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Siervo, M., Oggioni, C., Jakovljevic, D.G. et al. (9 more authors) (2016) Dietary nitrate does not affect physical activity outcomes in health older adults in a randomized, crossover trial. Nutrition Research, 36 (12). pp. 1361-1369. ISSN 0271-5317

https://doi.org/10.1016/j.nutres.2016.11.004

DIETARY NITRATE DOES NOT HAVE AN EFFECT ON PHYSICAL ACTIVITY OUTCOMES IN HEALTHY OLDER ADULTS: A RANDOMIZED, CROSS-OVER TRIAL

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The material presented in this manuscript is original and it has not been submitted for publication elsewhere while under consideration for Nutrition Research

Conflict of interest statement: The authors have no conflict of interest to declare.

Type of Manuscript: Research paper

Abstract word count: 249

Main text word count: 3616

References: 40

Table: 3

Figures: 3

Online Supplementary Material: 1

Abbreviations

BMI= body mass index; HGS= hand-grip strength; TUG= time-up-and-go; RCRT=repeated-chair-rising-test; WLS=10m walking speed; NO= nitric oxide; ATP= Adenosine triphosphate; PAD= peripheral arterial disease; COPD= chronic obstructive pulmonary disease; BP= blood pressure; eNOS= endothelial Nitric Oxide Synthase; ROS= reactive oxygen species; ECG = electrocardiography; CHO= carbohydrate; PRO= protein; FAT= fat; BIA= Bioelectrical impedance analyses; FM= fat mass; FFM= fat free mass; WC= waist circumference; IPAQ= International Physical Activity Questionnaire; EPIC= European Prospective Investigation into Cancer and Nutrition; FFQ= Food Frequency Questionnaire; RER= respiratory exchange ratio; GC-MS= gas chromatography mass spectrometry; GLM= General Linear Models; HOMA-IR= Homeostatic Model of Insulin Resistance; MET=Metabolic Equivalent of Task.

ABSTRACT

Dietary nitrate (NO_3^-) ingestion appears to enhance exercise capacity and performance in young individuals whereas inconclusive findings have been reported in older people. We conducted a double-blind, cross-over randomized clinical trial in older normal weight and overweight healthy participants testing whether beetroot juice (a rich source of NO_3^-) for one week may increase nitric oxide bioavailability via the non-enzymatic pathway and enhance 1) exercise capacity during an incremental exercise test, 2) physical capability and 3) free-living physical activity.

Twenty non-smoking healthy participants aged 60-75y and BMI 20.0-29.9kg/m^2 were included. Pre and post supplementation resting, sub-maximal, maximal and recovery gas exchanges were measured. Physical capability was measured by hand-grip strength (HGS), time-up-and-go (TUG), repeated-chair-rising-test (RCRT), and 10m walking speed (WLS). Free-living physical activity was assessed by triaxal accelerometry. Changes in urinary and plasma NO_3^- concentrations were measured by gas chromatography mass spectrometry.

Nineteen participants (M/F=9/10) completed the study. Beetroot juice increased significantly both plasma and urinary NO_3^- concentrations ($p<0.001$) compared to placebo. Beetroot juice did not influence resting, sub-maximal and maximal oxygen consumption during the incremental exercise test. In addition, measures of physical capability and physical activity levels measured in free-living conditions were not modified by beetroot juice ingestion.

The positive effects of beetroot juice ingestion on exercise performance seen in young individuals were not replicated in healthy, older adults. Whether aging represents a modifier of the effects of dietary NO_3^- on muscular performance is not known and mechanistic studies and larger trials are needed to test this hypothesis.

Keywords: inorganic nitrate, nitric oxide, exercise, oxygen consumption, aging

1. INTRODUCTION

Aging is characterized by a progressive decline in muscle mass and strength which are risk factors for physical disability[1]. Aging is also associated with modifications of mitochondrial bioenergetics with consequent effects on muscular performance[2]. Dietary nitrate (NO_3^-) supplementation enhances muscular efficiency in humans[3, 4], a finding which can be explained by increased nitric oxide (NO) bioavailability and the role of NO in modulating mitochondrial coupling and bioenergetics of muscular activity[5, 6]. However, the majority of NO_3^- supplementation studies have been conducted in healthy, physically active young adults[7, 8] and few studies have evaluated the effects of dietary NO_3^- on physical or muscular function in older people[9-11]. Larsen et al in 2007[12] was the first to report reduced sub-maximal O_2 uptake in young healthy adults after three-day oral supplementation with potassium NO_3^-. Kenjale et al[10] observed delayed onset of claudication after three days of oral NO_3^- supplementation in older patients with peripheral arterial disease (PAD). However, subsequent studies reported contrasting results for the effects of dietary NO_3^- on exercise performance in healthy older people[9, 13] as well as in at risk populations (i.e., those with diabetes[11], heart failure[14], and chronic obstructive pulmonary disease (COPD)[15, 16]). All studies employed a double-blind randomized cross-over study design and administered beetroot juice to increase NO_3^- intake. However, differences in study duration, NO_3^- dose or assessment of exercise capability likely contributed to the observed heterogeneous responses. For example, outcomes have included sub-maximal [12, 15, 17] or maximal oxygen (O_2) uptake [18-20] assessed with incremental standardised tests [12, 15, 21] as well as time trials [22-24] or physical capability tests [9, 25], all of which were performed in controlled settings. No study has investigated the effects of dietary NO_3^- supplementation on free living physical activity.

We hypothesized that dietary NO_3^- supplementation would increase NO bioavailability, muscular energetics and exercise performance – with significant changes expected in sub-maximal, maximal and recovery O_2 uptake – which may translate into beneficial effects on physical capability and free living physical activity. To test these hypotheses, we conducted a double-blind, cross-over, placebo controlled RCT in older healthy adults to investigate the effects of beetroot juice, chosen as a rich source of dietary NO_3^-, on physical activity outcomes measured in research (O_2 uptake during incremental cycle ergometer exercise, walking speed, time-up-and-go, repeated chair rising test and hand grip strength) and free living (accelerometry) settings.

2. METHODS and MATERIALS

The trial was approved by the North of Scotland Research Ethics committee (14/NS/0061) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants. The study was a double-blind, cross-over, placebo-controlled RCT which took place between May and August 2014 across two sites (Newcastle upon Tyne and Sheffield). The duration of the each intervention was one week with a wash-out period between treatments of at least one week. This trial was registered in the International Standard Randomized Controlled Trial Number Register (ISRCTN19064955).

2.1 Participants: Twenty male and female, older (60-75 y) non-obese adults (BMI range: 18.5 - 29.9 kg/m^2) were enrolled in the study. Participants were non-smokers and weight stable. Participants were included in the study if they did not have medical conditions or were not taking medications that might influence the study outcomes. A full list of the inclusion and exclusion criteria is provided in the **Online Supplementary Material**. Participants were asked to maintain their habitual diet and to avoid using chewing gum or mouth wash for at least 48 prior to the baseline visits (first and third visit) and during each of the one-week supplementation periods.

2.2 Randomization: A randomization list for each site was generated by a member of staff not involved in the study using www.sealedenvelopes.com. Each participant was randomized to the cross-over interventions (i.e., placebo ➔ NO_3^- or NO_3^- ➔ placebo). Intervention agents were dispensed at each baseline visit by two members of staff not involved in the study who had access to the stored beetroot juice and ensured the correct treatment allocation.

2.3 Study Overview: A telephone screening was performed to check eligibility according to the trial inclusion and exclusion criteria. Eligible participants were invited for a further screening visit at the research facilities including measurement of BMI, resting BP and resting 12-lead electrocardiography (ECG). Participants were asked to arrive after a 12-hour overnight fast and having avoided strenuous physical activity for three days preceding the visit. If eligible, participants were randomized to a cross-over intervention and the baseline assessment continued with the measurement of body composition, collection of blood and urine samples and assessment of physical capability. Participants then rested for one hour and consumed a meal providing approximately 300kcal (CHO=85%, PRO=3%, FAT=12%). In addition, during this one-hour rest period, participants completed a series of questionnaires to assess dietary intake and physical activity. After the one-hour rest, participants were explained the exercise test while they accustomized to the ergometer. The exercise protocol is described in **Figure S1 of the Online Supplementary Material**. After the exercise test, instructions were provided for self-administration of the nutritional intervention (14 bottles of either NO_3^- -rich or NO_3^- -depleted beetroot juice; 70ml x 2/day; Beet It, James White Ltd, UK) and asked to consume one bottle of beetroot juice each morning and evening for the subsequent 7 days. The daily dose of NO_3^- -rich (intervention) or NO_3^- -depleted (placebo) beetroot juice contained ~12mmol and ~0.003mmol of NO_3^-, respectively. Participants were provided with instructions and forms for recording wearing time of the accelerometer. This concluded Visit 1 of the trial. Participants returned to the research facilities in the morning of

154 day eight after they had completed a seven-day supplementation period. A detailed medical
155 interview was conducted to ascertain any side effects experienced during the supplementation
156 period. A resting 12-lead ECG was performed and, if normal, the study visit was completed
157 by repeating the same assessments as performed during Visit 1. At the end of the second visit,
158 participants were asked to resume their habitual diet and physical activity. After a wash out
159 period of at least seven days the second phase (including Visits 3 and 4) was conducted
160 similar to the first phase with the exception that participants crossed-over experimental arms
161 i.e. consumed the other intervention agent.
162 *2.4 Body Composition:* Bioelectrical impedance analyses (BIA) (Newcastle: TANITA
163 418MA, Tanita Ltd, Japan; Sheffield: InBody 720 Analyser, InBody Bldg, Korea) was used
164 to assess fat mass (FM) and fat free mass (FFM). Body weight, height and waist
165 circumference (WC) were measured using standardized protocols.
166 *2.5 Resting Blood Pressure*: Resting BP was measured in triplicate using an automated BP
167 monitor (Omron M3, Omron Healthcare, UK) with the participant seated comfortably for 15
168 min prior to measurement and the arm supported at the level of the heart. The recorded value
169 was calculated as the mean of the three measurements.
170 *2.6 Physical Capability:* A battery of tests (hand grip strength (HGS), timed up and go
171 (TUG), repeated chair rise test (RCRT) and 10m walking speed (WLS)), performed in the
172 same order at each visit, was completed at baseline and at the end visit of each phase.
173 Triplicate measurements of HGS were performed in both arms at baseline and after
174 intervention using a digital dynamometer (Takei 5401, Takei, Japan). The average of six
175 measurements was calculated. To complete the TUG, participants were asked to stand up
176 from a chair, walk three meters at a self-selected comfortable speed, cross a line on the floor,
177 turn around, walk back, and sit down again. The RCRT was completed using a standard chair
178 without armrests. Participants had both arms crossed against the chest, starting from the

