

Dietary nitrate does not have an effect on physical activity outcomes in healthy older adults: a randomized, cross-over trial

SIERVO, M, OGGIONI, C, GJAKOVLJEVIC, D.G., TRENELL, M, MATHERS, J.C., HOUGHTON, D, CELIS-MORALIS, C, ASHOR, A.W., RUDDOCK, Alan http://orcid.org/0000-0001-7995-9115, KLONIZAKIS, Markos http://orcid.org/0000-0002-8864-4403 and WILLIAMS, E.A.

Available from Sheffield Hallam University Research Archive (SHURA) at:

https://shura.shu.ac.uk/14003/

This document is the Accepted Version [AM]

Citation:

SIERVO, M, OGGIONI, C, GJAKOVLJEVIC, D.G., TRENELL, M, MATHERS, J.C., HOUGHTON, D, CELIS-MORALIS, C, ASHOR, A.W., RUDDOCK, Alan, RANCHORDAS, Mayur, KLONIZAKIS, Markos and WILLIAMS, E.A. (2016). Dietary nitrate does not have an effect on physical activity outcomes in healthy older adults: a randomized, cross-over trial. Nutrition Research, 36 (12), 1361-1369. [Article]

Copyright and re-use policy

See http://shura.shu.ac.uk/information.html

ONLINE SUPPLEMENTARY MATERIAL

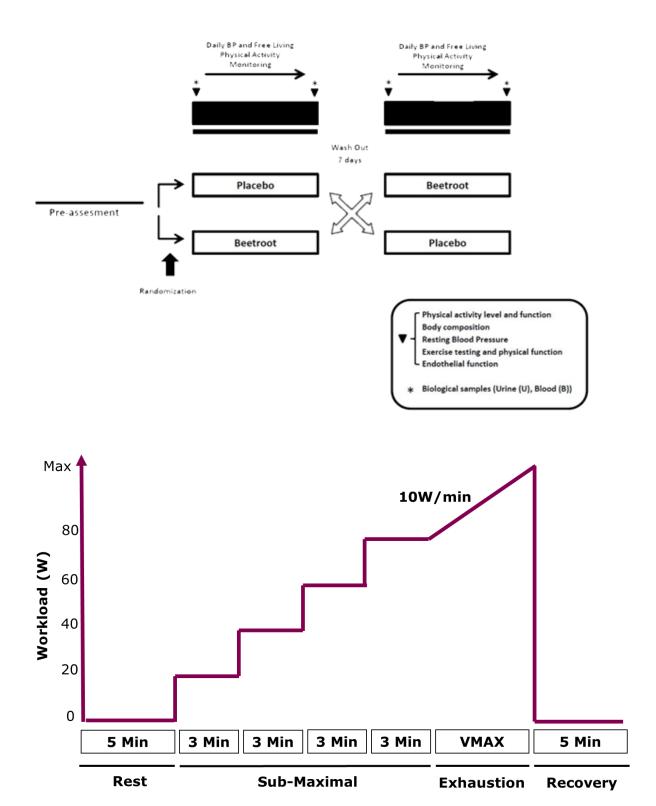


Figure S1: Study design (upper panel) and protocol of standardised exercise test (lower panel). After an initial screening participants were randomised to either placebo or nitraterich beetroot juice in cross-over fashion for one week. After a one-week wash out period, participants were invited to return 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 cycloergometer 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/m²). 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 Δ}
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	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
(mmHg)							
Resting Diastolic BP	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
(mmHg)							
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 \pm 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

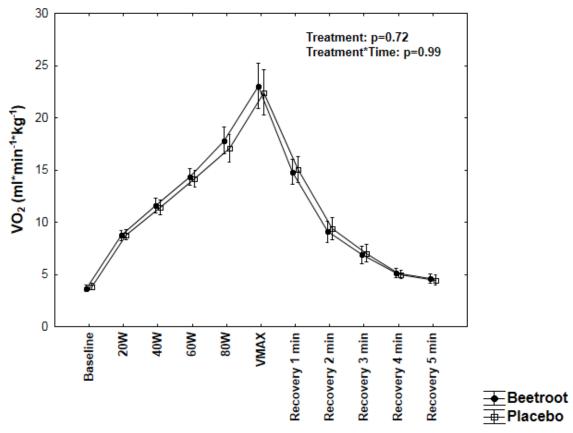


Figure S2: Differences in oxygen consumption after one-week supplementation with either nitrate-rich or nitrate-depleted (placebo) beetroot juice in 19 older healthy adults. Data presented as means $\pm 95\%$ CI. A repeated-measure ANOVA model was applied to test differences between the two interventions in 19 older healthy adults. VO₂= 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.

