FULL PROTOCOL TITLE

Thiamine to improve stem cell function in patients undergoing bypass surgery: a randomised controlled trial

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SYNOPSIS

1.1 *Study Title*

Thiamine to improve stem cell function in patients undergoing bypass surgery: a randomised controlled trial

1.2 *Objectives*

To determine whether high dose oral thiamine administered before coronary artery bypass surgery improves resident cardiac progenitor cell function.

1.3 *Background*

Heart failure is a major cause of morbidity and mortality in New Zealand. Over 10,000 hospital admissions per year are due to heart failure in New Zealand, with a median survival after admission of only 3.5 years, and no reduction in mortality in recent years. There is a large impact on quality of life – the obvious impact on physical health is matched by mental health issues in advanced heart failure comparable to major depression. There are negative effects on other members of the community as well, with family caregivers suffering from emotional distress and impaired health.

Even in mildly symptomatic patients with systolic (pump) dysfunction on optimal treatment, there is a substantial mortality rate. There are still no proven treatments for diastolic (relaxation) dysfunction. Over the past decade, it has become clear that the heart has an intrinsic ability to regenerate itself, orchestrated by progenitor or stem cells. However, cell therapies are still restricted to the research sector, where only modest results have been seen. With the growing epidemic of heart failure, there is an urgent need for new therapies, especially in patients with coronary artery disease.

Patients with diabetes mellitus are at higher risk of developing heart failure compared to those without diabetes. Furthermore we have shown previously that diabetics have reduced resident cardiac progenitor cell number and function, and that this can be improved *in vitro* and *in vivo* (in animal models) using a thiamine analogue. Although the thiamine analogue improved progenitor cell function in cell cultures from both diabetic and non-diabetic animals, the diabetic animals had worse function initially.

1.4 *Design and Outcomes*

This is a single centre, randomised, double-blind, controlled phase 2b trial, built on the infrastructure of an ongoing observational trial. Participants listed for inpatient urgent coronary artery bypass grafting (CABG) will be randomised to thiamine or placebo, for 3-5 days before their procedure. At the time of CABG, the right atrial appendage, which is discarded as a normal part of the procedure, will be collected as well as a small left ventricular biopsy (both of these techniques being a routine part of our current observational study).

The primary outcome is the proliferation ability of resident cardiac progenitor cells, as measured by BrdU activity.

Secondary outcomes are other functional measures, such as the ability to differentiate into cardiomyocytes, as measured by the number of GATA-4 and connexin-43 positive cells after exposure to differentiation medium, and measurement of the level of transketolase to determine the activation of pentose phosphate pathway.

Our primary hypothesis to be tested is that treatment with thiamine will improve the primary outcome in diabetics and non-diabetics. We also wish to test the hypotheses that treatment with thiamine will improve the secondary outcomes in diabetics and non-diabetics, and that diabetics receiving placebo have reduced resident cardiac progenitor cell number and function compared to non-diabetics receiving placebo.

1.5 *Interventions and Duration*

Patients will be randomised to oral thiamine (2g/day) or matching placebo once listed for inpatient CABG. Total length of time on intervention treatment will be 3-5 days.

1.6 *Sample Size and Population*

The number of patients to be randomised in total is 40. Randomisation will be stratified based on diabetic status (non-diabetic/diabetic). The study population will be those patients admitted to hospital and awaiting urgent (i.e. inpatient) coronary artery bypass surgery, without valvular heart disease requiring intervention.

2 BACKGROUND

2.1 *Rationale*

Background and need for the study

Heart failure is a major cause of morbidity and mortality in New Zealand. Over 10,000 hospital admissions per year are due to heart failure in New Zealand, with a median survival after admission of only 3.5 years, and no reduction in mortality in recent years.[1] There is a large impact on quality of life – the obvious impact on physical health is matched by mental health issues in advanced heart failure comparable to major depression.[2] There are negative effects on other members of the community as well, with family caregivers suffering from emotional distress and impaired health.[3]

Even in mildly symptomatic patients with systolic (pump) dysfunction on optimal treatment, there is a mortality rate of 11% over less than two years.[4] There are still no proven treatments for diastolic (relaxation) dysfunction. Over the past decade, it has become clear that the heart has an intrinsic ability to regenerate itself, orchestrated by progenitor or stem cells (despite their differences, we use the widely recognised term stem cell interchangeably in those parts of the study likely to read by a wide audience, such as title and abstract).[5] However, cell therapies are still restricted to the research sector, where only modest results have been seen.[6] With the growing epidemic of heart failure, there is an urgent need for new therapies, especially in patients with coronary artery disease.

Rationale for patient population chosen

This is a phase 2, randomised control trial with a real-world design. The patient population consists of patients admitted to hospital awaiting urgent in-hospital coronary artery bypass grafting (CABG). In general, these patients have suffered an acute coronary syndrome, and therefore, as patients with the highest grade coronary disease, they have among the highest risks of development of subsequent heart failure. We are stratifying by diabetic status as these patients are at highest risk of developing heart failure [7] and because our previous studies, described below, have identified these patients as having reduced resident cardiac progenitor cell number and function.

