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Improvement in Cardiac Energetics by Perhexiline in Heart Failure Due to Dilated Cardiomyopathy

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ABSTRACT

OBJECTIVES The aim of this study was to determine whether short-term treatment with perhexiline improves cardiac energetics, left ventricular function, and symptoms of heart failure by altering cardiac substrate utilization.

BACKGROUND Perhexiline improves exercise capacity and left ventricular ejection fraction (LVEF) in patients with heart failure (HF). ^{31}P cardiac magnetic resonance spectroscopy can be used to quantify the myocardial phosphocreatine/adenosine triphosphate ratio. Because improvement of HF syndrome can improve cardiac energetics secondarily, we investigated the effects of short-term perhexiline therapy.

METHODS Patients with systolic HF of nonischemic etiology (n = 50, 62 ± 1.8 years of age, New York Heart Association functional class II to IV, LVEF: 27.0 ± 1.44%) were randomized to receive perhexiline 200 mg or placebo for 1 month in a double-blind fashion. Clinical assessment, echocardiography, and ^{31}P cardiac magnetic resonance spectroscopy were performed at baseline and after 1 month. A substudy of 22 patients also underwent cross-heart blood sampling at completion of the study to quantify metabolite utilization.

RESULTS Perhexiline therapy was associated with a 30% increase in the phosphocreatine/adenosine triphosphate ratio (from 1.16 ± 0.39 to 1.51 ± 0.51; p < 0.001) versus a 3% decrease with placebo (from 1.36 ± 0.31 to 1.34 ± 0.31; p = 0.37). Perhexiline therapy also led to an improvement in New York Heart Association functional class compared with placebo (p = 0.036). Short-term perhexiline therapy did not change LVEF. Cross-heart measures of cardiac substrate uptake and respiratory exchange ratio (which reflects the ratio of substrates used) did not differ between patients who received perhexiline versus placebo.

CONCLUSIONS Perhexiline improves cardiac energetics and symptom status with no evidence of altered cardiac substrate utilization. No change in LVEF is seen at this early stage. (Metabolic Manipulation in Chronic Heart Failure; NCT00841139) (J Am Coll Cardiol HF 2015;■:■–■) © 2015 by the American College of Cardiology Foundation.

Despite recent advances in treatment, heart failure (HF) has devastating effects on survival (1) and quality of life (2). Pharmacological therapy consists of diuretics and neurohormonal modulating agents. It is well established that energy deficiency contributes to the

syndrome of HF (3), and this has been proposed as a therapeutic target.

In previous phase 2 studies, beneficial effects of perhexiline have been observed in refractory angina (4), HF (5), and hypertrophic cardiomyopathy (6). This is subject to variable metabolism by P4502D6, of which there are several polymorphic variants. Sustained high plasma levels may lead to hepatotoxicity and neurotoxicity due to phospholipid accumulation in these tissues. It has been shown that toxicity is avoided by plasma monitoring with dose titration (7).

The therapeutic effects of perhexiline have been believed to relate to altered cardiac substrate utilization. Perhexiline has been shown to inhibit carnitine palmitoyltransferase 1 and, to a lesser extent, carnitine palmitoyltransferase 2 (8). These mitochondrial enzymes facilitate entry of medium- and long-chain fatty acids into mitochondria to undergo beta-oxidation and subsequently produce adenosine triphosphate (ATP). This inhibition of fatty acid metabolism may be expected to lead to a reciprocal increase in carbohydrate activation (via allosteric activation of the enzyme pyruvate dehydrogenase) and thus an improvement in myocardial efficiency and phosphocreatine (PCr)/ATP ratio.

^{31}P cardiac magnetic resonance spectroscopy (^{31}P MRS) allows noninvasive quantification of the PCr/ATP ratio, which is an accepted measure of cardiac energetic status. It has been shown that the PCr/ATP ratio is reduced in the failing human heart (9,10). Therefore, we sought to determine whether the therapeutic benefit of perhexiline in HF is related to an improved cardiac PCr/ATP ratio and whether this is a consequence of altered cardiac substrate utilization. Effective therapy for HF that does not act in the first instance by direct effects on cardiac metabolism may nevertheless in the longer term result in improvements in the cardiac PCr/ATP ratio via an improvement in HF syndrome, leading in turn to molecular reverse remodeling of maladaptive changes in genes involved in cardiac metabolism and calcium handling and thereby potentially to a virtuous cycle of improved cardiac function (11-13). In this study, we hypothesized that perhexiline therapy would improve the cardiac PCr/ATP ratio in patients with HF by altering cardiac substrate utilization. To assess whether these changes occur early and are by inference directly responsible for the subsequent improvement in cardiac function, we used a short-term (1-month) regimen of perhexiline that typically resulted in plasma perhexiline levels in the low therapeutic range for approximately 2 weeks.