179 seated position and standing up (legs straight) and sitting down (full weight on the chair) and
180 the test calculates the time required (in seconds) to complete five repeated chair stands. For
181 the WLS, a 10-m path with a flying start was used to avoid acceleration/deceleration effects
182 associated with starting and stopping during this assessment. The middle 6-m of this path
183 were used for the measurement. Patients were instructed to "walk as fast as they can" and the
184 time (in seconds) to complete the 6-m path was recorded.
185 *2.7 Objective Measurement of Free Living Physical Activity:* Participants were asked to wear
186 a triaxial accelerometer (GT3X ActiGraph accelerometer (Pensacola, FL, USA)) above the
187 right hip for eight consecutive days during waking hours and to remove it only for water
188 activities (for example, swimming or bathing). Accelerometery data were collected in one-
189 minute epochs. Non-wear time was defined as 60 min or more of consecutive zero counts.
190 One participant experienced a device malfunction and data were excluded from subsequent
191 analysis. Counts per minute were converted into minutes of sedentary time (less than or equal
192 to 100 counts per min), light (100-759 counts per min), moderate (1952–5724 counts per
193 min) and vigorous-intensity (5725+ counts per min) physical activity[26]. Physical activity
194 energy expenditure was calculated using the Freedson approach[26].
195 *2.8 Dietary and Lifestyle Questionnaires:* The 9-item short form of the International Physical
196 Activity Questionnaire (IPAQ) was used to record duration of four intensity levels of
197 physical activity: 1) vigorous-intensity activity, 2) moderate-intensity activity, 3) walking,
198 and 4) sitting. A combined total physical activity score was calculated and expressed in
199 MET-minutes/week[27]. The EPIC Food Frequency Questionnaire (FFQ) was administered
200 at baseline and the FETA software used to extract dietary (energy and nutrient)
201 information[28].
202 *2.9 Exercise Test*: An incremental exercise test was performed at baseline and at the end of
203 each intervention period to assess pulmonary gas exchange variables at rest, during sub-

maximal and maximal intensities and in the post-exercise recovery phase. Briefly, each participant underwent cardiopulmonary exercise testing on an electronically-braked cycle ergometer. The protocol included a five-minute resting phase followed by a 20 watts stepwise increase in workload every three minutes while they were invited to maintain a stable pedalling rate (60-70 rpm). After reaching 80 watts, participants were asked to exercise until exhaustion (ramp protocol: 10 watts/minute), which was followed by a five-minute passive recovery period. A graphical description of the protocol is described in **Figure S1 of the Online Supplementary Material**. Pulmonary gas exchange and ventilation were measured (Newcastle: MetaMax 3B, Cortex Biophysik, Leipzig, Germany; Ultima CardiO2, Medgraphics, St Paul, MN, USA). Heart rate (HR) was measured during all tests using cardio-thoracic impedance. Oxygen uptake ($\dot{V}O_2$), minute ventilation ($\dot{V}E$), carbon dioxide excretion rate ($\dot{V}CO_2$), and respiratory exchange ratio (RER) were assessed. $\dot{V}O_2$ assessed during the last minute of the incremental exercise test was recorded as $\dot{V}O_{2peak}$. Ventilatory threshold was calculated using the V-slope method[29].

2.10 Blood and Urine Collection: Fasting blood samples were collected at the beginning of each visit and centrifuged at 3,000rpm for 10 min at 4 °C within 30min of collection. Aliquots of plasma and serum were frozen and stored at –80 °C for subsequent analyses. Mid-stream urine samples were collected, in fasting conditions, into sterile containers and stored at –20 °C for subsequent analyses.

2.11 Biomarker Analysis: A modified version of the gas chromatography mass spectrometry (GC-MS) method proposed by Tsikas et al[30] was used to determine NO_3^- concentrations in urine and plasma samples. The protocol and validation of the modified GC-MS method have been described elsewhere[31]. This method showed good repeatability, with coefficients of variation for replicate analyses of 7.8%, 8.6% and 12.0% for saliva, urine and plasma samples, respectively.

2.12 Sample size: The primary outcome of the study was the effect of NO_3^- supplementation on VO_2 consumption during sub-maximal exercise. Data on the expected effect size were obtained from a previous cross-over design study testing the effects of incremental exercise on sub-maximal and maximal O_2 consumption in young adults after a six-day nitrate supplementation[32] which showed that $\dot{V}O_2$ during moderate exercise was 1.53±0.12 L·min^{-1} and 1.45±0.12 L·min^{-1} in the placebo and nitrate groups respectively. On this basis, 20 participants were needed in a cross-over randomized trial to detect a difference of 0.08±0.12 L·min^{-1} with a power of 0.80 and alpha of 0.05.

2.13 Statistical Analyses: Repeated-Measures General Linear Models (GLM) were used to test the effect at the end of each intervention of NO_3^- supplementation on measures of exercise performance and physical capability. Treatment (NO_3^- vs placebo) was entered as a group factor (Tr) and the time points of the incremental exercise test as the repeated factor (Ti). Post-hoc comparison between treatment groups at each time point was performed using the Fisher LSD test. The area under the curve (AUC) for $\dot{V}O_2$ consumption during the incremental exercise test was calculated at baseline and end of study using the trapezoidal method. A paired t test was used to compare differences between the two interventions for the AUCs and free living physical activity outcomes. Data were presented as means ± SD or means ± 95% confidence intervals (95%CI). Analyses were conducted using Statistica 10 for Windows (StatSoft.Inc, Tulsa, OK, USA). Statistical significance was set at <0.05.

3. RESULTS

3.1 Participants' characteristics, safety and Compliance with Interventions: Twenty participants were randomized to the intervention. One person developed an ischemic event during the physical exercise testing performed at the second visit and he was excluded from the study (**Figure 1**). The remaining 19 participants (mean age 64.7±3.0 years (range: 60 - 75 years)) reported no side effects apart for the expected urine discoloration related to the

excretion of beetroot juice pigment (beeturia). All participants reported that they consumed all the intervention drinks provided and all of them completed all the measurements included in the study protocol. This included high compliance with wearing of the accelerometer (total wear time: ~7.5-8.0 days out of maximum 8 days).

3.2 Dietary Intake and Self-Reported Physical Activity: Energy intake was 2728±1430 kcal/day with 47±8%, 35±7% and 18±4% of energy provided by carbohydrates, fats and protein respectively. Self-reported physical activity was again not different between the placebo and the NO_3^- arms as participants in both groups reported an average increase in total physical activity of approximately 300 METs/week (p=0.99) (**Table 1 and Table S2 of the Online Supplementary Material**).

3.3 Body Composition: Mean baseline BMI was 25.6±3.4 kg/m^2 with 12 participants being in the overweight category (25≤BMI<30kg/m^2). Body weight was stable across the study with changes of 0.01±0.85 kg in the placebo and -0.16±0.57 kg in the intervention group (p=0.51). Similarly, no statistically significant between-treatment differences were found for FFM (0.02±1.00 kg vs 0.11±0.77 kg, p=0.65) and FM (-0.03±0.79 kg vs 0.27±0.75 kg, p=0.86) (**Table 1 and Table S2 of the Online Supplementary Material**).

3.4 Resting Blood Pressure: Baseline resting systolic and diastolic BP ranged from 100.0 to 168.0 mmHg and 62.0 to 97.0 mmHg, respectively. The decrease in systolic BP (-5.05±9.45 mmHg) with NO_3^- supplementation was approximately double that observed with the placebo (-2.64±9.04 mmHg) but this difference was not significant (p=0.48). Both interventions produced similar falls in diastolic BP (-3.70±5.59 vs -3.49±6.42 mmHg, p=0.90) (**Table 1 and Table S2 of the Online Supplementary Material**).

3.5 Laboratory biomarkers: Concentrations of nitrite plus nitrate (NO_2^-+NO_3^-, NOx) in plasma and urine increased substantially after NO_3^- supplementation by 150±77% and 979±488% but not after the placebo intervention (-9±33% and -13±34%, respectively).

3.6 Gas-Exchange during Standardized Exercise: Nitrate supplementation had no significant effect on pulmonary gas exchange (O_2 and CO_2) measured during resting, sub-maximal, maximal and recovery phases of the incremental exercise test. O_2 consumption increased linearly with the intensity of the workload and O_2 consumption at exhaustion was 1.67±0.51 and 1.64±0.55 L·min^{-1} following NO_3^- and placebo interventions (p=0.86), respectively. There was a steady and comparable decline in O_2 consumption during the 5-minute recovery phase with return to baseline resting values for both interventions (**Figure 3A**). The AUCs for O_2 consumption for both treatments were similar (p=0.89, data not showed). Similarly, weight-adjusted O_2 consumption did not significantly different between the NO_3^- and placebo groups (p=0.99, **Figure S2 of the Online Supplementary Material**). O_2 consumption at ventilatory threshold was similar for the NO_3^- (0.90±0.39 L·min^{-1}) and placebo (0.91±0.39 L*min^{-1}) treatments (p=0.35) and no differences between the two interventions were observed for CO_2 production, RER, V̇E and HR (**Figure 3B to 3E**). Time to exhaustion was shorter following the NO_3^- intervention but the difference was not significant (p=0.10, **Figure 3F**). The adjustment of the analyses for baseline values of gas exchanges did not modify the results (data not showed). A summary of the data for each time point is provided in **Table S3 of the Online Supplementary Material**.

