Protocol
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Dietary Nitrates for Heart Failure

Principal Investigator: Linda R. Peterson, MD

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A Introduction

A1 Study Abstract

This study has several arms. The overall objective of the study is to evaluate the improvements in cardiac, skeletal muscle, and whole body performance after treatment with dietary nitrate (NO_3^-) derived from beet root juice (BRJ). The rationale for this study is that it was fairly recently discovered that NO_3^- from the diet can be a source for nitric oxide (NO) production in the body through an 'enterosalivary pathway.' NO has pleiotropic effects in the body including relaxing smooth muscle (e.g., around arteries causing vasorelaxation) and, paradoxically, stimulating skeletal muscle contraction. Subjects with heart failure have reduced bioavailability of NO, so they may particularly benefit from increased NO production via the dietary pathway.

Substudy #1 ($\dot{V}O_{2peak}$ study). The purpose of this study is to assess the effects of acute ingestion of a single dose of dietary nitrate vs. placebo on exercise performance (6 min walk test; $\dot{V}O_{2peak}$ test; short physical performance battery), skeletal muscle (energetics, blood flow and/or oxygen levels), and cardiac muscle function (using magnetic resonance spectroscopy and imaging). Breath NO, plasma NO_3^- and nitrite (NO_2^-) will also be measured before and after nitrate ingestion.

Subjects with HF or controls (without HF) will be studied. The study design is a randomized, double-blind crossover study with at least a 7 d washout in between the 2 study days (placebo [BRJ depleted of nitrate] and BRJ).

Substudy #2 (Neuromuscular study). The purpose of this study is to assess the effects of acute ingestion of a single dose of dietary nitrate on exercise performance (6 min walk; muscle power and speed of contraction as measured using a Biodex machine; short physical performance battery). Breath NO, plasma NO_3^- and nitrite (NO_2^-) will also be measured before and after nitrate ingestion. There is an optional muscle biopsy as a part of this substudy. The purpose of this is to evaluate the molecular changes after nitrate ingestion v. placebo.

Subjects with heart failure or controls (without heart failure) will be studied. The study design is a randomized, double-blind crossover study with at least a 7 d washout in between the 2 study days (placebo [BRJ depleted of nitrate] and BRJ).

A2 Primary Hypothesis

The primary hypothesis is that patients with heart failure will have improvements in exercise performance (primary endpoint is neuromuscular power, secondary is $\dot{V}O_{2peak}$) after ingestion of BRJ containing dietary nitrate as compared to after ingestion with placebo. We further hypothesize that breath NO, plasma nitrate and nitrite as well as cardiac or skeletal muscle nitrate/nitrite and cGMP will increase after ingestion of BRJ as compared with after placebo.

A3 Purpose of the Study Protocol

The primary purpose of this study protocol is to outline the procedures for each of the above-listed Substudies that comprise the overall dietary nitrate intervention study.

B Background

B1 Prior Literature and Studies

• **Significance of Heart Failure (HF)**: HF is a major public health problem affecting 5.8 million Americans. Moreover, adults over age 40 have a 1 in 5 chance of developing HF in their lifetime. Total HF costs are ~$39.1 billion. The human cost is great as well: 40% of patients die within 1 y of diagnosis.[1] Moreover, the number affected by HF and the number of HF deaths continue to increase. In addition to being a major cause of death, HF is a significant cause of disability. Indeed, the New York Heart Association classification system of HF is based on the severity of patients' inability to exercise and to perform normal activities of daily living. Patients with class II HF have mildly limited physical activity; those with Class III have markedly limited physical activity; and those with Class IV cannot carry out any physical activity without discomfort and have symptoms even at rest. Given the disabling nature of HF and its tremendous impact on quality of life, any improvement in exercise capacity or tolerance would be of enormous benefit to patients with this disease.

• **Significance of Nutrition and Diet in the Management of HF**: Compared to what is known about pharmaceutical treatments for HF, relatively little is known about how diet, nutrition, and the microbiota may improve HF. The main nutritional guidelines for HF recommend avoidance of high sodium and promoting 'healthy' diets. Guidelines on specific nutrients are mostly limited to advice to include adequate vitamin and mineral intake to avoid rare causes of HF (e.g., Beriberi and Keshan's disease). Overall, there is a dearth of studies related to nutrition in HF patients. Our proposed study would help fill this gap in our knowledge.