METHODS

STUDY DESIGN. This randomized, double-blind, placebo-controlled, parallel-group study explored the effects of perhexiline over 1 month (32 [29 to 35] days) in patients with symptomatic, nonischemic cardiomyopathy. All participants provided written informed consent, and research was performed in accordance with the Declaration of Helsinki. The predefined primary endpoint was an increase in the cardiac PCr/ATP ratio with perhexiline therapy compared with placebo. The secondary endpoints were improvement in New York Heart Association (NYHA) functional class, quality of life (Minnesota Living With Heart Failure Questionnaire [MLWHFQ]), and left ventricular ejection fraction (LVEF). In a subgroup of 22 patients, cross-heart respiratory exchange ratio (RER) was measured to assess cardiac substrate utilization. The study protocol and methodology have been previously reported (14). The study was approved by the South Birmingham Research Ethics Committee (06/Q2707/7) and the Medicines and Healthcare Products Regulatory Agency (21761/0003/001) and was registered with ClinicalTrials.gov (NCT00841139).

PATIENT SELECTION. Patients (n = 50) were consecutively recruited from the Advanced HF Programme at the University Hospitals Birmingham NHS Foundation Trust (Birmingham, England) and the University Hospitals of Wales NHS Trust (Cardiff, Wales) between February 2008 and June 2011. All patients were receiving maximal tolerated doses of evidence-based HF therapy (angiotensin-converting enzyme inhibitors, beta-blockers, and mineralocorticoid receptor antagonists). Inclusion criteria were nonischemic dilated cardiomyopathy, symptoms of HF despite optimal tolerated medical therapy, and LVEF <40% on echocardiography. Patients with a history of coronary heart disease and with cardiac rhythms other than sinus were not included. Exclusion criteria were a history of liver disease or liver function test measurements greater than twice the upper limit of normal; concomitant use of amiodarone, quinidine, haloperidol, or selective serotonin reuptake inhibitors that inhibit the CYP2D6 enzyme; pre-existing evidence of peripheral neuropathy; women of childbearing potential; and contraindications to MRS.

Patients underwent a clinical assessment, including assessment of NYHA functional class and quality of life (MLWHFQ). They also underwent venous blood sampling, ^{31}P MRS, and

echocardiography before and after the intervention. A subgroup of patients underwent invasive testing with cross-heart sampling at the end of the study to investigate on-treatment differences in cardiac substrate utilization between the perhexiline and placebo groups. These patients satisfied the same inclusion criteria but gave consent for the additional invasive component of the study.

INTERVENTION. After baseline studies were performed, patients were randomized in a double-blind fashion with a block size of 5 to receive either perhexiline 200 mg once daily (n = 25) or placebo (n = 25). 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 a therapeutic level (therapeutic range: 0.15 to 0.6 mg/l) and to avoid drug toxicity according to a protocol devised by Horowitz et al. (7). Identical dosage adjustments were made by the unblinded observer for patients receiving placebo to ensure that blinding of the investigators was maintained as described previously (14).

VENOUS BLOOD. All venous samples obtained before and after treatment were analyzed for levels of glucose, glycerol, lactate, nonesterified fatty acids (NEFA), triglycerides, pyruvate, insulin, and N-terminal pro–B-type natriuretic peptide (NT-proBNP). A Kone 20i selective chemistry analyzer (Thermo-Fisher Scientific UK Ltd., Loughborough, England) was used for measurement of glucose, glycerol, lactate, NEFA, lactate, and triglyceride levels using a colorimetric method. Pyruvate levels were determined by enzymatic assay (kit ref ab65342, Abcam, Cambridge, England). Insulin levels were quantified using an enzyme-linked immunosorbent assay kit (kit ref EZHI-14K, Merck Millipore, Watford, England). Plasma concentrations of NT-proBNP were determined using an immunoluminometric assay (15). Patients who had plasma triglyceride levels >2.5 mmol/l were assumed not to have fasted, and their samples were excluded from analysis of insulin and metabolites.

Myocardial energetics. Cardiac high-energy phosphate metabolism was measured using ^{31}P MRS on a 3-T Philips Achieva whole-body magnet (Philips Healthcare, Reigate, Surrey, England) using a linearly polarized transmit-and-receive P-31 coil with a diameter of 14 cm. Localization was achieved by image-selected in vivo spectroscopy volume selection. The participants were placed in a supine position with the coil directly over the precordium. The coil was secured in place by straps around the upper body and coil. The participants were then positioned inside the