We are excluding those with impaired left ventricular systolic function (ejection fraction < 50%) as these patients are a heterogenous group with potentially widely varying exposures to myocardial ischaemia in the past. Also excluded are those with valve disease severe enough to require intervention at the time of cardiac surgery, as again these are a heterogenous group of patients with different aetiologies of valvular heart disease. We are excluding those below the age of 50 years or having CABG for non-atherosclerotic disease (e.g. coronary dissection) as again patients with these conditions often have additional less common risk factors for coronary disease. Finally those patients already requiring thiamine supplementation or at high risk for requiring thiamine supplementation due to alchohol use will be excluded.

This trial is being built around the framework of an ongoing observational study entitled “Effects of catecholamines on function and signalling in the human diabetic heart”, ethics reference LRS/12/01/001, referred to hereafter as “the observational study”. This study involves collection of atrial appendage and left ventricular tissue

from volunteers undergoing cardiac surgery and has made information delivery, the consent process, and tissue collection relatively routine for a large number of participants undergoing cardiac surgery in Dunedin Hospital. Information about this study is widely available on the cardiology and cardiac surgery wards. In this randomised trial, we will randomise participants to treatment for 3-5 days before surgery, monitor them before and after surgery, but the intra-operative procedures remain those normally followed by the observational study. As described below, the additional risk due to being randomised to active treatment is minimal. In addition, participants who desire to be involved in this randomised trial but do not meet inclusion criteria will be given the opportunity to take part in the observational study.

Rationale for interventional regime chosen
Thiamine is known to be depleted by coronary artery bypass grafting (CABG) [8] and is also reduced in heart failure.[9] We wish to determine if short term (3-5 days) treatment with thiamine improves the function of resident cardiac progenitor cells.

The trial is based on our previous research showing that treatment with benfotiamine, a fat-soluble analogue of thiamine, increases the proliferation of resident cardiac progenitor cells (CPCs).[10] In this study, CPCs from animals treated with benfotiamine had an improvement in proliferation of these progenitor cells as measured by the BrdU assay. This was seen in both diabetic and non-diabetic animals, but CPCs from diabetic animals had impaired proliferation ability at baseline. In addition, diabetic animals had lower number of CPCs than non-diabetic animals, and this also improved with benfotiamine. In CPCs isolated from humans, benfotiamine protected CPC proliferation ability when cultured in high glucose medium.

While benfotiamine, a lipid-soluble analogue of thiamine, was used for our preclinical studies, this has not been used in high doses in humans before. Although previous studies have not shown any major adverse effects from benfotiamine use even up to a dose of 400mg/day, a report for the European Food Safety Authority concluded that there was insufficient data to demonstrate the safety of benfotiamine as a source of vitamin B1 to be added to food supplements (which is how over the counter vitamins are regulated in the European Union).[11] We elected to use oral free thiamine, given its long history of safe use and assessment of toxicity as generally being viewed as “very low”.[12]

We have chosen a “real world” duration of intervention. Approximately 20-50% of coronary artery bypass surgery procedures are performed on patients with an acuity level of “urgent”, where the patient has been admitted to hospital with an acute unscheduled presentation and is to remain in hospital until the procedure is completed. These patients are necessarily higher risk than elective patients, but are generally stable enough to allow a period of 3-5 days of medical intervention prior to surgery. It is during this period, after the patient has been listed for surgery, that consenting patients will be randomised in the trial.

2.2 *Supporting Data*

2.2.1 Thiamine dose

In our previous study, the dose of benfotiamine administered to study animals was 70mg/kg body weight/day. The bioavailability of benfotiamine, which is fat soluble, is higher than that of oral soluble thiamine and there is corresponding higher plasma concentrations found for similar doses.

In one study of health volunteers, a single 40mg oral dose of benfotiamine lead to a plasma area under the concentration time curve (AUC) of 240 (standard deviation (sd) 22.9) h.ng/ml compared to 154.3 (sd 18.7) h.ng/ml for a single 100mg oral dose of thiamine mononitrate.[13] Maximum plasma concentration was 64.9 (sd 37.7) ng/ml for benfotiamine compared to 38.9 (sd 22.7) ng/ml for thiamine. Half life was not significantly different, at 2.9 (sd 2.8) hours for benfotiamine compared to 2.6 (2.5) hours for thiamine. This study implies that the thiamine dose should be 4.12 times the benfotiamine dose to achieve the same AUC.

A study in patients with end-stage renal disease found an AUC of 1491 h.ng/ml after 100mg oral benfotiamine compared to 355.1 after 100mg oral thiamine nitrate.[14] Maximum plasma concentration was 81.9 ng/ml after benfotiamine compared to 21.3 ng/ml after thiamine, while the half life was 7.6 hours for benfotiamine compared to 8.3 hours for thiamine. This gives a very similar value to before, of a 4.3 times higher AUC for benfotiamine than thiamine at the same dose.

Assuming a ratio of 4.2:1 thiamine dose:benfotiamine dose would give a similar AUC, this equates to 294mg/kg/day for our subjects. For an 80kg participant, this equates to 23,520mg/day. This is far in excess of the current maximum reported doses (7 – 8g/day). We therefore chose a lower dose of 1000mg twice a day (total daily dose 2000mg) to provide a dose higher than most replacement or treatment regimes, but still within previously reported safe dose limits.