3.7 Physical Capability and Objective Assessment of Free Living Physical Activity: Physical performance was assessed using a battery of tests measuring strength, performance and balance. NO_3^- supplementation produced small improvements in performance for all tests but the effects were not statistically significant (**Table 2**). Similarly, NO_3^- supplementation had no significant effect on total energy physical activity or on each type of physical activity (i.e., sedentary, light, moderate, vigorous) (**Table 3**).

4. DISCUSSION

4.1 Summary of Research Findings: This is the first study to evaluate the effects of dietary NO_3^- supplementation on physical performance assessed in research settings and free-living conditions in healthy older participants. Contrary to the large body of evidence supporting a positive effect of dietary NO_3^- supplementation on exercise performance, our study showed no effects of NO_3^- supplementation on O_2 consumption during sub-maximal and maximal exercise performance in older healthy participants. In addition, there were no significant effects of dietary NO_3^- supplementation on measures of physical capability and free-living physical activity.

4.2 Comparison with Body of Evidence: Research into the effects of dietary NO_3^- on exercise performance has been influenced by two significant events: 1) first paper published by Larsen et al in 2007[12] reporting a reduced sub-maximal O_2 consumption after three-day oral NO_3^- supplementation and 2) development of a NO_3^--depleted and NO_3^--enriched concentrated beetroot juice which has allowed the design of robust double-blind, randomized nutritional interventions[11]. Since 2007, several RCTs have tested the effects of dietary NO_3^- on exercise performance in humans. A small number of these trials supplemented participants with pharmacological preparation (sodium or potassiumNO_3^-)[3, 12, 19, 21, 33-35] whereas the majority of the trials used beetroot juice as a way to increase dietary NO_3^- intake[9-11, 16, 23, 25, 36]. Most of the studies recruited mainly young, physically fit participants and only a few trials [9-11, 13, 15-17, 37, 38] have tested the effects of dietary NO_3^- in older participants (mean age range: 63 – 70 years). The first study in older participants was conducted in eight patients with PAD who received 3.5 hours before the exercise testing either 500ml of beetroot juice or orange juice[10]. The study found an increased exercise time before onset of claudication pain and time to exhaustion. The remaining studies in older participants have reported contrasting results, which may be explained by differences in the duration of

supplementation (range: 2.5 hours[15] to 14 days[11]), type of population (healthy[9, 13], PAD[11], COPD[15, 16], type 2 diabetes[11], heart failure[14, 17]), dose of NO_3^- (range: ~ 300 – ~ 700mg) or exercise test (walking test[9, 10, 16, 25], incremental exercise[10, 14], forearm exercise[13]). Overall, the results have showed a reduced responsiveness of older participants to dietary NO_3^- supplementation. Negative results were seen in healthy older participants[9] and patients with diabetes[25] and COPD[16], whereas improved exercise performance was observed in patients with heart failure[14] and PAD[10]. Our study confirmed that dietary NO_3^- supplementation for one week in older adults produced no beneficial effects on physical capability or exercise performance measured in standardized clinical settings. In addition, we reported for the first time a lack of effect of NO_3^- supplementation on free living physical activity, which may entail a re-examination of the usefulness of dietary NO_3^- supplementation as a viable nutritional population strategy to enhance physical performance.

4.3 Biological Mechanisms: Dietary NO_3^- is converted to NO in a two-step reduction process proceeding via the intermediate formation of NO_2^-. The first step is performed by saprophytic bacteria with reductase activity colonizing the dorsal area of the tongue. NO_2^- is then either converted to NO in the acidic gastric environment or transported in blood and reduced enzymatically in areas of tissues with lower oxygen tension and pH where metabolic demands are higher[39]. The latter conditions are frequently encountered in areas of contracting muscles, which favour the NO_2^- conversion into NO to enhance coupling between muscle perfusion and metabolic activities[5]. The improved metabolic activity reported in previous studies appears to be related to an increased mitochondrial efficiency and/or reduction of the energetic cost of muscle contractions[6]. This raises important questions about why NO_3^- supplementation does not improve physical capability or function in older

people and stimulate future studies to investigate mechanisms that may explain the reduce effects of NO_3^- supplementation on muscular performance with aging. Putative mechanisms may involve altered reductase capacity to convert NO_3^- into NO or reduced effects of NO on skeletal muscle mediated by age-related changes in mitochondrial function and contractile efficiency. Whether higher doses or longer supplementation periods may overcome the alleged age-related decline in muscular response to dietary NO_3^- supplementation is currently not known.

4.4 Limitations: The small sample size and the relatively short duration of the intervention are important limitations of this study and therefore the results may require a careful interpretation. While we measured plasma NO_2^- concentrations using GCMS, due to logistic constraints it was not possible to process the samples immediately after collection to minimise NO_2^- degradation. These results are therefore unavailable. However, previous studies involving dietary NO_3^- supplementation in older participants where plasma NO_2^- concentration was measured, an increase in plasma NO_3^- concentrations similar to the amount observed in this study occurred alongside a significant rise in plasma NO_2^- concentrations [40].

5. CONCLUSIONS

We tested for the first time the ergogenic effects of dietary NO_3^- supplementation in older participants on exercise performance and free-living physical activity and found that, overall, dietary NO_3^- supplementation had no effects. The results seem to indicate that aging may modify the muscular response to dietary NO_3^- supplementation. However, these results await confirmation in future studies with larger samples size and in targeted populations with impaired muscular performance.

Author contributions

M.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. M.S. and E.W. designed the study. M.S. wrote the manuscript and researched data; C.O., D.J., D.H., C.C., A.W.A., A.R., M.R., M.K., E.W. researched data. All authors contributed to discussion and reviewed/edited manuscript.

Acknowledgements

We would like to thank first the study participants. We are very grateful to the staff at the Clinical Research Facilities at Royal Victoria Infirmary Newcastle University Hospitals for their support. In particular, we would like to thank Vikki Bridgett for her help with the study. We thank Dr Tom Hill for the analysis of the vitamin D concentrations. We thank Dr Kirsten Brandt and Mr Othman Qadir for the GCMS analyses of nitrate and nitrite. We would like to thank Femke van der Velde, Chi Teng Lei and Sneha B Jain for their help with the study. We would like to thank Dr Jose Lara for the useful discussion and advice on data analysis.

Funding

This study was supported by MRC – Arthritis Research UK Centre for Integrated research into Musculoskeletal Ageing (CIMA), Institute of Cellular Medicine, Newcastle University, and Human Nutrition Centre, Sheffield University.

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FIGURE LEGENDS

Figure 1: Description of recruitment phases

Figure 2: Changes in plasma and urinary nitrate after either one-week supplementation of nitrate-rich or nitrate-depleted beetroot juice in 19 older healthy adults. Data presented as means±95%CI. A paired t test was applied to test differences between the two interventions at baseline and end of the study.

Figure 3: Differences in gas exchanges and heart rate after one-week supplementation with either nitrate-rich or nitrate-depleted (placebo) beetroot juice in 19 older healthy adults. Data presented as means±95%CI. A repeated-measure ANOVA model was applied to test differences between the two interventions at the end of each intervention. $\dot{V}O_2$ = oxygen volume; $\dot{V}CO_2$ = carbon dioxide volume; RER= respiratory exchange ratio; $\dot{V}E$= pulmonary ventilation; HR= heart rate.

Table 1: Baseline characteristics (N=19)		
	Means	*SD*
M/F	9/10	
Age (years)	64.7	3.0
BMI (kg/m^2)	25.6	3.4
WC (cm)	88.5	13.9
FM (kg)	22.0	6.3
FFM(kg)	50.2	11.5
Resting Systolic BP (mmHg)	127.4	16.1
Resting Diastolic BP (mmHg)	76.2	9.6
Energy Intake (Kcal/day)	2728	1431
CHO (g/day)	308	152
FAT (g/day)	107	73
PRO (g/day)	103	57
Saturated Fat (g/day)	35.6	26.5
Unsaturated Fat (g/day)	14.1	10.4
Fibre (g/day)	23.9	13.0

N= number of participants; M= Male; F= Female; Body mass index= body mass index; WC= waist circumference; FM= fat mass; FFM= fat free mass; BP= blood pressure; CHO= carbohydrate; FAT= fat; PRO= protein;

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Table 2: Measures of physical capability before and after supplementation with either nitrate-rich or nitrate-depleted (placebo) beetroot juice for one week.					
	Placebo		**Nitrate**		
	Baseline	**End**	**Baseline**	**End**	**Main Effect**
Hand-Grip Strength (kg)	28.92±9.09	29.49±9.26	29.24±9.34	29.51±9.92	0.53
Time Up and Go (seconds)	5.44±0.76	5.62±0.76	5.67±1.07	5.58±1.00	0.53
Repeated Chair Standing (seconds)	8.03±2.24	7.65±1.73	7.73±1.77	7.60±1.73	0.41
10m Walking Test (seconds)	2.83±0.60	2.80±0.44	2.94±0.53	2.84±0.54	0.79

