

**Treatment of Barth Syndrome by CARDIolipin MANipulation (CARDIOMAN): A
randomised placebo controlled pilot trial conducted by the nationally commissioned
Barth Syndrome Service**

CARDIOMAN

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Glossary / abbreviations

ADR	Adverse drug reaction
AE	Adverse event - any undesirable event in a subject receiving treatment according to the protocol, including occurrences which are not necessarily caused by or related to administration of the research procedures.
AR	Adverse reaction – any undesirable experience that has happened a subject while taking a drug that is suspected to be caused by the drug or drugs
ATP	Adenosine 5' triphosphate – a multifunctional nucleotide that plays an important role in cell biology as a coenzyme involved in intracellular energy transfer
BDS	Bis die sumendum: twice daily
BEZA	Bezafibrate
BHI	Bristol Heart Institute
BNF	British National Formulary
BP	Blood pressure
BRI	Bristol Royal Infirmary
BRU	Biomedical Research Unit
BSF	Barth Syndrome Foundation
BSS	Barth Syndrome Service
BTHS	Barth syndrome
CK	Creatinine Kinase
CL	Cardiolipin
CRF	Case report form
CRIC	Clinical Research and Imaging Centre
CTEU	Clinical Trials and Evaluation Unit
DCM	Dilated cardiomyopathy
DMSC	Data monitoring and safety committee
EBV	Epstein-Barr virus
ECG	Graphical representation of electrical activity of the heart over time, as recorded by an electrocardiograph
ECMO	Extracorporeal membrane oxygenation
EF	Ejection Fraction
EME	Efficacy and mechanisms evaluation programme
EMA	European Medicines Agency
FS	Fractional shortening
GCSF	Granulocyte colony stimulating factor
GOSH	Great Ormond Street Hospital

GP	General Practitioner
HDL	High density lipoprotein
HHb	Deoxyhaemoglobin
HMG CoA	3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors (Statins)
HR	Heart rate
HSG	Health Service Guidelines
ICH-GCP	International conference for harmonisation of good clinical practice
IMP	Investigational medicinal product
LA	Left atrial
LDL	Low density lipoprotein
LFT	Liver function test
LV	Left ventricular
LVEF	Left ventricular ejection fraction
LVNC	Left ventricular non-compaction
MedRA	Medical Dictionary for Regulatory Activities
MHRA	Medicines and healthcare products regulatory agency
MLCL	Monolysocardiolipin
MRC	Medical Research Council
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
NHS	National Health Service
NIHR	National Institute for Health Research
NIRS	Near infrared spectroscopy
NOS	Not otherwise specified
OD	Omne in die: once daily
PCr	Phosphocreatine
Peds QL™	Paediatric quality of life inventory
PIL	Patient information leaflet
PKB	Patients Know Best website (https://www.patientsknowbest.com)
PW-TDI	Pulse Wave-Tissue Doppler Imaging
RA	Right atrial
RCT	Randomised controlled trial
REC	Research ethics committee
SAE	Serious adverse event - events which result in death, are life threatening, require hospitalisation or prolongation of hospitalisation, result in persistent or significant disability or incapacity.
SAR	Serious adverse reaction
SmPC	Summary of Product Characteristics
SOP	Standard operating procedure
SPC	Summary of Product Characteristics
SSAR	Suspected serious adverse reaction
SUSAR	Suspected unexpected serious adverse reaction - an untoward medical occurrence suspected to be related to a medicinal product that is not consistent with the applicable product information and is serious.
TDS	Ter die sumendum: three times a day
TSC	Trial steering committee
UH Bristol	University Hospitals Bristol NHS Foundation Trust
UK	United Kingdom
UoB	University of Bristol
USA	United States of America

VO₂ Volume of oxygen
WBC White blood cell count

1. Trial summary

Barth syndrome is a rare, life threatening, genetic disease which affects young males. It is caused by abnormal fats (lipids) in the powerhouses of cells (mitochondria) and those who suffer with it often develop heart failure, heart rhythm abnormalities, bacterial infections, poor growth or feeding, weak muscles, developmental delay, severe exercise intolerance, lethargy and fatigue; all of which affect their daily life. Low white blood cell counts occur frequently due to intermittent or persistent reduction in numbers of the neutrophils that are responsible for fighting bacterial infections. This requires expensive and distressing injections to stimulate the bone marrow to produce more neutrophils. In addition, approximately one third of all males living with this disease in the UK have required heart transplantation.

Scientific research has shown that several treatments can improve the fat abnormalities in cells affected by Barth syndrome from either mice or humans; one of which is a drug called bezafibrate. Bezafibrate is particularly attractive as it has been safely administered for over 20 years in the UK to both adults and children for the treatment of high blood fats. The purpose of this study is to see if bezafibrate can be given safely and effectively to people with Barth syndrome in a blinded randomised trial.

Bezafibrate or an inactive (placebo) treatment will be given for 4 months, followed by a one month break, and then followed by 4 months of the alternate treatment (e.g. placebo if bezafibrate taken for the first 4 months and vice versa). Half of the participants will take bezafibrate first, the other half will take placebo first; participants and research staff will not know which order the treatments are given in (double-blind trial).

Once the participants have completed the first phase of treatment, the study statisticians will be unblinded to the treatment arms and will then assess whether or not there is evidence of efficacy of bezafibrate for the primary outcome.

Tests will be performed at commencement of the study and after each 4 month treatment period, looking for benefit in blood cells, exercise capacity, heart function or quality of life. The clinical studies of bezafibrate will run alongside laboratory work at Bristol University and Great Ormond St Hospital to see the effect of bezafibrate on participant's cells and mitochondria (powerful electron microscopes show that these are abnormal in Barth syndrome). This is to see whether we can predict any improvement in symptoms in order to tell us which patients would benefit from this treatment in future. Laboratory work will also be carried out on participant's cells using another drug called resveratrol, which has also shown promise in laboratory tests, to see if this could provide an alternative treatment.

We wish to study up to 18 Barth syndrome boys and young men; all of those with Barth syndrome in the UK aged 6 years or over. We aim to use a national children's research network and/or local GPs to perform monthly blood tests in order to minimise the amount of travel to Bristol for participants. Safety will be monitored by an independent committee with the authority to recommend stoppage if obvious problems are seen.

If promising results are obtained with either or both drugs, we would share this information with American/European teams so that this work will have worldwide benefit. For this, we have the complete backing of our patients, their families and the respective UK and USA charities.

2. Background

Barth Syndrome is a life threatening genetic disease which affects young males. It is caused by abnormal lipids in mitochondria. Those affected may be stillborn or develop heart failure during the first decade of life, suffer from bacterial infections, poor growth or feeding problems during childhood. 22 males are currently alive with the disease in the UK. This disease carries many risks and problems for its sufferers, as well as major healthcare costs.

Boys and men living with the disease have severe exercise intolerance, lethargy and fatigue which affect their daily life; many use wheelchairs intermittently to aid mobility. Furthermore, children continue to die from this disease despite best conventional therapy including granulocyte colony stimulating factor (G-CSF) to stimulate neutrophil production, drugs to treat cardiomyopathy, and cardiac transplantation where required.

Scientific research has shown that several treatments can improve the fat abnormalities in cells affected by Barth syndrome in mice or humans [1]. One is a drug (bezafibrate) that has previously been used in children to lower blood fats as well as in adults with a muscle disease resulting from mitochondrial problems and the other is a food supplement (resveratrol). Both treatments have shown promising effects in a mouse that has heart problems like those seen in Barth syndrome [1].

There are no specific treatments for Barth syndrome other than supportive care provided for acute symptoms. Supplements such as coenzyme Q, carnitine and antioxidants are frequently used to treat other mitochondrial diseases but have proven ineffective in Barth syndrome [2] and there is no evidence that standard medications for cardiomyopathy ameliorate long-term outcome.

30% of living UK patients have undergone cardiac transplantation and several of these have died of cardiac rejection or post-transplant lymphoproliferative disease; others deteriorated too fast to undergo transplantation or died of complications of ventricular assist devices or ECMO whilst awaiting transplantation. In the past four years four babies/infants have been candidates for cardiac transplantation, requiring cumulatively approximately 20 months of intensive care bed stays. Sadly only one has undergone successful transplantation due to factors including shortage of donor hearts. Even those who have not received transplants can develop life-threatening heart rhythm disturbances.

Neutropenia is another life-threatening issue due to attendant serious bacterial infection. As a result, two thirds of UK patients require chronic subcutaneous injection therapy with granulocyte colony stimulating factor (G-CSF), a distressing and expensive medication. Furthermore, the management of neutropenia in this disease is challenging since patients have highly variable neutrophil counts, preventing administration of a consistent daily dose and requiring repeated blood counts and physician oversight. They often remain intermittently neutropaenic even when receiving G-CSF.

Lethargy and fatigue are universal, interfering with schoolwork and play and often necessitating the use of wheelchairs. Similarly patients cannot hold down strenuous or demanding jobs. Many

patients have major feeding problems from the neonatal period through into adult life and require long term supplemental feeding via gastrostomies.

There can be rapid deterioration during periods of stable health and when under expert medical care. Ventricular arrhythmia (tachycardia or fibrillation) affects 10% of adolescents and can cause sudden cardiac death at any stage of childhood, including the neonatal period [3]. These seemingly random acute crises are not predictable by genotype or recent medical history, producing a need for cardiac resuscitation training and chronic anxiety in affected families.