magnet with the center of the coil at the isocenter of the magnet. Survey images were obtained to check the position of the coil. The subjects and/or the coil were repositioned if required to ensure that the distance between the coil and septum and apex of the heart was minimized and signal strength maximized. Localized iterative first-order shimming was performed over the entire heart using the unsuppressed water signal acquired with the body coil as reference. The shimming process involves an automated hydrogen-1 spectral acquisition to test the quality of the shim, which is expressed as full width at half maximum. This was repeated until a full width at half maximum of <40 Hz was achieved. A short-axis cine scan was performed to calculate the trigger delay for electrocardiographic triggering and to check the quality of shimming and F_0 determination. The trigger delay was calculated such that the spectra were acquired in the diastolic period, when the heart is as still as possible. The 3-dimensional in vivo spectroscopy voxel of acquisition was planned to include most of the septum and apex of the heart within the shimmed area. Care was taken to minimize blood contamination from the right ventricle, liver, and skeletal muscle. The voxel size was kept constant at 89.54 ml (44 × 55 × 37 mm^3). After this, the ^{31}P spectrum was acquired with a repetition time of 10,000 ms, 136 averages, and 512 samples. A repetition time of 10,000 ms was found to be optimal for adequate reduction of saturation effects without greatly increasing the scan time. The average scan time was 23 min. The spectra were analyzed and quantified using the Java-based Magnetic Resonance User Interface (jMRUI) software. Post-processing was performed with 15-Hz Gaussian line broadening and Fourier transformation. Phase correction was performed with the PCr peak as the reference peak. Quantification was performed with AMARES (Advanced Method for Accurate, Robust and Efficient Spectral fitting), a time domain fitting program, which involves selecting peaks and defining their line width. The concentrations of PCr, ATP (γ, α, and β), and 2,3-diphosphoglycerate were calculated as the area under the peaks. The PCr/ATP ratio was determined after correcting the γATP peak for blood contamination as described previously on the basis of the quantity of 2,3-diphosphoglycerate in the derived spectrum (16). A single, blinded operator experienced in the technique performed the analysis.

Cramer-Rao lower bounds (CRLBs) were calculated to assess the quality of the spectral fit. The CRLBs are the lowest possible SDs of all unbiased model parameter estimates obtained from the data and are widely used as a measure of attainable precision of parameter estimates (17).

Symptom status. NYHA functional class was determined in all patients by the same blinded cardiologist asking a standard set of questions (18). Quality of life was assessed with the standard 21-question MLWHQ. All patients completed questionnaires alone and without assistance.

Echocardiography. Standard transthoracic echocardiography (Vivid 7, GE Vingmed Ultrasound, Horten, Norway) was performed by an experienced echocardiographer using second harmonic imaging and an M3S multifrequency transducer. All parameters were measured in triplicate and averaged according to the recommendations of the American Society of Echocardiography (19). Analysis was performed offline by a single blind observer using an EchoPAC workstation (GE Vingmed Ultrasound).

Grayscale images for 2-dimensional left ventricular strain were acquired from the apical 4-, 2-, and 3-chamber views and parasternal short-axis views at basal, papillary, and apical ventricular levels at end expiration at frame rates >70 Hz for speckle tracking echocardiography. Analysis was performed offline using commercially available software (GE Vingmed Ultrasound, Horten, Norway). Peak systolic velocities, strain, strain rate, rotation, and twist were measured for each myocardial segment in triplicate and averaged for global estimates.

Cross-heart sampling. A coronary sinus thermodilution catheter was inserted via a 7-F sheath placed in either the right internal jugular vein or right femoral vein under local anesthesia. The catheter was guided into place using fluoroscopy and small injections of

FIGURE 1 CONSORT Trial Flow Diagram
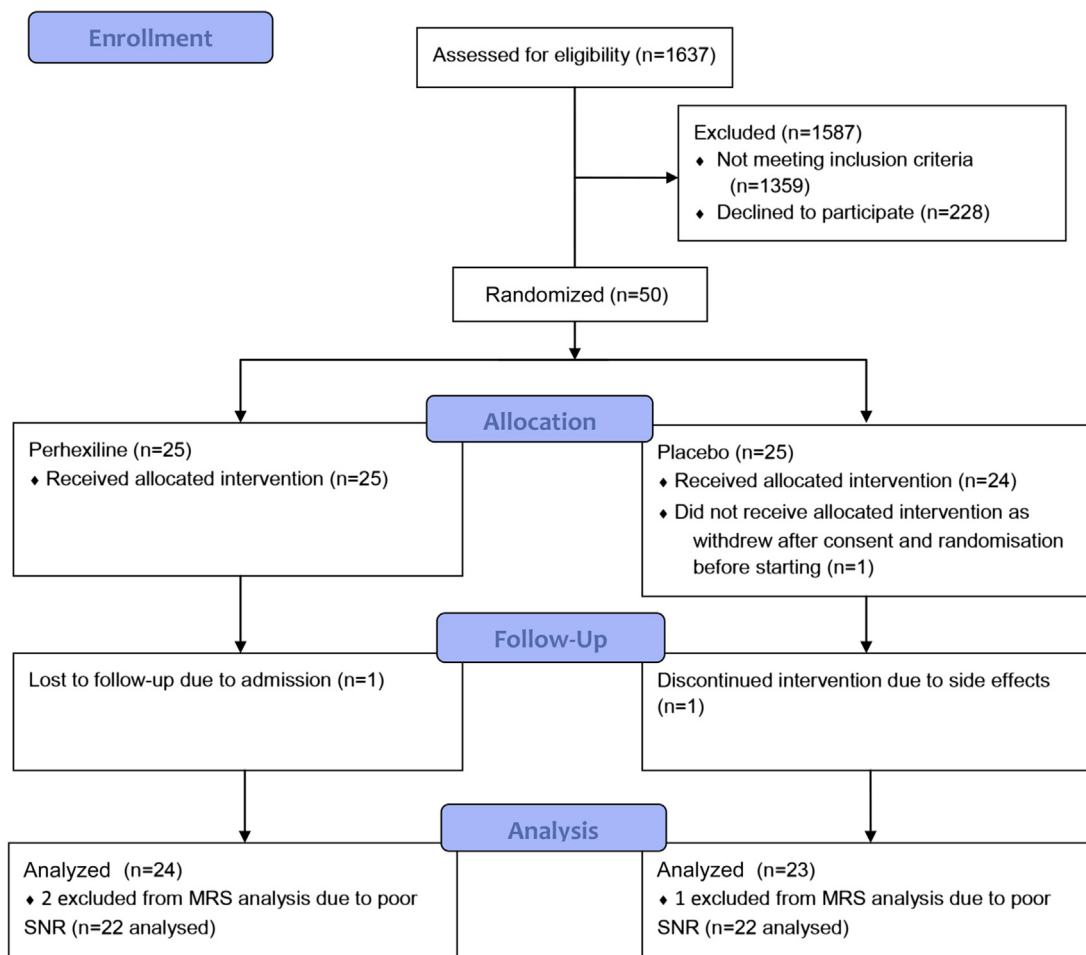
A total of 1,637 patients were screened for inclusion, 278 met the inclusion criteria, and 50 were randomized. One patient was lost to follow-up. Twenty-two patients in each group underwent cardiac ^{31}P MRS. CONSORT = Consolidated Standards of Reporting Trials; MRS = magnetic resonance spectroscopy; SNR = signal-to-noise ratio.

