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No effect of acute and chronic dietary nitrate supplementation on muscle oxygen consumption-recovery following exercise

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Abstract

AIM: The purpose of this study was to investigate the effect of acute and chronic ingestion of BR on mVO2-recovery of the vastus lateralis following exercise. **METHOD:** Nine healthy recreationally active men participated in a double-blind, placebo control, cross over design study, and ingested either 2x70 ml BR (2 x 450-500 mg nitrate) or PL (2 x 0.7 – 2.52 mg nitrate) per day for six days. MuscleVO2-recovery was measured using near infrared spectroscopy (NIRS) on vastus lateralis and rapid repeated occlusions on the right thigh immediately after cycling bouts on LI- and HI-intensity. Muscle-VO2 values were fit to a mono-exponential curve in order to calculate a time-constant and look for changes between BR and PL. **RESULTS:** No effect was found on mVO2-recovery following a seven minute cycling bout on LI after acute and chronic ingestion of BR. No effect was found on BP, and on pVO2 and HR from end-exercise, after acute and chronic supplementation. **Discussion:** No analysis on mVO2-recovery after cycling on HI were done in this study. This was due to an unforeseen prolonged elevation of mVO2-values, not seen on LI, making it difficult to fit a mono exponential curve and calculate a TC. Further research is necessary to investigate mVO2-recovery on higher intensity whole body exercise. Effect of BR on mVO2-recovery might not be expected due to no effect found on pVO2, BP and HR. This study is one of the first investigate mVO2-recovery following a whole body exercise, the effect of BR is uncertain at the time.

Abstrakt

MÅL: Hensikten med denne studien var å undersøke effekten av akutt og kronisk inntak av BR på mVO2-gjennoppretting av vastus lateralis etter trening. **METODE:** Ni friske aktive menn på et mosjonistnivå deltok i en dobbelblindet, placebokontroll, kryssover studie, og inntok enten 2x70 ml BR (2 x 450-500 mg nitrat) eller PL (2 x 0,7 til 2,52 mg nitrat) per dag i seks dager. mVO2-gjennoppretting ble målt ved hjelp av nær infrarød spektroskopi (NIRS) på vastus lateralis og raske gjentatte okklusjoner på høyre lår umiddelbart etter sykkeløkter på LI og HI. Muskel-VO2-verdier ble tilpasset en mono-eksponentiell kurve for å beregne en tidskonstant og se etter endringer mellom BR og PL. **RESULTATER:** Ingen effekt ble funnet på mVO2-gjenoppretting etter en syv minutters sykkeløkt på LI etter akutt og kronisk inntak av BR. Ingen effekt ble funnet på BP, og på pVO2 og HR fra slutt-økt, etter akutt og kronisk tilskudd. **Diskusjon:** Ingen analyse på mVO2-gjenoppretting etter sykling på HI ble gjort i denne studien. Dette skyldes en uforutsett vedvarende høye mVO2-verdier, ikke sett på LI, noe som gjør det vanskelig å tilpasse en mono eksponentiell kurve og beregne en TC. Videre forskning er nødvendig for å undersøke mVO2-gjennoppretting på helkropps-aktivitet med høg intensitet. Effekt av BR på mVO2-gjennoppretting er kanskje ikke forventet på grunn av ingen effekt funnet på pVO2, BP og HR. Denne studien er en av de første til undersøke mVO2-gjennoppretting etter helkropps-aktivitet, effekt av BR er uvisst for øyeblikket.

Preface

This study is part of a bigger project conducted at NTNU. Between the vascular occlusion test and the cycling tests, there were also a dynamic handgrip test. There were also one occlusion during each cycling bout, and NIRS measurements were also done on tibialis anterior and flexor digitorium superficialis through the whole session. This study will not go in depth of these procedures.

Acknowledgement

I would like to thank my supervisor, Mireille van Beekvelt for all her help, patience and great knowledge. Further, I would like to thank my mother and father for all their support through my whole life. I would also like to thank my dear grandmother, Ruth, who passed away on December 16. 2015. Lastly, I would like to thank my fellow students, Lars Oddvar Hagen and Simen Eggerud, for good teamwork during the data collection.

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Abbreviations

BP: Blood pressure

BR: Beetroot juice

DNS: Dietary nitrate supplementation

DP: Diastolic blood pressure

HI: High intensity, 70% of max aerobic power

HR: Heart rate

LI: Low intensity, 50% of onset blood lactate accumulation

mVO2: Muscle oxygen consumption

mVO2-recovery: Muscle oxygen consumption recovery

NO: Nitric oxide

PL: Placebo

SP: Systolic blood pressure

Introduction

In the present world of sport and fitness, new and better ways to further improve performance are constantly sought after. Relatively recently, DNS has been suggested as a potential performance booster in activities that are endurance dependent, and has thus become increasingly popular. Several studies have now proved that ingestion of DNS can help reduce resting blood pressure, and can significantly increase physical efficiency in endurance exercises because of an increase in NO [1, 2, 8, 13, 14, 15, 16, 29, 30, 31]. Both acute and chronic effect of DNS have been reported [29]. There are however also studies that have not found any improvements, and often these studies have been done with subjects on an elite endurance trained level [3, 5, 20]. Literature tends to indicate that an effect is not expected from individuals with a VO2-max around 60 ml/min/kg and above, but there are also studies that reports no effect on less endurance trained subjects [4].

