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(54) Title: TREATMENT OF HEART FAILURE WITH NORMAL EJECTION FRACTION

(57) Abstract: The invention relates to perhexiline, or a pharmaceutically acceptable salt thereof, for use in the treatment of HfπEF, as well as to a method of treating HfnEF, which comprises administering to an animal in need thereof an effective amount of perhexiline, or a pharmaceutically acceptable salt thereof, to treat said HFnEF. The invention further relates to a treatment programme for treating HFnEF, which involves the co-use or co-administration of perhexiline with one or more other compounds that are advantageous in treating HFnEF or the symptoms thereof.

TREATMENT OF HEART FAILURE WITH NORMAL EJECTION FRACTION

The invention relates to treatment of heart failure with normal left ventricular (LV) ejection fraction syndrome (HFnEF).

Background of the Invention

Significant advances in therapy for heart failure (HF) with impaired systolic function have improved quality of life, and increased survival. However up to 50% of patients who have clinical evidence of HF are found to have a relatively (or near) normal left ventricular ejection fraction (HF with normal left ventricular (LV) ejection fraction syndrome (HFnEF), also referred to as HF with preserved left ventricular ejection fraction syndrome (HFpEF). Patients with HFnEF represent a rapidly increasing proportion of patients hospitalised and suffering mortality from heart failure.

Despite a normal EF, HFnEF patients manifest subtle systolic dysfunction but the principal abnormality in most is a disorder of active relaxation and/or passive filling of the LV. However resting measures of active relaxation and filling relate poorly to symptoms and exercise capacity therefore no 'gold standard' diagnostic echocardiographic test exists for HFnEF. Effective ventricular filling results from a highly energy dependent active relaxation process and from passive filling which is dependent on loading conditions as well as the intrinsic (passive) properties of the LV. Since both these parameters change markedly during exercise due to sympathetic activation, it is not surprising that these resting parameters are so poorly predictive of exercise capacity and symptoms.

The treatment of patients with HFnEF is discussed in Hunt et al., "ACC/AHA 2005 Guideline Update for the Diagnosis and Management of Chronic Heart Failure in the Adult", 2005, Section 4.3.2, available at www.acc.org.

Perhexiline (2-(2,2-dicyclohexylethyl) piperidine) is a known anti-anginal agent that operates principally by virtue of its ability to shift metabolism in the heart from free fatty acid metabolism to glucose, which is more energy efficient.

WO-A-2005/087233 discloses the use of perhexiline for the treatment of chronic heart failure (CHF) where the CHF is a result of an initial inciting influence of ischaemia or where the CHF is a result of an initial non-ischaemic inciting influence.

Summary of the Invention

According to a first aspect of the present invention, there is provided a method of treating HFnEF, which comprises administering to an animal in need thereof an effective amount of perhexiline, or a pharmaceutically acceptable salt thereof, to treat said HFnEF. The animal is preferably a mammal and most preferably a human.

According to another aspect of the present invention, perhexiline, or a pharmaceutically acceptable salt thereof, is provided for use in the treatment of HfnEF.

According to a further aspect of the invention there is provided a treatment programme for treating HFnEF, which involves the co-use or co-administration of perhexiline or pharmaceutically acceptable salt thereof with one or more other compounds that are advantageous in treating HFnEF or the symptoms thereof, for example a diuretic, an angiotensin receptor blocker or a calcium channel blocker.

Brief Description of the Drawings

Figure 1 displays variables correlating with Aerobic Exercise Capacity (VO_2max) in HFnEF patients and controls.

Figure 2 shows MR images of a patient with HFpEF lying prone over a ^{31}P surface coil (Panel A) and the corresponding localized ^{31}P MR spectra from the left ventricle (Panel B). Panel C is Individual PCr/ γ- ATP ratio in Patients with HfpEF and Controls.

Figure 3 is a flow chart of a study carried out to establish a causative role for energy deficiency and to evaluate the impact of perhexiline on cardiac energy status in HCM.

Figures 4A-4D represent the baseline data of HCM vs controls, more particularly:

Figure 4A represents the peak oxygen consumption (peak V_{O2}) results;

Figure 4B represents the diastolic ventricular filling results (nTTPF, normalized for heart rate Time To Peak Filling) and shows that PCr/ATP ratio (a measure of cardiac energetic state) is lower in HCM patients versus controls.;

Figure 4C is an example of ^{31}P cardiac spectra of a HCM patient in which Point 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, and shows that nTTPF (a measure of the rate of

active relaxation of the LV) is essentially unchanged on exercise in the controls bu abnormally slows in the HCM patients; and

Figure 4D represent the myocardial energetic results (PCr/ γ ATP ratio) and shows that exercise capacity (peak VO2) is lower in HCM patients versus controls.

Figures 5A and 5B respectively represent the effect of Placebo and Perhexiline on peak oxygen consumption (peak V_{O2}), p = 0.003 and myocardial energetic (PCr/ γ ATP ratio), p = 0.003, where the p value represents the significant difference between perhexiline and placebo response. Peak VO2 (exercise capacity) increases with Perhexiline (Figure 5A). Perhexiline improves PCr/ATP ratio (energetic status of heart), but this was unchanged in the placebo group (Figure 5B).

Figures 5C and 5D respectively represent nTTPF changes in the placebo group (3C) and the perhexiline group (3D), p = 0.03, where the p value represents the significant difference between perhexiline and placebo response. In the placebo group nTTPF (a measure of the rate of LV active relaxation) abnormally lengthened at baseline and on treatment. The response in healthy controls is shown in dotted lines. Perhexiline (Figure 5D) normalises the response to similar to that seen in healthy controls (also shown in dotted lines).

Figure 5E and 5F illustrate that NYHA score (of breathlessness) falls (improves) with perhexiline (5E) and Minnesota living with heart failure questionnaire score falls (= improved quality of life) on perhexiline (5F).

Figure 6 illustrate the causative role for energy deficiency in the pathophysiology of HCM.

Detailed description of the invention

The findings of the study reported in Example 1 herein are that a) HfpEF patients manifest a significant reduction in PCr/ATP ratio at rest, indicating impairment of myocardial energy 'reserves' and b) during exercise, the energetically demanding active relaxation stage of diastole lengthened in patients (vs. a shortening in controls) and there was also a failure of contractile function to increase in patients. These abnormalities together resulted in a lower stroke volume on exercise. It was also found that HfpEF patients demonstrated chronotropic incompetence on exercise.

These findings correlate closely with findings in a study of patients with hypertrophic

cardiomyopathy (HCM), which is reported in Example 2. The study of Example 2 also demonstrated the effectiveness of the agent perhexiline in the treatment of patients with HCM. Because of the related pathophysiology of HfnEF and HCM, the inventors are able to predict, based on the effectiveness of perhexiline in the treatment of HCM, that this same agent will be an effective therapeutic agent for treatment of HFnEF.

In aspects of the present invention, the perhexiline exists in the form of a salt of perhexiline, preferably the maleate salt. The perhexiline may be used at doses titrated to achieve therapeutic but non-toxic plasma perhexiline levels (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). Typical doses for a normal patient would be 100mg to 300mg daily, although smaller doses may be appropriate for patients who are slow metabolisers of perhexiline.

Physiologically acceptable formulations, such as salts, of the compound perhexiline, may be used in the invention. Additionally, a medicament may be formulated for administration in any convenient way and the invention therefore also includes within its scope use of the medicament in a conventional manner in a mixture with one or more physiologically acceptable carriers or excipients. Preferably, the carriers should be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof. The medicament may be formulated for oral, buccal, parental, intravenous or rectal administration. Additionally, or alternatively, the medicament may be formulated in a more conventional form such as a tablet, capsule, syrup, elixir or any other known oral dosage form.

The invention is illustrated by the following non-limiting examples.

Example 1

The role of exercise related changes was evaluated in left ventricular (LV) relaxation and of vasculo-ventricular coupling as the mechanism of exercise limitation in patients with heart failure with normal (or preserved) LV ejection fraction (HFnEF) and whether cardiac energetic impairment may underlie these abnormalities.

The study involved 37 patients with HFpEF and 20 matched controls. Vasculo-ventricular coupling (VVC) and Time to Peak LV Filling (a measure of LV active

relaxation) (nTTPF) were assessed at rest and on exercise by Multiple Uptake Gated Acquisition scanning. Cardiac energetic status (PCr/ATP ratio) was assessed by ^{31}P Magnetic Resonance Spectroscopy. At rest nTTPF and VVC were similar in patients and controls. Cardiac PCr/ATP ratio was reduced in patients vs. controls (1.57±0.52 vs.2.14±0.62; P=0.003). VO_2max was lower in patients vs. controls (19±4 vs.36±8 ml/kg/min; P<0.001). During maximal exercise the heart rate increased less in patients vs. controls (52±16 vs.81±14 bpm; p<0.001) and the relative changes in stroke volume and cardiac output during submaximal exercise were lower in patients vs. controls (0.99±0.34 vs. 1.25±0.47, P=0.04; 1.36±0.45 vs. 2.13±0.72, P<0.001). nTTPF fell during exercise in controls, but increased in patients (-0.03±12 sec vs. +0.07±0.11; P=0.005). VVC decreased on exercise in controls but was unchanged in patients (-0.01±0.15 vs. -0.25±0.19; p<0.001). Heart rate, VVC and nTTPF were independent predictors of VO_2max.