radio-opaque contrast when required to confirm the position of the catheter. This catheter was used to take venous blood samples from the coronary sinus as well as to measure flow by thermodilution. Arterial samples were taken simultaneously from an arterial sheath. No heparin was used until these samples had been taken. Samples were spun immediately in ethylenediaminetetraacetic acid, and plasma was frozen in liquid nitrogen and stored at −80°C. These samples were analyzed in a single batch at the end of the study for glucose, lactate, NEFA, and pyruvate. Blood samples for oxygen were taken in blood gas syringes, and measurement of oxygen was performed using a Bayer Rapidlab 800 series blood gas analyzer (Bayer Healthcare LLC, East Walpole, Massachusetts). Measurement of carbon dioxide was performed off site with isotope-ratio mass spectrometry using a Thermo Finnigan Delta XP isotope ratio mass spectrometer (Thermo Fisher Scientific UK, Lough-borough, England).

Statistical analysis. Variables are expressed as mean ± SD when normally distributed or median and interquartile range when not normally distributed. Continuous variables were compared between baseline data for perhexiline and placebo using unpaired Student t test (2-tailed) if variables were normally distributed and the Mann-Whitney U test if the data were non-normally distributed. The Kolmogorov-Smirnov test and normality plots were used to assess normality of continuous variables. Categorical variables were compared with the Pearson chi-square test. NYHA functional class was analyzed between groups using Kendall tau-b test. A 2-way repeated-measures analysis of variance was used for others to compare the effect of the intervention between the 2 groups. For non-normally distributed variables that failed normality testing with log transformation, the changes between groups were compared by an unpaired Student t test (2-tailed) or Mann-Whitney U test as appropriate. Correlations were performed with a bivariate Pearson r test. A p value of 0.05 was considered to indicate statistical significance. The p values were not corrected for multiple comparisons. Statistical analyses were performed using IBM SPSS version 20 (IBM, Portsmouth, United Kingdom).

SAMPLE SIZE. The primary endpoint was the change in the PCr/ATP ratio after 4 weeks of treatment. Pilot work from our group using the same ^{31}P MRS technique with perhexiline revealed an improvement of 0.4 in the PCr/ATP ratio in the treatment group by comparing means. The response was normally distributed with an SD of 0.5. Using these data, 26

| TABLE 1	Clinical Characteristics of the Patient Groups		
	Perhexiline Group	Placebo Group	p Value
Age, yrs	62 ± 1.8	60 ± 2.43	0.49
Male	22 (88)	16 (64)	0.10
Body mass index, kg/m²	27 ± 1.1	29 ± 0.9	0.23
Body surface area, m²	1.9 ± 0.04	1.9 ± 0.04	0.88
Heart rate, beats/min	70 ± 2.92	69 ± 3.10	0.92
Systolic blood pressure, mm Hg	120 [35]	120 [34]	0.59
Diastolic blood pressure, mm Hg	60 [20]	60 [10]	0.72
Diabetes	4	2	0.38
Loop diuretics	16	20	0.21
ACE-I/ARBs	25	25	–
Beta-blockers	21	19	0.48
Calcium channel blockers	0	1	0.31
Spironolactone	14	13	0.78
Digoxin	7	4	0.31
Statins	13	9	0.24
Serum perhexiline level, mg/l			
Week 1	0.17 ± 0.04	0	–
Week 4	0.33 ± 0.06	0	–
PCr/γATP ratio	1.16 ± 0.08	1.38 ± 0.07	0.04
NYHA functional class (class)	11 (II), 9 (III), 5 (IV)	14 (II), 10 (III), 1 (IV)	0.21
MLWHFQ score	25 [34]	36 [48]	0.79
LVEF, %	27 ± 1.44	30 ± 1.34	0.21
NT-proBNP level, pg/ml	620 [2,342]	241 [508]	0.02

Values are mean ± SD, n (%), or median [interquartile range].

