Short Title: Neuromuscular fatigability in individuals with heart failure

Full Title: The influence of active muscle mass and nitrate supplementation on fatigability in individuals with heart failure

Research Protocol

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NCT Number:

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Background

Chronic heart failure (CHF) is a complex clinical syndrome associated with structural and functional abnormalities, which lead to reduced cardiac output at rest and/or in response to stress [1]. Exercise intolerance is a hallmark symptom of chronic heart failure (CHF) and is associated with reduced quality of life as well as being a strong prognostic indicator [2, 3]. Research in recent years has attempted to better understand the aetiology of exercise intolerance to provide therapeutic targets to improve physical capacity and quality of life, with studies primarily focusing on maximal oxygen uptake, metabolic thresholds and oxygen uptake kinetics [4-7]. Another important determinant of exercise intolerance is neuromuscular fatigability, defined as the reduction in neuromuscular function measured after exercise of a discrete time period [8, 9]. At present, the few studies that have assessed neuromuscular fatigability in individuals with CHF have utilised exercise involving a small muscle mass, such as isometric knee extension. However, one limitation of this approach is that it does not reflect the types of activity performed on a daily basis, and thereby lacks ecological validity. Specifically, activities of daily living (e.g. walking, gardening, housework or climbing stairs) involve dynamic, large muscle mass exercise and in turn a substantially higher cardiorespiratory demand relative to isometric tasks. At present, there is limited research assessing neuromuscular fatigability in individuals with CHF during large muscle mass exercise. Research characterising fatigability and determining its underlying mechanisms can help better understand the aetiology of exercise intolerance, and in turn provide therapeutic targets aimed at improving physical capacity and quality of life in individuals with CHF.

One potential strategy to attenuate fatigability is through nitrate supplementation (i.e. beetroot juice). Specifically, the consumption of nitrate-rich beetroot juice promotes increased nitric oxide bioavailability, which in turn can enhance local perfusion and oxygenation, skeletal muscle contractility, and muscle efficiency [10]. Given that individuals with CHF have impaired nitric oxide bioavailability and reduced local perfusion and oxygenation [6], which likely contribute to impaired fatigability, nitric oxide represents an attractive intervention to mitigate fatigability and improve exercise tolerance. To date, no study has assessed the effect of nitrate supplementation on neuromuscular fatigability in individuals with CHF.

Aim and Objectives:

The aims of this project are to, 1) characterise neuromuscular fatigability in individuals with CHF during exercise involving a small and large active muscle mass (Part I), 2) determine the effect of nitrate supplementation on neuromuscular fatigability during large muscle mass exercise in individuals with CHF (Part II), and 3) understand the impact of exercise intolerance on quality of life in individuals with CHF (Part III).

Methods/Design:

Study Design

Part I is a cross-sectional, observational study, which will characterise neuromuscular fatigability in individuals with CHF. Part II is a randomised crossover interventional study, which will assess neuromuscular fatigability following nitrate supplementation and a sham-control in individuals with CHF. For part II, participants will consume nitrate-rich beetroot juice in the lead up to one visit, and beetroot juice with nitrate extracted as a sham-control in the lead up to another visit, with the order of the conditions randomised. Part III is a qualitative study, which will evaluate the impact of exercise intolerance on quality of life in individuals with CHF.

Sample size

A total of 20 patients will be recruited into the study, with the same 20 patients participating in Parts I-III.

Eligibility criteria

Inclusion criteria:

- Patients with a left ventricular ejection fraction < 40%;
- Diagnosed for at least 3 months;
- Classified according to New York Heart Association (NYHA) class II-III;
- Clinically stable and receiving an optimal medical treatment;
- Aged ≥ 45 years old;

- Willingness to participate in a semi-structured interview (this is optional and the participant will be able to participate in the study if they choose not to take part in the interview);
- Ability to read, write and converse in English without the support of an interpreter;
- Willingness to undertake physical activity;
- No contraindications to physical activity and capable of performing activities of daily living independently, without the use of a walking aid;
- Able to provide written informed consent.

