

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

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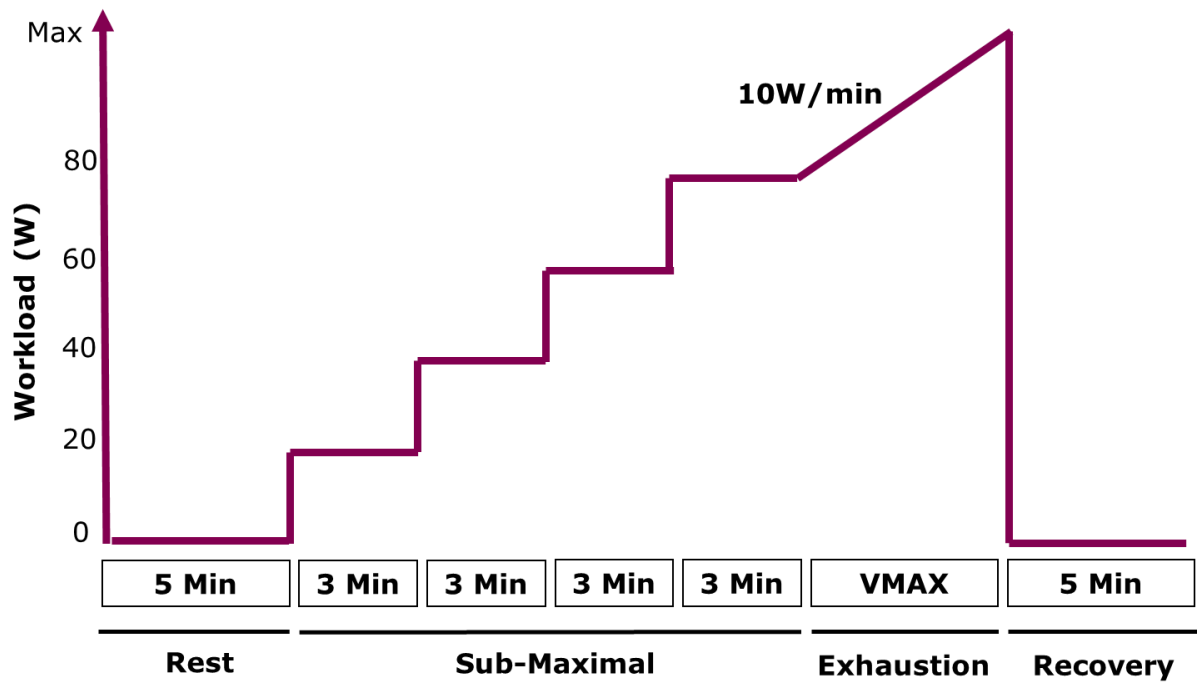
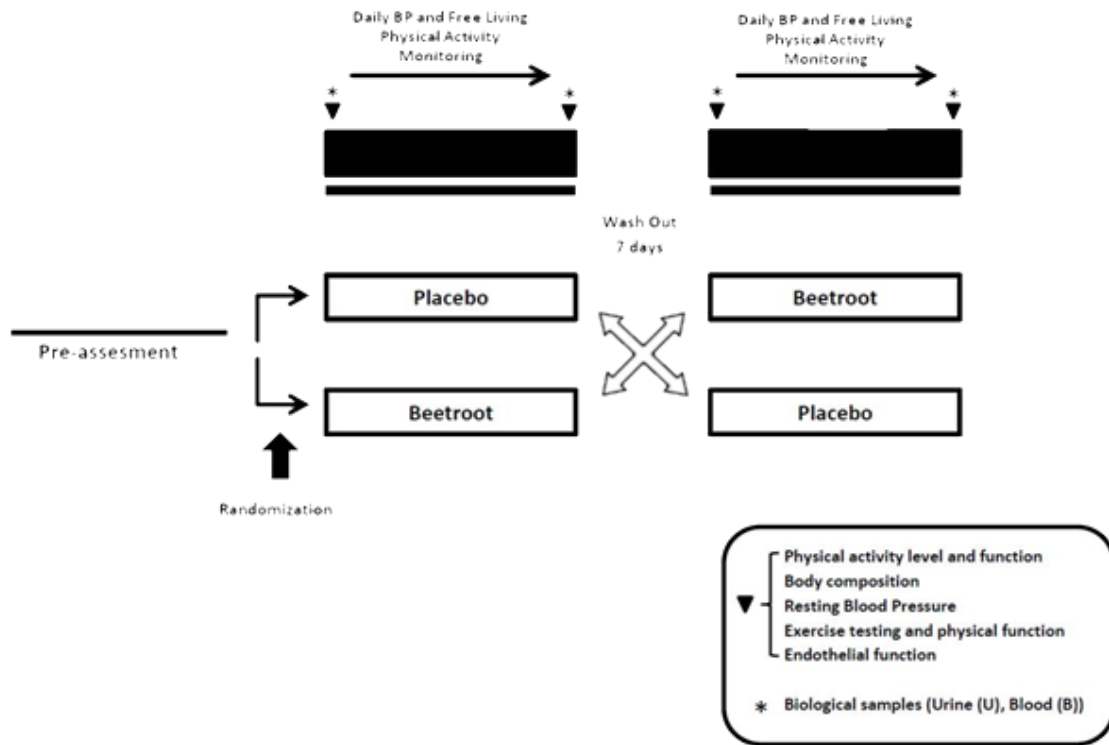
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# ONLINE SUPPLEMENTARY MATERIAL



**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 cycle-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 every 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<sup>2</sup>). Subjects will be non-smokers and weight stable.