539 Data presented as means±SD. A repeated-measure ANOVA model was applied to test differences between the two interventions at the end of
540 each intervention in 19 older healthy adults.
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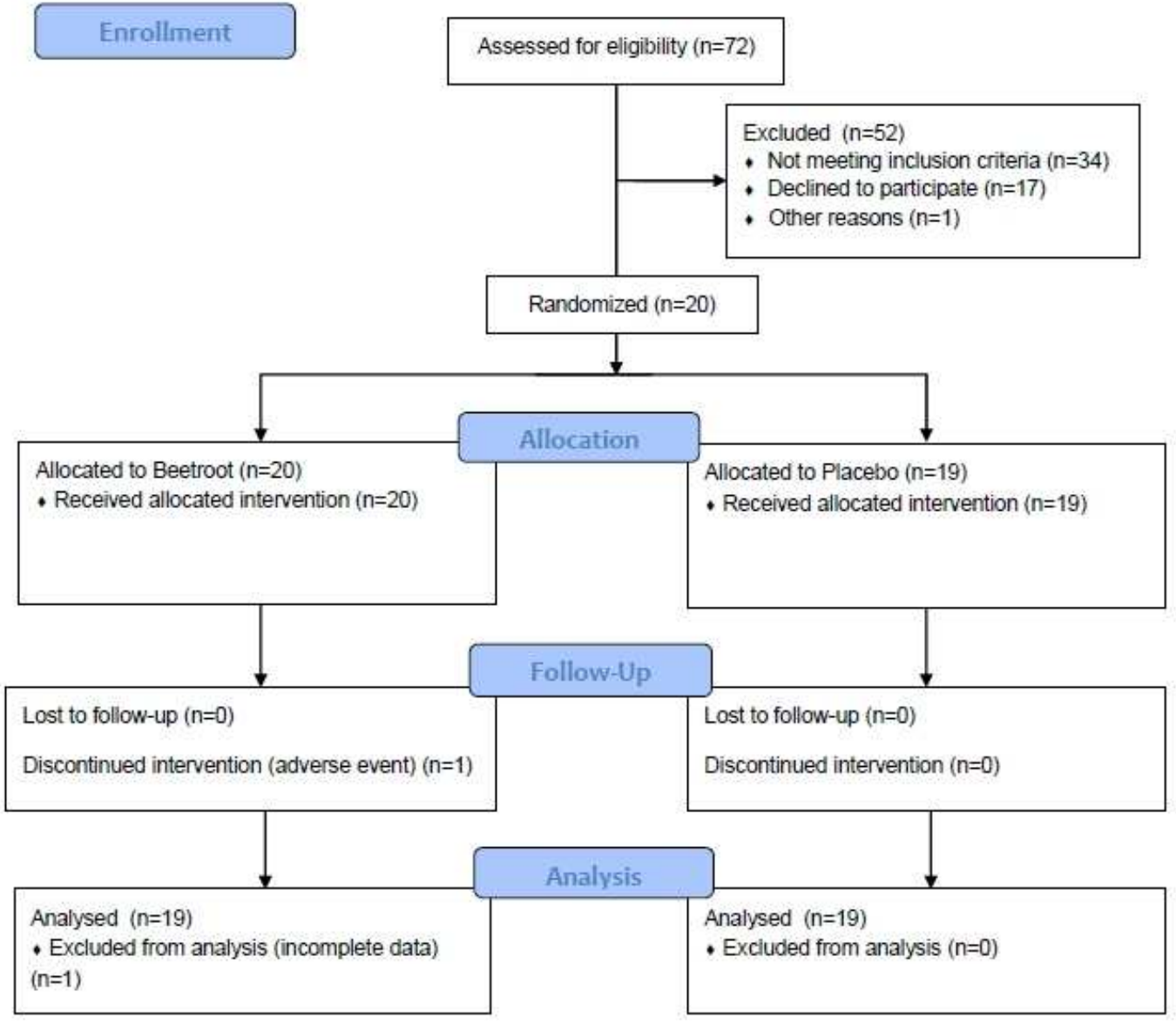
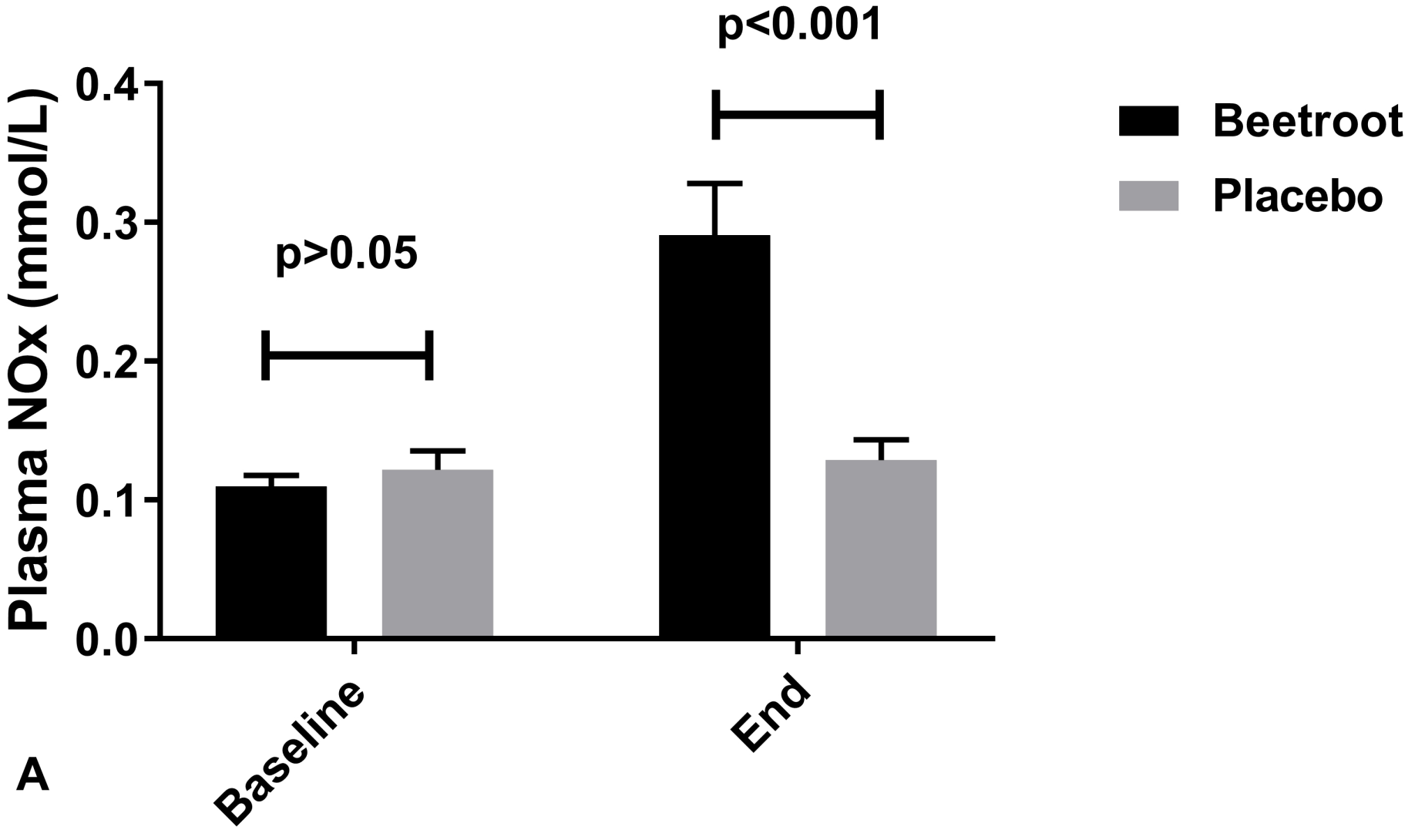
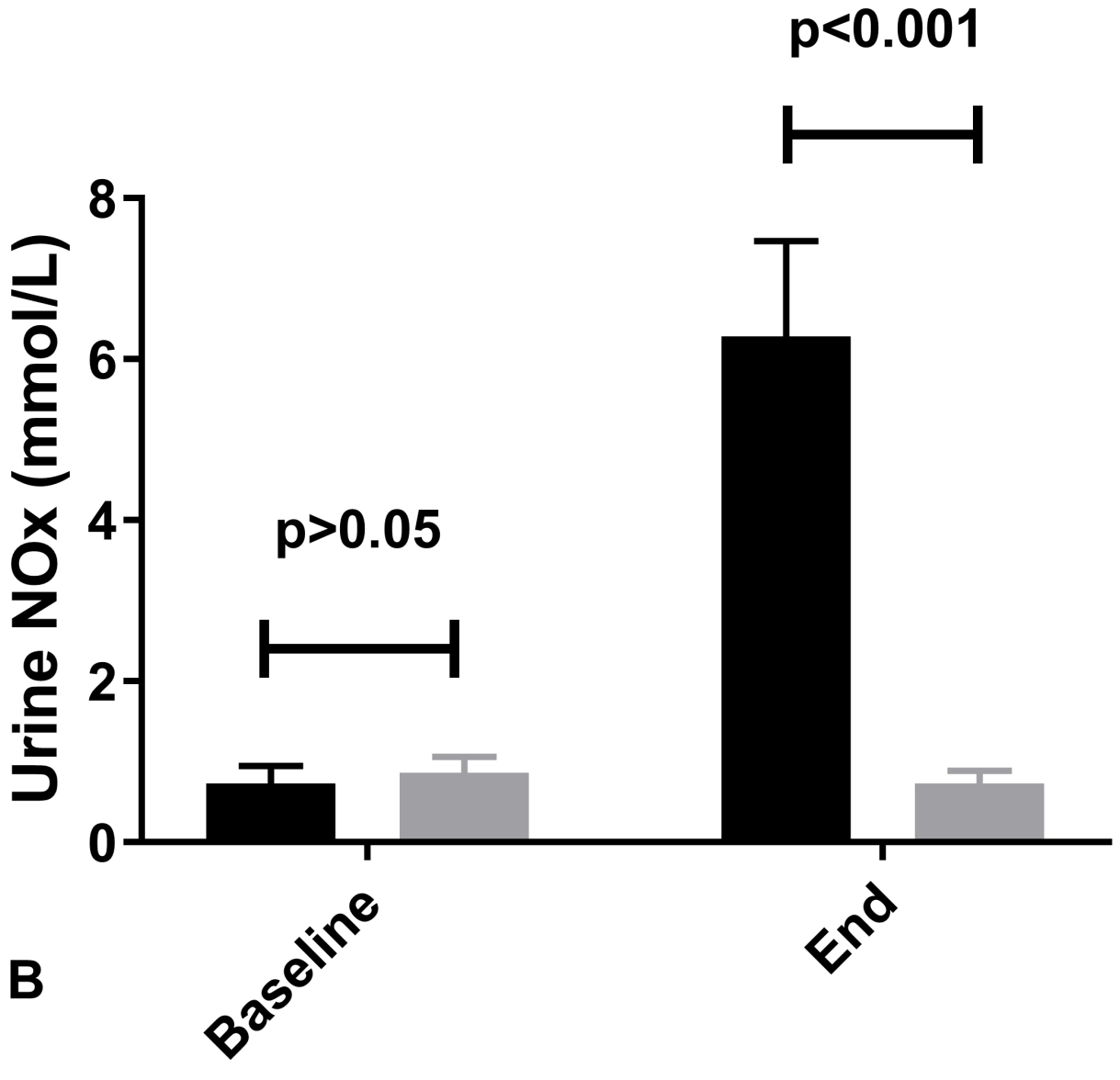
Table 3: Measures of free living physical activity after supplementation with either nitrate-rich or nitrate-depleted (placebo) beetroot juice measured over each one-week intervention with either placebo or nitrate.