Exclusion criteria:

- Electrically implanted device (e.g. pacemaker, left ventricular assist device);
- Uncontrolled cardiac arrhythmias, myocardial infarction, percutaneous coronary intervention and/or bypass graft surgery up to 3 months previously;
- Receiving antacids or proton pump, xanthine oxidase, or phosphodiesterase inhibitors which affect the reduction of nitrate to nitrite and nitrite to nitric oxide;
- Treated with organic nitrates (e.g. trinitroglycerin)
- Major multi-morbidity or other alternative diagnoses of no obvious acute and self-limiting cause (e.g. patients with a terminal diagnosis of cancer, patients in receipt of oxygen therapy or oxygen saturation at rest <92%);
- Obesity (body mass index > 30 kg/m^2);
- Current smoker;
- Presented with severe symptoms requiring urgent assessment and stabilisation (e.g. breathless at rest, hypotension, confusion);
- Severe physical disability preventing them from functioning independently;
- Unable to provide informed consent;
- Currently taking part in any other study;
- Unable to communicate in English.

Recruitment procedures

Patients will be identified from the Heart Failure Diagnostic Clinic run at the Royal Victoria Infirmary and Freeman Hospital, Newcastle upon Tyne, by Consultant Cardiologists, Dr MacGowan, Dr Bailey, Dr Williams, Dr Fernandez and Dr Nelson, all of whom are part of the research team and play a vital role in identifying patients who meet the inclusion criteria. The consultant cardiologist will then invite the patient to talk to a member of the research team (Dr Callum Brownstein, Dr Sarah Charman, Miss Emily Dodd) who all have honorary contracts with the Newcastle upon Tyne Hospitals NHS Trust and sufficient level of competence and permission to assess patients' medical records using computerised systems in place. This will take place at the end of the patient's consultation, after which a member of the research team will meet the patient to explain the study. If the patient is interested, they will be provided with an information sheet. To avoid coercion, the patient will not be asked to make a decision in the presence of a member of the research team, and instead will be afforded 48 hours to read the information sheet and decide whether they would like to participate. The research team will request to call the patient after this 48 hour period to discuss the study and answer any questions. If the patient is happy to proceed when they will be invited to the Clinical Research Facility for Visit 1, where they will provide informed consent if they are willing to become a participant in the study.

Research Visits

Parts I and II: Quantitative work package

Individuals with CHF (n = 20) will visit the NIHR Clinical Research Facility on 4 occasions across Parts I and II of the study. Visit 1 will involve screening and consent, completion of questionnaires, familiarisation with study procedures, and performing a non-invasive measurement of mitochondrial function using near-infrared spectroscopy (NIRS). Visits 2-4 will be randomised in order, and will include, 1) incremental single-leg cycling to exhaustion with intermittent assessments of neuromuscular function, 2) incremental double-leg cycling to exhaustion with intermittent assessments of neuromuscular function, following a period of supplementation with nitrate-rich beetroot juice and, 3) incremental double-leg cycling to exhaustion with intermittent

assessments of neuromuscular function, following a period of supplementation with a nitrate-extracted beetroot juice placebo. For Visits 2-4, during which exhaustive exercise will take place, a consultant cardiologist will be present. The cardiologist will monitor ECG at rest prior to exercise and throughout the exercise protocol. Exercise will be discontinued if any abnormalities in the ECG trace are observed, or if the participant begins to feel unwell. Furthermore, the cardiologists involved in the study are trained in emergency care, and will be able to provide immediate assistance if the participant required medical care. Further details on these visits are provided below.

Visit 1 – Screening, consent, questionnaires, familiarisation, and NIRS-derived measure of mitochondrial function (1 hour 30 min)

The following procedures will be performed during Visit 1:

1) An investigator will discuss aspects of the study with the participant, including the measurements involved and the supplementation process, and the participant will be provided with the opportunity to ask further questions and if they are happy to participate then they will be requested to provide written informed consent. A Medical History Questionnaire will be completed after written informed consent has been received (20 min).
2) Physical examination performed, including body weight, height, and blood pressure (10 min).
3) Participants will complete four validated questionnaires. These will include the Minnesota Living with Heart Failure (MLHF) Questionnaire [11], Short-Form Health Survey (SF-36) [12], Pittsburgh Fatigability Sale [13], Godin Leisure-Time Physical Activity questionnaire [14] and food frequency questionnaire (20 min).
4) Participants will be familiarised with performing assessments of neuromuscular function on a customised semi-recumbent cycle ergometer, consisting of isometric maximal voluntary contractions (MVCs) of the knee extensors and supramaximal single (1 ms) electrical stimuli to the femoral nerve during and following MVCs. They will subsequently be familiarised with performing single-leg cycling at low-intensity, followed by immediate re-assessments of neuromuscular function (20 min).