Buesline	healthy older adults.	Placebo			Beetroot			
Vol. Chimis			End	Δ		End	Δ	P _{between}
Baseline	ŸO₂ (L*min -¹)					-		DetweenA
2007		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
Fig. 2007								
60W 1.19.02 1.09.015 1.008.018 1.02-02 1.19.013 0.008.010 0.009.011 VMAX 1.21.02.02 1.21.02.02 1.23.02.21 1.25.02.01 1.25.02.11 1.05.01 0.022.01 0.37 VAMAX 1.61.038 1.36.05.5 0.0021.01 0.01.027 1.07.031 0.05.01.1 0.05.01.1 0.009.01 1.07.031 0.05.01.1 0.05.01.1 0.009.01 1.07.031 0.05.01.1 0.009.01 0.009.02 0.009.03 0.009.02 0.009.03 0.009.0								
VAAX Recovery - Junis 1.00-041 1.08-023 1.07-022 0.01-027 1.06-024 1.07-031 0.064-025 0.07-022 0.01-027 0.05-011 0.064-025 0.07-022 0.01-027 0.05-011 0.064-025 0.07-022 0.01-027 0.05-011 0.064-025 0.07-022 0.01-027 0.05-011 0.064-025 0.07-022 0.01-027 0.05-011 0.064-025 0.07-022 0.01-027 0.05-011 0.064-025 0.07-022 0.02-011 0.05-011 0.064-025 0.07-022 0.07-022 0.07-021 0.008-0.09 0.03-011 0.03-010 0.06-018 0.05-017 0.008-0.09 0.03-011 0.03-010 0.06-018 0.05-017 0.008-0.09 0.03-011 0.03-010 0.064 0.07-017 0.06-018 0.06-019 0.06-018 0.06-0								
Recovery - Jamin								
Recovery - Junis 0.64-0.25								
Recovery - Jamin 0.48-0.17 0.51-0.18 0.03-0.12 0.54-0.18 0.39-0.09 0.37-0.10 0.01-0.08 Recovery - Jamin 0.37-0.14 0.36-0.09 0.01-0.08 0.39-0.09 0.33-0.11 0.33-0.10 0.004-0.06 0.64 0.65 0.0								
Recovery - Junis 3.39.01 0.37.01.01 0.39.01.01 0.39.01.01 0.39.01.01 0.39.01.01 0.39.01.01 0.39.01.01 0.30.01.01 0.39.01 0.39.01.01 0.39.01 0.39.01.01 0.39.01 0.39.01.01 0.39.01 0.								
Recovery - Jamin								
No.980' (nl.2*min**9kg*) S.709.075 S.88:0.00 O.13±0.68 S.609.063 S.68:0.79 O.77-0.52 O.73 20W S.95±0.97 S.79-0.75 O.16±0.21								
Baseline		0.33±0.12	0.32±0.10	0.006±0.09	0.33±0.11	0.33±0.10	-0.004±0.00	0.04
20W		2.70 : 0.75	2.02.0.60	0.12.0.60	2 60 . 0 62	2.60.0.70	0.07.0.52	0.72
agw 11.38-1.06 11.40:1.34 -0.02:-0.22 11.71:1.60 11.59:1.64 -0.12:1.11 0.60 60W 12.12:1.26 14.15:1.55 0.06:-0.32 14.33:2.19 14.32:2.19 0.01:1.79 0.01:1.79 0.01:1.79 0.02:0.41 17.25:2.56 17.73:2.29 0.02:1.73 0.01:1.79 0.01:1.79 0.02:0.41 17.25:2.26 -0.57:1.83 0.38 0.89 0.02:0.41 17.25:2.26 -0.57:1.83 0.38 0.89 0.02:0.41 17.25:2.26 -0.57:1.83 0.38 0.08 0.02:0.29 1.45:6.1.96 18.82:2.69 -0.57:1.83 0.89 0.02:0.29 0.02:0.								
60W 14,21+1.26 14,15+1.50 0.06e0.30 14,33+2.04 14,32+1.92 0.01+1.79 0.01 80W 17.06e2.07 17.09e2.00 -0.024.41 17.52+2.56 18.32.96 -0.05±1.83 0.28 VMAX 21.99±5.20 22.43±4.82 -0.43±0.62 21.94±3.11 23.05±4.40 -1.10±2.38 0.49 Recovery - Jmin 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 - Jmin 5.06±1.15 4.98±0.74 -0.08±0.26 5.39±0.62 5.14±0.98 0.41±1.42 0.15 Recovery - Jmin 4.72±1.09 4.46±0.96 0.26±0.04 4.00±0.93 4.59±0.99 -0.18±0.04 0.22±1.11 0.64 Recovery - Jmin 4.72±1.09 0.02±0.00 0.000±0.05 0.000±0.05 0.25±0.07 0.26±0.07 0.000±0.03 0.59 VCO, (2min**) 0.27±0.00 0.00±0.01 0.000±0.05 0.25±0.07 0.000±0.03 0.59 20W 0.00±0.01 0.00±0.01 0.00±0.01 0.00±0.01								
MAX 1.99-5.20 22.43+3.82 -0.43+0.62 21.94+3.31 23.05+4.40 -1.10-2.38 0.49 0								
Recovery - Junin								
Recovery - 2mm								
Recovery - mm 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 - mm 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								