	Placebo	**Nitrate**	**Δ**	**P**
Total Physical Activity (kcal)	3378.66±1615.62	3066.11±1274.17	-312.55 ± 904.17	0.14
Average Length of Sedentary Bouts (minutes)	170.15±41.57	175.73±68.76	5.57±68.73	0.72
Daily Average of Sedentary Bouts (minutes)	184.10 ± 194.84	136.10 ± 155.60	-48.01 ± 85.25	0.40
Average Length of Sedentary Breaks (minutes)	110.31±42.81	129.10±86.42	18.78±100.36	0.42
Daily Average of Sedentary Breaks (minutes)	331.05 ± 102.62	322.68 ± 94.62	-8.38 ± 56.28	0.79
Time in Sedentary Activity (minutes)	8993.68±984.47	8473.15±2139.85	-520.52±1782.42	0.21
Time in Light Activity (minutes)	2690.31±1194.68	2520.63±1171.47	-169.68±806.39	0.37
Time in Moderate Activity (minutes)	249.42±149.13	222.42±144.92	-26.94±97.83	0.24
Time in Vigorous Activity (minutes)	32.94±94.86	20.52±56.40	-12.42±54.82	0.19

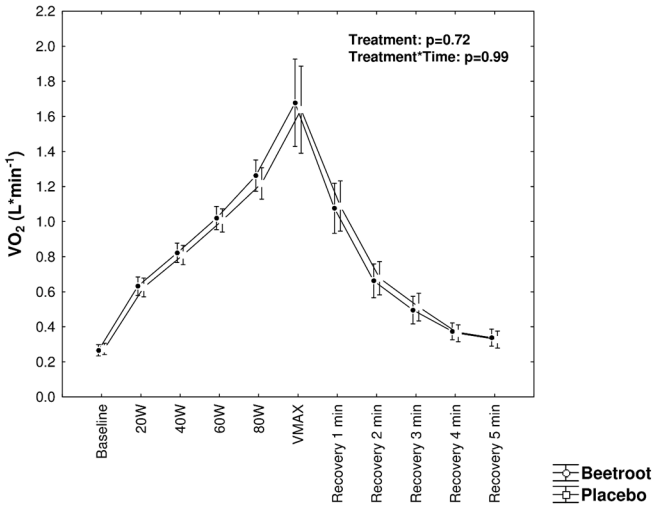
Data presented as means±SD. Δ= difference between placebo and beetroot juice groups. A paired t test was used to compare differences between the two interventions for free living physical activity outcomes in 19 older healthy adults.


Enrollment
Assessed for eligibility (n=72)
Excluded (n=52)
♦ Not meeting inclusion criteria (n=34)
♦ Declined to participate (n=17)
♦ Other reasons (n=1)
Randomized (n=20)
Allocation
Allocated to Beetroot (n=20)
♦ Received allocated intervention (n=20)
Allocated to Placebo (n=19)
♦ Received allocated intervention (n=19)
Follow-Up
Lost to follow-up (n=0)
Discontinued intervention (adverse event) (n=1)
Lost to follow-up (n=0)
Discontinued intervention (n=0)
Analysis
Analysed (n=19)
♦ Excluded from analysis (incomplete data) (n=1)
Analysed (n=19)
♦ Excluded from analysis (n=0)

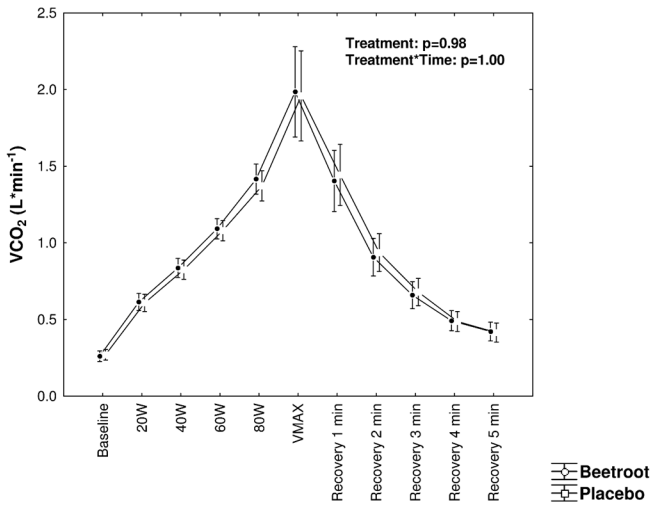

p<0.001
p>0.05
Plasma NOx (mmol/L)
0.4
0.3
0.2
0.1
0.0
Beetroot
Placebo
Baseline
End
A



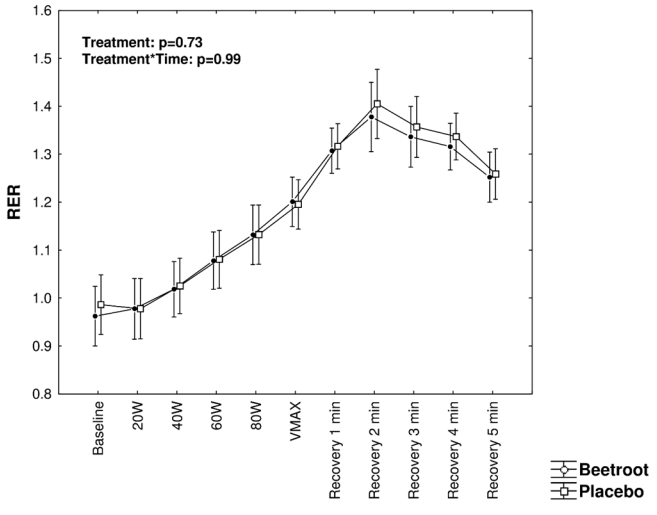
B


Treatment: p=0.72
Treatment*Time: p=0.99
VO2 (L*min-1)
Baseline
20W
40W
60W
80W
VMAX
Recovery 1 min
Recovery 2 min
Recovery 3 min
Recovery 4 min
Recovery 5 min
Beetroot
Placebo

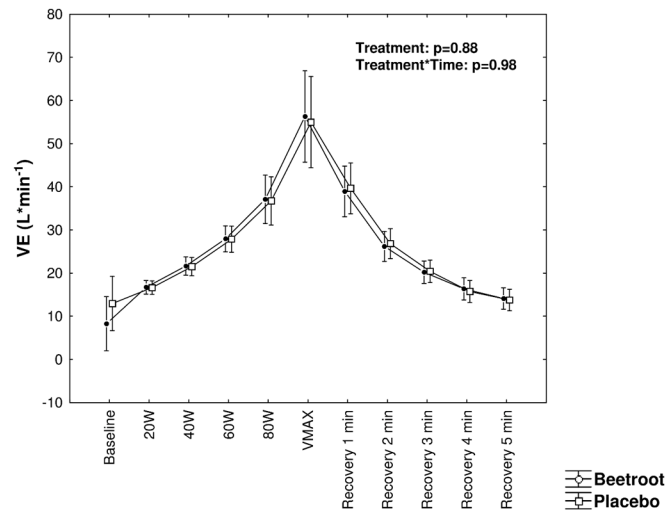
A


Treatment: p=0.98
Treatment*Time: p=1.00
VCO2 (L*min-1)
Baseline
20W
40W
60W
80W
VMAX
Recovery 1 min
Recovery 2 min
Recovery 3 min
Recovery 4 min
Recovery 5 min
Beetroot
Placebo

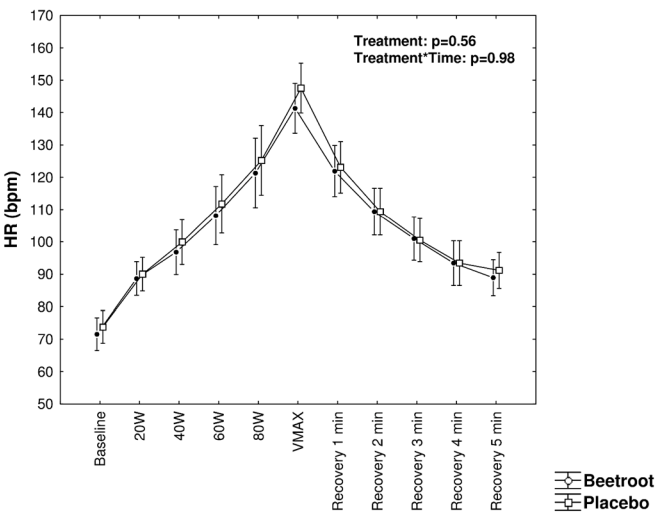
B


Treatment: p=0.73
Treatment*Time: p=0.99
RER
Baseline
20W
40W
60W
80W
VMAX
Recovery 1 min
Recovery 2 min
Recovery 3 min
Recovery 4 min
Recovery 5 min
Beetroot
Placebo