It is clear that more disease-specific therapy is required to prevent morbidity, mortality, psychological distress and disruption of quality of life, potentially producing major savings for the NHS. We in the UK are uniquely well-positioned to explore such therapy, having the world's highest density of diagnosed patients and the only national multidisciplinary service. The NHS Specialised Services Barth syndrome service currently cares for 22 living boys from England, Scotland and Wales (from approximately 200 ever diagnosed worldwide), of whom 18 are above six years of age and candidates for the trial proposed.

Several of the laboratory and clinical readouts proposed in this study (cardiolipin profiling, cardiac MRS and electron microscopic evaluation of mitochondrial morphology) could have importance for assessing genotype/phenotype correlation and future therapies in Barth syndrome beyond the scope of this randomised trial. Patients with unexplained conditions very closely allied to Barth syndrome are known to this team. The technical expertise developed here may allow better investigation of these patients and those with other unexplained conditions such as idiopathic neutropenia or cardiomyopathy.

'Gold standard' evidence from a randomised controlled trial is now required to investigate the potential risks and benefits of bezafibrate treatment in this population. Prepublication data from Dr Ren *et al* at New York University (2012) demonstrated clear effects of bezafibrate and resveratrol in partially normalising the highly skewed cardiolipin (CL) ratio in Barth mouse knockdown cells [1]. They also improved oxygen utilisation in these cells and isoproterenol cardiac stress responses in a Barth mouse model. Ren *et al* hypothesise that these agents could be therapeutic in humans and this accords with experience in some males seen at Bristol clinics who have relatively normal L4-CL (though aberrant monolysocardiolipin (MLCL)) and hence CL ratios intermediate between typical Barth syndrome and those with normal lipid function. Despite categorical proof of Barth syndrome, these boys have a less severe phenotype - several are good runners and their neutropenia is mild/non-existent – suggesting that normalisation of the cardiolipin ratio of cells in humans may result in less severe disease.

Bezafibrate is an European Medicines Agency (EMA) approved lipid-lowering drug with established use in adults and children and a good safety record in long-term use [4]. The BNF recommends doses of 200mg OD to TDS for 10-18 year olds, and Bezafibrate use is well tolerated in younger children in the dose range 5-20 mg/kg/day [5-7] and in adults up to 600-900mg/day [8]. Bezafibrate has been reported to significantly ameliorate a mitochondrial myopathy (CPT2 deficiency) in adults [9] as well as giving promising results in a range of animal/human cellular models of mitochondrial disease.

Resveratrol, a naturally occurring food supplement available from nutraceutical companies, affects energy metabolism and mitochondrial function. Previous human clinical trial data is limited, with mild gastrointestinal symptoms occurring at doses above 1g/day [10]. In a 2011 double-blind crossover trial in healthy obese men Resveratrol (at 150mg/day for 30 days)

produced a range of statistically significant beneficial clinical effects [11]. Resveratrol has been included for comparative *in vitro* studies because - although Bezafibrate and Resveratrol had equivalent effects in the mouse model/cells - it had the significantly more convincing effect on improving CL ratio in human Barth syndrome fibroblasts. However, it has not been used in this trial due to NIHR EME Board concerns that its use could aggravate pre-existing neutropenia.

Barth syndrome is characterised by many private gene mutations; more than 120 have been identified in only 200 affected persons [2]. There is profound phenotypic variability between affected patients within families and those in different families, both in severity of cardinal problems (e.g. cardiomyopathy, neutropenia) and the number of different organs that are significantly affected. There has been no definitive proof of any genotype/phenotype correlation in Barth syndrome.

3. Aims and objectives

We hypothesise that bezafibrate will modify the cellular ratio of monolysocardiolipin to L4-cardiolipin, ameliorating disease phenotype in those living with the disease, providing the drug is free of significant side-effects at clinically effective doses.

We also hypothesise that bezafibrate (and/or resveratrol *in-vitro*) may have beneficial effects independent of any effect on cardiolipin ratio due to their abilities to influence a range of mitochondrial pathologies.

The major questions to be answered are as follows:

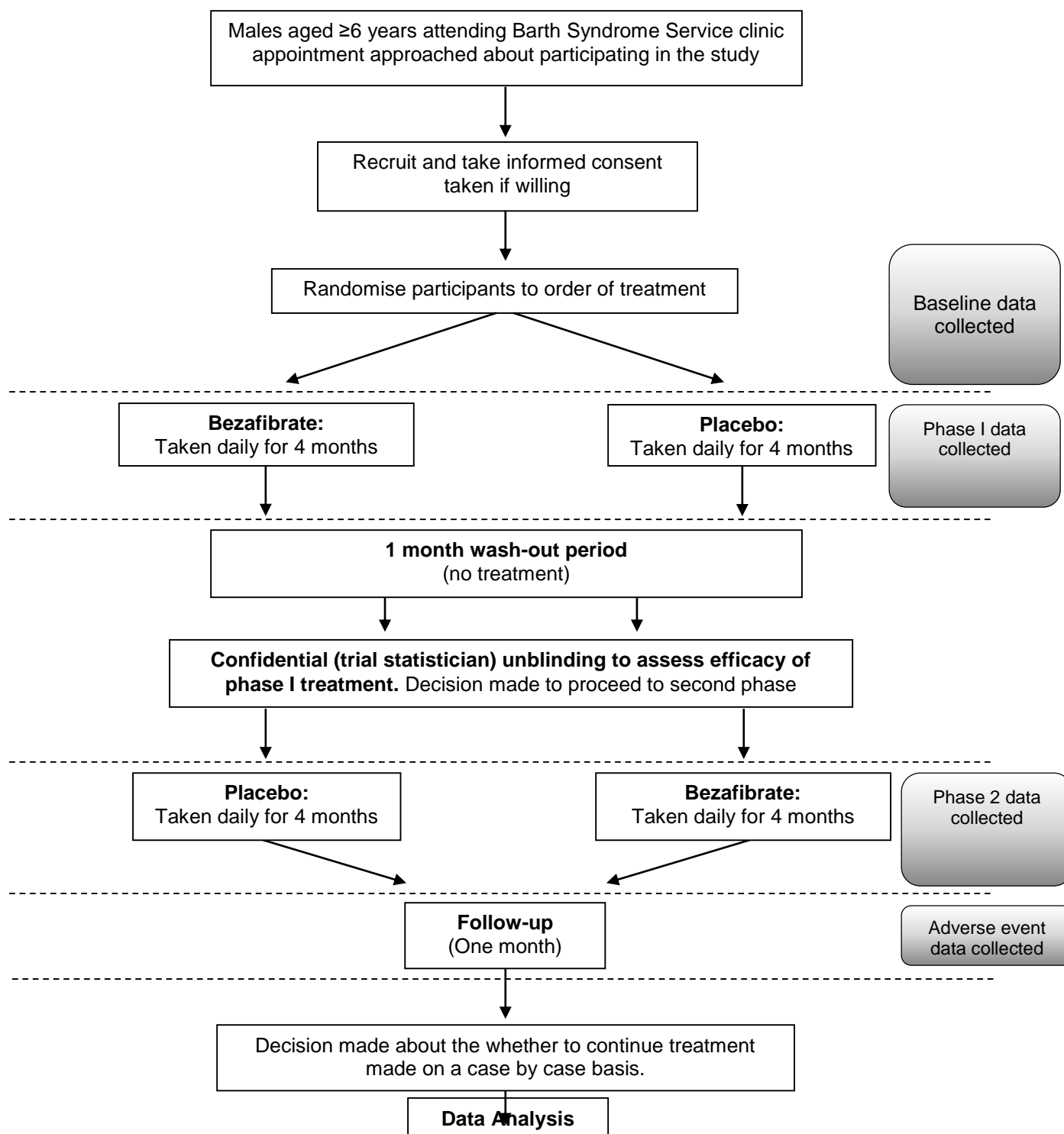
1. What is the effect on biochemical/clinical outcome measures and quality of life of bezafibrate treatment in comparison to placebo in Barth syndrome?
2. Is it possible to correlate clinical improvements with *in vitro* analysis of cardiolipin ratio/profile and mitochondrial morphology in each participant's cells when exposed to bezafibrate in laboratory culture? How does this contrast with an *in vitro* response to resveratrol exposure? This could indicate whether a trial examining the efficacy of resveratrol may be required in the future to determine the optimal choice of drug.
3. What are the most feasible methods and standardised outcome measures that may allow better conduct of future trials (e.g. larger multinational trials) and evaluations in Barth syndrome?
4. Is it possible to create a research infrastructure which optimises recruitment, retention and communication with families and people with Barth syndrome?
5. What are the participant and family perceptions of research and any important potential barriers to participation?

The primary outcome to be measured is whole body oxygen consumption during peak bike ergometry exercise (i.e. peak VO_2). Improved quality of life is an overarching goal, principally through improved muscle function and is an important secondary outcome. A broad range of biochemical, mitochondrial and clinical readouts has also been selected as secondary outcome measures (see section 4.6) including cardiolipin ratio on which this work is predicated.

This study would also aim to look at many aspects of the practicality and robustness of study design.

