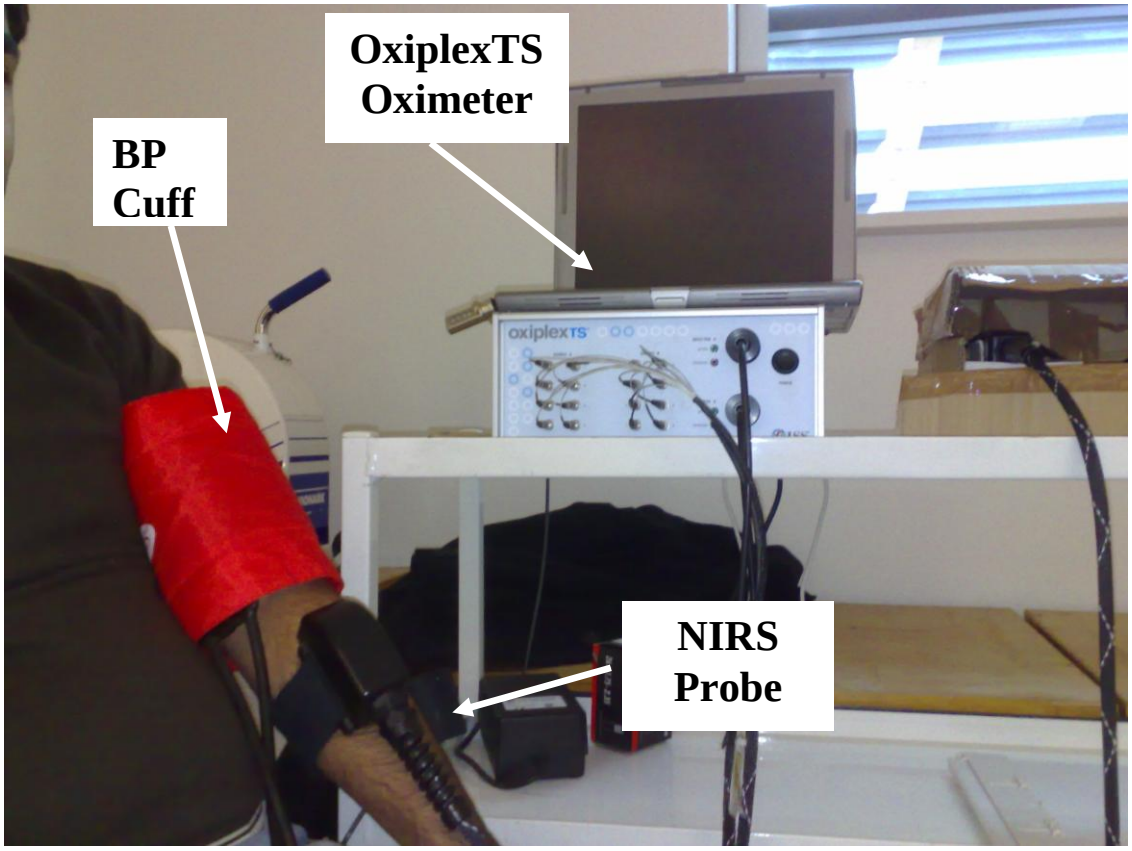


Figure 17: NIRS Experiment Setting.

NIRS exploits the difference in optical absorption spectra between the [HbT] and [Deoxy-Hb]. At a wavelength of 834 nm, [Oxy-Hb] and [Deoxy-Hb] exhibit similar absorption coefficients. Therefore, absorption of light at this wavelength is proportional to [HbT] in the muscle under examination. At a wavelength of 692 nm, absorption is primarily by the deoxygenated Hb. Changes in light absorption at 692 nm provide assessment of changes in [Deoxy-Hb]. The difference between absorption at 834 and 692 nm gives the [Oxy-Hb].

A blood pressure cuff was applied around the proximal part of the arm as described previously [166]. The experiment involved continuous measurements of [Oxy-Hb], [Deoxy-Hb] and [HbT] at rest for 2 minutes. Then the cuff was inflated to 220 mm Hg for 1 minute, to induce forearm arterial occlusion. [Oxy-Hb], [Deoxy-Hb] and [HbT] were again recorded continuously during the 1 minute forearm arterial occlusion (Figure 18).

The resting muscle oxygen consumption rate was determined by the rate of decline of the difference between [Oxy-Hb] and [Deoxy-Hb] during occlusion and was then expressed per 100 gram of forearm muscle tissue. The conversion of oxygen consumption rate to ml O_2/min/100g was determined as previously described [166, 167].

Initially, the oxygen consumption NIRS value (µmol/litre/s) was converted into minutes. 1.04 kg/L was used for muscle density to convert 1 litre to 100 g of skeletal muscle. 1 mole of Hb carries 4 moles of O2. Then, 1 mole of gas was converted into 1 litre with value of 1 mole gas = 22.4 L standard temperature and pressure, dry (STPD) conditions. Oxygen saturation was calculated from the ratio between [Oxy-Hb] and [HbT].

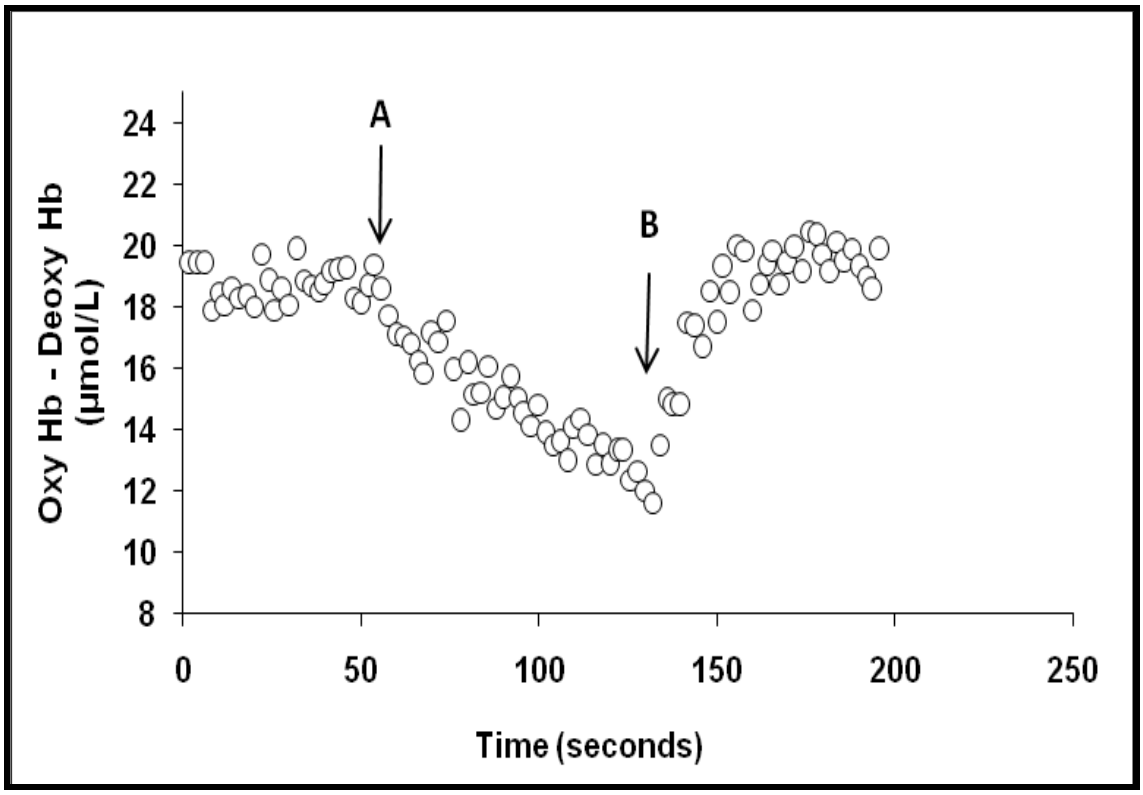


Figure 18: NIRS measurement of oxygen consumption.

This graph shows continuous NIRS measurements at rest, during 1 minute arterial occlusion and during recovery in a health subject. The resting skeletal muscle oxygen consumption rate was determined by the rate of decline of the difference between [Oxy-Hb] and [Deoxy-Hb] during arterial occlusion. A represents the start of arterial occlusion whereas B represents the end of arterial occlusion.

Symptomatic status assessment

We used the New York Heart Association (NYHA) class and Minnesota living with heart failure (MLHF) questionnaire to assess symptoms.

NYHA functional classification:

Symptom status was determined according to NYHA functional classification as shown in Table 3.

Class	Patient Symptoms
Class I (Mild)	No limitation of physical activity.
Class II (Mild)	Slight limitation of physical activity.
Class III (Moderate)	Marked limitation of physical activity.
Class IV (Severe)	Unable to carry out any physical activity without discomfort.

Table 3: NYHA functional classification.

Minnesota Living with heart failure (MLHF) questionnaire

Symptom status was determined by asking patients to complete MLHF questionnaire, Figure 19.

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**
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

Figure 19: MLHF questionnaire.

CHAPTER 4: REDUCED PERIPHERAL METABOLIC RATE IN PATIENTS WITH CHF– A STUDY USING NEAR INFRARED SPECTROPHOTOMETER (NIRS)

Abstract

Background: We used Near Infrared Spectrophotometer (NIRS) during arterial occlusion to measure resting skeletal muscle oxygen consumption in CHF patients and to compare it with that in age-matched HVs.

Methods: Fifteen CHF patients (ten males) and eleven HVs (six males) had echocardiographic evaluation followed by measurement of the oxygen consumption of the brachioradialis muscle using NIRS. This involved continuous measurement of the [Oxy-Hb] and [Deoxy-Hb] with an Oxiplex TS NIRS probe first under basal overnight fasted resting conditions followed by 1 minute of forearm arterial occlusion. A linear decline was observed in [Oxy-Hb-Deoxy-Hb] during the arterial occlusion and the oxygen consumption rate was calculated from the initial slope observed.

Results: CHF patients were 59 ± 2.8 years old with LVEF 31% ± 2.2 and the HVs were 52 ± 4.8 years old with LVEF 62% ± 2.5. The resting muscle oxygen consumption rate was significantly reduced in CHF patients versus HVs (0.04 ± 0.01 mlO2/min/100g versus 0.07 ± 0.01 mlO2/min/100g) $p < 0.005$.

Conclusions: There is a significant reduction in resting oxygen consumption per gram of tissue in skeletal muscle of patients with CHF.

Introduction

CHF is a common condition and associated with considerable morbidity and mortality [168]. Clinical features include muscle weakness, general fatigue and dyspnoea [169, 170]. It is clear that central haemodynamics correlate poorly with exercise capacity in CHF [170]. Therefore, attention has been focused on peripheral factors such as intrinsic skeletal muscle abnormalities and skeletal muscle underperfusion. Several studies in patients [171-176] and animal models of CHF [177-182] have described skeletal muscle histological abnormalities, including fibre-type transformation toward faster phenotype, fibre atrophy, and reduced oxidative enzyme activities. PCr and creatine kinase (CK) are involved in the fine regulation between energy production and energy utilization in muscle cells [38, 52, 183]. ^{31}P Magnetic Resonance Spectroscopy (MRS) has revealed a decreased content of mitochondrial CK in skeletal muscle of patients with CHF. However, whether these skeletal muscle abnormalities result in reduced peripheral oxygen consumption has never been directly assessed. The goal of the present study was therefore to examine whether skeletal muscle oxygen consumption is reduced in CHF patients as compared to age matched HVs.

Resting skeletal muscle oxygen consumption is the ultimate measure of resting muscle metabolic rate, and is affected by environmental temperature, body temperature, the microvascular blood flow and nutrition [184]. In the absence of blood flow the oxygen content of the tissue diminishes as oxygen is consumed by the mitochondria. Therefore oxygen consumption can be determined by measuring the decline in oxygen saturation of haemoglobin and myoglobin following total arterial occlusion. Assuming the duration of occlusion is insufficient to cause a significant shift to anaerobic glycolysis, this will be a

reliable indicator of resting oxygen consumption. This work represents a clinical application of non-invasive technique to assess and compare resting skeletal muscle oxygen consumption in CHF patients and HVs using NIRS.

Methods

Fifteen CHF patients (ten males) and eleven HVs (six males), who provided written informed consent, were included in the study. Characteristics and treatment of participants are shown in Table 4. The experiment was approved by the local Research Ethics Committee at the University of Birmingham, UK and the investigation conforms to the principles outlined in the Declaration of Helsinki.

Patient selection

Inclusion criteria:

- LVEF $\leq$ 50%.
- History of dyspnoea on exertion (NYHA II or III).
- Sinus rhythm.

Exclusion criteria:

- Unstable clinical condition in the last 3 months.

Controls selection

Inclusion criteria:

- Normal ECG.

- Normal Echocardiogram (LVEF ≥ 55%).

Exclusion criteria:

- History or symptoms of any medical illness.

CHF patients and HVs were matched regarding age, sex and body surface area. All study participants had ECG, echocardiographic evaluation and measurement of oxygen consumption using NIRS.

Resting Echocardiography

Echocardiography was performed with participants in the left lateral decubitus position with a Vivid 7 echocardiographic machine and a 2.5-MHz transducer. Measurements were averaged for 3 beats. Resting scans were acquired in standard echocardiographic windows for LVEF. LV volumes were obtained by biplane echocardiography, and LVEF was derived from a modified Simpson's formula. Studies were stored digitally and analyzed off-line.

NIRS

Subjects were investigated in the overnight fasted state. Skeletal muscle [Oxy-Hb], [Deoxy-Hb] and [HbT] were measured at rest and during 1 minute forearm arterial occlusion (Figure 20). The resting muscle oxygen consumption rate was determined by the rate of decline of the difference between [Oxy-Hb] and [Deoxy-Hb] during occlusion and was then expressed per 100 gram of forearm muscle tissue.

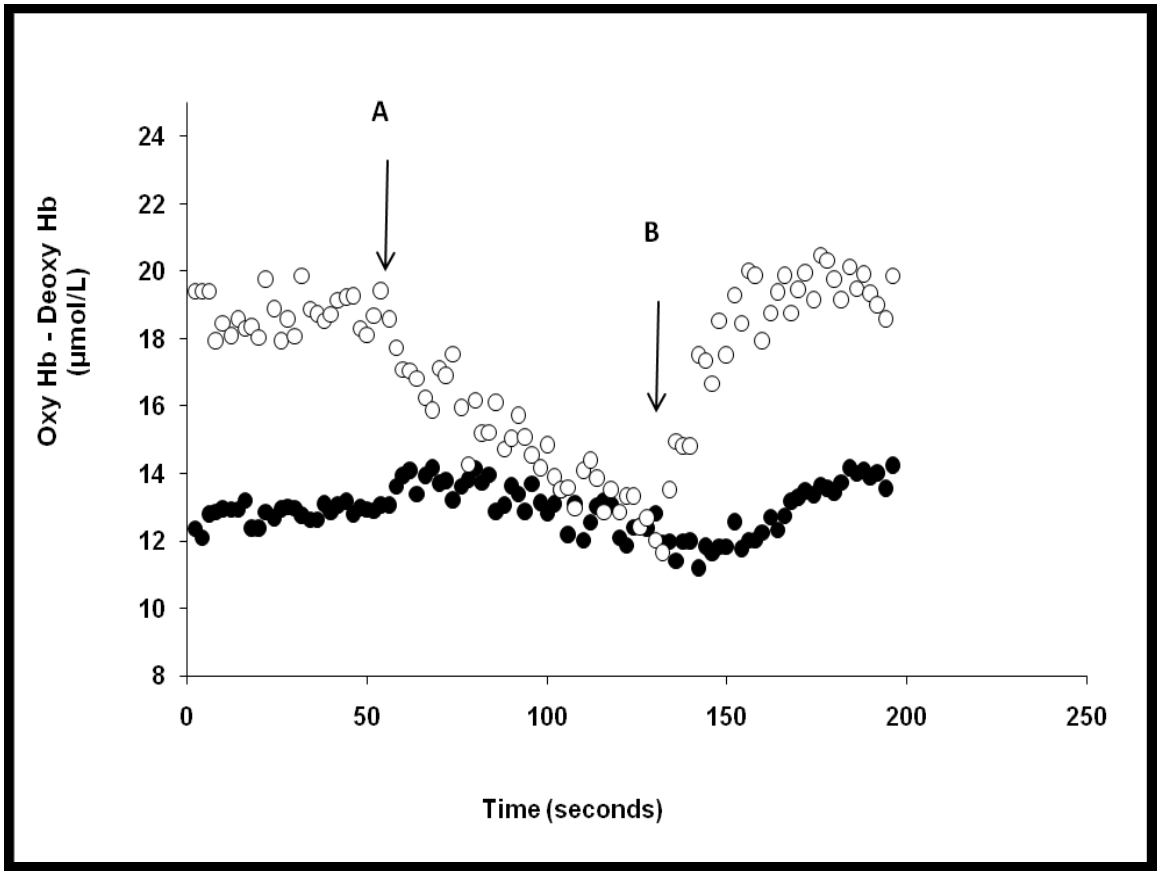


Figure 20: Oxygen Consumption in HVs & CHF patients.

This graph shows continuous NIRS measurements at rest, during 1 minute arterial occlusion and during recovery in HVs (white circle) and CHF patients (black circle). The resting skeletal muscle oxygen consumption rate was determined by the rate of decline of the difference between [Oxy-Hb] and [Deoxy-Hb] during arterial occlusion. A represent the start of arterial occlusion whereas B represent the end of arterial occlusion.

Statistics

Comparison of oxygen consumption in HVs with CHF patients was determined by a 2–sided Student's t-test. Data were analyzed with SPSS 14 for Windows and expressed as mean ± standard deviation (SD). Variances of data sets were determined using F-test. A difference of a p value of < 0.05 was taken to indicate statistical significance.

Results

CHF patients and HVs were well matched with respect to age and gender (Table 4). However, LVEF was significantly lower in CHF patients (Table 5).

Parameter	*CHF patients*	*H Vs*	*P value*
Age (mean ± SD)	**59.33 ± 2.82**	**52 ± 4.8**	**ns**
Number (male)	**15 (10)**	**11 (6)**	**ns**
NYHA	**II-IV**	**……**	**……**
Diabetes	**3**	**0**	**……**
ACE inhibitors	**10**	**0**	**……**
AT2 receptor blockers	**4**	**0**	**……**
ß-Blockers	**8**	**0**	**……**
Aspirin	**6**	**0**	**……**
Diuretics	**13**	**0**	**……**
Warfarin	**8**	**0**	**……**
Calcium channel blockers	**1**	**0**	**……**
Spironolactone	**3**	**0**	**……**
Amiodarone	**1**	**0**	**……**
Statins	**10**	**0**	**……**
Oral Hypoglycemic agents	**3**	**0**	**……**
Insulin	**1**	**0**	**……**

Table 4: CHF patients and HVs characteristics and treatments - NIRS study.

Parameter	*CHF patients*	*H Vs*	*P value*
BMI (Kg/m^2)	**25.6 ± 4.2**	**24.9 ± 3.7**	**ns**
Skin fold thickness (mm)	**8.01 ± 3.1**	**6.5 ± 4.3**	**ns**
LVEF (%)	**31.35 ± 2.15**	**62.15 ± 2.46**	**< 0.001**
LVEDV (ml)	**162 ± 25**	**76 ± 4.5**	**< 0.05**
LVESV (ml)	**113 ± 19**	**29 ± 3**	**< 0.05**
Stroke Volume (ml)	**49 ± 8**	**47 ± 4**	**ns**
Resting O_2 sat (%)	**62.85 ± 1.96**	**64.18 ± 2.50**	**ns**
Resting [Hb-T] (µmol)	**47.86 ± 4.27**	**59.66 ± 4.46**	**< 0.05**
Resting [Oxy-Hb] (µmol)	**30.13 ± 2.95**	**37.68 ± 2.45**	**< 0.05**
Resting [Deoxy-Hb] (µmol)	**17.73 ± 1.68**	**21.97 ± 2.88**	**ns**

Table 5 : Baseline investigations of CHF patients and HVs – NIRS study.

Resting NIRS measurements showed no significant difference in resting oxygen saturation in CHF versus HVs (62.85 ± 1.96 versus 64.18 ± 2.50 %, p=0.67). However the resting [HbT] was significantly lower in CHF patients (47.86 ± 4.27 µmol) versus HVs (59.66 ± 4.46 µmol, $p < 0.05$). The [Oxy-Hb] was also significantly lower in CHF patients (30.13 ± 2.95 vs 37.68 ± 2.45 µmol, $p < 0.05$). There was a non-significant trend towards a lower [Deoxy-Hb], probably as a result of lower [HbT] (17.73 ± 1.68 vs 21.97 ± 2.88 µmol, $p = 0.094$) (Table 5). Oxygen consumption at rest in CHF patients was significantly reduced versus HVs (0.04 ± 0.01 mlO_2/min/100g versus 0.07 ± 0.01 mlO_2/min/100g) $p < 0.005$.

There were no correlations between oxygen consumption and LVEF in patients with CHF (r = 0.09, p=0.74) (Figure 21).

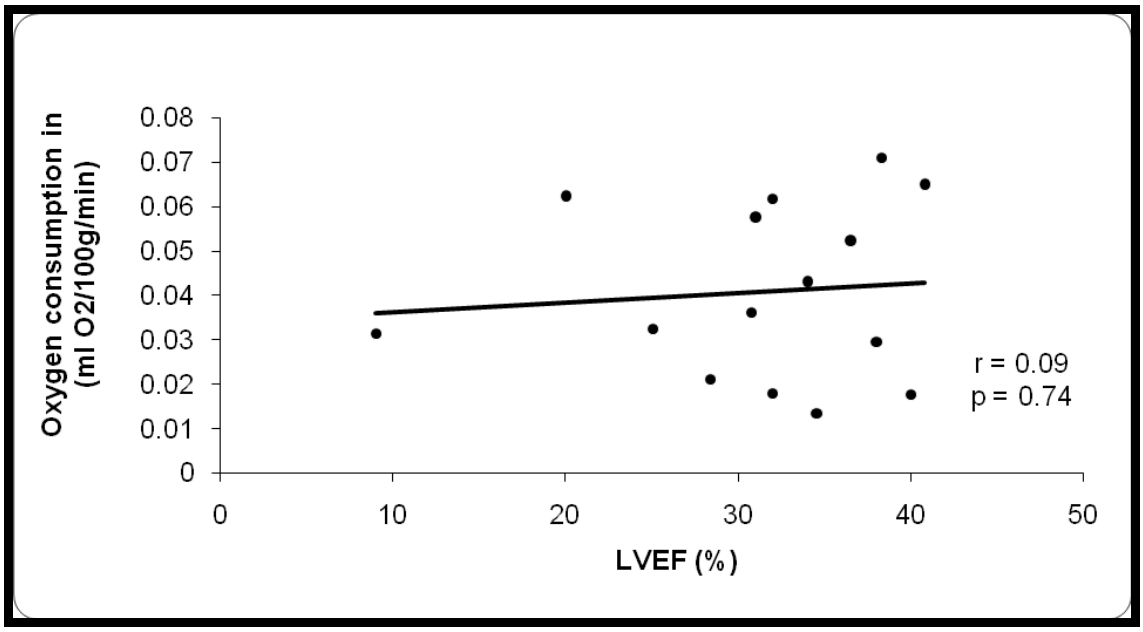


Figure 21: Correlation between skeletal muscle oxygen consumption and LVEF in CHF patients.

There is no correlation between peripheral oxygen consumption, as expressed in ml/100g/min, and LVEF in patients with CHF.

Measurements of oxygen consumption correlated positively with [Oxy-Hb] (r = 0.59, p = 0.001), [Deoxy-Hb] (r = 0.71, p < 0.001) and [HbT] (r = 0.73, p < 0.001) (Figure 22 A, B and C). However, there was no significant correlation between oxygen consumption and oxygen saturation (r = 0.28, p = 0.17) (Figure 22D).

Discussion

In the present study, we have used a non-invasive tool (NIRS) to measure peripheral oxygen consumption in CHF patients and age matched HVs. Whilst a reduction in peak oxygen consumption during exercise is a hallmark of heart failure, our finding of reduced resting peripheral oxygen consumption is to the best of our knowledge novel. Both central and skeletal muscle factors play a role in exercise limitation in CHF, though the relative importance of each has been controversial [185, 186].

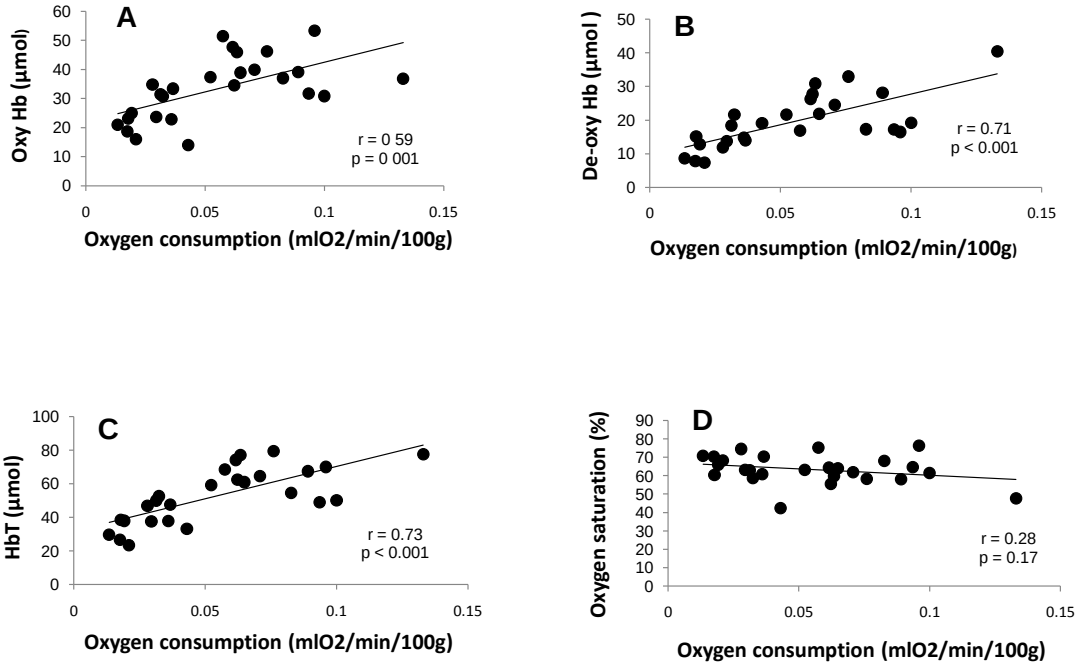


Figure 22: Correlation between skeletal muscle oxygen consumption and resting NIRS measurements.

There were positive correlation between peripheral oxygen consumption and resting [Oxy-Hb] (graph A), [Deoxy-Hb] (graph B) and [HbT] (graph C). However, there was no significant correlation between peripheral oxygen consumption and resting oxygen saturation (graph D).

Skeletal muscle underperfusion is a recognized feature in CHF patients [187]. This leads to a reduction in oxygen supply to skeletal muscle that might result in adaptation of the skeletal muscle to consume less oxygen at rest. NIRS measurement of [Oxy-Hb] and [HbT] had been used as a surrogate measurement of skeletal muscle blood flow [187, 188]. Our resting data of [Oxy-Hb] and [HbT] were reduced at rest in CHF patients as compared to age matched HVs (Table 5), which is in agreement with Wilson JR and co-workers [187]. Interestingly, Massie and co-workers [189] showed no relationship between skeletal muscle blood flow and the metabolic findings in CHF patients. However, it is important to note that we measured skeletal muscle oxygen consumption under conditions of temporary circulatory occlusion therefore reduction in oxygen delivery cannot be the direct cause of the observed reduction in skeletal muscle oxygen consumption. Chronic skeletal muscle hypoperfusion may nevertheless induce adaptive changes in skeletal muscle function that are responsible for the reduced oxygen consumption. Furthermore, it is important to note that the oxygen consumption measurements are expressed per 100 mls forearm tissue therefore these findings cannot be explained in terms of a reduced skeletal muscle mass in heart failure patients. The mechanisms that may lead to this reduction in resting oxygen consumption are discussed below.

Possible mechanisms underlying reduced skeletal muscle oxygen consumption in CHF patients

Heart failure is associated with altered metabolism within the skeletal muscle [190] with reduction in mitochondrial density and oxidative enzymes resulting in reduced aerobic capacity [171, 191, 192]. Evidence of a reduction in mitochondrial density originates from

studies showing that CHF patients had increased PCr breakdown, intracellular acidosis [193], decreased rate of ATP resynthesis during exercise and recovery [193, 194] and activity of selected oxidative enzymes [174, 175]. Furthermore, other studies showed that abnormal skeletal muscle metabolism contributes to the reduced functional capacity in CHF patients [193-195]. Further studies are required to examine the contribution of these mitochondrial factors to the observed reduction in skeletal muscle oxygen consumption in CHF patients.

Previous reported studies have shown that skeletal muscle energetic status at rest is not significantly impaired in heart failure, in contrast there is accelerated PCr depletion and slower recovery of PCr following exercise [196]. These indicate a reduced ability to generate ATP during the increased demands associated with exercise. Accordingly our observations can only be explained either by a reduced metabolic rate in skeletal muscle (i.e. a reduced resting requirement for ATP production) or by an increase in metabolic efficiency (i.e. an increase in ATP production per unit oxygen consumption). The former paradigm would imply a situation analogous to 'hibernation' seen in cardiac muscle in which repetitive myocardial stunning induces a downregulation of basal metabolic processes. Heart muscle contractile function can be recruited to hibernating myocardium by the infusion of low dose dobutamine but further increments in dobutamine infusion rate result in a loss of contractile function again [197]. In the same way it is possible to conceive of a reduction in basal metabolic rate with a capacity to upregulate metabolism during low level exercise but rapid fatigue at higher exercise loads. The second paradigm (increased metabolic efficiency) might theoretically be explained by a change in substrate utilization. Skeletal muscle is capable of using various sources to generate energy such as carbohydrates, fats (including ketones derived from fats) and proteins. The oxidation of glucose generates

approximately 12% more ATP per unit oxygen consumption than the oxidation of fats [38, 52, 183]. At rest in the fasting state skeletal muscle metabolizes principally free fatty acids (FFAs) and they are also the main form of substrate during low to medium intensity exercise [198]. However, Sidossis and colleagues showed that FFAs oxidation is limited during high-intensity exercise, which suggest a shift to an energy efficient substrate such as glucose [198]. Theoretically a shift towards greater glucose utilization might result in greater metabolic efficiency, which leads to less oxygen consumed to generate same amount of ATP as compared to FFAs. However, this shift could not on its own explain the magnitude of the difference in oxygen consumption that we observed.

Skeletal muscle atrophy and fibrosis

In the present study, both CHF and HVs groups had comparable body mass index (Table 5). There was an insignificant small increase in the forearm skin thickness in CHF patients as compared to HVs (8.01 mm ± 3.10 vs. 6.50 mm ± 4.30, respectively). However, the NIRS probe used in this study had source-detector distances of 30-44 mm to limit the contribution of skin and subcutaneous non-muscle tissue. Skeletal muscle atrophy has been observed in patients with CHF [194, 199], which may result from different mechanisms such as inactivity, inflammation, apoptosis and an imbalance in catabolic/anabolic processes [200]. Our measurement of oxygen consumption was expressed as per 100 gram of forearm muscle tissue. Therefore, skeletal muscle atrophy and increased interstitial fat content and fibrosis in CHF patients may contribute to the reduction in oxygen consumption, but seems unlikely to explain the magnitude of the difference.

CHAPTER 5: LEFT VENTRICULAR STRAIN AND UNTWIST IN HCM: RELATION TO EXERCISE CAPACITY

Abstract

Background: HCM is often associated with reduced exercise capacity. This study examined the importance of left ventricular strain, twist and untwist as predictors of exercise capacity in HCM patients.

Method: 56 HCM patients (31 male and mean age of 52 years) and 43 age and gender matched controls were enrolled. We measured peak oxygen consumption (peak V_{O2}) and acquired standard echocardiographic images in all participants. 2-D speckle tracking was applied to measure rotation, twist, untwist rates, strain and strain rate.