Finally, previous views held that absorption of thiamine is by a saturable active transport mechanism in the small intestine, which limits oral absorption of high doses of thiamine. However a recent study examining the pharmacokinetics of high doses thiamine in healthy subjects showed that, contrary to these views, both saturable and non-saturable absorption exists.[15] Doses up to 1500mg of thiamine hydrochloride (the formulation used in this study) led to a higher plasma AUC compared to doses of 500mg or 100mg, being 2046 h.ng/ml for the 1500mg dose, 623 for the 500mg dose, and 214 for the 100mg dose. Although this study was performed in healthy individuals, it is likely that similar non-saturable absorption would occurs in participants in our trial.

2.2.2 Potential risks of thiamine

Thiamine is a vitamin, being available in many different food groups as well as being available to buy without a prescription. Dietary supplementation is generally viewed as being low risk, with no specific toxic effects identified.[12]

A number of small studies performed in patients with Alzheimer's disease showed that high doses produce minimal side effects.[16–18] The majority of the participants in these trials were treated with 3g/day, at which dose no adverse effects were seen. A small follow-on study of 17 patients were treated at doses of 4-8g/day over 3 to 13

months.[18] No adverse effects were seen at doses up to 6g/day. A number of participants at higher doses (6g/day or higher) had some degree of neuromuscular blocking detected on electomyography (>10% of motor units), but this was asymptomatic and not clinically detectable. Above 7g/day, 2 participants developed nausea and indigestion, which settled on reducing the dose by 0.5g/day. Given the lack of toxicity at the proposed dose (2g/day), it is not thought likely that toxicity will develop over the 3-5 days of the study.

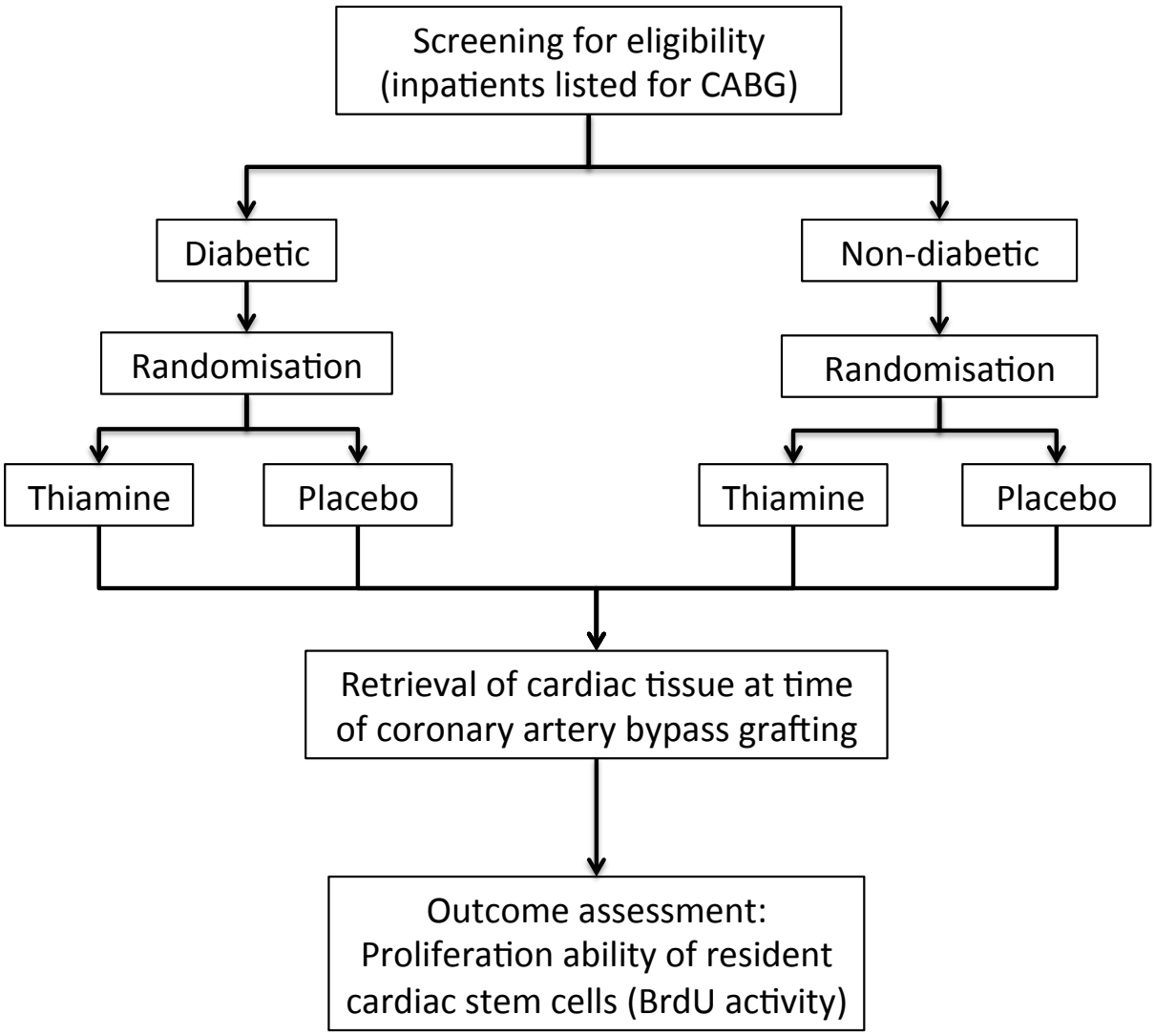
A report from 2003 by the Food Standards Agency in the United Kingdom reported that "Reports of thiamin-associated toxicity in humans are rare and most relate to incidents following parenteral administration of the vitamin. High doses (≥5000 mg) of thiamin hydrochloride may cause headache, nausea, irritability, insomnia, rapid pulse and weakness; these symptoms are relieved following cessation of treatment or reduction of dose."[12]