Ingestion of DNS leads to a rise in levels of plasma nitrate and nitrite, which tends to be dose-dependent [13, 31]. Nitrate is reduced to nitrite and finally to NO through the entero-salivary circulation. The reduction of nitrate to nitrite is made possible by bacteria located on the tongue [18]. Some studies [4, 10] proved this by successfully attenuated the rise of nitrite, and further benefits from NO [10], by using antibacterial mouthwash. NO is a molecule which is produced in the body, and it has a vital role to maintain normal body function. It is involved in functions like neurotransmission, vasodilation, mitochondrial respiration, contraction of skeletal muscle, etc.[12].

Numerous studies have investigated the effects of DNS, but many of these studies have only looked upon the effects on whole body measurements, such as pVO2 and BP. The exact physiological mechanism behind the effect of DNS is still not completely determined, so more and more studies are investigating the effects of DNS peripherally. Some of the suggested potential physiological mechanisms behind this effect are increased mitochondrial efficiency [15], reduced cost of ATP production [1], or changes in blood flow [26].

One way to measure peripheral changes, is to incorporate the use of near-infrared spectroscopy (NIRS). NIRS is a non-invasive optical method that makes it possible to monitor relative concentration changes of oxy- and deoxyhemoglobin / myoglobin within tissue. This makes NIRS a potential method to investigate some of the suggested mechanisms behind the effects from DNS that occur in muscle tissue. Studies have successfully used NIRS to measure oxygen consumption of skeletal muscle during rest and exercise [11, 27]. Few studies have looked upon what happens with mVO2 immediately after exercise and in what manner it

recovers to its resting value [6, 9, 19, 21-25]. In these studies they conclude that measuring mVO2 by using NIRS and RRO is a valid method to evaluate mitochondrial function. Looking for changes in mVO2-recovery after ingestion of DNS, might give new insight to the potential mechanisms behind the expected effect.

The aim of this study is therefore to investigate the acute and chronic effect of DNS on mVO2-recovery on vastus lateralis following exercise.

Method

Test subjects

Nine healthy, moderately active, non-smoking men participated in this project. The test subjects had no prior history of cardiovascular or pulmonary disease, no orthopaedic problems that might have worsened due to the test procedures, and were not using medication or dietary supplements that might affect the results. Test subjects who reached a peak Vo2 above 60 ml/min/kg were excluded from further testing. This is due to little to no effect expected from nitrate supplementation at that physical level. Recruitment was done through posters located at several different locations in Trondheim, mainly at school or sport facilities. Before commencing pre-tests on the first day, the test subjects were informed of the following test procedures and written informed consent was submitted. Permission to perform this study was given by the regional ethical committee.

Study design and protocol

Each test subject attended on five days of testing over a period of four weeks. The study consisted of an initial test, two supplementation phases of six days and an eight-day washout period between each phase. This study used a double blind placebo controlled crossover design with random allocation in a counterbalanced order to investigate the effect of BR.Test subjects were given BR or PL at the first supplementation phase and opposite in the second supplementation phase. Tests were performed at the first and last day of the supplementation phases. Apart from the initial tests, all test sessions had the same contents and consisted of two different tests performed in the following order: vascular occlusion test and dynamic cycling test. An overview of the study design is displayed in figure 1.

Supplementation

Supplementation consisted of a daily dosage of either 2x70 ml concentrated beetroot juice: BR (2x450-500 mg nitrate, 2x 6.5 mmol, Beet It, James White Drinks, Ipswich, UK) or placebo: PL (0.7-2.52 mg nitrate, 0.006-0.02 mmol, Beet It, James White Drinks, Ipswich, UK). Both supplementation are identical in taste and consistency, and might give beeturia (red urine) and red stools.