Results: HCM patients exhibited marked exercise limitation compared to controls (peak V_{O2} 23.28 ± 6.31 vs 37.70 ± 7.99 ml/kg/min, $p < 0.0001$). LVEF in HCM patients and controls was similar (62.76 ± 9.05 % vs 62.48 ± 5.82 %**,** p =0.86**)**. Longitudinal, radial and circumferential strain and strain rate were all significantly reduced in HCM patients compared to controls. There was a significant delay in 25 % of untwist in HCM compared to controls. Both systolic and diastolic apical rotation rates were lower in HCM patients. Longitudinal systolic and diastolic strain rate correlated significantly with peak V_{O2} ($r = -0.34$, $p = 0.01$ and $r = 0.36$, $p = 0.006$ respectively). 25% untwist correlated significantly with peak V_{O2} ($r = 0.36$, $p = 0.006$).

Conclusion: There are widespread abnormalities of both systolic and diastolic function such as strain and untwist in HCM patients and that these abnormalities are significant determinants of exercise capacity.

Introduction

Patients with HCM often complain of breathlessness and/or fatigue and most have reduced exercise capacity [15]. HCM patients typically exhibit diastolic dysfunction, which is thought to contribute significantly to the genesis of breathlessness in these patients. Active relaxation is slowed and filling rate is typically diminished at rest in HCM patients [14]. Furthermore, our group previously showed that there is frequently a failure of the normal increase in rate of active relaxation (as measured by TTPF) on exercise, indeed in some patient's active relaxation paradoxically slowed on exertion [15].

Resting echocardiographic Doppler parameters of LV filling have been used to measure diastolic function [201]. However, these parameters are dependent on loading conditions. The LV twists during systole as a result of counter clockwise rotation of the apex and clockwise rotation of the base with corresponding untwisting during diastole. The resulting untwisting may represent a useful marker of diastolic function, which is largely independent of loading conditions [202, 203]. The velocity of LV untwisting has been correlated with invasive measurements of LV relaxation under a variety of loading and inotropic conditions in animal models. Dong and colleagues confirmed that LV untwisting is a measure of LV relaxation which is independent of preload as reflected in left atrial pressure [204].

The development of STE has allowed LV strain, twisting and untwisting rates to be measured relatively easily with reasonably high temporal resolution [157]. In this study, we used STE to measure LV strain, strain rate, twist and untwist rates in HCM patients and controls and we sought to assess their relationship with exercise capacity.

Methods

56 HCM patients and 43 age and gender matched controls were included in this study all of whom provided written informed consent. The study was approved by the local ethics committee and the investigations conform to the principles outlined in the Declaration of Helsinki. All study participants had ECG, echocardiogram and cardiopulmonary exercise test.

Patients selection

Inclusion criteria:

- Aged 18 years or above.
- Fulfilled conventional echocardiographic criteria for the diagnosis of HCM (LV wall thickness greater than 1.5 cm in the absence of another cause for hypertrophy and without cavity dilatation) [205, 206].
- Sinus rhythm.

Exclusion criteria:

- A resting or provocable LVOTO gradient > 30mmHg. Those patients were excluded because LVOTO is an important cause of symptoms and exercise limitation.

Controls selection

Inclusion criteria:

- Normal ECG.

- Normal Echocardiogram (LVEF ≥ 55%).

Exclusion criteria:

- History or symptoms of any medical illness.

Cardiopulmonary Exercise Test

This was performed using a Schiller CS-200 Ergo-Spiro exercise machine which was calibrated before every study. Subjects underwent spirometry and this was followed by symptom-limited erect treadmill exercise testing. Participants were encouraged to exercise to exhaustion with a minimal requirement of RER > 1. Peak oxygen consumption (peak V_{O2}) was defined as the highest V_{O2} achieved during exercise and was expressed in ml/min/kg.

Resting Echocardiography

LV volumes were obtained by biplane echocardiography, and LVEF was derived from a modified Simpson's formula [150]. A pulse wave Doppler sample volume was placed at the mitral valve tips to record 3 cardiac cycles. Mitral annulus velocities [PW-TDI] were recorded from basal anterolateral and basal inferoseptal segment in apical 4-chamber view. LA volumes were measured by area length method from apical 2 and 4 chambers as previously described [150] and indexed to body surface area to derive LA volume index (LAVI). LV mass was measured by area length method and indexed to body surface area to derive LV mass index as previously described [151]. The greatest thickness measured in the LV wall at any short axis parasternal view was considered to represent maximal LV wall thickness (MWT) in HCM patients [13].

Speckle Tacking Echocardiography (STE)

LV peak strain and strain rate in each view were calculated with the use of the entire length of the LV myocardium. The basal and apical LV global rot and rot-r STE data were measured from basal and apical short axis view respectively and were then exported to a spreadsheet program (Excel 2003, Microsoft Corp, Seattle, Washington) and then exported to DPlot Graph Software (2001-2008 by HydeSoft Computing, Inc) to calculate LV twisting and untwisting rate [157].

Statistics

Data were analyzed using SPSS ver. 15.0 for Window and Microsoft Office Excel 2007, and expressed as mean ± standard deviation (SD). Comparison of variables between HCM patients and controls were by unpaired Student's t-test (2-tail) if variables were normally distributed and the Mann-Whitney U-test if the data were non-normally distributed. A difference of $p < 0.05$ was taken to indicate statistical significance. Pearson's correlation coefficient was used to examine correlations between exercise capacity and echocardiographic parameters in HCM patients.

Results

The clinical characteristics and cardiopulmonary exercise test results of HCM patients and controls are shown in Table 6. Both groups were well matched with respect to age and gender. HCM patients exhibited marked exercise limitation as compared to controls whereas mean EF was comparable in both groups. Heart rate was lower in the HCM group, as compared to controls, due to rate limiting medication (beta blockers or/and calcium channel blockers).

Conventional Echocardiographic Measurements

Standard, Doppler and PW-TDI echocardiographic findings of HCM and controls groups are listed in Table 7. Transmitral Doppler measurements showed no significant difference in peak E and A, and E/A ratio in HCM versus controls (Table 7). However, PW-TDI measurements of Sm, Em, Am and E/Em of the inferoseptal, anterolateral and average basal segments were significantly lower in HCM patients compared to controls (Table 7).

Two Dimensional STE Measurements

STE measurements of strain and strain rate are shown in Table 8. Longitudinal strain (SL) and strain rate (SrL) during both systole and diastole were all significantly reduced in HCM patients as compared to controls. Similarly, radial strain (SR) and strain rate (SrR) were also significantly lower in HCM patients than in controls. Circumferential strain (SC) and diastolic strain rate (SrC) were also significantly lower in HCM patients as compared to controls. There was a non-significant trend to lower systolic circumferential strain rate (SrC) in HCM patients compared to controls.

Twist and Untwist Rate

STE measurements of LV rotation, twist and untwist in both groups are shown in Table 9 . There was no significant difference in the LV ability to twist in systole and untwist in diastole between HCM and controls. However, 25% untwist was significantly delayed in HCM versus controls. Unlike the base, there was a significant reduction in apical rotation rates during systole and diastole in HCM patients as compared to controls.

Parameter	*HCM*	*Controls*	*P value*
Age [years]	**52 ± 11**	**51 ± 16**	**0.74**
Number (Female)	**56 (16)**	**43 (12)**	**0.64**
Heart Rate [bpm]	**68.58 ± 13.19**	**80.47 ± 15.71**	**0.0002***
Systolic BP [mm Hg]	**125.67 ± 19.42**	**124.51 ± 19.8**	**0.78**
Diastolic BP [mm Hg]	**77.58 ± 10.77**	**78.17 ± 10.72**	**0.80**
Peak V_{O2} [ml/kg/min]	**23.28 ± 6.31**	**37.70 ± 7.99**	**< 0.0001***
Drug therapy – no. (%)			
Diuretic	**10 (18)**	**0**	**-**
ACE inhibitor	**6 (11)**	**0**	**-**
ARB	**1 (2)**	**0**	**-**
Beta-blocker	**17 (31)**	**0**	**-**
Calcium channel blocker	**26 (47)**	**0**	**-**
Aspirin	**13 (24)**	**0**	**-**
Warfarin	**5 (9)**	**0**	**-**
Nitrate	**2 (4)**	**0**	**-**
Statin	**15 (27)**	**0**	**-**

Table 6: The clinical characteristics and cardiopulmonary exercise test results of HCM patients and controls – STE study.

Parameter	*HCM*	*Controls*	*P value*
EF Biplane (%)	**62.76 ± 9.05**	**62.48 ± 5.82**	**0.86**
Biplane LA Volume (ml)	**72.69 ± 29.22**	**35.09 ± 9.83**	**< 0.0001***
LA Volume Index (ml/m^2)	**33.92 ± 14.62**	**11.76 ± 10.17**	**< 0.0001***
LV Mass Index (g/m^2)	**181.92 ± 84.09**	**71.67 ± 23.82**	**< 0.0001***
MV E (m/s)	**0.69 ± 0.16**	**0.67 ± 0.15**	**0.49**
MV A (m/s)	**0.64 ± 0.19**	**0.59 ± 0.15**	**0.17**
MV E/A	**1.18 ± 0.48**	**1.20 ± 0.38**	**0.83**
MV Dec Time (ms)	**231.12 ± 71.66**	**260.10 ± 68.24**	**0.06**
Antlat Sm (cm/s)	**0.06 ± 0.02**	**0.10 ± 0.03**	**< 0.0001***
Antlat Em (cm/s)	**0.08 ± 0.03**	**0.11 ± 0.04**	**< 0.0001***
Antlat Am (cm/s)	**0.07 ± 0.03**	**0.09 ± 0.02**	**< 0.0001***
Infsep Sm (cm/s)	**0.06 ± 0.02**	**0.08 ± 0.02**	**0.0001***
Infsep Em (cm/s)	**0.05 ± 0.02**	**0.08 ± 0.03**	**< 0.0001***
Infsep Am (cm/s)	**0.07 ± 0.02**	**0.09 ± 0.02**	**< 0.0001***
Average Sm (cm/s)	**0.06 ± 0.02**	**0.09 ± 0.02**	**< 0.0001***
Average Em (cm/s)	**0.06 ± 0.02**	**0.09 ± 0.04**	**< 0.0001***
E/Em $_{antlat}$	**9.70 ± 3.86**	**6.71 ± 2.35**	**0.0001***
E/Em $_{infsep}$	**17.05 ± 7.38**	**9.64 ± 3.34**	**< 0.0001***
E/Em $_{average}$	**11.81 ± 4.28**	**8.04 ± 2.92**	**< 0.0001***

Table 7: Standard, Doppler and PW-TDI Echocardiographic characteristics of HCM vs controls – STE study.

Parameter	*HCM*	*Controls*	*P value*
Longitudinal			
SL Peak S (%)	**-12.74 ± 4.21**	**-18.38 ± 2.99**	**< 0.0001***
SrL Peak S (1/s)	**-0.92 ± 0.25**	**-1.14 ± 0.2**	**< 0.0001***
SrL Peak E (1/s)	**0.94 ± 0.35**	**1.33 ± 0.3**	**< 0.0001***
SrL Peak A (1/s)	**0.78 ± 0.28**	**1.07 ± 0.29**	**< 0.0001***
Radial			
SR Peak S (%)	**17.52 ± 7.86**	**29.63 ± 13.26**	**< 0.0001***
SrR Peak S (1/s)	**1.22 ± 0.34**	**1.39 ± 0.34**	**0.02***
SrR Peak E (1/s)	**-1.0 ± 0.39**	**-1.46 ± 0.52**	**< 0.0001***
SrR Peak A (1/s)	**-0.77 ± 0.38**	**-1.09 ± 0.61**	**< 0.01***
Circumferential			
SC Peak S (%)	**-16.50 ± 4.29**	**-19.99 ± 5.53**	**< 0.001***
SrC Peak S (1/s)	**-1.4 ± 0.35**	**-1.54 ± 0.34**	**0.05**
SrC Peak E (1/s)	**1.34 ± 0.42**	**1.8 ± 0.65**	**< 0.001***
SrC Peak A (1/s)	**0.87 ± 0.34**	**1.09± 0.47**	**0.02***

Table 8: STE results of longitudinal, radial & circumferential strain and strain rates in HCM patients vs controls.

Predictors of Exercise Capacity

In HCM patients, exercise capacity (peak V_{O2}) was independent of LV mass index (r = 0.01, p = 0.97, Figure 23A), MWT (r = 0.04, p = 0.8, Figure 23B) or LA volume index (r = - 0.08, p = 0.6, Figure 23C). However, it significantly correlated with the following STE parameters: longitudinal strain rate (SrL) during systole (peak S) (r = -0.34, p = 0.01, Figure 24A), during early diastole (peak E) (r = 0.36, p = 0.006, Figure 24B) and during late diastole (peak A) (r = 0.31, p = 0.02, Figure 24C), and it also correlated with 25% untwist (r = 0.36, p = 0.006, Figure 24D).

Discussion

In this study we assessed myocardial strain using an ultrasound speckle tracking technique that avoids the angle-dependency of Doppler-based techniques. Longitudinal, radial and circumferential strain and strain rates, twist and untwist rates were measured in HCM patients and in age and gender matched controls. Whilst HCM patients typically exhibit hyperdynamic systolic function as assessed by conventional echocardiography, our STE findings of reduced longitudinal strain rate and delayed 25% untwist as significant predictors of exercise capacity are to the best of our knowledge novel.

STE tracks characteristic speckle patterns created by interference of ultrasound beams in the myocardium which is based on grayscale B-mode images, and is angle independent [152].

Parameter	*HCM*	*Controls*	*P value*
Apical Rot and Rot-R			
Peak Rot (deg)	**7.65 ± 6.90**	**9.48 ± 5.40**	**0.18**
Peak S Rot R (deg/s)	**49.77 ± 49.49**	**74.56 ± 42.25**	**0.01***
Peak E Rot R (deg/s)	**-41.19 ± 39.14**	**-72.96 ± 38.00**	**< 0.001***
Peak A Rot R (deg/s)	**-26.24 ± 33.94**	**-39.87 ± 27.01**	**<0.05***
Basal Rot and Rot-R			
Peak Rotation (deg)	**-6.25 ± 3.73**	**-5.80 ± 2.81**	**0.55**
Peak S Rotation Rate (deg/s)	**-57.89 ± 28.94**	**-61.24 ± 17.82**	**0.54**
Peak E Rotation Rate (deg/s)	**53.07 ± 25.96**	**48.88 ± 26.42**	**0.47**
Peak S Rotation Rate (deg/s)	**42.39 ± 24.52**	**44.67 ± 20.68**	**0.65**
Twist and Untwist			
Peak Twist (deg)	**13.37 ± 7.14**	**13.69 ± 6.55**	**0.83**
25% Untwist (deg)	**9.85 ± 5.47**	**10.27 ± 4.91**	**0.71**
Time 25% Untwist (R-R %)	**11.34 ± 5.63**	**7.21 ± 8.01**	**0.01***

Table 9: STE measurement of LV rotation, twist and untwist in HCM and controls.

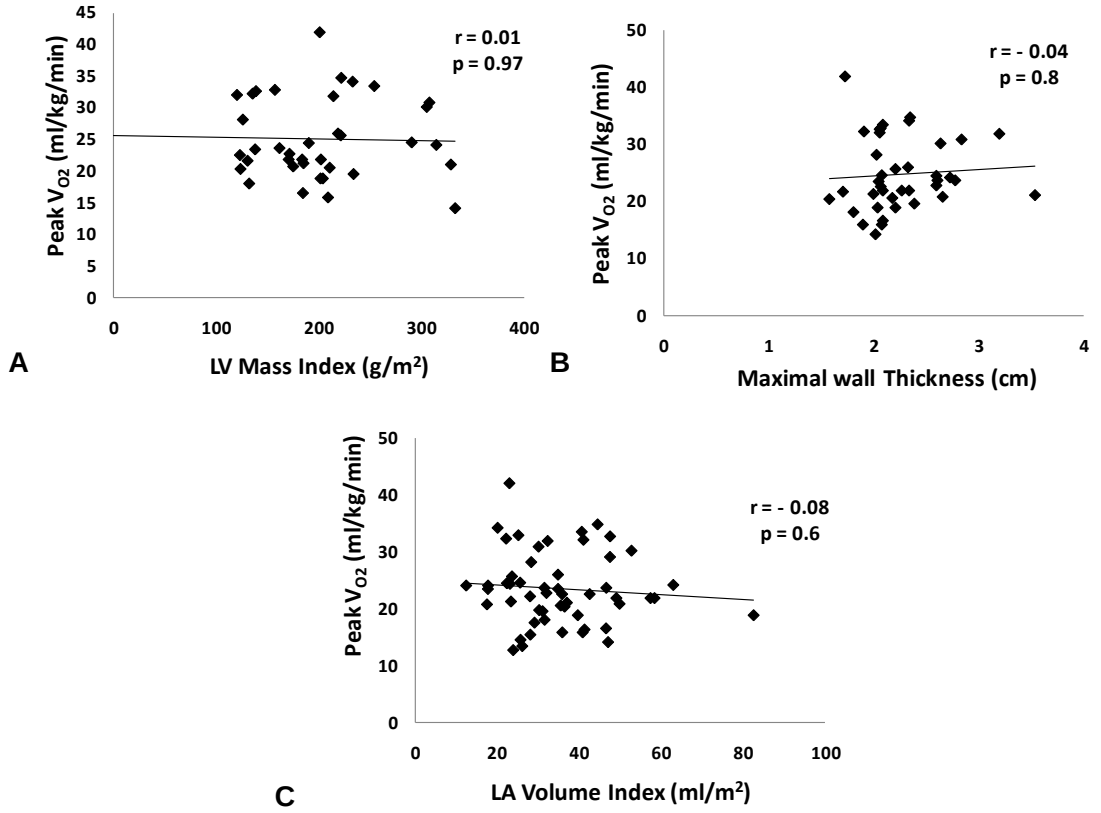


Figure 23: Correlation between exercise capacity (peak V02) and (A) LV Mass Index, (B) MWT and (C) LV Volume Index.

In humans, measurement of LV long-axis strain by STE correlated well with MRI tagging (r=0.87)[207]. We are now able to characterize myocardial function in greater detail breaking down global function into the individual components that make up the whole. This can now be done for both the diastolic phase and systolic phases of myocardial function quickly and reproducibly using a relatively new technique of speckle tracking.

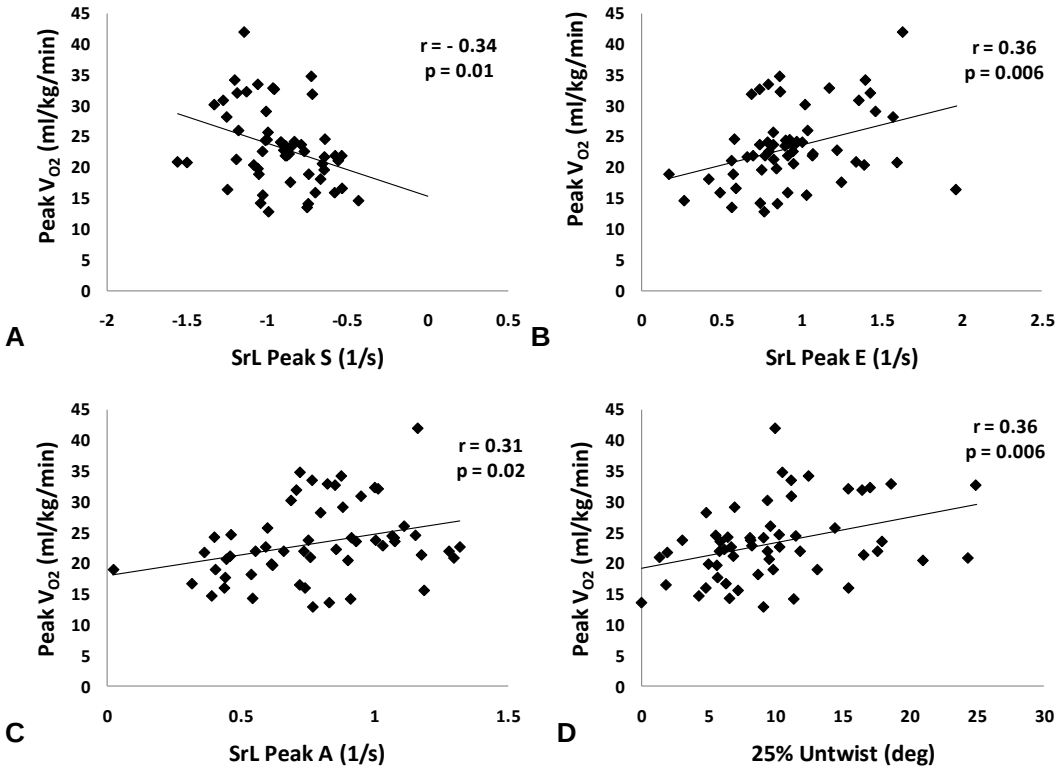


Figure 24: Correlation between exercise capacity (peak V02) and (A) Longitudinal strain rate (SrL) Peak S, (B) SrL Peak E, (C) SrL Peak A and (D) 25% Untwist.

Our findings of reduced longitudinal, circumferential and radial strains are in agreement with previously published works [208]. However the clinical relevance of these alterations in function has not been previously assessed.

Systolic function in HCM

The function of the LV is often described as hyperdynamic in HCM. This is based on the frequent observation of high normal or supra-normal EF. In this study, we showed that HCM patients had reduced systolic STE-derived longitudinal, circumferential and radial strain and strain rates despite normal LVEF. These results are consistent with

TDI-derived data which have shown that systolic long-axis function is reduced in patients with HCM [209]. Carasso and colleagues showed reduced longitudinal but higher circumferential STE-derived strain and strain rate in HCM patients compared to controls [210]. Our findings of reduced circumferential strain are consistent with previous tagged MRI studies [211, 212].

Diastolic function in HCM

The majority of HCM patients have some degree of diastolic dysfunction [213-216] independent of symptoms, presence of ventricular outflow tract obstruction, the magnitude of hypertrophy, or a combination of these [12, 209, 214, 217]. The mechanisms responsible for the diastolic dysfunction are probably multifactorial. The hypertrophy, myocyte disarray and fibrosis may increase passive left ventricular stiffness. Several studies had shown abnormalities of active relaxation in HCM patients [218, 219] and these may relate to abnormal calcium handling [17], to microvascular myocardial ischaemia and mechanical dyssynchrony [18], or to impaired myocardial energy utilisation [19, 38, 52].

There are several echo measurements that can quantify diastolic dysfunction in HCM patient but there remain some controversies about their accuracy. Our data showed no significant difference in transmitral Doppler derived parameters between HCM patients and controls. However, there were significant abnormalities of other echocardiographic measurements of diastolic function (such as LA Volume, LA Volume Index and TDI-derived indexes) in HCM patients. More importantly, untwist (a marker of diastolic function) was significantly delayed in these patients which is in agreement with a previous study [220].

Exercise limitation in HCM

Traditionally it has been believed that LV diastolic dysfunction is the predominant cause of breathlessness and exercise limitation in HCM patients [15]. Interestingly, we found that markers of both left ventricular systolic and diastolic function significantly correlated with exercise capacity.

Our colleagues have previously [15] demonstrated that exercise capacity in patients with HCM is limited by the failure to increase stroke volume on peak exercise. Furthermore, the normal inverse relationship of TTPF of the LV with increasing heart rate was lost. A similar pattern has also been shown in patients with ischaemic heart disease [221]. The mechanism of this limitation of left ventricular filling has not been fully elucidated.

Left ventricular untwist is increasingly being recognized as an important component of diastolic function, contributing significantly to suction. Knudtson et al [222] have shown that myocardial ischaemia impairs untwist. Untwisting, a recoil phenomenon occurs during the isovolumetric relaxation period, tending towards geometric restoration of the zero twist point (end diastole) with concurrent maximum left ventricular volume. Twist and untwist rates appear to be closely related. During systole myocardial deformation (twist) results in elastic energy being stored in compressed titin [223] and transmural shear between myofibril sheets [224]. This energy is spent during early diastole, before the onset of left ventricular filling [225] which leads to untwisting of the LV that contributes to the generation of suction. The basis of twist and untwist abnormalities in HCM patients is possibly due to regional abnormalities of systolic and diastolic function [226]. Peak ventricular untwist rate has been correlated with the time constant of LV pressure decay

and intraventricular pressure gradients, both markers of diastolic function [227]. Rovner A and colleagues showed a significant improvement in intraventricular pressure gradients in patients with obstructive HCM after alcohol ablation [228]. Reduced untwist rate may lead to impairment in diastolic filling, which becomes more notable with increased heart rates [153] due to short filling period. Notomi and colleagues showed that greater untwisting rate produced an increase in suction during exercise in healthy subjects, which is able to fill the ventricle more despite a shorter filling period. This suggest that untwist rate is an important mechanism augmenting LV filling.

The delayed untwist in HCM patients observed in this study may lead to reduced left ventricular suction and filling, which will reduce the ability to utilize the Starling mechanism, leading to a failure to increase cardiac output, and as a result will limit exercise capacity.

In summary, although HCM patients often had normal/supranormal LVEF as measured by conventional echocardiography, STE imaging demonstrated a marked impairment of both systolic and diastolic LV function and they were both significant determinants of exercise capacity.

CHAPTER 6: VALIDATION AND REPRODUCIBILITY OF HIGH ENERGY PHOSPHATE KINETICS IN THE MYOCARDIUM USING ^{31}P CARDIAC SPECTROSCOPY AT 3 TESLA

Abstract

Background: ^{31}P magnetic resonance spectroscopy (MRS) allows measurement of *in vivo* high energy phosphate kinetics in the myocardium. While traditionally ^{31}P cardiac spectroscopy is performed at 1.5 Tesla, cardiac MRS at higher field strength can theoretically increase SNR and spectral resolution therefore improving sensitivity and specificity of the cardiac spectra. The reproducibility and feasibility of performing cardiac spectroscopy at 3 Tesla is presented here in this study in HVs and patients with HCM.

Methods: Cardiac spectroscopy was performed using a Phillips 3T Achieva scanner in 37 HVs and 26 patients with HCM to test the feasibility of the protocol. To test the reproducibility a single volunteer was scanned eight times on separate occasions. A single voxel ^{31}P MRS was performed using Image Selected *In vivo* Spectroscopy (ISIS) volume localisation.

Results: The mean PCr/Adenosine Triphosphate (PCr/ATP) ratio of the eight measurements performed on one individual was 2.11±0.25. Bland Altman plots showed a variance of 12% in the measurement of PCr/ATP ratios. The PCr/ATP ratio was significantly reduced in HCM patients compared to controls, 1.42 ± 0.51 and 2.11 ± 0.57, respectively, $P<0.0001$. (All results are expressed as mean ± standard deviation).

Conclusions: Here we demonstrate that cardiac ^{31}P MRS at 3T is a reliable method of measuring *in vivo* high energy phosphate kinetics in the myocardium for clinical studies

and diagnostics. Based on our data an impairment of cardiac energetic state in patients with HCM is indisputable.

Introduction

Phosphorus Spectroscopy is a non-invasive method of studying cardiac *in vivo* high energy phosphate kinetics [229]. It allows for determination of PCr, ATP, Adenosine Diphosphate (ADP) and inorganic phosphate (Pi) concentrations in the myocardium. The concentrations of these substances and the ratio of PCr/ATP are measures of the cardiac energetic status. PCr is an important short-term reserve energy source that maintains a high phosphorylation potential under conditions of increased energy demand like exercise and ischaemia. The conversion of ADP to ATP by transfer of a phosphoryl group from PCr is catalysed by creatine kinase. This reaction occurs 10 times faster than ATP production via oxidative phosphorylation [79].

In patients with mild to moderate heart failure, cardiac ATP flux mediated by creatine kinase is reduced by approximately 50% [230]. Animal and human studies have demonstrated that a progressive reduction of the creatine pool is directly related to the severity of heart failure [84]. This is largely due to a decrease in the number of creatine transporters at sites of energy production and utilisation [87]. In normal myocardium two thirds of the creatine pool is phosphorylated via creatine kinase reaction to form PCr [38, 52, 183] and the expression and activity of this enzyme is reduced in heart failure [231, 232]. Therefore in heart failure the available PCr is markedly diminished. The depletion of PCr occurs to a greater extent compared to ATP resulting in reduced PCr/ATP ratio in heart failure as measured by MRS [85]. Reduced PCr/ATP ratio is associated with increased mortality in heart failure patients [86]. ^{31}P cardiac spectroscopy can also be used to monitor disease progress in heart failure patients. It has been used as an objective marker to show benefits of various treatments such as metabolic modulators [233].

Reduced PCr/ATP ratio is also seen in other conditions including ischaemic heart disease [234], left ventricular hypertrophy secondary to hypertension [235], valvular heart disease (mitral regurgitation and aortic valve disease) [85, 86], diabetes [236] and HCM [34, 237, 238]. Interestingly, patients with genotypic HCM who do not yet have hypertrophy have a similar degree of impairment of cardiac PCr/ATP ratio as do patients with marked hypertrophy, implying that the disturbance may be an early feature of the disease and is not simply due to the hypertrophy [32].

Traditionally cardiac spectroscopy in humans has been performed using 1.5 Tesla magnets. Previous studies in animals [239] and humans demonstrated that higher field strength such as 3T [240] or 4.7T offer higher SNR. The increased sensitivity can be traded for either increased spatial resolution or improved quantification precision and hence might improve specificity of ^{31}P cardiac MRS as a diagnostic tool. Hence it is desirable to perform cardiac MRS at 3T MR scanners. However, higher field strength also result in inhomogeneities of transmit and receive B1 field along with restrictions of the maximum achievable B1 field strength that result in larger chemical shift displacements. Furthermore susceptibility differences between adjacent tissues have a greater effect on B0 homogeneity, which results in line broadening. Hence in this study we test the feasibility and reliability of ^{31}P cardiac spectroscopy at 3 Tesla for clinical diagnostics using standard methods pre-implemented on a clinical MR scanner.

Methods

37 Controls (22 males) and 26 HCM patients (21 males) with symptomatic non-obstructive cardiomyopathy, who provided written informed consent, were included in

the study. The experiment was approved by the Regional Ethics Committee at Birmingham, UK. All HVs were screened with history, echocardiography and metabolic exercise testing to rule out any structural heart diseases. All patients were recruited from cardiomyopathy clinics and had clinically proven diagnosis of non-obstructive HCM.

Patients selection

Inclusion criteria:

- Aged 18 to 80 years.
- Fulfilled conventional echocardiographic criteria for the diagnosis of HCM (LV wall thickness greater than 1.5 cm in the absence of another cause for hypertrophy and without cavity dilatation) [205, 206].
- Sinus rhythm.
- Exertional symptoms.
- Peak V_{O2} < 75% of predicted for age and gender.

Exclusion criteria:

- A resting or provocable LVOTO gradient > 30mmHg. Those patients were excluded because LVOTO is an important cause of symptoms and exercise limitation.

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Controls selection

Inclusion criteria:

- Normal ECG.

- Normal Echocardiogram (LVEF ≥ 55%).

Exclusion criteria:

- History or symptoms of any medical illness.

The subject characteristics are presented in Table 10. The mean age of the HVs was 48 ± 16 years and that of HCM patients was 55 ± 13 (p = ns).

Parameter	Controls (N=37)	HCM (N=26)	P Value
Age (years)	48 ± 16	55 ± 13	ns
Male sex –no (%)	22 (59)	21 (81)	ns
EF (%)	64 ± 6	64 ± 9	ns
Peak V_{O2} (ml/kg/min)	39 ± 8	24 ± 6	<0.0001
RER	1.2 ± 0.2	1.1 ± 0.1	ns
Heart rate (bpm)	79.5 ± 11.7	67.5 ± 12.5	<0.01
QTc interval (msec)	421.8 ± 16.0	455.9 ± 35.0	<0.01
Systolic BP (mm Hg)	127.0 ± 20.4	126.1 ± 20.1	ns
Diastolic BP (mm Hg)	79.5 ± 9.6	75.6 ± 10.7	ns

Table 10: Baseline characteristics of HCM patients & controls - MRS reproducibility study.

The mean EF on echocardiography was 64 ± 6% in controls and 64 ± 9% (p = ns) in HCM patients. None of the HVs had any structural heart disease or ECG abnormalities. ^{31}P cardiac spectroscopy was performed eight times in one participant both on the same and on different days - to test the reproducibility and coefficient of variation of the test.

^{31}P cardiac magnetic resonance spectroscopy was performed using a Phillips Achieva 3T scanner and a linearly polarized transmit and receive ^{31}P coil with a diameter of 14 cm. Localization was achieved by ISIS ^{160} volume selection.