• **Significance of Dietary NO3- for Improving Exercise Tolerance in Patients with HF**: Pharmaceutical nitrates (in combination with hydralazine) improve quality of life and survival[2] in HF patients. By investigating the efficacy of dietary NO3- on exercise tolerance in a well-designed and controlled study, our study would not only help improve our knowledge of nutrition in HF, but also potentially point towards a novel, relatively inexpensive "nutraceutical" that could be widely applied to HF treatment. There is already a burgeoning literature demonstrating the effectiveness of dietary NO3-, from beet root juice (BRJ) (from a standardized BRJ drink from James White Drinks Inc.), in enhancing exercise tolerance in normal subjects,[3-6] in athletes,[7] and in patients with peripheral arterial disease.8 Peak power during an incremental exercise test4, time to fatigue during constant intensity exercise in normoxia[3,5] or hypoxia,[6] average power during a simulated time trial7, and distance walked before the onset of claudication[8] all improve after BRJ supplementation. If these findings hold true for patients with HF, it would demonstrate that BRJ supplementation improves exercise capacity and tolerance thereby likely improving quality of life and decreasing disability and cost of HF. Documenting an improvement in submaximal performance such as is demonstrated in a 6 minute walk test (in addition to peak performance) would be important since most activities of daily living and work do not require a maximal exercise capacity but rather the ability to tolerate sustained exercise.

• **Significance of Dietary NO_3^- as a Substrate for the Alternative NO Production Pathway**: One of the most powerful efficiency-enhancing molecules is nitric oxide (NO).

However, the classical pathway of endothelial NO production from L-arginine, mediated by endothelial NO synthase, is dependent upon the presence of oxygen (O_2).[9] In contrast, in the alternative pathway for NO production (i.e., NO3- → NO2- → NO) dietary NO3- is first reduced to NO2- by oral facultative anaerobic bacteria. After NO2- (and residual NO3-) are swallowed, stomach acid, and/or ischemic/deoxygenated conditions further reduce these compounds to NO. This process is O2 independent. Details of this enterosalivary pathway for NO2- and NO production are expanded upon in recent reviews.[9,10] This alternative pathway is not only a parallel system but an important back-up providing NO in conditions when the classical pathway cannot. These conditions include local ischemic and acidic conditions, which in HF patients can result from increased left ventricular (LV) wall stress, coronary artery disease, or rigorous exertion. Importantly, as listed in Figure 1, tolerance does not appear to develop following repeated stimulation of this alternative pathway, in contrast to treatment with pharmacologic nitrates (e.g., nitroglycerin).9 In sum, this is a heretofore under-appreciated NO production system that may be exploited through the use of dietary NO3- to improve energy efficiency and, hence, function in patients with HF.

• **Significance of Oxygen Uptake Inefficiency in HF**: Inefficiency (a poor cardiac work:O2 use ratio) is a hallmark of HF.[11] In HF, both the heart and skeletal muscle are inefficient.[11,12] It is an unfortunate irony that in HF, a condition in which it is especially important to get the most work from a given amount of O_2, the normally tight coupling between the two is impaired. This inefficiency feeds into a vicious cycle in HF: decreased function leads to inefficiency, which further impairs function.[11] Clearly, inefficiency in HF is an attractive target for the development of novel therapies. If this important input that contributes to continuing progression of HF can be improved, more energy should be available for work for the heart, skeletal muscle, and potentially other organs.

• **Significance of Dietary NO_3- as a Treatment of Inefficiency in HF**: There are limited therapeutic options for improving efficiency in HF. A few medications exist that may improve efficiency by altering substrate metabolism preference (e.g., trimetazidine and perhexiline), but they are not available in the U.S., and the latter an unattractive side effects. However, studies of dietary NO3- supplementation show that it reduces the O_2 cost of exercise, i.e., after dietary NO3- supplementation peak exercise time and output increase, but $\dot{V}O_2$ at a given power decreases.[3-5,13-15] An in vitro study of mitochondria from healthy volunteers who were supplemented with BRJ demonstrated improved oxidative phosphorylation efficiency (i.e., an improved P:O ratio) and a decrease in state 4 respiration.[15] The improved P:O ratio correlated with a decrease in the O_2 cost of whole-body exercise. Thus, thermodynamic efficiency and coupling of muscle mitochondria was improved by BRJ in normals. Whether BRJ-derived NO can also improve the whole body inefficiency of HF is unknown. Our study will help fill this gap in our knowledge.