Methods

Patients

The study involved 37 HFpEF patients prospectively recruited from heart failure clinics. Also studied were twenty age-gender-matched healthy controls with no cardiac history or diabetes mellitus. Study participants had clinical examination, 12-lead electrocardiogram, pulmonary function test, echocardiogram, metabolic exercise test, radionuclide ventriculography and a subgroup underwent cardiac ^{31}P MRS studies to assess cardiac energetic status. All controls had a normal cardiovascular examination, 12-lead electrocardiogram and echocardiogram. HFpEF patients were defined in accordance with ACC/AHA recommendation (1): i) symptoms and signs of heart failure, ii) ejection fraction ≥50%, iii) no valvular abnormalities. In addition it was stipulated that patients should have iv) VO_2max <80% of age and gender predicted with a pattern of gas exchange on metabolic exercise testing indicating a cardiac cause for limitation, v) absence of objective evidence of lung disease on formal lung function testing and/or absence of arterial desaturation during exercise and with a ventilatory reserve at peak exercise ≥15L. This definition was chosen in order to have robust evidence that patients had exercise limitation that was cardiac rather than non cardiac in origin and so as not to prejudge the underlying pathophysiology by stipulating the presence of resting diastolic abnormalities because mild diastolic abnormlities are frequently present also in healthy elderly subjects and moderate to

severe resting diastolic abnormalities are frequently not present in patients with clear evidence of HfpEF. Patients with rhythm other than sinus were excluded.

Echocardiography

Echocardiography was performed with participants in the left lateral decubitus position with a Vivid 7 echocardiographic machine using a 2.5-MHz transducer. Cardiac quantifications were determined in accordance with European Association of Echocardiography. (2) LV end-systolic elastance (E_{es}), a measure of LV contractility, was determined using the non-invasive single-beat technique. (3) Arterial elastance (E_a), a measure of the stiffness of the entire arterial tree, was calculated as the ratio of LV end-systolic pressure/stroke volume. Studies were stored digitally and analyzed off-line.

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

In vivo myocardial energetics was measured by MRS at 3-Tesla (4). ^{31}P cardiac magnetic resonance spectroscopy was performed using a Phillips Achieva 3T scanner and a linearly polarized transmitter and receiver ^{31}P coil with a diameter of 14 cm. The repetition time was 10000 ms with 136 averages and 512 samples. Acquisition was ECG gated and the trigger delay was set to acquire in diastole. Total scan time was 23 minutes (5). Java magnetic resonance user interface v3.0 (jMRUI) was used for analysis. PCr and γ-ATP was used to determine the PCr/ATP ratio which is a measure of cardiac energetic state (6). Patients with ischemic heart disease and diabetes (N=7) were excluded from the MRS studies because these conditions are known to have impaired cardiac energetics (7,8). Patients with contraindications were also excluded from the MRS study (N=5). One patient's spectra was excluded from the analysis due to poor quality. Three controls had contraindication to MRS study. Data were analysed separately by an investigator unaware of participants' clinical status.

Radionuclide Ventriculography

LV ejection fraction and diastolic filling were assessed by radionuclide ventriculography at rest and during graded semi erect exercise on a cycle ergometer as previously described. (9,10) Three minutes of data were acquired at rest and during exercise after a 30-second period for stabilisation of heart rate at the commencement of each stage. Exercise was performed at 50% workloads of heart rate reserve. Data were analysed using LinkMedical MAPS software, Sun Microsystems (Hampshire, UK). Peak left

ventricular filling rate in terms of end-diastolic count per second (EDC/s) and time to peak filling normalised for R-R interval (nTTPF) in milliseconds after end systole were calculated from the first derivative of the diastolic activity-time curve. Venous blood samples were obtained for weighing and for counting of blood gamma activity during each scan in order to correct for physical and physiological decay as well as for determination of relative volume changes. (11) The validity of these radionuclide measures of diastolic filling at high heart rates has been established previously. (12)

All gated blood pool scan-derived volumes were normalized to body surface area, yielding their respective indexes: end-diastolic volume index (EDVI), end-systolic volume index (ESVI), stroke volume index (SVI), and cardiac index. The following indexes were calculated: a) arterial elastance index (E_{aI}) = ESP/SVI; b) LV end-systolic elastance index (E_{LVI}) = ESP/ESVI and c) vasculo-ventricular coupling ratio (VVC) = E_{aI}/E_{LVI} = (1/EF)–1. (13)

Metabolic Exercise Test

All participants underwent a symptom-limited erect treadmill exercise using a standard ramp protocol with simultaneous respiratory gas analysis. (14)

Statistics

Continuous variables are expressed as means±SD. Unpaired Student's t-test (2-tail) was used to assess differences between mean values. Categorical variables were compared with Pearson Chi-Square test. All reported P values were calculated on the basis of two sided tests and a P value of <0.05 was considered to indicate statistical significance. Variances of data sets were determined using F-test. Pearson correlation coefficient (r) was used to describe the relationship between variables. All subjects were included into the model. Variables of interest that were found to correlate with the dependent variable on univariate analysis were included in a stepwise linear regression analysis to identify independent variables. SPSS (v15.0) was used to perform the statistical operations.

Results

The results obtained are set forth in Tables 1-3 below and in Figures 1 and 2.

In Figure 1 variables correlating with Aerobic Exercise Capacity (VO_2max) are shown. Panel A: VO_2max correlated negatively with Exercise-induced Changes in nTTPF.

Panel B: VO_2max correlated negatively with Exercise-induced Changes in Vasculo-Ventricular Coupling Ratio. Panel C: VO_2max correlated directly with Exercise-induced Changes in Heart Rate. Black circles indicate patients with HFpEF, and Open circles represents healthy controls. When patients on beta blockers were excluded from analysis, the level of significance were similar.

In Figure 2, Panel A shows an MR images of a patient with HFpEF lying prone over a ^{31}P surface coil and the corresponding localized ^{31}P MR spectra from the left ventricle is shown in panel B. The resonances derive from PCr and the γ-, α-, and β-phosphate Resonances of the ATP. Panel C Individual PCr/ γ- ATP ratio in Patients with HfpEF and Controls. The PCr/ γ- ATP ratio was significantly reduced in patients with HfpEF compared to healthy controls, P= 0.003

Table 1. Baseline Characteristics of the Subjects

Variable	Patient (N = 37)	Control (N = 20)	P Value
Age - yr	67±9	63±7	0.51
Female sex - no. (%)	28 (76)	10 (50)	0.05
Body Mass Index	30±4	26±5	<0.01
Left Ventricular Hypertrophy - no. (%)	19 (51)	5 (25)	0.05
Diabetes mellitus - no. (%)	4 (11)	0	-
Hypertension - no. (%)	27 (73)	0	-
Ischemic Heart Disease - no. (%)	4 (11)	0	-
NYHA functional class – no.			
I	10	0	-
II	18	0	-
III	8	0	-
Drug therapy – no. (%)			
Diuretic	10 (27)	0	-
ACE inhibitor	20 (54)	0	-
ARB	6 (16)	0	-
Beta-blocker	8 (22)	0	-
Calcium blocker	10 (27)	0	-

Alpha Blocker	4 (11)	0	-
Spironolactone	2 (5)	0	-
Nitrate	3 (8)	0	-
VO2max (ml/kg/min)	19±4	36±8	<0.001
Respiratory Exchange Ratio (RER)	1.06±0.07	1.13±0.10	0.003
Breathing Reserve - L/min	36±15	43±18	0.16
Exercise Time - min	6±2	7±1	0.03
Resting HR - beats/min	74±14	83±17	0.03
Peak HR - beats/min	127±20	166±11	<0.001
ΔHR – beats/min	52±16	81±14	<0.001
Rest SBP (mmHg)	138±19	131±23	0.23
Rest DBP (mmHg)	81±11	81±12	0.98
Rest MABP (mmHg)	100±12	96±15	0.30
Peak SBP (mm/Hg)	182±26	190±30	0.30
Peak DBP (mmHg)	81±13	84±10	0.36
Peak MABP (mmHg)	113±17	114±25	0.91
Left ventricular ejection fraction - %	64±14	63±6	0.77
Mitral E-wave velocity - m/sec	0.72±0.19	0.61±0.12	0.02
Mitral A-wave velocity - m/sec	0.80±0.20	0.59±0.17	<0.001
Ratio of E-wave: A-wave velocity	0.96±0.35	1.03±0.32	0.47
Mitral E-wave deceleration - msec	274±70	269±73	0.82
E/E' (septum)	15±5	11±3	0.003
E/E' (lateral)	12±4	8±2	<0.001
E_{es}	3.07±1.07	2.60±.53	0.09
E_a	2.22±0.63	2.28±0.48	0.69

Plus-minus values are means ± SD. When patients on beta blockers were excluded from analysis, the level of significance were similar apart from resting HR (P=0.14). NYHA denotes New York Heart Association, ACE angiotensin-converting enzyme, ARB angiotensin II receptor blockers, BMI body mass index, SBP systolic blood pressure, DBP diastolic blood pressure, MABP mean arterial blood pressure, LA left atrium, E/E' mitral E-wave velocity-E' tissue velocity (PW-TDI) at basal inferoseptum

ratio, E_{es} denotes Left Ventricular End-Systolic Elastance and Ea is Arterial elastance. The bodymass index is the weight in kilograms divided by the square of the height in meters.