The participants were positioned supine with the coil directly over the precordium. The 3-D voxel of acquisition was planned to include most of the septum and apex of the heart. Care was taken to minimize blood contamination from the RV as much as possible. The voxel size was kept constant at 89.54ml (44 x 55 x 37 mm^3) so that comparisons could be made between different subjects and scans. Following this the ^{31}P spectra were acquired with a repetition time of 10000 ms, 136 averages and 512 samples. A repetition time of 10000 ms was found to be optimal to adequately reduce saturation effects without increasing the scan time greatly. The spectral acquisition was ECG gated and the trigger delay was set to acquire spectra mainly in diastole. The trigger delay was measured by subtracting 250-300 msec from the total length of the cardiac cycle which allowed 250-300 msec of the cardiac cycle left for spectral acquisition (acquisition time is 170 msec). The total scan time was 23 minutes.

MRS Analysis

The spectra were analysed and quantified on jMRUI software using AMARES a time domain fitting program [161]. Cramer Rao lower bounds [162] and PCr/ATP ratio were calculated as previously described.

Results

A typical cardiac spectrum in HV as compared to HCM is shown in Figure 25.

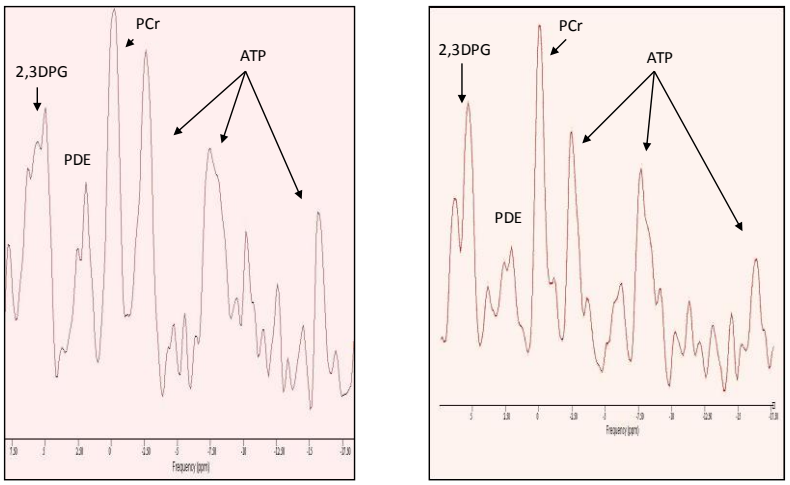

Figure 25: Typical cardiac spectra in HCM and control.

The PCR/ATP ratio was significantly lower in HCM (1.42 ± 0.51) as compared to healthy controls (2.11 ± 0.57, P < 0.0001) (Figure 26). The mean PCr/ATP ratio for the one participant with eight measurements was 2.11 ± 0.25 (Table 11). Bland Altman plots were used as a test of reproducibility (Figure 27). The distribution of all the data

points showed a good reproducibility with a variance of 12% in the measurement of PCr/ATP ratios which is within limits of agreement of repeated measurements.

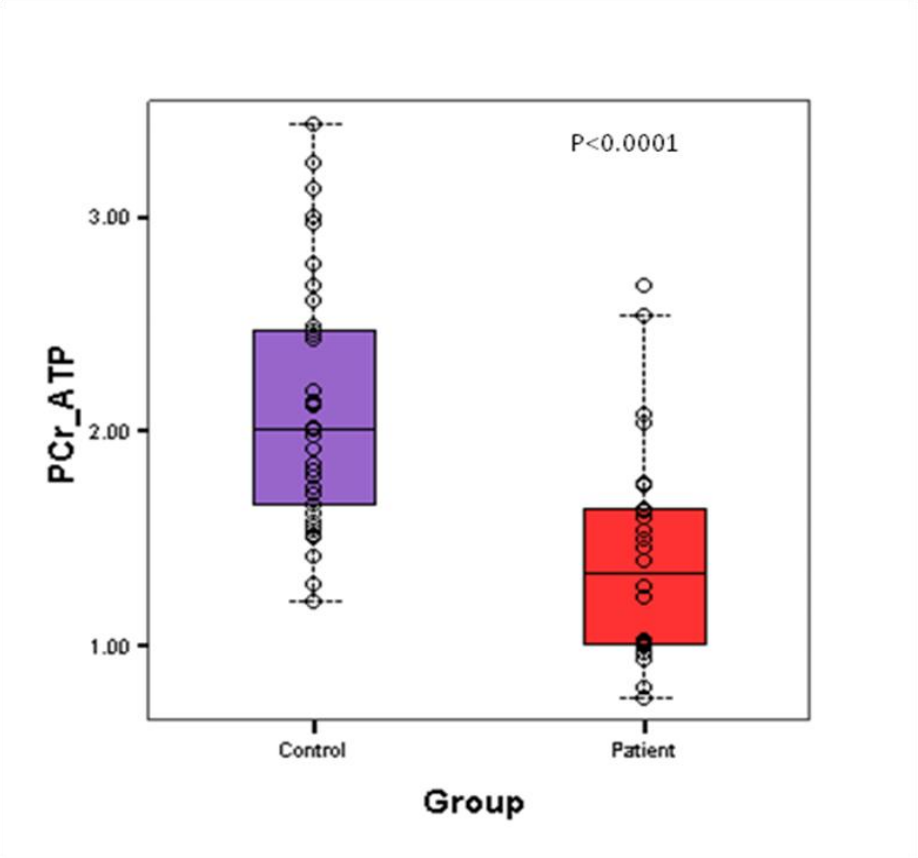

Figure 26: Box-plots of PCr/ATP ratios in controls and HCM patients.

We also measured the line width of PCr peaks in the one participant who had 8 repeated scans. The mean line width was 1.36 ± 0.07 parts per million (ppm), Figure 28. The standard deviation of the line width was low which again confirmed the fact that the spectra were of good reproducible quality.

Subject No	2,3 DPG sum	PCr	γ ATP	γ ATP (C)	PCr/γ ATP Ratio	PCr/ γ ATP Ratio (C)
Subject 1A	6.72E-04	4.90E-04	3.57E-04	2.45 E-04	1.37	2.00
Subject 1B	4.27E-04	4.32E-04	3.21E-04	2.5 E-04	1.34	1.72
Subject 1C	7.32E-04	6.53E-04	4.77E-04	3.55 E-04	1.36	1.83
Subject 1D	1.00E-03	8.52E-04	5.12E-04	3.45 E-04	1.66	2.47
Subject 1E	9.39E-04	5.53E-04	3.86E-04	2.3 E-04	1.43	2.40
Subject 1F	8.29E-04	6.34E-04	4.26E-04	2.88 E-04	1.48	2.20
Subject 1G	8.66E-04	5.36E-04	3.90E-04	2.46 E-04	1.37	2.18
Subject 1H	8.32E-04	5.55E-04	4.05E-04	2.66 E-04	1.37	2.08

Table 11: Intra-subject MRS measurements.

Table depicting the concentrations of 2, 3-DPG, ATP and PCr and the calculated PCr/ATP ratios from these measurements in the subject with eight measurements. (C): corrected for blood contamination.

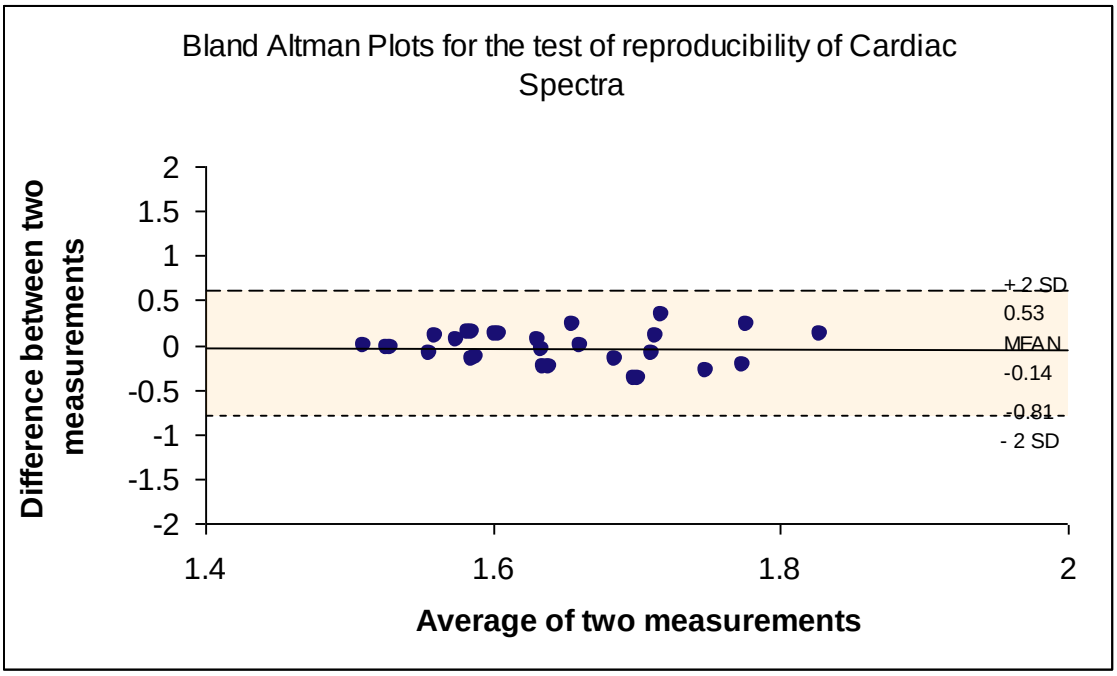

Figure 27: Bland-Altman plots of ^{31}P cardiac Intra-subject MRS measurements.

Graph plotted as difference in two measurements against mean of the same two measurements.

As a further measure of the quality of spectra, we calculated the Cramer Rao lower bounds 162, which was 6 ± 1% for the PCr peak and 10 ± 1% for the gamma ATP peak. Cramer Rao lower bounds were calculated for the whole group, which was 12 ± 6% for the PCr peak and 17 ± 9% for the gamma ATP peak (Table 12). Due to an increase of susceptibility differences between heart muscle tissue, blood and air in the lungs at high field strength shim quality was slightly decreased at 3T in comparison to values reported for 1.5T cardiac spectroscopy. Hence multiplet splitting of the ATP resonances due to J-coupling is hardly visible.

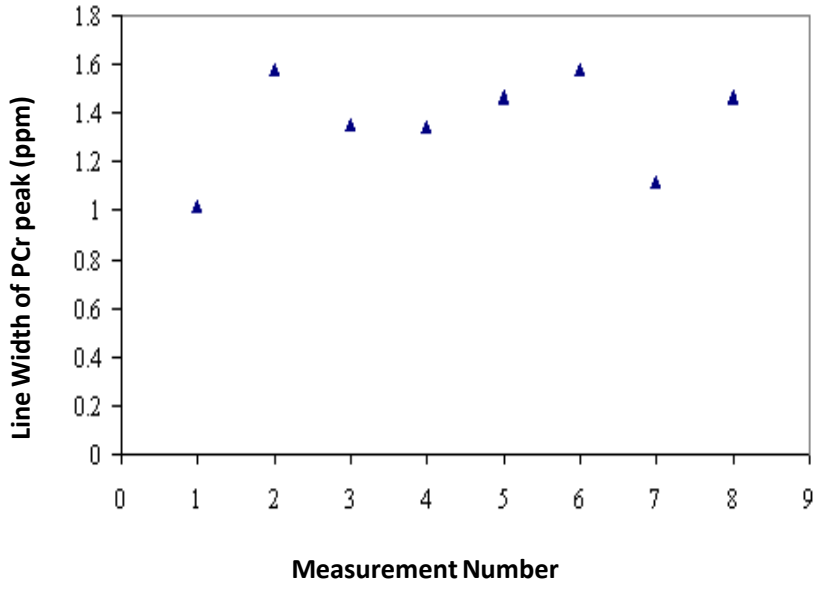

Figure 28: Intra-subject line width of PCr peaks.

Line width: 1.3625 ± 0.072 ppm.

Groups	PCr peak	γ ATP Peak
Healthy control (n = 1) **(8 Measurements)**	6% ± 1	10% ± 1
Whole group (n = 63) **(Controls + HCM)**	12% ± 6	17% ± 9
Controls (n = 37)	11% ± 5	16% ± 8
HCM (n = 26)	12% ± 6	17% ± 9

Table 12: Signal to Noise Ratio (SNR) assessment.

Cramer Rao Lower bounds measured to test the quality of spectra.

Discussion

Here we demonstrate that in-vivo cardiac ^{31}P MRS at 3T is a reliable method of measuring high energy phosphate kinetics in the myocardium. We also show reduced PCr/ATP ratio in HCM patients known to have impaired cardiac energetics as measured in 1.5T systems [32, 34]. The spectra show good reproducibility indicating that ^{31}P cardiac MR spectroscopy is feasible on a clinical 3T MR system. The standard deviation for the PCr/ATP ratio for the whole group was low and comparable to previous published data on cardiac spectroscopy at 1.5 Tesla [241]. This basic method of acquiring ^{31}P spectra at 3T using pre-implemented methods such as ISIS volume localisation and iterative shimming promises to be an important diagnostic and research tool. Possible applications are the comparison of PCr/ATP ratios in conditions like heart failure, ischaemic heart disease and valvular heart disease. It might also be used to monitor disease progression and study effects of medications like metabolic modulators in heart disease.

Cardiac spectroscopy at 3T continues to face some challenges which are present at 1.5T as well, but some of them are even more pronounced at high field strength. One main drawback of cardiac spectroscopy is the effect of respiratory motion and movement of the heart itself. This can result in contamination from liver and skeletal muscle of the chest wall. Careful localization of the voxel ensures minimal contamination. We particularly paid attention to this aspect while planning our voxel of acquisition. Provided the voxel is positioned carefully, no significant advantages to the quality of spectra acquired with multiple outer volume suppression bands was observed. None of our spectra show any significant contamination with skeletal muscle or liver. It is also

important to ensure that the spectral acquisition is done when the heart motion is at its minimum. This is during diastole and we set the trigger delay such that all of the spectral acquisition happened during diastole. One other problem is the respiratory motion. This could be partially negated by acquiring the spectra with respiratory gating as well as cardiac gating (double triggered) and volume tracking [242, 243]. Further developments in these techniques should improve quality of cardiac spectra and reduce the contamination from surrounding structures.

Higher field strength of 3T offers better spatial resolution and SNR. These effects have been particularly noted in proton spectroscopy of the human brain [244]. However, increasing susceptibility differences between muscle tissue, blood and air in the lungs cause increased B0 field inhomogeneities and hence problems with shim convergence. Various shimming techniques like iterative and FASTMAP based [245] higher order shimming were compared. Localized iterative 1st order shimming based on a volume including the entire heart offered the best and most reproducible shim quality. However, there is definitely scope for further improvement in shimming techniques. One possibility might be localized shimming based on cardiac triggered B0-mapping as reported earlier for cardiac imaging at 3T [246]. Another problem is the shortened transverse relaxation times (T2) at 3T in comparison to 1.5T. This causes an additional increase in line width of the various peaks.

No significant advantages to the quality of spectral acquisition using proton decoupling or Nuclear Overhauser Enhancement (NOE) were found. Therefore these techniques were not used. Although NOE can increase the SNR by up to 40%, this is not useful for

experiments where quantification is required. NOE in particular imparts different amount of energy to phosphates in ATP and PCr which can result in altered PCr/ATP ratio. This is particularly important as the effect of NOE is different for distinct molecules and depends on conditions as the pH which might be changed in the diseased myocardium.

Due to a decrease of the maximum achievable B1 field strength at 3T together with an increase in spectral separation compared to 1.5T, the bandwidth of the excitation pulse was limited and hardly sufficient to excite the entire frequency range of interest. Using a simple surface coil this is especially a problem in large penetration depth. In addition, the excitation profile was not homogeneous. Hence different flip angles were applied to spins with different offset frequencies. This resulted in a frequency dependent weighting of peak intensities. Hence, the intensity of the beta ATP peak, which has a large frequency offset compared to PCr, was always significantly decreased or even lost in obese subjects, where the distance between coil and VOI is large. However the determined PCr/gamma-ATP ratios were not affected, because the frequency difference between both resonances is small and the pulse frequency offset was chosen to be in-between of both.

CHAPTER 7: MYOCARDIAL ENERGY DEFICIENCY IN HCM: RELATION TO EXERCISE CAPACITY AND DYNAMIC SYSTOLIC AND DIASTOLIC DYSFUNCTION

Abstract

Introduction: Previous studies had shown that hypertrophic cardiomyopathy (HCM) patients manifest impaired cardiac energetics. We hypothesized that reduced cardiac energetic status would result in dynamic impairment of both diastolic and systolic function upon exercise and contribute to exercise limitation.

Methods: 51 symptomatic non-obstructive HCM patients (39 male and mean age of 54 years) and 33 age and gender matched controls were enrolled. All participants had an ECG, echocardiogram and cardiopulmonary exercise test. We measured radionuclide indices of left ventricular diastolic filling (Time To Peak Filling [TTPF]) and normalised this for heart rate (nTTPF), LV end systolic elastance index ($E_{LV}I$) and vasculoventricular coupling (VVC) ratio at rest and during submaximal exercise (50% of heart rate reserve). Myocardial energetic status (PCr/γ ATP ratio) was measured by ^{31}P magnetic resonance spectroscopy (MRS).

Results: HCM patients exhibited marked exercise limitation vs healthy controls (peak V_{O2} 24 ± 6 vs 38 ± 8 ml.kg-1.min-1, $p < 0.0001$), and reduced PCr/γATP ratio (1.41 ± 0.48 vs 2.26 ± 0.59 , $p < 0.0001$). nTTPF (normalised for heart rate) fell during submaximal exercise in controls (0.19 ± 0.09 to 0.16 ± 0.08 sec), but increased significantly in patients (0.17 ± 0.07 to 0.32 ± 0.09 sec) , ($p < 0.0001$). The increase in $E_{LV}I$ was greater in controls (relative increase 1.7±0.7 vs 1.24±0.68, $p<0.05$). The VVC ratio decreased on exercise in controls but was unchanged in HCM patients (0.60 ± 0.21 vs 0.41 ± 0.15, $p = 0.003$ and 0.55 ± 0.35 vs 0.46 ± 0.24, $p = 0.28$, respectively). PCr/ γ

ATP ratio correlated significantly with nTTPF during exercise ($r = -0.47$, $p = 0.003$), with peak V_{O2} ($r = 0.52$, $p < 0.001$) and with δVVC ratio ($r = -0.4$, $p = 0.02$).

Conclusion: HCM patients have impaired myocardial energetics which correlates significantly with exercise capacity, and is associated with dynamic diastolic and systolic dysfunction. This study provides a rationale for further consideration of metabolic therapies in HCM.

Introduction

Biochemical and biophysical analyses of the mutant sarcomeric proteins that cause HCM led Watkins and colleagues to propose that energy depletion may underlie HCM [91-93]. Consistent with this, unexplained left ventricular hypertrophy is a feature of disorders associated with mutations in a variety of metabolic genes that lead to defects in energy production in the heart [91]. In a recent study using *in vivo* cardiac MR spectroscopy, resting PCr/γATP ratio was diminished in patients with sarcomeric HCM, indicating reduced energy availability [34]. Importantly, patients with genotypic HCM who did not yet have hypertrophy had a similar degree of impairment of cardiac PCr/γATP ratio as seen in patients with marked hypertrophy, implying that the disturbance may be an early feature of the disease and is not simply due to the hypertrophy [32].

Diastolic dysfunction contributes significantly to the genesis of breathlessness in HCM patients. Active relaxation is typically slowed and filling rate diminished at rest in these patients [14]. Furthermore, our group previously showed that, on exercise there is frequently a failure of the normal increase in the rate of active relaxation on exercise, indeed in some patient's active relaxation paradoxically slowed on exertion [15]. Whilst resting measures of diastolic function were rather poorly related to exercise capacity, TTPF (an indirect measure of the rate of LV active relaxation) assessed during submaximal exercise was strongly related to peak oxygen consumption [15]. Izawa and colleagues subsequently confirmed these non invasive observation using invasive measures of active relaxation [16].

In this study, we assessed the relationship between exercise capacity, cardiac energetic and diastolic and systolic function at rest and during exercise in HCM patients and controls. We used *in vivo* cardiac ^{31}P MR spectroscopy to measure resting PCr/γATP ratio as a marker of cardiac energetic status and we used rest and exercise radionuclide ventriculography to measure diastolic filling of the left ventricle, LV end systolic elastance index ($E_{LV}I$) and vasculoventricular coupling (VVC) at rest and during exercise.

Methods

51 HCM patients (39 males) and 33 controls (20 males), who provided written informed consent, were included in the study. Characteristics and treatment of participants are shown in Table 13. The study was approved by the South Birmingham Research Ethics Committee and the investigation conforms to the principles outlined in the Declaration of Helsinki. All study participants had ECG, echocardiogram and metabolic exercise test. 5 healthy controls were excluded from MRS studies due to claustrophobia. 15 HCM patients were excluded from MRS studies – 8 due to claustrophobia, 4 had poor quality spectra either due to inadequate ECG gating or shimming, and 3 were deemed unsafe to have MRS studies due to potential risk of metal bodies in eyes.

Patient selection

Patients were consecutively recruited from cardiomyopathy clinics at the Heart Hospital, University College London Hospitals, London and Queen Elizabeth Hospital, Birmingham, UK between 2006 and 2008.

Inclusion criteria:

- Age 18 to 80 years.
- Exertional symptoms.
- Sinus rhythm.
- Peak V_{O2} < 75% of predicted for age and gender.

Exclusion criteria:

- Presence of resting or provocable LVOT obstruction (peak gradient > 30 mm Hg).
- Presence of epicardial coronary artery disease.
- Diabetic patients.

Controls selection

Inclusion criteria:

- Normal ECG.
- Normal Echocardiogram (LVEF ≥ 55%).

Exclusion criteria:

- History or symptoms of any medical illness.

Cardiopulmonary Exercise Test

All study participants underwent symptom-limited erect treadmill exercise testing (Schiller CS-200 Ergo-Spiro exercise machine) using a standard ramp protocol with simultaneous respiratory gas analysis [158]. Peak oxygen consumption (peak V_{O2}) was

defined as the highest peak V_{O2} achieved during exercise (with RER >1) expressed in ml/min/kg.

Transthoracic Echocardiography

Echocardiography was performed with participants in the left lateral decubitus position with a Vivid 7 echocardiographic machine (GE Healthcare) and a 2.5-MHz transducer. LVEF was derived from a modified Simpson's formula. LA volume index and MWT were measured. Pulse and continuous wave Doppler were used to assess resting LVOTO gradient.

Radionuclide Ventriculography

Diastolic filling was assessed by equilibrium R-wave gated blood pool scintigraphy at rest and during graded semi-erect exercise on a cycle ergometer as previously described [221, 247]. Peak left ventricular filling (end-diastolic count per second (EDC/s)) and time to peak filling normalised for R-R interval (nTTPF), an indirect measure of the rate of LV active relaxation were assessed at rest and during exercise (50% of heart rate reserve). The validity of these radionuclide measures of diastolic filling at high heart rates has been established previously [221, 247]. The following indexes were calculated: arterial elastance index (EaI) and LV end-systolic elastance index ($E_{LV}I$) as previously described by our group [248] and others [249]. VVC is an index of the interaction between arterial and ventricular elastic properties, which is an important determinant of cardiac performance. It is defined by the ratio of arterial elastance (EaI) to left ventricular systolic elastance ($E_{LV}I$) [249]. δVVC Ratio was defined as the net difference between exercise and resting VVC ratio.

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

In vivo myocardial energetics were measured using a MRS at 3-Tesla Phillips Achieva 3T scanner, as previously validated [250]. A Java magnetic resonance user interface v3.0 (jMRUI) was used for analysis [251]. PCr and γ-ATP peaks were used to determine the PCr/γATP ratio which is a measure of the cardiac energetic state. Data were analyzed by an investigator who was blinded to the participants' clinical status. Cramér–Rao ratios were used to assess signal: noise ratio [250]. Example of HCM ^{31}P cardiac spectra is shown in Figure 29.

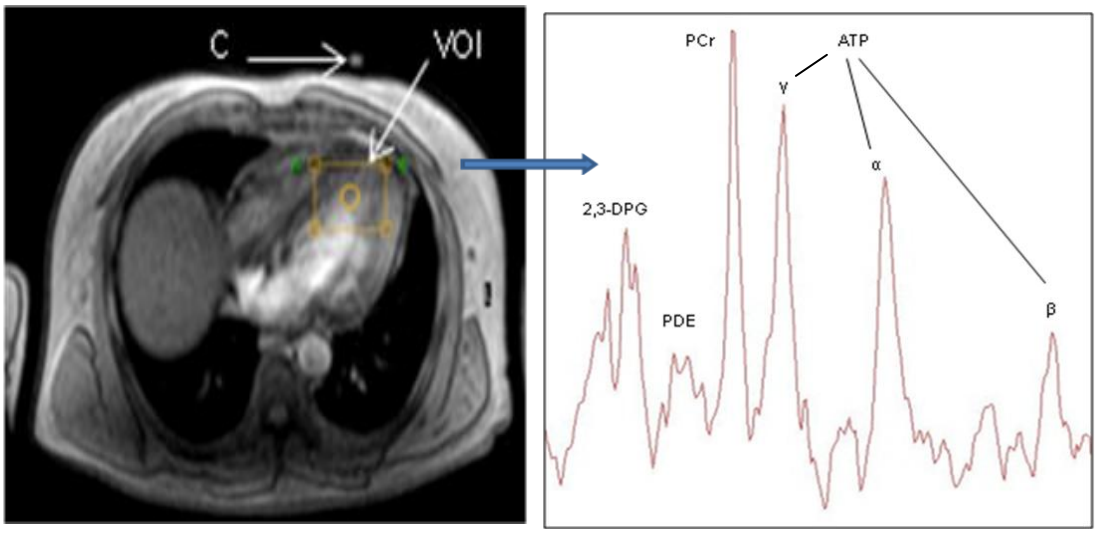

Figure 29: An example of ^{31}P cardiac spectra of a HCM patient.

C indicates centre of phosphorus coil, VOI; voxel of interest, 2, 3-DPG indicates 2, 3-diphosphoglycerate; PDE, phosphodiesters; PCr, phosphocreatine; α, β, γ indicate the three phosphorus nuclei of ATP.

Statistics

Data were analyzed using SPSS ver. 15.0 for Window and Microsoft Office Excel 2007, and expressed as mean ± standard deviation (SD). Comparisons of variables were determined by Student's t-test (2-tail) if variables were normally distributed and the Mann-Whitney U-test if the data were non-normally distributed. Categorical variables were compared with the Pearson chi-square test. A difference of $p < 0.05$ was taken to indicate statistical significance. Pearson's correlation coefficient was used to describe the relationship between variables**.** Another analysis was performed after excluding HCM patients who were on beta blockers therapy.

Results

The clinical characteristics and cardiopulmonary exercise test results of HCM patients and controls are shown in Table 13. Both groups were well matched with respect to age and gender. HCM patients exhibited marked exercise limitation as compared to controls whereas mean EF was comparable in both groups. Heart rate was lower in the HCM group, as compared to controls, probably due to rate limiting medication (beta blockers or/and calcium channel blockers).

Conventional Echocardiographic Measurements

Standard, Doppler and PW-TDI echocardiographic findings of HCM and controls groups are listed in Table 14. Transmitral Doppler measurements showed no significant difference in peak E and A, and E/A ratio in HCM versus controls (Table 14). However, PW-TDI measurements of Sm, Em and Am of the inferoseptal and

anterolateral basal segments were significantly lower in HCM patients compared to controls (Table 14).

Parameter	*HCM*	*Controls*	*P value*
Age [years]	**54 ± 12**	**52 ± 15**	**0.4**
Number (Male)	**51 (35)**	**33 (20)**	**0.64**
Heart Rate [bpm]	**69 ± 14**	**82 ± 16**	**< 0.001***
Systolic BP [mm Hg]	**126 ± 21**	**126± 21**	**0.91**
Diastolic BP [mm Hg]	**75 ± 12**	**78 ± 11**	**0.3**
Peak Vo_2 [ml/kg/min]	**24 ± 6**	**38 ± 8**	**< 0.0001***
% Predicted peak Vo2 [%]	**66 ± 14**	**101 ± 18**	**< 0.0001***
Drug therapy – no. (%)			
Diuretic	**10 (20)**	**0**	**-**
ACE inhibitor	**6 (12)**	**0**	**-**
ARB	**1 (2)**	**0**	**-**
Beta-blocker	**20 (39)**	**0**	**-**
Calcium channel blocker	**26 (51)**	**0**	**-**
Aspirin	**13 (25)**	**0**	**-**
Warfarin	**5 (10)**	**0**	**-**
Nitrate	**2 (4)**	**0**	**-**
Statin	**15 (29)**	**0**	**-**

Table 13: The clinical characteristics of HCM patients and controls – Correlation study.

Parameter	*HCM*	*Controls*	*P value*
EF Biplane (%)	**65 ± 9**	**63 ± 6**	**0.2**
Biplane LA Volume (ml)	**78 ± 30**	**39 ± 20**	**< 0.0001***
LA Volume Index (ml/m^2)	**37 ± 17**	**15 ± 13**	**< 0.0001***
MV E (m/s)	**0.70 ± 0.17**	**0.66 ± 0.15**	**0.34**
MV A (m/s)	**0.67 ± 0.24**	**0.59 ± 0.14**	**0.06**
MV E/A ratio	**1.16 ± 0.5**	**1.17 ± 0.38**	**0.89**
MV Dec Time (ms)	**239 ± 76**	**260 ± 70**	**0.27**
Antlat Sm (cm/s)	**0.06 ± 0.02**	**0.09 ± 0.02**	**< 0.0001***
Antlat Em (cm/s)	**0.08 ± 0.03**	**0.10 ± 0.04**	**< 0.0001***
Antlat Am (cm/s)	**0.06 ± 0.03**	**0.10 ± 0.03**	**< 0.0001***
Infsep Sm (cm/s)	**0.06 ± 0.02**	**0.08 ± 0.02**	**0.0001***
Infsep Em (cm/s)	**0.05 ± 0.02**	**0.07± 0.03**	**< 0.0001***
Infsep Am (cm/s)	**0.07 ± 0.02**	**0.09 ± 0.02**	**0.0001***
Average Sm (cm/s)	**0.06 ± 0.02**	**0.08 ± 0.02**	**< 0.0001***
Average Em (cm/s)	**0.06 ± 0.02**	**0.09 ± 0.04**	**< 0.001***
E/Em $_{antlat}$	**9.84 ± 4.09**	**6.94 ± 2.28**	**0.001***
E/Em $_{infsep}$	**17.28 ± 7.32**	**9.55 ± 3.79**	**< 0.0001***
E/Em $_{average}$	**11.93 ± 4.39**	**8.03 ± 3.28**	**< 0.0001***

Table 14: Standard, Doppler and PW-TDI Echocardiographic characteristics of HCM vs controls - Correlation study.

In vivo Cardiac Energetics

At rest, cardiac PCr/γATP ratio in all HCM patients and excluding those on beta blockers were significantly reduced compared to controls (1.41 ± 0.48 vs 2.26 ± 0.59, $p < 0.0001$ and 1.35 ± 0.48 vs 2.26 ± 0.59, $p < 0.0001$ respectively) (Figure 30).