ACE-I/ARBs = angiotensin-converting enzyme inhibitors/angiotensin receptor blockers; ATP = adenosine triphosphate; LVEF = left ventricular ejection fraction; MLWHFQ = Minnesota Living With Heart Failure Questionnaire; NT-proBNP = N-terminal pro-B-type natriuretic peptide; NYHA = New York Heart Association; PCr = phosphocreatine.

FIGURE 2 Monitoring of Perhexiline Levels
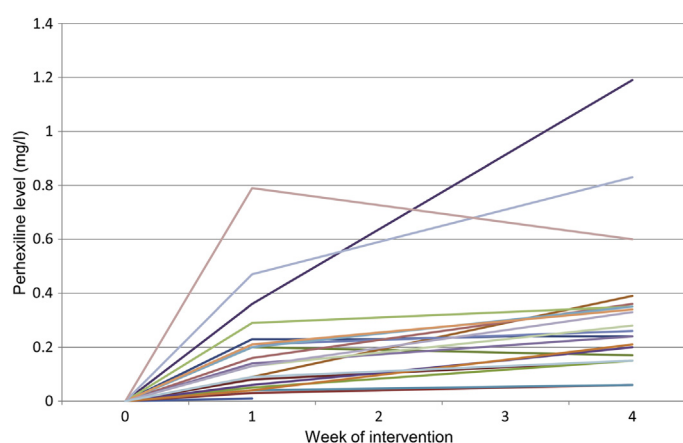
Perhexiline levels were measured in all patients at the end of the first week and again at the conclusion of the intervention period. The dose of perhexiline was adjusted according to the level in the first week.

FIGURE 3   Relationship Between PCr/ATP Ratio and NYHA Functional Class
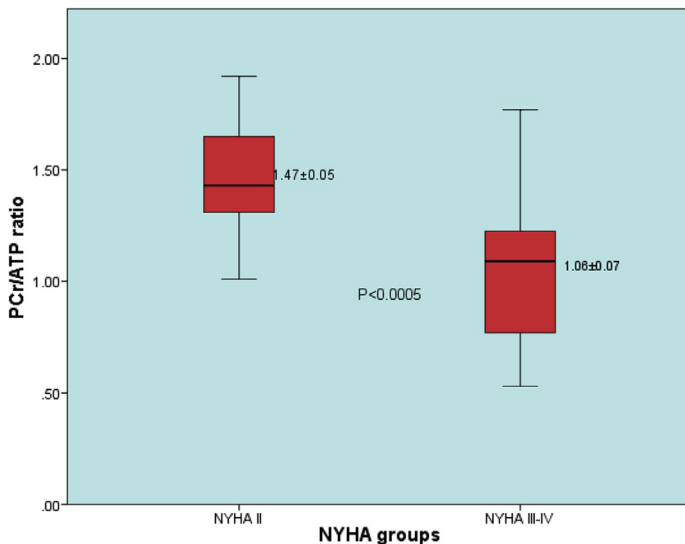
The cardiac ³¹P MRS PCr/ATP ratio was markedly lower in those patients with higher NYHA functional class. ATP = adenosine triphosphate; NYHA = New York Heart Association; PCr = phosphocreatine; other abbreviation as in **Figure 1**.

FIGURE 4   Change in PCr/ATP Ratio Between Baseline and Follow-Up

Placebo 1.36±0.31 to 1.34±0.31

Perhexiline 1.16±0.39 to 1.51±0.51

The PCr/ATP ratio was not well matched between groups at baseline. There was a significant increase in the PCr/ATP ratio with perhexiline therapy from 1.16 ± 0.39 to 1.51 ± 0.51 compared with placebo ($p < 0.001$). Abbreviations as in **Figure 3**.

subjects in each arm provided at least 80% power for detecting a change of 0.4 in the PCr/ATP ratio.
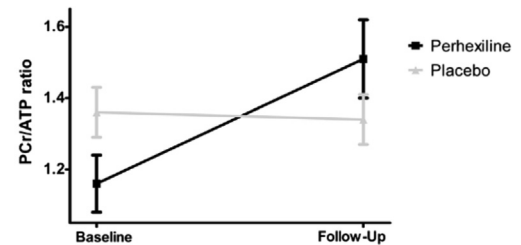
RESULTS

STUDY POPULATION. Fifty patients were randomized, of whom 47 were included in the analysis (**Figure 1**). One patient from the perhexiline group was lost to follow-up due to a hospital admission with intercurrent pneumonia at the time of follow-up. One patient in the placebo group withdrew from the study after randomization but before starting an intervention. Another patient in the placebo group withdrew due to adverse effects (headaches and lethargy). The baseline clinical characteristics of the 2 groups are shown in **Table 1**. The groups were matched for age, sex, NYHA functional class, MLWHFQ scores, and conventional medical therapy. The PCr/ATP ratio was lower at baseline in the perhexiline group (1.16 ± 0.08 vs. 1.38 ± 0.07; $p = 0.036$) and the NT-proBNP level was higher (620 [2,342] pg/ml vs. 241 [508] pg/ml).