Table 2. MUGA at Rest and on Exercise: Diastolic Filling Characteristics, Systolic Function, Relaxation, Stiffness, and Ventricular-Arterial Coupling

Variable	Patient (N = 37)	Control (N = 20)	P Value
Heart Rate – rest (beats/min)	71±12	68±15	0.40
Heart Rate – exercise (beats/min)	97±14	114±11	<0.001
Exercise SBP (mm/Hg)	204±26	198±27	0.45
Exercise DBP (mmHg)	95±15	97±7	0.56
Exercise MABP (mmHg)	132±15	131±9	0.85
Ejection fraction – rest (%)	65±9	64±9	0.61
Ejection fraction – exercise (%)	66±9	72±8	0.05
Peak emptying rates - rest (EDC/sec)	382±106	400±90	0.56
Peak emptying rates - exercise (EDC/sec)	477±123	563±144	0.04
Peak filling rates - rest (EDC/sec)	342±120	321±111	0.54
Peak filling rates - exercise (EDC/sec)	504±127	602±163	0.02
Time to peak filling - at rest (msec)	176±80	181±56	0.84
Time to peak filling - exercise (msec)	246±91	162±80	0.001
Relative Δ Stroke Volume Index	0.99±0.34	1.25±0.47	0.04
Relative Δ Cardiac Output Index	1.36±0.45	2.13±0.72	<0.001
Relative Δ E_{LV}l - exercise	1.35±0.50	1.85±0.63	0.01
Relative Δ E_al - exercise	1.52±0.48	1.28±0.44	0.17
Vasculo-Ventricular Coupling ratio (VVC) (E_al/E_{LV}l) – rest	0.57±0.20	0.62±0.22	0.36
Vasculo-Ventricular Coupling ratio (VVC) (E_al/E_{LV}l) – exercise	0.55±0.19	0.41±0.15	0.01
Δ VVC	-0.01±0.15	-0.25±0.19	<0.001

Plus-minus values are means ± SD. When patients on beta blockers were excluded from analysis, the level of significance were similar apart from peak filling rates during exercise (P=0.08). EDC end diastolic count. SBP systolic blood pressure, DBP diastolic blood pressure, MABP mean arterial blood pressure. Relative Δ Stroke Volume Index is $SVi_{EXERCISE}$ / SVi_{REST}, Relative Δ Cardiac Output Index is $COi_{EXERCISE}$ /

COi REST. Relative Δ ELVI is ELVIEXERCISE / ELVIREST. Relative Δ Eal is EalEXERCISE / EalREST. Δ Vasculoventricular coupling ratio is (Eal/ELVI)EXERCISE - (Eal/ELVI)REST. Δ **VVC** - 0.01±0.15 -0.25±0.19 <0.001

Table 3. Multivariate Predictors of VO_2max

Variable	R Square	P Value
Exercise-induced change in HR *	0.584	<0.001
Exercise-induced change in VVC†	0.696	0.003
Age ‡	0.728	0.018
Exercise-induced change nTTPF §	0.769	0.018

*Predictors: ΔHR, †Predictors: ΔHR and Δ VVC coupling ratio, ‡Predictors: ΔHR, Δ VVC coupling ratio and age, §Predictors: ΔHR, Δ VVC coupling ratio, age and ΔTTPF. Multivariate analysis was adjusted for the variable that some patients were on betablockers.

Characteristics of the Patients

HFpEF Patients were generally females, overweight, aged 67±9 years old with a history of hypertension, however blood pressure was well treated (systolic BP 138±19mmHg vs. 131±23mmHg; p=0.23, in patients vs. controls) (see Table 1 below). The tissue Doppler E/E' at the basal anterolateral left ventricular wall (a measure of left ventricular end-diastolic pressure) (15), was significantly higher in patients than controls. There was also a trend (non-significant) to higher Ees in patients than in the control group. HFpEF patients also had significantly reduced VO2max and reduced peak HR on metabolic exercise testing. There was a positive correlation between VO2max and ΔHR (HREXERCISE - HRREST) (r=0.7, P<0.001) (see Figure 1). During semi-erect cycle exercise the relative stroke volume (SVi EXERCISE /SVi REST) was lower in patients compared to controls (0.99±0.34 vs. 1.25±0.47; P=0.04), and relative cardiac output (COiEXERCISE/COiREST) was also lower (1.36±0.45 vs 2.13±0.72; p<0.001). (see Table 2)

Left Ventricular Active Relaxation

nTTPF is determined by the rate of active relaxation (16) and by transmitral pressure gradient at the time of mitral valve opening. nTTPF was similar at rest in HFpEF patients and controls. During exercise it shortened in controls, but lengthened in patients (Table 2). There was a negative correlation between VO2max and ΔnTTPF

($nTTPF_{EXERCISE}$ - $nTTPF_{REST}$) (r=-0.4, P=0.005) (see Figure 1). Furthermore, during exercise other radionuclide ventriculography diastolic filling variables such as peak filling rates as well as systolic function parameters e.g. EF and peak emptying rates, were significantly reduced in patients compared to controls. (see Table 2)

Left ventricular contractile function and Vasculo-Ventricular Coupling

VVC was similar at rest in HfpEF patients and controls. During exercise, LV arterial elastance, a measure of the stiffness of the entire arterial tree, increased in both patients and controls but tended to increase more in patients. LV end systolic elastance, a measure of LV contractile function, markedly increased on exercise in controls but increased substantially less in patients. Accordingly the vasculoventricular coupling ratio was essentially unchanged on exercise inpatients but fell substantially on exercise in healthy controls furthermore whilst resting LVEF and peak emptying rate were similar in patients and controls on exercise both were lower in patients. There was a negative correlation between VO2max and Δ VVC on exercise (r=-0.6, P<0.001) (Figure 1).

***In vivo* Myocardial Energetic state**

At rest, cardiac PCr/ATP ratio in HFpEF patients (N=24) was significantly reduced compared to healthy controls (N=17), 1.57±0.52 and 2.14±0.63, respectively, P=0.003 (see Figure 2).

Independent Predictors of Aerobic Exercise Capacity

In the multivariate analysis, a linear-regression model was used to examine VO_2max as the dependent variable and found that exercise-induced changes in HR, VVC and nTTPF were independent predictors of VO_2max. (see Table 3)

Discussion

The principal findings are: a) HFpEF patients manifest a significant reduction in PCr/ATP ratio at rest, indicating impairment of myocardial energy "reserve" that is likely to be exacerbated during exercise. b) As a corollary, during exercise, the energetically demanding active relaxation stage of diastole lengthened in patients (vs. a shortening in controls) and was accompanied with a failure to increase LV contractile function . These combined dynamic abnormalities of both diastolic and contractile function together resulted in a lower stroke volume on exercise. c) Consistent with

previous studies, HFpEF patients demonstrated chronotropic incompetence on exercise. (17). d) This study underlines the importance of dynamic (rather than resting) assessment of cardiac function to comprehensively characterise patients with HfpEF.

The pathophysiology of HFpEF has been the subject of considerable controversy. These patients are typically hypertensive and exhibit impaired LV active relaxation and/or increased passive left ventricular diastolic stiffness at rest. (18) This has led many to conclude that exercise limitation is primarily a result of impaired LV diastolic filling and to the use of the term 'diastolic heart failure' by some. (19) However, diastolic dysfunction is also a common finding at rest in healthy elderly subjects. (20) Furthermore, 'subtle' abnormalities of systolic function, in particular long axis systolic function, are also almost universally observed in HFpEF patients despite normal LV ejection fraction. (21) This has led others to propose that HFpEF is predominantly a disorder of contractile function. (22) In order to compare both of these possibilities, we defined HfpEF as a limitation of exercise with an unequivocally cardiac cause as assessed by VO2max (rather than using resting diastolic parameters) to avoid biasing our mechanistic studies to a select group of patients with HfpEF.

Little attention has been directed to changes in systolic and diastolic function during dynamic exercise, which is when the majority of patients experience most severe symptoms. In one study, ten patients with HFpEF were assessed with invasive pressure volume loops and compared with age-matched controls. (23) The former had increased arterial elastance (a measure of the stiffness of the entire arterial tree), and increased LV end-systolic elastance (a measure of the stiffness of the ventricle during systole, and the relatively load independent measure of the contractile state of the left ventricle. (24) Whilst diastolic abnormalities were not universally present in patients at rest, marked differences appeared during handgrip exercise. The rate of LV active relaxation increased in healthy subjects but it slowed in patients. (25) Another study from the same group, exercise-related symptoms in Afro-Caribbean hypertensive patients appeared to be strongly associated with chronotropic incompetence and an inadequate vasodilator reserve on exercise. (26)

The present study examined the patho-physiological mechanisms and predictors of exercise limitation in a substantially larger series of patients during a much more physiologically relevant form of exercise (dynamic leg exercise). There were marked dynamic abnormalities in both contractile and diastolic function of the left ventricle, and

a lower peak exercise HR in patients. The independent predictors of impaired exercise capacity were abnormal ventricular-arterial coupling on exercise, a reduced HR response on exercise and a 'paradoxical' slowing of the rate of LV active relaxation on exercise (manifest as a prolongation of nTTPF). Despite the relative robustness of these observations, deciding whether these changes are adaptive or maladaptive remains challenging. The independent value of an impaired chronotropic response in predicting exercise capacity in HfpEF exemplifies this challenge. For example, VO_2max is largely determined by cardiac output on exercise and the latter is simply the product of HR and SV. On this basis, the detrimental consequecnes of an impaired HR appear plausible. However, in the setting of a profound slowing of active relaxation and increased LV passive diastolic stiffness, a longer diastolic filling period might be expected to be beneficial, both by increasing SV and reducing the cardiac energy load. This in part explains the efficacy of β blocker therapy in hypertrophic cardiomyopathy, a classic cause of HFpEF. (27) The latter also seems plausible, since increasing heart rate by atrial pacing has been shown to reduce supine resting stroke volume and cardiac output in patients with HFpEF. (28) Nevertheless, despite a longer diastolic filling time, the relative change in SV was lower in our patients during sub-maximal exercise. However, this failure to increase cardiac workload through limiting HR may represent a strategy of energetic parsimony in a heart with limited energy reserves. Finally, an alternative explanation is that an inadequate chronotropic response is simply a consequence and/or contributor to heart failure. (29) Such incompetence is typically present in systolic heart failure and is in part a manifestation of impaired vagal tone. (30) Clearly it will be important to undertake further studies to assess whether heart rate plays a causal role in exercise limitation in HFpEF, because if so this may be amenable to rate responsive pacing.