2.2.3 Potential risks of study procedures (right atrial appendage retrieval/left ventricular biopsy)

As part of the study, the right atrial appendage and a left ventricular biopsy will be obtained. These procedures have been part of the observational study since 2012 (right atrial appendage) and 2013 (left ventricular biopsy). The right atrial appendage is removed as part of the normal operating procedure for CABG and so does not entail any additional risk – this tissue is otherwise simply discarded. Left ventricular biopsy is not part of the normal operating procedure for CABG but entails minimal risk. In the appendix are two documents submitted with the application for amendment to the observational study covering this procedure, a letter of support from Professor Konstantinov regarding the safety of the procedure, and a scientific statement regarding the procedure. Over the time period of the observational study, there have been no episodes of harm related to these procedures. As we are not changing any part of the procedures involved with this aspect of the observational study, there is no additional risk related to the procedures compared to the observational study alone.

3 STUDY DESIGN

The study design is that of a randomised trial, stratified by diabetes status, as shown in the following figure.



4 SELECTION AND ENROLLMENT OF SUBJECTS

4.1 *Inclusion Criteria*

1. Patients listed for inpatient coronary artery bypass surgery due to atherosclerotic coronary artery disease *and*
2. Aged 50 years and over *and*
3. Left ventricular systolic function normal on echocardiography (ejection fraction ≥ 50%) performed during this inpatient stay (or within three months if no myocardial infarction has occurred between date of echocardiogram and date of enrollment).

4.2 *Exclusion Criteria*

1. Left ventricular systolic function impaired (echocardiographic ejection fraction < 50%) *or*
2. Renal failure with creatinine > 200 umol/l or requiring renal replacement therapy *or*
3. Already taking thiamine supplementation *or*
4. Previously had an adverse reaction to thiamine, or a supplement or medication containing thiamine *or*

5. Previous or current history of alcohol related problems. This refers to a previous diagnosis of a medical condition thought to be caused or exacerbated by alcohol, or harmful use of alcohol (International Classification of Diseases version 10 codes F10.0 to F10.9) *or*
6. Other major medical conditions that, in the opinion of the investigator, would make randomisation of the participant to thiamine unsafe or undesirable.

4.3 *Study Enrollment Procedures*

- Potential participants will be identified on release of the inpatient cardiac surgery list.
- These potential participants will be approached by a study nurse with information about the study.
- Potential participants will be given an appropriate period of time to consider whether to take part, depending on their prior knowledge of the observational study.
- If willing to take part, the potential participant will be screened according to the criteria above, with the screening recorded for future use.
- If eligible for the study, the potential participant will then be asked to sign a consent form.
- After consenting, the study nurse will obtain a baseline history and record investigations obtained as part of routine clinical care.
- The participant will then be randomised, with the initial treatment to be given at 6pm the evening of enrollment and the final treatment to be given the morning of the procedure.
- Study drug will have been prelabelled by the Clinical Trials Pharmacist with a unique code, and so the study drug will be allocated sequentially by study nurses to participants, based on diabetes status. The Cardiology Research Nurses will record the medication label and add the participant's name and the date to the medication packaging.

5 STUDY INTERVENTIONS

5.1 *Interventions, Administration, and Duration*

If allocated to active treatment, 1000mg (2 x 500mg capsules, Pharmaceutical Compounding NZ Ltd, Auckland) of thiamine will be administered orally twice a day, with the initial dose to be at approximately 6pm no more than 5 days before CABG. No side effects are expected at this dose, but potential side effects seen at higher doses are nausea, headache, irritability, insomnia, rapid pulse and weakness. If these occur, the active treatment may be stopped. If allocated to placebo treatment, 2 x matching placebo capsules (containing maltodextrin 500mg each) will be administered following the same regime as active treatment (Pharmaceutical Compounding NZ Ltd, Auckland). Capsules will be prepacked into bottles of 20. Active and placebo medications will be identical, both medications being dry powder in size 00 natural gelatin capsules. The Thiamine HCl (USP) is been purchased by Pharmaceutical Compounding NZ Ltd from Medisca (Australia), a specialist supplier of ingredients for compounding pharmacies. Medisca hold a TGA Licence to Repack Therapeutic Goods; and as such operate a testing and quality assurance system to ensure that all materials supplied meet the appropriate specification.

In the event of predicted postponement of CABG after randomisation, four and a half days of study drug (active or placebo) should be completed, i.e. 9 administrations of study drug equal to 18 capsules. The final further single 1000mg dose of study drug (2 x 500mg thiamine capsule or 2 x placebo capsule) should then be administered the night before surgery. In the event of postponement of CABG after the full course has been taken (i.e. cancellation on the scheduled day of surgery), no further study drug needs to be taken.