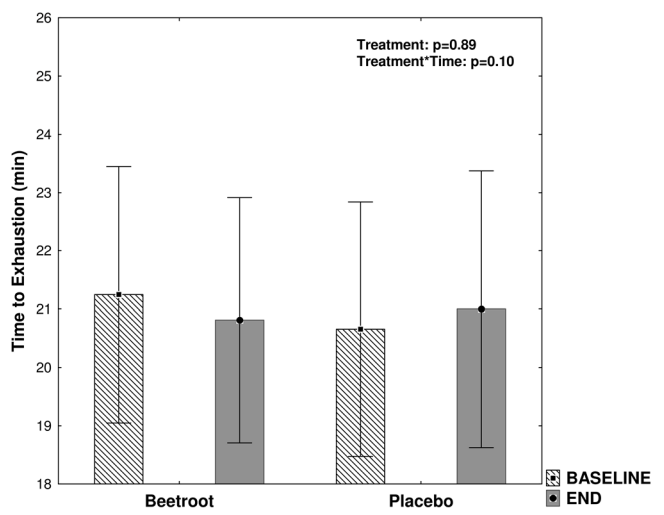
C


Treatment: p=0.88
Treatment*Time: p=0.98
VE (L*min-1)
Baseline
20W
40W
60W
80W
VMAX
Recovery 1 min
Recovery 2 min
Recovery 3 min
Recovery 4 min
Recovery 5 min
Beetroot
Placebo

D

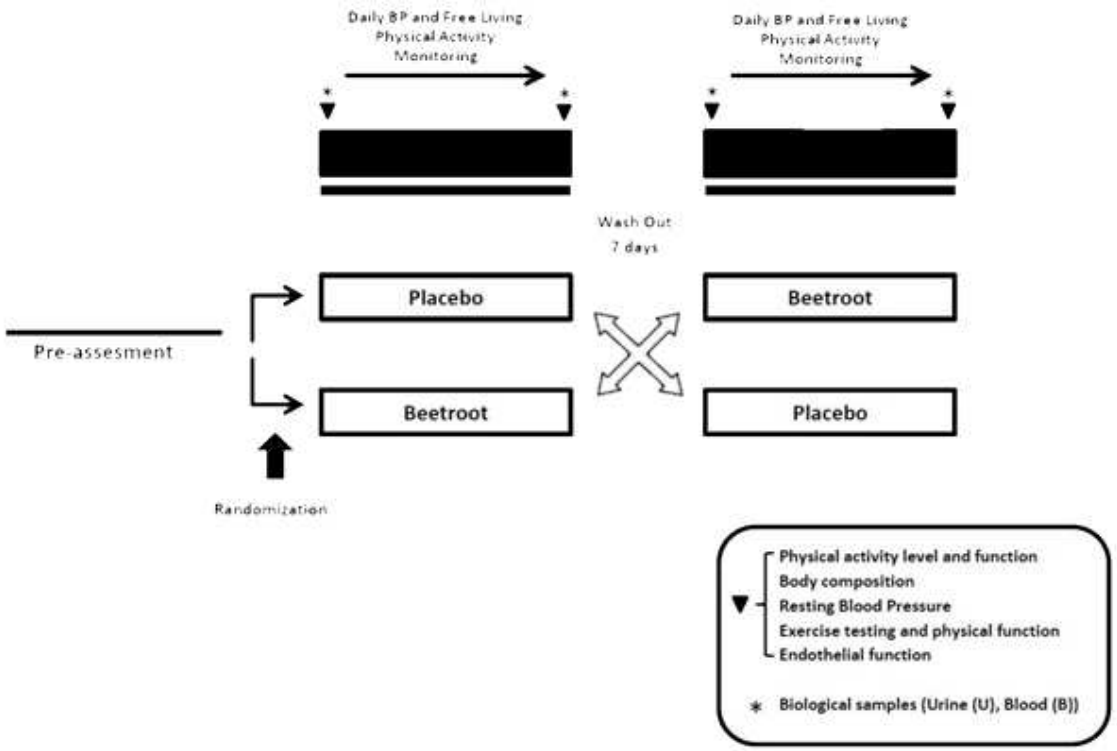

Treatment: p=0.56
Treatment*Time: p=0.98
HR (bpm)
Baseline
20W
40W
60W
80W
VMAX
Recovery 1 min
Recovery 2 min
Recovery 3 min
Recovery 4 min
Recovery 5 min
Beetroot
Placebo

E


Treatment: p=0.89
Treatment*Time: p=0.10
Time to Exhaustion (min)
Beetroot
Placebo
BASELINE
END

F

ONLINE SUPPLEMENTARY MATERIAL



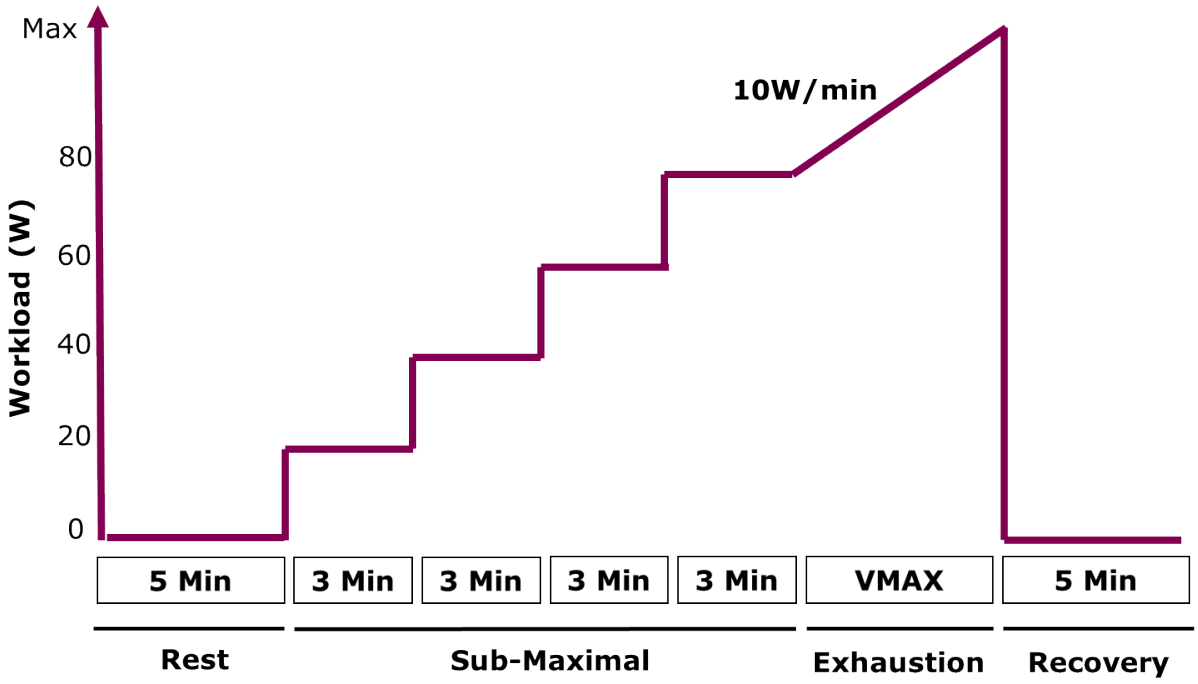


Figure S1: Study design (upper panel) and protocol of standardised exercise test (lower panel). After an initial screening participants were randomised to either placebo or nitrate-rich beetroot juice in cross-over fashion for one week. After a one-week wash out period, participants were invited to return to the research centre and crossed to the other intervention. Measurements were conducted at baseline and end of each intervention giving a total of four measurement sessions. Detailed measurements of physical performance were performed at the research centre while when at home physical activity was monitored by triaxial accelerometer. Blood and urine samples were collected at each visit for the measurement of various biomarkers. A standardised exercise test was performed at each visit using a cyclo-ergometer and continuous monitoring of gas exchanges. The test started with a 5-minute rest following by a stepwise increase in workload by 20 watts very three minutes until reaching a workload of 80 watts. After this value participants were invited to exercise exhaustion (ramping 10 watts per minute) which was then followed by a 5-minute recovery period. VMAX = peak of gas exchanges.

Table S1: List of inclusion and exclusion criteria applied in the recruitment of participants willing to enrol in the trial.

Inclusion criteria

We aim to recruit 20 male and female, older (60-75 y) non-obese subjects (BMI Range: 18.5 - 29.9 kg/m^2). Subjects will be non-smokers and weight stable.

Exclusion criteria are (*reason for exclusion*)

- ✓ Current participation in other research clinical studies
- ✓ Very high resting blood pressure readings (Systolic>180mmHg and/or Diastolic >110mmHg)
- ✓ Vegetarianism (*likely to have very high nitrate intake*)
- ✓ High physical activity level (>15000 steps per day; *may have BMI in overweight range but low fat mass*)
- ✓ Weight change more than 3.0kg in the last 2 months (*important influence on systemic metabolism and vascular function*).
- ✓ Active cancer and any diagnosis of malignant cancer in the last 5 years (*systemic effects on study outcomes*).
- ✓ Diagnosis of chronic and acute metabolic, cardiovascular and inflammatory conditions interfering with the study outcome (*systemic effects on study outcomes*). For example flu, Crohn's Disease, rheumatoid arthritis, heart disease.
- ✓ Weight loss medications (sibutramine, orlistat, rimonabant) and history of bariatric surgery (*weight loss related changes in systemic metabolism*).
- ✓ Previous diagnosis of type 1 or type-2 diabetes treated with insulin and oral hypoglycaemic agents (*modification of regulation of intermediate metabolism*). Type 2 diabetic patients treated with diet only will be included in the study.
- ✓ Drugs: corticosteroids, sildenafil, aspirin, NSAIDs, diuretics, beta-blockers, antacids, anti-hypertensive (Ca++ channel blockers, ACE inhibitors), statins and any other anti-dyslipidaemic agent,anticoagulants, nitrate-derived agents, anti-cholinergic, (*all drugs may have an effect on NO production via different mechanisms*).
- ✓ Subjects on hormonal therapies (oestrogens, thyroxine, progesterone) and psychiatric drugs (antidepressants, sedatives, antipsychotics) will be excluded if dose has been started/changed in the previous three months (*make sure that these disorders are under strict control to avoid interference with the study outcomes*).
- ✓ Haematological disorders including self-reported anaemia, (*risk for the participant and effects on the study outcomes*).
- ✓ Major surgical operations interfering with the study outcomes (*systemic effects on study outcomes*).
- ✓ Alcohol intake >21 units/week for men and >14 units/week women
- ✓ Blood donations in the previous 3 months.