The same challenges arise when interpreting the role of an impairment of vasculo-ventricular coupling in HfpEF. The patients in this study had a history of hypertension but were well treated with antihypertensives (in most cases including vasodilators) therefore resting blood pressure and arterial elastance were not significantly higher than in the control group. Consistent with prior studies (31), at rest, LV end-systolic elastance (a measure of contractility or systolic stiffness) tended to be higher in patients although this did not reach significance. The increase in arterial elastance during exercise tended to be greater in patients vs. controls (presumably reflecting a greater increase in large artery stiffness). However, whilst left ventricular end-systolic elastance almost doubled during exercise in controls, the increase was only 35% in

patients; hence VVC reduced by 33% during exercise in controls but was unchanged in patients. These findings indicate a blunting of the physiological increase in the contractile state of the left ventricle on exercise. As with heart rate, these changes may be interpreted to be either maladaptive or adaptive. A failure to adequately augment contractile function against a high "relative load" of disease and hence a failure to optimise cardiac energetic efficiency might be considered contributory to HfpEF. On the other hand, a smaller increment in LV end-systolic elastance will reduce the absolute increase in energy demand in an already energy constrained heart at the cost of an impaired dynamic increase in cardiac output.

Integrating these observations, we speculate that dynamic energy impairment may account for the slowing of LV active relaxation on exercise as well as the failure of LV contractile function to increase. To increase the generalisability of this hypothesis, we avoided positively biasing our study by excluding patients with established causes of cardiac energy deficiency (ischemic heart disease and diabetes) (32,33). Nevertheless, the PCr/ATP ratio was still substantially reduced in HfpEF patients vs controls at rest. The lower PCr/ATP ratio in patients indicates a reduction of high energy phosphates reserve at rest. (34,35) Although the time required for acquisition of Cardiac MRS signals precluded the measurement of high energy phosphate status on exercise, it is likely that any basal energetic impairment will be exacerbated dynamically. This exacerbation of dynamic energetic impairment would explain the prolongation of the energy demanding active relaxation as manifest by nTTPF. Moreover, the lower hearts rates and lesser increases in LV end-systolic elastance may represent strategies to limit dynamic cardiac energy demands. The cause for this resting energy deficit may relate to insulin resistance (36), to impaired mitochondrial function as a result of ageing (37), and to neuroendocrine activation and aberrant substrate metabolism. (38) Such observations provide a rationale to assess the therapeutic value of 'metabolic agents' that increase cardiac energetic status by altering cardiac substrate use (39). These agents have shown promise in patients with systolic heart failure. (40)

Study limitations

The radionuclide exercise protocol involved asking subjects to maintain a HR which was 50% of HR reserve above their resting HR. Since this HR reserve was calibrated to peak HR rate, the absolute workload in patients was lower. To have compared patients at the same workload would be inappropriate since this would represent a

higher relative workload in patients. Moreover, most changes in SV occur in the first part of exercise with subsequent increases in cardiac output being principally due to increases in HR. (41) A small proportion of patients were on β-blockers which may have affected their cardiovascular response to exercise, however, when these patients were excluded from the analysis the findings and the level of significance remained unchanged. In addition, some patients were on calcium blockers however these were all peripherally acting (dihydropyridines for hypertension) and therefore are not expected to affect the myocardium. Ideally we would have liked to measure cardiac energetics during exercise however cardiac MRS studies during exercise is currently quite challenging more so if we tried to replicate the same dynamic leg exercise in the confinement of a MR scanner. MRS and Radionuclide studies also require a regular rhythm, thus patients with atrial fibrillation were excluded from the study. In contrast, the strength of radionuclide studies is their increasing temporal resolution at higher heart rates. This obviates the confounding E:A fusion as is frequently experienced with exercise echocardiography. Radionuclide studies are thus not subject to systematically biasing mechanistic HfpEF towards a subgroup of patients without E:A fusion.

Conclusion

HFpEF patients have abnormal resting cardiac energetic status which when exacerbated dynamically may contribute to the abnormal active relaxation on exercise and to a failure to increase LV end-systolic elastance. In addition chronotropic response was markedly impaired on exercise in patients. The independent predictors of exercise capacity in patients with HFpEF are exercise-induced changes in active relaxation, heart rate and ventricular-arterial coupling.

Example 2

A study was carried out to establish a causative role for energy deficiency and to evaluate the impact of perhexiline on cardiac energy status in HCM.

The study was approved by the South Birmingham Research Ethics Committee and the investigation conforms with the principles outlined in the Declaration of Helsinki. All study participants provided written informed consent. The study was a randomized, double blind, placebo-controlled parallel-group design of minimum 3 months duration. Figure 3 represents a flow chart of the study. The pre-defined primary end point was peak oxygen consumption (peak VO2). Pre-defined secondary end points were symptomatic status, resting myocardial energetics (PCr/ γ-ATP ratio) and diastolic

function at rest and during exercise (nTTPF). 33 controls of similar age and gender distribution were recruited for comparison with baseline data of HCM patients. All controls had no history or symptoms of any cardiovascular disease with normal ECG and echocardiogram (LVEF ≥ 55%).

Patients were recruited from dedicated cardiomyopathy clinics at The Heart Hospital, University College London Hospitals, London and Queen Elizabeth Hospital, Birmingham, UK between 2006 and 2008. Inclusion criteria were 18 to 80 years old symptomatic HCM patients (predominant symptom breathlessness) in sinus rhythm with reduced peak VO2 (< 75% of predicted for age and gender) and no significant LVOT obstruction at rest (gradient < 30mmHg). Exclusion criteria were presence of epicardial coronary artery disease, abnormal liver function test, concomitant use of amiodarone or selective serotonin reuptake inhibitors (due to potential drug interactions with perhexiline), peripheral neuropathy and women of childbearing potential. Diabetic patients were also excluded to maintain the blindness of the study as Perhexiline may lead to a reduction in plasma glucose in such patients necessitating a reduction in anti-diabetic therapy. 46 consecutive consenting patients who met these entry criteria were recruited into the study.

Patients were subjected to a number of tests and assessments as follows.

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 using a standard ramp protocol with simultaneous respiratory gas analysis (Bruce RA, McDonough JR. Stress testing in screening for cardiovascular disease. Bull N Y Acad Med 1969; 45(12):1288-1305.; Davies NJ, Denison DM. The measurement of metabolic gas exchange and minute volume by mass spectrometry alone. Respir Physiol 1979;36(2):261-267). Peak oxygen consumption (peak VO2) was defined as the highest VO2 achieved during exercise and was expressed in ml/min/kg.

Symptomatic status assessment

All HCM patients filled in Minnesota Living with heart failure questionnaire and were also assessed for NHYA class.

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. Resting scans were acquired in standard apical 4-chamber and apical 2-chamber. LV volumes were obtained by biplane echocardiography, and LVEF was derived from a modified Simpson's formula (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.) Pulse wave doppler sample volume was used to assess resting LVOTO gradient.

Radionuclide Ventriculography

Diastolic filling were assessed by equilibrium R-wave gated blood pool scintigraphy using a standard technique at rest and during graded semi erect exercise on a cycle ergometer (Atherton JJ, Moore TD, Lele SS et al. Diastolic ventricular interaction in chronic heart failure. Lancet 1997;349 (9067):1720-1724; 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). Peak left ventricular filling rate in terms of end-diastolic count per second (EDC/s) and time to peak filling normalised for R-R interval (nTTPF) in milliseconds were measured 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 (Atherton et al. and Lele et al., see above).

31P cardiac Magnetic Resonance Spectroscopy (MRS)

In vivo myocardial energetics were measured using a MRS at 3-Tesla Phillips Achieva 3T scanner (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). A java magnetic resonance user interface v3.0 (jMRUI) was used for analysis (see Naressi A, Couturier C, Castang I, de Beer R, 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)). PCr and γ-ATP peaks was used to determine the PCr/ γ-ATP ratio which is a measure of the cardiac energetic state (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). Data were analyzed by an investigator who was blinded to the participants' clinical status. Carmeo-Rao ratio was used to assess signal to noise ratio. A typical example of cardiac 31P MRS spectra from a patient with HCM is shown in Figure 4C.

Intervention

Following baseline studies, patients were randomized in a double-blind fashion to receive either perhexiline (n = 25) or placebo (n = 21) 100 mg OD. 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 and to avoid drug toxicity. 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. At the end of study, patients were re-evaluated as described earlier.

Statistical Analysis

Data were analyzed using SPSS ver. 15.0 for Window and Microsoft Office Excel 2007, and expressed as Mean ± Standard Deviation (SD). 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. ANCOVA with baseline values as covariates was performed to test for the significance of differences in the perhexiline versus placebo group after treatment. For the primary end point, the sample size required to detect a change in peak Vo2 of 3 ml/kg/min versus placebo group with a power of 90% and probability of 5% is 44. 30 patients will be required to identify a 5 % change in cardiac PCr/ATP ratio with a power of 90% and a p value of <0.05. 40 patients will be required to detect a change ≥25 % in nTTPF with power of 0.99 with probability of 5%. Therefore, we aimed to study 50 patients including the drop-outs, 32 of them will take part in the MRS study.