VCO, d.*min*) D. 27±0.09 0.26±0.06 0.0008±0.05 0.25±0.07 0.26±0.07 0.08±0.03 0.59 2DW 0.60±0.11 0.82±0.10 0.01±0.09 0.007±0.07 0.61±0.12 0.61±0.14 4.007±0.08 0.99 4DW 0.80±0.14 0.82±0.17 0.01±0.09 0.82±0.17 0.61±0.14 4.007±0.08 0.99 6DW 1.07±0.15 1.07±0.13 -0.007±0.14 1.07±0.22 1.09±0.15 -0.01±0.15 0.88 8DW 1.34±0.23 1.37±0.22 -0.02±0.02 1.00 1.14±0.22 1.09±0.15 -0.01±0.15 0.85 PMAX 1.86±0.67 1.95±0.69 -0.09±0.25 1.86±0.88 1.98±0.55 -0.11±0.16 0.79 Recovery - Jmin 0.37±0.30 0.93±0.28 -0.08±0.19 0.90±0.22 0.90±0.22 0.90±0.22 0.90±0.22 0.90±0.22 0.90±0.22 0.90±0.22 0.90±0.22 0.90±0.22 0.90±0.22 0.90±0.22 0.90±0.22 0.90±0.22 0.90±0.22 0.90±0.22 0.90±0.22 0.90±0.22 0.90±0.22 0.90								
Baseline	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
Baseline	VCO₂ (L*min -¹)						1	
20W	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
60W								
60W 107±0.15 1.07±0.13 -0.007±0.02 1.09±0.15 -0.01±0.15 0.88 80W 1.34±0.23 1.37±0.22 -0.02±0.02 1.41±0.20 -0.04±0.15 0.84 0.84 0.44 0.09±0.25 1.86±0.58 1.98±0.55 -0.11±0.16 0.79 0.09±0.21 1.86±0.58 1.98±0.55 -0.11±0.16 0.79 0.02±0.12 1.86±0.58 1.98±0.55 -0.11±0.16 0.79 0.02±0.12 1.86±0.58 1.98±0.55 -0.11±0.16 0.02±0.10 0.02±0.11 0.02±0.13 0.04±0.11 0.02±0.11 0.00±0.13 0.04±0.11 0.02±0.11 0.09±0.18 0.05±0.01 0.02±0.11 0.02±0.11 0.02±0.11 0.02±0.11 0.02±0.10 0.17 Recovery - Smin 0.43±0.18 0.43±0.12 0.01±0.10 0.42±0.12 0.02±0.10 0.02±0.10 0.02±0.10 0.02±0.10 0.02±0.10 0.02±0.10 0.02±0.10 0.02±0.10 0.02±0.10 0.02±0.10 0.02±0.10 0.02±0.10 0.02±0.10 0.02±0.10 0.02±0.10 0.02±0.10 0.02±0.10 0.02±0.11 0.02±0.11 <t< td=""><td></td><td>0.80±0.14</td><td></td><td></td><td>0.82±0.17</td><td></td><td>-0.01±0.10</td><td></td></t<>		0.80±0.14			0.82±0.17		-0.01±0.10	
MAX								
MAX 186-067 1.95-0.69 -0.09+0.25 186-0.88 1.98+0.55 -0.11±0.16 0.79 0.79 0.78 0.79								
Recovery - Jmin								
Recovery - 2min								
Recovery - Jmin								
Recovery - 4min								
Recovery - Smin 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 0.05 60W 1.06±0.11 1.08±0.11 -0.01±0.11 1.05±0.12 1.07±0.14 -0.02±0.08 0.86 60W 1.10±0.11 1.13±0.11 -0.02±0.11 1.09±0.13 1.13±0.14 -0.02±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 1.07±0.14 -0.03±0.09 0.63 VERCOVERY - Imin 1.26±0.08 1.31±0.11 -0.02±0.13 1.35±0.12 1.30±0.07 -0.05±0.11 0.94 Recovery - Smin 1.38±0.15 1.40±0.18 -0.05±0.19 1.30±0.21 1.33±0.10 -0.02±0.13 1.33±0.10 -0.02±0.11 0.94 Recovery - Smin 1.23±0.12 1.25±0.0								
Baseline								
Baseline		0.43±0.13	0.41±0.12	0.01±0.11	0.42±0.12	0.42±0.13	-0.01±0.07	0.54
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 60W 1.06±0.11 1.02±0.10 -0.02±0.09 0.9±0.13 1.01±0.14 -0.02±0.09 0.86 60W 1.16±0.11 1.08±0.11 -0.01±0.11 1.05±0.12 1.07±0.14 -0.02±0.09 0.86 80W 1.16±0.09 1.19±0.09 -0.03±0.09 1.15±0.11 1.02±0.13 1.13±0.14 -0.03±0.09 0.63 Recovery - Imin 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±0.10 -0.02±0.11 1.33±0.10 -0.02±0.11 -0.02±0.11 -0.02±0.11 -0.02±0.11 -0.02±0.11 -0.02±0.11 -0.03±0.15 0.55 1.05±0.12 1.33±0.10 -0.03±0.11 1.28±0.16 1.31±0.10 -0.03±0.15 0.55 1.05±0.12 1.22±0.12 1.22±0.12 1.22±0.12 1.22±0.12 1.22±0.12 1.22±0.12 1.22±0.12 1.22±0.12<		0.07 : 0.17	0.00.0.10	0.000.016	0.06.0.11	0.06.0.12	0.005.0.11	0.74