5) A measurement of mitochondrial function will be taken using NIRS. This will involve participants performing 2 × 10 s contractions of the knee extensor at 40% MVCs separated by 10 s rest. Immediately following the second contraction, repeated, transient (5-20 s) occlusions of arterial circulation to the knee extensors will be performed to over a 3 min period using a rapid occlusion system (VenaPulse, VP-25, ACI Medical, USA) inflated to > 240 mmHg. This permits the measurement of muscle oxygen uptake recovery following muscle contractions by observing the slope of deoxygenation using NIRS, with the speed of oxygen uptake recovery well associated with gold-standard measurements of mitochondrial function [15] (20 min).

Depending on the order of randomisation, participants will be provided with their supplement for the subsequent visit, and instructed on supplementation.

Visits 2-4 – Neuromuscular fatigability during double-leg cycling following nitrate-extracted beetroot juice placebo, double-leg cycling following nitrate-rich beetroot juice supplementation, and single-leg cycling (2 hours)

Supplementation will only take place prior to the visits involving double-leg cycling (i.e. not prior to single-leg cycling). The supplementation will consist of 7 consecutive days of consuming either 2 × 70 ml of nitrate-rich beetroot juice (Beet It Sport, James White Drinks, UK) per day in the morning and evening, or 2 × 70 ml of nitrate-extracted beetroot juice as a placebo control (Beet It Sport, James White Drinks, UK). Testing will take place on the 7th day of supplementation, with participants asked to consume 2 bottles of the supplement 2 hours prior to testing taking place. Participants will be asked to avoid chewing gum or using mouthwash throughout the study period, as this can interfere with the conversion of nitrate to nitric oxide. Furthermore, in the 7 days during the supplementation periods, participants will be asked to complete a beetroot juice intake diary. A minimum washout period of 7 days will be given between periods of supplementation. Confirmation of supplementation will be performed by two means, 1) measurement of salivary nitrate concentrations and 2) having participants return empty, consumed bottles to the laboratory.

The following procedures will be performed during Visits 2-4:

1) Physical examination performed, including body weight, height and weight (10 min)
2) Salivary nitrate measurement via chewing on a cotton ball for 2 minutes, to subsequent be placed in an Eppendorf and frozen for later analysis (only during double-leg cycling visits) (2 min).
3) Participant set-up (45 min), including:
    a. Set-up for fatigability measurements, including placement of peripheral nerve stimulation electrodes on the femoral nerve (cathode) and gluteal fold (anode), and electromyography electrodes placed on the *vastus lateralis*. Stimulation of the femoral nerve via the cathode and anode permit the measurement of contractile function through resting twitch responses to supramaximal stimuli (i.e. stimuli eliciting a maximal twitch response) and voluntary activation of the quadriceps through supramaximal stimuli delivered during an isometric maximal voluntary contraction of the knee-extensors. These methods have been used extensively to quantify the neuromuscular mechanisms of fatigability in a range of populations [16], including individuals with CHF [17];
    b. Placement of electrocardiography electrodes;
    c. Placement of NIRS on the *vastus lateralis* (PortaLite, Artinis, The Netherlands). NIRS provides a non-invasive means of measuring muscle oxygenation by shining near-infrared light which penetrates the tissue. The degree of absorption of near-infrared light depends on the degree of oxygenation of haemoglobin, with non-absorbed light reflected back towards a receiver optode on the NIRS device. Accordingly, NIRS is able to measure relative changes in oxygenated and deoxygenated haemoglobin through changes in the absorption of near-infrared light [18].
    d. Placement of a bioreactance Non-Invasive Cardiac Output Monitor (NICOM, Cheetah Medical, USA) which we have previously evaluated [19-21] The method uses four pairs of electrodes applied at the front side of the upper and lower thorax (similar to ECG). Bioreactance is a novel method for continuous non-invasive cardiac function monitoring and estimates cardiac output by analysing the frequency of relative phase shift of electronic current delivered across the thorax.

e. Placement of face mask for measurement of gas exchange (i.e. oxygen consumption, carbon dioxide production) and standard metabolic analyser (Cortex, Germany).