4. Plan of Investigation

4.1 Study schema



4.2 Trial design

This study is a double-blinded, randomised, placebo-controlled crossover study. Treatment will be given in two 4 month long phases and a one month washout period between these where no treatment is given. Participants will be followed up as part of the study for one month after the end of second treatment period (drug or placebo).

After phase 1 treatment has been completed by participants, confidential unblinding will be performed by the study statistician to assess the response to bezafibrate. If an improved peak VO_2 (primary outcome) is demonstrated in $\geq 50\%$ of participants receiving bezafibrate, phase 2 of the study will commence as planned. If no response is demonstrated, or a response is demonstrated in less than 50% of participants receiving bezafibrate, phase 2 of the study will not go ahead and the study will finish.

4.3 Key design features to minimise bias

(a) Confounding/allocation bias (systematic differences between baseline characteristics of the groups that are compared)

Participants will act as their own controls providing that they complete both phases of the trial, precluding confounding. The allocation sequence (order of drug and placebo in the two phases) will be randomised and concealed making prediction of the next treatment allocation difficult, minimising confounding for any order effect; this will also minimise confounding of the treatment effect for phase 1 if phase 2 proves not to be feasible. However, the small numbers of participants available for this study may introduce imbalances between the groups by chance (see section 5.1).

(b) Performance bias (systematic differences between groups in the care that is provided, or in exposure to factors other than the interventions of interest)

This bias will be minimised by:

- Blinding of all participants and personnel (apart from dispensing pharmacist) and the success of blinding will be assessed (participants may become unblinded due to side effects);
- Administering the intervention, comparator and other procedures undertaken during the trial according to standard protocols;
- Pre-defining all procedures for participant follow-up and applying the procedures to all participants in the same way;
- Monitoring adherence to the protocol.

(c) Detection bias (systematic differences between groups in how outcomes are determined)

This bias will be minimised by:

- Blinding of all individuals assessing outcomes, including cardiologists/technicians conducting the bicycle ergometry tests (i.e. peak VO_2), and participants and assessing the success of blinding;

- Using an objective primary outcome measure of peak whole body oxygen consumption on bicycle ergometry which is a well validated indicator of skeletal muscle mitochondrial dysfunction in Barth syndrome.

(d) Attrition bias (systematic differences between groups in withdrawals from a study)

This bias will be minimised by:

- Maintaining contact with participating families throughout the duration of the trial to maximise the proportion of participants for whom all outcome data are available (see section 5.12), even if the drug or placebo intervention has to be stopped for a participant (this may be facilitated through the follow-up of patients through the Barth Syndrome Service if necessary), and the proportion of participants who receive the intervention to which they were allocated.
- Implementing measures to promote adherence to random allocations.
- Pre-specifying analysis of phase 1 only if a proportion of participants (to be specified) fail to complete phase 2.
- Using intention to treat analysis.

(e) Reporting bias

This type of bias will be minimised by having pre-specified outcomes (see section 4.6) and a pre-specified analysis plan (see section 6.0).

4.4 Trial population

The reference population is males with a confirmed diagnosis of Barth syndrome.

The study population is males aged ≥ 6 years in the UK, with a confirmed diagnosis of Barth syndrome with the following inclusion/exclusion criteria applied. Males under 6 years of age are not included in this patient population because of difficulties in obtaining data on the primary outcome through bicycle ergometry in young children.

4.4.1 Inclusion criteria

Participant may enter study if ALL of the following apply:

- Male aged ≥ 6 years old
- Clinical diagnosis of Barth syndrome with characteristic abnormality of the L4-cardiolipin/monolysocardioplin ratio plus identified mutation in the tafazzin gene
- Under the care of the NHS Barth Syndrome Service (BSS)
- Stable cardiac condition
- Able to swallow bezafibrate tablets (large size)

4.4.2 Exclusion criteria

Participant may not enter study if ANY of the following apply:

1. Known hypersensitivity to bezafibrate, to any component of the product or to other fibrates
2. Known photoallergic or phototoxic reactions to fibrates.

3. Hepatic dysfunction and/or liver function tests greater than 2x normal
4. A shortening fraction of <25 (or a significant drop in shortening fraction in the previous year)
5. Documented atrial or ventricular arrhythmia (atrial/ventricular tachycardia or atrial/ventricular fibrillation) that has not been stabilised with treatment.
6. Renal impairment (creatinine clearance < 90 mL/min)
7. Pre-existing known gallbladder disease.
8. Recent unspecified significant deterioration in general health
9. Prisoners and adults lacking capacity to provide informed consent

There are reports of rhabdomyolysis occurring in patients treated with a combination of Bezafibrate and statins. Four of the five males in this patient cohort who have undergone cardiac transplantation are maintained on the statin pravastatin. This has been discussed with the cardiology team at Great Ormond Street Hospital who are agreeable for this medication to be ceased during the trial period.

4.5 Trial interventions

Bezafibrate is classed as an Investigational Medicinal Product (IMP) and is therefore under the regulation of the Medicines and Healthcare products Regulatory Authority (MHRA).

All participants will receive 4 calendar months of the intervention (bezafibrate) **and** 4 calendar months of the placebo (if the trial proceeds to phase 2. See section 4.2 for details). The order in which they will be administered will depend on the allocated treatment arm at first randomisation. Those first allocated to the intervention at randomisation will be given bezafibrate followed by the placebo. Those first allocated to the placebo at randomisation will be given the placebo followed by bezafibrate. Both arms will have a 1 calendar month washout period between the intervention and placebo administered, where no treatment is given.

Participants will be dispensed at least a month's supply of either bezafibrate or placebo at any one time. For participants who live some distance away from Bristol, they will receive further dispensed medications via courier.

The study intervention will be prescribed once at the start of the study. As investigators and participants are blinded, the prescription will not specify which medication (bezafibrate or placebo) will be taken first: this will be determined by the randomisation list held by the UH Bristol Clinical Trials Pharmacy department. Future dispensing will be managed by UH Bristol Clinical Trials Pharmacy Department, including cross-over to the other study medication if the study proceeds to the second phase

Intervention: Bezafibrate taken orally in capsule formulation.

- Children aged 6-9 years: Commence on 100mg OD for the first month and if well tolerated increase to 100mg BD for the remaining 3 month treatment period
- Children aged 10-17 years: commence on 200mg OD for the first month and if well tolerated increase to 200mg BD for the remaining 3 month treatment period
- Adults (≥ 18 years): 200mg BD

Placebo: Capsule formulation with no active substance taken orally. The placebo will look, taste and smell as similar as possible to the intervention

Bezafibrate and placebo will be stored, packaged and labelled by UH Bristol Pharmacy Department. Drug accountability will also be managed and monitored by UH Bristol Pharmacy Department. Both will be performed according to their SOPs, which are in accordance with GCP.

Compliance with study treatment will be assessed by asking participants to return any unused medications.

Concomitant medication (including contraindicated medication/treatments) [12]:

- Care is required in administering bezafibrate to patients taking coumarin-type anti-coagulants, the action of which may be potentiated. The dosage of anti-coagulant should be reduced by up to 50% and readjusted by monitoring blood coagulation.
- As bezafibrate improves glucose utilisation the action of antidiabetic medication, including insulin, may be potentiated and should be considered.
- Should combined therapy with an ion-exchange resin be considered necessary, there should be an interval of 2 hours between the intake of the resin and bezafibrate the absorption of bezafibrate otherwise may be impaired.
- In isolated cases, a pronounced though reversible impairment of renal function (accompanied by a corresponding increase in serum creatinine level) has been reported in organ transplant patients receiving immuno-suppressant therapy and concomitant bezafibrate. Accordingly, renal function should be closely monitored in these patients and, in the event of relevant significant changes in laboratory parameters, bezafibrate, should if necessary, be discontinued. (NB creatinine will be measured at 2 weeks after commencing study medication in both phases of the study in participants who have received an organ transplant.)
- MAO-inhibitors (with hepatotoxic potential) should not be administered together with bezafibrate.
- Interaction between HMG CoA reductase inhibitors and fibrates may vary in nature and intensity depending on the combination of the administered drugs. A pharmacodynamic interaction between these two classes of drugs may, in some cases, also contribute to an increase in the risk of myopathy for specific dose recommendations of statins refer also to the SPC of the relevant product.

Granulocyte colony-stimulating factor (GCSF): Participants will continue to be prescribed GCSF as clinically indicated as we are not aware of any drug interactions with this treatment.

Blood count deterioration: if a patient is shown to have a neutrophil count below $0.5 \times 10^9/L$, either on their monthly monitoring counts or if a participant presents with sign and symptoms of infection and requires additional blood tests as part of their normal care, GCSF therapy will initiated or dose/frequency of dosing reviewed and possibly increased in line with routine care of these patients. Subsequent weekly full blood counts will be requested on post G-CSF days for the next two weeks (as this is the routine way of monitoring neutrophil counts in these patients). If the patient has three consecutive blood counts of less than $0.5 \times 10^9/L$, administration of the intervention will be terminated.

Post-trial treatment: Once the participants have completed the study treatment their medical care will revert to the standard care received from the Barth Syndrome Service. However, if

bezafibrate is shown to have a beneficial effect on individual participants, provision will be made for them to continue receiving bezafibrate, on a case-by-case basis

Current licensed indications:

- Treatment of severe hypertriglyceridaemia with or without low HDL cholesterol.
- Mixed hyperlipidaemia when a statin is contraindicated or not tolerated.