The characteristics and treatment of participants are shown in Table 1 below. Vo_2:

refers to peak oxygen consumption, ACE: refers to angiotensin-converting enzyme, and ARB refers to angiotensin II receptor blockers.

Table1: The clinical characteristics of HCM patients and controls.

	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	25 (19)	21 (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 Vo_2 [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
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

* indicates statistical significance

Baseline data (HCM versus Controls)

The clinical characteristics and cardiopulmonary exercise test results of all the HCM patients and controls are shown in Table 1. The groups were well matched with respect to age and gender. Heart rate was lower in the HCM group compared to controls due to medication use (beta blockers and/or calcium channel blockers).

The resting cardiac PCr/γATP ratio was lower in HCM patients than in controls (1.28 ± 0.01vs 2.26 ± 0.02, $p < 0.0001$) (see Figures 4A and B), 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, $p = 0.44$). During submaximal exercise (at a workload that achieved 50% of heart rate reserve) it remained relatively constant in controls (from 0.18 ± 0.003 sec to 0.16 ± 0.002 sec, [δnTTPF = - 0.02 ± 0.003 sec]), but lengthened in patients (from 0.17 ± 0.002 to 0.34 ± 0.002 sec, [δnTTPF = + 0.17 ± 0.002 sec]) $p < 0.0001$, (Fig. 4C). This pattern persisted after exclusion of patients on beta blockers and remained significantly different from controls ($p < 0.0001$). Patients exhibited marked exercise limitation compared to controls (23 ± 0.12 vs 38 ± 0.24 ml/kg/min, $p <0.0001$) (Fig. 4D).

Randomized, double blinded, placebo-controlled parallel-group

The perhexiline and placebo groups were well matched (see Table 1). Only one patient (on placebo) did not complete the study due to poor compliance. 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 during the study period.

Myocardial energetics

The PCr/γATP ratio increased with perhexiline (1.27 ± 0.02 to 1.73 ± 0.02) as compared with placebo (1.29 ± 0.01 to 1.23 ± 0.01), $p = 0.003$ (see Figure 5A). The mean Cramer-Rao ratios for PCr and γATP were 7.5 % and 10.8 % respectively. The effect of perhexiline on PCr/γATP ratio remained significant after inclusion of the 3 patients with Cramer Rao ratios >20 from the analysis ($p = 0.02$).

Diastolic ventricular filling

Whereas the placebo group showed similar prolongation of nTTPF during exercise before and after therapy (0.17 ± 0.004 to 0.35 ± 0.005 [δnTTPF 0.18 ± 0.006 sec] and 0.23 ± 0.006 to 0.35 ± 0.005 sec [δnTTPF 0.12 ± 0.006 sec], respectively), in the perhexiline group there was a substantial improvement on therapy with nTTPF at rest and exercise similar (0.19 ± 0.003 to 0.19 ± 0.004 sec[δnTTPF 0.00 ± 0.003 sec]) p = 0.03 between the perhexiline and placebo response (see Figures 5B and 5C).

Symptomatic Status

More patients in the perhexiline group than in the placebo group had improvements in NYHA classification (67 percent vs. 30 percent) and fewer had worsening (8 percent vs. 20 percent) ($p<0.001$). Minnesota Living with heart failure questionnaire score showed an improvement (fall in score) in the perhexiline group (from 36.13 ± 0.94 to 28 ± 0.75) but did not change in the placebo group ($p < 0.001$) (see Figures 5D and 5E).

Exercise capacity (peak oxygen consumption)

Peak V_{O2} at baseline was similar in the perhexiline and placebo groups (Table1). 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), $p = 0.003$ (see Figure 5F).

Discussion of Results

The study indicates 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). The metabolic modulator perhexiline resulted in significant myocardial energy augmentation. 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. These biochemical and physiological improvements translated into significant subjective (NYHA classification and QoL score) and objective (V_{O2}) clinical benefits in

symptomatic HCM patients already on optimal medical therapy (see Figure 6).

The content of all cited references is expressly incorporated herein by reference for all purposes.

References

1. Hunt SA, Abraham WT, Chin MH et al. ACC/AHA 2005 Guideline Update for the Diagnosis and Management of Chronic Heart Failure in the Adult: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure): developed in collaboration with the American College of Chest Physicians and the International Society for Heart and Page | 18 Lung Transplantation: endorsed by the Heart Rhythm Society. *Circulation*. 2005;112:e154-e235.
2. 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;18:1440-1463.
3. Chen CH, Fetics B, Nevo E et al. Noninvasive single-beat determination of left ventricular end-systolic elastance in humans. *J Am Coll Cardiol*. 2001;38:2028-2034.
4. 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.
5. Naressi A, Couturier C, Castang I, de Beer R, 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.
6. 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;86:1810-1818.

7. 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;86:1810-1818.
8. Scheuermann-Freestone M, Madsen PL, Manners D et al. Abnormal cardiac and skeletal muscle energy metabolism in patients with type 2 diabetes. Circulation. 2003;107:3040-3046.
9. Lele SS, Thomson HL, Seo H et al. Exercise capacity in hypertrophic cardiomyopathy. Role of stroke volume limitation, heart rate, and diastolic filling characteristics. *Circulation*. 1995;92:2886-2894.
10. Lele SS, Macfarlane D, Morrison S et al. 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;17:204-212.
11. Atherton JJ, Moore TD, Lele SS et al. Diastolic ventricular interaction in chronic heart failure. *Lancet*. 1997;349:1720-1724.
12. Bacharach SL GMBJSESE. Left ventricular peak ejection rate, filling rate and ejection fraction-frame requirements at rest and exercise: concise communication. Journal of Nuclear Medicine 20, 189-193. 1979.
13. Najjar SS, Schulman SP, Gerstenblith G et al. Age and gender affect ventricularvascular coupling during aerobic exercise. *J Am Coll Cardiol*. 2004;44:611-617.
14. Davies NJ, Denison DM. The measurement of metabolic gas exchange and minute volume by mass spectrometry alone. *Respir Physiol*. 1979;36:261-267.
15. Ommen SR, Nishimura RA, Appleton CP et al. Clinical utility of Doppler echocardiography and tissue Doppler imaging in the estimation of left ventricular filling pressures: A comparative simultaneous Doppler-catheterization study. *Circulation*. 2000;102:1788-1794.
16. Magorien DJ, Shaffer P, Bush C et al. Hemodynamic correlates for timing intervals, ejection rate and filling rate derived from the radionuclide angiographic volume curve. *Am J Cardiol*. 1984;53:567-571. Page | 20
17. Borlaug BA, Melenovsky V, Russell SD et al. Impaired chronotropic and vasodilator reserves limit exercise capacity in patients with heart failure and a preserved ejection fraction. *Circulation*. 2006;114:2138-2147.

18. Zile MR, Baicu CF, Gaasch WH. Diastolic heart failure--abnormalities in activerelaxation and passive stiffness of the left ventricle. N Engl J Med. 2004;350:1953-1959.
19. Zile MR, Brutsaert DL. New concepts in diastolic dysfunction and diastolic heart failure: Part I: diagnosis, prognosis, and measurements of diastolic function. *Circulation*. 2002;105:1387-1393.
20. Mantero A, Gentile F, Gualtierotti C et al. Left ventricular diastolic parameters in 288 normal subjects from 20 to 80 years old. *Eur Heart J*. 1995;16:94-105.
21. Yu CM, Lin H, Yang H et al. Progression of systolic abnormalities in patients with "isolated" diastolic heart failure and diastolic dysfunction. *Circulation*. 2002;105:1195-1201.
22. Burkhoff D, Maurer MS, Packer M. Heart failure with a normal ejection fraction: is it really a disorder of diastolic function? *Circulation*. 2003;107:656-658.
23. Kawaguchi M, Hay I, Fetics B et al. Combined ventricular systolic and arterial stiffening in patients with heart failure and preserved ejection fraction: implications for systolic and diastolic reserve limitations. *Circulation*. 2003;107:714-720.
24. Grossman W, Braunwald E, Mann T et al. Contractile state of the left ventricle in man as evaluated from end-systolic pressure-volume relations. *Circulation*. 1977;56:845-852.
25. Kawaguchi M, Hay I, Fetics B et al. Combined ventricular systolic and arterial stiffening in patients with heart failure and preserved ejection fraction: implications for systolic and diastolic reserve limitations. *Circulation*. 2003;107:714-720.
26. Borlaug BA, Melenovsky V, Russell SD et al. Impaired chronotropic and vasodilator reserves limit exercise capacity in patients with heart failure and a preserved ejection fraction. *Circulation*. 2006;114:2138-2147.
27. Nihoyannopoulos P, Karatasakis G, Frenneaux M et al. Diastolic function in hypertrophic cardiomyopathy: relation to exercise capacity. *J Am Coll Cardiol*. 1992;19:536-540.
28. Westermann D, Kasner M, Steendijk P et al. Role of left ventricular stiffness in heart failure with normal ejection fraction. *Circulation*. 2008;117:2051-2060.

