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The effect of perhexiline on myocardial protection during coronary artery surgery: a two-centre, randomized, double-blind, placebo-controlled trial

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Abstract

OBJECTIVES: Perhexiline is thought to modulate metabolism by inhibiting mitochondrial carnitine palmitoyltransferase-1, reducing fatty acid uptake and increasing carbohydrate utilization. This study assessed whether preoperative perhexiline improves markers of myocardial protection in patients undergoing coronary artery bypass graft surgery and analysed its effect on the myocardial metabolome.

METHODS: In a prospective, randomized, double-blind, placebo-controlled trial, patients at two centres were randomized to receive either oral perhexiline or placebo for at least 5 days prior to surgery. The primary outcome was a low cardiac output episode in the first 6 h. All pre-specified analyses were conducted according to the intention-to-treat principle with a statistical power of 90% to detect a relative risk of 0.5 and a conventional one-sided α -value of 0.025. A subset of pre-ischaemic left ventricular biopsies was analysed using mass spectrometry-based metabolomics.

RESULTS: Over a 3-year period, 286 patients were randomized, received the intervention and were included in the analysis. The incidence rate of a low cardiac output episode in the perhexiline arm was 36.7% (51/139) vs 34.7% (51/147) in the control arm [odds ratio (OR) 0.92, 95% confidence interval (CI) 0.56–1.50, $P = 0.74$]. Perhexiline was associated with a reduction in the cardiac index at 6 h [difference in means 0.19, 95% CI 0.07–0.31, $P = 0.001$] and an increase in inotropic support in the first 12 h (OR 0.55, 95% CI 0.34–0.89, $P = 0.015$). There were no significant differences in myocardial injury with troponin-T or electrocardiogram, reoperation, renal dysfunction or length of stay. No difference in the preischaemic left ventricular metabolism was identified between groups on metabolomics analysis.

CONCLUSIONS: Preoperative perhexiline does not improve myocardial protection in patients undergoing coronary surgery and in fact reduced perioperative cardiac output, increasing the need for inotropic support. Perhexiline has no significant effect on the mass spectrometry-visible polar myocardial metabolome *in vivo* in humans, supporting the suggestion that it acts via a pathway that is independent of myocardial carnitine palmitoyltransferase inhibition and may explain the lack of clinical benefit observed following surgery.

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Keywords: Cardiac output • Metabolism • Metabolomics • Myocardial reperfusion injury • Myocardial stunning

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INTRODUCTION

The population of patients undergoing cardiac surgery is becoming older, with more comorbidities and complex patterns of disease, requiring more urgent surgery [1]. Such factors are often associated with abnormal myocardial metabolism and may contribute towards increasing the length of ischaemic arrest, which remains an independent predictor of mortality in contemporary practice [2]. With current techniques for myocardial protection using blood cardioplegia, up to 30% of patients have a period of clinically detectable transient dysfunction due to myocardial stunning. An episode of non-fatal low cardiac output in the early post-operative period is associated with a significant reduction in late survival following coronary artery bypass graft (CABG) surgery [3] and inadequate protection may lead to permanent injury due to diffuse necrosis, fibrosis, remodelling and long-term impairment of ventricular function. The need for longer periods of ischaemia in more metabolically vulnerable patients necessitates further research to improve protection strategies [4].

Pharmacological manipulation of myocardial metabolism aims to induce a shift in substrate utilization from fatty acids to glucose, thereby increasing energy efficiency and decreasing the potentially harmful effects of β -oxidation. Suppression of fatty acid utilization improves coupling of glycolysis to glucose oxidation, reduces lactate production, diminishes mitochondrial proton leakage and increases the efficiency of adenosine triphosphate (ATP) production per mole of oxygen [5]. Metabolic drugs may have a role in myocardial protection as adjuncts to cardioplegia and hypothermia by increasing metabolic efficiency and reducing the impact of ischaemia-reperfusion injury, thereby translating into enhanced recovery of cardiac function during reperfusion, and improved clinical outcomes. We have previously shown that glucose-insulin-potassium (GIK) therapy improves myocardial protection in patients with multivessel disease undergoing CABG [6] and in those with left ventricular hypertrophy undergoing aortic valve replacement [7]. However, GIK therapy has not been widely adopted due to the lack of large multicentre randomized, controlled trials or a standardized protocol, increased monitoring requirements with high-dose insulin, the potential negative impact of perioperative hyperglycaemia and uncertainty over its mechanisms of action. An agent with a similar metabolic efficacy but without the complexity of administration is desirable although the evidence of benefit for such an agent in myocardial protection is lacking.

Perhexiline is a modulator of myocardial metabolism that is effective in patients with refractory angina unsuitable for revascularization [8] and chronic cardiac failure [9, 10]. However, marked interindividual variation in its metabolism via cytochrome p450 2D6 (CYP2D6) previously resulted in severe adverse events due to chronic toxicity in a small number of poor metabolizers; toxicity can be prevented by therapeutic drug monitoring and dose adjustment to achieve plasma concentrations within the therapeutic range (0.15–0.6 mg/l) [11]. In the rat heart, it has been shown to inhibit carnitine palmitoyltransferase-1 (CPT-1), the key uptake enzyme for long-chain fatty acids into mitochondria, and this is thought to be its primary mechanism of action [12]. We report a prospective, double-blind, randomized, placebo-controlled trial to investigate whether preoperative administration of oral perhexiline improves clinical markers of myocardial protection in patients undergoing CABG at two centres in the UK. To examine the impact of perhexiline on myocardial metabolism in humans, we analysed the polar metabolic profile of left ventricular biopsies

obtained prior to ischaemia from patients within the therapeutic range for perhexiline compared with controls using high-resolution mass spectrometry (MS) [13]. The polar metabolome was chosen to provide the greatest insight into energy metabolism, including glycolysis and the citric acid cycle.