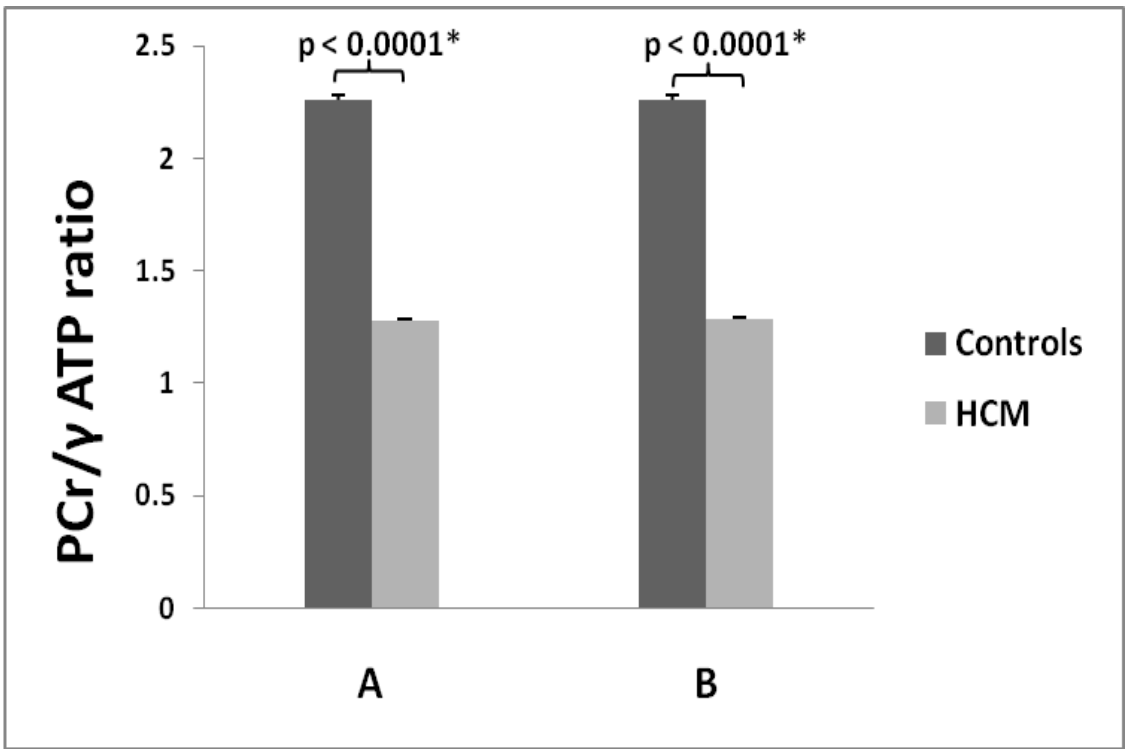

Figure 30: Cardiac PCr/γATP ratio in HCM patients and controls.

A represents all HCM patients and all controls whereas B represents HCM patients not on beta blockers and all controls. Both HCM groups exhibit significant reduction in cardiac energetics compared to controls.

Radionulcide Diastolic Indices

At rest, nTTPF was similar in HCM patients and controls (0.17 ± 0.07 sec vs 0.19 ± 0.09 sec) (p = 0.38). During submaximal exercise (at a workload that achieved 50% of heart rate reserve) it shortened in controls (from 0.19 ± 0.09 sec to 0.16 ± 0.08 sec), but lengthened in patients (from 0.17 ± 0.07 sec to 0.32 ± 0.09 sec) p < 0.0001 and this was similar after excluding HCM patients on beta blockers (Figure 31).

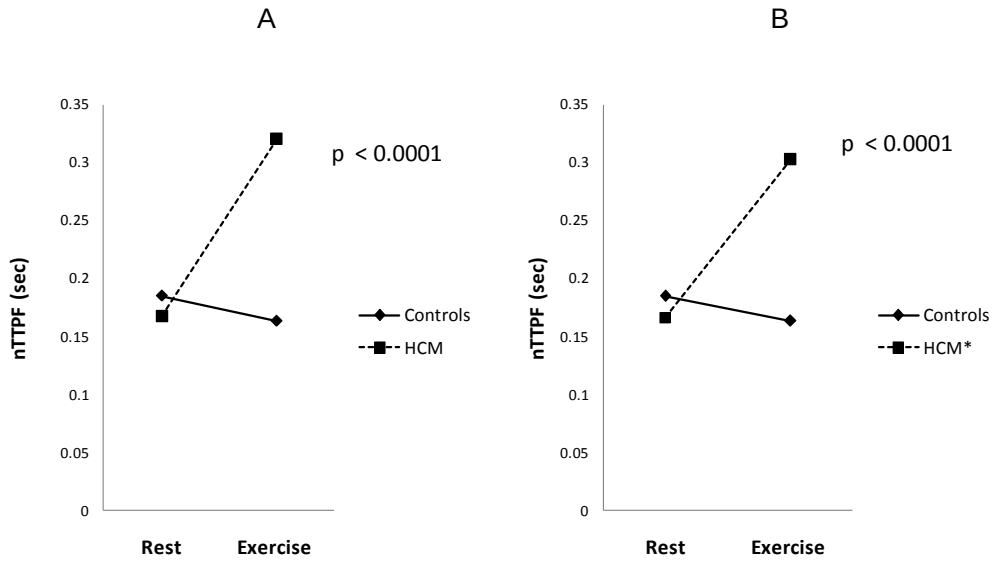

Figure 31: nTTPF at rest and during exercise (50% workloads of heart rate reserve).

A-represents comparison in nTTPF between all HCM patients and controls whereas B- represents comparison, between HCM* patients (excluding patients taking beta blockers) and controls. During exercise, nTTPF had shortened in controls but lengthened in HCM patients. There were similar results in both groups.

Vasculo-Ventricular Coupling

Consistent with a previous study [249], VVC ratio decreased significantly on exercise in healthy control subjects (0.60 ± 0.21 vs 0.41 ± 0.15, p = 0.003). However, there was a blunt response in HCM patients as with VVC essentially unchanged during exercise (0.55 ± 0.35 vs 0.46 ± 0.24, p = 0.28.) δVCC Ratio decreased significantly in the controls group as compared to the HCM group (Figure 32).

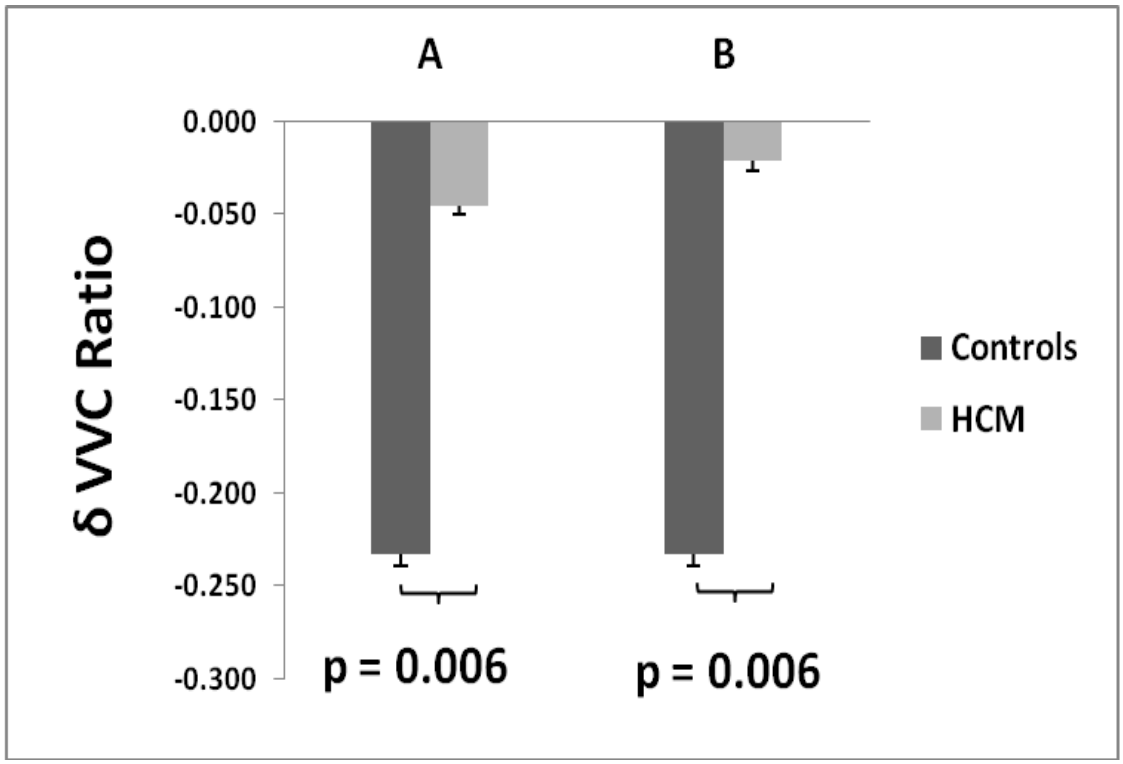

Figure 32: δVCC Ratio in HCM patients and controls.

A-represents comparison in δVCC between all HCM patients and controls whereas B- represents comparison, between HCM* patients (excluding patients taking beta blockers) and controls. There was a significant change in the control group whereas the HCM group showed a blunt response in VVC ratio. These results remained unchanged statistically even after excluding HCM patients on B-Blockers (panel B).

The difference in VVC responses was principally due to a significantly greater increase in LV contractility (relative Δ $E_{LV}I$) in controls during exercise compared to HCM patients (Figure 33). There was no significant difference in the change in arterial elastance during exercise (relative Δ Eal) between the two groups (Figure 34). These results remained unchanged statistically even after excluding HCM patients on B-Blockers.

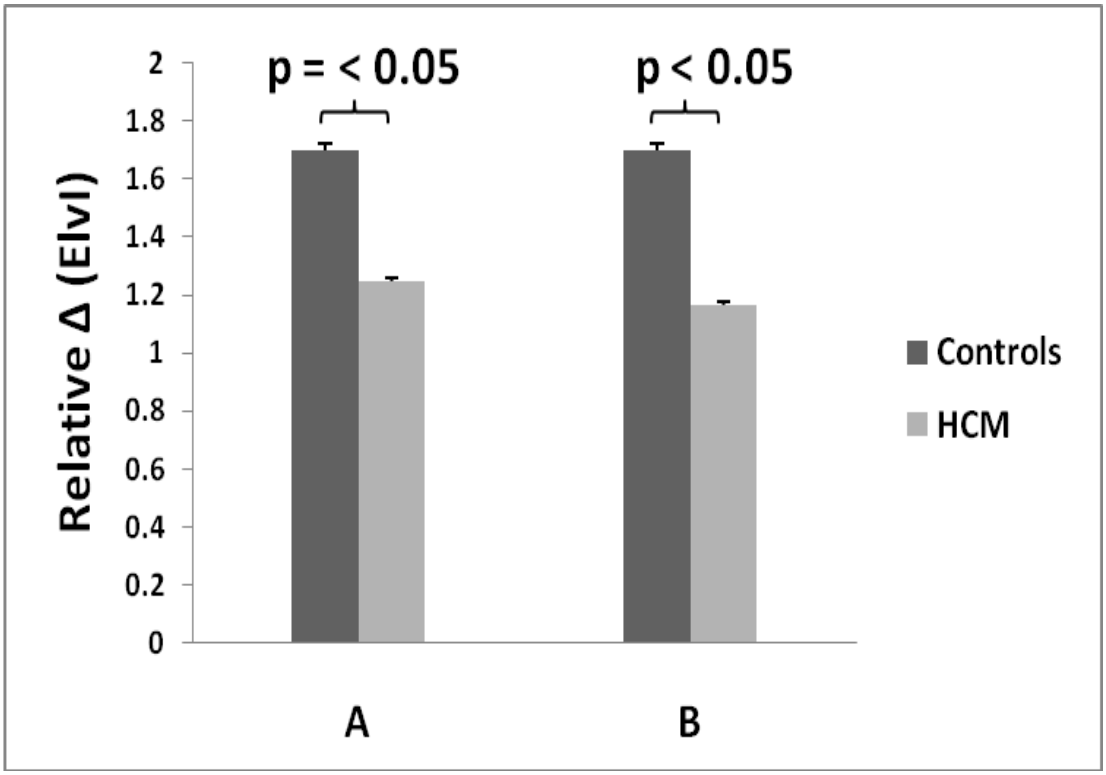

Figure 33: Relative Δ ($E_{LV}I$) in HCM patients and controls.

A-represents comparison in relative Δ ($E_{LV}I$) between all HCM patients and controls whereas B-represents comparison, between HCM patients (excluding patients taking beta blockers) and controls. Relative Δ $E_{LV}I$ = $E_{LV}I$ exercise / $E_{LV}I$ rest. There was a significant increase in the control group whereas the HCM group showed a blunt response in relative Δ ($E_{LV}I$). These results remained unchanged statistically even after excluding HCM patients on B-Blockers (panel B).

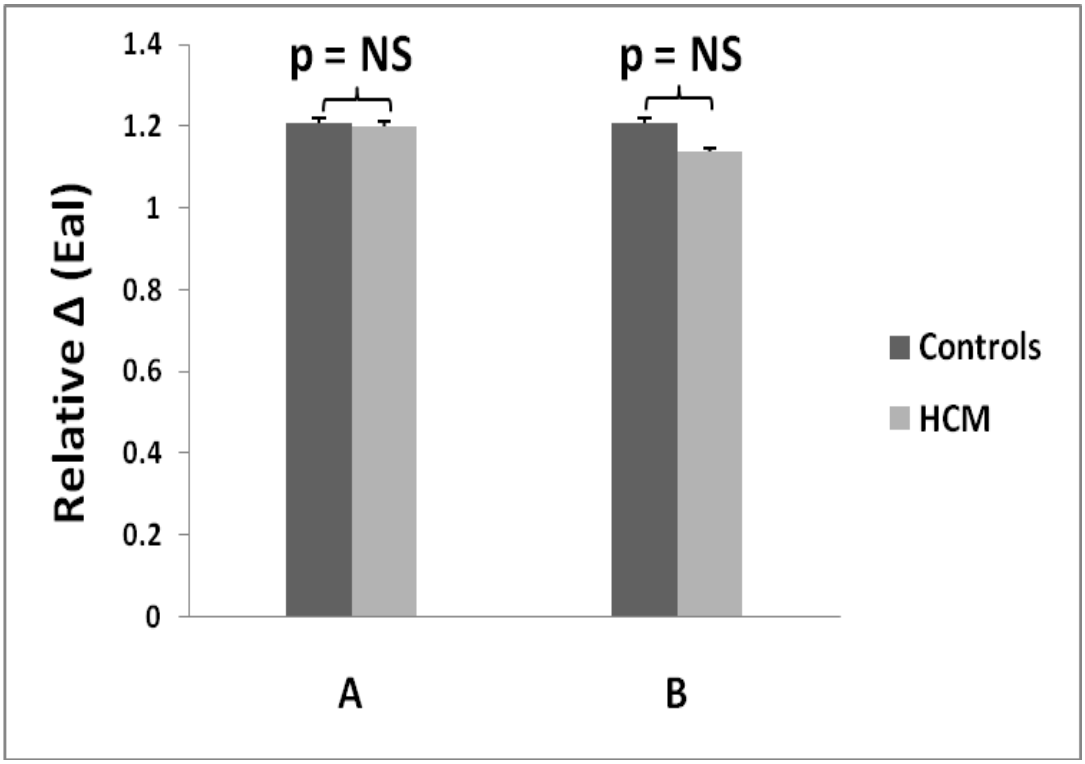

Figure 34: Relative Δ (EaI) in HCM patients and controls.

A-represents comparison in relative Δ (EaI) between all HCM patients and controls whereas B-represents comparison, between HCM patients (excluding patients taking beta blockers) and controls. Relative Δ EaI = EaI exercise / EaI rest. There was no significant difference in the change in arterial elastance during exercise (relative Δ Eal) between the two groups. These results remained unchanged statistically even after excluding HCM patients on B-Blockers (panel B).

Relationship to *in vivo* cardiac energetics

PCr/γATP ratio correlated inversely with nTTPF during exercise (r = - 0.48, p = 0.003) and with VVC ratio (r = - 0.4, p = 0.02), but positively with peak V_{O2} (r = 0.52, p < 0.001), (Figure 35). These correlations remained significant even after excluding patients on beta blockers (Figure 36).

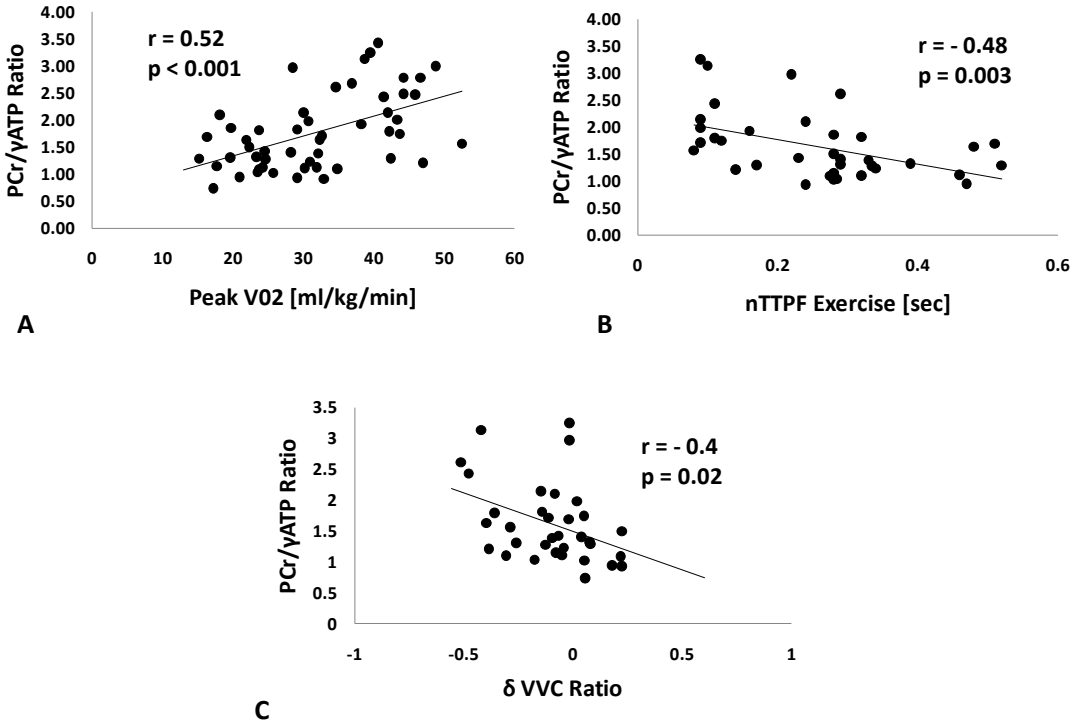

Figure 35: Relationship to *in vivo* cardiac energetics.

Panel A: Cardiac PCr/γATP ratio correlated positively with exercise capacity (peak V_{O2}). Panel B: Cardiac PCr/γATP ratio correlated negatively with nTTPF (exercise). Panel C: Cardiac PCr/γATP ratio correlated negatively with δ VVC ratio.

Discussion

The purpose of this study was to test the hypothesis that impaired cardiac eneregtic status is associated with the previously described dynamic disturbance of LV active relaxation occurring on exercise in patients with HCM, and also to assess whether there is also a dynamic impairment of contractile function on exercise. The principal findings are: a) Consistent with previous studies, HCM patients demonstrate reduced resting myocardial energetic status [32, 34, 252] and a paradoxical slowing of LV relaxation on

exercise [15]. b) Whereas in healthy controls LV contractile status increased upon exercise resulting in a marked reduction in the vasculo-ventricular coupling ratio, LV contractile status was not augmented in HCM and vasculo-ventricular coupling ratio was unchanged c) Cardiac energetic status correlated positively with exercise capacity and d) was inversely correlated with nTTPF during submaximal exercise (a measure of the rate of LV active relaxation) and with δVVC ratio.

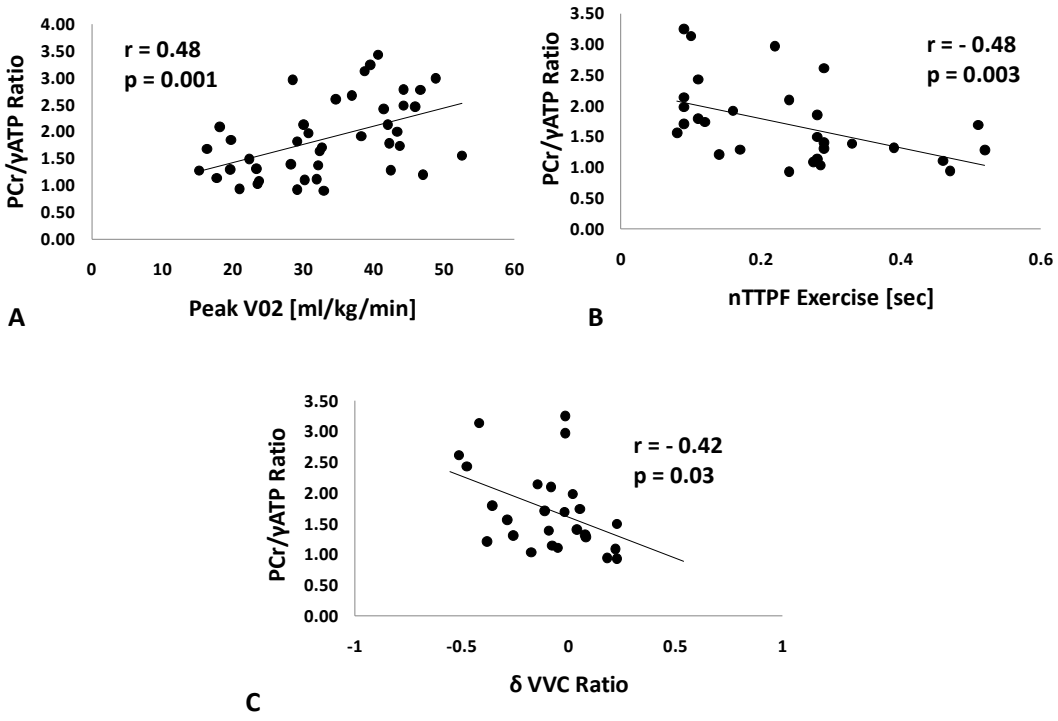

Figure 36: Relationship to *in vivo* cardiac energetics (excluding HCM patients on beta blockers).

Panel A: Cardiac PCr/γATP ratio correlated positively with exercise capacity (peak V_{O2}). Panel B: Cardiac PCr/γATP ratio correlated negatively with nTTPF (exercise). Panel C: Cardiac PCr/γATP ratio correlated negatively with δ VVC ratio. These correlations remained significant after excluding patients on beta blockers and were similar to the results in the whole HCM group shown in Figure 35.

The pathophysiology of HCM is complex. A substantial proportion of these patients complain of breathlessness and/or fatigue and most have reduced exercise capacity [15]. These patients typically exhibit impaired LV active relaxation at rest and/or during exercise. This has led many to conclude that exercise limitation is primarily a result of impaired LV diastolic filling. Somewhat surprisingly our data show no significant difference in nTTPR at rest between HCM and controls. This could be at least in part because the mean age of our population of patients (and in consequence controls) is rather older than previous studied populations [15, 253, 254]. Nevertheless, on exercise patients and controls behaved very differently, with nTTPF shortening in healthy controls during submaximal exercise but lengthening in patients, consistent with our previous study [15]. The change in nTTPF on exercise was inversely related to the resting cardiac PCr/ATP ratio. This parameter is a measure of cardiac energy 'reserves'. In both HCM and heart failure PCr is depleted to a greater extent than ATP resulting in a fall in the ratio of PCr/ATP [34, 86]. PCr represents a rapid source of ATP under conditions of stress such as exercise. The rate at which ATP can be regenerated from PCr is approximately 10 fold faster than the rate of *De Novo* synthesis of ATP. The presence of a reduced resting PCr/ATP ratio therefore indicates a reduction in energy 'reserves' likely to be exacerbated during exercise. Because of the duration of acquisition required to obtain MRS spectra it was not however possible to measure PCr/ATP ratio on exercise.

Patients with flow limiting coronary disease and diabetes were excluded to avoid the confounding influence of impaired cardiac energetic status associated with these disorders [255, 256]. MRS and Radionuclide studies also require a regular rhythm, thus

patients with atrial fibrillation were excluded from the study. In addition, we excluded patients with significant LVOT obstruction (gradients > 30 mmHg) which will significantly disrupt the acquisition of radionuclide indices of diastolic filling. A proportion of patients were on β-blockers which may have affected their cardiovascular response to exercise, however, all heart rate dependent parameters were corrected. Furthermore, the level of significance remained similar when these patients on β-blockers were excluded from the analysis.

In summary, in the present study, exercise led to paradoxical slowing of the rate of active relaxation of the LV compared to a shortening in controls. Furthermore, there was a failure of the normal increase in contractility during exercise. Resting cardiac energetic status was substantially reduced in HCM patients, which is supported by other studies [32, 34]. We propose that energetic impairment may underlie these dynamic changes in active relaxation and contractility and contribute significantly to exercise limitation. These findings may indicate the potential for therapeutic benefit from 'metabolic agents' that improve myocardial energetic status by altering cardiac substrate use [257]. These agents have shown promise in ischaemic heart disease [67, 114, 258-260]and in heart failure [261, 262].

CHAPTER 8: METABOLIC MODULATION WITH PERHEXILINE CORRECTS ENERGY DEFICIENCY AND IMPROVES EXERCISE CAPACITY IN SYMPTOMATIC HCM

Abstract

Background Patients with HCM exhibit myocardial energetic impairment, but a causative role for this energy deficiency in the pathogenesis of HCM remains unproven. We hypothesized that the metabolic modulator, perhexiline would ameliorate myocardial energy deficiency and thereby improve diastolic function and exercise capacity.

Methods Forty six consecutive patients with symptomatic exercise limitation (peak oxygen consumption - peak V_{O2} <75% of predicted) due to non-obstructive HCM (mean age 55 ± 0.26 years old) were randomized to perhexiline (n=24) or placebo (n=22). Myocardial energetics (PCr/γATP ratio) measured by ^{31}P magnetic resonance spectroscopy, left ventricular diastolic filling [heart rate normalized time to peak filling nTTPF] at rest and during exercise using radionuclide ventriculography, peak V_{O2}, symptoms and QOL were assessed at baseline and at the end of the study (4.6 ± 1.8 months).

Results Perhexiline improved the PCr/γATP ratio (from 1.27 ± 0.02 to 1.73 ± 0.02; p = 0.003) and normalised the abnormal prolongation of nTTPF between rest and exercise (δnTTPF – 0.01 ± 0.005 vs + 0.11 ± 0.008 sec; p = 0.03). These changes were accompanied by improvement in peak V_{O2} (22.2 ± 0.2 to 24.29 ± 0.2 ml/kg/min; p = 0.003) and symptoms (NYHA class by 0.8 units p <0.001) (all p values ANCOVA).

Conclusions Modulation of myocardial metabolism with perhexiline in patients with HCM ameliorates cardiac energetic impairment, corrects diastolic dysfunction and

increases exercise capacity. This study supports the hypothesis that energy deficiency contributes to the pathogenesis and provides a rationale for further consideration of metabolic therapies in HCM.

Introduction

HCM is the commonest inherited cardiac condition (prevalence ~0.2%).[4] Symptoms arising from left ventricular outflow tract obstruction (LVOTO), are present in ~30% of HCM patients, are amenable to drug therapies and to interventions such as surgical septal myectomy or alcohol septal ablation [263]. However, treatment options in symptomatic patients without obstruction are less successful, mandating a better understanding of the mechanisms underlying symptoms in HCM with the intention of identifying and testing novel therapies [264, 265].

HCM is a disease of the sarcomere, with >400 mutations having been identified in genes encoding cardiac contractile proteins [2]. HCM-causing mutations increase sarcomeric Ca2+ sensitivity, ATPase activity and the energetic cost of myocyte contraction [266-269]. These abnormalities led to the proposal that the pathophysiology of HCM is attributable, at least in part, to excessive sarcomeric energy use [270]. The resulting hypertrophy of HCM, accompanied by significant microvascular dysfunction, [271] may limit myocyte oxygen delivery and further exacerbate the primary energy deficiency [235]. Consistent with a functional role for the observed energy deficiency of HCM [32, 34], LV relaxation (a highly energy requiring process), paradoxically slows on exercise and is correlated with exercise limitation [15]. To assess a causative role for energy deficiency and to test a potentially novel therapeutic strategy for HCM, we evaluated whether perhexiline, an agent thought to improve cardiac energetics by shifting myocardial substrate utilization to more efficient carbohydrate metabolism [66, 99, 272, 273], would increase exercise capacity by improving cardiac energetics and diastolic filling.

Methods

Study Design

The study was approved by the South Birmingham Research Ethics Committee and conforms to the principles outlined in the Declaration of Helsinki. All participants provided written informed consent. The study was a randomized, double blind, placebo-controlled parallel-group design of minimum 3 months duration (Figure 37). The pre-defined primary end point was peak oxygen consumption (peak V_{O2}) and secondary end points were symptomatic status, resting myocardial energetics (PCr/γATP ratio) and diastolic function at rest and during exercise (nTTPF). Thirty three controls of similar age and gender distribution were recruited for comparison of baseline data. Controls were asymptomatic and had normal ECGs and echocardiograms.

Patient selection

Patients were consecutively recruited from cardiomyopathy clinics at the Heart Hospital, University College London Hospitals, London and Queen Elizabeth Hospital, Birmingham, UK between 2006 and 2008.

Inclusion criteria:

- Age 18 to 80 years.
- Exertional symptoms.
- Sinus rhythm.
- Peak V_{O2} < 75% of predicted for age and gender.
- Absence of LVOT obstruction at rest (peak gradient < 30mmHg).

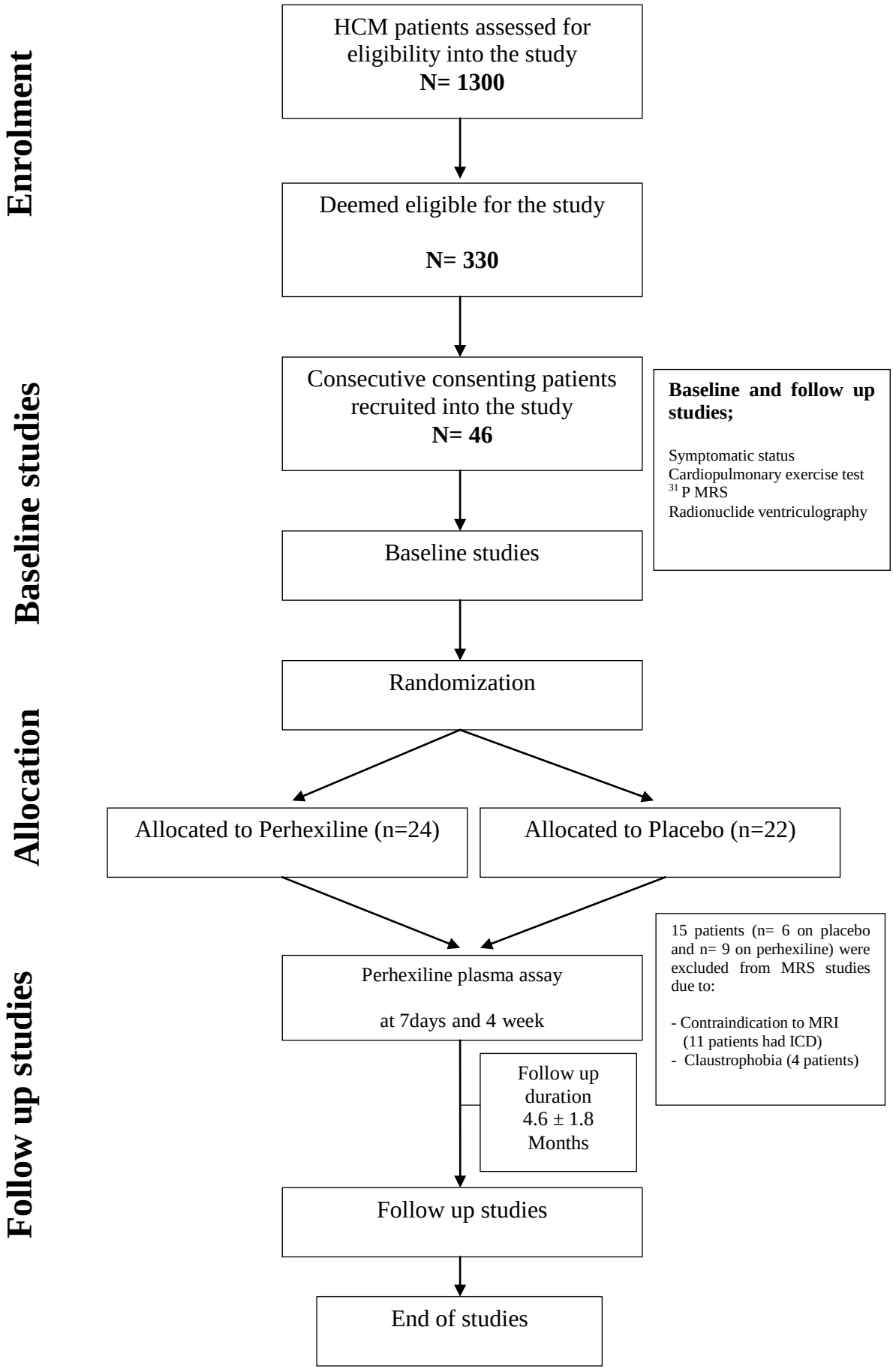

Figure 37: Perhexiline Trial profile.