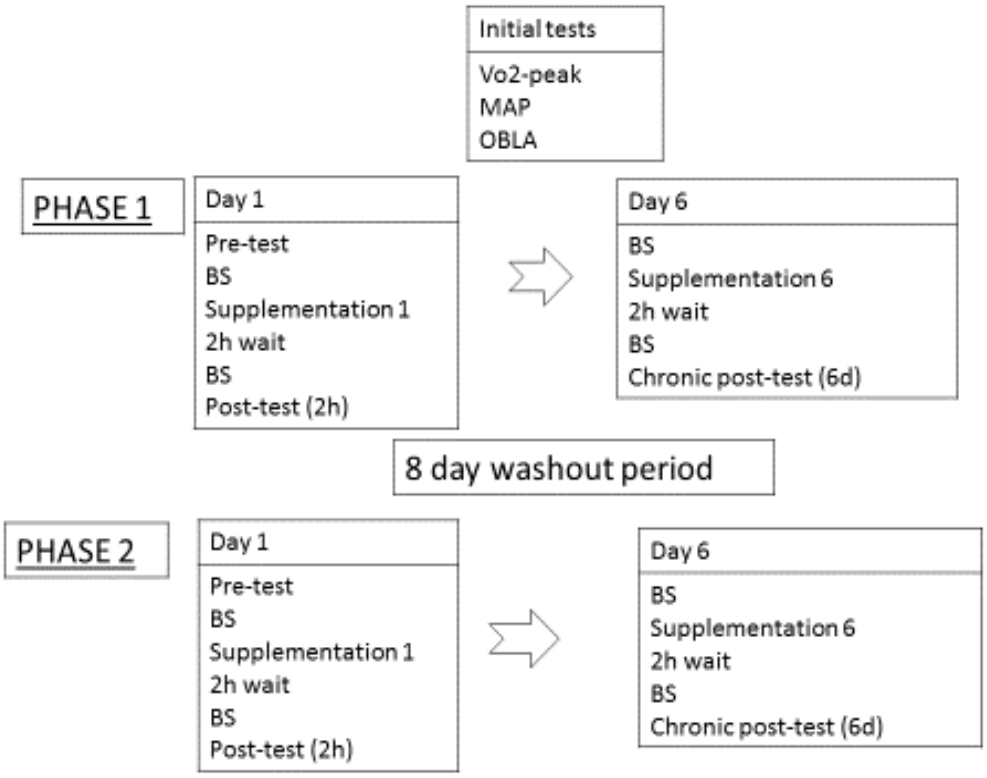


Figure 1. Representation of the cross-over design. Subjects were given either BR or PL in phase 1 and opposite in phase 2. BS: blood sample.

Acute test days

These days consisted of a pre- and post-test. The subject started with performing a test session, then gave a blood sample and consumed 2x70ml of either PL or BR under supervision. The test subjects returned after 2 hours for a new blood sample, and then performed the post-test. At the end of the day, the subjects were handed the remaining doses of either PL or BR. The subjects were told to take the supplementation between 9 and 11 in the morning, to avoid use of mouthwash, and to avoid eating excessive amounts of nitrate-rich vegetables (beetroot, spinach, etc.) during the supplementation phase.

Chronic test days

The subject returned after six days, started with giving a blood sample and then consumed the last 2x70ml of either PL or BR under supervision. The test subjects then returned after 2 hours for a new blood sample, and then one test session was performed (post-test 2).

Initial tests

Prior to the tests of the cross-over design, an initial test day was done to determine the intensity levels for the following main cycling tests. This test day consisted of a lactate profile test, VO2max-test and anthropometric measurements. The subjects arrived to the lab in a rested and hydrated condition. No caffeine was consumed at least six hours prior to the test. The subjects were informed of the study and the tests to come and a questionnaire was filled out before commencing the tests. Estimated skeletal muscle mass was calculated by using four measured points of skinfold thickness, three circumference measurements, height and weight.

Lactate profile and VO2max test

Cycling was done on an electrical braked ergometer bike. Vo2 and HR were measured during the test periods and subjects were told to maintain a cadence of 80-100 RPM during both cycling tests. The test started after a warm up of 5-10 min on 75 W. Start workload was set to 100 W and each period lasted four minutes. The workload increased with 25 W, and RPE and blood lactate were measured after each period. The test ended after the period where blood lactate levels exceeded four mmol/l. (onset of blood lactate accumulation; OBLA). Five minutes of active recovery separated the lactate profile test and the VO2max test. The VO2max test started with a workload set to OBLA minus 25 W and increased every minute by 25 W. The test lasted to exhaustion or failure to maintain the acquired RPM. Lactate was measured at the end of the test, and Vo2peak was calculated by using a mean from the three last measurements (before failure to maintain sufficient RPM). After approx. one week, the test subjects returned to the lab to start the main tests.

Vascular occlusion test

This test was done in order to define mVo2 at rest. After five minutes of baseline measurements, two occlusions were performed with the subject in a semi-supine position, fitted with a NIRS optode on top of the muscle belly of the vastus lateralis and an inflatable cuff placed proximally on the right thigh. The cuff pressure was set to 300 mmHg for all occlusions. The first occlusion, one minute in duration, was initiated after approximately five minutes of baseline NIRS-measurement. Subjects were informed to remain as calm as possible both during and after the occlusions. The second occlusion, 10 minutes in duration and with a 10 min recovery, was initiated after a five minute recovery period from the first occlusion. The first occlusion was performed in order for the subjects to get used to the procedure and to check for any flaws in signal quality. Resting mVO2 was calculated from the 10-min occlusion.

Dynamic cycling test

After the vascular occlusion test, the subjects carefully changed from the patient bed onto an ergometer bike, and were fitted with a hear rate sensor. The leg cuff was refitted and held in place with an additional wrap, to prevent movement of the cuff during cycling. Blood lactate and RPE were measured during the warm up and after each bout. The test consisted of two cycling bouts of seven minutes with 10 min recovery, the first on LI (50 % of OBLA) and the second on HI (70% of MAP). The subjects were informed to maintain a cadence between 80-100 RPM. Immediately following the end of each bout rapid repeated occlusions were initiated (RRO), which consisted of five seconds occlusion and five seconds rest for three minutes and then five seconds occlusion and 15 seconds rest for three min. The subjects were informed to remain as calm as possible in a standardized position with hands on the steer wheel. A block was placed under the right pedal of the bike in order to help maintain a standardized position for the RRO, with the crank in horizontal position and the thigh in a slightly less than 90-degree angle. Measurements of pVO2 continued through the end of RRO. NIRS-measurements continued throughout the whole test. After completion of each test session, adipose tissue thickness (ATT) at the optode location was measured. The outlining of the optode was also marked in order to place the optode at the same location at the next test session. The subject were encouraged to refresh the marks between the test days.