B2 Rationale for this Study

The rationale for this study is that it was fairly recently discovered that NO_3^- from the diet can be a source for nitric oxide (NO) production in the body through an 'enterosalivary pathway.' NO has pleiotropic effects in the body including relaxing smooth muscle (e.g., around arteries causing vasorelaxation) and, paradoxically, stimulating skeletal muscle

contraction. Subjects with heart failure have reduced bioavailability of NO, so they may particularly benefit from increased NO production via the dietary pathway. This increase in NO bioavailability should translate into improved skeletal and cardiac muscle and blood vessel function. These improvements in organ function should, in turn, result in improved exercise performance, especially in patients with heart failure.

C Study Objectives

C1 Primary Aim

The primary aim of this study is to determine if dietary nitrate (NO_3^-), in this case from BRJ, is efficacious in improving exercise performance ($\dot{V}O_{2peak}$ and muscle power and speed) in patients with heart failure.

Rationale for the Selection of Outcome Measures

The primary outcome measures are the $\dot{V}O_{2peak}$ and peak leg muscle power and speed. These were chosen because they have been correlated with survival in patients with heart failure. Furthermore, these endpoints directly impact a patient's ability to perform activities of daily living.

Note: there are other **Tertiary Aims** including assessment of the mechanisms by which dietary nitrate may improve exercise performance. To this end, the Tertiary Aims are to determine the effects of BRJ on plasma nitrate and nitrite levels, breath NO.

D Investigational Agent

D1 Preclinical Data

In 2004, Webb et al. (Proc Natl Acad Sci USA 2004;101:13683–13688.) reported protective effects of nitrite in isolated perfused heart preparations subjected to IR injury. Under ischaemic conditions, both rat and human myocardium generated NO from nitrite. Although NO was produced in a dose-dependent manner (10–100 mM), the degree of protection did not differ, suggesting that the beneficial effect of nitrite treatment was reached already at low concentrations. Furthermore, the authors showed that the conversion to NO was dependent on xanthine oxidoreductase (XOR), since co-administration of allopurinol or BOF-4272 attenuated nitrite-dependent NO formation. XOR is generally thought to contribute to IR injuries via production of reactive oxygen species (ROS), including superoxide (O − 2). However, the findings by Webb et al. suggest that during hypoxic conditions, nitrite supplementation may partly shift the activity of XOR from generation of damaging
O −2 to protective NO.

Soon after, Duranski et al. (J Clin Invest 2005;115:1232–1240.) demonstrated potent cytoprotective effects of low dose nitrite using in vivo mouse models of myocardial infarction and liver ischaemia. The nitrite-mediated effects

were independent of NOS and abolished by co-administration of the NO scavenger carboxy-PTIO (cPTIO), suggesting NO as an active mediator.

D2 Clinical Data to Date

Based on the results from this study, we have shown that dietary nitrate (in the form of BRJ) has improved: 1) muscle power, 2) speed of muscle contraction, 3) nitrate, nitrite, and NO levels, 4) $\dot{V}O_{2peak}$. The improvements in muscle power and speed and nitrate/nitrite levels was shown in patients with HF and in controls. Our preliminary data also suggest that muscle blood flow and oxygen levels are increased in patients with heart failure.

D3 Dose Rationale and Risk/Benefits

Initially, we had started off with giving one bottle of BRJ with ~5-6mmol of dietary nitrate. However, we were not seeing any differences between the BRJ studies and the placebo studies. After increasing the dose to ~ 11.2 mmol, we showed a difference between the BRJ and placebo studies. Despite this increase in dose, we have had no episodes of increased side effects including hypotension.

E Study Design

E1 Overview or Design Summary

Please see above for specifics of the designs of each of the studies. All studies are prospective and interventional. All substudies are of a cross-over design.

E2 Subject Selection and Withdrawal

Subjects will include patients with heart failure with reduced ejection fraction.