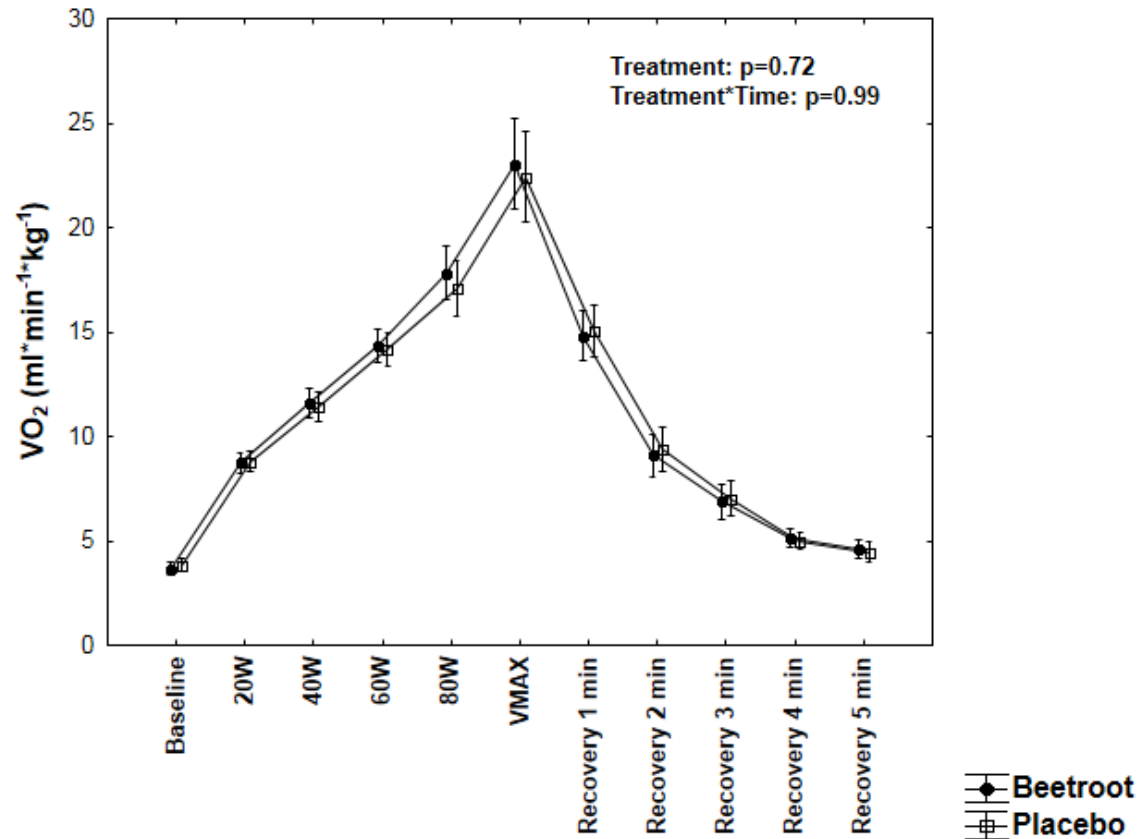
**Exclusion criteria are (reason for exclusion)**

- ✓ 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<sup>++</sup> 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			P <sub>between Δ</sub>
	Baseline	End	Δ	Baseline	End	Δ	
<b>Weight (kg)</b>	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
<b>FFM (kg)</b>	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
<b>FM (kg)</b>	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
<b>Resting Systolic BP (mmHg)</b>	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
<b>Resting Diastolic BP (mmHg)</b>	75.93±10.07	72.44±8.67	-3.49±6.42	76.54±9.55	72.83±8.87	<b>-3.70±5.59</b>	0.90
<b>IPAQ (METs/week)</b>							
<i>Walking</i>	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
<i>Moderate</i>	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
<i>Vigorous</i>	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
<i>Total</i>	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  $\pm$  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			P <sub>betweenΔ</sub>
	Baseline	End	Δ	Baseline	End	Δ	
<b><math>\dot{V}O_2</math> (L*min<sup>-1</sup>)</b>							
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
<b><math>\dot{V}O_2</math> *BW<sup>-1</sup> (mL*min<sup>-1</sup>*kg<sup>-1</sup>)</b>							
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
<b><math>\dot{V}CO_2</math> (L*min<sup>-1</sup>)</b>							
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
<b>RER</b>							
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
<b>VE (L*min<sup>-1</sup>)</b>							
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
<b>HR (bpm)</b>							
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