60W 0.99±0.09 1.02±0.10 -0.02±0.09 0.99±0.13 1.01±0.13 -0.02±0.09 0.86 80W 1.10±0.11 1.13±0.11 -0.01±0.11 1.05±0.12 1.07±0.14 -0.02±0.09 0.80 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 0.07 Recovery - Imin 1.26±0.08 1.31±0.11 -0.04±0.13 1.25±0.12 1.30±0.17 -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 1.31±0.12 1.33±0.10 -0.02±0.11 1.32±0.01 1.33±0.10 -0.03±0.15 0.58 Recovery - 4min 1.31±0.12 1.33±0.10 -0.02±0.11 1.22±0.14 1.25±0.13 -0.02±0.14 0.90 VE (L*min*) 1.22±0.12 1.25±0.09 -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								
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 NWAX 1.10±0.09 1.19±0.09 -0.03±0.09 1.13±0.14 -0.02±0.13 1.13±0.14 -0.04±0.12 -0.04±0.11 0.67 Recovery - Imin 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 - Smin 1.36±0.18 1.35±0.16 -0.02±0.14 1.35±0.19 1.37±0.11 -0.02±0.13 0.94 Recovery - Smin 1.36±0.18 1.35±0.16 -0.05±0.11 1.32±0.10 -0.03±0.15 0.55 Recovery - Smin 1.22±0.12 1.25±0.09 -0.03±0.10 1.22±0.14 1.25±0.13 -0.03±0.15 0.55 Recovery - Smin 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*min³) Baseline 9.34±2.69 1.29±18.99 -3.60±18.73 8.10±1.64 8.25±1.84 -0.14±1.01 0.42 20W 1.713±3.58 1.663±3.06 0.49±8.73 </td <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>								
80W 1.10±0.11 1.13±0.11 -0.02±0.11 1.09±0.13 1.13±0.12 -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 - Imin 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 - 4min 1.31±0.12 1.33±0.10 -0.02±0.11 1.28±0.16 1.31±0.10 -0.03±0.15 0.55 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 0.85 Recovery - 5min 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 1.13±3.58 16.72±3.70 0.40±1.54 0.35 80W 28.08±5.87 27.86±6.72 0.21±3.85 28.39±7.49 27.9±6.27								
MAX								
Recovery - Imin								
Recovery - 2min								
Recovery - 3min 1.36±0.18 1.35±0.16 0.005±0.19 1.30±0.12 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*min¹) D.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								
Recovery - 4min Recovery - 5min 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 VE (L*min¹) VA (D*L*L*) VA (D*L*L*) <td>Recovery – 2min</td> <td></td> <td></td> <td></td> <td>1.35±0.19</td> <td>1.37±0.11</td> <td></td> <td></td>	Recovery – 2min				1.35±0.19	1.37±0.11		
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*) 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 - 2min 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 - 3min 19.39±5.73 20.41±5.40 -1.01±5.18 20.40	•							
VE (L*min¹) 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 NMAX 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 - Imin 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 - 3min 19.39±5.73 20.41±5.40 -1.01±5.18 20.40±5.42 20.20±8.83 -0.95±5.13 0.43 Recovery - 4min 16.15±5.78 15.73±5.51 0.42±3.79 16								
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 - Imin 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 - Smin 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 - Smin 14.46±5.61 13.76±5.48 0.70±4.58 14.09±4.89 14.0		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
$ \begin{array}{c} 20W \\ 40W \\ 21.58\pm 4.27 \\ 20W \\ 21.58\pm 4.27 \\ 21.49\pm 3.86 \\ 21.49\pm 3.86 \\ 0.08\pm 2.01 \\ 0.21\pm 3.85 \\ 0.08\pm 2.01 \\ 22.06\pm 4.91 \\ 21.63\pm 5.13 \\ 0.43\pm 1.87 \\ 0.48 \\ 0.48\pm 1.87 \\ 0.48 \\ 0.08 \\ 0.$	VE (L*min ⁻¹)							