Elimination half-life of drug in the body: 1-2 hours

For further information about bezafibrate please see the Summary of Product Characteristics (SmPC): Bezalip 200mg tablets.

4.6 Primary and secondary outcomes

4.6.1 Primary outcome

The primary outcome measure is peak oxygen consumption on bicycle ergometry (i.e. peak VO_2), because this outcome is strongly associated with activity intolerance and may well correlate with subjective fatigability which we believe to be the most important determinant of quality of life in these patients. Peak VO_2 will be assessed at baseline and in the final week of each treatment phase.

4.6.2 Secondary outcomes

1. Monolysocardiolipin/tetralinoleoyl-cardiolipin MLCL/L4-CL ratio/cardioliipin profile
2. PCr/ATP ratio in cardiac muscle on ^{31}P Magnetic Resonance Spectroscopy
3. Skeletal muscle oxidative function/ on ^{31}P Magnetic Resonance Spectroscopy
4. Quality of life (QoL) assessed using age-appropriate PedsQL questionnaires
5. Absolute neutrophil count
6. Amino acid expression (serum arginine and cysteine levels)
7. Cardiac function (LVEF and shortening fraction)
8. Mitochondrial size in lymphocytes
9. Numbers of mitochondria (per lymphocyte)
10. Total area of mitochondria per lymphocyte
11. Area of mitochondria as proportion of cytoplasm
12. Mitochondria function and cristae organisation in lymphocytes
13. Arrhythmia profile from 12 lead ECG at rest and during exercise (for potential rhythm abnormalities)

See Section 5.6 Data Collection for the timing of these assessments

The work around the immortalised cell lines does not form part of this protocol and will be described in a separate protocol.

In addition to the data collected from the clinical trial we propose to integrate qualitative research methods in order to explore patients' and families' experiences of the different interventions. Parents of younger patients (<18 yrs) and patients (>14yrs, suitability for interviewing to be determined on advice from the Clinical Psychologist known to the patient) will be invited to take part in semi-structured one-to-one interviews in the assessment periods after the first and second interventions. The first interview will last approximately 20 minutes and

explore the parents'/participant's experience of the intervention. The second interview will last approximately 40 minutes and will explore the parents'/participant's experience of the second intervention and their perception of participating in the trial as a whole. Interviews with patients and parents will be digitally recorded with their consent and transcribed verbatim. Data from interviews will be analysed using framework analysis methodology [13] with the aid of the NVivo software package.

4.7 Sample size calculation

This is a single centre study to be performed in patients attending the NHS National Barth Syndrome Service. A total of 18 males aged between six and 24 years currently attend the service. We anticipate that between 12 and 15 UK patients are likely to consent to take part.

In a simple crossover trial (a single intervention and control comparison), the standard deviation (SD) of the within subject difference between treatments is given as: $\sqrt{2 \times \text{within subject SD}}$ [14]. In a crossover trial, all participants constitute their own control.

In this trial, the difference in mean peak volume of O₂ consumption between placebo and bezafibrate phases will be tested, assuming a 2-tailed alpha of 0.05. For a sample size of 12 participants, the trial will be able to detect a difference of 0.90 (within subject) SDs with 80% power, or 1.05 SDs with 90% power.

5. Trial methods

5.1 Description of randomisation

The random sequence allocations of bezafibrate or placebo will be generated prior to the start of the study by a CTEU statistician, using blocks of undisclosed size. The sequence will then be 'attached' to a list of consecutive study IDs and provided to the clinical trials pharmacy department. This method does not comply with the requirement of an internet based randomisation system supplied by an MHRA approved provider (e.g. Sealed Envelope) as described in the Bristol CTEU SOP ST 005 'Development of a Randomisation System' but it was felt using an internet-based system would be prohibitively expensive for so few patients.

The allocations of drug or placebo in the second phase of the trial will be the opposite of the first phase. Once a participant has given consent and eligibility has been documented and confirmed, he will be allocated to the next consecutive study ID. A doctor will prescribe the study 'medication' and the pharmacy will dispense the appropriate intervention given the participant's study ID and phase in the trial.

Allocation concealment will minimise selection bias. The sequence of random allocations will be generated by computer and will be concealed from all clinical and research personnel.

5.2 Blinding and code breaking

This study is double blinded: neither the participants nor investigators (or any other member of the research team apart from the pharmacist) will have knowledge of the allocated treatment being given, or the order in which it is given.

The capsules containing Bezafibrate or placebo do not have a particularly strong or unusual smell or taste, so we do not anticipate unblinding will occur due to the characteristics of the drug. The capsules for active drug and placebo will look identical, which should help to minimise the unblinding of participants and subsequently investigators. We are aware that bezafibrate may induce side-effects in some patients that will inadvertently unblind participants and we acknowledge this may be a limitation of this study.

The patient information leaflet and the process of informed consent will explain the uncertainty around the potential beneficial effects of bezafibrate over a placebo. Therefore, in the event of inadvertent unblinding of a participant, the participant should not have a strong expectation that one or other method should lead to a more favourable result.

Participants will be made aware before entering the study that they will not be told which treatment they will receive in each phase. Doctors will prescribe the 'study medication' rather than specifically bezafibrate or placebo and the prescription for the 'study medication' will be sent to pharmacy, who will then provide the medication as specified according to the pre-determined list drawn up by the study statistician prior to recruitment (as described earlier). The allocations will only be known by pharmacy and the study statistician and will not be disclosed to any other member of the research team.

Members of the health care team can request unblinding of the study medication in either phase in the event of a serious adverse event (SAE), if they consider that this information will alter their management of the SAE. During office hours CTEU Bristol should be contacted for requests to unmask. Out of office hours UH Bristol Pharmacy Department should be contacted using their on-call system and a request made to contact a member of Clinical Trials Pharmacy team. The on call pharmacist will have access to the unblinding list. All instances of unblinding will be documented, including who requested the unblinding, why it was required, the time and date and who performed the unblinding.

5.3 Research procedures

Detailed assessments will be performed in Bristol at baseline and at each end-of-treatment assessment. These assessments will be timed during the final week of therapy so that patients are still receiving interventions/placebo at the time of testing but will have had maximum cumulative exposure to the drug or placebo. These will include:

- Anthropometric data (height and weight)
- Medical history and examination (including resting blood pressure, heart rate and oxygen saturation)
- Trans-thoracic echocardiographic determination of cardiac function at rest and during exercise (stress Echo) (see Appendix 1).
- Tissue Doppler studies (see Appendix 1).
- Modified McMaster exercise protocol to assess peak oxygen consumption using an electronically braked GE Healthcare exercise echocardiography
- Muscle deoxygenation (HHb) during exercise using near infrared spectroscopy (NIRS) over the vastus lateralis muscle.
- Ventricle volumetrics using cardiac Magnetic Resonance Imaging (MRI)
- PCr/ATP ratio obtained from Magnetic Resonance Spectroscopy
- Quality of Life questionnaires (PedsQL)

- Qualitative assessments (semi-structured one-to-one interviews) to assess patient experiences of the intervention and participating in a trial as a whole, along with a dietary evaluation
- Cardiac arrhythmia profile from 12 lead ECG at rest and during exercise

In those who can tolerate an MRI scan lasting approximately one hour without general anaesthetic, we will use ³¹P magnetic resonance spectroscopy (performed on a Siemens 3T Magnetom Skyra MRI scanner) to assess phosphocreatine/adenosine triphosphate ratio (PCr/ATP) in myocardium and oxidative function (PCr recovery kinetics) in skeletal muscle. Cardiac MRI imaging will assess ventricle volumetrics. Detailed MRI/S methods will be documented in a separate protocol.

Imaging tests will be performed at the Clinical Research Imaging Centre (CRIC, a joint UoB/UHBristol facility).

A total of 20 mL of blood will be taken at each assessment visit for the following tests:

1. At baseline only: establishment of an EBV transformed lymphoblast line for *in vitro* incubation with Resveratrol or Bezafibrate and assessment of cardiolipin ratio.
2. At baseline and end-of-treatment assessments: Full blood count, absolute neutrophil count, urea/electrolytes, liver function tests, creatinine kinase, plasma arginine/cysteine, full lipid profile (total cholesterol, high density lipoprotein, triglycerides), brain natriuretic peptide (as a blood marker of LV function).
3. Mitochondrial tests: assessment of detailed cardiolipin profiling (to be performed by Prof Simon Heales' laboratory at Great Ormond Street Hospital) and calculation of MLCL/L4-CL ratio. Mitochondria of the blood cells will be examined by electron microscopy of lymphocytes and measurements of size, number and shape recorded. This work will be performed at the University of Bristol in collaboration with Dr Paul Verkade. Lymphocytes extracted from 5 mL whole blood will undergo analysis for mitochondrial function studies including analysis of respiratory chain enzyme complexes using established spectrophotometric methods. This work will be performed in the Department of Clinical Biochemistry, Bristol Royal Infirmary in collaboration with Ann Bowron.

Subjective quality-of-life will be assessed using age appropriate Peds QLTM Paediatric Quality of Life Inventory assessment forms and parental questionnaires. These include forms suitable for young adults (18-25).

5.4 Duration of treatment period

The duration of the treatment period will be for 9 months in total: 4 months of bezafibrate followed by 4 months of placebo (or vice versa), with a one month washout period where no treatment is given between the two treatments.