29. Borlaug BA, Melenovsky V, Russell SD et al. Impaired chronotropic and vasodilator reserves limit exercise capacity in patients with heart failure and a preserved ejection fraction. *Circulation.* 2006;114:2138-2147.
30. Eckberg DL, Drabinsky M, Braunwald E. Defective cardiac parasympathetic control in patients with heart disease. *N Engl J Med.* 1971;285:877-883.
31. Kawaguchi M, Hay I, Fetics B et al. Combined ventricular systolic and arterial stiffening in patients with heart failure and preserved ejection fraction: implications for systolic and diastolic reserve limitations. *Circulation.* 2003;107:714-720.
32. 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;86:1810-1818.
33. Scheuermann-Freestone M, Madsen PL, Manners D et al. Abnormal cardiac and skeletal muscle energy metabolism in patients with type 2 diabetes. *Circulation.* 2003;107:3040-3046.
34. Lamb HJ, Beyerbacht HP, van der LA et al. Diastolic dysfunction in hypertensive heart disease is associated with altered myocardial metabolism. *Circulation.* 1999;99:2261-2267.
35. Smith CS, Bottomley PA, Schulman SP et al. Altered creatine kinase adenosine triphosphate kinetics in failing hypertrophied human myocardium. *Circulation.* 2006;114:1151-1158.
36. Scheuermann-Freestone M NSCK. Abnormal cardiac muscle function in heart failure is related to insulin resistance. Cardiovasc J S Afr. 15, s12.
37. Szibor M, Holtz J. Mitochondrial ageing. *Basic Res Cardiol.* 2003;98:210-218.
38. Ashrafian H, Frenneaux MP, Opie LH. Metabolic mechanisms in heart failure. *Circulation.* 2007;116:434-448. Page | 23
39. Abozguia K, Clarke K, Lee L et al. Modification of myocardial substrate use as a therapy for heart failure. *Nat Clin Pract Cardiovasc Med.* 2006;3:490-498.
40. 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;112:3280-3288.
41. Higginbotham MB, Morris KG, Williams RS et al. Regulation of stroke volume during submaximal and maximal upright exercise in normal man. *Circ Res.* 1986;58:281-291.

CLAIMS:

1. Perhexiline, or a pharmaceutically acceptable salt thereof, for use in the treatment of HfnEF.

2. Perhexiline for use as claimed in claim 1, wherein the perhexiline is in the form of a pharmaceutically acceptable salt.

3. Perhexiline for use as claimed in claim 2, wherein the perhexiline is in the form of the maleate salt.

4. A method of treating HfnEF, which comprises administering to an animal in need thereof an effective amount of perhexiline, or a pharmaceutically acceptable salt thereof, to treat said HFnEF.

5. The method of claim 4, wherein the animal is a mammal.

6. The method of claim 5, wherein the mammal is a human.

7. A treatment programme for treating HFnEF, which involves the co-use or co-administration of perhexiline with one or more other compounds that are advantageous in treating HFnEF or the symptoms thereof.

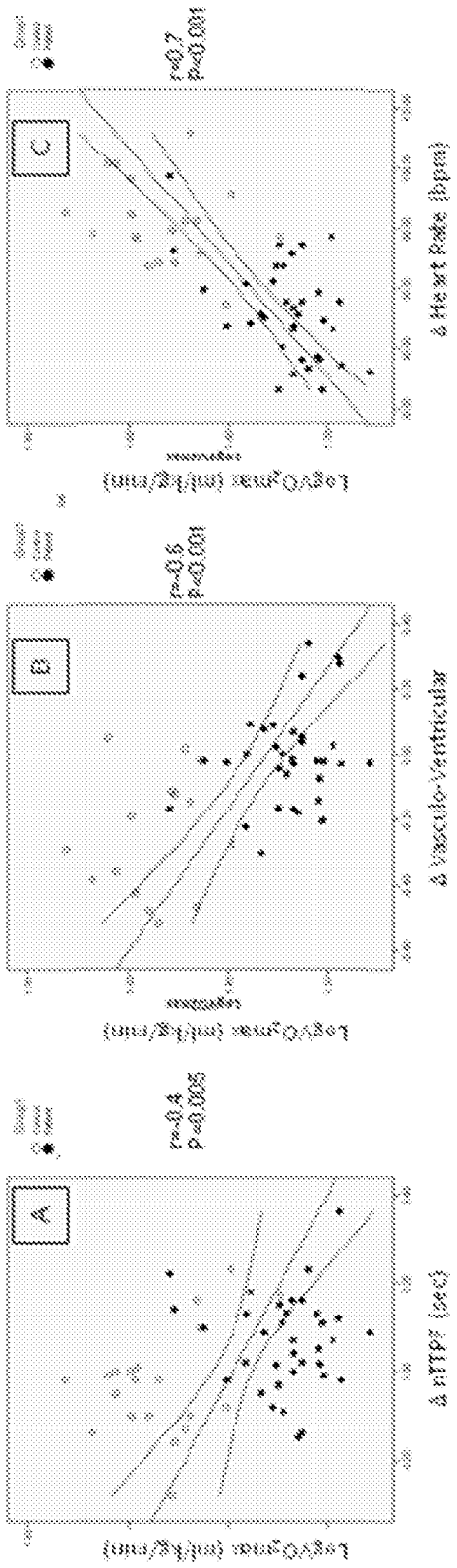

A
B
C
r=-0.4
P<0.0005
r=-0.6
P<0.001
r=-0.7
P<0.001
Δ PTTP (sec)
Δ Vascular-Ventricular Coupling Ratio
Δ Heart Rate (bpm)
LogVO2max (ml/kg/min)