$\begin{array}{c} 40W \\ 60W \\ 28.08\pm 5.87 \\ 80W \\ 35.98\pm 10.39 \\ 8.632\pm 12.98 \\ 8.0W \\ 28.08\pm 5.87 \\ 27.86\pm 6.72 \\ 80W \\ 35.98\pm 10.39 \\ 36.72\pm 12.98 \\ -0.74\pm 6.16 \\ 36.44\pm 11.55 \\ 37.10\pm 11.03 \\ 36.44\pm 11.55 \\ 37.10\pm 11.03 \\ -0.66\pm 3.61 \\ -0.95 \\ -0.77 \\ 8ccovery - Imin \\ 8.63\pm 14.79 \\ 39.64\pm 11.79 \\ -1.00\pm 11.92 \\ 36.84\pm 0.58 \\ -1.41\pm 6.14 \\ 26.12\pm 5.68 \\ 26.17\pm 8.03 \\ -0.05\pm 5.13 \\ -0.05\pm 5.21 \\ -0.05\pm 0.27 \\ -0.0$		9.34±2.69	12.94±18.99	-3.60±18.73	8.10±1.64		-0.14±1.01	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	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
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 - Imin 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 -1.01 ± 5.18 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 32.3 ± 10.85 38.81 ± 11.64 38.65 ± 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 0.44 ± 6.96 0.44 60W 118.15 ± 22.99 111.76 ± 16.27 6.39 ± 21.64 112.73 ± 19.55 108.13 ± 20.04 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 </td <td>40W</td> <td>21.58±4.27</td> <td>21.49±3.86</td> <td>0.08 ± 2.01</td> <td>22.06±4.91</td> <td>21.63±5.13</td> <td>0.43±1.87</td> <td>0.48</td>	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
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 – Imin 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 -1.01 ± 5.18 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 32.3 ± 10.85 88.81 ± 11.64 88.65 ± 11.46 0.15 ± 9.19 0.41 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 ± 0.00 122.47 ± 15.98 121.89 ± 8.67 0.57 ± 9.52	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
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	80W			-0.74±6.16				
Recovery – Imin 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 ± 0.85 88.81 ± 11.64 88.65 ± 11.46 0.15 ± 9.19 0.41 40W 102.23 ± 15.09 99.4 ± 12.96 2.28 ± 13.19 101.26 ± 18.55 96.81 ± 6.56 4.44 ± 6.96 0.44								
$\begin{array}{cccccccccccccccccccccccccccccccccccc$								
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$								
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$								
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 $Recovery - Imin$ 122.00 ± 14.76 123.11 ± 15.26 -1.10 ± 10.68 122.47 ± 15.98 121.89 ± 18.67 0.57 ± 9.52 0.69 $Recovery - 2min$ 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								
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 $Recovery - Imin$ 122.00 ± 14.76 123.11 ± 15.26 -1.10 ± 10.68 122.47 ± 15.98 121.89 ± 18.67 0.57 ± 9.52 0.69 $Recovery - 2min$ 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	2							
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 $Recovery - Imin$ 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			22.70_2.10	3.7.0_ 1.50		,_5.21	00_1.77	0.01
$\begin{array}{cccccccccccccccccccccccccccccccccccc$		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
$\begin{array}{cccccccccccccccccccccccccccccccccccc$								
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$								
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$								
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$								
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$								
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$								
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$								
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 – 3min	99.89±13.90						
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	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