Equipment and measurements

All tests were performed in the same lab. Cycling was performed on an Electro-magnetically braked cycle ergometer (Velotron, RacerMate Inc., Washington, USA). Measurements of pVO2 were done with open-circuit indirect calorimetry with a mouthpiece and nose clip set up. (Oxycon Pro, Jaeger GmbH, Hoechberg, Germany) The apparatus was calibrated at the start of each test day using a gas of known concentration (16.0% O2 and 5.85% CO2, Riessner-Gase GmbH & Co, Lichtenfels, Germany) and a 3-liter syringe (Hans Rudolph Inc, Kansas City, MO, USA). Heart rate was measured with a heart rate monitor (Polar RS800, Polar Electro OY, Kempele, Finland). Vo2 and HR for each test subject were summarized to a mean per min and sorted to group means after the supplementation key was available. BP was measured with a portable automatic blood pressure monitor (OSZ 5 easy, Welch Allyn Jungingen, Germany) Measurements were performed two times within the five minute baseline period before the vascular occlusion test, and the mean value from the two measurements were used in analysis . Blood lactate was measured from small blood samples taken from the fingers with a lactate analyzer (Lactate Pro LT-1710, Arkray, Kyoto, Japan).

NIRS-measurements on the vastus lateralis were done with a continuous-wave near-infrared spectrophotometer with one optode (Oxymon MKIII, Artinis Medical Systems, The Netherland). Wavelengths of the light from the optode was 766 and 856 nm. NIRS is based on the relative tissue transparency for light in the near-infrared region and can detect changes in oxygenation of both hemoglobin (Hb) and myoglobin (Mb). Because of identical spectral characteristics, it is not possible to distinguish between Hb and Mb [27]. An algorithm described by Livera et al. (1991), makes it possible to convert the changes in absorbed light into concentration changes of oxy-Hb and deoxy-Hb. The sum of oxy-Hb and deoxy-Hb reflects the total amount of Hb. The difference between oxy-Hb and deoxy-Hb (Hb-diff) is used for the calculations of mVO2 during AO [27]. Resting mVO2 was calculated by using a modified Lambert-Beer law and AO to control inflow and outflow of the limb [27]. An algorithm for blood volume correction was used in order to remove potential artifacts due to small shifts in blood volume during RRO [22]. A mono-exponential curve was fitted to the mVO2- values from each RRO in order to calculate mVO2-recovery in form of a time constant [19]. Figure 2 shows an example of NIRS-raw signals from a 10 minute AO and RRO following exercise. Arterial occlusions and rapid repeated arterial occlusions (RRO) were done with a pneumatic cuff (Hokanson SC12L; Marcom Medical ApS, Denmark) and a rapid automatic inflation system (Hokanson E20 Rapid Cuff Inflator Marcom Medical ApS, Denmark) combined with a

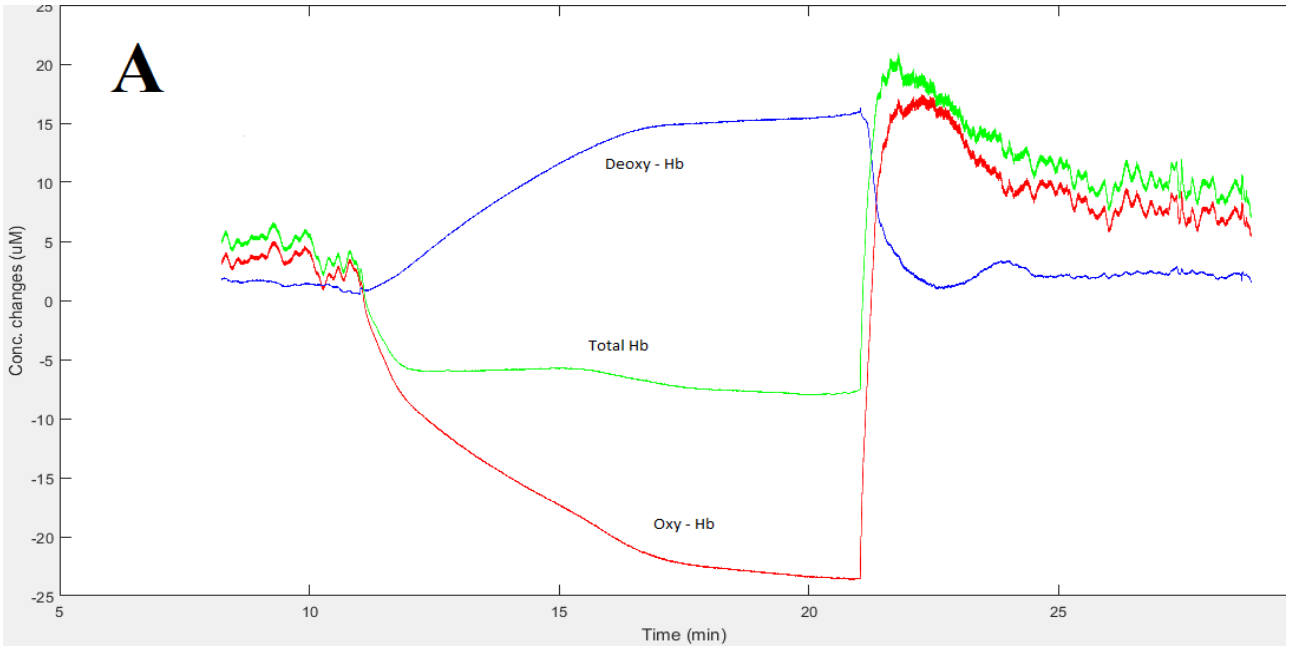
separate air source. A separate air source was needed to perform and maintain sufficient pressure for the RRO. Skinfold thickness, measured with a skinfold caliper (Holtain, Crymmych, UK), was used to calculate bodyfat percentage and skeletal muscle mass, but also to measure adipose tissue thickness (ATT) at the NIRS-optode location. NIRS-data were processed through several steps with MATLAB R2015b.