Exclusion criteria:

- Presence of epicardial coronary artery disease.
- Abnormal liver function tests.
- Concomitant use of amiodarone or selective serotonin reuptake inhibitors (due to potential drug interactions with perhexiline).
- Peripheral neuropathy.
- Women of childbearing potential.
- Diabetic patients were also excluded to maintain the blinding of the study as perhexiline may lead to a reduction in plasma glucose necessitating a reduction in anti-diabetic therapy.

Controls selection

Inclusion criteria:

- Normal ECG.
- Normal Echocardiogram (LVEF $\geq$ 55%).

Exclusion criteria:

- History or symptoms of any medical illness.

Cardiopulmonary Exercise Test

All study participants underwent symptom-limited erect treadmill exercise testing (Schiller CS-200 Ergo-Spiro exercise machine) using a standard ramp protocol with simultaneous respiratory gas analysis [158, 159]. Peak oxygen consumption (peak V_{O2}) was

defined as the highest peak V_{O2} achieved during exercise (with RER >1) expressed in ml/min/kg.

Symptomatic status assessment

Symptom status was determined by a single investigator (KA) using the NYHA functional classification. All patients completed a MLHF questionnaire at baseline and at the end of the treatment phase.

Transthoracic Echocardiography

Echocardiography was performed with participants in the left lateral decubitus position with a Vivid 7 echocardiographic machine (GE Healthcare) and a 2.5-MHz transducer. LVEF was derived from a modified Simpson's formula. LA volume index and MWT were measured. Pulse and continuous wave Doppler were used to assess resting LVOTO gradient.

Radionuclide Ventriculography

Diastolic filling was assessed by equilibrium R-wave gated blood pool scintigraphy at rest and during graded semi-erect exercise on a cycle ergometer as previously described [221, 247]. Peak left ventricular filling (end-diastolic count per second (EDC/s)) and time to peak filling normalised for R-R interval (nTTPF), an indirect measure of the rate of LV active relaxation were assessed at rest and during exercise (50% of heart rate reserve). The validity of these radionuclide measures of diastolic filling at high heart rates has been established previously [221, 247].

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

In vivo myocardial energetics were measured using a MRS at 3-Tesla Phillips Achieva 3T scanner, as previously validated [250]. A Java magnetic resonance user interface v3.0 (jMRUI) was used for analysis [251]. PCr and γ-ATP peaks were used to determine the PCr/γATP ratio which is a measure of the cardiac energetic state. Data were analyzed by an investigator who was blinded to the participants' clinical status. Cramér–Rao ratios were used to assess signal: noise ratio [250].

Intervention

Following baseline studies, patients were randomized in a double-blind fashion to receive either perhexiline 100 mg OD (n = 24) or placebo (n = 22). Serum perhexiline levels were obtained at 1 and 4 weeks after initiation of the drug. Dose adjustments were advised by an unblinded physician according to serum level to achieve therapeutic level (therapeutic range 0.15-0.6 mg/l) and to avoid drug toxicity (Table 15 & Table 16). Identical dosage adjustments were also made for randomly allocated placebo-treated patients by the unblinded observer to ensure that blinding of the investigators was maintained.

Statistical Analysis

Data were analyzed using SPSS version 15.0 for Window and Microsoft Office Excel 2007, and expressed as Mean ± Standard Error of Mean (SEM). Comparison of continuous variables between perhexiline and placebo baseline data were determined by unpaired Student's t-test (2-tail) if variables were normally distributed and the Mann-Whitney U-test if the data were non-normally distributed.

Perhexiline concentration (mg/L)	Recommended new daily dose (mgs)
0.00-0.05	300
0.05-0.15	250
0.15-1.00	200
1.00-1.50	100
1.50-2.00	50
>2.00	Cease for 1 week then 50 mg on alternative days

Table 15: Dose planning based on perhexiline assays result on day 7

Perhexiline concentration(mg/L)	Recommended new daily dose (mgs)
<0.15	Double the daily dose
0.15-0.60	No change
0.60-0.90	Reduced dose by 25%
0.90-1.20	Halve the daily dose
>1.20	Cease for 1 week then reduce the daily dose 25% of the previous dose

Table 16: Dose planning based on perhexiline assays result at ≥ 4 weeks.

ANCOVA with baseline values as covariates was performed to test for the significance of differences in the perhexiline versus placebo group after treatment. A p value of 0.05 was taken to indicate statistical significance. For the primary end point, forty four patients are required to detect a change in peak V_{O2} of 3 ml/kg/min in the treatment versus the placebo group with a power of 90% and a p value of <0.05. Thirty patients are required to identify a 5 % change in cardiac PCr/γATP ratio with a power of 90% and a p value of <0.05. Forty patients are required to detect a change ≥25 % in nTTPF with power of 0.99 with probability of 5%.

Results

Baseline data (HCM versus Controls)

The clinical characteristics and cardiopulmonary exercise test results of the HCM patients and controls are shown in Table 17. The groups were well matched with respect to age and gender. Heart rate was lower in the HCM group compared to controls probably due to medications used (beta blockers and/or calcium channel blockers).

Resting cardiac PCr/γATP ratio was lower in HCM patients than in controls (1.28 ± 0.01 vs 2.26 ± 0.02, $p < 0.0001$) (Figure 38), and this remained so after excluding patients taking beta blocker therapy ($p < 0.0001$). At rest, nTTPF, a sensitive marker of LV relaxation, was similar in HCM patients and controls (0.17 ± 0.002 vs 0.18 ± 0.003 sec, $p = 0.44$).

Parameter	HCM	Controls	P value	HCM (Perhexiline)	HCM (Placebo)	P value
Age [years]	55 ± 0.26	52 ± 0.46	0.2	56 ± 0.46	54 ± 0.64	0.42
Number (Male)	46 (34)	33 (20)	0.64	24 (19)	22 (17)	0.69
Heart Rate [bpm]	69 ± 0.27	82 ± 0.47	< 0.001*	69 ± 0.53	69 ± 0.52	0.97
Systolic BP [mm Hg]	126 ± 0.64	126± 0.44	0.93	123 ± 0.84	130± 0.92	0.2
Diastolic BP [mm Hg]	76 ± 0.25	78 ± 0.34	0.33	74 ± 0.45	78 ± 0.57	0.24
Peak V_{O2} [ml/kg/min]	23 ± 0.12	38 ± 0.24	< 0.0001*	22.2 ± 0.2	23.56 ± 0.27	0.42
Resting nTTPF (sec)	0.17± 0.002	0.18± 0.003	0.44	0.19± 0.003	0.17± 0.004	0.52
PCr/γATP ratio	1.28 ± 0.01	2.26 ± 0.02	< 0.0001*	1.27 ± 0.02	1.29 ± 0.01	0.86
LAV Index [ml/m2]	35.37 ± 0.33	14.68 ± 0.39	< 0.0001*	35.9 ± 0.38	39.45 ± 0.77	0.4
MWT [cm]	2.31 ± 0.01	-	-	2.32 ± 0.02	2.25 ± 0.01	0.58
F H/O HCM	19	0	-	11	8	0.51
F H/O SCD	9	0	-	4	5	0.52
Drug therapy – no.						
Beta-blocker	17	0	-	10	7	0.21
CC-blocker	24	0	-	11	8	0.53
Diuretic	10	0	-	4	5	0.49
ACE inhibitor	6	0	-	3	2	0.84
ARB	4	0	-	3	1	0.41
Warfarin	5	0	-	2	3	0.48
Statin	15	0	-	7	7	0.9

Table 17: The clinical characteristics of HCM patients and controls – Perhexiline trial.

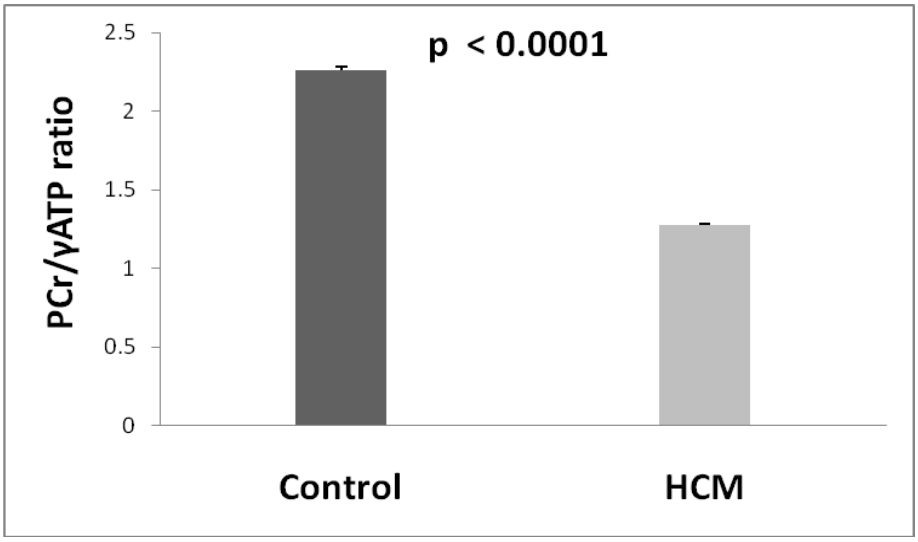

Figure 38: Baseline data of myocardial energetic in HCM vs controls.

During submaximal exercise (at a workload that achieved 50% of heart rate reserve), nTTPF remained relatively constant in controls (from 0.18 ± 0.003 sec to 0.16 ± 0.002 sec, [δnTTPF = - 0.01 ± 0.004 sec]), but lengthened in patients (from 0.17 ± 0.002 to 0.34 ± 0.002 sec, [δnTTPF = + 0.13 ± 0.003 sec]), p < 0.0001, (Figure 39).

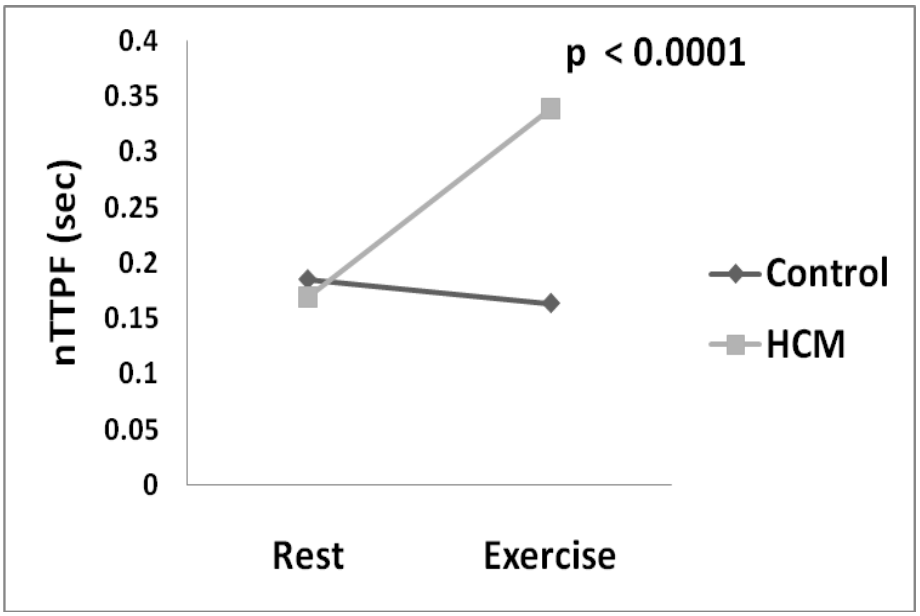

Figure 39: Baseline data of Diastolic ventricular filling in HCM vs controls.

Patients exhibited marked exercise limitation compared to controls (peak oxygen consumption 23 ± 0.12 vs 38 ± 0.24 ml/kg/min, p <0.0001) (Figure 40). This remained so after excluding patients taking beta blockers (p < 0.0001).

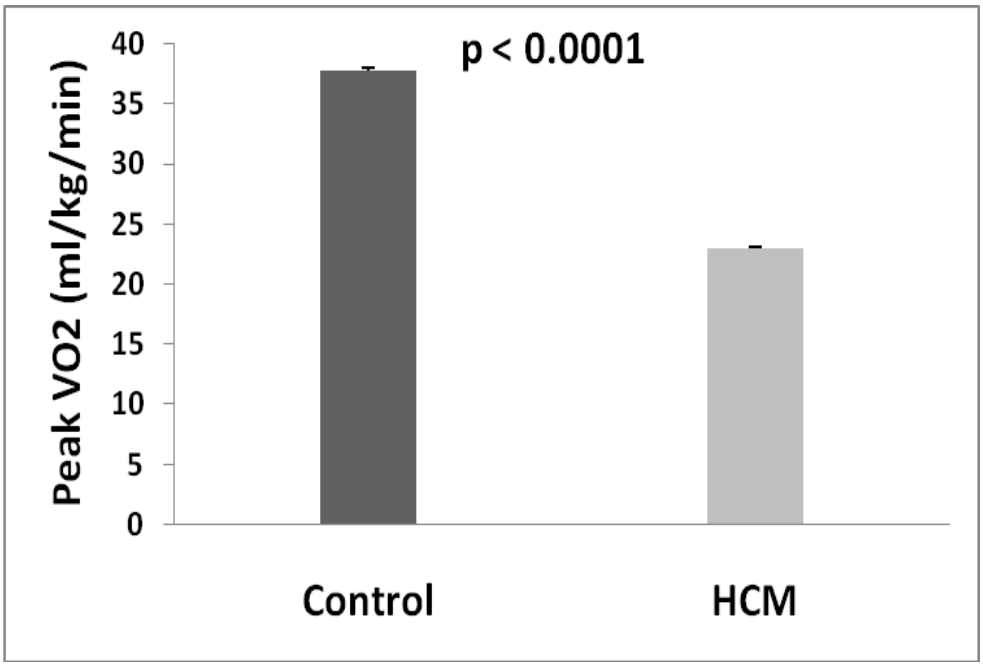

Figure 40: Baseline data of peak oxygen consumption in HCM vs controls.

Randomized, double blind, placebo-controlled parallel-group study

The perhexiline and placebo groups were well matched (Table 17). One patient (on placebo) did not complete the study due to poor compliance. 6 patients were subtherapeutic and none above the therapeutic range at the end of the study.

Side effects were restricted to transient nausea (n = 3) and dizziness (n = 2) in the perhexiline group and transient nausea (n = 2) and headache (n =1) in the placebo group

during the first week of treatment. There were no deaths or major adverse events during the study period.

Myocardial energetics

The mean Cramer-Rao ratios for PCr and ATP for the entire group were 7.5 % and 10.8 % respectively, indicating satisfactory SNR. 3 patients were excluded from the initial analysis due to poor SNR (Cramer Rao ratios >20 %). The PCr/γATP ratio increased with perhexiline (1.27 ± 0.02 to 1.73 ± 0.02) vs placebo (1.29 ± 0.01 to 1.23 ± 0.01), p = 0.003 (Figure 41). The effect of perhexiline on PCr/γATP ratio remained significant with inclusion of the 3 patients with poor SNR (p = 0.02).

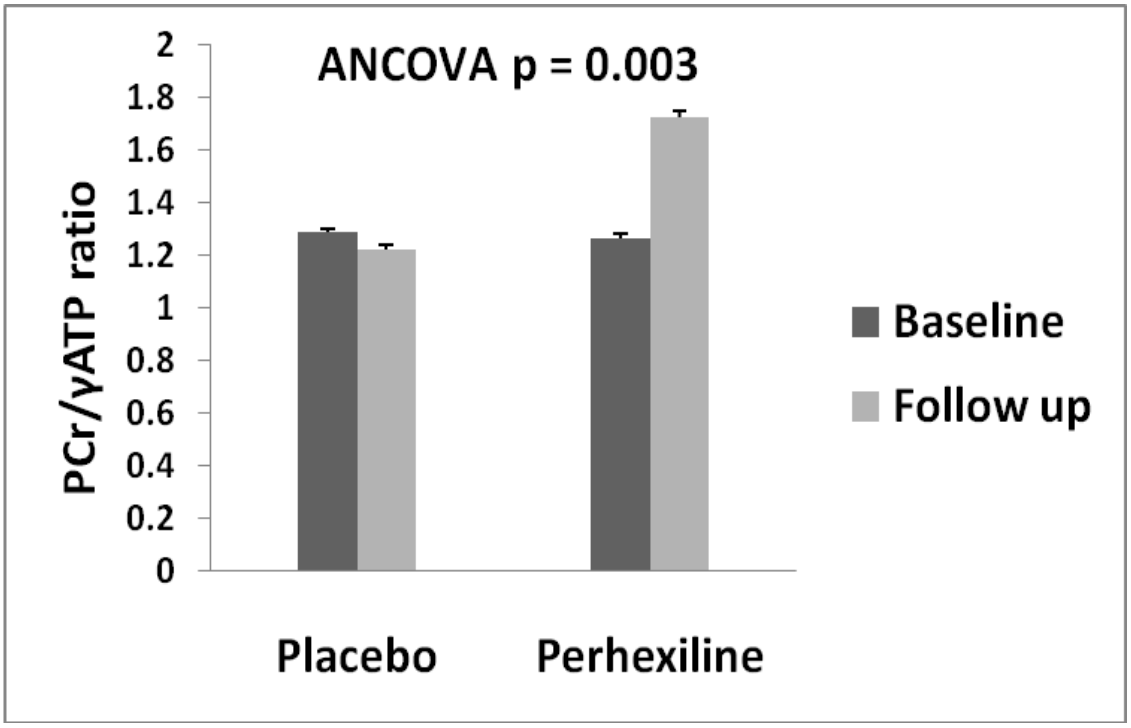

Figure 41: The effects of placebo and perhexiline on myocardial energetic.

Diastolic ventricular filling

Whereas the placebo group showed prolongation of nTTPF during exercise before and after therapy (by 0.15 ± 0.007 and 0.11 ± 0.008 sec respectively), in the perhexiline group nTTPF lengthened at baseline (by 0.11 ± 0.008 sec) but shortened on treatment (by - 0.01 ± 0.005sec) ; p = 0.03 for difference between the perhexiline and placebo response (Figure 43 and Figure 42).

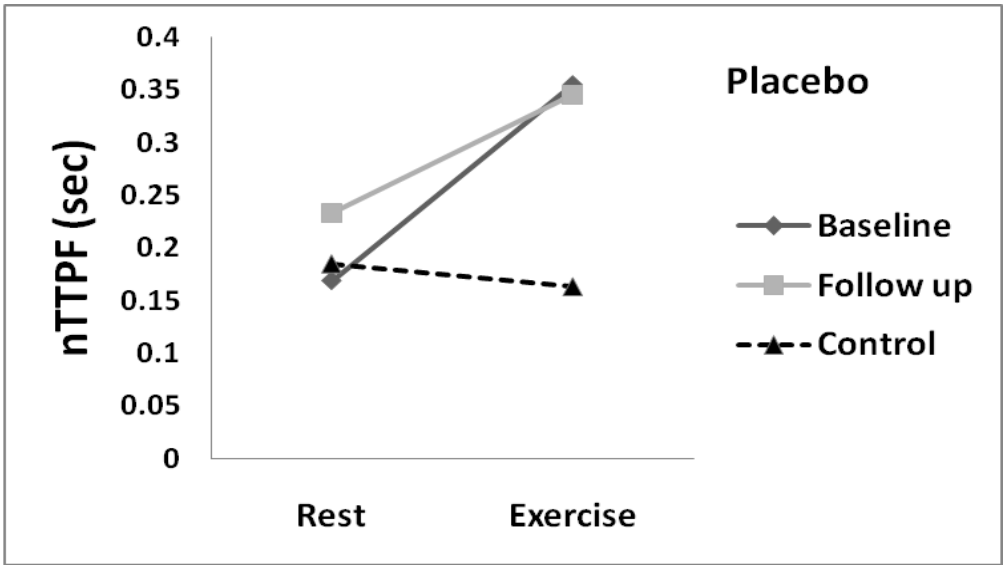

Figure 42: The effects of Placebo on diastolic ventricular filling.
nTTPF changes in healthy controls (dotted lines) are shown for comparison.

Exercise capacity (peak oxygen consumption)

Peak V_{O2} at baseline was similar in the perhexiline and placebo groups (Table 17). After treatment, Peak V_{O2} fell by 1.23 ml/kg/min in the placebo group (from 23.56 ± 0.27 to 22.32 ± 0.27 ml/kg/min), but increased by 2.09 ml/kg/min in the perhexiline group (from 22.2 ± 0.2 to 24.29 ± 0.2 ml/kg/min), p = 0.003 (Fig. 3D).

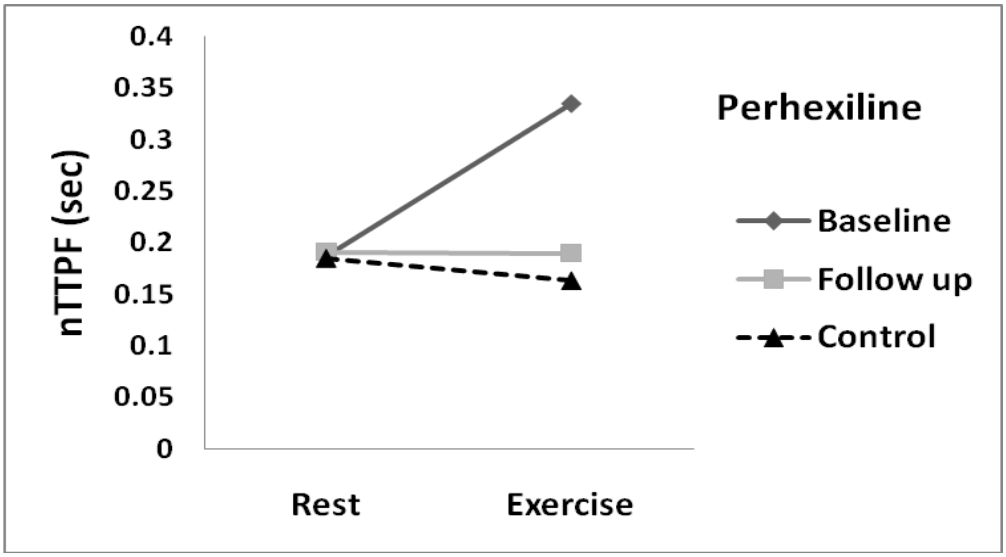

Figure 43: The effects of perhexiline on diastolic ventricular filling.

nTTPF changes in healthy controls (dotted lines) are shown for comparison.

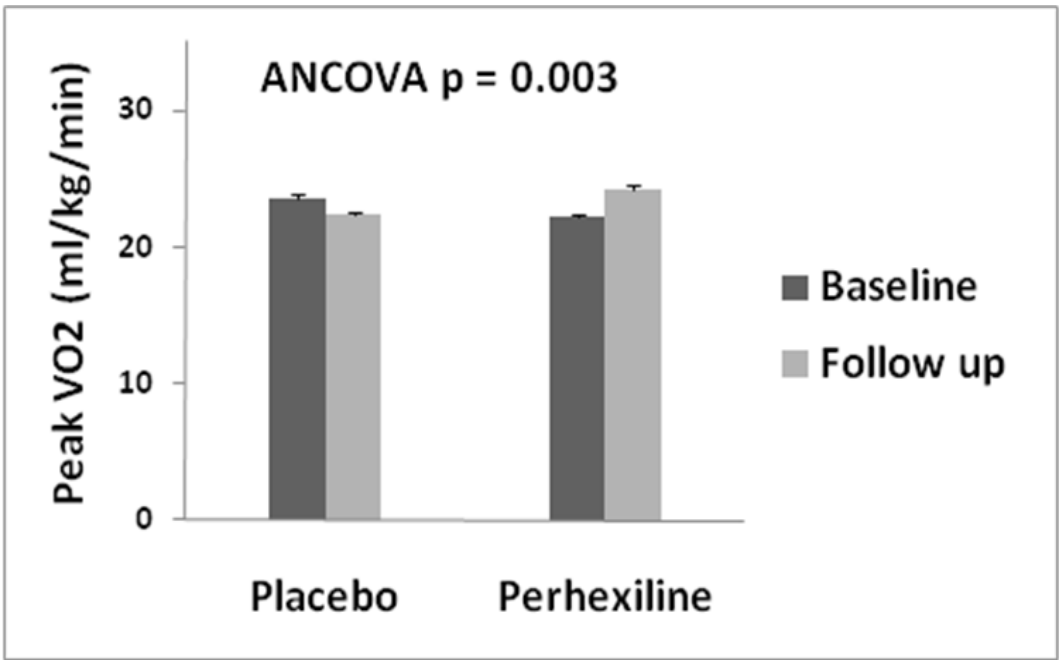

Figure 44: The effects of placebo and perhexiline on exercise capacity.

Symptomatic Status

MLHF questionnaire score showed an improvement in the perhexiline group (from 36.13 ± 0.94 to 28 ± 0.75) but did not change in the placebo group (from 36.86 ± 1.21 to 33.6 ± 1.25 p=0.69), $p < 0.001$ for difference in response between placebo and perhexiline. NYHA score showed an improvement in the perhexiline group (from 2.67 ± 0.02 to 1.92 ± 0.02 $p < 0.001$) but did not change in the placebo group (from 2.52 ± 0.2.04 to 2.45 ± 0.04 p=0.78), $p < 0.001$. More patients in the perhexiline than in the placebo group had improvements in NYHA (67% vs. 30%) and fewer had worsening (8% vs. 20%), $p<0.001$, (Figure 45 and Figure 46).

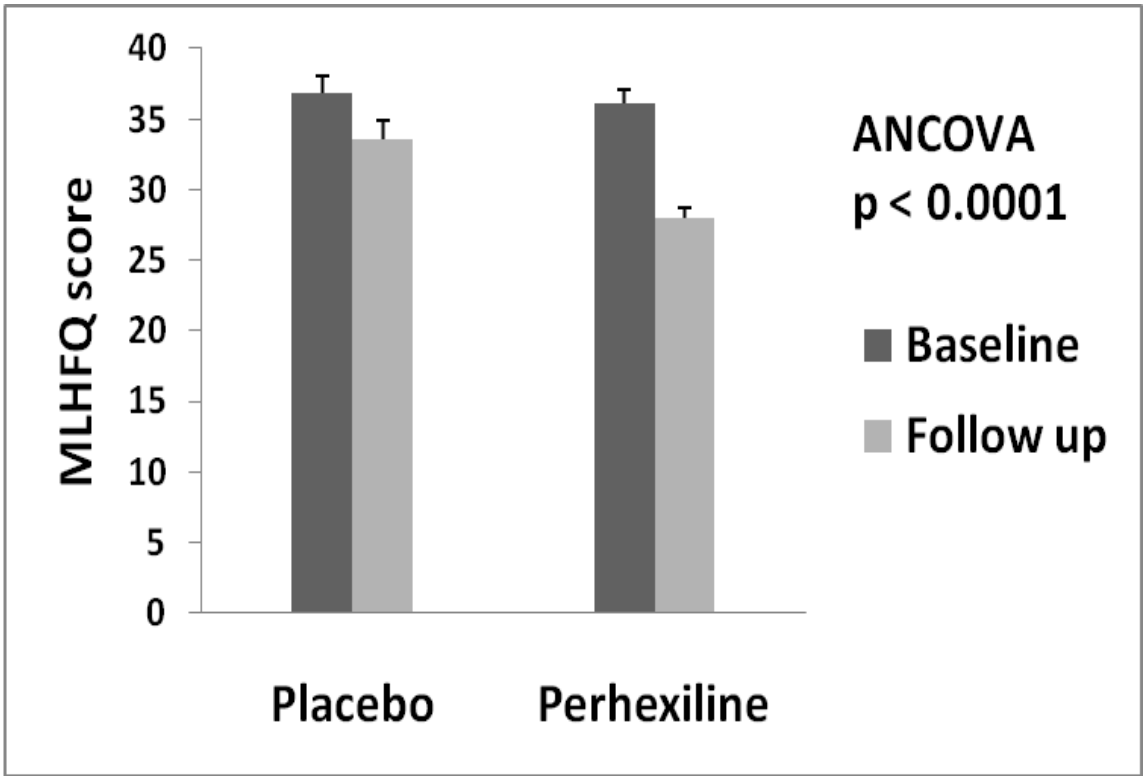

Figure 45: The effects of placebo and perhexiline on MLHF questionnaire score.

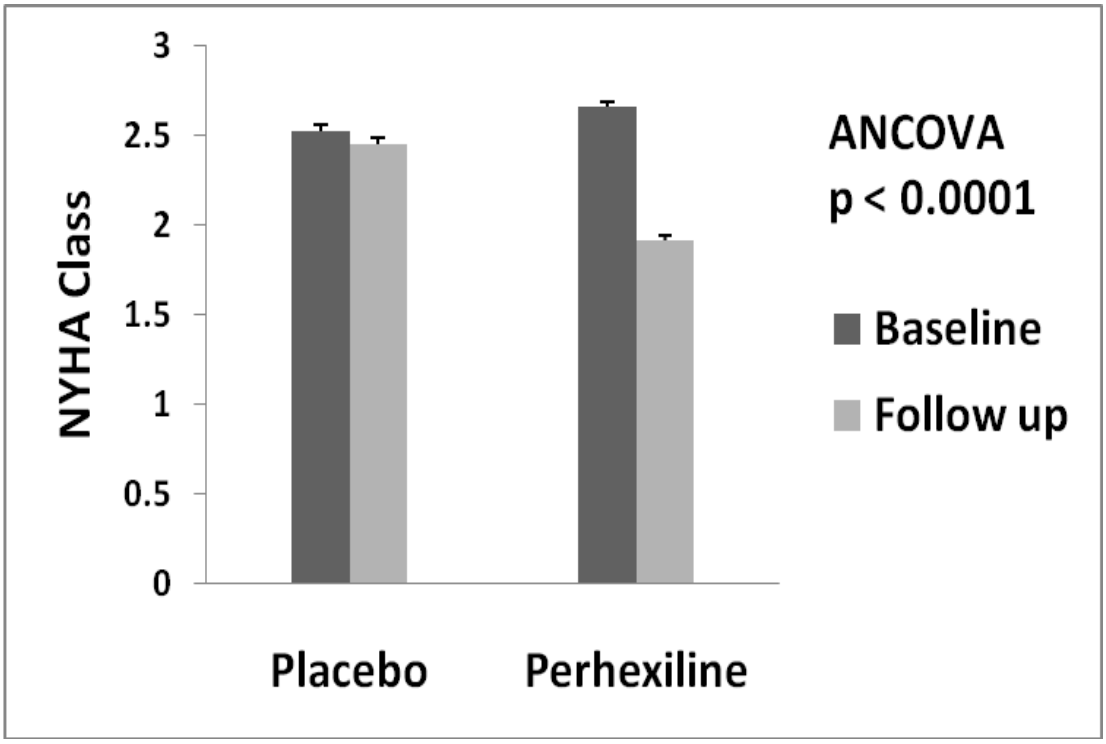

Figure 46: The effects of placebo and perhexiline on NYHA score.

Discussion

This study confirms that patients with symptomatic non-obstructive HCM manifest a cardiac energy defect (reduced PCr/γATP ratio) accompanied by a slowing of the energy-dependent early diastolic LV active relaxation during exercise (prolongation of nTTPF). Consistent with the hypothesis tested, the metabolic modulator perhexiline augmented myocardial energetics, corrected the abnormal prolongation of nTTPF on exercise, improved symptoms and increased the functional capacity (peak V_{O2}).

The observation that symptomatic patients with HCM manifest impaired myocardial energetics (Figure 38) is an expected consequence of the biophysical properties of HCM mutations and accords with previous animal and human studies [32-36]. Thus although the

pathogenesis of HCM has been varyingly attributed to increased sarcomeric Ca2+ sensitivity, ATPase activity, and aberrant cross-bridge dynamics [266, 268] with complications such as oxidative stress [274] and altered sarcomeric phosphorylation [275, 276], a common feature of such studies is the excessive energetic cost of tension generation by sarcomeric mutations [267, 269]. Moreover, the failure to preserve myocardial high-energy phosphates in asymptomatic [34] and pre-hypertrophic HCM [32], is consistent with the proposal that energy deficiency contributes to the pathogenesis of disease [270]. The resulting energy deficiency may contribute to left ventricular hypertrophy in HCM, as seen in other primary disorders of myocardial energy deficiency (e.g. mitochondrial disorders and Friedreich's ataxia) [277].