If no effect of bezafibrate is found after the first treatment phase of four months, the study may not continue on to the second phase. If this happens, the duration of treatment will be four months.

5.5 Definition of end of trial

The end of the trial for participants will be after 10 months when they have completed the treatment period and a one month follow-up period (or have been lost to follow-up or died). The end of the trial as a whole will be when all recruited participants have completed the treatment and follow-up period **and** all data has been collected, data queries answered and all laboratory samples have been processed and analysed.

5.6 Data collection

Data collection will occur at four different time-points: baseline, end of treatment phase 1, end of treatment phase 2 and after completion of one month follow-up.

A schedule of investigations and assessments over the 10 months of the trial is shown below.

Table 1 Schedule of Events

Event		Baseline	Phase 1				Washout Period	Phase 2				Follow-up
Month		0	1	2	3	4	5	6	7	8	9	10
Informed Consent		✓										
Height & weight		✓				✓					✓	
Medical history		✓										
Clinical examination*		✓				✓					✓	
Bicycle ergometry (exercise bike test)	Peak oxygen consumption	✓				✓					✓	
	Muscle deoxygenation (HHb) (NIRS)	✓				✓					✓	
	Tissue Doppler studies	✓				✓					✓	
Echocardiogram (at rest and during exercise)*		✓				✓					✓	
12 Lead ECG at rest and during exercise (during echo)		✓				✓					✓	
Blood sample (20-30 ml total)#	Transformed lymphoblast line for in vitro incubation with Bezafibrate and Resveratrol	✓										
	FBC, absolute neutrophil count, urea/electrolytes, LFTs, CK, plasma arginine/ cysteine, full lipid profile (total cholesterol, high density lipoprotein, triglycerides), brain natriuretic peptide	✓				✓					✓	
	Mitochondrial assessment#	✓				✓					✓	
Blood sample (safety assessment) done locally to patient	FBC including absolute neutrophil count, routine renal and liver function tests, plasma triglyceride/total cholesterol/LDL-		✓	✓	✓		✓	✓	✓	✓		

Event		Baseline	Phase 1				Washout Period	Phase 2				Follow-up
	cholesterol and Creatine Kinase, Creatinine**											
Month		0	1	2	3	4	5	6	7	8	9	10
Cardiac/skeletal muscle MRI/MRS scan [§]		✓				✓					✓	
QoL: /Paeds QoL		✓				✓					✓	
Adverse events						✓					✓	✓
Drug prescribing		✓										
Drug dispensing		✓		✓	✓	✓		✓	✓	✓	✓	
Qualitative Interview						✓ (20 mins)					✓ (40 mins)	

+ Including resting blood pressure, heart rate and oxygen saturation

* Cardiac status: LVEF and shortening fraction

Samples to go to Prof Simon Heales at GOSH for detailed cardioplipin profiling and calculation of MLCL/L4-CL ratio;

Dr Paul Verkade at UoB for examination of mitochondria of the blood cells by electron microscopy and measurements of size, number and shape recorded; Ann Bowron at UH Bristol labs for analysis of mitochondrial function studies including analysis of respiratory chain enzyme complexes.

**to be measured at 2 weeks after initiated study drug in both phases for participants who have had an organ transplant

\$ Cardiac MRI will be used to assess:

- Right and left ventricle volumetrics.

MRS will be used to assess:

- Phosphocreatine/adenosine triphosphate ratio (PCr/ATP) in myocardium and oxidative function
- ATP production in skeletal muscle.

Monthly blood tests will be performed throughout the study at the patients' local hospital or GP surgery to assess the continuing safety of the bezafibrate in this population. An additional blood test for creatinine will be carried out at 2 weeks after the initiation of study drug in both phases in participants who had had an organ transplant. Absolute neutrophil count, routine renal and liver function tests, plasma triglycerides/total cholesterol/LDL-cholesterol and creatine phosphokinase will be tested. The study research nurses will facilitate the arrangement of these tests with the patients/families and their local provider and will obtain the results from the place of testing for our monitoring.

5.7 Source data

The primary data source will be the participant's medical notes for clinical data, including peak oxygen consumption (primary outcome), cardiac function parameters and NIRS data. The laboratory reports will be the primary data source for the results of the blood tests, whilst records from laboratory books, images, print-outs and electronic data will form the source data for mitochondrial structure and function.

The MRI/MRS outputs with associated reports will form the source data for imaging tests such as MRS and stress echocardiogram. The completed patient questionnaires will be the primary data source for these measures.

Source data for some adverse events will be on the 'Patients Know Best' web-based service that some of the Barth syndrome patients already use. This is a NHS-contracted certified private IT provider of a patient information site to allow patients and medical carers easy online access to their personal medical information and treatment plans.

5.8 Planned recruitment rate

Eligible patients will be recruited from the national clinics held in Bristol. All patients in the UK with Barth syndrome use our national specialist multi-disciplinary service and we expect to recruit approximately 12-15 of the 18 eligible patients, from several clinic dates over a short period of time (expected to be approximately 2-4 weeks).

5.9 Participant recruitment

Potential participants will be identified through the Barth Syndrome Service, which is a centralised NHS Specialist Service run in Bristol. Potential recruits are already well known to the research team due to the small numbers with the disease. The research team is led by Dr Colin Steward, who is also the clinical lead for the NHS Barth Syndrome Service.

An invitation letter and Participant Information Leaflet (PIL) will be sent to the participant, or their parents/guardians, well in advance of their clinic visit (likely to be at least once month) to provide enough time for the participant or their families to read and digest the information about the study.

In order to arrange for participants and their families to attend the clinic in Bristol we will need some prior indication as to whether the patient is likely to participate in the study. Therefore we

will contact the patient or their family by telephone before their clinic appointment to determine if they are interested in participating on the understanding that they may change their minds.

Patients will be approached to participate in the clinic appointment by Dr Colin Steward and/or a research nurse who will discuss the study in detail and will answer any questions patients or their families may have about the study. If the patient and/or the family is willing, informed consent will be taken during this clinic visit

5.10 Discontinuation/withdrawal of participants

Each participant has the right to withdraw at any time by informing any member of the research team and without needing to give a reason. In addition, the investigator may withdraw the participant from their allocated treatment arm if they do not feel it is in the best interest of the participant to continue with the treatment due to adverse side-effects.

If a participant wishes to withdraw, we will continue to analyse any data already collected, unless the participant expresses a wish for their samples and any associated data to be destroyed. We will not collect further data on them after the time of withdrawal but will monitor adverse events for reporting where necessary.

Participants will be followed-up for one month after withdrawal of treatment for assessment of safety. A withdrawal form will be completed at the time of the withdrawal, documenting the reason for the withdrawal (if known) and the wishes of the participant with respect to their data.

5.11 Frequency and duration of follow up

Follow up for this study will continue for one month after the study treatment has ended in order to assess the safety of bezafibrate in this patient population.

Monthly blood tests will be performed throughout the study to assess full blood count including absolute neutrophil count, routine renal and liver function tests, plasma triglyceride/total cholesterol/LDL-cholesterol and creatinine kinase. The latter is included since bezafibrate can induce rhabdomyolysis, although this risk is most significant in those being additionally treated with statins [15]. An additional blood test for creatinine will be carried out at 2 weeks after the initiation of study drug in both phases in participants who had had an organ transplant. Where patients are receiving GCSF for management of severe neutropenia these tests will follow our usual testing protocol and be performed on the day following GCSF administration. This method of assessment is used since some patients will show unpredictably exuberant responses to GCSF, best detected in order to allow dose manipulation by monitoring of "peak" rather than "trough" counts.

These safety monitoring blood tests will be performed at a GP surgery/hospital local to the participant, or an appropriate assessment visit to Bristol.

The study research nurses will have regular contact with participants to collect adverse event data: after the first week of starting treatment and then monthly thereafter until end of follow-up. Patients will be asked to report (by telephone or via the Patients Know Best web service which the Barth Syndrome Service utilise for secure electronic communication with patients / parents / guardians) any unexpected symptoms or hospital visits/admissions to the Barth Syndrome

Service clinical and nursing team, or the research team. If required, out of normal service hours participants may contact the on-call haematology registrar or Consultant at Bristol Royal Hospital for Children who will be able to contact one of the clinicians allied to the Barth Syndrome Service.

5.12 Likely rate of loss to follow-up

As all of the potential participants are already well known to the research team and would use the Barth Syndrome Service outside of this study, we do not anticipate there to be any loss to follow-up. Therefore there are no special features to minimise attrition bias. Established CTEU methods will be used to maximise the proportion of participants for whom all outcome data are available and the proportion of participants who receive the intervention to which they were allocated.

5.13 Expenses

Participants will be reimbursed for their travel expenses to and from Bristol, plus 1-2 overnight stays for participants who live further away.

6. Statistical analyses

6.1 Plan of analysis

It is hoped that all patients will complete and contribute data to both phases of the crossover study, although we recognise that some missing data are likely due to the fragile nature of Barth syndrome.

We aim to retain all participants in the trial for the full duration of 10 months follow-up, irrespective of any periods when an intervention has to be suspended because of ill health. The only exceptions will be when a participant expressly requests to end all aspects of his participation in the trial or cannot continue because of deteriorating health. We propose that primary analyses should respect the intention-to-treat principle but envisage that additional secondary or sensitivity analyses may be specified in a detailed analysis plan, written prior to locking the database for final analyses.