5.2 *Handling of Study Interventions*

The interventions (thiamine/placebo) will be bought by the Southern District Health Board Clinical Trials pharmacist from Pharmaceutical Compounding NZ Ltd, Auckland. The capsules will be packed into 20 capsule bottles by Pharmaceutical Compounding NZ Ltd (Licenced Pharmacy). Deliveries to the Southern District Health Board will be in batches of 15 bottles of active intervention and 15 bottles of placebo at a time. They will be stored in the Cardiology Research Nurses office in a designated locked area designed for dispensing of study medication. This room is kept at 21-22 degrees Celsius, which should be sufficient to supply the supplied 6 month shelf life for the study drugs.

5.3 *Randomisation procedure*

Prior to delivery of study drug to the Cardiology Research Nurses, the Clinical Trials Pharmacist will label the study drugs. This will be based on a randomisation list generated from http://www.randomization.com, where we will use 5 blocks of 4 participants each for the diabetic and nondiabetic arms of the trial. After labeling the study drugs, the Clinical Trials Pharmacist will hold both hard and soft copies of the randomisation lists, which will only be made available to the study investigators at the time of statistical analysis, i.e. unblinding.

5.4 *Concomitant Interventions*

None

5.5 *Adherence Assessment*

Study nurses will assess the participants prior to CABG to determine adherence with medication, by means of a pill count and patient questioning. In addition, functional B1 assays will be performed as described in the laboratory evaluations below.

6 CLINICAL AND LABORATORY EVALUATIONS

6.1 *Schedule of Evaluations*

Evaluation	Screen-ing	Pre-Entry	Entry	Day 3	Day 4	Day 5/day of CABG	Day 6 post CABG
Informed Consent		X					
Documentation of Disease/Disorder	X	X					
Medical/Treatment History	X	X					X
Clinical Assessment		X					
Laboratory results assessment		X					
Progenitor cell isolation						X	
Stored Plasma		X				X	X
Stored blood		X				X	X
Adherence Assessments			X	X	X		
Questionnaires - safety			X	X	X		X

6.2 *Timing of Evaluations*

This section should include definitions of the column headings in the Schedule of Evaluations and any special instructions.

6.2.1 Pre-Randomisation Evaluations

Screening will be performed as described above. No fixed time limit is required for this as long as a participant, if randomised to active treatment, will receive 3-5 days of treatment.

6.2.2 On-Study/On-Intervention Evaluations

At baseline, we will obtain 40ml of blood. This may be used for analysis of variables not already examined as part of the participant's clinical care (e.g. HbA1c). The majority of this blood will be stored for later comparison with the sample taken immediately prior to CABG. We will also obtain a standardized assessment of patient's health status and history. Routine laboratory test results already taken will be recorded as will echocardiogram and angiogram results.

On day three after randomisation and each subsequent day prior to the procedure, medication adherence and assessment of toxicity will be assessed. This will necessarily be a different number of days depending on when surgery has been scheduled.

Immediately before surgery (on the day of the procedure), a 40 ml blood sample will be taken. To ensure adequate absorption of oral thiamine has been achieved, a vitamin B1 assay will be carried out in all patients.

From this point on, the evaluation follows the same process as that already established in the observational study, which is included below under Laboratory Evaluations (Section 6.1.11).

6.2.3 Intervention Discontinuation Evaluations

If the participant wishes to discontinue active treatment, the adherence questionnaire and assessment of toxicity will be completed. Reason for discontinuation will be specified, and the participant will be invited to remain on study but off-intervention.

6.2.4 On Study/Off-Intervention Evaluations

If the participant is on study but off-intervention prior to CABG we will still retrieve the second blood sample prior to CABG as well as tissue for which consent has been granted. No active treatment is planned for after the time of CABG.

6.2.5 Final On-Study Evaluations

Prior to discharge on day 6 (i.e. the following Monday for those having CABG on Tuesday) the patient will be assessed for early inhospital outcomes to monitor safety, in particular atrial fibrillation. Another blood sample will be taken for storage, especially to assess thiamine levels.

6.2.6 Off-Study Requirements

Once off-study, no further follow-up is planned, although consent will be requested for possible longer term follow-up using electronic records.

6.3 *Special Instructions and Definitions of Evaluations*

6.3.1 Informed Consent

Once identified, potential participants will be given the Patient Information Leaflet to read by one of the study nurses. Any questions regarding the trial will be directed to one of the study nurses in the first instance. If the potential participant decides to take part, an investigator or an appropriate person delegated by the investigator to take consent will check understanding and, if satis-

fied that consent is valid, will countersign the consent form after the participant. “Appropriate person” refers to someone with sufficient experience and knowledge of the trial and trial processes to ensure accurate information is available to the potential participant – in practice, this is likely to be a cardiology study nurse, cardiology registrar, or cardiology consultant. Signed consent forms will be stored along with other study documentation by the cardiology research nurses.

6.3.2 Documentation of inclusion criteria

Presence on the CABG inpatient waiting list along with an angiogram report documenting atherosclerotic coronary artery disease suffices to demonstrate the disease inclusion criteria.