MATERIALS AND METHODS

Study design

A prospective, two-centre, double-blind, randomized, placebo-controlled trial of preoperative perhexiline (PEXSIG, Aspen Australia, Croydon, Victoria, Australia) was conducted in patients undergoing elective or urgent (during the same hospital admission) isolated, first-time CABG for multivessel coronary artery disease. The study was approved by the Cambridgeshire 1 Research Ethics Committee (06/Q0104/41) and the Medicines and Healthcare products Regulatory Agency (2006-003164-62). The trial was registered with ClinicalTrials.gov (NCT00845364), and patients were enrolled between February 2007 and April 2010 at the Queen Elizabeth Hospital Birmingham and the Royal Sussex County Hospital, Brighton, UK. Informed consent was obtained from each participant and all research was performed in accordance with the Declaration of Helsinki and the UK Human Tissue Act 2004 within a research governance framework.

The exclusion criteria were diabetes mellitus, significant renal or hepatic impairment, peripheral neuropathy, porphyria, atrial fibrillation, recent amiodarone therapy, emergency surgery (before the next scheduled operating list), known hypersensitivity to perhexiline or prospectively identified as requiring a significant deviation from the protocol on clinical grounds. Patients were randomized to either perhexiline or placebo in a 1:1 ratio using a computer-generated random allocation sequence with minimization for surgeon, priority of surgery and left ventricular function. All tablets were identical in appearance to conceal allocation. Trial medication was commenced a minimum of 5 days prior to the planned date of surgery and continued for up to 31 days. A standardized loading and maintenance regime was used: 200 mg b.i.d. for 3 days followed by 100 mg b.i.d. until the morning of surgery. Blood was drawn prior to anaesthesia and stored; serum perhexiline concentrations were determined by high-performance liquid chromatography at the end of the trial.

Surgery, anaesthesia, cardiopulmonary bypass and myocardial protection

Anaesthesia, cardiopulmonary bypass (CPB) and myocardial protection with intermittent antegrade cardioplegia using St Thomas' solution buffered in cold blood were all standardized as previously described [6], except that phenylephrine was used as the first-line vasoconstrictor. Distal anastomoses were performed during cardioplegic arrest and proximal anastomoses during partial aortic occlusion. A pulmonary artery catheter was used to assess haemodynamic variables, and the thermodilution technique was used to measure cardiac function. Baseline haemodynamic studies were performed prior to sternotomy. Following weaning from CPB, further measurements were taken before and ~10 min after the administration of protamine, and then at 2, 4, 6, 9 and 12 h after release of the aortic

cross-clamp. Standardized protocols were used to guide perioperative management including heart rate, volume expansion, inotrope use, temperature and glycaemic control. Serial blood samples were drawn at baseline at 6, 12 and 24 h to determine troponin-T by ECLIA (Roche Diagnostics, Burgess Hill, UK) and plasma non-esterified free fatty acids by enzymatic colorimetric assay (Wako Chemicals, Neuss, Germany).

End-points

The primary end-point was the incidence of a low cardiac output episode (LCOE), defined as a cardiac index of <2.2 l/min/m² refractory to appropriate intravascular volume expansion after correction of dysrhythmias in the first 6 h after cross-clamp release. A blinded end-points committee assessed all episodes of LCOE and a consensus opinion was reached. A planned exploratory analysis compared patients in the perhexiline arm who reached the lower threshold of the therapeutic range (0.15 mg/l) with propensity score-matched controls.

Secondary end-points were a comparison of cardiac index and inotropic support in the first six and 12 h, peak and area under the concentration-time curve (AUC) for troponin-T in the first 24 h and perioperative myocardial injury on 12-lead electrocardiogram (ECG). ECG changes were assessed by an independent blinded cardiologist and defined as new Q waves ≥ 2 mm in two or more contiguous leads, new bundle branch block or loss of R wave progression by the 4th postoperative day.

Metabolomic analysis

Transmural biopsies of the left ventricular free wall between the left anterior descending artery and the first diagonal branch were obtained prior to application of the aortic cross-clamp and immediately snap-frozen. Metabolites were extracted using a methanol : water : chloroform solvent system [14], dried using a centrifugal concentrator and stored at -80°C . Subsequently, each dried polar extract was re-dissolved in 80 : 20 methanol:water containing 20 mM ammonium acetate, vortexed and centrifuged prior to MS. Quality control (QC) samples were prepared by pooling an aliquot of each sample.