Table S2: Changes in body composition, resting blood pressure (BP), self-reported physical activity and nitrate intake after one-week supplementation with either nitrate-rich or nitrate-depleted (placebo) beetroot juice in 19 older healthy adults.

	Placebo			**Beetroot**			
	Baseline	**End**	**Δ**	**Baseline**	**End**	**Δ**	**$P_{between\ \Delta}$**
Weight (kg)	72.31±14.26	72.29±14.22	0.01±0.85	72.3±14.20	72.4±14.23	-0.16±0.57	0.51
FFM (kg)	50.25±11.72	50.27±11.64	0.02±1.00	50.23±11.70	50.12±11.85	0.11±0.77	0.65
FM (kg)	22.05±6.16	22.01±6.04	-0.03±0.79	22.07±6.36	22.35±6.14	0.27±0.75	0.86
Resting Systolic BP (mmHg)	125.78±15.37	123.13±15.19	-2.64±9.04	129.09±17.17	124.04±15.47	-5.05±9.45	0.48
Resting Diastolic BP (mmHg)	75.93±10.07	72.44±8.67	-3.49±6.42	76.54±9.55	72.83±8.87	**-3.70±5.59**	0.90
IPAQ (METs/week)							
Walking	1850.60±1719.84	2136.31±1937.68	285.71±1548.69	1596.15±1613.84	1694.28±1791.14	98.13±1371.98	0.66
Moderate	674.73±2175.92	626.31±1515.81	48.42±713.46	642.10±1334.29	829.47±1627.58	187.36±1221.66	0.49
Vigorous	400.00±858.21	468.63±1239.44	68.63±833.11	168.42±457.35	190.73±481.69	22.31±291.39	0.38
Total	2925.34±2238.93	3231.26±2004.45	305.92±1958.07	2406.68±1762.43	2714.50±1993.12	307.81±1590.87	0.99

Data presented as means±SD. Δ= difference between baseline and end of study. A paired t test was used to compare differences (Δ) between the two interventions. IPAQ= international physical activity questionnaire

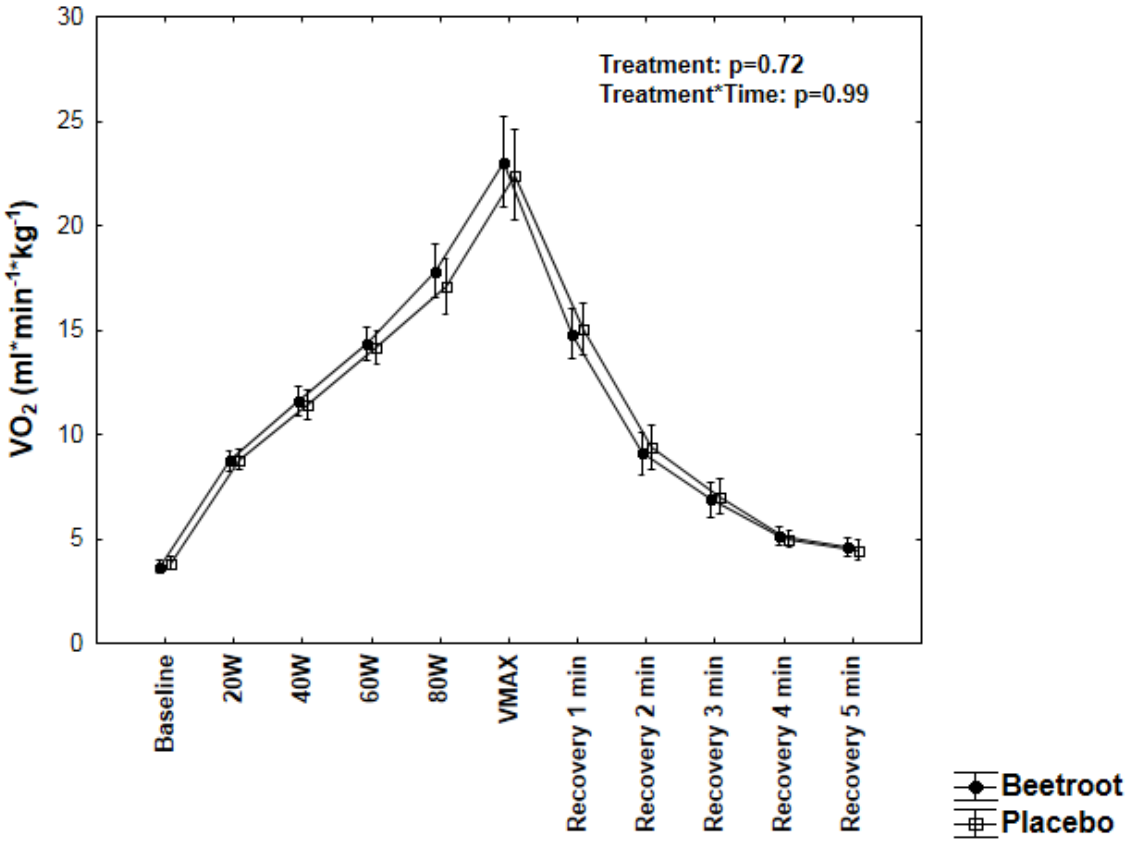
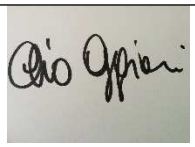
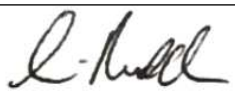
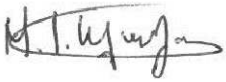
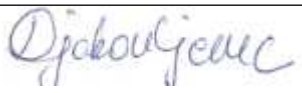
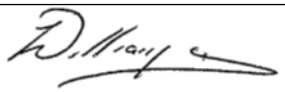


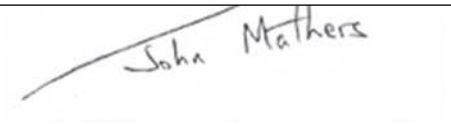
Figure S2: Differences in oxygen consumption after one-week supplementation with either nitrate-rich or nitrate-depleted (placebo) beetroot juice in 19 older healthy adults. Data presented as means±95%CI. A repeated-measure ANOVA model was applied to test differences between the two interventions in 19 older healthy adults. VO_2= oxygen volume adjusted for body weight;

Table S3: Changes in gas exchanges and heart rate after one-week supplementation with either nitrate-rich or nitrate-depleted (placebo) beetroot juice in 19 healthy older adults.