Inclusion Criteria

HF Subjects:

- Men and women with a diagnosis of heart failure with reduced ejection fraction
- Age > or = 18 y

2.a Exclusion Criteria

HF and Control Subjects:

- Age <18 y
- Those taking phosphodiesterase inhibitors (e.g., Viagra) will be excluded, as these can potentiate NO effects
- Those taking prescription hormones (e.g., birth control pills, HRT), as these may block conversion of nitrate to nitric oxide
- Women who are pregnant or intend to become pregnant during the study
- Individuals who are not able to perform the exercise tests
- Individuals who can not give informed consent

2.b Ethical Considerations

Vulnerable populations, such as children and prisoners, will not be enrolled. All study procedures will be conducted with the highest ethical standards, in accordance with IRB and HIPAA regulations.

2.c Subject Recruitment Plans and Consent Process

We will recruit subjects from the following resources:

- the Washington University Volunteer for Health database
- Dr. Peterson's $\dot{V}O_2$max database (which contains subjects who have already signed an informed consent document to be contacted about future studies)
- Dr. Mann's heart failure registry
- the Washington University Heart Failure and Cardiology clinics
- Dr. Peterson's previously approved study #201107366 (Heart failure and myocardial metabolism) who have agreed (in their signed consent form) to allow us to contact them for future studies, if they meet the entry criteria

2.d Randomization Method and Blinding

Subjects are randomized by the dietician in the Clinical Research Unit (Suzanne Waller). It is she who hands out the BRJ and the placebo. The BRJ and placebo are packaged the same and have similar taste, texture, and color.

2.e Risks and Benefits

See this description in the HRPO website for this study.

2.f Early Withdrawal of Subjects

Subjects may withdraw from this study at their discretion or the discretion of the PIs.

2.g When and How to Withdraw Subjects

Subjects are withdrawn from the study at the discretion of the PIs based on patient safety considerations and/or compliance.

2.h Data Collection and Follow-up for Withdrawn Subjects

If subjects are withdrawn, this is reported in publications according to the Study flow charts.

E3 Study Drug

3.a Description

Beet IT Sport shot and Beet IT organic stamina shot.

3.b Treatment Regimen

See above for the particular treatment regimen for each substudy.

3.c Method for Assigning Subjects to Treatment Groups

For all substudies that are cross-over, Suzanne Waller randomly assigns the treatment order. For the substudies in which the BRJ or placebo are randomly assigned, Suzanne performs the randomization and assigns subjects to a treatment group.

3.d Preparation and Administration of Study Drug

The BRJ or placebo are placed in a cup and the patient is to ingest it orally.

3.e Subject Compliance Monitoring

We watch the subjects ingest the BRJ/placebo on the study days. For the longer-term studies, we ask the subjects about compliance.

3.f Prior and Concomitant Therapy

Subjects may stay on all allowed previously prescribed medications.

3.g Packaging

Per James White Drinks, LTD (Ipswitch, UK)

3.h Blinding of Study Drug

Per Suzanne Waller of the CRU

3.i Receiving, Storage, Dispensing and Return

Study BRJ and placebo are shipped to Dr. Peterson and may be stored at room temp. or at refrigerated temperatures. BRJ/placebo are dispensed by Suzanne Waller. No BRJ/placebo is returned due to high costs of shipment to the UK.

F Study Procedures

F1 Screening for Eligibility

Subjects' medical chart will be reviewed after they sign consent to assess for eligibility.

F2 Schedule of Measurements

For all studies and sub-studies:

- Subjects will be consented.
- Subjects will give permission for the investigators to review their medical records.
- Subjects for the main VO2 study, and the neuromuscular function testing will be HF patients.
- **I. BRJ main study (1-2 shot BRJ intervention):**

1) After consenting to participate, subjects will be instructed to refrain from spitting or the use of an antibacterial mouthwash, antacids, proton pump inhibitors, or chewing gum during the study.

2) Subjects will be asked to answer questionnaires regarding their medical health (basic health questionnaire, Minnesota Living with Heart Failure questionnaire, a combined fatigue questionnaire, a magnetic resonance safety questionnaire -i.e., questions regarding implanted pacemakers, etc.). Subjects will have a physical examination.