The mean serum perhexiline level at the end of study in the perhexiline arm was 0.33 ± 0.3 mg/l, with 2 patients (8.3%) falling below the lower threshold of the therapeutic range (0.15 mg/ml) (**Figure 2**). There were no significant differences in venous metabolites and insulin levels between the perhexiline and placebo groups.

MYOCARDIAL ENERGETICS. At baseline, the PCr/ATP ratio correlated negatively with the MLWHFQ score ($r = -0.361$, $p < 0.05$). There was a significant difference in mean PCr/ATP ratio between patients in lower and higher NYHA functional classes (NYHA functional class II mean PCr/ATP ratio: 1.47 ± 0.05; NYHA functional class III and IV mean PCr/ATP ratio: 1.06 ± 0.07; $p = 0.0005$) (**Figure 3**). There was no significant correlation between the PCr/ATP ratio and LVEF ($r = 0.1$, $p = 0.52$) or NT-proBNP ($r = 0.01$, $p = 0.99$) levels.

At follow-up, the myocardial PCr/ATP ratio increased by 30% versus baseline in the perhexiline group (1.16 ± 0.39 to 1.51 ± 0.51; $p < 0.001$) but did not change in the placebo group (1.36 ± 0.31 to 1.34 ± 0.31; $p = 0.37$) ($p < 0.001$; 2-way repeated-measures analysis of variance) (**Table 2**, **Figure 4**). The ³¹P MRS spectra of a patient before and after the intervention

TABLE 2   Effect of Perhexiline and Placebo

Parameter	Perhexiline Group		Placebo Group		p Value
	Before Treatment	After Treatment	Before Treatment	After Treatment	
PCr/γATP ratio	1.16 ± 0.39	1.51 ± 0.51	1.36 ± 0.31	1.34 ± 0.31	<0.001
NHYA functional class, (class)	11 (II), 9 (III), 5 (IV)	2 (I), 16 (II), 3 (III), 3 (IV)	14 (II), 10 (III), 1 (IV)	15 (II), 8 (III)	0.031
MLWHFQ score	25 [34]	20 [37]	36 [48]	27 [50]	0.20
NT-proBNP level, pg/ml	620 [2,342]	528 [1,670]	241 [508]	307 [455]	0.93

Values are mean ± SD or mean [interquartile range].
  Abbreviations as in **Table 1**.

are shown in **Figure 5**. The mean CRLBs for PCr and ATP for the entire group were 10.7% and 12.7%, respectively, indicating a satisfactory signal-to-noise ratio [20]. Three patients were excluded from the initial analysis because of a poor signal-to-noise ratio (CRLBs >20%).

SYMPTOMS. At follow-up, there was an improvement in NHYA functional class in the perhexiline group compared with the placebo group (p = 0.036). Thirteen patients in the perhexiline group improved by 1 NHYA functional class (52%) compared with 5 patients (20%) in the placebo group (p = 0.02). No patient changed by more than 1 NYHA functional class (**Table 2**).
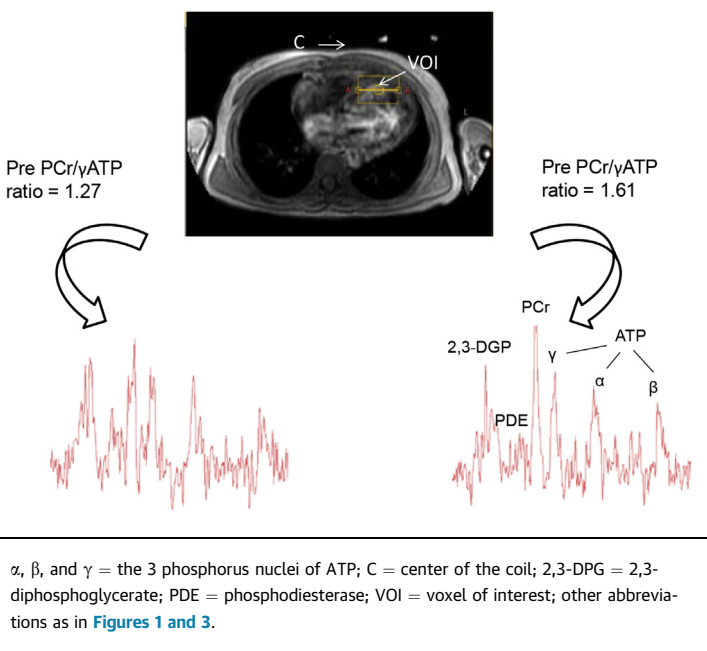
ECHOCARDIOGRAPHY. There was no change in the secondary endpoint of LVEF in the perhexiline group (27 ± 1.44% to 26 ± 1.77%) compared with the placebo group (30 ± 1.34% to 29 ± 1.96%) (p = 0.68). More sensitive measures of left ventricular function were sought by analyzing results derived from longitudinal strain measurements. Of the 47 patients who underwent echocardiography before and after the intervention, only 27 had adequate images for longitudinal strain and strain rate analysis (perhexiline: 12; placebo: 15) from speckle tracking echocardiography. This showed no change in peak global systolic strain or strain rate between the groups. Rotational strain analysis was available in 24 patients (perhexiline: 13; placebo: 11) and showed no change in either absolute twist or twist rate (data not shown).