MS analyses were conducted using a hybrid 7-Tesla linear ion trap Fourier transform ion cyclotron resonance (FT-ICR) MS (LTQ FT Ultra, Thermo Fisher Scientific, Germany) equipped with a Triversa chip-based nano-electrospray ion source (Advion Biosciences, NY, USA) using conditions as described previously [15]. Three mass spectra for each sample were collected using a selected-ion-monitoring stitching method from m/z (mass-to-charge ratio) 70–740 in negative ion mode [15, 16], processed, normalized and generalized log-transformed as reported previously [17, 18]. This produced a peak intensity matrix representing the metabolic profile of each extracted biopsy. Using the MI-Pack software [19], m/z measurements were putatively annotated (see [Supplementary material](#)).

Statistical analysis

Clinical trial. All analyses were prespecified and conducted according to the intention-to-treat principle. The trial had a statistical power of 90% to identify a relative risk of 0.50, which was statistically significant, assuming an incidence of LCOE in the control group of 0.37 and a conventional one-sided α of 0.025.

Analyses were conducted with SAS software version 9.1 or above (SAS Institute, Inc., Cary, NC, USA). Continuous data are presented as mean (standard deviation, SD) or median (interquartile range, IQR). P -values other than for the primary end-point were nominal. All analyses were stratified for left ventricular function and urgency of surgery as patient-level covariates, and surgeon as a random effect. Dichotomous outcomes were analysed with the use of nonlinear mixed models and continuous data with mixed models. Propensity scoring approaches were used to identify matched controls, based on age, race, weight, days of trial therapy, left ventricular function and priority of surgery. Supportive analyses were conducted using generalized linear models and repeated measures.

Metabolomics. Student's t -tests were conducted on the non-generalized log-transformed peak intensity matrix, using a false discovery rate (FDR) of 5%, to determine if individual peaks changed significantly between groups [20]. In addition, principal components analyses (PCA) and partial least squares-discriminant analyses (PLS-DA) were conducted to discover metabolic differences between groups. Internal cross-validation was applied to assess for over-fitting of the optimal PLS-DA model.

Additional methods appears in the [Supplementary material](#).

RESULTS

Study population

Three hundred and twenty-seven patients were randomized of whom 286 were included in the analysis (Fig. 1), 139 randomized to perhexiline and 147 to placebo (see [Supplementary material](#)). Baseline preoperative characteristics were similar between the groups (Table 1) including median logistic EuroSCORE: 1.82 (1.23–3.06) in the treatment arm and 1.82 (1.07–2.94) in the control arm. Participants took the trial medication for a median of 10 days (6–12 days) prior to surgery. Eighteen (6.3%) patients reported potential side effects of perhexiline, principally nausea or dizziness, of whom 15 were in the treatment group and three in the placebo group; 6 (4.3%) patients in the perhexiline group reported non-compliance with the dosing protocol due to side effects. Serum perhexiline at the time of surgery was measured in 280 patients including 135/139 (97.1%) in the treatment group in whom the median serum concentration was 0.24 mg/l (0.33 mg/l) with a range of 0.04–1.97 mg/l. In the perhexiline arm, 37 (27.4%) patients were below the lower threshold of the therapeutic range (0.15 mg/l); there was no correlation between serum perhexiline level and length of therapy. In the placebo group, all patients measured had a serum perhexiline level of zero, confirming the difference between the groups.

All patients underwent multivessel CABG. One patient in the perhexiline and 3 in the control group had an additional mitral valve procedure and 1 further patient in the control group underwent aortic valve replacement. Operative variables including CPB and aortic cross-clamp times were similar between groups (Table 2).

Primary outcome

A LCOE was diagnosed in 102 of 286 (35.7%) patients analysed; however, there was no significant difference in incidence between the two groups: 51/139 (36.7%) in the perhexiline arm and 51/147 (34.7%) in the control arm (OR 0.92, 95% CI 0.56–1.50, $P=0.74$) (Table 3). In the exploratory analysis, patients in the perhexiline arm