	Placebo			**Beetroot**			
	Baseline	**End**	**Δ**	**Baseline**	**End**	**Δ**	**$P_{betweenΔ}$**
$\dot{V}O_2$ (L*min^{-1})							
Baseline	0.26±0.07	0.27±0.06	-0.006±0.04	0.25±0.06	0.26±0.06	-0.006±0.02	0.99
20W	0.64±0.12	0.62±0.10	0.02±0.06	0.63±0.10	0.63±0.11	0.007±0.07	0.55
40W	0.81±0.13	0.80±0.11	0.004±0.05	0.82±0.14	0.82±0.12	0.007±0.07	0.88
60W	1.01±0.15	1.00±0.15	0.008±0.08	1.02±0.20	1.01±0.13	0.003±0.10	0.86
80W	1.21±0.20	1.21±0.20	0.0009±0.11	1.23±0.22	1.26±0.18	-0.02±0.10	0.37
VMAX	1.61±0.58	1.63±0.55	-0.02±0.20	1.62±0.48	1.67±0.51	-0.05±0.14	0.69
Recovery – 1min	1.10±0.41	1.08±0.29	0.01±0.27	1.06±0.24	1.07±0.31	-0.01±0.23	0.74
Recovery – 2min	0.64±0.25	0.67±0.22	-0.02±0.15	0.68±0.20	0.66±0.18	0.02±0.12	0.26
Recovery – 3min	0.48±0.17	0.51±0.18	-0.03±0.12	0.54±0.18	0.49±0.15	0.05±0.10	0.06
Recovery – 4min	0.37±0.14	0.36±0.09	0.01±0.08	0.39±0.09	0.37±0.10	0.01±0.09	0.85
Recovery – 5min	0.33±0.12	0.32±0.10	0.008±0.09	0.33±0.11	0.33±0.10	-0.004±0.06	0.64
$\dot{V}O_2$ *BW^{-1} (mL*min^{-1}*kg^{-1})							
Baseline	3.70±0.75	3.83±0.60	-0.13±0.68	3.60±0.63	3.68±0.79	-0.07±0.52	0.73
20W	8.95±0.97	8.79±0.75	0.16±0.21	9.08±1.25	8.75±1.31	0.33±1.14	0.64
40W	11.38±1.06	11.40±1.34	-0.02±0.22	11.71±1.60	11.59±1.64	0.12±1.11	0.66
60W	14.21±1.26	14.15±1.50	0.06±0.30	14.33±2.04	14.32±1.92	0.01±1.79	0.91
80W	17.06±2.07	17.09±2.60	-0.02±0.41	17.25±2.56	17.83±2.96	-0.57±1.83	0.28
VMAX	21.99±5.20	22.43±4.82	-0.43±0.62	21.94±4.31	23.05±4.40	-1.10±2.38	0.49
Recovery – 1min	14.98±3.83	15.08±2.60	-0.10±0.87	14.56±1.96	14.82±2.69	-0.25±2.99	0.89
Recovery – 2min	8.81±2.50	9.40±2.45	-0.58±0.56	9.40±2.01	9.08±1.96	0.31±1.74	0.17
Recovery – 3min	6.51±1.75	7.00±2.02	-0.49±0.46	7.28±1.86	6.86±1.58	0.41±1.42	0.15
Recovery – 4min	5.06±1.15	4.98±0.74	0.08±0.26	5.39±0.62	5.14±0.98	0.25±1.11	0.64
Recovery – 5min	4.72±1.09	4.46±0.96	0.26±0.24	4.40±0.93	4.59±0.99	-0.18±0.94	0.22
$\dot{V}CO_2$ (L*min^{-1})							
Baseline	0.27±0.09	0.26±0.06	0.0008±0.05	0.25±0.07	0.26±0.07	-0.008±0.03	0.59
20W	0.60±0.11	0.60±0.09	-0.007±0.07	0.60±0.12	0.61±0.14	-0.007±0.08	0.99
40W	0.80±0.14	0.82±0.10	-0.01±0.09	0.82±0.17	0.83±0.16	-0.01±0.10	0.88
60W	1.07±0.15	1.07±0.13	-0.007±0.14	1.07±0.22	1.09±0.15	-0.01±0.15	0.85
80W	1.34±0.23	1.37±0.22	-0.02±0.20	1.41±0.26	1.41±0.20	-0.06±0.18	0.43
VMAX	1.86±0.67	1.95±0.69	-0.09±0.25	1.86±0.58	1.98±0.55	-0.11±0.16	0.79
Recovery – 1min	1.39±0.52	1.44±0.45	-0.04±0.29	1.40±0.33	1.40±0.40	-0.07±0.28	0.77
Recovery – 2min	0.87±0.30	0.93±0.28	-0.05±0.19	0.90±0.22	0.90±0.24	-0.005±0.13	0.34
Recovery – 3min	0.65±0.21	0.67±0.19	-0.02±0.14	0.65±0.19	0.65±0.18	0.02±0.10	0.17
Recovery – 4min	0.49±0.18	0.48±0.13	0.10±0.10	0.49±0.11	0.49±0.14	0.005±0.09	0.87
Recovery – 5min	0.43±0.15	0.41±0.12	0.01±0.11	0.42±0.12	0.42±0.13	-0.01±0.07	0.34
RER							
Baseline	0.97±0.17	0.98±0.12	-0.009±0.16	0.96±0.11	0.96±0.13	0.005±0.11	0.74
20W	0.93±0.10	0.97±0.11	-0.03±0.09	0.95±0.12	0.97±0.15	-0.02±0.10	0.58
40W	0.99±0.09	1.02±0.10	-0.02±0.09	0.99±0.13	1.01±0.13	-0.02±0.08	0.86
60W	1.06±0.11	1.08±0.11	-0.01±0.11	1.05±0.12	1.07±0.14	-0.02±0.09	0.80
80W	1.10±0.11	1.13±0.11	-0.02±0.11	1.09±0.13	1.13±0.14	-0.03±0.09	0.63
VMAX	1.16±0.09	1.19±0.09	-0.03±0.09	1.15±0.11	1.20±0.12	-0.04±0.11	0.67
Recovery – 1min	1.26±0.08	1.31±0.11	-0.04±0.13	1.25±0.12	1.30±0.07	-0.05±0.11	0.94
Recovery – 2min	1.38±0.15	1.40±0.18	-0.02±0.14	1.35±0.19	1.37±0.11	-0.02±0.13	0.94
Recovery – 3min	1.36±0.18	1.35±0.16	0.005±0.19	1.30±0.21	1.33±0.10	-0.03±0.15	0.55
Recovery – 4min	1.31±0.12	1.33±0.10	-0.02±0.11	1.28±0.16	1.31±0.10	-0.03±0.14	0.85
Recovery – 5min	1.22±0.12	1.25±0.09	-0.03±0.10	1.22±0.14	1.25±0.13	-0.02±0.14	0.90
VE (L*min^{-1})							
Baseline	9.34±2.69	12.94±18.99	-3.60±18.73	8.10±1.64	8.25±1.84	-0.14±1.01	0.42
20W	17.13±3.58	16.63±3.06	0.49±1.87	17.13±3.58	16.72±3.70	0.40±1.54	0.87
40W	21.58±4.27	21.49±3.86	0.08±2.01	22.06±4.91	21.63±5.13	0.43±1.87	0.48
60W	28.08±5.87	27.86±6.72	0.21±3.85	28.39±7.49	27.93±6.27	0.45±2.52	0.83
80W	35.98±10.39	36.72±12.98	-0.74±6.16	36.44±11.55	37.10±11.03	-0.66±3.61	0.95
VMAX	51.95±20.27	54.99±21.88	-3.03±9.14	52.48±20.55	56.31±23.57	-3.82±7.22	0.77
Recovery – 1min	38.63±14.79	39.64±11.79	-1.00±11.92	36.84±10.58	38.93±13.40	-2.08±9.85	0.74
Recovery – 2min	25.43±7.47	26.84±6.75	-1.41±6.14	26.12±5.68	26.17±8.03	-0.05±5.13	0.43
Recovery – 3min	19.39±5.73	20.41±5.40	-1.01±5.18	20.40±5.42	20.20±5.82	0.19±3.07	0.35
Recovery – 4min	16.15±5.78	15.73±5.51	0.42±3.79	16.01±4.39	16.34±5.45	-0.33±3.38	0.54
Recovery – 5min	14.46±5.61	13.76±5.48	0.70±4.58	14.09±4.89	14.09±5.21	-0.45±1.97	0.31
HR (bpm)							
Baseline	76.15±14.00	73.75±12.74	2.39±6.49	73.15±9.57	71.52±8.60	1.62±4.92	0.72
20W	93.23±14.37	90.00±10.81	3.23±10.85	88.81±11.64	88.65±11.46	0.15±9.19	0.41
40W	102.23±15.09	99.94±12.96	2.28±13.19	101.26±18.55	96.81±16.56	4.44±6.96	0.44
60W	118.15±22.99	111.76±16.27	6.39±21.64	112.73±19.55	108.13±22.04	4.60±8.54	0.75
80W	128.07±27.09	125.21±21.04	2.86±25.23	121.36±21.85	121.31±25.07	0.05±16.19	0.69
VMAX	143.18±14.97	147.55±15.65	-4.36±20.00	143.26±15.43	141.34±17.38	1.92±8.32	0.29
Recovery – 1min	122.00±14.76	123.11±15.26	-1.10±10.68	122.47±15.98	121.89±18.67	0.57±9.52	0.60
Recovery – 2min	103.11±26.72	109.37±14.04	-6.26±25.51	108.74±14.02	109.37±16.74	-0.63±10.63	0.38
Recovery – 3min	99.89±13.90	100.53±13.71	-0.63±13.45	99.53±14.34	101.00±15.06	-1.47±12.00	0.84
Recovery – 4min	92.89±11.82	93.42±13.76	-0.52±9.36	92.58±12.37	93.42±15.81	-0.84±10.63	0.92
Recovery – 5min	90.89±12.01	91.16±12.25	-0.26±8.90	90.47±11.82	88.89±11.69	1.57±4.97	0.53

$\dot{V}O_2$ = oxygen volume; $\dot{V}CO_2$ = carbon dioxide volume; RER= respiratory exchange ratio; VE= pulmonary ventilation; HR= heart rate. Data presented as means±SD. Δ= difference between baseline and end of study. A paired t test was used to compare differences (Δ) between the two interventions

Title: Dietary Nitrate does not Have an Effect on Physical Activity Outcomes in Healthy Older Adults: A Randomised, Cross-Over Trial Manuscript Number: NR_2016_154		
Author	Signature	Date
Mario Siervo		18 October 2016
Mike Trenell		19 October 2016
Carlos Celis		19 October 2016
Clio Oggioni		19 October 2016
Alan Ruddock		19 October 2016
Markos Klonizakis		19 October 2016
Mayor Ranchordas		19 October 2016
Djordje Jakovljevic		19 October 2016
Elizabeth Williams		19 October 2016

David Houghton		19 October 2016
John Mathers		19 October 2016
Ammar Ashor		19 October 2016

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4
	2b	Specific objectives or hypotheses	5
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	5-6
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	N/A
Participants	4a	Eligibility criteria for participants	5-6, OSM
	4b	Settings and locations where the data were collected	5
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	5-7
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	7-9
	6b	Any changes to trial outcomes after the trial commenced, with reasons	N/A
Sample size	7a	How sample size was determined	9
	7b	When applicable, explanation of any interim analyses and stopping guidelines	N/A
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	5-6
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	5-6
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	5-6

Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	5-6
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	5-6
	11b	If relevant, description of the similarity of interventions	6
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	9
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	N/A
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	10
	13b	For each group, losses and exclusions after randomisation, together with reasons	10, OSM
Recruitment	14a	Dates defining the periods of recruitment and follow-up	10/OSM
	14b	Why the trial ended or was stopped	N/A
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	10
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	10-12
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	N/A
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	N/A
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	10
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	14-15
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	14

Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	12-15
Other information			
Registration	23	Registration number and name of trial registry	5
Protocol	24	Where the full trial protocol can be accessed, if available	On request
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	16

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

- Dietary nitrate supplementation, administered either as a supplement or by consuming nitrate-rich foods such as beetroot juice, has been associated with improved exercise performance
- Limited evidence is available on the effects of dietary nitrate supplementation on exercise performance in older populations
- This study reported a lack of effect of dietary nitrate on physical performance measured in research settings and free-living conditions