4) Arterial occlusion of the knee extensors for 5 min, followed by 10 min passive recovery. This is performed in conjunction with NIRS to provide a physiological calibration of the minimum and maximum NIRS signal through the lowest muscle oxygenation (during occlusion) and highest muscle oxygenation (following the hyperaemic response after the release of occlusion), respectively (15 min).
5) Figure 1 below displays a schematic of the exercise protocol. The exercise protocol will consist of incremental cycling during single- or double-leg cycling, depending on visit and randomisation. Double-leg cycling will consist of 3 min stages of incremental cycling beginning at 16 W, with a 16 W increment each stage up until stage 5 (80 W). If stage 5 is reached, the increment will increase to 26 W. During the single-leg cycling visit, the protocol will be the same apart from the power output of each stage being half that of double-leg cycling (i.e. 8 W increment for stages 1-5, 13 W beyond stage 5). At baseline, between each stage, and at task-failure, an assessment of neuromuscular function will be performed, comprising isometric MVCs of the right knee extensor, and the assessment of % voluntary activation (VA) of the knee extensors using supramaximal femoral nerve stimulation during the MVCs, and contractile function through the assessment of the force evoked following supramaximal femoral nerve stimulation delivered at rest (potentiated twitch force, $Q_{tw,pot}$). Exercise will continue until participants are unable to achieve the target power output, or voluntarily disengage from the task. Following exercise cessation, the same neuromuscular function assessment will be performed at 1, 3, 5, 10, and 15 min post-exercise in order to determine the rate of recovery of neuromuscular function (30 min).