VENOUS BLOOD METABOLITES AND NT-proBNP LEVELS. After the intervention, there were no differences in venous metabolites, NT-pro-BNP levels, or insulin levels between the groups (**Tables 2 and 3**).

CROSS-HEART METABOLISM. Cross-heart sampling from the invasive studies did not show a group difference in metabolite extraction (**Table 4**). The respiratory quotient was similar between the groups (perhexiline: 0.86 ± 0.06; placebo: 0.90 ± 0.16; p = 0.63). In the cross-heart sampling group, perhexiline therapy was associated with an increase in the PCr/ATP ratio of 0.51 ± 0.18, whereas placebo was associated with a reduction in the PCr/ATP ratio of 0.1 ± 0.16 (p < 0.005 for between-group difference).

ADVERSE EFFECTS. Adverse effects included nausea (n = 3), dizziness (n = 1), and diarrhea (n = 1) in the perhexiline group and headaches (n = 1), lethargy (n = 1), and metallic taste in the mouth (n = 1) in the placebo group. There were no instances of hepatotoxicity and no deaths or major adverse events during the study period. In the perhexiline group, 2 patients had subtherapeutic levels, and 2 patients had levels

FIGURE 5 An Example of ^{31}P MRS Before and After Treatment With Perhexiline

Pre PCr/γATP ratio = 1.27

Pre PCr/γATP ratio = 1.61

α, β, and γ = the 3 phosphorus nuclei of ATP; C = center of the coil; 2,3-DPG = 2,3-diphosphoglycerate; PDE = phosphodiesterase; VOI = voxel of interest; other abbreviations as in **Figures 1 and 3**.

above the therapeutic level at the end of the study period (**Figure 2**).

DISCUSSION

This is the first study to show that short-term perhexiline therapy leads to an improvement in cardiac energetics without a shift in substrate utilization. Importantly, this was associated with an improvement in NYHA functional class.

We have previously shown that perhexiline leads to an improvement in the PCr/ATP ratio in patients with hypertrophic cardiomyopathy after 5 months of therapy [6]. In the present study, the effects of perhexiline on the PCr/ATP ratio were observed by 1 month of therapy with a regimen that typically takes

TABLE 3 Effect of Perhexiline on Venous Metabolites and Insulin

Parameter	Perhexiline Group		Placebo Group		p Value
	Before Treatment	After Treatment	Before Treatment	After Treatment	
Glucose, mmol/l	5.81 ± 0.79	5.85 ± 0.79	6.42 ± 1.11	6.43 ± 0.37	0.75
Glycerol, mmol/l	75.02 ± 63.2	87.36 ± 68.71	74.98 ± 40.00	68.50 ± 30.34	0.55
Lactate, mmol/l	1.34 ± 0.49	1.42 ± 0.69	1.35 ± 0.49	1.49 ± 0.85	0.99
NEFA, mmol/l	0.52 ± 0.36	0.54 ± 0.29	0.56 ± 0.35	0.39 ± 0.11	0.20
Triglycerides, mmol/l	1.25 ± 0.37	1.23 ± 0.21	1.31 ± 0.43	1.30 ± 0.59	0.68
Pyruvate, nmol/ml	17.95 ± 6.76	19.50 ± 8.32	19.23 ± 9.96	16.86 ± 9.83	0.35
Insulin, μU/ml	9.49 ± 4.70	10.02 ± 1.96	17.27 ± 14.31	14.03 ± 6.74	0.11

Values are mean ± SD.
NEFA = nonesterified fatty acids.

TABLE 4 Cross-Heart Differences in Metabolites

Parameter	Perhexiline Group		Placebo Group		p Value
	Arterial	Venous	Arterial	Venous	
Glucose, mmol/l	5.20 ± 0.20	5.04 ± 0.31	5.12 ± 0.33	4.98 ± 0.13	0.24
Lactate, mmol/l	0.79 ± 0.11	0.63 ± 0.11	0.52 ± 0.08	0.47 ± 0.10	0.20
NEFA, mmol/l	1.07 ± 0.52	0.82 ± 0.51	0.77 ± 0.14	0.57 ± 0.16	0.12
Pyruvate, nmol/ml	4.52 ± 3.14	3.82 ± 4.87	6.42 ± 1.19	5.78 ± 1.58	0.48

Values are mean ± SD.
Abbreviation as in Table 3.

approximately 2 weeks to achieve therapeutic levels. This marked change in energetics is thus an early phenomenon, occurring before any demonstrable effect on resting cardiac performance, which is consistent with this being a direct effect of the drug on cardiac energetics rather than an improvement due to a longer-term improvement in the HF syndrome with consequent molecular reverse remodeling.