Our plan for analyses assumes that the continuously scaled outcome data (or transformed data) will be distributed satisfactorily to allow parametric methods to be applied. We propose to analyse the data using mixed effects regression methods that will allow for participants' data to be included if not complete. The regression models will estimate effects for both the treatment factor (2 levels) and time period of intervention (2 levels), adjusting for baseline assessments of outcomes at the time of recruitment.

Participants will be fitted as random effects. We will test for the presence of a carry-over effect (i.e. the possibility that results obtained during the second treatment phase are affected by what happened in the first treatment phase) by including a treatment-by-time period interaction in the model. We expect that this interaction effect will not be statistically significant.

Similar methods will be used for analyses of the additional objectives to ensure that (a) the data hierarchy is respected (i.e. repeated measurements within subjects) and (b) available data for all participants can be included. Regression models will be fitted to estimate differences in secondary outcomes when treated with Bezafibrate vs. placebo and, additionally, to quantify the extent to which more 'proximal' biomarkers are associated with more 'distal' clinical and symptomatic outcomes.

Non-adherence to random allocations will be documented and every effort will be made to include all randomised participants.

6.2 Subgroup analyses

No subgroup analysis is planned

6.3 Frequency of analyses

The primary analysis will take place when follow-up is complete for all recruited participants. An interim analysis is also planned after phase 1 treatment has been completed by all participants. If an improved peak VO_2 (primary outcome) is demonstrated in $\geq 50\%$ of participants receiving bezafibrate, phase 2 of the study will commence as planned. If no response is demonstrated, or a response is demonstrated in less than 50% of participants receiving bezafibrate, phase 2 of the study will not go ahead and the study will finish.

The DMSC will receive periodic reports on all adverse events to a timetable that the committee will set (together with the information about the medicinal product received if desired), together with any additional analyses the committee request.

6.4 Criteria for the termination of the trial

The trial may be terminated early on the instruction of the DMSC or if the interim analyses after the first treatment phase does not show an improved peak VO_2 demonstrated in $\geq 50\%$ of participants receiving bezafibrate.

6.5 Economic issues

No economic analyses are planned

7. Trial management

The trial will be managed by the Clinical Trials and Evaluation Unit Bristol (CTEU Bristol); an UK Clinical Research Collaboration registered Clinical Trials Unit. The CTEU Bristol will prepare and submit all the relevant trial documentation for regulatory and governance approvals and prepare data collection forms, specify and deliver the randomisation scheme, develop and maintain the study database, check data quality as the trial progresses, monitor recruitment and carry out trial analyses in collaboration with the clinical investigators.

7.1 Day-to-day management

A Trial Management Group (TMG) will comprise: Chief Investigator, co-investigators and the trial manager. Members of the research team will be co-opted as required at different stages of the study. This group will meet at a frequency determined by the stage of the project and whether or not particular challenges in delivery need to be addressed, at least monthly; we expect that it will meet weekly during some periods where key decisions about the management of the study will have to be made.

The trial manager will be responsible for day-to-day management of the trial, which will be guided by the standard operating procedures and other infrastructure provided by the CTEU Bristol.

Potential trial participants will be identified through the Barth Syndrome Service run here in Bristol by a member of the research team. Dr Colin Steward will seek informed consent from participants and their families.

A research nurse will be responsible for collecting trial data. All members of the research team are responsible for ensuring the trial protocol is adhered to.

Trial Steering (TSC) and Data Monitoring and Safety Committees (DMSC) will be established to oversee the trial. We will identify appropriate nominees for these committees.

7.2 Monitoring of sites

7.2.1 Initiation visit

Before the study commences training session(s) will be organised by CTEU Bristol for research team members and any other staff groups as appropriate. These sessions will ensure that personnel involved fully understand the protocol, CRFs and the practical procedures for the study.

7.2.2 Site monitoring

As this is a single centre study there will be no site monitoring conducted by the CTEU Bristol.

7.3 Trial Steering Committee and Data Monitoring and Safety Committee

The TSC will have an independent Chair (an external Professor of Paediatrics with an interest in trial design), an independent statistician, an adult with experience of Barth syndrome (Mrs Michaela Damin, Chairperson, Barth Syndrome Trust), and a lay member.

The DMSC will also be established to monitor recruitment of study participants, ethical issues of consent, quality of data, the incidence of adverse events, and any other factors that might compromise satisfactory completion of the study.

The DMSC will have an independent Chair. Membership will cover trial, statistics and relevant clinical expertise; all members will be completely independent of the trial research team. We will invite several international experts on Barth syndrome and national expert cardiac/haematology consultants to join. The DMSC will meet at least quarterly either face-to-face or by

teleconference but this may be increased if necessary. The Committee will make a recommendation to the TSC about continuation of the study after each meeting.

8. Safety reporting

Serious and other adverse events will be recorded and reported in accordance with the International Conference for Harmonisation of Good Clinical Practice (ICH GCP) guidelines and the Sponsor's Research Related Adverse Event Reporting Policy (see Figure 2).

In Barth syndrome, adverse events are not unexpected and are not infrequent. However, because the study medication is not currently licenced for use in this patient population, all Serious Adverse Events (SAEs) that are not as a result of Barth syndrome itself will be reported to the Trial Sponsor. SAEs that require reporting to the Sponsor will be reported within 24 hours of the investigator becoming aware of the event. The sponsor will report SUSARs to regulatory authorities and copy all reports to CTEU Bristol.

Serious adverse events may prompt immediate withdrawal of the drug for a period at the discretion of the treating physician.

Note: Elective *treatment* taking place during the follow-up period that was planned prior to recruitment to the trial will not be reported as an unexpected SAE.

8.1 Expected adverse events

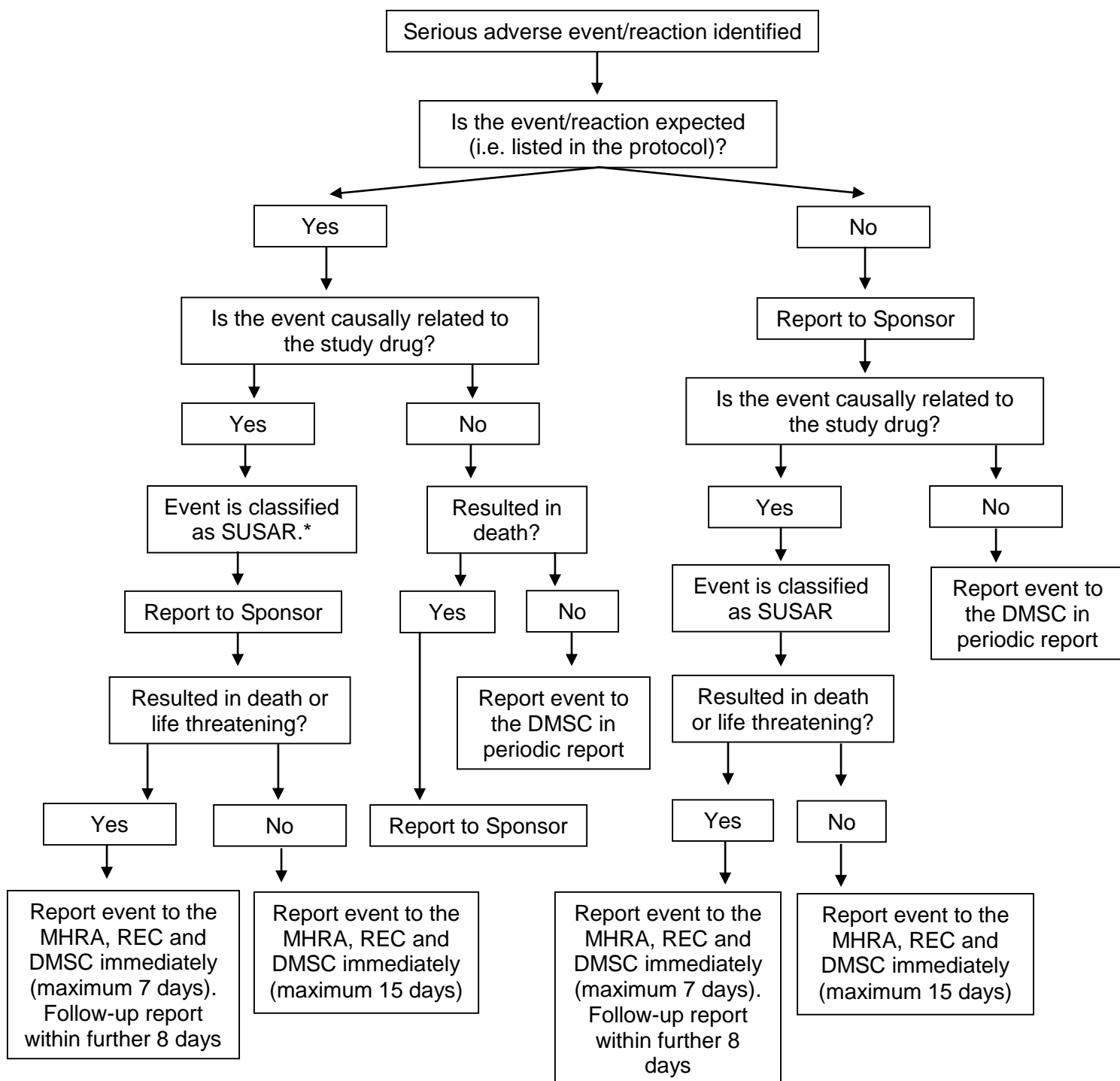
The following list of events are 'expected' as part of the signs and symptoms of Barth syndrome and will not be reported to the Sponsor, REC or MHRA if the event is classified as serious, unless deemed related to bezafibrate. Data on all serious adverse events (including events in the list below) and their relatedness will be recorded in the patient's CRF and reported periodically to the DMSC for review.