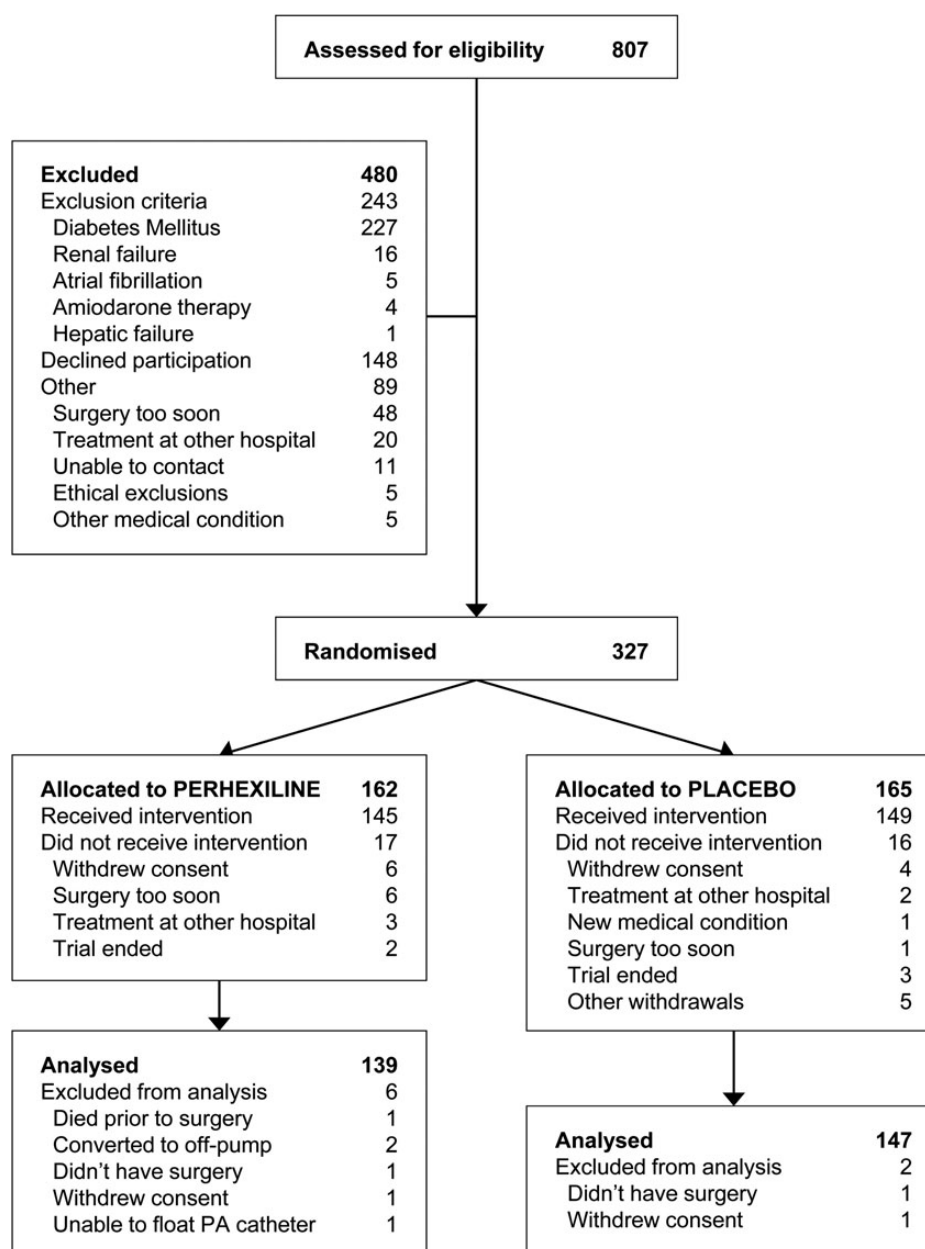


Figure 1: Participant flow diagram.

who were above the lower threshold of the therapeutic range (≥ 0.15 mg/l) at the time of surgery were compared with propensity score-matched controls ($n = 97$ in each group, 1 patient excluded due to missing data). There remained no significant difference in the incidence of LCOE between groups: 38/97 (39.2%) for perhexiline and 31/97 (32.0%) for controls (OR 0.73, 95% CI 0.40–1.33, $P = 0.30$).

Secondary outcomes

Haemodynamic data. At 6 h following reperfusion, the mean cardiac index was significantly lower in the perhexiline group (2.51 l/min/m², SD 0.43) than in the control group (2.70 l/min/m², SD 0.54) (difference in means 0.19, 95% CI 0.07–0.31, $P = 0.001$) (Fig. 2). By 12 h after reperfusion, there was no difference between the groups: 2.73 l/min/m² (SD 0.54) for perhexiline vs 2.79 l/min/m²

(SD 0.48) for controls (difference in means 0.058, 95% CI –0.06–0.18, $P = 0.34$). Throughout the study, heart rate, mean arterial pressure, central venous pressure and pulmonary artery wedge pressure were similar between groups.

Cardiac index measured prior to ischaemia was found to be significantly lower in the perhexiline group (2.09 l/min/m², SD 0.57) than in the control group (2.31 l/min/m², SD 0.53) (difference in means 0.22, 95% CI 0.09–0.35, $P = 0.001$). Using repeated measures, the cardiac index was found to be significantly affected by perhexiline therapy (difference in means 0.13, 95% CI 0.08–0.17, $P < .0001$).

Inotrope and vasoconstrictor use. There was no difference in the prevalence of inotropic support in the first 6 h after reperfusion: perhexiline 39/139 (28.1%) vs control 36/147 (24.5%) (OR 0.84, 95% CI 0.49–1.44, $P = 0.52$). However, by 12 h, inotrope use was significantly more frequent in the perhexiline group (67/139, 48.2%) versus the control group (50/147, 34.0%) (OR 0.55,