Since the heart has a very high energy requirement [278], cardiac energy deficiency would be expected to translate into physiological defects such as a slowing of energy-dependent [279] early diastolic relaxation. Thus whilst in control subjects, the nTTPF remained approximately constant between rest and exercise, in HCM nTTPF paradoxically lengthened as heart rate increased (Figure 39). This slower filling in the context of the reduced diastolic filling time reduces cardiac output and limits exercise capacity [15].

Perhexiline inhibits the metabolism of free fatty acids and enhances myocardial carbohydrate utilisation by suppressing carnitine palmitoyl transferase (CPT) I and II, which are crucial for the uptake of long chain fatty acids into mitochondria [38, 99, 272]. While perhexiline's mechanism(s) of action is likely to be complex, its primary action in diverting myocardial metabolism towards carbohydrates, especially in the context of

myocardial oxygen limitation (e.g. due to microvascular dysfunction), [271] is predicted to enhance the efficiency of myocardial energy generation [280, 281]. Exploiting this effect, agents which modify substrate utilization (e.g. trimetazidine and perhexiline) and enhance myocardial energetics, have clinical efficacy in the energy starved state of heart failure [66, 233, 278]. The coexistence of augmented energetic status with substantial improvements in diastolic filling, symptoms and exercise performance (~2 ml/kg/min in peak V_{O2}) are consistent with a causal relationship. These improvements are clinically important, and are comparable in magnitude to those seen with successful gradient reduction in obstructive HCM (~3 ml/kg/min).[282, 283]

In this study perhexiline (or placebo) was added to standard medical therapy. Such therapy (e.g. beta-blockers), might have affected the cardiovascular response of participants to exercise. To account for this potential confounder all pertinent physiological parameters were corrected for heart rate. In addition, the statistical significance of the results remained unchanged irrespective of whether patients on beta-blockers were included or excluded from the analysis. Similarly, patients with flow limiting epicardial coronary disease and diabetes were excluded to avoid the confounding influence of impaired cardiac energetic status associated with these disorders [236]. Moreover, multiple objective and independent parameters (i.e. PCr/ATP, nTTPF and V_{O2}) were assessed, to obviate the biases that may confound interpretation of phase 2 trials in uncommon disorders.

The PCr/γATP ratio was measured at rest (current technology does not permit reliable dynamic measurements). We speculate however that the impact of metabolic

modulation on PCr/γATP ratios would be more striking during exercise. To further substantiate the ^{31}P MRS findings, analyses were performed both including and excluding studies with unacceptable signal-to-noise ratios (Cramer-Rao >20%); the results remained statistically similar under both circumstances.

CHAPTER 9: GENERAL DISCUSSION

The myocardium is a metabolic omnivore, which mainly uses fatty acids and glucose for generation of Adenosine-5'-triphosphate (ATP), Figure 47. Myocardial metabolism is altered in heart diseases and plays a key role in the pathogenesis and progression of these diseases. Recently, there has been a great deal of interest in the role of disturbances in cardiac and skeletal energetics in the pathophysiology of heart diseases and of therapeutic potential of metabolic modulation.

The aim of this PhD work was to assess the myocardial and peripheral metabolic status and to examine their causative role in the pathophysiology of heart diseases especially HCM patients. Furthermore, we focused on the potential role of metabolic modulator, perhexiline, in the management of these symptomatic HCM patients.

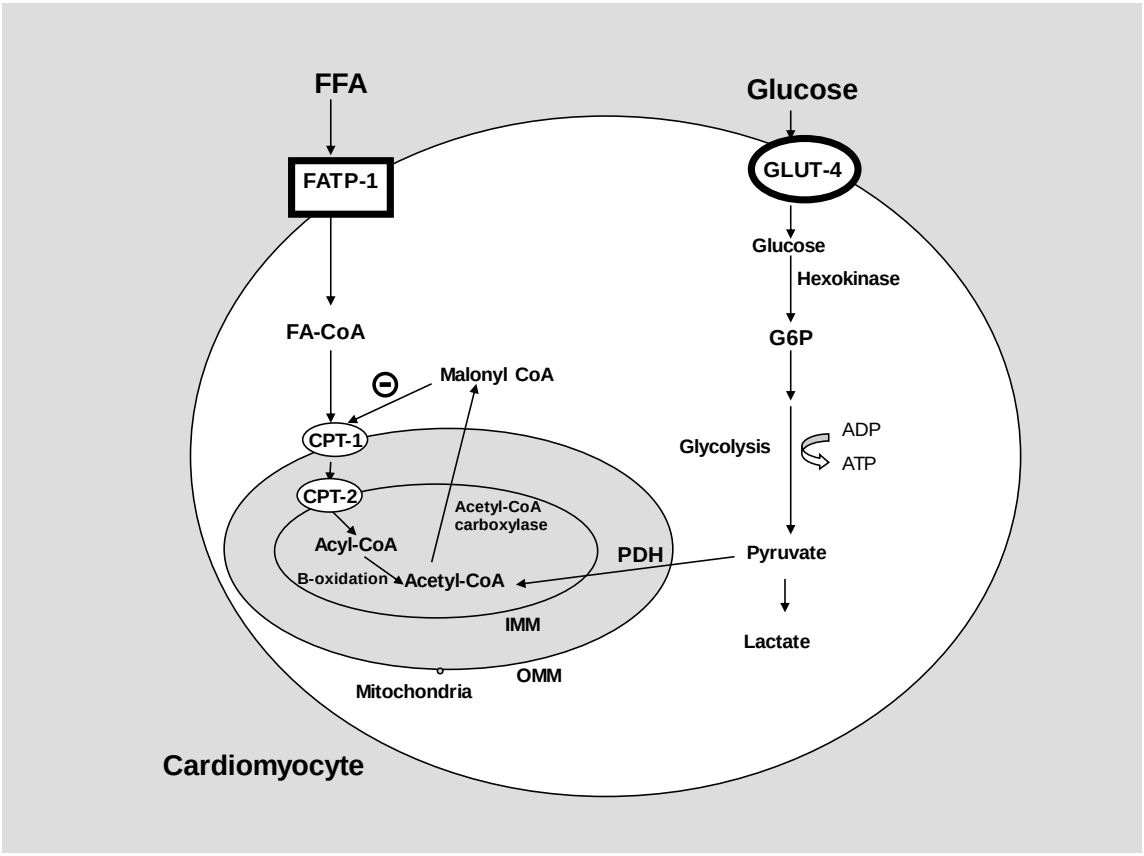

Figure 47: Myocardial ATP production.

Initially, we used NIRS to provide a non-invasive assessment of peripheral oxygen kinetics in CHF patients. [Oxy-Hb] and [HbT] were significantly reduced at rest in CHF patients as compared to age matched controls (Table 5). However, there were no significant differences in resting oxygen saturation or [Deoxy-Hb] between the two groups (Table 5). We showed that there is a significant reduction in resting oxygen consumption per gram of tissue in skeletal muscle of patients with CHF. This is unlikely to be due to skeletal muscle underperfusion as we measured oxygen consumption under conditions of arterial occlusion. More importantly, we expressed oxygen consumption measurements per 100 grams of forearm tissue to account for reduced skeletal muscle mass in heart failure patients as a potential confounder. We speculate that reduced skeletal muscle oxygen consumption observed in this study could be due to combination of intrinsic factors such as mitochondrial dysfunction, altered skeletal muscle substrate utilisation and skeletal muscle composition. Further histological and biochemical studies of muscle biopsy will be needed to examine the underlying mechanisms responsible for these observations. We conclude that NIRS has the potential to be an important clinical tool in assessing the severity of skeletal muscle metabolic impairment in heart failure, the progression and also response to treatment.

HCM is often associated with reduced exercise capacity. We used STE to examine the importance of left ventricular strain, twist and untwist rates as predictors of exercise capacity in HCM patients. Despite normal LVEF, there was a significant impairment of strain in all directions (longitudinal, circumferential and radial) and delayed untwisting in 56 HCM patients compared to 43 age and gender matched controls (Table 8 and

Table 9). While LV diastolic dysfunction is believed to be the predominant cause of exercise limitation in HCM patients [15], we found that peak V_{02} correlates significantly with markers of both left ventricular systolic and diastolic function (Figure 24). Interestingly, there were no significant correlations between peak V_{02} and other resting echocardiographic parameters such as LV mass index, MWT and LV volume index (Figure 23). In summary, STE imaging demonstrated a marked impairment of both systolic and diastolic LV function and both were significant determinants of exercise capacity. These data provide new insights for the mechanism of exercise limitation in patients with HCM using resting echocardiographic parameters.

In order to assess *in vivo* cardiac energetic status, we used MRS technology for the measurement of the PCr/γATP ratio. Conventionally, ^{31}P magnetic resonance spectroscopy (MRS) at 1.5 Tesla is used to measure *in vivo* high energy phosphate myocardial kinetics. Recently, we acquired the state of art MRI machine (Philips Achieva), which operates at a higher magnetic field (3 Tesla). This enables us to improve the sensitivity and specificity of the cardiac spectra by increasing the SNR and spectral resolution. In fact, our present study showed that ^{31}P MRS of the myocardium at 3T is feasible and allows for reliable determination of high energy phosphate kinetics. Our reproducibility data showed that the method is robust and might be used for clinical diagnostics as well as for clinical studies. However, to take full advantage of increased SNR and spectral separation at 3T as compared to 1.5T advances in shimming, coil and RF pulse design are necessary.

Using MRS technology, we showed that HCM patients have reduced cardiac energetic status (Figure 30). Consistent with previous study, we showed a paradoxical slowing of LV relaxation on exercise in HCM patients as comparing to shortening in controls [15]. Furthermore, we demonstrated a failure in VVC response on exercise in these HCM patients (Figure 32). Impaired cardiac energetics inversely correlated with VVC response and with abnormal prolongation of LV relaxation whereas it correlated positively with exercise capacity (peak V_{02}) (Figure 35). We conclude that reduced cardiac energetics contribute significantly to the abnormal active relaxation and impaired VVC response on exercise resulting in reduced exercise capacity. Based on these findings, we proposed that augmenting cardiac metabolism may have a potential role in the management of symptomatic non-obstructive HCM patients who are refractory to current treatment and not suitable for any other therapeutic options.

The pharmacologic treatment of HCM patients has remained the same for over 30 years since beta blockers and calcium blockers were introduced to treat this condition. The only other significant addition has been the implantable cardioverter defibrillator (ICD) for patients at high risk of sudden death. Treatment options in symptomatic patients with the non obstructive form of the disease are more limited. Most treatments in this group aim to improve diastolic filling by lowering heart rate, but this can reduce the cardiac output response to exercise in some patients and as a result increase exertional limitation. Furthermore, these therapies do not prevent the progressive deterioration in left ventricular diastolic and systolic function that occurs in significant minority of patients. Hence, there is a strong need for a novel pharmacological approach especially

for symptomatic non-obstructive patients who are not suitable for any surgical or percutaneous intervention.

We designed a placebo controlled, double blind, randomized study to evaluate the impact of the metabolic modulator, perhexiline [280, 284], on cardiac energy status in HCM, and to assess whether any ensuing energetic changes were accompanied by correction of the diastolic abnormalities and improvement in exercise capacity in symptomatic non-obstructive HCM patients. Our results support the hypothesis that HCM is, at least in part, a disease of energy deficiency (Figure 48).

We showed that patients with symptomatic HCM manifest a cardiac energy defect at rest (reduced PCr/γATP ratio). This defect was accompanied by a slowing of the energy-requiring early diastolic LV active relaxation during exercise (prolongation of nTTPF (Figure 39). The metabolic modulator perhexiline resulted in significant myocardial energy augmentation (Figure 41). Supporting a causative role for energy deficiency in the pathophysiology of HCM, this energy augmentation was accompanied by striking normalisation of HCM's characteristic "paradoxical" nTTPF-prolongation in exercise (Figure 43). Further supporting the hypothesis that energy deficiency contributes to the sequelae of HCM, perhexiline significantly improved the symptoms (by ~ 0.8 NYHA units), and myocardial oxygen consumption (by ~ 2 ml/kg/min) in symptomatic HCM patients on standard therapy (Figure 46 and Figure 44, respectively). These improvements are substantial and are similar to the benefits seen with septal myectomy and ablation in obstructive HCM in some studies (~3 ml/kg/min).[282, 285]

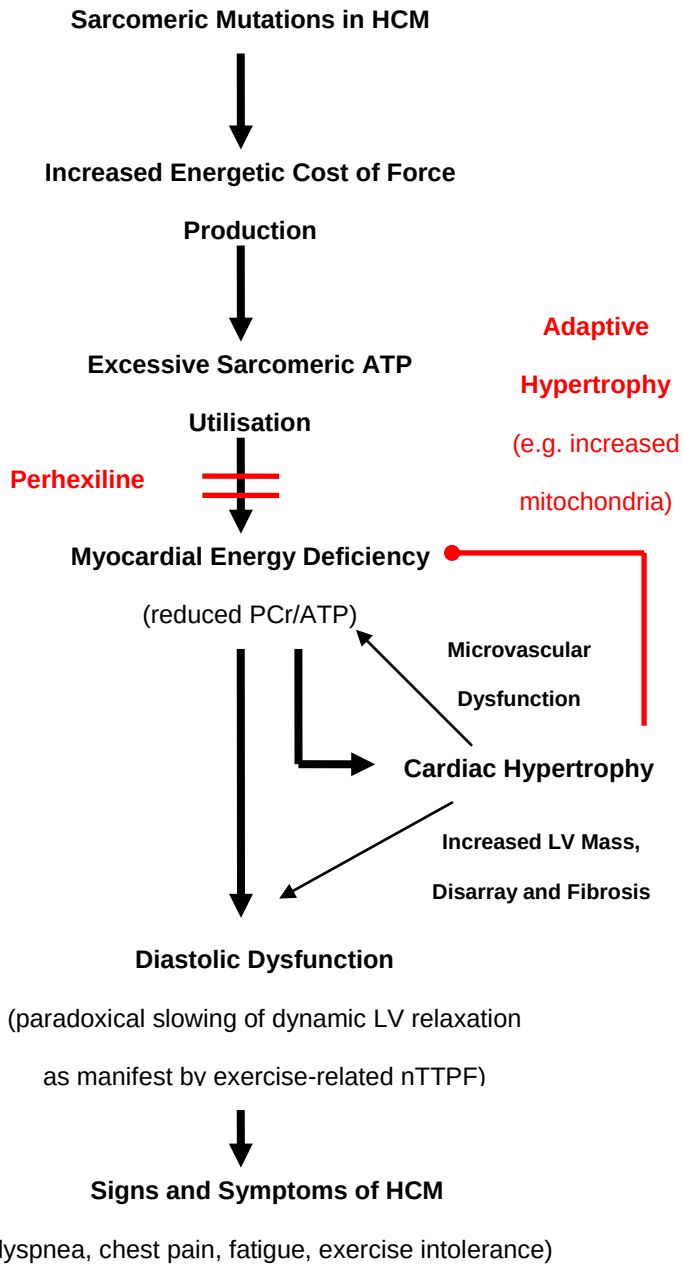

Figure 48: The causative role for energy deficiency in the pathophysiology of HCM.

In contrast, although pharmacological therapies such as beta-blockers and calcium channel antagonists are extensively used [264, 265], evidence for their use, is largely derived from small, uncontrolled, observational studies with diverse (hemodynamic) endpoints

in heterogeneous patients (often with obstruction). Our study, which is comparable to the most rigorous of existing trials [286], is the only double-blind randomised controlled (RCT) study with robust biochemical, physiological and clinical endpoints, supporting a novel therapeutic strategy in symptomatic HCM.

In summary, metabolic modulation by perhexiline augmented myocardial energetics, and normalised diastolic ventricular filling which translated into significant subjective (NYHA classification and MLHFQ score) and objective (Peak V_{O2}) clinical improvement in patients with symptomatic non obstructive HCM, a condition for which there are currently limited therapeutic options. Importantly, this encouraging efficacy was achieved with few side effects, perhexiline plasma monitoring and dose titration prevents the longer term hepatotoxicity and neuropathy that may occur in slow drug metabolisers [280].

Limitation of studies

The relevance of changes in twist and untwist during exercise would be interesting to examine, however we have demonstrated that even resting measurements of strain and untwist were significant predictors of exercise capacity. We have been unable to reliably measure these parameters during exercise due to several technical difficulties. First of all, it was hard to maintain the same heart rate during exercise while acquiring apical and basal short axis echocardiographic images which are crucial for the measurement of twist and untwist. Furthermore, exercise exaggerates the issue of through-plane motion, particularly at the basal short axis level which is again vital for twist measurement. In addition, the current STE analysis software has insufficient

temporal resolution for higher heart rate during exercise. It optimally operates at frame rate of 40-100 frames per second which is adequate only for resting studies. Moreover, excessive respiratory movement may degrade the quality of images as compared to the resting images. Further advances in the STE technique (e.g. 3D Speckle Tracking with higher temporal resolution) are required to resolve these issues.

In the STE study, we report that there are no significant differences in twist between non-obstructive HCM patients and controls (Table 9). The absence of significant differences in LV twist observed in this study could be explained by the increased through-plane motion at the base of the heart. However, we showed a significant reduction in apical rotation rates during both systole and diastole. Previous study showed that apical rotation represents the major contributions to LV twist [287].

Although the heart rate is slower in the HCM group (Table 6), all longitudinal, radial and circumferential strain parameters were independent of heart rate because we measured peak values rather than timing. Untwist timing parameters were corrected for heart rate by converting R-R interval to 100 % as previously described [288].

Thus although the consistent improvement cardiac status using multiple reliable independent parameters (i.e. PCr/ATP, nTTPF, NYHA, MLHFQ and V_{O2}) in our randomised clinical trial provides reassurance against the possibility of a false positive result, a notable feature of this study is the contrast between the robust biochemical, physiological and symptomatic improvements and the clinically significant but less prominent improvement in V_{O2}. The causes for this dissociation are likely to be multi-

factorial but probably relate, in part, to the relatively short period of perhexiline (~4.6 months) used herein to elucidate the mechanisms underlying HCM. Thus despite the improvements observed in cardiac physiology with perhexiline, there may have been inadequate time for extra-cardiac improvement (i.e. detraining of skeletal muscle persisted).[282] In this context, HCM hearts may no longer have been the limitation to exercise (V_{O2}).

To mitigate some of the challenges encountered by studies of this size in disorders of relatively low prevalence and to be maximally informative, the present study focussed on as homogenous a group of patients as possible. For example, one source of heterogeneity derived from the fact that perhexiline (or placebo) was added to standard medical therapy. Such therapy (e.g. beta-blockers), might have affected the cardiovascular response of participants to exercise. To account for this potential confounder, firstly, all pertinent physiological parameters were corrected for heart rate. In addition, the statistical significance of our results remained unchanged irrespective of whether patients on beta-blockers were included or excluded from the analysis. Similarly, patients with flow limiting epicardial coronary disease and diabetes were excluded to avoid the confounding influence of impaired cardiac energetic status associated with these disorders [289]. Moreover, multiple objective and independent parameters (i.e PCr/ATP, nTTPF and V_{O2}) were assessed, as previously advocated, to obviate the biases that have confounded existing therapeutic HCM studies [282, 286]. However, in contrast to the nTTPF and $V_{O2,}$ the PCr/γATP ratio, representing a surrogate of cardiac energetic status, was measured at rest (current technology does not permit reliable dynamic measurements). We speculate that the impact of metabolic

modulation on PCr/γATP ratios would be more striking during exercise. To further substantiate the ^{31}P MRS findings, analyses were performed both including and excluding studies with unacceptable signal-to-noise ratios (Cramer-Rao >20%); the results remained statistically similar under both circumstances. Finally, while it is formally possible that the pathway(s) through which perhexiline augmented energetics were distinct from those which led to an improvement in physiology, by virtue of perhexiline's predominant mode of action being to modify metabolism, perhexiline's benefits are likely to be, as assumed, through energy augmentation.

Future research

One interesting area of future research is to examine the mechanisms involved in the beneficial response of perhexiline on cardiac energetics. It would be important to demonstrate a reduction in myocardial fat oxidation concurrent with an increase in glucose utilization. It be would be necessary to have a measure of myocardial substrate utilization such PET scan to non-invasively evaluate myocardial glucose and FA utilization and myocardial V_{O2}. Alternatively, this could be achieved invasively using cardiac catheterisation to assess the cardiac respiratory quotient. Such studies would directly demonstrate if the mechanisms for the beneficial impact of perhexiline were the result of a shift in substrate metabolism and an increase in cardiac efficiency. Furthermore, perhexiline could have a multitude of effects in addition to inhibiting fatty acid oxidation. It would be helpful to know what happens to circulating levels of glucose, triglyceride, insulin and free fatty acids before and after perhexiline treatment. The limited duration of the drug treatment in this study does not allow ascertaining if, as

is the case of secondary LVH where treatment with drugs such as ACE inhibitors results in regression of tissue hypertrophy, treatment with perhexiline induces any regression in LVH in patients with HCM. Although double-blind randomised controlled clinical trial of this size are uncommon in HCM pharmacological studies,[286, 290] and despite the consistently positive hence promising results across diverse cardiac parameters in symptomatic HCM, the clinical implementation of metabolic therapy in HCM must await further confirmation. Larger formal trials in a broader range of patients will be necessary to establish the long-term efficacy and safety of rapidly implementable metabolic modulators (e.g. with perhexiline, trimetazidine or other metabolic modulators [38]). Furthermore, successful metabolic modulation in HCM may act as an exemplar for assessing the physiological and clinical benefits of energy augmentation through metabolic modulation in more common myocardial disorders, also recognised to be associated with energy deficiency (e.g. HF with normal ejection fraction).

To address some of these important issues, we wrote a research proposal which was successfully awarded a British Heart Foundation project grant. In this proposal we will assess the cardiac function, remodelling/reverse remodelling (by cardiac Magnetic Resonance Imaging MRI) and cardiac energetic status (by cardiac Magnetic Resonance spectroscopy MRS), cardiac efficiency (via pressure-volume loops) and substrate utilization (left and right heart catheterization) pre and post six months of perhexiline therapy in patients on optimal conventional medications for heart failure (Prof M Frenneaux, Prof A Wagenmakers and Dr K Abozguia. British Heart Foundation project grant [PG/06/105/21468] entitled 'Modification of Myocardial Substrate Utilisation as a Therapy for Heart Failure' [£235,616], Aug 2006).

APPENDIX I: PARTICIPANTS INFORMATION SHEET,

CONSENT AND RESPONSE FORMS

Information sheet for participants

Study title: **Perhexiline Therapy in Patients with Hypertrophic Cardiomyopathy**

Researchers: **Prof M Frenneaux, Prof H Watkins, Dr P Elliott, Dr Z Yousef and Dr K Abozguia**

I am writing to invite you to take part in a study we are conducting. We intend to investigate a drug that may be beneficial to you. Your treatment will not be affected in any way if you decide not to take part in this study.

What is the purpose of this study?

We have previously shown that a drug called perhexiline improves symptoms of breathlessness and also improves the heart's performance in certain types of heart disease. We now wish to test whether it is also effective in a heart condition called Hypertrophic Cardiomyopathy.

Why have I been chosen?

You have been chosen because you have Hypertrophic Cardiomyopathy and you are limited in your everyday activities by breathlessness or fatigue despite your current treatment.

Who is organising the study*?*

This is a multi-centre study organised by University of Birmingham, University of Oxford, University Hospital of Wales and University College London Hospitals. The Department of Cardiovascular Medicine at University of Birmingham is leading the study. Prof Michael Frenneaux (Head of Department) is the chief investigator.

What will happen to me if I take part?

You will be asked to arrive between 9 am to 3 pm at the Department of Cardiovascular Medicine, University of Birmingham, which is on the site of Queen Elizabeth Hospital. You will receive a clinical examination and a blood sample (total of 15 ml) will be taken. In addition, an echocardiogram (a non-invasive ultrasound scan of the heart) will be performed. You will then be asked to exercise on a treadmill wearing a mask to measure the amount of oxygen you consume. You will also be asked to fill in a questionnaire, which gives an indication as to the severity of your symptoms. These tests will take a total of one and a half hours. Then you will have a nuclear heart scan. This is a test in which your red blood cells will be labelled with a very small amount of radioactivity and a special 'Gamma Camera' is used to obtain pictures of your heart at rest and during exercise. The test will last for 1 ½ hours and you may feel slight discomfort during venflon (Needle) insertion in your arm, the insertion of the venflon and its associated pain will take less than two minutes. Otherwise it's a painless test and side effects are extremely rare. The method of administration has been performed safely by our team for a number of years now without complications in just under four hundred subjects. The amount of radiation that you will be exposed to is approximately the same as the background of radiation you will receive if you lived in Cornwall (UK) for one year and is the same as the amount you would receive from a CT scan of your

chest. Then you will have Magnetic Resonance Scans of your heart if appropriate. These Magnetic Resonance Scans will be carried either in Birmingham University Imaging Centre or John Radcliffe Hospital, Oxford (optional). Scanners that are used for the Magnetic Resonance tests are shaped like a polo mint and some patients find this scanner slightly claustrophobic. The first test (Magnetic Resonance Imaging scan of the heart) will measure the heart size and function at rest. For this test, you will lie comfortably on your back, and we will ask you to hold your breath for a few seconds during the scan. This test will take approximately 40 minutes. In the next test (Magnetic Resonance Spectroscopy of the heart) we will look at the metabolism of the heart. We will ask you to lie on your stomach, and you do not need to hold your breath. This study will last about 45 minutes.

You will then be given either perhexiline or a placebo (a sugar tablet that does not contain any active treatments) to be taken daily for three months. The allocation to perhexiline or placebo is random (50:50). Neither the doctor conducting the study nor you will know whether you are taking perhexiline or placebo. A code that tells us which tablet you are taking will be broken at the end of the study, or sooner should the need arise. While on the tablet you will need to have blood tests taken in order to avoid any potential side effects of the treatment. The blood tests will be taken 7 days after you start taking the treatment to measure the concentration of the drug in your blood and this will be followed by another blood test after 4 weeks. If the level of the drug reaches a steady state then the blood tests will only be repeated at 3 months. If the level of the drug has yet to reach a steady state we will continue to monitor your blood levels every 4 weeks until the level of the drug reaches a steady state. These blood tests can be

carried out in your GP surgery (provided they agree to do so), otherwise it will be carried out by a member of our research team in one of the three centres taking part in this study (the centre which is most convenient for you). The doses of perhexiline will be changed according to the results of the blood tests. Dose adjustments will also be made in patients taking placebo tablets in order that neither you nor we know which treatment you are taking. At the end of three months you will be seen again at the Department of Cardiovascular Medicine, University of Birmingham, for a final set of blood tests, echocardiography, nuclear scan and exercise test. Another set of MR scans will be carried out in the same centre you had your initial set. After the last scan the medication will be discontinued.

What do I have to do?

Study Day 1: Baseline investigations. These are blood tests, heart tracing, Ultrasound heart scan, exercise test, nuclear heart scan , completing a questionnaire and Magnetic Resonance scans (MRI and MRS) of the heart.

We will be happy to organise these studies over 2 days if you wish to do so. You will then be randomised to receive either the active drug (perhexiline) or a placebo. You will be required to take this drug daily for three months. While you are taking the drug, you will need monitoring of the blood level of the drug. This will be done through blood tests, which you will have by attending the hospital or by seeing your General

Practitioner. This is explained in detail above. Having been on treatment for 3 months, you will then be asked to attend for the following studies:

Study Day2 : Repeating of Baseline investigations. These are blood tests, heart tracing, Ultrasound heart scan, exercise test, nuclear heart scan, completing a questionnaire and Magnetic Resonance scans (MRI and MRS) of the heart.

We will be happy to organise these studies over 2 days if you wish to do so.

End of Study: Following completion of all studies, the drug will be discontinued, and the study will end.

We will pay your travel and accommodation expenses. If you require a carer for medical reasons to accompanying you during this study, we will also reimburse the carer travel and accommodation expenses.

What is perhexiline?

In the 1970s, perhexiline was shown to be an effective drug at relieving symptoms of angina and breathlessness in some forms of heart disease. There were cases of liver or peripheral nerve damage which eventually resulted in the withdrawal of perhexiline in many countries around the world. In approximately 1980, it was found that the

probability of developing toxic reactions to perhexiline was closely related to plasma drug levels. A simple assay for drug levels is available. It has been shown that with regular monitoring of perhexiline levels, the risk of either liver damage or of peripheral nerve damage can be reduced to virtually zero. In our experience with perhexiline in Cardiff and the experience of its widespread use in Australia, no serious adverse effects have been observed. Recently, there have been studies performed suggest that it works by making the heart muscle more efficient and so reducing the amount of oxygen required. It is through that mechanism that we suspect it could also be useful at reducing breathlessness caused by Hypertrophic Cardiomyopathy.

What are the potential side effects of perhexiline?

Minor nausea and dizziness can occur in the first few days but usually pass. Long-term side effects (liver damage and nerve damage) are extremely rare and are avoided by monitoring blood levels and adjusting the dose if necessary. Perhexiline has been shown to be a safe drug provided blood levels are monitored on a regular basis and drug dosage is adjusted accordingly. A doctor will inform you on the correct dose adjustment based on your blood levels. You will be asked to report any symptoms of muscle weakness, limb numbness or jaundice to the doctor. A 24-hour phone number will be provided in case of emergencies.

What are the possible benefits of taking part?

You may find perhexiline useful at relieving your symptoms. However, there is a 50% chance that you will be on Placebo which may not affect your symptoms. Your participation in this study will help to broaden our understanding of Hypertrophic Cardiomyopathy, which is a condition affecting many people in the United Kingdom. We also hope that the study will demonstrate to us how this treatment works, so that we may develop similar treatments in the future which do not require regular blood tests to avoid side effects.

Who will have access to the data and study results?

Prof M Frenneaux will be ultimately responsible for the data and study results. The research staff at the Departments of Cardiovascular Medicine, within the three study sites will have control of the data. Ethics Committees, national and international regulatory authorities, which will review information on the drug and study, may also inspect the results. The data will be kept in a locked filing cabinet in the Department of Cardiovascular Medicine, University of Birmingham. In addition, information about you will be kept confidential, and your medical records will not be made public. We aim to publish the research in peer reviewed journals and the abstracts are usually available on the internet to the public. If the results from this trial should be published, your identity will be kept completely confidential. If at the end of the study you would like a copy of the published paper, this can be sent to you on request.

Are there compensation arrangements if something goes wrong?

In the unlikely event of your health deteriorating in any way as a result of negligence, you may submit a claim to the University Hospital Birmingham NHS Trust for compensation to be considered. If you are harmed due to someone's negligence, then you may have grounds for a legal action. There is no cover for non-negligent harm but 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 NHS complaints mechanisms are available to you. If you are covered by Private Medical Insurance you should contact them to determine whether your cover would be affected in any way by taking part in this research.

What happens if I do not want to participate or I change my mind during the research study?

You do not have to participate in this study. If you decide not to participate or change your mind later on; you can withdraw at any time. Your withdrawal will not affect your subsequent treatment in any way.

What if new information becomes available?

You would be informed by telephone of any new developments.

What happens at the end of the study?

At the end of the study the tablet you were taking for three months will be discontinued. We can't guarantee that this medication will be available to you at the end of the study.

If this study produces positive results, we will negotiate with the Hospital Trust to try to continue the drug.

Will my GP be informed*?*

Yes. Your GP will receive a letter explaining the objectives of the study.

If you have any complains during the study please do not hesitate to let me know.

If you are unhappy about our approach, manners, or the way we have dealt with any of your requests, please feel free to contact the chief investigator Professor M Frenneaux in the Department of Cardiovascular medicine, University of Birmingham (Tel.0121–4146926).

If you are interested in taking part or if you have more questions or do not understand something please contact:

Dr Khalid Abozguia

Department of Cardiovascular Medicine

University of Birmingham

Edgbaston, Birmingham

B15 2TT

Tel: 01214145916

In cases of emergencies Dr K Abozguia can also be contacted on his mobile phone [redacted]

Finally, thank you very much for taking the time to consider taking part in this study and we will be grateful if you could send the response form in the self-addressed paid for envelope.