Figure 1

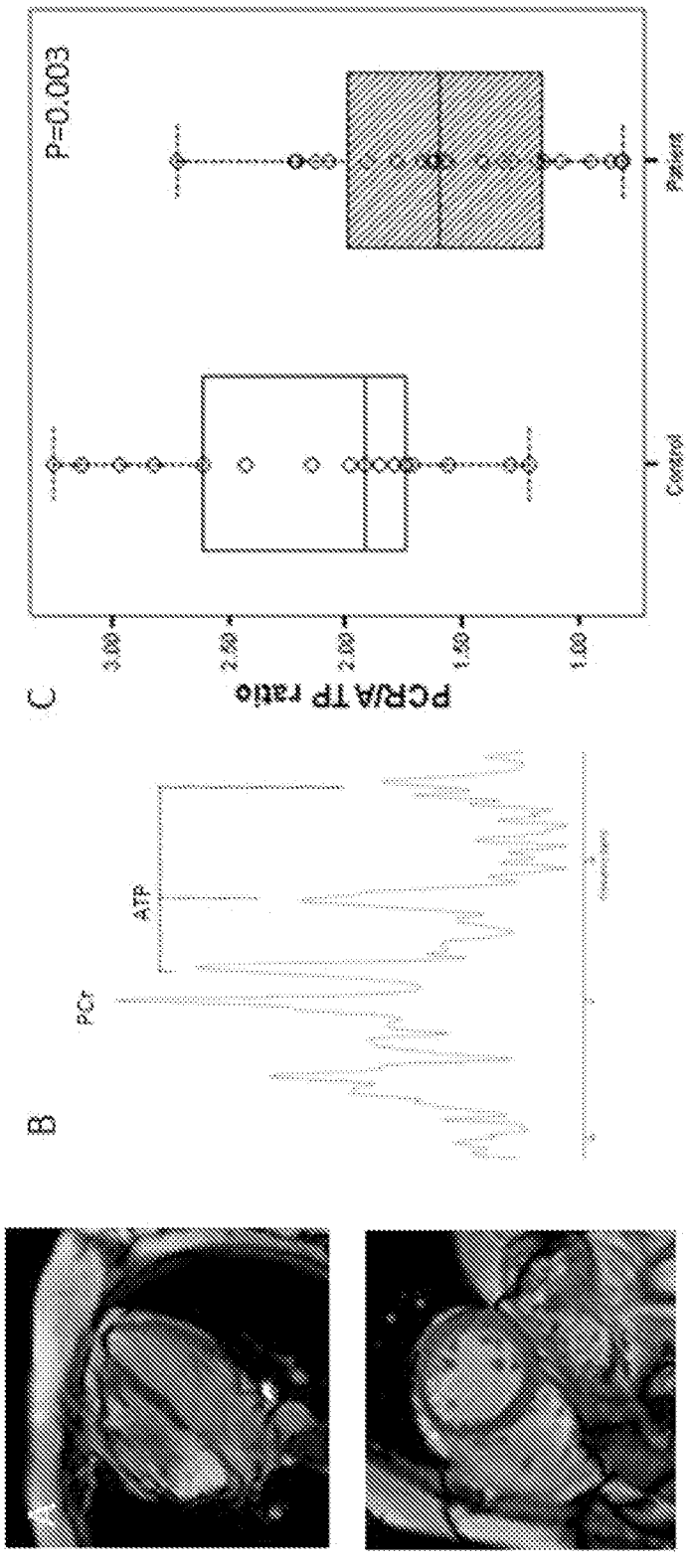

C
PCR/ATP ratio
3.00
2.50
2.00
1.50
1.00
P=0.003
Patient
Control
B
PCr
ATP
A

Figure 2

Figure 3

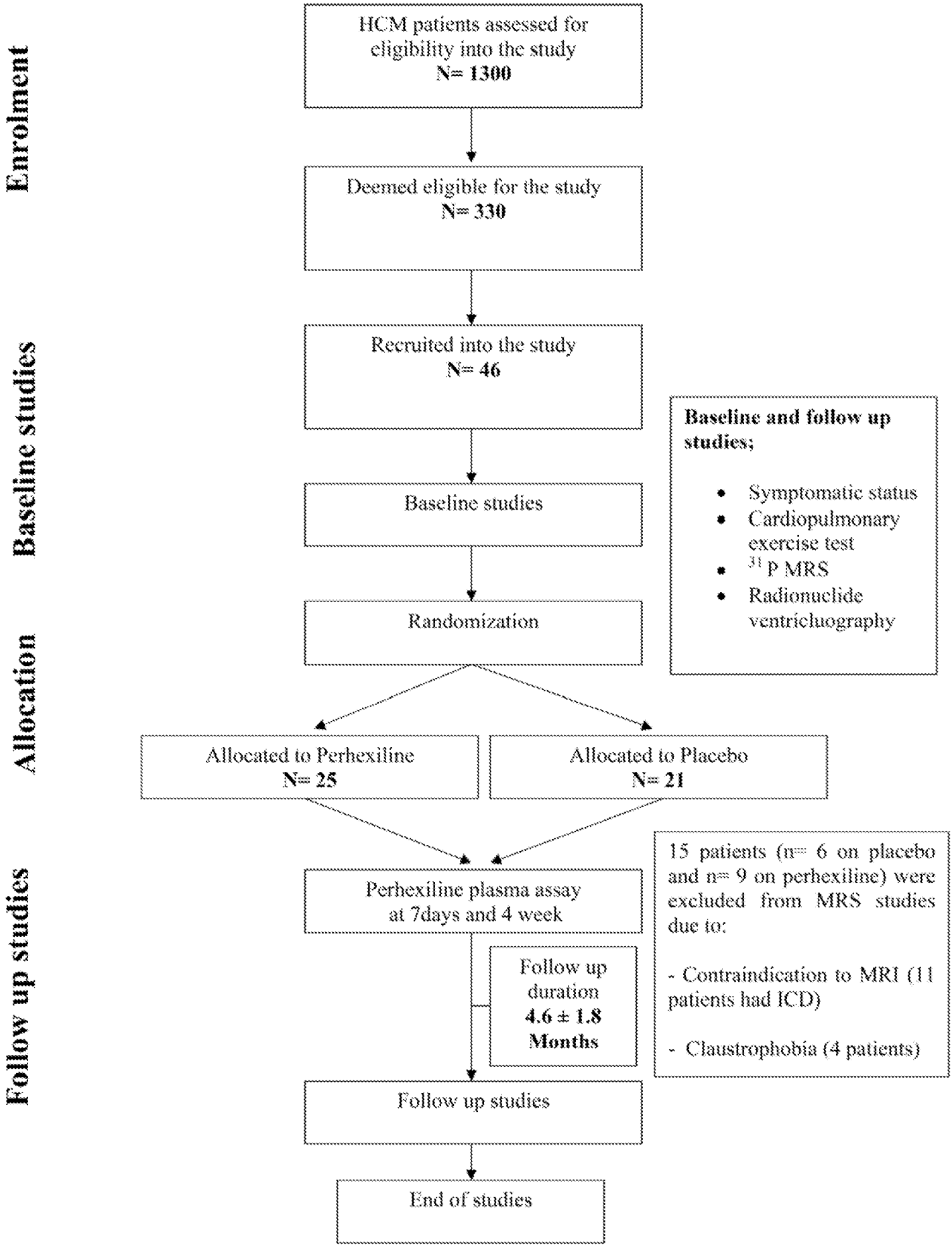
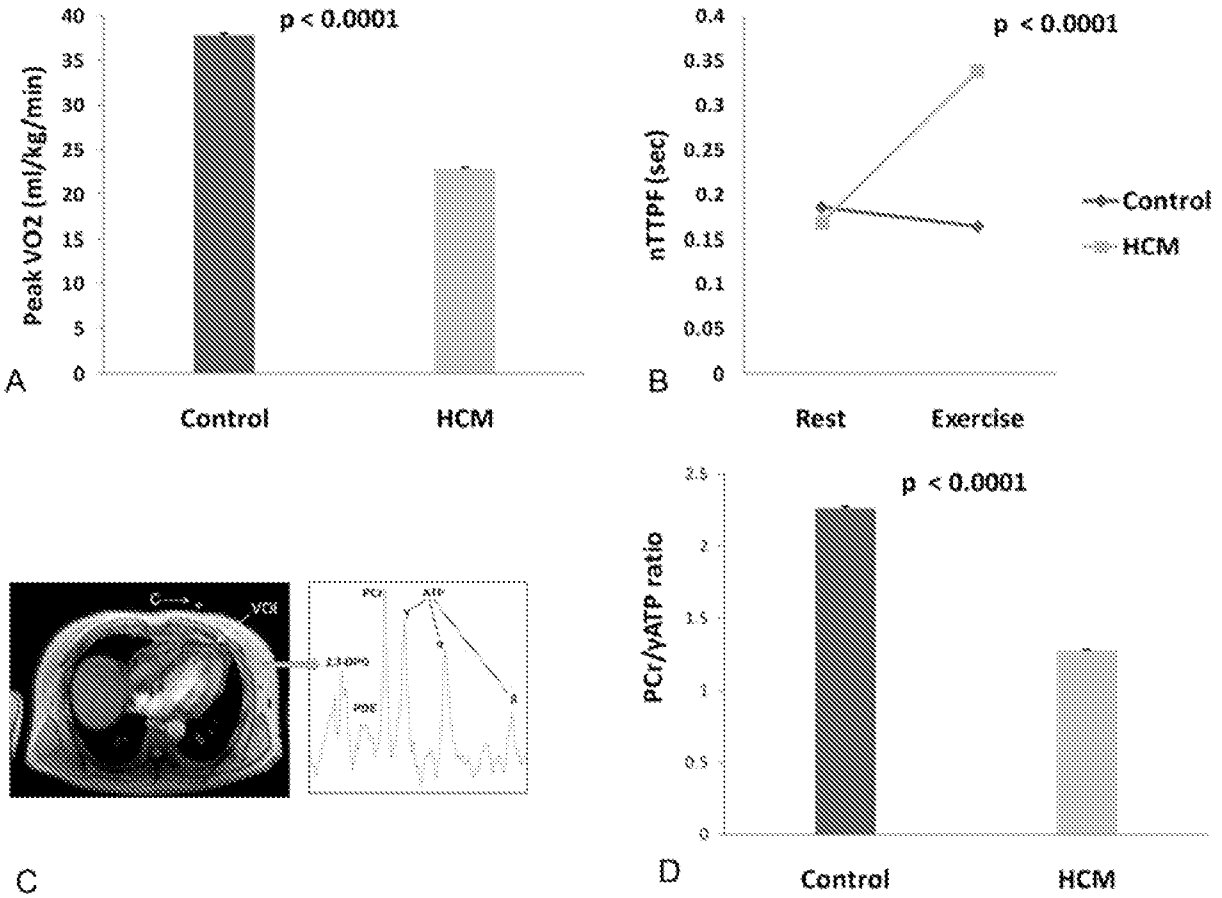

Enrolment
Baseline studies
Allocation
Follow up studies
HCM patients assessed for eligibility into the study N= 1300
Deemed eligible for the study N= 330
Recruited into the study N= 46
Baseline studies
Randomization
Baseline and follow up studies;
Symptomatic status
Cardiopulmonary exercise test
31 P MRS
Radionuclide ventricluography
Allocated to Perhexiline N= 25
Allocated to Placebo N= 21
Perhexiline plasma assay at 7days and 4 week
Follow up duration 4.6 ± 1.8 Months
15 patients (n= 6 on placebo and n= 9 on perhexiline) were excluded from MRS studies due to:
- Contraindication to MRI (11 patients had ICD)
- Claustrophobia (4 patients)
Follow up studies
End of studies

Figure 4


p < 0.0001
Peak VO2 (ml/kg/min)
40
35
30
25
20
15
10
5
0
Control
HCM
A
p < 0.0001
nTTPF (sec)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Control
HCM
Rest
Exercise
B
VCG
PCr
ATP
PDE
C
p < 0.0001
PCr/γATP ratio
2.5
2
1.5
1
0.5
0
Control
HCM
D