Exclusion criteria will be determined by examination of echocardiograph report and medical history. Inclusion and exclusion criteria will be completed on the screening log entry for each potential participant.

6.3.3 Medical/Treatment History

Medical history will be obtained by examining the medical record of the patient and discussion with the participant. A standard reporting form will be completed, documenting in particular previous history (especially presence and duration of diabetes), details of current presentation (especially non-ST elevation myocardial infarction vs unstable angina), and relevant tests (especially high sensitivity troponin, HbA1c). Documentation of current medications will be made.

Prior to discharge, brief assessment of development of post-operative complications, such as atrial fibrillation, will be made.

6.3.4 Clinical Assessments

At time of entry to study, brief assessment of physical status will be made, using the NYHA scale for heart failure symptoms and the Canadian Cardiovascular Society scale for angina symptoms. Heart rate, blood pressure, height and weight will be obtained from the medical charts.

6.3.5 Laboratory Evaluations

During the surgical procedure right atrial appendages are standardly excised for the insertion of a venous cannula to institute cardiac pulmonary bypass before cross clamping of the ascending aorta. Thus, collection of right atrial appendages does not require a change from the standard surgical protocol.

For the ventricular samples, an area of the anterior wall of the left ventricle (LV), free from any epicardial scarring following previous myocardial

infarction, will be identified. A wedge (1.5 x 1.5 x 10 mm) from the anterior wall of LV will be biopsied. The minimal amount of tissue needed for the protein analysis is 5 mg wet weight. The site will be sutured using a single pledgeted or non-pledgeted 4-0 prolene suture.

The protocol for isolation and culture of cardiac progenitor cells has been successfully established in the our laboratory and published.[10] In brief, after serial washing to remove the blood, atrial appendage tissue will be digested with a mixture of collagenase type II and collagenase type IV (Worthington Biochem, USA) to prepare the single cell suspension. Next, the CD34 positive population, which indicates the circulating progenitor cells, will be depleted using a commercially available magnetic beads separation system (MACS separation columns, Miltenyi). CD90 and CD105 positive cells will then be enriched from the CD34 depleted population using the same system as above. The isolated cells will be cultured in the commercially available stem cells maintenance medium (Mitenyi). Using this protocol we regularly extract $0.5X10^4 – 1X10^4$ $CD34^-CD90^+CD105^+$ cells per 100mg of tissue which can then be expanded for the required experiments. Importantly, these cells can be freeze stored for later use in the experiments. BrdU incorporation by the progenitor cells, a measure of cell proliferation, will be measured using a BrdU immunofluorescence assay kit from Roche, according to the manufacturer's instructions. Transketolase activity will be measured using a commercially available kit (Sigma).

6.3.6 Other Laboratory Studies

Other laboratories studies are likely to be carried out in the future as substudies, but are not part of the primary or secondary outcome measures of the trial. These may include, but are likely not to be limited to, further assessment of the metabolic changes in the tissue and blood of those receiving active treatment, relationship between genomic and epigenomic factors (such as specific single nucleotide variants and specific methylation patterns, respectively) and response to treatment, differences in expression due to active treatment of molecules and proteins relevant to diabetic cardiomyopathy (such as advanced glycation end-products or extracellular collage) or other diseases. After unblinding, the samples taken from those participants may be included with other participants for analysis in the observational study.

6.3.7 Toxicity Assessment

A brief questionnaire assessing potential side effects related to active treatment will be completed prior to participants undergoing CABG. Prior to discharge, a brief assessment of post-operative complications will be recorded.

6.3.8 Adherence Assessments

Adherence will be assessed by a brief questionnaire and a pill count.

7 MANAGEMENT OF ADVERSE EXPERIENCES

Adverse experiences related to the study intervention are expected to unlikely, for reasons discussed in section 2.2.2. These are:

- headache,
- nausea,
- irritability,
- insomnia, rapid pulse
- weakness

These can be treated in the usual form, as decided by the clinical team caring for the participant.

8 CRITERIA FOR INTERVENTION DISCONTINUATION

If the clinical team treating the patient believe any of these adverse experiences are related to the study intervention, they may stop the study intervention without further discussion with the study team. Study nurses will document details of cessation of treatment, including reason and progress after cessation, during their daily patient assessment prior to CABG. No unblinding is expected to be performed as a result of study intervention cessation, and further evaluations, in particular right atrial appendage and left ventricular biopsy, will be carried out as before.

9 STATISTICAL CONSIDERATIONS

9.1 *Outcomes*

9.1.1 Primary outcome

The primary outcome measure is the proliferation ability of resident cardiac stem cells, as measured by BrdU activity.

9.1.2 Secondary outcomes

Secondary outcomes are other functional measures, such as the ability to differentiate into cardiomyocytes, as measured by the number of GATA-4 and connexin-43 positive cells after exposure to differentiation medium, and measurement of the level of transketolase to determine the activation of pentose phosphate pathway

9.2 *Outcomes discussion*

Primary and secondary outcomes will be measured without knowledge of treatment assignment (i.e. blinded).