Blood sampling procedures

Blood samples were taken at NTNU, Trondheim by trained personnel. One 6ml EDTA glass tube and two 3,5ml citrate glass tubes were filled for each blood sample. The tubes were centrifuged at 3000 rpm for 10 min. Blood plasma was then separated in cryotubes and centrifuged at 3000 rpm for 10 min. The cryotubes containing EDTA plasma were shock frozen in liquid nitrogen. EDTA plasma were stored at -32 degree Celsius after the shock freeze, while the ones with citrate were slow frozen in a freezer at -32 degree Celsius. At the end of the test period, all samples were shipped to the UK for analysis of plasma nitrate- and nitrite-levels.

Statistics

A two way ANOVA for repeated measures in SPSS were used to test the effect of BR on end-exercise pVO2 and HR, TC and BP between pre-test, post-test 1 and post-test 2. Greenhouse-Geisser was used whenever sphericity was violated in all statistics.



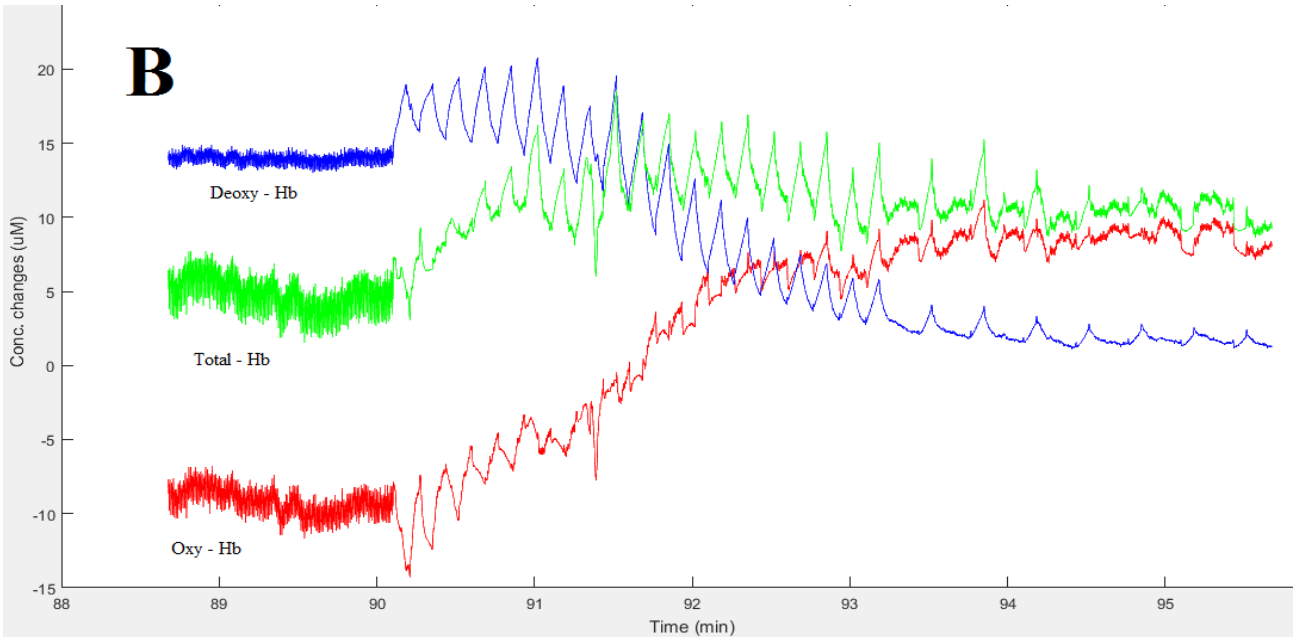


Figure 2. Raw signal from one subject during a 10 min AO on vascular occlusion test (A) and RRO following cycling on HI (B). Oxy – Hb (red), deoxy – Hb (blue) and total Hb (green).

Results

Subjects

In total, 12 subjects were recruited to participate in this study. Three test subjects were removed from the project. One was excluded due to too high Vo2-max at initial pre-test, one due to sickness after phase 1, and one due to injury before start of phase 1. Characteristics of the remaining nine subjects can be seen in table 1. All variables are from the initial test, except ATT, which is presented as a group mean from all tests.