- Small changes of dilated cardiomyopathy (DCM) (change in EF of <15%)
- Left ventricular non-compaction (LVNC)
- Prolonged corrected QT interval
- Proximal myopathy/weakness/fatigue
- Exercise intolerance
- Neutropaenia
- Aphthous ulcers and sore gums
- Bacterial skin infections
- Hypcholesterolaemia
- Hypoglycaemia (primarily in infants)
- Episodic or chronic diarrhoea

Adverse drug reactions (ADRs) as listed in the SmPC for bezafibrate (see section 9.2) are not included in the list of 'expected' events as the drug has not yet been tested in Barth syndrome patients. Although similar ADRs may be seen in patients affected by Barth syndrome, a conservative reporting approach will be taken so that adverse events which are not a primary result of the disease shall be reported. Therefore, expected events that are deemed possibly, probably or definitely related to the study drug become unexpected, by virtue of their relatedness, and should be reported as SUSARs.

Adverse event data will be collected from the time of consent until the end of the 1 month follow-up period.

Figure 2 Serious adverse event reporting flow chart



8.2 Period for recording serious adverse events

Data on serious adverse events will be collected from consent until the end of the follow-up period.

9. Ethical considerations

9.1 Review by an NHS Research Ethics Committee

Ethics review of the protocol for the trial and other trial related essential documents (e.g. PIL and consent form) will be carried out by a UK NHS Research Ethics Committee (REC).

Any amendments to these documents, after a favourable opinion from the REC has been given, will be submitted to the REC for approval prior to implementation.

9.2 Risks and anticipated benefits

Potential benefits to participants: There is no current treatment for Barth syndrome itself and manifested symptoms are treated individually on a clinical basis. Therefore the use of bezafibrate in our clinical trial is the first potential treatment available for the alleviation of the aberrant cardiolipin ratio demonstrated in these patients and those affected by Barth syndrome have never had the opportunity of participation in clinical trials previously. Barth syndrome patients, parents and guardians who attend the NHS Barth Syndrome Service clinics in Bristol are already aware of the potential benefits of bezafibrate, but the beneficial effects of bezafibrate have not yet been proven in this population and care will be taken to ensure that potential participants are aware of the equipoise of the study.

Potential harms to participants: Bezafibrate has a number of side-effects that may be experienced by participants taking the drug, as detailed below. In addition to this, participants are required to make additional visits to Bristol as part of the study, which will cause some inconvenience and participants will also be required to undergo additional tests that they would not usually be asked to do.

Dr Todd Cade has previously conducted extensive exercise testing (n>50) in both children and adults with Barth syndrome and no untoward events have occurred. Therefore we do not have reason to believe that exercise testing will result in potential harm to participants, but a paediatric cardiologist will be present during the exercise testing during echocardiography and other appropriately medically qualified staff will attend the exercise testing during MRI/MRS scanning. Additional safety monitoring during bicycle exercise will be provided by 12-lead ECG ("stress ECG".) This non-invasive method is routinely used in paediatric cardiology clinical practice.

Participants able to tolerate the MRS assessments will be required to spend approximately an hour in the scanner, which may be uncomfortable.

Possible adverse effects of each intervention: (Taken from the SmPC)

The overall safety profile of bezafibrate is based on a combination of clinical study data and post-marketing experience.

The frequency of adverse drug reactions (ADRs) according to MedDRA System Organ Class is displayed in the table below.

Frequency of reporting: Common ($\geq 1/100$ and $<1/10$), uncommon ($\geq 1/1,000$ and $<1/100$), rare ($\geq 1/10,000$ and $<1/1000$), very rare ($<1/10,000$).

Blood and lymphatic system disorders:

Very rare: Pancytopenia, thrombocytopenic purpura.

Immune system disorders:

Uncommon: Hypersensitivity reactions including anaphylactic reactions.

Metabolism and nutrition disorders:

Common: Decreased appetite.

Nervous system disorders:

Uncommon: Dizziness, headache.

Rare: Peripheral neuropathy, paraesthesia.

Psychiatric disorders:

Rare: Depression, insomnia.

Gastrointestinal disorders:

Common: Gastrointestinal disorders.

Uncommon: Abdominal pain, constipation, dyspepsia, abdominal distension, diarrhoea, nausea.

Rare: Pancreatitis

Hepatobiliary disorders:

Uncommon: Cholestasis.

Very rare: Cholelithiasis.

Skin and subcutaneous tissue disorders:

Uncommon: Pruritus, urticaria, photosensitivity reaction, alopecia, rash.

Very rare: Erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis.

Musculoskeletal and connective tissue disorders:

Uncommon: Muscular weakness, myalgia, muscle cramp.

Very rare: Rhabdomyolysis.

Renal and urinary disorders:

Uncommon: Acute renal failure.

Reproductive system and breast disorders:

Uncommon: Erectile dysfunction Not Otherwise Specified.

Respiratory, thoracic and mediastinal disorders:

Very rare: Interstitial lung disease.

Investigations:

Uncommon: Increased blood creatinine phosphokinase, blood creatinine increased, decreased gamma-glutamyl transferase and in parallel alkaline phosphatase
Very rare: Haemoglobin decreased, platelet increased, white blood cell count decreased, gamma-glutamyl transferase increased, transaminase increased.

No adverse effects are expected from the placebo intervention

Benefits to society: This trial can be expected to more effectively educate those affected by the disease and their families about the importance of effective trial design, treatment adherence and regular assessment in establishing future therapies to benefit both their personal health and that of others with the same disease. The results of the study will also advance knowledge of the treatment of Barth syndrome patients and help to inform future studies.

9.3 Informing potential study participants of possible benefits and known risks

Information about possible benefits and risks of participation will be described in the PIL.

9.4 Obtaining informed consent from participants

Participants aged 16 years and older will be required to complete a written informed consent form before being enrolled on the study. For participants **under** the age of 16 years (≤ 15 years), written informed consent will be obtained from the child's parents/legal guardian but information will be given to the child about the study and its risks and benefits, according to their level of understanding and by staff with experience of recruiting children. The wishes of the child will be taken into consideration by the investigator in deciding whether or not they should be included in the trial. Children with sufficient understanding and capacity (usually aged 11-15) may be asked to formally document their 'assent' in written format if this is deemed appropriate.

The Chief Investigator and/or a research nurse will be responsible for the consent process, which will be described in detail in the Trial Manual.

9.5 Co-enrolment

Potentially eligible participants will be excluded from the study if they are already participating in another interventional research study, due to effects of the other intervention that may be observed on the objectives of our study and also for participant safety. In addition, the small number of patients we will recruit will not enable equal distribution of participants involved in other interventional research across the groups.

Participants already enrolled on this study should not be enrolled on any other interventional research study until after they have completed the follow-up period.

Participants can be enrolled in observational studies during their time in CARDIOMAN.

10. Research governance

This study will be conducted in accordance with:

- The Medicine for Human Use (Clinical Trial) Regulations 2004
- International Conference for Harmonisation of Good Clinical Practice (ICH GCP) guidelines
- Research Governance Framework for Health and Social Care
- European Union Directive 2001/20/EC on clinical trials

10.1 Sponsor approval

Any amendments to the trial documents must be approved by the sponsor prior to submission to the REC.

10.2 NHS approval

Approval from the local NHS Trust is required prior to the start of the trial.

Any amendments to the trial documents approved by the REC will be submitted to the Trust for information or approval as required.

10.3 Investigators' responsibilities

Investigators will be required to ensure that local research approvals have been obtained and that any contractual agreements required have been signed off by all parties before recruiting any participant. Investigators will be required to ensure compliance to the protocol and study manual and with completion of the CRFs. Investigators will be required to allow access to study documentation or source data on request for monitoring visits and audits performed by the Sponsor or CTEU Bristol or any regulatory authorities.

Investigators will be required to read, acknowledge and inform their trial team of any amendments to the trial documents approved by the REC that they receive and ensure that the changes are complied with.

10.4 Monitoring by sponsor

The study will be monitored and audited in accordance with the Sponsor's policy, which is consistent with the Research Governance Framework and the Medicines for Human Use (Clinical Trials) Regulations 2004. All study related documents will be made available on request for monitoring and audit by the sponsor, the relevant REC and for inspection by the MHRA or other licensing bodies.

10.5 Indemnity

This is an NHS-sponsored research study. For NHS sponsored research HSG(96)48 reference no. 2 refers. If there is negligent harm during the clinical trial when the NHS body owes a duty of care to the person harmed, NHS Indemnity covers NHS staff, medical academic staff with honorary contracts, and those conducting the trial. NHS Indemnity does not offer no-fault compensation and is unable to agree in advance to pay compensation for non-negligent harm. Ex-gratia payments may be considered in the case of a claim.