Bromodeoxyuridine (BrdU) is a thymidine analogue that is taken up by cells actively replicating DNA. As such it is widely used as an assay to determine proliferation of cells, and has appeared in over 20,000 biomedical science articles.[19] In addition, we have demonstrated accuracy in our laboratory, with our animal studies demonstrating

a coefficient of variation of 9% in non-diabetic animals and 13-14% in diabetic animals. As such, it is a valid test to use to assess the function of resident CPCs.

9.3 *Sample Size and Accrual*

The primary outcome will initially be assessed in treated vs non-treated groups (combining diabetics with non-diabetics) and then stratified by diabetic status. We therefore calculated a sample size powered by subgroup (diabetic vs non-diabetic) status. Based on our previous data, with a sample size of 10 in each group (non-diabetic no active treatment, non-diabetic active treatment, diabetic no active treatment, diabetic active treatment) giving a total of 40 patients in total, we will have 99% power and 91% power, in non-diabetics and diabetics respectively, to detect a two-tailed 30% difference in primary outcome with treatment at a significance level of 0.05. Here we use a t-test to compare groups, with data coming from assessment of BrdU activity in CPCs isolated from treated and untreated animals.[10] Note that we may have higher power, as we detected a 79% and 106% increase in mean proliferation activity of stem cells in treated non-diabetic and diabetic animals, respectively, compared with control. However given the uncertainty moving from an animal model to humans, we felt it prudent not to limit groups to less than 10 patients each.

Accrual rate is expected to be approximately 1-2 participants per fortnight. This is based on current accrual rates of 2-4 participants per fortnight in the observational study – many of these will be eligible for inclusion in this trial and are likely to volunteer based on the low toxicity of the intervention. Given the exclusion of the highest risk cases (those with impaired left ventricular systolic function or those undergoing cardiac surgery on an emergency basis) and the short duration of treatment, we expect the vast majority of those enrolled to complete allocated treatment until CABG, i.e. a low loss to follow-up rate.

9.4 *Data Monitoring*

An interim blinded analysis of the toxicity data will be performed after recruitment of 20 patients by the study team. We expect there to be few adverse experiences on or off active treatment. If more than 20% of participants report adverse experiences, then a separate Data and Safety Monitoring Board will be recruited consisting of at least two clinicians independent of the investigators. These will be supplied with the toxicity data and the unblinding codes, if required. If, in their opinion, excess toxicity is seen with active treatment, a recommendation to halt the trial can be made to the study investigators.

An interim analysis of study recruitment will be made one year after randomisation of the first subject. If recruitment is less than 20 subjects, we will attempt to assess the cause of this slow recruitment. This will be done by comparing with recruitment to the observational study and analysis of screening logs. If study validity can be maintained, an amendment to the study will be made to rectify perceived causes of slow recruitment.

No interim analysis will be made for primary or secondary outcomes given the small number of study participants.

9.5 *Data Analyses*

A standard two-tailed t-test will be used to compare the primary outcome between treated and non-treated groups, using an intention-to-treat analysis. Separate analysis of the primary outcomes stratified by diabetic status will be performed using a t-test. For secondary analyses, differences across multiple groups will be assessed using an analysis of variance (ANOVA) test followed by a Šidák-Holm multiple comparison test. Comparison between two groups will be assessed using t-tests.

Missing data will be removed from individual analyses, while not removing the entirety of the participant's data from further analysis. As mentioned above, loss to follow-up is expected to be low as participants are inpatients with short study duration.

10 DATA COLLECTION, SITE MONITORING, AND ADVERSE EXPERIENCE REPORTING

10.1 *Records*

Cardiology research nurses will keep records of screening, consent forms, medical/interventional history, adherence, and toxicity. Department of Physiology, University of Otago will keep records of blood samples obtained, tissue samples obtained, and results of blood and tissue analysis. All of these will be kept in locked areas, with no access to the public. Records will be kept for a minimum of 15 years in case these data require re-examination.

10.2 *Role of Data Management*

At study completion, study investigators will combine the records kept by the Cardiology research nurses and the Department of Physiology. The database will then be unblended and statistical analyses described above carried out.

10.3 *Quality Assurance*

The Coordinating Investigator will ensure the cardiology research nurses are trained and equipped to perform their duties in the study. Laboratory protocols will follow standard laboratory methods for quality assurance. A random sample of participants clinical, echocardiographic and angiographic data will be checked for accuracy.

10.4 *Adverse Experience Reporting*

Serious adverse events will almost certainly be related to the participant's underlying diagnosis (i.e. admission with acute coronary syndrome) rather than the use of the interventional medicine. Adverse experiences are therefore reported to the Coordinating Investigator as soon as possible, within one month for non-serious adverse events and within one week for serious adverse events. Under their Standard Operat-

ing Procedures (May 2012) adverse events are not, in general, individually reported to the Health and Disability Ethics Committee, but are reported annually. The exceptions are in the event of urgent safety measures being required or a temporary halt to the study is required, in which case the Committee will be notified immediately (with seven days being the time limit specified by the Committee).

11 OTHER ISSUES

11.1 *Health and Disability Ethics Committee Review and Informed Consent*

This protocol, the informed consent document, the patient information leaflet, and any subsequent modifications will be reviewed and approved by the Health and Disability Ethics Committee. A signed consent form will be obtained from the subject. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation.