3) Subjects will be instructed by study personnel to minimize dietary intake of foods that are high in dietary nitrates the day before each study visit.

4) During study visit #1, subjects will be randomized to receive either one to two "shots" of BRJ (James White Drinks) or placebo (BRJ without nitrates).

5) Before and at 3 time points after receiving the BRJ or placebo, subjects will undergo phlebotomy for plasma nutrient/hormone levels and genetic testing and will have their blood pressure checked and will blow into a tube connected to a small machine that will measure the amount of nitric oxide in their breath.

6) Subjects will undergo transthoracic echocardiographic imaging at rest. They will also have their body composition determined via DXA.

7) ~2 h after ingestion, subjects will blow into another machine to measure their respiratory muscle function.

8) Subjects will pedal a cycle ergometer for 6 min at ~50, ~60, and ~70% of $\dot{V}O_2$peak while $\dot{V}O_2$ is measured using a ParvoMedics TrueOne metabolic cart. The average $\dot{V}O2$ during the last 2 min of each stage will be used to calculate gross and delta efficiency using stoichiometric equations as previously described. Following a 10 min rest period, peak power and $\dot{V}O_2$peak will determined during a continuous exercise test performed using a 10 W/min ramp protocol. Subjects will have their blood pressure, heart rate and rhythm monitored during this study. They will also have their muscle oxygen levels measured using small sensors taped to their thighs.

11) Subjects will undergo a 7 d washout period.

12) Subjects will come in for study day 2 in which they will receive whichever treatment (BRJ or placebo) they did not receive at first and then repeat the studies listed above 5)-11).

II. BRJ neuromuscular function testing sub-study:

1) Subjects will be asked to undergo steps 1-7 of the main study described above.

2) Subjects will undergo a test of neuromuscular function using an isokinetic dynamometer (a device that measures voluntary muscle force production while controlling the speed of movement).

3) Subjects will undergo a 7 d washout period.

4) Subjects will come in for Study day 2 in which they will receive whichever treatment (BRJ or placebo) they did not receive at first and then repeat the studies listed above 1-5.

F3 Safety and Adverse Events

3.a Safety and Compliance Monitoring

We have a Data and safety monitoring board meeting at least yearly and should any AEs or SAEs arise, we may have them more frequently.

3.b Medical Monitoring

i Investigator only

ii Independent expert to monitor

iii Institutional Data and Safety Monitoring Board

Dr. Geltman is the head of the Data and Safety Monitoring Board.

iv Independent Data and Safety Monitoring Board

3.c Definitions of Adverse Events

Any adverse event that occurs during the study. This includes patient complaints or medically-recognized events, e.g., hypotension.

3.d Classification of Events

i Relationship

We have only had on AE during the study. The only AE we had was one person had abdominal discomfort and urgency after drinking the BRJ. This was added to the expected risks in the consent forms.

ii Severity

We have had no SAEs.

iii Expectedness

Our consent forms include all of our expected risks and their likelihood of occurring. We have had no SAEs. The only AE we had was one person had abdominal discomfort and

urgency after drinking the BRJ. This was added to the expected risks in the consent forms.

3.e Data Collection Procedures for Adverse Events

We will follow the HRPO-outlined procedures for collecting and reporting adverse events.

3.f Reporting Procedures

We will follow the HRPO-outlined procedures for collecting and reporting adverse events.

3.g Adverse Event Reporting Period

Per the HRPO suggestions, AEs should be reported within 24h

3.h Post-study Adverse Event

There is no long-term follow-up of these very short-term, low morbidity studies. We have also not heard of any post-study adverse events.

F4 Study Outcome Measurements and Ascertainment

G Statistical Plan

G1 Sample Size Determination and Power

These are first-in-human, exploratory proof of concept studies. Thus a formal power analysis was not performed.

G2 Interim Monitoring and Early Stopping

NA – if the DSMB determines there is reason for early stopping, any of the Substudies may be stopped.

G3 Analysis Plan

See our publications for the specific statistical methods used for each particular substudy.

G4 Statistical Methods

Please see our publications for the specific statistical methods used for each particular substudy.

G5 Missing Outcome Data

This is reported in all publications.

G6 Unblinding Procedures

After a particular substudy is complete, we unblind the data.