Consent form

Patient Identification/Study Number: _____

Project Title**: Perhexiline therapy in patients with Hypertrophic Cardiomyopathy.**

Researchers: **Prof M Frenneaux, Prof H Watkins, Dr P Elliott, Dr Z Yousef and Dr K Abozguia**

Please initial box

1. I confirm that I have read and understand the information sheet dated **01-05-2007** Version **4** 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 sections of my medical notes relating to participation in the study may be inspected by responsible individuals from the three study centres mentioned above or from regulatory authorities. All personal details will be treated as STRICTLY CONFIDENTIAL. I give permission for these individuals to have access to my records. ☐
4. I am willing that my general practitioner is notified of my participation in this research. ☐
5. I agree to take part in the above study and am satisfied that I have been given sufficient time and information to reach this decision. ☐

_____ _____ _____

Name of Research Subject Date Signature

(Please print)

_____ _____ _____

Name of Witness to Signature Date Signature

(Must **not** be member of research team)

(Please print)

_____ _____ _____

Name of Research Team member Date Signature

(Please print)

Response Form

Thank you for reading the enclosed information sheet. Once you have reached a decision I would be grateful if could complete and return this form, indicating whether you would like 1) To participate, 2) To have further information or 3) To not participate, by ticking the appropriate box below. Complete as many details as you can. Please return the completed form in the enclosed stamped addressed envelope.

Name: _____

DOB: _____

Address: _____

_____

_____

Tel: _____Business Tel: _____

Mobile: _____ Email address:_____

GP Name: _____

GP Address: _____

_____

Tel: _____ Fax: _____

I agree to participate in the study ☐ (Please tick one box)

I would like further information ☐

I do not wish to participate in the study ☐

REFERENCES

Reference List

1. Frenneaux MP. Assessing the risk of sudden cardiac death in a patient with hypertrophic cardiomyopathy. Heart 2004;90(5):570-575.

2. Seidman JG, Seidman C. The genetic basis for cardiomyopathy: from mutation identification to mechanistic paradigms. Cell 2001;104(4):557-567.

3. Spirito P, Seidman CE, McKenna WJ, Maron BJ. The management of hypertrophic cardiomyopathy. N Engl J Med 1997;336(11):775-785.

4. Maron BJ, Gardin JM, Flack JM, Gidding SS, Kurosaki TT, Bild DE. Prevalence of hypertrophic cardiomyopathy in a general population of young adults. Echocardiographic analysis of 4111 subjects in the CARDIA Study. Coronary Artery Risk Development in (Young) Adults. Circulation 1995;92(4):785-789.

5. Elliott PM, Poloniecki J, Dickie S et al. Sudden death in hypertrophic cardiomyopathy: identification of high risk patients. J Am Coll Cardiol 2000;36(7):2212-2218.

6. Elliott PM, Gimeno B, Jr., Mahon NG, Poloniecki JD, McKenna WJ. Relation between severity of left-ventricular hypertrophy and prognosis in patients with hypertrophic cardiomyopathy. Lancet 2001;357(9254):420-424.

7. Maki S, Ikeda H, Muro A et al. Predictors of sudden cardiac death in hypertrophic cardiomyopathy. Am J Cardiol 1998;82(6):774-778.

8. Maron BJ, Roberts WC, Epstein SE. Sudden death in hypertrophic cardiomyopathy: a profile of 78 patients. Circulation 1982;65(7):1388-1394.

9. Maron BJ, Kogan J, Proschan MA, Hecht GM, Roberts WC. Circadian variability in the occurrence of sudden cardiac death in patients with hypertrophic cardiomyopathy. J Am Coll Cardiol 1994;23(6):1405-1409.

10. Maron BJ, Shirani J, Poliac LC, Mathenge R, Roberts WC, Mueller FO. Sudden death in young competitive athletes. Clinical, demographic, and pathological profiles. JAMA 1996;276(3):199-204.

11. Maron BJ, Olivotto I, Spirito P et al. Epidemiology of hypertrophic cardiomyopathy-related death: revisited in a large non-referral-based patient population. Circulation 2000;102(8):858-864.

12. Spirito P, Maron BJ. Relation between extent of left ventricular hypertrophy and occurrence of sudden cardiac death in hypertrophic cardiomyopathy. J Am Coll Cardiol 1990;15(7):1521-1526.

13. Spirito P, Bellone P, Harris KM, Bernabo P, Bruzzi P, Maron BJ. Magnitude of left ventricular hypertrophy and risk of sudden death in hypertrophic cardiomyopathy. N Engl J Med 2000;342(24):1778-1785.

14. Bonow RO, Rosing DR, Bacharach SL et al. Effects of verapamil on left ventricular systolic function and diastolic filling in patients with hypertrophic cardiomyopathy. Circulation 1981;64(4):787-796.

15. Lele SS, Thomson HL, Seo H, Belenkie I, McKenna WJ, Frenneaux MP. Exercise capacity in hypertrophic cardiomyopathy. Role of stroke volume limitation, heart rate, and diastolic filling characteristics. Circulation 1995;92(10):2886-2894.

16. Izawa H, Yokota M, Takeichi Y et al. Adrenergic control of the force-frequency and relaxation-frequency relations in patients with hypertrophic cardiomyopathy. Circulation 1997;96(9):2959-2968.

17. Gwathmey JK, Warren SE, Briggs GM et al. Diastolic dysfunction in hypertrophic cardiomyopathy. Effect on active force generation during systole. J Clin Invest 1991;87(3):1023-1031.

18. Betocchi S, Hess OM, Losi MA, Nonogi H, Krayenbuehl HP. Regional left ventricular mechanics in hypertrophic cardiomyopathy. Circulation 1993;88(5 Pt 1):2206-2214.

19. Ashrafian H, Watkins H. Myocardial dysfunction in hypertrophic cardiomyopathy. Circulation 2001;104(25):E165.

20. HARRISON DC, BRAUNWALD E, GLICK G, MASON DT, CHIDSEY CA, ROSS J, Jr. EFFECTS OF BETA ADRENERGIC BLOCKADE ON THE CIRCULATION WITH PARTICULAR REFERENCE TO OBSERVATIONS IN PATIENTS WITH HYPERTROPHIC SUBAORTIC STENOSIS. Circulation 1964;29:84-98.

21. Cohen LS, BRAUNWALD E. Chronic beta adrenergic receptor blockade in the treatment of idiopathic hypertrophic subaortic stenosis. Prog Cardiovasc Dis 1968;11(3):211-221.

22. Pollick C. Muscular subaortic stenosis: hemodynamic and clinical improvement after disopyramide. N Engl J Med 1982;307(16):997-999.

23. Rosing DR, Kent KM, Maron BJ, Epstein SE. Verapamil therapy: a new approach to the pharmacologic treatment of hypertrophic cardiomyopathy. II. Effects on exercise capacity and symptomatic status. Circulation 1979;60(6):1208-1213.

24. Rosing DR, Kent KM, Borer JS, Seides SF, Maron BJ, Epstein SE. Verapamil therapy: a new approach to the pharmacologic treatment of hypertrophic cardiomyopathy. I. Hemodynamic effects. Circulation 1979;60(6):1201-1207.

25. Jeanrenaud X, Goy JJ, Kappenberger L. Effects of dual-chamber pacing in hypertrophic obstructive cardiomyopathy. Lancet 1992;339(8805):1318-1323.

26. Fananapazir L, Epstein ND, Curiel RV, Panza JA, Tripodi D, McAreavey D. Long-term results of dual-chamber (DDD) pacing in obstructive hypertrophic cardiomyopathy. Evidence for progressive symptomatic and hemodynamic improvement and reduction of left ventricular hypertrophy. Circulation 1994;90(6):2731-2742.

27. Nishimura RA, Trusty JM, Hayes DL et al. Dual-chamber pacing for hypertrophic cardiomyopathy: a randomized, double-blind, crossover trial. J Am Coll Cardiol 1997;29(2):435-441.

28. Nishimura RA, Hayes DL, Ilstrup DM, Holmes DR, Jr., Tajik AJ. Effect of dual-chamber pacing on systolic and diastolic function in patients with hypertrophic cardiomyopathy. Acute Doppler echocardiographic and catheterization hemodynamic study. J Am Coll Cardiol 1996;27(2):421-430.

29. Betocchi S, Losi MA, Piscione F et al. Effects of dual-chamber pacing in hypertrophic cardiomyopathy on left ventricular outflow tract obstruction and on diastolic function. Am J Cardiol 1996;77(7):498-502.

30. Lim DS, Lutucuta S, Bachireddy P et al. Angiotensin II blockade reverses myocardial fibrosis in a transgenic mouse model of human hypertrophic cardiomyopathy. Circulation 2001;103(6):789-791.

31. Patel R, Nagueh SF, Tsybouleva N et al. Simvastatin induces regression of cardiac hypertrophy and fibrosis and improves cardiac function in a transgenic rabbit model of human hypertrophic cardiomyopathy. Circulation 2001;104(3):317-324.

32. Crilley JG, Boehm EA, Blair E et al. Hypertrophic cardiomyopathy due to sarcomeric gene mutations is characterized by impaired energy metabolism irrespective of the degree of hypertrophy. J Am Coll Cardiol 2003;41(10):1776-1782.

33. Javadpour MM, Tardiff JC, Pinz I, Ingwall JS. Decreased energetics in murine hearts bearing the R92Q mutation in cardiac troponin T. J Clin Invest 2003;112(5):768-775.

34. Jung WI, Sieverding L, Breuer J et al. 31P NMR spectroscopy detects metabolic abnormalities in asymptomatic patients with hypertrophic cardiomyopathy. Circulation 1998;97(25):2536-2542.

35. Luedde M, Flogel U, Knorr M et al. Decreased contractility due to energy deprivation in a transgenic rat model of hypertrophic cardiomyopathy. J Mol Med 2009;87(4):411-422.

36. Spindler M, Saupe KW, Christe ME et al. Diastolic dysfunction and altered energetics in the alphaMHC403/+ mouse model of familial hypertrophic cardiomyopathy. J Clin Invest 1998;101(8):1775-1783.

37. Sack MN, Harrington LS, Jonassen AK, Mjos OD, Yellon DM. Coordinate regulation of metabolic enzyme encoding genes during cardiac development and following carvedilol therapy in spontaneously hypertensive rats. Cardiovasc Drugs Ther 2000;14(1):31-39.

38. Abozguia K, Clarke K, Lee L, Frenneaux M. Modification of myocardial substrate use as a therapy for heart failure. Nat Clin Pract Cardiovasc Med 2006;3(9):490-498.

39. Liedtke AJ. Alterations of carbohydrate and lipid metabolism in the acutely ischemic heart. Prog Cardiovasc Dis 1981;23(5):321-336.

40. Stanley WC, Lopaschuk GD, Hall JL, McCormack JG. Regulation of myocardial carbohydrate metabolism under normal and ischaemic conditions. Potential for pharmacological interventions. Cardiovasc Res 1997;33(2):243-257.

41. Sack MN, Rader TA, Park S, Bastin J, McCune SA, Kelly DP. Fatty acid oxidation enzyme gene expression is downregulated in the failing heart. Circulation 1996;94(11):2837-2842.

42. Ingwall JS, Weiss RG. Is the failing heart energy starved? On using chemical energy to support cardiac function. Circ Res 2004;95(2):135-145.

43. Hajri T, Ibrahimi A, Coburn CT et al. Defective fatty acid uptake in the spontaneously hypertensive rat is a primary determinant of altered glucose metabolism, hyperinsulinemia, and myocardial hypertrophy. J Biol Chem 2001;276(26):23661-23666.

44. Shimojo N, Miyauchi T, Iemitsu M et al. Effects of medium-chain triglyceride (MCT) application to SHR on cardiac function, hypertrophy and expression of endothelin-1 mRNA and other genes. J Cardiovasc Pharmacol 2004;44 Suppl 1:S181-S185.

45. Iemitsu M, Shimojo N, Maeda S et al. The benefit of medium-chain triglyceride therapy on the cardiac function of SHRs is associated with a reversal of metabolic and signaling alterations. Am J Physiol Heart Circ Physiol 2008;295(1):H136-H144.

46. Young ME, Laws FA, Goodwin GW, Taegtmeyer H. Reactivation of peroxisome proliferator-activated receptor alpha is associated with contractile dysfunction in hypertrophied rat heart. J Biol Chem 2001;276(48):44390-44395.

47. Nikolaidis LA, Sturzu A, Stolarski C, Elahi D, Shen YT, Shannon RP. The development of myocardial insulin resistance in conscious dogs with advanced dilated cardiomyopathy. Cardiovasc Res 2004;61(2):297-306.

48. Hardy CJ, Weiss RG, Bottomley PA, Gerstenblith G. Altered myocardial high-energy phosphate metabolites in patients with dilated cardiomyopathy. Am Heart J 1991;122(3 Pt 1):795-801.

49. Tian R, Musi N, D'Agostino J, Hirshman MF, Goodyear LJ. Increased adenosine monophosphate-activated protein kinase activity in rat hearts with pressure-overload hypertrophy. Circulation 2001;104(14):1664-1669.

50. Marsin AS, Bertrand L, Rider MH et al. Phosphorylation and activation of heart PFK-2 by AMPK has a role in the stimulation of glycolysis during ischaemia. Curr Biol 2000;10(20):1247-1255.

51. Merrill GF, Kurth EJ, Hardie DG, Winder WW. AICA riboside increases AMP-activated protein kinase, fatty acid oxidation, and glucose uptake in rat muscle. Am J Physiol 1997;273(6 Pt 1):E1107-E1112.

52. Abozguia K, Nallur Shivu G, Phan TT, Ahmed I, Maher AR, Frenneaux MP. Potential of metabolic agents as adjunct therapies in heart failure. Future Cardiology 2007;3(No.5):525-535.

53. Wu Z, Puigserver P, Andersson U et al. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 1999;98(1):115-124.

54. St Pierre J, Lin J, Krauss S et al. Bioenergetic analysis of peroxisome proliferator-activated receptor gamma coactivators 1alpha and 1beta (PGC-1alpha and PGC-1beta) in muscle cells. J Biol Chem 2003;278(29):26597-26603.

55. Osorio JC, Stanley WC, Linke A et al. Impaired myocardial fatty acid oxidation and reduced protein expression of retinoid X receptor-alpha in pacing-induced heart failure. Circulation 2002;106(5):606-612.

56. Sack MN, Disch DL, Rockman HA, Kelly DP. A role for Sp and nuclear receptor transcription factors in a cardiac hypertrophic growth program. Proc Natl Acad Sci U S A 1997;94(12):6438-6443.

57. Kelly DP, Strauss AW. Inherited cardiomyopathies. N Engl J Med 1994;330(13):913-919.

58. Labarthe F, Khairallah M, Bouchard B, Stanley WC, Des RC. Fatty acid oxidation and its impact on response of spontaneously hypertensive rat hearts to an adrenergic stress: benefits of a medium-chain fatty acid. Am J Physiol Heart Circ Physiol 2005;288(3):H1425-H1436.

59. Hajri T, Ibrahimi A, Coburn CT et al. Defective fatty acid uptake in the spontaneously hypertensive rat is a primary determinant of altered glucose metabolism, hyperinsulinemia, and myocardial hypertrophy. J Biol Chem 2001;276(26):23661-23666.

60. Liao R, Jain M, Cui L et al. Cardiac-specific overexpression of GLUT1 prevents the development of heart failure attributable to pressure overload in mice. Circulation 2002;106(16):2125-2131.

61. Huss JM, Kelly DP. Mitochondrial energy metabolism in heart failure: a question of balance. J Clin Invest 2005;115(3):547-555.

62. Lommi J, Kupari M, Yki-Jarvinen H. Free fatty acid kinetics and oxidation in congestive heart failure. Am J Cardiol 1998;81(1):45-50.

63. Taylor M, Wallhaus TR, Degrado TR et al. An evaluation of myocardial fatty acid and glucose uptake using PET with [18F]fluoro-6-thia-heptadecanoic acid and. J Nucl Med 2001;42(1):55-62.

64. Wisniacki N, Taylor W, Lye M, Wilding JP. Insulin resistance and inflammatory activation in older patients with systolic and diastolic heart failure. Heart 2005;91(1):32-37.

65. Nikolaidis LA, Sturzu A, Stolarski C, Elahi D, Shen YT, Shannon RP. The development of myocardial insulin resistance in conscious dogs with advanced dilated cardiomyopathy. Cardiovasc Res 2004;61(2):297-306.

66. Lee L, Campbell R, Scheuermann-Freestone M et al. Metabolic modulation with perhexiline in chronic heart failure: a randomized, controlled trial of short-term use of a novel treatment. Circulation 2005;112(21):3280-3288.

67. Di Napoli P, Taccardi AA, Barsotti A. Long term cardioprotective action of trimetazidine and potential effect on the inflammatory process in patients with ischaemic dilated cardiomyopathy. Heart 2005;91(2):161-165.

68. How OJ, Aasum E, Kunnathu S, Severson DL, Myhre ES, Larsen TS. Influence of substrate supply on cardiac efficiency, as measured by pressure-volume analysis in ex vivo mouse hearts. Am J Physiol Heart Circ Physiol 2005;288(6):H2979-H2985.

69. Kjekshus JK, Mjos OD. Effect of free fatty acids on myocardial function and metabolism in the ischemic dog heart. J Clin Invest 1972;51(7):1767-1776.

70. Mjos OD, Kjekshus JK, Lekven J. Importance of free fatty acids as a determinant of myocardial oxygen consumption and myocardial ischemic injury during norepinephrine infusion in dogs. J Clin Invest 1974;53(5):1290-1299.

71. J.F.Hutter HMPaPGS. Effect of fatty acid oxidation on efficiency of energy production in rat heart. Am J Physiol Heart Circ Physiol 1985;249:723-728.

72. Boehm EA, Jones BE, Radda GK, Veech RL, Clarke K. Increased uncoupling proteins and decreased efficiency in palmitate-perfused hyperthyroid rat heart. Am J Physiol Heart Circ Physiol 2001;280(3):H977-H983.

73. Schrauwen P, Hoeks J, Schaart G et al. Uncoupling protein 3 as a mitochondrial fatty acid anion exporter. FASEB J 2003;17(15):2272-2274.

74. Murray AJ, Anderson RE, Watson GC, Radda GK, Clarke K. Uncoupling proteins in human heart. Lancet 2004;364(9447):1786-1788.

75. Mjos OD. Effect of free fatty acids on myocardial function and oxygen consumption in intact dogs. J Clin Invest 1971;50(7):1386-1389.

76. BING RJ, SIEGEL A, UNGAR I, GILBERT M. Metabolism of the human heart. II. Studies on fat, ketone and amino acid metabolism. Am J Med 1954;16(4):504-515.

77. Shen W, Asai K, Uechi M et al. Progressive loss of myocardial ATP due to a loss of total purines during the development of heart failure in dogs: a compensatory role for the parallel loss of creatine. Circulation 1999;100(20):2113-2118.

78. Liao R, Nascimben L, Friedrich J, Gwathmey JK, Ingwall JS. Decreased energy reserve in an animal model of dilated cardiomyopathy. Relationship to contractile performance. Circ Res 1996;78(5):893-902.

79. Bittl JA, Ingwall JS. Reaction rates of creatine kinase and ATP synthesis in the isolated rat heart. A 31P NMR magnetization transfer study. J Biol Chem 1985;260(6):3512-3517.

80. Wallimann T, Dolder M, Schlattner U et al. Some new aspects of creatine kinase (CK): compartmentation, structure, function and regulation for cellular and mitochondrial bioenergetics and physiology. Biofactors 1998;8(3-4):229-234.

81. Neubauer S, Remkes H, Spindler M et al. Downregulation of the Na(+)-creatine cotransporter in failing human myocardium and in experimental heart failure. Circulation 1999;100(18):1847-1850.

82. Tian R and Ingwall JS. The Molecular Energetics of the Failing Heart from Animal ModelsSmall Animal Models. Heart Failure Reviews 1999;4(3):245-253.

83. Zhang J and Bache R. The Molecular Energetics of the Failing Heart from Animal Models—Large Animal Models. Heart Failure Reviews 1999;4(3):255-267.

84. Nakae I, Mitsunami K, Omura T et al. Proton magnetic resonance spectroscopy can detect creatine depletion associated with the progression of heart failure in cardiomyopathy. J Am Coll Cardiol 2003;42(9):1587-1593.

85. Conway MA, Allis J, Ouwerkerk R, Niioka T, Rajagopalan B, Radda GK. Detection of low phosphocreatine to ATP ratio in failing hypertrophied human myocardium by 31P magnetic resonance spectroscopy. Lancet 1991;338(8773):973-976.

86. Neubauer S, Horn M, Cramer M et al. Myocardial phosphocreatine-to-ATP ratio is a predictor of mortality in patients with dilated cardiomyopathy. Circulation 1997;96(7):2190-2196.

87. De Sousa E, Veksler V, Minajeva A et al. Subcellular creatine kinase alterations. Implications in heart failure. Circ Res 1999;85(1):68-76.

88. Gross WL, Bak MI, Ingwall JS et al. Nitric oxide inhibits creatine kinase and regulates rat heart contractile reserve. Proc Natl Acad Sci U S A 1996;93(11):5604-5609.

89. Gordon A, Hultman E, Kaijser L et al. Creatine supplementation in chronic heart failure increases skeletal muscle creatine phosphate and muscle performance. Cardiovasc Res 1995;30(3):413-418.

90. Andrews R, Greenhaff P, Curtis S, Perry A, Cowley AJ. The effect of dietary creatine supplementation on skeletal muscle metabolism in congestive heart failure. Eur Heart J 1998;19(4):617-622.

91. Ashrafian H, Redwood C, Blair E, Watkins H. Hypertrophic Cardiomyopathy: A Paradigm for myocardial energy depletion. Trends in Genetics. 2000.

92. Redwood CS, Moolman-Smook JC, Watkins H. Properties of mutant contractile proteins that cause hypertrophic cardiomyopathy. Cardiovasc Res 1999;44(1):20-36.

93. Sweeney HL, Feng HS, Yang Z, Watkins H. Functional analyses of troponin T mutations that cause hypertrophic cardiomyopathy: insights into disease pathogenesis and troponin function. Proc Natl Acad Sci U S A 1998;95(24):14406-14410.

94. Nagueh SF, McFalls J, Meyer D et al. Tissue Doppler imaging predicts the development of hypertrophic cardiomyopathy in subjects with subclinical disease. Circulation 2003;108(4):395-398.

95. Dong SJ, MacGregor JH, Crawley AP et al. Left ventricular wall thickness and regional systolic function in patients with hypertrophic cardiomyopathy. A three-dimensional tagged magnetic resonance imaging study. Circulation 1994;90(3):1200-1209.

96. Thompson CH, Kemp GJ, Taylor DJ et al. Abnormal skeletal muscle bioenergetics in familial hypertrophic cardiomyopathy. Heart 1997;78(2):177-181.

97. Caforio AL, Rossi B, Risaliti R et al. Type 1 fiber abnormalities in skeletal muscle of patients with hypertrophic and dilated cardiomyopathy: evidence of subclinical myogenic myopathy. J Am Coll Cardiol 1989;14(6):1464-1473.

98. Hermann HP, Pieske B, Schwarzmuller E, Keul J, Just H, Hasenfuss G. Haemodynamic effects of intracoronary pyruvate in patients with congestive heart failure: an open study. Lancet 1999;353(9161):1321-1323.

99. Kennedy JA, Unger SA, Horowitz JD. Inhibition of carnitine palmitoyltransferase-1 in rat heart and liver by perhexiline and amiodarone. Biochem Pharmacol 1996;52(2):273-280.

100. Kennedy JA, Kiosoglous AJ, Murphy GA, Pelle MA, Horowitz JD. Effect of perhexiline and oxfenicine on myocardial function and metabolism during low-flow ischemia/reperfusion in the isolated rat heart. J Cardiovasc Pharmacol 2000;36(6):794-801.

101. Bouche P, Bousser MG, Peytour MA, Cathala HP. Perhexiline maleate and peripheral neuropathy. Neurology 1979;29(5):739-743.

102. Pessayre D, Bichara M, Degott C, Potet F, Benhamou JP, Feldmann G. Perhexiline maleate-induced cirrhosis. Gastroenterology 1979;76(1):170-177.

103. Roberts RK, Cohn D, Petroff V, Seneviratne B. Liver disease induced by perhexiline maleate. Med J Aust 1981;2(10):553-554.

104. Vitale C, Wajngaten M, Sposato B et al. Trimetazidine improves left ventricular function and quality of life in elderly patients with coronary artery disease. Eur Heart J 2004;25(20):1814-1821.

105. Fragasso G, Palloshi A, Puccetti P et al. A randomized clinical trial of trimetazidine, a partial free fatty acid oxidation inhibitor, in patients with heart failure. J Am Coll Cardiol 2006;48(5):992-998.

106. Schmidt-Schweda S, Holubarsch C. First clinical trial with etomoxir in patients with chronic congestive heart failure. Clin Sci (Lond) 2000;99(1):27-35.

107. Holubarsch CJ, Rohrbach M, Karrasch M et al. A double-blind randomised, multi-centre clinical trial to evaluate the efficacy and safety of two doses of Etomoxir in comparison to placebo in patients with moderate congestive heart failure: The ERGO-study, Etomoxir and ergometry. Clin Sci (Lond) 2007.

108. Omran H, Illien S, MacCarter D, St Cyr J, Luderitz B. D-Ribose improves diastolic function and quality of life in congestive heart failure patients: a prospective feasibility study. Eur J Heart Fail 2003;5(5):615-619.

109. Anand I, Chandrashekhan Y, De Giuli F et al. Acute and chronic effects of propionyl-L-carnitine on the hemodynamics, exercise capacity, and hormones in patients with congestive heart failure. Cardiovasc Drugs Ther 1998;12(3):291-299.

110. Study on propionyl-L-carnitine in chronic heart failure. Eur Heart J 1999;20(1):70-76.

111. Sokos GG, Nikolaidis LA, Mankad S, Elahi D, Shannon RP. Glucagon-like peptide-1 infusion improves left ventricular ejection fraction and functional status in patients with chronic heart failure. J Card Fail 2006;12(9):694-699.

112. Morgan MY, Reshef R, Shah RR, Oates NS, Smith RL, Sherlock S. Impaired oxidation of debrisoquine in patients with perhexiline liver injury. Gut 1984;25(10):1057-1064.

113. Cole PL, Beamer AD, McGowan N et al. Efficacy and safety of perhexiline maleate in refractory angina. A double-blind placebo-controlled clinical trial of a novel antianginal agent. Circulation 1990;81(4):1260-1270.

114. Horowitz JD, Sia ST, Macdonald PS, Goble AJ, Louis WJ. Perhexiline maleate treatment for severe angina pectoris--correlations with pharmacokinetics. Int J Cardiol 1986;13(2):219-229.

115. Klassen GA, Sestier F, L'Abbate A, Mildenberger RR, Zborowska-Sluis DT. Effects of perhexiline maleate on coronary flow distribution in the ischemic canine myocardium. Circulation 1976;54(1):14-20.

116. Pepine CJ, Schang SJ, Bemiller CR. Proceedings: Alteration of left ventricular responses to ischemia with oral perhexiline. Postgrad Med J 1973;49:Suppl-6.

117. Pepine CJ, Schang SJ, Bemiller CR. Effects of perhexiline on coronary hemodynamic and myocardial metabolic responses to tachycardia. Circulation 1974;49(5):887-893.

118. Unger SA, Kennedy JA, McFadden-Lewis K, Minerds K, Murphy GA, Horowitz JD. Dissociation between metabolic and efficiency effects of perhexiline in normoxic rat myocardium. J Cardiovasc Pharmacol 2005;46(6):849-855.

119. Lionetti V, Linke A, Chandler MP et al. Carnitine palmitoyl transferase-I inhibition prevents ventricular remodeling and delays decompensation in pacing-induced heart failure. Cardiovasc Res 2005;66(3):454-461.

120. Greaves P, Martin J, Michel MC, Mompon P. Cardiac hypertrophy in the dog and rat induced by oxfenicine, an agent which modifies muscle metabolism. Arch Toxicol Suppl 1984;7:488-493.

121. Bachmann E, Weber E. Biochemical mechanisms of oxfenicine cardiotoxicity. Pharmacology 1988;36(4):238-248.

122. Reaven GM, Chang H, Hoffman BB. Additive hypoglycemic effects of drugs that modify free-fatty acid metabolism by different mechanisms in rats with streptozocin-induced diabetes. Diabetes 1988;37(1):28-32.

123. Abdel-aleem S, Li X, Anstadt MP, Perez-Tamayo RA, Lowe JE. Regulation of glucose utilization during the inhibition of fatty acid oxidation in rat myocytes. Horm Metab Res 1994;26(2):88-91.

124. Turcani M, Rupp H. Modification of left ventricular hypertrophy by chronic etomixir treatment. Br J Pharmacol 1999;126(2):501-507.

125. Lopaschuk GD, Spafford M. Response of isolated working hearts to fatty acids and carnitine palmitoyltransferase I inhibition during reduction of coronary flow in acutely and chronically diabetic rats. Circ Res 1989;65(2):378-387.

126. Bristow MR, Gilbert EM, Abraham WT et al. Carvedilol produces dose-related improvements in left ventricular function and survival in subjects with chronic heart failure. MOCHA Investigators. Circulation 1996;94(11):2807-2816.

127. Packer M, Colucci WS, Sackner-Bernstein JD et al. Double-blind, placebo-controlled study of the effects of carvedilol in patients with moderate to severe heart failure. The PRECISE Trial. Prospective Randomized Evaluation of Carvedilol on Symptoms and Exercise. Circulation 1996;94(11):2793-2799.

128. Wallhaus TR, Taylor M, Degrado TR et al. Myocardial free fatty acid and glucose use after carvedilol treatment in patients with congestive heart failure. Circulation 2001;103(20):2441-2446.

129. Al Hesayen A, Azevedo ER, Floras JS, Hollingshead S, Lopaschuk GD, Parker JD. Selective versus nonselective beta-adrenergic receptor blockade in chronic heart failure: differential effects on myocardial energy substrate utilization. Eur J Heart Fail 2005;7(4):618-623.

130. Kantor PF, Lucien A, Kozak R, Lopaschuk GD. The antianginal drug trimetazidine shifts cardiac energy metabolism from fatty acid oxidation to glucose oxidation by inhibiting mitochondrial long-chain 3-ketoacyl coenzyme A thiolase. Circ Res 2000;86(5):580-588.

131. MacInnes A, Fairman DA, Binding P et al. The antianginal agent trimetazidine does not exert its functional benefit via inhibition of mitochondrial long-chain 3-ketoacyl coenzyme A thiolase. Circ Res 2003;93(3):e26-e32.

132. Szwed H, Sadowski Z, Elikowski W et al. Combination treatment in stable effort angina using trimetazidine and metoprolol: results of a randomized, double-blind, multicentre study (TRIMPOL II). TRIMetazidine in POLand. Eur Heart J 2001;22(24):2267-2274.

133. Rosano GM, Vitale C, Sposato B, Mercuro G, Fini M. Trimetazidine improves left ventricular function in diabetic patients with coronary artery disease: a double-blind placebo-controlled study. Cardiovasc Diabetol 2003;2(1):16.

134. Thrainsdottir IS, von Bibra H, Malmberg K, Ryden L. Effects of trimetazidine on left ventricular function in patients with type 2 diabetes and heart failure. J Cardiovasc Pharmacol 2004;44(1):101-108.

135. McCormack JG, Barr RL, Wolff AA, Lopaschuk GD. Ranolazine stimulates glucose oxidation in normoxic, ischemic, and reperfused ischemic rat hearts. Circulation 1996;93(1):135-142.

136. Clarke B, Wyatt KM, McCormack JG. Ranolazine increases active pyruvate dehydrogenase in perfused normoxic rat hearts: evidence for an indirect mechanism. J Mol Cell Cardiol 1996;28(2):341-350.

137. Antzelevitch C, Belardinelli L, Zygmunt AC et al. Electrophysiological effects of ranolazine, a novel antianginal agent with antiarrhythmic properties. Circulation 2004;110(8):904-910.

138. McCullough PA. Chronic angina: new medical options for treatment. Rev Cardiovasc Med 2005;6(3):152-161.

139. Chaitman BR, Skettino SL, Parker JO et al. Anti-ischemic effects and long-term survival during ranolazine monotherapy in patients with chronic severe angina. J Am Coll Cardiol 2004;43(8):1375-1382.

140. Chaitman BR, Pepine CJ, Parker JO et al. Effects of ranolazine with atenolol, amlodipine, or diltiazem on exercise tolerance and angina frequency in patients with severe chronic angina: a randomized controlled trial. JAMA 2004;291(3):309-316.