Table 1: Baseline patient characteristics

	Perhexiline (n = 139)	Control (n = 147)
Age, median (IQR) (years)	66.1 (59.4–73.2)	65.7 (60.2–73.6)
Male gender, n (%)	128 (92.1)	134 (91.2)
Race, n (%)		
Caucasian	133 (95.7)	136 (92.5)
South Asian	5 (3.6)	10 (6.8)
Black	1 (0.7)	1 (0.7)
CCS class, n (%)		
0	12 (8.6)	7 (4.8)
I	9 (6.5)	14 (9.5)
II	70 (50.4)	71 (48.3)
III	39 (28.1)	43 (29.3)
IV	9 (6.5)	12 (8.2)
NYHA class, n (%)		
I	58 (41.7)	59 (40.1)
II	68 (48.9)	79 (53.7)
III	13 (9.4)	9 (6.1)
IV	0 (0)	0 (0)
Previous MI, n (%)	49 (35.3)	48 (32.7)
Previous coronary stent, n (%)	13 (9.4)	10 (6.8)
Left main stem disease ≥50%, n (%)	54 (38.9)	47 (32.0)
Left ventricle function, n (%)		
Good	118 (84.9)	122 (83.0)
Moderate	20 (14.4)	24 (16.3)
Poor	1 (0.7)	1 (0.7)
Priority, n (%)		
Elective	120 (86.3)	124 (84.4)
Urgent	19 (13.7)	23 (15.7)
Smoker, n (%)		
Non	38 (27.3)	55 (37.4)
Current	14 (10.1)	16 (10.9)
Ex-smoker	87 (62.6)	76 (51.7)
Pulmonary disease, n (%)	17 (12.2)	12 (8.2)
Peripheral vascular disease, n (%)	14 (10.1)	10 (6.8)
Recent MI (last 90 days), n (%)	19 (13.7)	17 (11.6)
Number of antianginal agents, n (%)		
0	3 (2.2)	2 (1.4)
1	68 (48.9)	65 (44.2)
2	48 (34.5)	55 (37.4)
3	15 (10.8)	19 (12.9)
4	5 (3.6)	6 (4.1)
Haemoglobin, mean (SD) (g/dl)	14.32 (1.3)	14.11 (1.2)
Creatinine, mean (SD) (mg/dl)	1.20 (0.2)	1.12 (0.2)
EuroSCORE, median (IQR)	3 (1 to 4)	3 (1 to 4)
Logistic EuroSCORE, median (IQR)	1.82 (1.23 to 3.06)	1.82 (1.07 to 2.94)

CCS: Canadian Cardiovascular Society; NYHA: New York Heart Association; MI: myocardial infarction; SD: standard deviation; IQR: interquartile range.

95% CI 0.34–0.89, $P = 0.015$). There were no differences between groups in the dose requirements for phenylephrine ($P = 0.26$), norepinephrine ($P = 0.12$) or insulin ($P = 0.43$) in the first 12 h.

Myocardial injury. There was no difference in the mean peak serum troponin-T concentration during the first 24 h after surgery: 0.78 ng/ml (SD 0.71) in perhexiline patients vs 0.89 ng/ml (SD 0.92) in control patients (difference in means 0.11, 95% CI –0.09–0.30, $P = 0.28$). The AUC in the first 24 h was also not significantly

different between groups: perhexiline 3.98 ng h/ml (SD 3.79) vs control 4.71 ng h/ml (SD 5.32) ($P = 0.12$) (Fig. 3). There was no difference in the frequency of ECG changes consistent with new myocardial injury: 23/139 (16.6%) in the perhexiline group vs 25/147 (17.0%) in the control group (OR 1.02, 95% CI 0.55–1.92, $P = 0.94$).

Plasma non-esterified free fatty acids were measured in 35 patients (perhexiline $n = 16$, control $n = 19$) and found to be significantly higher prior to ischaemia in the perhexiline group (0.75 mmol/l, SD 0.08) than in the control group (0.50 mmol/l, SD 0.13) ($P < 0.001$). This difference was lost by 6 h into reperfusion: 0.73 mmol/l (SD 0.15) in the perhexiline group vs 0.70 mmol/l (SD 0.21) in the control group ($P = 0.64$). No significant relationship between plasma free fatty acid concentration and pre-ischaemic cardiac index was observed on regression ($R^2 = 0.09$, $P = 0.08$).

There were no significant differences in the prespecified safety end-points of death, stroke, the need for renal replacement therapy, reoperation or length of ICU or hospital stay between groups (Table 4).

Metabolomic analysis

Polar extracts from preischaemic left ventricular biopsies were analysed from 43 patients (perhexiline $n = 22$, control $n = 21$). All biopsies from the perhexiline group were from patients above the lower end of the therapeutic range (median 0.36 mg/l, IQR 0.24–0.58). The median spectral relative standard deviation, a benchmark to assess the reproducibility in metabolomics [21], for each sample analysed in triplicate by MS was relatively small and consistent across all samples (mean 11.6%, SD 1.6%). The final intensity matrix after data processing consisted of 4039 peak intensity measurements for each sample. All peaks were examined using univariate statistics to determine if any intensity changed significantly in response to perhexiline treatment. No significant peak intensity changes were found (FDR 5%). Multivariate PCA was used to reduce the dimensionality of the data and visualize the metabolic similarities and differences between the two groups; the plot of PCA scores did not show any metabolic effects due to perhexiline treatment (Fig. 4). The clustering of the QC samples demonstrates the consistency in instrument performance over time. Additionally, PLS-DA was conducted to maximize the separation of the metabolic profiles of the two groups (see [Supplementary material](#)). The control and treatment samples were minimally separated, and the associated mean classification error rates of the model for predicting class membership were high at 43 and 47%, respectively, suggesting no metabolic differences between groups. More than 200 m/z measurements in the FT-ICR MS dataset were assigned to at least one putative named metabolite including ATP, creatine, phosphocreatine, glycolytic and citric acid cycle intermediates (see [Supplementary material](#)).