10.6 Clinical Trial Authorisation

Bezafibrate is classed as an investigational medicinal product (IMP) and a Clinical Trial Authorisation from the MHRA must be in place before starting the trial.

11. Data protection and participant confidentiality

11.1 Data protection

Data will be collected and retained in accordance with the UK Data Protection Act 1998.

11.2 Data handling, storage and sharing

11.2.1 Data handling

Data will be collected on paper Case Report Forms (CRFs) and entered onto a purpose designed database with data validation and cleaning carried out on an ongoing basis throughout the trial. Standard operating procedures (SOPs) for database use, data validation and data cleaning will be available and regularly maintained.

11.2.2 Data storage

All study documentation will be retained in a secure location during the conduct of the study and until the youngest participant reaches their 25th birthday when all patient identifiable paper records will be destroyed by confidential means. Prior to destruction, paper records will be scanned and stored on the University server with limited password controlled access. Where trial related information is documented in the medical records, these records will be identified by a label bearing the name and duration of the trial in accordance to UHBristol policy. In compliance with the MRC Policy on Data Preservation, relevant 'meta'-data about the trial and the full dataset, but without any participant identifiers other than the unique participant identifier, will be held indefinitely (University server). A secure electronic 'key' with a unique participant identifier, and key personal identifiers (e.g. name, date of birth and NHS number) will also be held indefinitely, but in a separate file and in a physically different location (NHS hospital server). These will be retained because of the potential for the raw data to be used subsequently for secondary research.

11.2.3 Data sharing

Data will not be made available for sharing until after publication of the main results of the study. Thereafter, anonymised individual patient data will be made available for secondary research, conditional on assurance from the secondary researcher that the proposed use of the data is compliant with the MRC Policy on Data Preservation and Sharing regarding scientific quality, ethical requirements and value for money. A minimum requirement with respect to scientific quality will be a publicly available pre-specified protocol describing the purpose, methods and analysis of the secondary research, e.g. a protocol for a Cochrane systematic review. The second file containing patient identifiers would be made available for record linkage or a similar purpose, subject to confirmation that the secondary research protocol has been approved by a UK REC or other similar, approved ethics review body.

12. Dissemination of findings

We will report our intention to conduct this study and major aspects of the trial design through the websites of the Barth Syndrome Trust and Barth Syndrome Service and the Journal of the Barth Syndrome Foundation (BSF, USA). Progress reports on design and recruitment would be presented to the biennial BSF Medical, Scientific and Family Conference in 2016.

When all of the data has been collected and analysed we will hold a social gathering after one of the Barth Syndrome Service clinics to feed the information back to the patient/families directly and to thank them for their participation. We will send a lay summary to all people/families in the UK with Barth Syndrome.

The Barth Syndrome Service uses a certified private provider patient information site (Patients Know Best) to allow patients and medical carers easy online access to their personal medical information and treatment plans. A lay summary of the results will be posted to each patient's personal pages, for both trial participants and non-participants, as a permanently available, easily accessible record.

Decisions on the most appropriate leads for specific dissemination/ exploitation activities will be made by the Steering Group. Information will be made freely available through web sites. Suitable summaries of the research findings will be licenced to individual web sites to ensure that the material remains available for a fixed period of time regardless of hit rates.

Findings from the study will be submitted for publication in a peer-reviewed journal, selecting one with open access arrangements, under the NIHR Terms of Agreement, in order to allow rapid dissemination to the Barth syndrome community. We would also present the findings at national/international paediatric, paediatric cardiology and metabolic disease conferences.

The conduct and findings of this trial will be discussed by videoconference/meetings of the Scientific and Medical Advisory Board of the Barth Syndrome Foundation, of which Drs Toth and Steward are members. If it were decided that further trials were necessary, for example if important endpoints showed promising trends but not statistical significance or detailed dose finding studies were deemed necessary, we would discuss the potential for international collaboration at those meetings. We are cognisant that it would have been ideal to conduct this trial in the first instance as an international collaboration (e.g. for sample size, statistical power) but this has not been possible due to the unavailability of bezafibrate in the USA and other considerations. We are hopeful that such barriers will be removed in the future to allow more effective European/US cooperation on this and other very rare diseases.

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14. Amendments to protocol

Amendment number	Version number of new protocol	Date of new version of protocol	Brief summary of change	Date of ethical approval (or NA if non-substantial)	Date of MHRA approval (or NA if non-substantial)

Appendix 1

Echocardiography Protocol:

- 1.) Participants and parents welcomed to exercise laboratory by research staff and familiarised with facilities.
- 2.) Functional trans-thoracic echocardiography will be performed at rest in a semi-recumbent position
- 3.) Start of exercise practise and familiarisation on exercise bike with appropriate rest thereafter
- 4.) 10 ECG stickers will be attached to the patient's chest and connected to an ECG monitor that is linked to the metabolic exercise software platform during exercise and recovery
- 5.) Start of exercise test and acquisition of echocardiographic, oxygen consumption and NIRS data. A full exercise protocol will take approx. 21 mins to complete. Oxygen consumption and NIRS data will be recorded continuously, echocardiographic data will be acquired during exercise at every exercise stage, that is every 3 minutes, at maximal 7 exercise stages. Each acquisition will take approximately one minute. For detailed exercise protocol please see below. Heart rate, blood pressure, oxygen saturations and NIRS will be acquired at each stage by standard non-invasive recording.
- 6.) Cessation of exercise if protocol completed, participant wishes to stop or research staff determine the test deleterious.
- 7.) Continuous oxygen consumption and NIRS data acquisition until 10 min recovery. Echocardiographic data acquisition at rest at 2 min and 10 min recovery
- 8.) Participants can recover and have a drink/ food as requested
- 9.) End of visit

Participant's baseline heart rate (HR) and blood pressure (BP) are recorded at rest and at peak exercise (Dinamap Pro 100V2, GE Medical Systems Information Technologies 2002, Tampa, Florida, USA).

Near infrared spectroscopy (NIRS) is used to measure muscle deoxygenation (HHb) during exercise (Artinis PortaMon near-infrared spectrometer, Artinis Medical Systems, The Netherlands). The device is secured over the vastus lateralis muscle, using the midpoint of the anterior superior iliac spine and the head of the fibula as a reference point.

Exercise stress test is performed on an electronically braked GE Healthcare exercise echocardiography eBike EL (GE Medical Systems Information Technologies GmbH, Freiburg, Germany) whilst echocardiography is simultaneously performed by a Cardiologist. A step protocol is used, with each child beginning on a baseline of 0 watts, and the intensity increased by 25 watts every 3 minutes (modified McMaster protocol). Exercise stages of three minutes are used to obtain detailed "steady state" information for each exercise stage and facilitate echocardiographic image acquisition. Children are encouraged to cycle at 60 revolutions per minute (rpm) throughout. Children are told to cycle until completion/ the point of voluntary

exhaustion. The exercise test is terminated when the child is unable to continue due to exhaustion or when they cannot sustain a cadence of 60 ± 5 rpm.

Oxygen consumption is measured using a gas cart (Metalyzer 3B Cortex, Biophysik, Leipzig, Germany). The device was calibrated using a known reference gas before testing and a 3 litre calibration syringe was used to calibrate the turbine volume transducer (Hans Rudolph, Kansas City, MO). The gas cart provided breath-by-breath gas exchange analysis, averaged to 10 second time intervals. The highest 10 second average VO_2 represented that individual's $\text{VO}_{2\text{peak}}$. Average data for each stage is expressed as the average of the last 30 seconds of each workload. The Borg Scale is used to measure the rating of perceived exertion (RPE) at the end of each 3 minute interval, with the children asked to subjectively indicate which grading (scale out of 10) is relevant to them at that point in the test.

Functional trans-thoracic echocardiography at rest is performed by a Cardiologist following the guideline of the American Society of Echocardiography (1) and using the following hardware: Vivid Q or E9, General Electric, cardiac transducers 4-6 Mhz, GE Healthcare, Little Chalfont, UK). Left Ventricular (LV) systolic function during rest and exercise is assessed using short axis and long axis fractional shortening (FS), Pulse Wave –Tissue Doppler Imaging (PW-TDI) derived myocardial systolic velocities and 2-D myocardial strain analysis (speckle tracking). For longitudinal and radial strain analysis images of the left ventricle are obtained in apical 4 chamber and parasternal short axis views. Images for strain analysis are obtained at a rate of 40-90 frames per minute during rest and exercise. Diastolic LV function is assessed using Pulse Wave –Tissue Doppler Imaging (PW-TDI) derived myocardial diastolic velocities and PW Doppler mitral valve inflow velocities. Fast image acquisition during exercise is facilitated using the GE exercise stress protocol GE software. Functional echocardiographic quantification of left ventricular function is processed offline using the Q analysis research tool of ECHO PAC offline software (EchoPac, GE Healthcare, Little Chalfont, UK).

Echocardiography protocol:

Rest:

Exercise and recovery: 7 images per exercise stage:

1. 2-D 4 Chamber view LV
2. Tissue Doppler Imaging (TDI) 4 Chamber view
3. Pulse Wave (PW) TDI lateral LV 4 chamber view
4. Mitral valve inflow PW Doppler trace
5. Aortic outflow PW Doppler trace
6. 2-D parasternal short-axis view (base)
7. 2-D parasternal short axis view (apex)

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