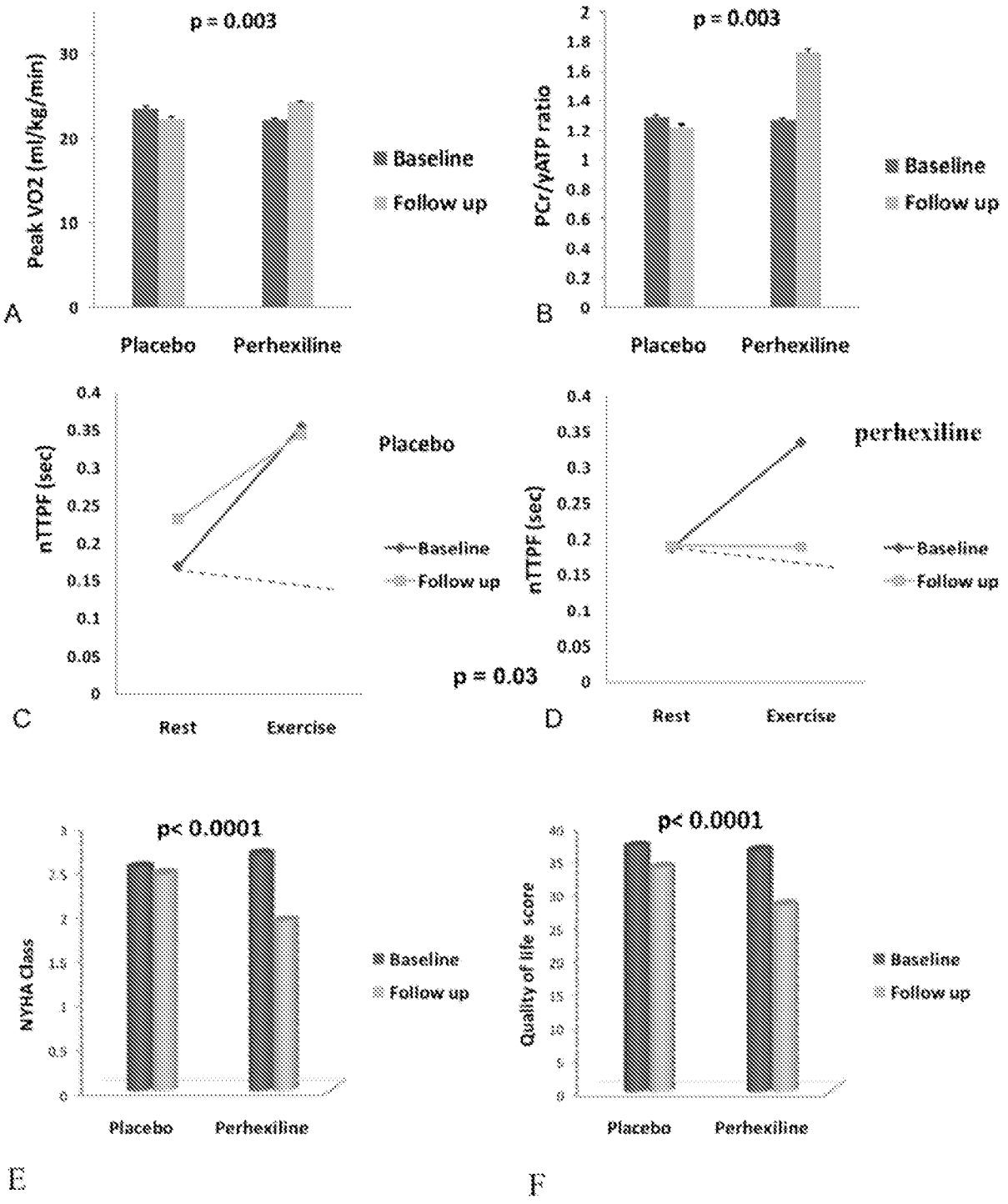

p = 0.003
Peak VO2 (ml/kg/min)
30
20
10
0
Placebo
Perhexiline
Baseline
Follow up
A
p = 0.003
PCr/γATP ratio
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Placebo
Perhexiline
Baseline
Follow up
B
nTTPF (sec)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Rest
Exercise
Placebo
Baseline
Follow up
C
nTTPF (sec)
perhexiline
p = 0.03
Rest
Exercise
Baseline
Follow up
D
p< 0.0001
NYHA Class
Placebo
Perhexiline
Baseline
Follow up
E
p< 0.0001
Quality of life score
Placebo
Perhexiline
Baseline
Follow up
F

Figure 5

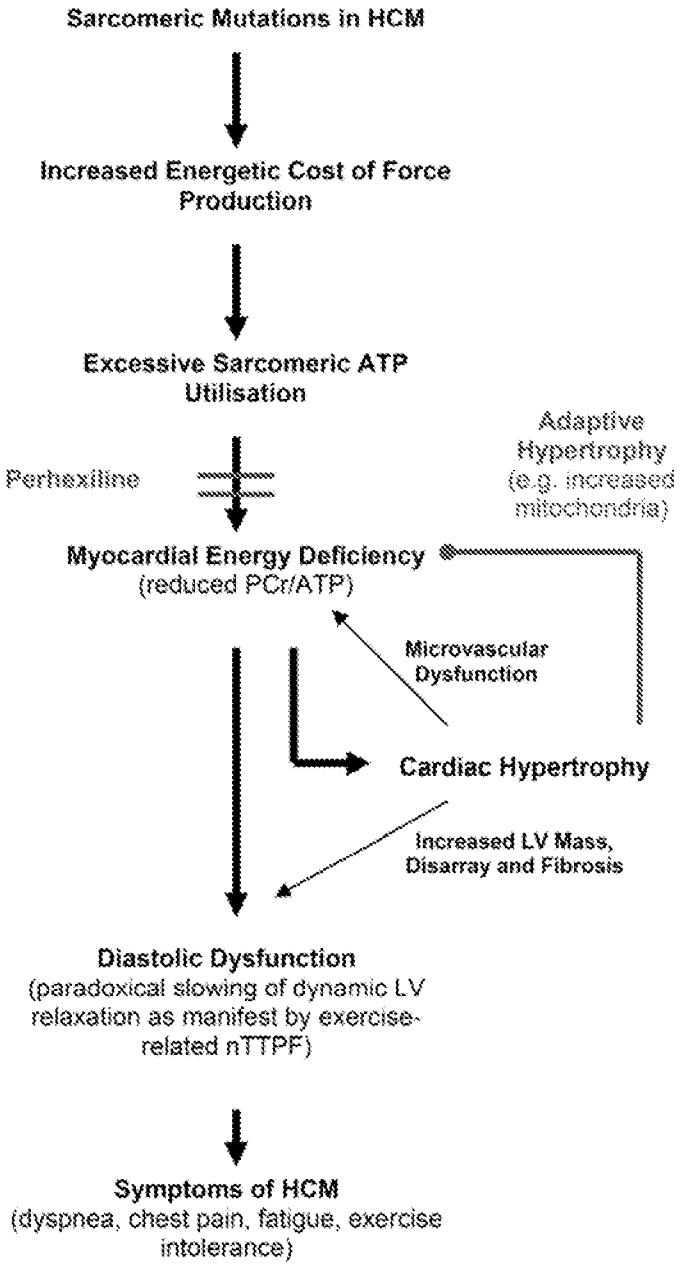

Sarcomeric Mutations in HCM
Increased Energetic Cost of Force Production
Excessive Sarcomeric ATP Utilisation
Perhexiline
Adaptive Hypertrophy (e.g. increased mitochondria)
Myocardial Energy Deficiency (reduced PCr/ATP)
Microvascular Dysfunction
Cardiac Hypertrophy
Increased LV Mass, Disarray and Fibrosis
Diastolic Dysfunction (paradoxical slowing of dynamic LV relaxation as manifest by exercise-related nTTPF)
Symptoms of HCM (dyspnea, chest pain, fatigue, exercise intolerance)

Figure 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2009/050539

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/4458 A61P9/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/087233 A (HEART METABOLICS LTD [GB]; FRENNEAUX MICHAEL PAUL [GB]) 22 September 2005 (2005-09-22) cited in the application claims 1-15	1-7
Y	----- -/--	1-7

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 October 2009	09/11/2009

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Fax: (+31–70) 340–3016	Madalinska, K

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2009/050539

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PELTIER M ET AL: "Treatment practices in heart failure with preserved left ventricular ejection fraction: A prospective observational study" INTERNATIONAL JOURNAL OF CARDIOLOGY, vol. 118, no. 3, 12 June 2007 (2007-06-12), pages 363-369, XP025320041 ISSN: 0167-5273 [retrieved on 2007-05-05] abstract; tables 1,4 page 368, left-hand column, paragraph CONCLUSION	1-7
Y	METRA ET AL: "Treatment of advanced chronic heart failure with normal left ventricular ejection fraction. Response to the letter by Dr. Martinez-Selles" EUROPEAN JOURNAL OF HEART FAILURE, vol. 9, no. 12, 19 November 2007 (2007-11-19), pages 1224-1225, XP022361135 ISSN: 1388-9842 page 1224, left-hand column, paragraphs 1,2	1-7
Y	TEO K K ET AL: "Perhexiline during exercise training in coronary heart disease." CLINICAL PHARMACOLOGY AND THERAPEUTICS, vol. 34, no. 6, December 1983 (1983-12), pages 744-748, XP009114152 ISSN: 0009-9236 abstract	1-7
A	METRA M ET AL: "Advanced chronic heart failure: A position statement from the Study Group on Advanced Heart Failure of the Heart Failure Association of the European Society of Cardiology" EUROPEAN JOURNAL OF HEART FAILURE,, vol. 9, no. 6-7, 10 May 2007 (2007-05-10), pages 684-694, XP022069148 ISSN: 1388-9842 page 687, left-hand column, paragraphs 3.2,SYSTOLIC,VERSUS...	1-7

-/--

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2009/050539

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ROSSI A ET AL: "Chronic heart failure with preserved left ventricular ejection fraction: Diagnostic and prognostic value of left atrial size" INTERNATIONAL JOURNAL OF CARDIOLOGY, vol. 110, no. 3, 28 June 2006 (2006-06-28), pages 386-392, XP025034882 ISSN: 0167-5273 [retrieved on 2006-06-28] page 386, left-hand column, paragraph 1 - page 386, right-hand column, paragraph 1	1-7
A	HOLDEN K R: "Chronic Heart Failure and Disability"[Online] 7 August 2007 (2007-08-07), XP002520273 Retrieved from the Internet: URL:http://www.disabilitydoc.com/chronic-heart-failure-and-disa/> [retrieved on 2009-03-19] the whole document	1-7
E	WO 2009/066085 A (HEART METABOLICS LTD [GB]; ASHRAFIAN HOUMAN [GB]) 28 May 2009 (2009-05-28) the whole document	1-7

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2009/050539

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005087233 A	22-09-2005	CN 1950084 A	18-04-2007
		EP 1732551 A1	20-12-2006
		JP 2007528378 T	11-10-2007
		US 2007275997 A1	29-11-2007
WO 2009066085 A	28-05-2009	NONE	