H Data Handling and Record Keeping

H1 Confidentiality and Security

Per HRPO guidelines.

H2 Training

NA

H3 Case Report Forms and Source Documents

The data collection sheets are kept in the subjects' hardcopy folders, which are kept in a secure location.

H4 Records Retention

All records are retained in compliance with the HRPO guidelines under double-lock and password protected, encrypted computers.

H5 Performance Monitoring

NA

I Study Monitoring, Auditing, and Inspecting

I1 Study Monitoring Plan

The study is monitored by the PI, the study coordinators, and the DSMB.

I2 Auditing and Inspecting

NA for this single center, small study

J Study Administration

J1 Organization and Participating Centers

WUSM only

J2 Funding Source and Conflicts of Interest

CCIR JIT funding is current. Dr. Peterson also has funds to continue these studies. These funds are separate from a grant. There are no conflicts of interest

J3 Committees

NA

J4 Subject Stipends or Payments

Subjects are reimbursed for their time for each Substudy as outlined in the consent forms for that particular Substudy.

J5 Study Timetable

The study is ongoing. It is not limited by

K Publication Plan

We already have several publications from this study:

1. Coggan AR, Leibowitz JL, Kadkhodayan A, Thomas DT, Ramamurthy S, Anderson-Spearie C, Waller S, Farmer M, **Peterson LR.** Effect of acute dietary nitrate intake on maximal knee extensor speed and power in healthy men and women. *Nitric Oxide: Biol and Chem* 2015;48:16-21. PMCID: PMC4362985 (http://dx.doi.org/doi:10.1016/j.niox.2014.08.014).

2. Coggan AR, Leibowitz JL, Anderson Spearie C, Kadkhodayan A, Thomas DP, Ramamurthy S, Mahmood K, Park S, Waller S, Farmer M, **Peterson LR.** Acute dietary nitrate intake improves muscle contractile function in patients with heart failure: a double-blind, placebo-controlled, randomized trial. *Circ Heart Fail* 2015;8:914-20. (the most cited article from this journal in the lay press per Altmetrics). PMCID: PMC4573847 http://circheartfailure.ahajournals.org/content/early/2015/07/15/CIRCHEARTFAILURE.115.002141.full.pdf?ijkey=81kIxpBz5FebFXK&keytype=ref

3. Rimer EG, **Peterson LR,** Coggan AR, Martin JC. Acute dietary nitrate supplementation increases maximal cycling power of trained athletes. *Int J Sports Physiol Perform* 2016; 11:715-20. PMCID: PMC4889556

4. Coggan AR, Broadstreet SR, Mahmood K, Mikhalkova D, Madigan M, Bole I, Park S, Leibowitz J, Kadkhodayan A, Thomas D, Thies D, **Peterson LR.** Dietary nitrate increases VO_2 peak and performance but does not alter ventilation or efficiency in patients with heart failure with reduced ejection fraction. *J Card Fail* (in press).

5. Coggan AR, Broadstreet S, Mikhalkova D, Bole I, Leibowitz J, Kadkhodayan A, Park S, Thomas D, Thies D, **Peterson LR.** Dietary nitrate-induced increases in human muscle power: high vs. low responders. *Physiol Rev* (in review).

6. Coggan AR, **Peterson LR**. Dietary nitrate and skeletal muscle contractile function in heart failure. *Curr Heart Fail Rep* 2016;13:158-165. PMCID: PMC Journal PMID: 27271563.

We will continue to publish our findings as they become available.

L Attachments

L1 Tables

L2 Informed consent documents

See the consents attached to the HRPO website for this study.

L3 Patient education brochures

NA

L4 Special procedures protocols

NA

L5 Questionnaires or surveys

See questionnaires that are attached to the HRPO website for this study.