DISCUSSION

In this double-blind, randomized, placebo-controlled trial of pre-operative oral perhexiline as an adjunct to cold blood cardioplegia for myocardial protection during CABG, there was no effect on the primary end-point, the incidence of a LCOE. This was confirmed in a propensity score-matched analysis in which patients in the treatment group who were below the therapeutic range were excluded. In addition, there was no difference in the use of

Table 2: Operative variables

	Perhexiline (n = 139)	Control (n = 147)
Number of grafts, mean (SD)	3.30 (0.73)	3.29 (0.82)
Internal mammary artery graft used, n (%)	132 (95.0)	136 (92.5)
Additional procedure, n (%)	1 (0.7)	4 (2.7)
Operation performed by trainee, n (%)	38 (27.3) ^a	50 (34.0)
CPB time, median (IQR) (min)	109 (93–131) ^b	110 (90–134)
Reinstitution of CPB, n (%)	3 (2.2)	6 (4.1)
Aortic cross-clamp time, median (IQR) (min)	56 (45–70) ^b	57 (45–72)
Total cardioplegia dose, median (IQR) (l)	1.95 (1.70–2.30)	1.90 (1.62–2.24)
Reperfusion VF/VT, n (%)	3 (2.2)	3 (2.0)
Antifibrinolytic used, n (%)	97 (69.8)	100 (68.0)
Cell salvaged blood, median (IQR) (ml)	470 (260–700)	485 (300–700)
Intra-aortic balloon pump used, n (%)	10 (7.2) ^a	21 (14.3)
Preop for unstable angina	1 (0.7)	0 (0)
Preop elective in theatre	3 (2.2)	6 (4.1)
Pre-CPB for intraoperative instability	2 (1.4)	0 (0)
During CPB for anticipated instability	1 (0.7)	4 (2.7)
Post-CPB for instability	3 (2.2) ^a	11 (7.6)

Reinstitution of CPB relates to episodes of unsuccessful weaning from CPB requiring a second period of CPB to prevent cardiovascular collapse.

CPB: cardiopulmonary bypass; VF: ventricular fibrillation; VT: ventricular tachycardia; SD: standard deviation; IQR: interquartile range.

^aNot significant with Fisher's exact test.

^bNot significant with Student's *t*-test.

Table 3: Study outcomes, the perhexiline group compared with the control group

Outcomes	Perhexiline	Control	Odds ratio (95% CI)	P-value
Primary outcome, n (%)	139	147		
Low cardiac output episode	51 (36.69%)	51 (34.69%)	0.92 (0.56 to 1.50)	0.74
Secondary outcomes, categorical, n (%)	139	147		
Inotrope use in first 6 h ^a	39 (28.06%)	36 (24.49%)	0.84 (0.49 to 1.44)	0.52
Inotrope use in first 12 h	67 (48.20%)	50 (34.01%)	0.55 (0.34 to 0.89)	0.015
New myocardial injury on ECG	23 (16.55%)	25 (17.01%)	1.02 (0.55 to 1.92)	0.94
Secondary outcomes, continuous, mean (SD)	135	144		
Cardiac index at 6 h (l/min/m ²)	2.51 (0.43)	2.70 (0.54)	0.19 (0.07 to 0.31) ^b	0.001
Peak troponin-T (ng/ml)	0.78 (0.71)	0.89 (0.92)	0.11 (–0.09 to 0.30) ^b	0.28
AUC troponin-T (ng h/ml)	3.98 (3.79)	4.71 (5.32)	0.73 (–0.40 to 1.86) ^b	0.12 ^c

Accounting for baseline left ventricular function and priority of surgery, with surgeon as a random effect.

SD: standard deviation; AUC: area under the concentration-time curve.

^aBefore cardiopulmonary bypass, during cardiopulmonary bypass and 0–6 h.

^bDifference in means (95% confidence interval).

^cOne-tailed.

inotropes in the first 6 h or the incidence of myocardial injury determined by troponin release or significant ECG changes between groups. Indeed, cardiac index was found to be lower in the treatment arm compared with controls both prior to ischaemia and at 6 h after release of the aortic cross-clamp. On studying the polar metabolome of the left ventricle prior to ischaemia using MS-based metabolomics, we found that the metabolic profiles of patients on perhexiline, with confirmed therapeutic plasma levels, were indistinguishable from those in the placebo group.

Metabolic therapy provides an opportunity to improve myocardial protection by inducing a shift in substrate utilization from fatty acids to glucose, thereby increasing the efficiency of energy

production and reducing the harmful effects of β -oxidation during reperfusion [4]. The most extensively studied metabolic strategy is altering the availability and use of substrates using intravenous GIK solution. Insulin has multiple metabolic and non-metabolic effects on the myocardium but their relative importance is unclear. These include suppression of lipolysis with reduced uptake and β -oxidation of fatty acids; increased glucose flux, myocardial oxygen efficiency and glycolytic ATP production; replenishment of citric acid cycle intermediates via anaplerosis; and activation of pro-survival reperfusion injury salvage kinases (RISK) including Akt and AMPK [7]. GIK has been shown to improve postoperative haemodynamics, decrease inotrope requirements and reduce myocardial injury in patients undergoing cardiac surgery [6, 7, 22], but has not

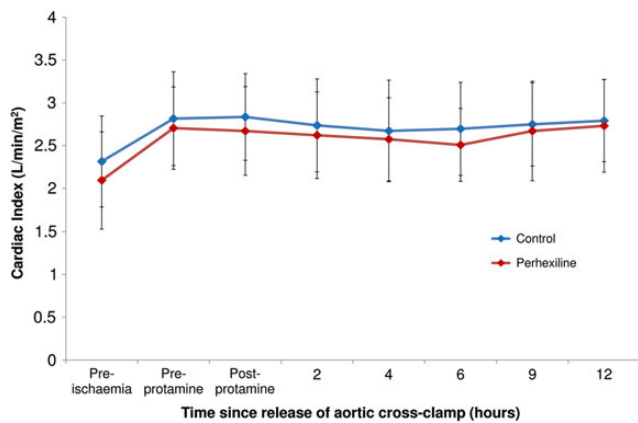


Figure 2: Mean cardiac index (l/min/m²) between treatment groups. Error bars represent 95% confidence intervals (CI) for the mean ($P = 0.018$ at 6 h).