11.2 *Subject Confidentiality*

All laboratory specimens, evaluation forms, reports, and other records (including images such as echocardiographic or angiographic images) that leave the care of the investigators will be identified only by the Study Identification Number (SID) to maintain subject confidentiality. All records will be kept in a file protected by a locked door. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the Southern District Health Board, the Health and Disability Ethics Committee, the sponsor, or the sponsor's designee.

11.3 *Study Modification/Discontinuation*

The study may be modified or discontinued at any time by the Health and Disability Ethics Committee, the sponsor, Southern District Health Board, or the investigators, as part of their duties to ensure that research subjects are protected.

12 PUBLICATION OF RESEARCH FINDINGS

Scientific publication of results will be organised and determined by the study investigators. In particular, there will be no role of the study funder or sponsors in the analysis of results, or in writing or submission of manuscripts. A lay summary will be provided to participants and funding organisations along with a copy of scientific publication generated.

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Appendix

A1. Letter of support for left ventricular biopsy

18 November 2012

To whom it may concern,

The method to obtain a tiny (1.5 x 1.5 x 10 mm) left ventricular epicardial biopsy during cardiac surgery as proposed by Regis Lamberts et al. in the amendment to their Human Ethics application (LRS/12/01/001) is a recognised technique for obtaining myocardial tissue. Inherently to take a biopsy, it will carry a degree of risk, however, for the proposed procedure it is expected to be minimal. To my knowledge, no side effects have been reported with this procedure in the medical literature. The biopsy will be done intra-operatively under direct vision and unlikely to result in any adverse event. In my opinion, this is a very low risk procedure. Therefore I support the approval of this study as it is likely to contribute significantly to the advancement of medical knowledge in the field that this research group is working on and the risk of biopsy is minimal.

Sincerely,

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A2. Scientific statement on LV epicardial biopsy – for amendment to project LRS-12-01/001

S Coffey, P Saxena, MJA. Williams, R Katare, J C Baldi, P P Jones, R R Lamberts
21th September 2012

A2.1. Introduction

This brief statement examines the use of epicardial left ventricular biopsies in the context of the project entitled "Effect of catecholamines on functions and signaling in obese human diabetics (LRS-12-01/001)". Full details regarding the project, including background, hypotheses, and protocol can be found in the other documents accompanying the request for approval of amendment to the project.

A2.2. How is an epicardial left ventricular biopsy obtained?

In patients undergoing coronary artery bypass grafting (CABG) who have consented to having an epicardial biopsy, cardiopulmonary bypass (CPB) will be instituted using standard anticoagulation protocol and cannulation. An area of the anterior wall of the left ventricle (LV), free from any epicardial scarring following previous myocardial infarction, will be identified. A wedge (1.5 x 1.5 x 10 mm) will be biopsied. The site will be repaired using a single pledgeted or non-pledgeted 4-0 prolene suture. In total, the additional operating time is expected to be approximately one or two minutes.

A2.3. What is the indication for epicardial left ventricular biopsy?

Diabetes is associated with a significantly increased risk of heart failure, both with impaired and preserved ejection fraction. One of the primary goals is to further characterise diabetic heart disease. To this point, we have used atrial tissue removed routinely during surgery. Our atrial tissue findings to date with regard to calcium handling in the hearts with preserved ejection fraction have been unexpected. However, calcium handling in atrial and ventricular myocytes is known to be different in animal studies (see, for example references [1, 2]). As clinical heart failure is due predominantly to disease in the ventricle, analysis of ventricular tissue is clearly required.

Another technique for obtaining ventricular myocytes is via a percutaneous endomycardial approach. However independent advice from interventional cardiologists in Dunedin and Auckland recommended considering an epicardial biopsy at the time of CABG as the safest approach.

A2.4. What are the risks of epicardial left ventricular biopsy?

Obtaining left ventricular epicardial biopsies is an internationally established technique with no reported adverse effects. On the other hand, many cardiac surgeons (including in Dunedin) undertake decompression of the left ventricle routinely during valve procedures, which by necessity requires a transmyocardial ventriculotomy. The proposed epicardial biopsy taken

under direct vision is a less invasive procedure with a similar technique both for obtaining the biopsy and subsequent repair, meaning that there should not be any significant learning curve for the procedure.

A number of international centres have obtained left ventricular epicardial biopsies and reported their results. A study from 1992 included six patients, and noted that there were no complications [3]. A 1995 study using epicardial biopsies did not give the number of biopsies taken for their study, but reported that the procedure had been found to be safe in over 40 procedures [4], A 2005 study included 26 patients, and did not report any complications [5]. A 2010 study from the same group in Burlington, Vermont included a further 21 patients, and again did not note any complications [6]. Most recently a group from Oxford performed both atrial and epicardial ventricular biopsies, without note of any complications [7]. So, to the best of our knowledge, no complication has ever been reported for this procedure.

A2.5. Summary

In conclusion, while left ventricular epicardial biopsy is a procedure that must logically carry some small degree of risk, there have been no reported complications in the medical literature as far as we know. In addition, the longevity of the research project and ongoing studies performed by the Vermont team provide a degree of reassurance about the lack of long-term complications.

A2.6. References

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