M References

1. Bleumink GS, Knetsch AM, Sturkenboom MC, Straus SM, Hofman A, Deckers JW, Witteman JC, Stricker BH. Quantifying the heart failure epidemic: prevalence, incidence rate, lifetime risk and prognosis of heart failure The Rotterdam Study. Eur Heart J 2004;25:1614-1619.
2. Taylor AL, Ziesche S, Yancy C, Carson P, D'Agostino RJ, Ferdinand K, Taylor M, Adams K, Sabolinski M, Worcel M, Cohn JN. Combination of isosorbide dinitrate and hydralazine in blacks with heart failure. N Engl J Med 2004;351:2049-2057.
3. Bailey SJ, Winyard P, Vanhatalo A, Blackwell JR, Dimenna FJ, Wilkerson DP, Tarr J, Benjamin N, Jones AM. Dietary nitrate supplementation reduces the O2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. J Appl Physiol 2009;107:1144-1155.
4. Bailey SJ, Fulford J, Vanhatalo A, Winyard PG, Blackwell JR, DiMenna FJ, Wilkerson DP, Benjamin N, Jones AM. Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans. J Appl Physiol 2010;109:135-148.
5. Lansley KE, Winyard PG, Fulford J, Vanhatalo A, Bailey SJ, Blackwell JR, DiMenna FJ, Gilchrist M, Benjamin N, Jones AM. Dietary nitrate supplementation reduces the O2 cost of walking and running: a placebo-controlled study. J Appl Physiol 2011;110:591-600.
6. Vanhatalo A, Fulford J, Bailey SJ, Blackwell JR, Winyard PG, Jones AM. Dietary nitrate reduces muscle metabolic perturbation and improves exercise tolerance in hypoxia. J Physiol 2011 Sep 12. [Epub ahead of print].
7. Lansley KE, Winyard PG, Bailey SJ, Vanhatalo A, Wilkerson DP, Blackwell JR, Gilchrist M, Benjamin N, Jones AM. Acute dietary nitrate supplementation improves cycling time trial performance. Med Sci Sports Exerc 2011;43:1125-1131.
8. Kenjale AA, Ham KL, Stabler T, Robbins JL, Johnson JL, Vanbruggen M, Privette G, Yim E, Kraus WE, Allen JD. Dietary nitrate supplementation enhances exercise performance in peripheral arterial disease. J Appl Physiol 2011;110:1582-1591.
9. Lundberg JO, Weitzberg E, Gladwin MT. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. Nat Rev Drug Discov 2008;7:156-167.

10. Gilchrist M, Winyard PG, Benjamin N. Dietary nitrate--good or bad? Nitric Oxide. 2010;22:104-109.
11. Opie LH. The metabolic vicious cycle in heart failure. Lancet 2004;364:1733-1734.
12. Witte KK, Levy WC, Lindsay KA, Clark AL. Biomechanical efficiency is impaired in patients with chronic heart failure Eur J Heart Fail. 2007;9:834-838.
13. Bescós PB, Rodríguez FA, Iglesias X, Ferrer MD, Iborra E, Pons A. Acute administration of inorganic nitrate reduces VO2peak iun endurance athletes. Med Sci Sports Exerc 2011;43:1979-1986.
14. Larsen FJ, Weitzbeg E, Lundberg JO, Ekblom B. Dietary nitrate reduces maximal oxygen consumption while maintaining work performance in maximal exercise. Free Rad Biol Med 2010;48:342-347.
15. Larsen FJ, Schiffer TA, Borniquel S, Sahlin K, Ekblom B, Lundberg JO, Weitzberg E. Dietary inorganic nitrate improves mitochondrial efficiency in humans. Cell Metab 2011;13:149-159.
16. Peterson LR, Schechtman KB, Ewald GA, Geltman EM, de las Fuentes L, Meyer T, Krekeler P, Moore ML, Rogers JG. Timing of cardiac transplantation in patients with heart failure receiving beta-adrenergic blockers. J Heart Lung Transplant 2003;22:1141-1148.
17. Peterson LR, Waggoner AD, Schechtman KB, Meyer T, Gropler RJ, Barzilai B, Davila-Roman VG. Alterations in left ventricular structure and function in young healthy obese women: assessment by echocardiography and tissue Doppler imaging. J Am Coll Cardiol 2004;43:1399-1404.
18. Peterson LR, Schechtman KB, Ewald GA, Geltman EM, Meyer T, Krekeler P, Rogers JG. The effect of beta-adrenergic blockers on the prognostic value of peak exercise oxygen uptake in patients with heart failure. J Heart Lung Transplant 2003;22:70-77.
19. Stolker JM, Heere B, Geltman EM, Schechtman KB, Peterson LR. Prospective comparison of ventilator equivalent versus peak oxygen consumption in predicting outcomes of patients with heart failure. Am J Cardiol 2006;97:1607-1610.
20. Peterson LR, Rinder MR, Schechtman KB, Spina RJ, Glover KL, Villareal DT, Ehsani AA. Peak exercise stroke volume: associations with cardiac structure and diastolic function. J Appl Physiol 2003;94:1108- 1114.
21. Spina RJ, Ogawa T, Coggan AR, Holloszy JO, Ehsani AA. Exercise training improves left ventricular contractile response to beta-adrenergic agonist. J Appl Physiol 1992;72:307-311.
22. Spina RJ, Ogawa T, Martin WHr, Coggan AR, Holloszy JO, Ehsani AA. Exercise training prevents decline in stroke volume during exercise in young healthy subjects. J Appl Physiol 1992;72:2458-2462.
23. Coggan AR, Habash DL, Mendenhall LA, Swanson SC, Kien CL. Isotopic estimation of CO2 production during exercise before and after endurance training. J Appl Physiol 1993;75:70-75.
24. Ehsani AA, Spina RJ, Peterson LR, Rinder MR, Glover KL, Villareal DT, Binder EF, Holloszy JO. Attenuation of cardiovascular adaptations to exercise in frail octogenarians. J Appl Physiol 2003;95:1781-1788.
25. Ruby BC, Coggan AR, Zderic TW. Gender differences in glucose kinetics and substrate oxidation during exercise near the lactate threshold. J Appl Physiol 2002;92:1125-1132.
26. Peterson LR, Courtois M, Peterson LF, Peterson MR, Davila-Roman VG, Spina RJ, Barzilai B. Estrogen increases hyperemic microvascular blood flow velocity in postmenopausal women. J Gerontol A Biol Sci Med Sci 2000;55:M174-9.
27. O'Connor RD, Gropler RJ, Peterson L, Schaffer J, Ackerman JJ. Limits of a