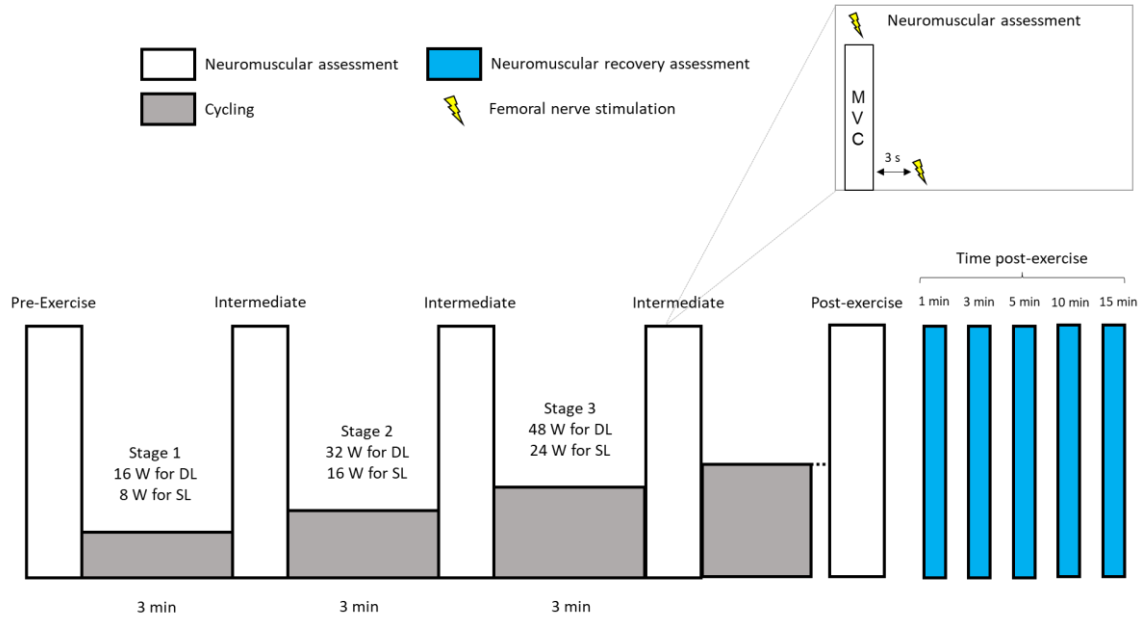

Figure 1. Protocol schematic displaying incremental cycling to exhaustion with stages interspersed with neuromuscular assessments, and post-exercise recovery measurements. DL, double leg; SL, single leg; MVC, maximal voluntary contraction.

Part III: Qualitative Work Package

While Parts I and II will further our understanding on the aetiology of exercise intolerance and the effect of nitrate supplementation in improving exercise tolerance in individuals with CHF, it is important to clarify the impact of exercise intolerance on the lives of these individuals. As such, the aim of Part III is to understand the symptoms associated with performing exercise and the impact of exercise intolerance on social, functional and emotional functioning on individuals with CHF.

One optional semi-structured interviews will be conducted with the participants who provide consent to participate in this part of the study (n = 20 or until saturation in findings) once they have completed the Parts I and II. Participants may choose to opt out from this part of the study and this option will be included in the consent form. An interview specific topic guide has been developed on symptoms associated with performing physical activity and on the impact of exercise intolerance on quality of life. Data from the semi-structured interviews will be analysed thematically using an inductive approach [13]. All interviews will be transcribed verbatim. The interviews will be conducted remotely and participants will be given the option to use the zoom platform or receive a telephone call. If the participant becomes distressed or upset from completing the interview then we have requested they contact the research study

team who will initiate referral to our clinical psychology department at the Freeman Hospital or Royal Victoria Infirmary, Newcastle upon Tyne.

End of the Study

At the end of the study all participants will be provided with information about the study's major findings. This will be communicated by letter mailed to the participants' home address and will also include an invitation to attend a volunteer feedback evening at the Clinical Research Facility where the overall results of the study will be presented. The study will be considered as completed when participants have completed the proposed clinical procedures.

Data storage

All digital data will be securely stored on the University's Filestore Service and will be pseudonymised with study identification numbers. The data will be accessible only to authorised project staff and backed up daily with a highly available offsite mirror. The audio recordings for the qualitative work package will be destroyed once they have been transcribed.

Data archive and sharing

Research data that supports publications and unpublished data of value at project end will be archived with supporting documentation in data.ncl (https://data.ncl.ac.uk/), Newcastle's Research Data Repository. The datasets will be made public under a Creative Commons licence to ensure credit is given when the data is reused and access provided for at least ten years. Data deposited will also be assigned a persistent identifier (i.e. DOI) that can be included in project outputs, including publications, to detail how and where the data can be accessed. At this stage all study identification numbers will be removed and all data will be anonymised before being archived and shared through the repository. Where there is a risk to data being re-identified the dataset will be archived to make the record findable but access will be controlled and dependent on the future use of the data in question.

Sample size and statistical analysis

The target sample size is based on a power calculation using the expected effect moderate effect size for a positive effect of nitrate supplementation on exercise tolerance derived from previous research [22]. For an *a* of 0.05 and a power of 0.80, a total sample size of 24 is required. To account for potential drop-outs, an additional 4 participants will be recruited, based on drop-out rate of ~20% in studies assessing the effect of nitrate supplementation on exercise tolerance [23, 24]. For Part I, the analysis will include a two-way repeated measures analysis of variance (ANOVA) to determine the effect of the magnitude of active muscle mass (small vs. large) on neuromuscular fatigability and other physiological variables measured during cycling. For Part II, the analysis will include a two-way repeated measures ANOVA to determine the effect of nitrate supplementation vs. placebo on neuromuscular fatigability and other physiological variables measured during cycling. For Part II, an inductive approach to analysis will be made and data saturation of themes will be determined at the analysis stage [25]. Two independent reviewers in the research study team will code and extract segments of the data to identify key themes. Inclusion of supporting quotes from each of the themes will be included in the write up and publication.

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