An important finding of this study is that the improvement in cardiac energetics with perhexiline occurs without any evidence of altered whole blood substrate levels. Furthermore, we did not see any difference in cross-heart substrate gradients or in cross-heart RER between the perhexiline and placebo groups. This is important because the latter provides a measure of relative substrate utilization (carbohydrate vs. fatty acids) derived from both uptake from plasma and from cardiac stores. A lower fatty acid oxidation and a greater reliance on carbohydrate oxidation would be expected to have manifested as a higher cross-heart RER. There was no significant difference between the groups, but the mean RER was actually lower in the perhexiline group. These findings are consistent with those of a study in the working rat heart model in which very short-term treatment with perhexiline was associated with a substantial improvement in cardiac mechanical efficiency at a stage before the demonstration of reduced palmitate uptake (21). However, this might have been attributable to an early reduction in use of fatty acids derived from myocyte triglyceride stores before a reduction in myocardial fatty acid uptake.

The mechanism(s) responsible for our observations of improved energetics with perhexiline are unclear. Although longer duration and/or higher plasma concentrations may well alter substrate use, our observations suggest additional or alternate mechanisms for improved energetics. Treatment with perhexiline has been reported to inhibit NADH/NADPH, thereby reducing reactive oxygen species generation (22), and to inhibit mTORC1 and thereby potentially increase autophagy, including mitophagy (23). It may

potentially have other pleotropic actions. A recent study reported a direct effect of trimetazidine on the mitochondrial electron transport chain in an experimental HF model (24), but this has not been assessed with perhexiline.

The lack of improvement in LVEF with perhexiline therapy conflicts with a prior study of perhexiline and with several studies of the metabolic modulator trimetazidine. A recent meta-analysis of trimetazidine in patients with HF showed an improvement in LVEF in patients with both ischemic and nonischemic HF (25). Lee et al. (5) showed an unprecedented 10% improvement in LVEF with perhexiline. The likely explanation for this is related to the purpose of this study, which was intended to ensure only short-term therapeutic plasma perhexiline levels to assess whether cardiac energetic improvement was a very early finding. We postulate that if treatment with perhexiline were continued in these patients, we would likely have seen a corresponding improvement in LVEF and NT-proBNP levels.

Despite the lack of change in LVEF, there was an improvement in NYHA functional class in the perhexiline-treated patients. This has been shown previously with both perhexiline (5) and trimetazidine (26). In a randomized, double-blind study, Lee et al. (5) showed that perhexiline therapy for 2 months led to a 21% reduction in NHYA functional class. This is comparable to the 17% reduction observed in the present study. Fragasso et al. (26) also showed a 19% reduction in NHYA functional class with trimetazidine. Such improvements in functional class have previously been attributed to both improvements in skeletal muscle function due to the effects of perhexiline on skeletal muscle or to improvements in cardiac output. Lee et al. (5) also performed ^{31}P MRS on skeletal muscle and showed improvement in PCr recovery time after exercise. The present study suggests that these changes occur before a demonstrable improvement in resting echocardiographic measures of LVEF and that they are either due to skeletal muscle effects and/or that improved cardiac performance during exercise precedes improvements in resting parameters.

STUDY LIMITATIONS. The number of patients was small and was not well matched at baseline, with those randomized to perhexiline having more severe HF, which could have had a significant impact on the baseline substrate utilization. Furthermore, for ethical reasons, it was not possible to submit patients to invasive studies before and after the intervention, which clearly would have been more robust. Nevertheless, given the substantial improvement in cardiac

energetics observed in these patients, we would have expected to see a difference in the on-treatment cross-heart substrate uptake and/or respiratory quotient if perhexiline were working through such a mechanism.

In our hands, the coefficient of variation for the measurement of RER was 9%. Although this was a secondary endpoint and our power calculations were on the basis of the primary endpoint, our sample size would have 80% power to detect a difference in RER between the 2 groups of 0.15. Accordingly, although we cannot exclude the possibility of a type II error resulting in failure to detect a modest difference in RER between the 2 groups, it seems unlikely that a shift in substrate utilization of sufficient magnitude to entirely explain the substantial effect of perhexiline on the cardiac PCr/ATP ratio would have been missed.

CONCLUSIONS

We have shown that short-term perhexiline therapy leads to improved cardiac energetics and NYHA functional class without altering substrate utilization or left ventricular function. This study supports the hypothesis of energy deficiency in HF and further consideration of metabolic therapies in the management of HF. Alternative mechanisms of action for perhexiline to explain these findings need to be explored.

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