141. Chandler MP, Stanley WC, Morita H et al. Short-term treatment with ranolazine improves mechanical efficiency in dogs with chronic heart failure. Circ Res 2002;91(4):278-280.

142. Sabbah HN, Chandler MP, Mishima T et al. Ranolazine, a partial fatty acid oxidation (pFOX) inhibitor, improves left ventricular function in dogs with chronic heart failure. J Card Fail 2002;8(6):416-422.

143. Leotta G, Maule S, Rabbia F et al. Relationship between QT interval and cardiovascular risk factors in healthy young subjects. J Hum Hypertens 2005;19(8):623-627.

144. Vilsboll T, Agerso H, Krarup T, Holst JJ. Similar elimination rates of glucagon-like peptide-1 in obese type 2 diabetic patients and healthy subjects. J Clin Endocrinol Metab 2003;88(1):220-224.

145. Ahren B, Simonsson E, Larsson H et al. Inhibition of dipeptidyl peptidase IV improves metabolic control over a 4-week study period in type 2 diabetes. Diabetes Care 2002;25(5):869-875.

146. Suzuki Y, Masumura Y, Kobayashi A, Yamazaki N, Harada Y, Osawa M. Myocardial carnitine deficiency in chronic heart failure. Lancet 1982;1(8263):116.

147. Regitz V, Bossaller C, Strasser R, Muller M, Shug AL, Fleck E. Metabolic alterations in end-stage and less severe heart failure--myocardial carnitine decrease. J Clin Chem Clin Biochem 1990;28(9):611-617.

148. Regitz V, Shug AL, Fleck E. Defective myocardial carnitine metabolism in congestive heart failure secondary to dilated cardiomyopathy and to coronary, hypertensive and valvular heart diseases. Am J Cardiol 1990;65(11):755-760.

149. Notomi Y, Setser RM, Shiota T et al. Assessment of left ventricular torsional deformation by Doppler tissue imaging: validation study with tagged magnetic resonance imaging. Circulation 2005;111(9):1141-1147.

150. Lang RM, Bierig M, Devereux RB et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005;18(12):1440-1463.

151. Park SH, Shub C, Nobrega TP, Bailey KR, Seward JB. Two-dimensional echocardiographic calculation of left ventricular mass as recommended by the American Society of Echocardiography: correlation with autopsy and M-mode echocardiography. J Am Soc Echocardiogr 1996;9(2):119-128.

152. Bohs LN, Trahey GE. A novel method for angle independent ultrasonic imaging of blood flow and tissue motion. IEEE Trans Biomed Eng 1991;38(3):280-286.

153. Notomi Y, Martin-Miklovic MG, Oryszak SJ et al. Enhanced ventricular untwisting during exercise: a mechanistic manifestation of elastic recoil described by Doppler tissue imaging. Circulation 2006;113(21):2524-2533.

154. Becker M, Hoffmann R, Kuhl HP et al. Analysis of myocardial deformation based on ultrasonic pixel tracking to determine transmurality in chronic myocardial infarction. Eur Heart J 2006;27(21):2560-2566.

155. Helle-Valle T, Crosby J, Edvardsen T et al. New noninvasive method for assessment of left ventricular rotation: speckle tracking echocardiography. Circulation 2005;112(20):3149-3156.

156. Reisner SA, Lysyansky P, Agmon Y, Mutlak D, Lessick J, Friedman Z. Global longitudinal strain: a novel index of left ventricular systolic function. J Am Soc Echocardiogr 2004;17(6):630-633.

157. Notomi Y, Lysyansky P, Setser RM et al. Measurement of ventricular torsion by two-dimensional ultrasound speckle tracking imaging. J Am Coll Cardiol 2005;45(12):2034-2041.

158. Bruce RA, McDonough JR. Stress testing in screening for cardiovascular disease. Bull N Y Acad Med 1969;45(12):1288-1305.

159. Davies NJ, Denison DM. The measurement of metabolic gas exchange and minute volume by mass spectrometry alone. Respir Physiol 1979;36(2):261-267.

160. Ordidge RJ, Van de Vyver FL. Re: Separate water and fat MR images. Radiology 1985;157(2):551-553.

161. Vanhamme L, Sundin T, Hecke PV, Huffel SV. MR spectroscopy quantitation: a review of time-domain methods. NMR Biomed 2001;14(4):233-246.

162. Cavassila S, Deval S, Huegen C, van Ormondt D, Graveron-Demilly D. Cramer-Rao bounds: an evaluation tool for quantitation. NMR Biomed 2001;14(4):278-283.

163. Conway MA, Bottomley PA, Ouwerkerk R, Radda GK, Rajagopalan B. Mitral regurgitation: impaired systolic function, eccentric hypertrophy, and increased severity are linked to lower phosphocreatine/ATP ratios in humans. Circulation 1998;97(17):1716-1723.

164. Mancini DM, Bolinger L, Li H, Kendrick K, Chance B, Wilson JR. Validation of near-infrared spectroscopy in humans. J Appl Physiol 1994;77(6):2740-2747.

165. Piantadosi CA, Duhaylongsod FG. Near infrared spectroscopy: in situ studies of skeletal and cardiac muscle. Adv Exp Med Biol 1994;361:157-161.

166. van Beekvelt MC, Colier WN, Wevers RA, van Engelen BG. Performance of near-infrared spectroscopy in measuring local O(2) consumption and blood flow in skeletal muscle. J Appl Physiol 2001;90(2):511-519.

167. van Beekvelt MC, van Engelen BG, Wevers RA, Colier WN. In vivo quantitative near-infrared spectroscopy in skeletal muscle during incremental isometric handgrip exercise. Clin Physiol Funct Imaging 2002;22(3):210-217.

168. O'Connell JB, Bristow MR. Economic impact of heart failure in the United States: time for a different approach. J Heart Lung Transplant 1994;13(4):S107-S112.

169. Coats AJ. Heart failure: What causes the symptoms of heart failure? Heart 2001;86(5):574-578.

170. Volterrani M, Clark AL, Ludman PF et al. Predictors of exercise capacity in chronic heart failure. Eur Heart J 1994;15(6):801-809.

171. Drexler H, Riede U, Munzel T, Konig H, Funke E, Just H. Alterations of skeletal muscle in chronic heart failure. Circulation 1992;85(5):1751-1759.

172. Mancini DM, Coyle E, Coggan A et al. Contribution of intrinsic skeletal muscle changes to 31P NMR skeletal muscle metabolic abnormalities in patients with chronic heart failure. Circulation 1989;80(5):1338-1346.

173. Massie BM, Simonini A, Sahgal P, Wells L, Dudley GA. Relation of systemic and local muscle exercise capacity to skeletal muscle characteristics in men with congestive heart failure. J Am Coll Cardiol 1996;27(1):140-145.

174. Opasich C, Aquilani R, Dossena M et al. Biochemical analysis of muscle biopsy in overnight fasting patients with severe chronic heart failure. Eur Heart J 1996;17(11):1686-1693.

175. Sullivan MJ, Green HJ, Cobb FR. Skeletal muscle biochemistry and histology in ambulatory patients with long-term heart failure. Circulation 1990;81(2):518-527.

176. Sullivan MJ, Duscha BD, Klitgaard H, Kraus WE, Cobb FR, Saltin B. Altered expression of myosin heavy chain in human skeletal muscle in chronic heart failure. Med Sci Sports Exerc 1997;29(7):860-866.

177. Arnolda L, Brosnan J, Rajagopalan B, Radda GK. Skeletal muscle metabolism in heart failure in rats. Am J Physiol 1991;261(2 Pt 2):H434-H442.

178. Bernocchi P, Ceconi C, Pedersini P, Pasini E, Curello S, Ferrari R. Skeletal muscle metabolism in experimental heart failure. J Mol Cell Cardiol 1996;28(11):2263-2273.

179. Sabbah HN, Hansen-Smith F, Sharov VG et al. Decreased proportion of type I myofibers in skeletal muscle of dogs with chronic heart failure. Circulation 1993;87(5):1729-1737.

180. Simonini A, Long CS, Dudley GA, Yue P, McElhinny J, Massie BM. Heart failure in rats causes changes in skeletal muscle morphology and gene expression that are not explained by reduced activity. Circ Res 1996;79(1):128-136.

181. Simonini A, Massie BM, Long CS, Qi M, Samarel AM. Alterations in skeletal muscle gene expression in the rat with chronic congestive heart failure. J Mol Cell Cardiol 1996;28(8):1683-1691.

182. Delp MD, Duan C, Mattson JP, Musch TI. Changes in skeletal muscle biochemistry and histology relative to fiber type in rats with heart failure. J Appl Physiol 1997;83(4):1291-1299.

183. Abozguia K, Shivu GN, Ahmed I, Phan TT, Frenneaux MP. The heart metabolism: pathophysiological aspects in ischaemia and heart failure. Curr Pharm Des 2009;15(8):827-835.

184. Davis SL, Fadel PJ, Cui J, Thomas GD, Crandall CG. Skin blood flow influences near-infrared spectroscopy-derived measurements of tissue oxygenation during heat stress. J Appl Physiol 2006;100(1):221-224.

185. Jondeau G, Katz SD, Zohman L et al. Active skeletal muscle mass and cardiopulmonary reserve. Failure to attain peak aerobic capacity during maximal bicycle exercise in patients with severe congestive heart failure. Circulation 1992;86(5):1351-1356.

186. Magnusson G, Kaijser L, Rong H, Isberg B, Sylven C, Saltin B. Exercise capacity in heart failure patients: relative importance of heart and skeletal muscle. Clin Physiol 1996;16(2):183-195.

187. Wilson JR, Mancini DM, McCully K, Ferraro N, Lanoce V, Chance B. Noninvasive detection of skeletal muscle underperfusion with near-infrared spectroscopy in patients with heart failure. Circulation 1989;80(6):1668-1674.

188. Nioka S, Kime R, Sunar U et al. A novel method to measure regional muscle blood flow continuously using NIRS kinetics information. Dyn Med 2006;5:5.

189. Massie B, Conway M, Yonge R et al. Skeletal muscle metabolism in patients with congestive heart failure: relation to clinical severity and blood flow. Circulation 1987;76(5):1009-1019.

190. Andrews R, Walsh JT, Evans A, Curtis S, Cowley AJ. Abnormalities of skeletal muscle metabolism in patients with chronic heart failure: evidence that they are present at rest. Heart 1997;77(2):159-163.

191. Ning XH, Zhang J, Liu J et al. Signaling and expression for mitochondrial membrane proteins during left ventricular remodeling and contractile failure after myocardial infarction. J Am Coll Cardiol 2000;36(1):282-287.

192. Schaper J, Froede R, Hein S et al. Impairment of the myocardial ultrastructure and changes of the cytoskeleton in dilated cardiomyopathy. Circulation 1991;83(2):504-514.

193. Mancini DM, Wilson JR, Bolinger L et al. *In vivo* magnetic resonance spectroscopy measurement of deoxymyoglobin during exercise in patients with heart failure. Demonstration of abnormal muscle metabolism despite adequate oxygenation. Circulation 1994;90(1):500-508.

194. Clark AL, Poole-Wilson PA, Coats AJ. Exercise limitation in chronic heart failure: central role of the periphery. J Am Coll Cardiol 1996;28(5):1092-1102.

195. Massie BM, Conway M, Rajagopalan B et al. Skeletal muscle metabolism during exercise under ischemic conditions in congestive heart failure. Evidence for abnormalities unrelated to blood flow. Circulation 1988;78(2):320-326.

196. Massie BM, Conway M, Yonge R et al. 31P nuclear magnetic resonance evidence of abnormal skeletal muscle metabolism in patients with congestive heart failure. Am J Cardiol 1987;60(4):309-315.

197. Chen C, Li L, Chen LL et al. Incremental doses of dobutamine induce a biphasic response in dysfunctional left ventricular regions subtending coronary stenoses. Circulation 1995;92(4):756-766.

198. Sidossis LS, Gastaldelli A, Klein S, Wolfe RR. Regulation of plasma fatty acid oxidation during low- and high-intensity exercise. Am J Physiol 1997;272(6 Pt 1):E1065-E1070.

199. Lang CC, Chomsky DB, Rayos G, Yeoh TK, Wilson JR. Skeletal muscle mass and exercise performance in stable ambulatory patients with heart failure. J Appl Physiol 1997;82(1):257-261.

200. Mettauer B, Zoll J, Garnier A, Ventura-Clapier R. Heart failure: a model of cardiac and skeletal muscle energetic failure. Pflugers Arch 2006;452(6):653-666.

201. Oh JK, Hatle L, Tajik AJ, Little WC. Diastolic heart failure can be diagnosed by comprehensive two-dimensional and Doppler echocardiography. J Am Coll Cardiol 2006;47(3):500-506.

202. Hansen DE, Daughters GT, Alderman EL, Ingels NB, Stinson EB, Miller DC. Effect of volume loading, pressure loading, and inotropic stimulation on left ventricular torsion in humans. Circulation 1991;83(4):1315-1326.

203. Dong SJ, Hees PS, Huang WM, Buffer SA, Jr., Weiss JL, Shapiro EP. Independent effects of preload, afterload, and contractility on left ventricular torsion. Am J Physiol 1999;277(3 Pt 2):H1053-H1060.

204. Dong SJ, Hees PS, Siu CO, Weiss JL, Shapiro EP. MRI assessment of LV relaxation by untwisting rate: a new isovolumic phase measure of tau. Am J Physiol Heart Circ Physiol 2001;281(5):H2002-H2009.

205. Maron BJ, Gottdiener JS, Epstein SE. Patterns and significance of distribution of left ventricular hypertrophy in hypertrophic cardiomyopathy. A wide angle, two dimensional echocardiographic study of 125 patients. Am J Cardiol 1981;48(3):418-428.

206. Shapiro LM, Kleinebenne A, McKenna WJ. The distribution of left ventricular hypertrophy in hypertrophic cardiomyopathy: comparison to athletes and hypertensives. Eur Heart J 1985;6(11):967-974.

207. Amundsen BH, Helle-Valle T, Edvardsen T et al. Noninvasive myocardial strain measurement by speckle tracking echocardiography: validation against sonomicrometry and tagged magnetic resonance imaging. J Am Coll Cardiol 2006;47(4):789-793.

208. Serri K, Reant P, Lafitte M et al. Global and regional myocardial function quantification by two-dimensional strain: application in hypertrophic cardiomyopathy. J Am Coll Cardiol 2006;47(6):1175-1181.

209. Nagueh SF, Bachinski LL, Meyer D et al. Tissue Doppler imaging consistently detects myocardial abnormalities in patients with hypertrophic cardiomyopathy and provides a novel means for an early diagnosis before and independently of hypertrophy. Circulation 2001;104(2):128-130.

210. Carasso S, Yang H, Woo A et al. Systolic myocardial mechanics in hypertrophic cardiomyopathy: novel concepts and implications for clinical status. J Am Soc Echocardiogr 2008;21(6):675-683.

211. Maier SE, Fischer SE, McKinnon GC, Hess OM, Krayenbuehl HP, Boesiger P. Evaluation of left ventricular segmental wall motion in hypertrophic cardiomyopathy with myocardial tagging. Circulation 1992;86(6):1919-1928.

212. Beache GM, Wedeen VJ, Weisskoff RM et al. Intramural mechanics in hypertrophic cardiomyopathy: functional mapping with strain-rate MR imaging. Radiology 1995;197(1):117-124.

213. Maron BJ, Epstein SE. Hypertrophic cardiomyopathy. Recent observations regarding the specificity of three hallmarks of the disease: asymmetric septal hypertrophy, septal disorganization and systolic anterior motion of the anterior mitral leaflet. Am J Cardiol 1980;45(1):141-154.

214. Nihoyannopoulos P, Karatasakis G, Frenneaux M, McKenna WJ, Oakley CM. Diastolic function in hypertrophic cardiomyopathy: relation to exercise capacity. J Am Coll Cardiol 1992;19(3):536-540.

215. Shapiro LM, Gibson DG. Patterns of diastolic dysfunction in left ventricular hypertrophy. Br Heart J 1988;59(4):438-445.

216. Spirito P, Maron BJ, Chiarella F et al. Diastolic abnormalities in patients with hypertrophic cardiomyopathy: relation to magnitude of left ventricular hypertrophy. Circulation 1985;72(2):310-316.

217. Nagueh SF, Kopelen HA, Lim DS et al. Tissue Doppler imaging consistently detects myocardial contraction and relaxation abnormalities, irrespective of cardiac hypertrophy, in a transgenic rabbit model of human hypertrophic cardiomyopathy. Circulation 2000;102(12):1346-1350.

218. Sanderson JE, Gibson DG, Brown DJ, Goodwin JF. Left ventricular filling in hypertrophic cardiomyopathy. An angiographic study. Br Heart J 1977;39(6):661-670.

219. Sanderson JE, Wang M, Yu CM. Tissue Doppler imaging for predicting outcome in patients with cardiovascular disease. Curr Opin Cardiol 2004;19(5):458-463.

220. Xie MX, Zhang L, Lu Q et al. [Left ventricular rotation and twist in patients with hypertrophic cardiomyopathy evaluated by two-dimensional ultrasound speckle-tracking imaging]. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 2008;30(1):58-62.

221. Lele SS, Macfarlane D, Morrison S, Thomson H, Khafagi F, Frenneaux M. Determinants of exercise capacity in patients with coronary artery disease and mild to moderate systolic dysfunction. Role of heart rate and diastolic filling abnormalities. Eur Heart J 1996;17(2):204-212.

222. Knudtson ML, Galbraith PD, Hildebrand KL, Tyberg JV, Beyar R. Dynamics of left ventricular apex rotation during angioplasty: a sensitive index of ischemic dysfunction. Circulation 1997;96(3):801-808.

223. Helmes M, Lim CC, Liao R, Bharti A, Cui L, Sawyer DB. Titin determines the Frank-Starling relation in early diastole. J Gen Physiol 2003;121(2):97-110.

224. Ashikaga H, Criscione JC, Omens JH, Covell JW, Ingels NB, Jr. Transmural left ventricular mechanics underlying torsional recoil during relaxation. Am J Physiol Heart Circ Physiol 2004;286(2):H640-H647.

225. Rademakers FE, Buchalter MB, Rogers WJ et al. Dissociation between left ventricular untwisting and filling. Accentuation by catecholamines. Circulation 1992;85(4):1572-1581.

226. Gibson DG, Sanderson JE, Traill TA, Brown DJ, Goodwin JF. Regional left ventricular wall movement in hypertrophic cardiomyopathy. Br Heart J 1978;40(12):1327-1333.

227. Notomi Y, Popovic ZB, Yamada H et al. Ventricular untwisting: a temporal link between left ventricular relaxation and suction. Am J Physiol Heart Circ Physiol 2008;294(1):H505-H513.

228. Rovner A, Smith R, Greenberg NL et al. Improvement in diastolic intraventricular pressure gradients in patients with HOCM after ethanol septal reduction. Am J Physiol Heart Circ Physiol 2003;285(6):H2492-H2499.

229. Bottomley PA. Noninvasive study of high-energy phosphate metabolism in human heart by depth-resolved 31P NMR spectroscopy. Science 1985;229(4715):769-772.

230. Weiss RG, Gerstenblith G, Bottomley PA. ATP flux through creatine kinase in the normal, stressed, and failing human heart. Proc Natl Acad Sci U S A 2005;102(3):808-813.

231. Smith CS, Bottomley PA, Schulman SP, Gerstenblith G, Weiss RG. Altered creatine kinase adenosine triphosphate kinetics in failing hypertrophied human myocardium. Circulation 2006;114(11):1151-1158.

232. Ye Y, Gong G, Ochiai K, Liu J, Zhang J. High-energy phosphate metabolism and creatine kinase in failing hearts: a new porcine model. Circulation 2001;103(11):1570-1576.

233. Fragasso G, Perseghin G, De CF et al. Effects of metabolic modulation by trimetazidine on left ventricular function and phosphocreatine/adenosine triphosphate ratio in patients with heart failure. Eur Heart J 2006;27(8):942-948.

234. Weiss RG, Bottomley PA, Hardy CJ, Gerstenblith G. Regional myocardial metabolism of high-energy phosphates during isometric exercise in patients with coronary artery disease. N Engl J Med 1990;323(23):1593-1600.

235. Maslov MY, Chacko VP, Stuber M et al. Altered high-energy phosphate metabolism predicts contractile dysfunction and subsequent ventricular remodeling in pressure-overload hypertrophy mice. Am J Physiol Heart Circ Physiol 2007;292(1):H387-H391.

236. Scheuermann-Freestone M, Madsen PL, Manners D et al. Abnormal cardiac and skeletal muscle energy metabolism in patients with type 2 diabetes. Circulation 2003;107(24):3040-3046.

237. de RA, Doornbos J, Luyten PR, Oosterwaal LJ, van der Wall EE, den Hollander JA. Cardiac metabolism in patients with dilated and hypertrophic cardiomyopathy: assessment with proton-decoupled P-31 MR spectroscopy. J Magn Reson Imaging 1992;2(6):711-719.

238. Sakuma H, Takeda K, Tagami T et al. 31P MR spectroscopy in hypertrophic cardiomyopathy: comparison with Tl-201 myocardial perfusion imaging. Am Heart J 1993;125(5 Pt 1):1323-1328.

239. Lee J, Hu Q, Nakamura Y et al. Open-chest 31P magnetic resonance spectroscopy of mouse heart at 4.7 Tesla. J Magn Reson Imaging 2006;24(6):1269-1276.

240. Tyler DJ, Hudsmith LE, Clarke K, Neubauer S, Robson MD. A comparison of cardiac (31)P MRS at 1.5 and 3 T. NMR Biomed 2008;21(8):793-798.

241. Metzler B, Schocke MF, Steinboeck P et al. Decreased high-energy phosphate ratios in the myocardium of men with diabetes mellitus type I. J Cardiovasc Magn Reson 2002;4(4):493-502.

242. Kozerke S, Schar M, Lamb HJ, Boesiger P. Volume tracking cardiac 31P spectroscopy. Magn Reson Med 2002;48(2):380-384.

243. Schar M, Kozerke S, Boesiger P. Navigator gating and volume tracking for double-triggered cardiac proton spectroscopy at 3 Tesla. Magn Reson Med 2004;51(6):1091-1095.

244. Barker PB, Hearshen DO, Boska MD. Single-voxel proton MRS of the human brain at 1.5T and 3.0T. Magn Reson Med 2001;45(5):765-769.

245. Gruetter R, Weisdorf SA, Rajanayagan V et al. Resolution improvements in in vivo 1H NMR spectra with increased magnetic field strength. J Magn Reson 1998;135(1):260-264.

246. Schar M, Kozerke S, Fischer SE, Boesiger P. Cardiac SSFP imaging at 3 Tesla. Magn Reson Med 2004;51(4):799-806.

247. Atherton JJ, Moore TD, Lele SS et al. Diastolic ventricular interaction in chronic heart failure. Lancet 1997;349(9067):1720-1724.

248. Phan TT, Abozguia K, Nallur SG et al. Heart failure with preserved ejection fraction is characterized by dynamic impairment of active relaxation and contraction of the left ventricle on exercise and associated with myocardial energy deficiency. J Am Coll Cardiol 2009;54(5):402-409.

249. Najjar SS, Schulman SP, Gerstenblith G et al. Age and gender affect ventricular-vascular coupling during aerobic exercise. J Am Coll Cardiol 2004;44(3):611-617.

250. Shivu GN, Abozguia K, Phan TT, Ahmed I, Henning A, Frenneaux M. (31)P magnetic resonance spectroscopy to measure in vivo cardiac energetics in normal myocardium and hypertrophic cardiomyopathy: Experiences at 3T. Eur J Radiol 2008.

251. Naressi A, Couturier C, Castang I, de BR, Graveron-Demilly D. Java-based graphical user interface for MRUI, a software package for quantitation of in vivo/medical magnetic resonance spectroscopy signals. Comput Biol Med 2001;31(4):269-286.

252. Shivu GN, Abozguia K, Phan TT, Ahmed I, Henning A, Frenneaux M. (31)P magnetic resonance spectroscopy to measure in vivo cardiac energetics in normal myocardium and hypertrophic cardiomyopathy: Experiences at 3T. Eur J Radiol 2008.

253. Betocchi S, Bonow RO, Bacharach SL, Rosing DR, Maron BJ, Green MV. Isovolumic relaxation period in hypertrophic cardiomyopathy: assessment by radionuclide angiography. J Am Coll Cardiol 1986;7(1):74-81.

254. Chen YT, Chang KC, Hu WS, Wang SJ, Chiang BN. Left ventricular diastolic function in hypertrophic cardiomyopathy: assessment by radionuclide angiography. Int J Cardiol 1987;15(2):185-193.

255. Neubauer S, Krahe T, Schindler R et al. 31P magnetic resonance spectroscopy in dilated cardiomyopathy and coronary artery disease. Altered cardiac high-energy phosphate metabolism in heart failure. Circulation 1992;86(6):1810-1818.

256. Scheuermann-Freestone M, Madsen PL, Manners D et al. Abnormal cardiac and skeletal muscle energy metabolism in patients with type 2 diabetes. Circulation 2003;107(24):3040-3046.

257. Abozguia K, Clarke K, Lee L, Frenneaux M. Modification of myocardial substrate use as a therapy for heart failure. Nat Clin Pract Cardiovasc Med 2006;3(9):490-498.

258. Horgan JH, O'Callaghan WG, Teo KK. Therapy of angina pectoris with low-dose perhexiline. J Cardiovasc Pharmacol 1981;3(3):566-572.

259. Horowitz JD, Mashford ML. Perhexiline maleate in the treatment of severe angina pectoris. Med J Aust 1979;1(11):485-488.

260. Rosano GM, Vitale C, Sposato B, Mercuro G, Fini M. Trimetazidine improves left ventricular function in diabetic patients with coronary artery disease: a double-blind placebo-controlled study. Cardiovasc Diabetol 2003;2(1):16.

261. Fragasso G, Perseghin G, De Cobelli F et al. Effects of metabolic modulation by trimetazidine on left ventricular function and phosphocreatine/adenosine triphosphate ratio in patients with heart failure. Eur Heart J 2006;27(8):942-948.

262. Lee L, Campbell R, Scheuermann-Freestone M et al. Metabolic modulation with perhexiline in chronic heart failure: a randomized, controlled trial of short-term use of a novel treatment. Circulation 2005;112(21):3280-3288.

263. Sorajja P, Valeti U, Nishimura RA et al. Outcome of alcohol septal ablation for obstructive hypertrophic cardiomyopathy. Circulation 2008;118(2):131-139.

264. Spirito P, Seidman CE, McKenna WJ, Maron BJ. The management of hypertrophic cardiomyopathy. N Engl J Med 1997;336(11):775-785.

265. Elliott P, McKenna WJ. Hypertrophic cardiomyopathy. Lancet 2004;363(9424):1881-1891.

266. Bottinelli R, Coviello DA, Redwood CS et al. A mutant tropomyosin that causes hypertrophic cardiomyopathy is expressed in vivo and associated with an increased calcium sensitivity. Circ Res 1998;82(1):106-115.

267. Frey N, Brixius K, Schwinger RH et al. Alterations of tension-dependent ATP utilization in a transgenic rat model of hypertrophic cardiomyopathy. J Biol Chem 2006;281(40):29575-29582.

268. Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and alpha-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res 2007;101(12):1266-1273.

269. Belus A, Piroddi N, Scellini B et al. The familial hypertrophic cardiomyopathy-associated myosin mutation R403Q accelerates tension generation and relaxation of human cardiac myofibrils. J Physiol 2008;586(Pt 15):3639-3644.

270. Ashrafian H, Redwood C, Blair E, Watkins H. Hypertrophic cardiomyopathy:a paradigm for myocardial energy depletion. Trends Genet 2003;19(5):263-268.

271. Petersen SE, Jerosch-Herold M, Hudsmith LE et al. Evidence for microvascular dysfunction in hypertrophic cardiomyopathy: new insights from multiparametric magnetic resonance imaging. Circulation 2007;115(18):2418-2425.

272. Jeffrey FM, Alvarez L, Diczku V, Sherry AD, Malloy CR. Direct evidence that perhexiline modifies myocardial substrate utilization from fatty acids to lactate. J Cardiovasc Pharmacol 1995;25(3):469-472.

273. Ashrafian H, Horowitz JD, Frenneaux MP. Perhexiline. Cardiovasc Drug Rev 2007;25(1):76-97.

274. Lombardi R, Rodriguez G, Chen SN et al. Resolution of established cardiac hypertrophy and fibrosis and prevention of systolic dysfunction in a transgenic rabbit model of human cardiomyopathy through thiol-sensitive mechanisms. Circulation 2009;119(10):1398-1407.

275. Jacques AM, Copeland O, Messer AE et al. Myosin binding protein C phosphorylation in normal, hypertrophic and failing human heart muscle. J Mol Cell Cardiol 2008;45(2):209-216.

276. van Dijk SJ, Dooijes D, dos RC et al. Cardiac myosin-binding protein C mutations and hypertrophic cardiomyopathy: haploinsufficiency, deranged phosphorylation, and cardiomyocyte dysfunction. Circulation 2009;119(11):1473-1483.

277. Yaplito-Lee J, Weintraub R, Jamsen K, Chow CW, Thorburn DR, Boneh A. Cardiac manifestations in oxidative phosphorylation disorders of childhood. J Pediatr 2007;150(4):407-411.

278. Neubauer S. The failing heart--an engine out of fuel. N Engl J Med 2007;356(11):1140-1151.

279. Kagaya Y, Weinberg EO, Ito N, Mochizuki T, Barry WH, Lorell BH. Glycolytic inhibition: effects on diastolic relaxation and intracellular calcium handling in hypertrophied rat ventricular myocytes. J Clin Invest 1995;95(6):2766-2776.

280. Ashrafian H, Horowitz JD, Frenneaux MP. Perhexiline. Cardiovasc Drug Rev 2007;25(1):76-97.

281. Ashrafian H, Frenneaux MP, Opie LH. Metabolic mechanisms in heart failure. Circulation 2007;116(4):434-448.

282. Firoozi S, Elliott PM, Sharma S et al. Septal myotomy-myectomy and transcoronary septal alcohol ablation in hypertrophic obstructive cardiomyopathy. A comparison of clinical, haemodynamic and exercise outcomes. Eur Heart J 2002;23(20):1617-1624.

283. Faber L, Welge D, Fassbender D, Schmidt HK, Horstkotte D, Seggewiss H. One-year follow-up of percutaneous septal ablation for symptomatic hypertrophic obstructive cardiomyopathy in 312

patients: predictors of hemodynamic and clinical response. Clin Res Cardiol 2007;96(12):864-873.

284. Lee L, Campbell R, Scheuermann-Freestone M et al. Metabolic modulation with perhexiline in chronic heart failure: a randomized, controlled trial of short-term use of a novel treatment. Circulation 2005;112(21):3280-3288.

285. Ommen SR, Nishimura RA, Squires RW, Schaff HV, Danielson GK, Tajik AJ. Comparison of dual-chamber pacing versus septal myectomy for the treatment of patients with hypertrophic obstructive cardiomyopathy: a comparison of objective hemodynamic and exercise end points. J Am Coll Cardiol 1999;34(1):191-196.

286. Maron BJ, Nishimura RA, McKenna WJ, Rakowski H, Josephson ME, Kieval RS. Assessment of permanent dual-chamber pacing as a treatment for drug-refractory symptomatic patients with obstructive hypertrophic cardiomyopathy. A randomized, double-blind, crossover study (M-PATHY). Circulation 1999;99(22):2927-2933.

287. Opdahl A, Helle-Valle T, Remme EW et al. Apical Rotation by Speckle Tracking Echocardiography: A Simplified Bedside Index of Left Ventricular Twist. J Am Soc Echocardiogr 2008.

288. Takeuchi M, Borden WB, Nakai H et al. Reduced and delayed untwisting of the left ventricle in patients with hypertension and left ventricular hypertrophy: a study using two-dimensional speckle tracking imaging. Eur Heart J 2007;28(22):2756-2762.

289. Scheuermann-Freestone M, Madsen PL, Manners D et al. Abnormal cardiac and skeletal muscle energy metabolism in patients with type 2 diabetes. Circulation 2003;107(24):3040-3046.

290. Olivotto I, Ommen SR, Maron MS, Cecchi F, Maron BJ. Surgical myectomy versus alcohol septal ablation for obstructive hypertrophic cardiomyopathy. Will there ever be a randomized trial? J Am Coll Cardiol 2007;50(9):831-834.