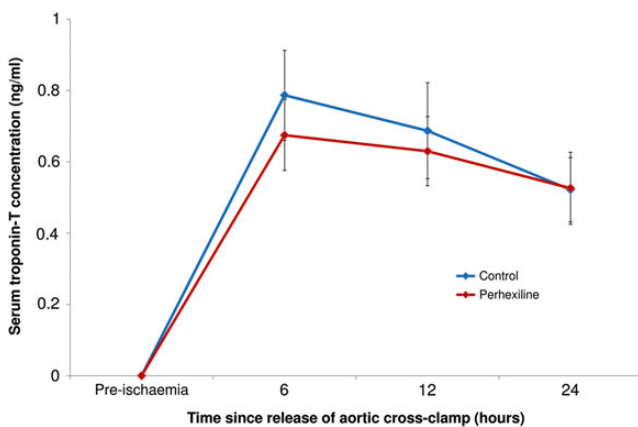


Figure 3: Area under the concentration–time curve for serum troponin-T (ng h/ml) ($P = 0.12$).

been widely adopted principally due to practical difficulties in its administration. While perhexiline has not previously been studied as an adjunct to myocardial protection in cardiac surgery, it has been shown to be clinically effective in the treatment of refractory angina [8], chronic cardiac failure [9] and symptomatic hypertrophic cardiomyopathy [10]. Its mechanism of action has been widely accepted as inhibition of mitochondrial CPT-1, leading to reduced uptake and β -oxidation of long-chain fatty acids [12]. A recent proteomic and metabolomic study in a murine model suggested that perhexiline activates the pyruvate dehydrogenase complex and may cause a complex rebalancing of carbon and nucleotide phosphate fluxes to increase metabolic flexibility [23]. However, our metabolomic analysis demonstrates that, in patients with ischaemic heart disease undergoing cardiac surgery, perhexiline has no significant effect on the polar myocardial metabolome with no change in the intermediates of energy transfer, glycolysis or the citric acid cycle. This lack of up-regulation in glucose metabolism may account for the lack of clinical benefit in this trial and suggests that the mechanism of action of perhexiline *in vivo* in humans may not be primarily metabolic. The possibility that perhexiline acts via a CPT-independent pathway has previously been raised following the observation that there is a temporal dissociation between its effects on cardiac efficiency and metabolism in a rat heart model [24]. Recent work has shown that it suppresses expression of thioredoxin-

Table 4: Other clinical outcomes and complications

	Perhexiline (n = 139)	Control (n = 147)
Postoperative death, n (%)	2 (1.4)	2 (1.4)
Stroke, n (%)	3 (2.2)	1 (0.7)
Neurological, n (%)	18 (12.9)	12 (8.2)
Type I (stroke, TIA)	3 (2.2)	2 (1.4)
Type II (confusion, disorientation)	15 (10.8)	10 (6.8)
Chest tube drainage at 12 h, mean (SD)	701 (498)	704 (448)
Reoperation, n (%)	9 (6.5) ^a	13 (8.8)
Bleeding	6 (4.3)	11 (7.5)
LCOE/tamponade	1 (0.7)	1 (0.7)
Arrest	2 (1.4)	1 (0.7)
Need for CPB at reoperation	1 (0.7)	1 (0.7)
Arrhythmia, n (%)	55 (39.6) ^a	66 (44.9)
Atrial fibrillation	53 (38.1)	64 (43.5)
Atrial flutter	2 (1.4)	2 (1.4)
Pneumonia, n (%)	15 (10.8)	14 (9.5)
Tracheostomy, n (%)	4 (2.9)	4 (2.7)
Any pulmonary complication, n (%)	33 (23.7)	34 (23.1)
Creatinine day 4, mean (SD) (mg/dl)	1.08 (0.35)	1.10 (0.45)
Creatinine peak, mean (SD) (mg/dl)	1.34 (0.51)	1.30 (0.54)
AKIN score, n (%)		
0	121 (87.1)	126 (85.7)
1	13 (9.4)	16 (10.9)
2	2 (1.4)	0 (0%)
3	3 (2.2)	5 (3.4)
RRT requirement, n (%)	3 (2.2)	2 (1.4)
Abdominal complication, n (%)	5 (3.6)	4 (2.7)
Gastrointestinal bleed	2 (1.4)	2 (1.4)
Prolonged paralytic ileus	0 (0)	2 (1.4)
Diarrhoea	3 (2.2)	0 (0)
Sternal infection, n (%)	5 (3.6)	5 (3.4)
Superficial infection/dehiscence	5 (3.6)	4 (2.7)
Deep infection requiring surgery	0 (0)	1 (0.7)
Any treated infective episode, n (%)	27 (19.4) ^a	25 (17.0)
Transfusion, mean (SD) (units)		
Blood	2.26 (2.62)	2.02 (2.58)
Platelets	0.82 (1.30)	0.85 (1.18)
Fresh frozen plasma	1.89 (2.55)	1.85 (2.64)
Fluid volume in 12 h, mean (SD) (ml/kg)	67.4 (27.5)	62.7 (31.3)
Discharge, n (%)	137 (98.6)	145 (98.6)
Home	130 (93.5)	138 (93.9)
Convalescence	5 (3.6)	6 (4.1)
Another hospital/department	2 (1.4)	1 (0.7)