Table 1. Subject characteristics from initial tests. Variables presented as a mean value with standard deviation and range (n = 9)

Age (y)	32.3± 8.8	21.0 – 45.0
Height (cm)	182.0±7.8	164.7 – 190.6
Weight (kg)	83.6±11.8	68.8 – 99.8
VO2peak (ml/min/kg)	49.3±5.2	42.0 – 57.8
HRpeak (bpm)	181.0±6.3	176 - 197
Body fat (%)	20.6±4.4	14.4 – 27.9
ATT (mm)*	5.0 ± 1.3	2.5 - 7.4
SM (kg)	35.5±5.9	28.2 – 41.8
Lactate threshold (W)	186.1±13.2	175 - 200
MAP (W)	327.8±36.3	275 - 375

*SM: Skeletal muscle mass; MAP: Max aerob power; * ATT: Adipose tissue thickness from all main tests.*

Nitrate supplementation

Plasma analysis was not completed at the time of publication of this thesis, so no further data analysis were done on plasma nitrate and nitrite levels. The nitrate supplementation was generally well tolerated, but there were some reports of upset stomach. No incidents of upset stomach were reported from the placebo supplementation. There were reports of beeturia and red stools from both supplements.

Work load

The work load on each intensity were the same for all tests. Group mean (SD) work load for LI was 101.1 ± 0.0 W and ranged from 90 – 150 W. Group mean (SD) work load for HI was 224.2 ± 0.6 and ranged from 190 – 250 W.

Baseline check

In order to ensure that baseline values were similar before each phase, analysis of pVO2 between each pre-test were conducted. Two-way ANOVA for repeated measures analysis of the first five minutes found no significant differences in pVO2 when comparing pre-tests on both workloads. Group mean (SD) pVO2 values per minute from both pre-tests on both workloads are shown in Fig.3. **LI:** No main effect of test ($F(1,8) = 0.630$, $p = 0.450$) was found. Significant main effect of time was found, simple contrast revealed that pVO2 increased in the initial part of exercise but remained stable after two minutes ($F(1.671, 13.369) = 55.572$, $p = 0.00$). No interaction between test x time ($F(1.223, 9.787) = 4.3$, $p = 0.060$) was found. **HI:** No main effect of test ($F(1,8) = 0.032$, $p = 0.862$) was found. Significant main effect of time was found, simple contrast revealed that pVO2 increased in the initial part of exercise but remained stable after two minutes ($F(1.671, 13.367) = 85.975$, $p = 0.00$). No interaction effect between test x time ($F(1.380, 11.043) = 0.126$, $p = 0.807$) was found. These findings indicates similar baseline values for all pre-tests.

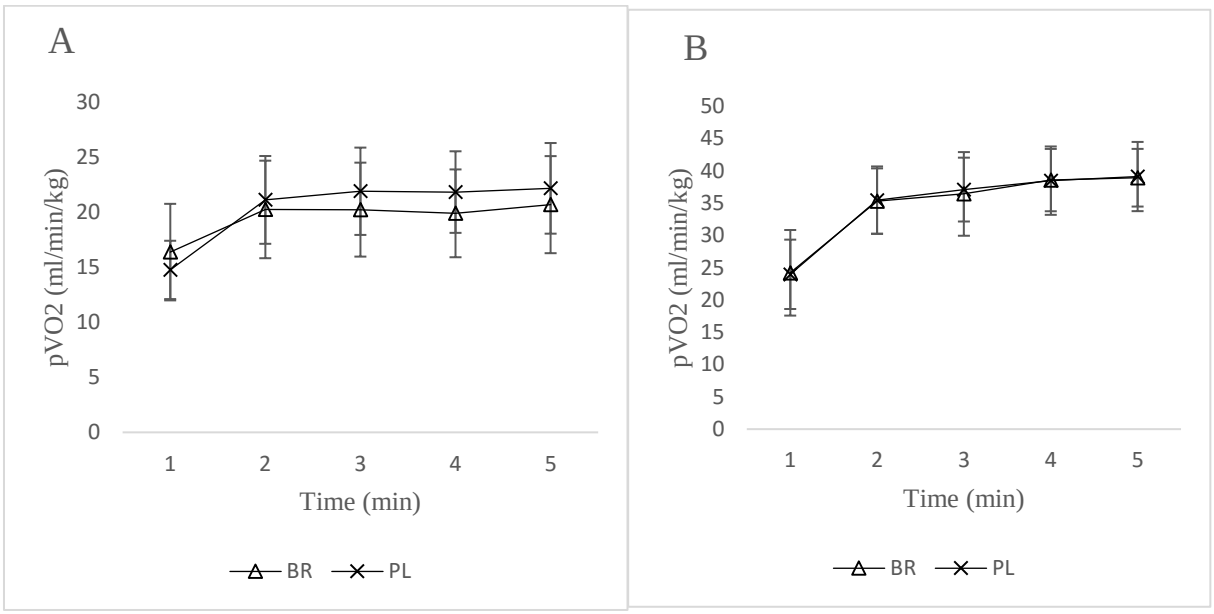


Figure 3. Group mean (SD) pVO2-values during the first five minutes of cycling from the pre-tests. LI (A) and HI (B)