localized magnetic resonance spectroscopy assay for ex vivo myocardial triacylglycerol. J Pharm Biomed Anal 2007;45:382-389.
28. Frimel TN, Deivanayagam S, Bashir A, O'Connor R, Klein S. Assessment of intrahepatic triglyceride content using magnetic resonance spectroscopy. J Cardiometab Syndr 2007;2:136-138.
29. Mancini DM, Coyle E, Coggan A, Beltz J, Ferraro N, Montain S, Wilson JR. Contribution of intrinsic skeletal muscle changes to 31P NMR skeletal muscle metabolic abnormalities in patients with chronic heart failure. Circulation 1989;80:1338-1346.
30. Kapil V, Milsom AB, Okorie M, Maleki-Toyserkani S, Akram F, Rehman F, Arghandawi S, Pearl V, Benjamin N, Loukogeorgakis S, Macallister R, Hobbs AJ, Webb AJ, Ahluwalia A. Inorganic nitrate supplementation lowers blood pressure in humans: role for nitrite-derived NO. Hypertension 2010;56:274-281.
31. Govoni M, Jansson EA, Weitzberg E, Lundberg JO. The increase in plasma nitrite after a dietary nitrate load is markedly attenuated by an antibacterial mouthwash. Nitric Oxide 2008;19:333-337.
32. Kemp GJ, Radda GK. Quantitative interpretation of bioenergetic data from 31P and 1H magnetic resonance spectroscopic studies of skeletal muscle: an analytical review. Magn Reson Q 1994;10:43-63.
33. Moseley L, Jeukendrup AE. The reliability of cycling efficiency. Med Sci Sports Exerc 2001;33:621-627.
34. Recommendations for exercise testing in chronic heart failure patients. Eur Heart J 2001;22:37-45.
35. Stamler J, Meissner G. Physiology of Nitric Oxide in Skeletal Muscle. Physiol Rev 2001;81:209-237.
36. van Heerebeek L, Hamdani N, Falcao-Pires I, Leite-Moreira AF, Begieneman MP, Bronzwaer JG, van der Velden J, Stienen GJ, Laarman GJ, Somsen A, Verheugt FW, Niessen HW, Paulus WJ. Low myocardial protein kinase G activity in heart failure with preserved ejection fraction. Circulation 2012;126:830-9.