TIA: transient ischaemic attack; LCOE: low cardiac output episode; CPB: cardiopulmonary bypass; AKIN: Acute Kidney Injury Network; RRT: renal replacement therapy; SD: standard deviation.

^aNot significant with Fisher's exact test.

interacting protein (TXNIP), the key regulator of the antioxidant thioredoxin system, and increases expression of the energy sensor AMPK and its downstream effector PGC-1 α [25], although the relevance of these observations remains to be proven. While its predominant mechanism of action in non-surgical patients remains unconfirmed, we found neither an improvement in contractility at any time point nor a reduction in myocardial injury following ischaemia–reperfusion.

Patients treated with perhexiline had a lower cardiac index prior to ischaemia although the mechanisms underlying this finding are unknown. Despite a weak calcium channel inhibitory effect [11], perhexiline has not previously been found to be negatively inotropic although none of the previous studies involved anaesthesia or surgery. Plasma free fatty acids prior to ischaemia were

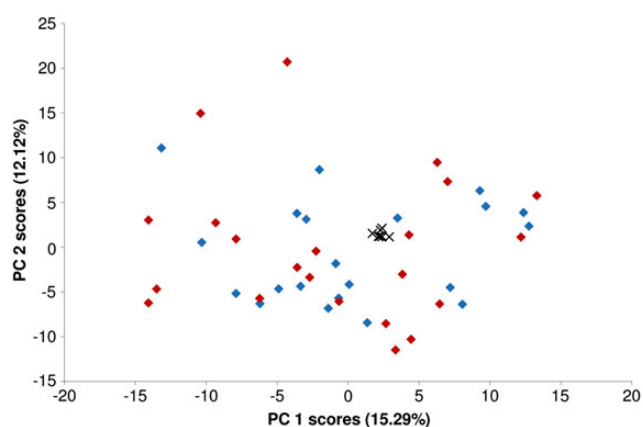


Figure 4: Principal components analysis scores plot from negative ion Fourier transform ion cyclotron resonance mass spectra of left ventricular extracts: 21 control samples (blue) and 22 perhexiline samples (red), shows no metabolic response to perhexiline along the PC1 and PC2 axes. All eight quality control samples (X) are in a narrow cluster, confirming the consistency of mass spectrometry instrument performance over time.

significantly higher in the perhexiline group than the control group; high levels of fatty acids are known to reduce cardiac efficiency although there was no correlation between plasma free fatty acids and preischemic cardiac index. In the design and conduct of the trial, randomization was stratified for left ventricular function, although it was not precisely quantified on echocardiogram prior to commencing the trial therapy. All patients in whom the primary end-point was available were analysed on an intention-to-treat basis and no differences between groups were observed for the primary end-point, other secondary outcomes apart from the cardiac index, safety end-points or any other post-operative variable.

One of the limitations of perhexiline is the marked interindividual variation in its metabolism mainly due to genetic polymorphisms of CYP2D6 [11]. The dosing regimen used in this trial attempted to achieve prompt but effective loading with perhexiline in the community with a low incidence of toxicity, especially in poor metabolizers of the drug. We found that ~27% of the perhexiline group were below the lower threshold of the therapeutic range (0.15 mg/l) at the time of surgery but there was no correlation with length of therapy, suggesting that the minimum period of loading was sufficient. These sub-therapeutic patients comprised all of those found to be ultrarapid metabolizers of perhexiline plus some of the patients who had reported side effects and were either known or presumed to have stopped taking the drug. While this failure to attain a therapeutic concentration at the time of surgery is a limitation of perhexiline use, the comparison of therapeutic patients with propensity score-matched controls confirmed that perhexiline had no benefit in reducing the incidence of LCOE in this trial.

In conclusion, the addition of oral perhexiline as an adjunct to cardioplegia in patients undergoing CABG did not improve clinical or biochemical end-points of myocardial protection or any other outcome measures, and was associated with a reduction in the perioperative cardiac index. Therefore, preoperative perhexiline does not improve the outcomes of patients undergoing cardiac surgery and may in fact impair cardiac function in the perioperative period, increasing the need for inotropic support. We also present novel human data suggesting that perhexiline has no significant effect on the MS-visible polar myocardial metabolome *in*

vivo at therapeutic serum concentrations; this supports the suggestion that perhexiline acts via a pathway that is largely or entirely independent of myocardial CPT-1 inhibition [24] and may explain the lack of clinical benefit observed following surgery.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *EJCTS* online.

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Conflict of interest: Michael P. Frenneaux is inventor of method of use patents for perhexiline in heart muscle diseases. The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree with the manuscript as written. Neither the funders nor the sponsor had any intellectual input into the design, analysis, report or submission for publication.

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