End exercise pVO2

Group mean (SD) values for end-exercise pVO2 at LI and HI are shown in Fig. 4. A two-way ANOVA for repeated measures was used on the last minute of exercise pVO_2 to test the effect of BR. **LI:** No main effect of supplementation (F(1,8) = 0.028, p = 0.870) was found. A significant main effect of test was found (F(2,16) = 4.026, p = 0.038). Contrasts revealed a significant reduction from post-test 1 (2h) to post-test 2 (6d) (p = 0.046), but no significant change from pre-test to post-test 1 (2h) (p = 0.179). No interaction effect between supplementation x test was found for end-exercise pVO2 (F(2,16) = 1.336, p = 0.291). **HI:** No main effect of supplementation (F(1,8) = 1.250, p = 0.296) was found. A significant main effect of test was found (F(2,16) =4.244, p = 0.033). Contrasts revealed a significant increase in pVO2 from pre-test to post-test 1 (2h) (p = 0.026), but no significant change from post-test 1 to post-test 2 (6D) (p = 0.068). No interaction effect between supplementation x test was found for end-exercise pVO2 (F(2,16) = 0.974, p = 0.399). These results indicate that BR had no acute or chronic effect on pVO2 at end-exercise on both workloads.

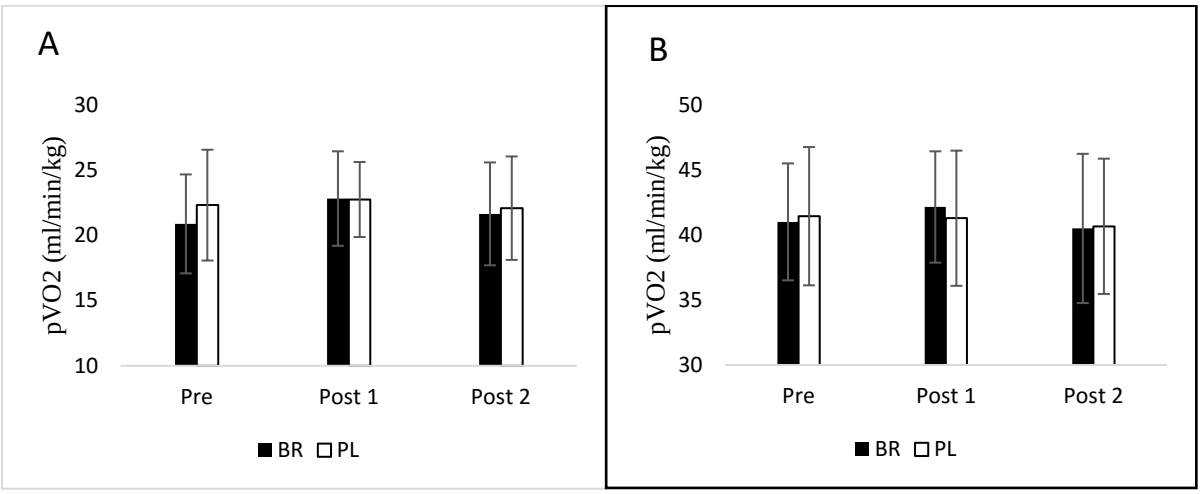


Figure 4. Effect of BR on pVO2 at end-exercise on LI (A) and HI (B). Pre: pre-test; Post 1: Acute post- test (2h); Post 2: Chronic post-test (6d).

Blood pressure

Figure 5 shows group mean (SD) of SP and DP from all tests **SP:** No main effect of supplementation (F(1,8) = 3.520, p = 0,097) was found. Main effect of time (F(2,16) = 5.455, p = 0.016), contrast revealed significant reduction in systolic blood pressure between pre-test and post-test 1 (p = 0.026). No interaction effect between supplementation x time (F(2,16) = 1.995, p = 0.168) was found. **DP:** No main effect of BR (F(1,8) = 0.370, p = 0.560) was

found. Significant main effect of time (F(2,16) = 4.523, p = 0.028) was found. Contrast revealed a significant reduction in diastolic blood pressure between pre-test and post-test 1 (p = 0.002), but no interaction effect between supplementation x time (F(2,16) = 3.115, p = 0.072) This shows that BR had no acute or chronic effect on BP in this study.

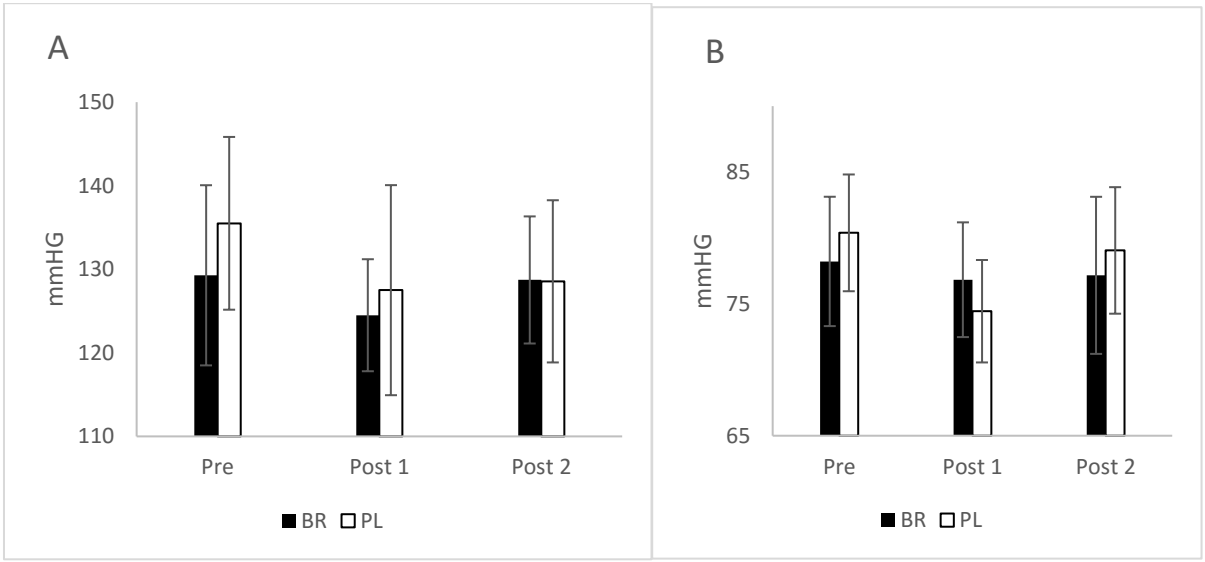


Figure 5. Group mean (SD) of systolic blood pressure (A) and diastolic blood pressure (B). Pre: pre-test; Post 1: Acute post- test (2h); Post 2: Chronic post-test (6d).

Heart rate

Data from one subject was removed from heart rate analysis due to missing data from one session. Group mean (SD) values for end-exercise HR at LI and HI are shown in Fig. 6. A two-way ANOVA for repeated measures were used on mean HR from the last minute of exercise to investigate the effect of BR. **LI:** No significant main effect of supplementation (F(1,7) = 0.276, p = 0.615) was found. Significant main effect of time was found (F(1.187, 8.311) = 12.5, p = 0.006) Contrast revealed significant elevation of HR between pre-test and post-test 1 (p = 0.001) and significant decreased HR between post-test 1 and post-test 2 (p = 0.008). No interaction effect between supplementation x test (F(2,14) = 0.081, p = 0.922) was found. **HI:** No significant main effect of supplementation on HR (F(1,7) = 2.364, p = 0.168) was found. No significant main effect of test (F(1.066, 7.459) = 1.220, p = 0.308), and no interaction effect of supplementation x test (F(1.085, 7.596) = 2.447, p = 0.158) was found. These results indicate that BR had no acute or chronic effect on HR on the last minute of exercise on both workloads.

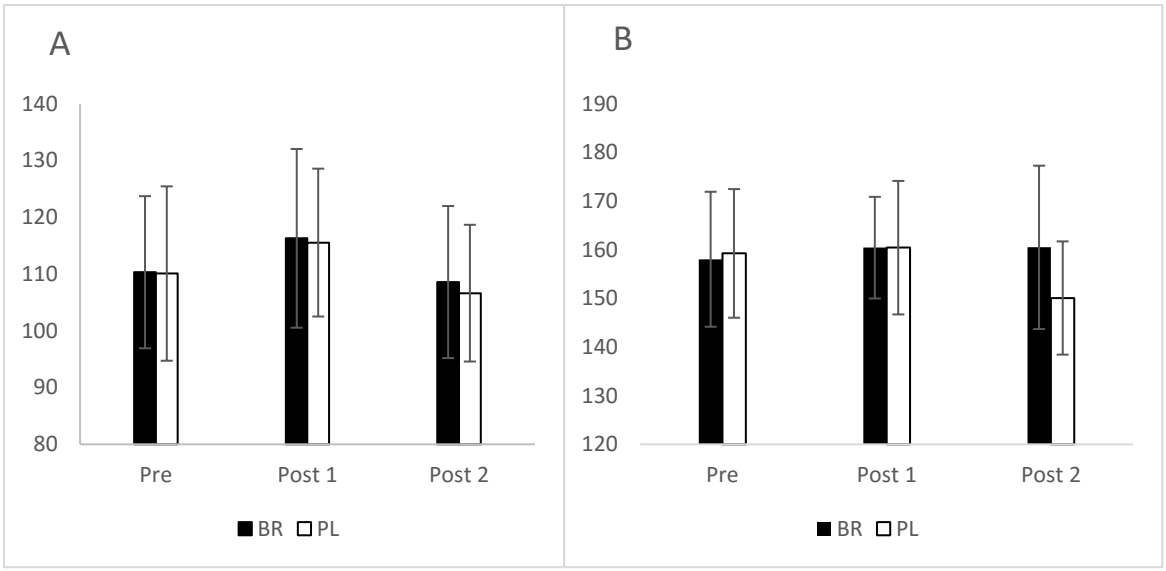


Figure 6. Effect of BR on HR at end-exercise on LI (A) and HI (B). Pre: pre-test; Post 1: Acute post- test (2h); Post 2: Chronic post-test (6d).

Muscle oxygen consumption recovery

The group mean (SD) values in figure 7 of mVO2-values during RRO reveals that fitting a mono-exponential curve to the values from HI is not ideal, due to prolonged elevation of mVO2-values at the first part of the RRO. Therefore, results of TC are only presented from LI in this thesis. Figure 8 shows group mean (SD) of TC from all tests after cycling on LI. No main effect of supplementation on TC ($F(1,8) = 0.074$, $p = 0{,}792$) was found, no main effect of test ($F(2,16) = 1.993$, $p = 0.169$) and no interaction effect between supplementation x test ($F(1.2, 9.8) = 0.386$, $p = 0.591$). Acute and chronic ingestion of BR did not improve TC after cycling on LI in this study.

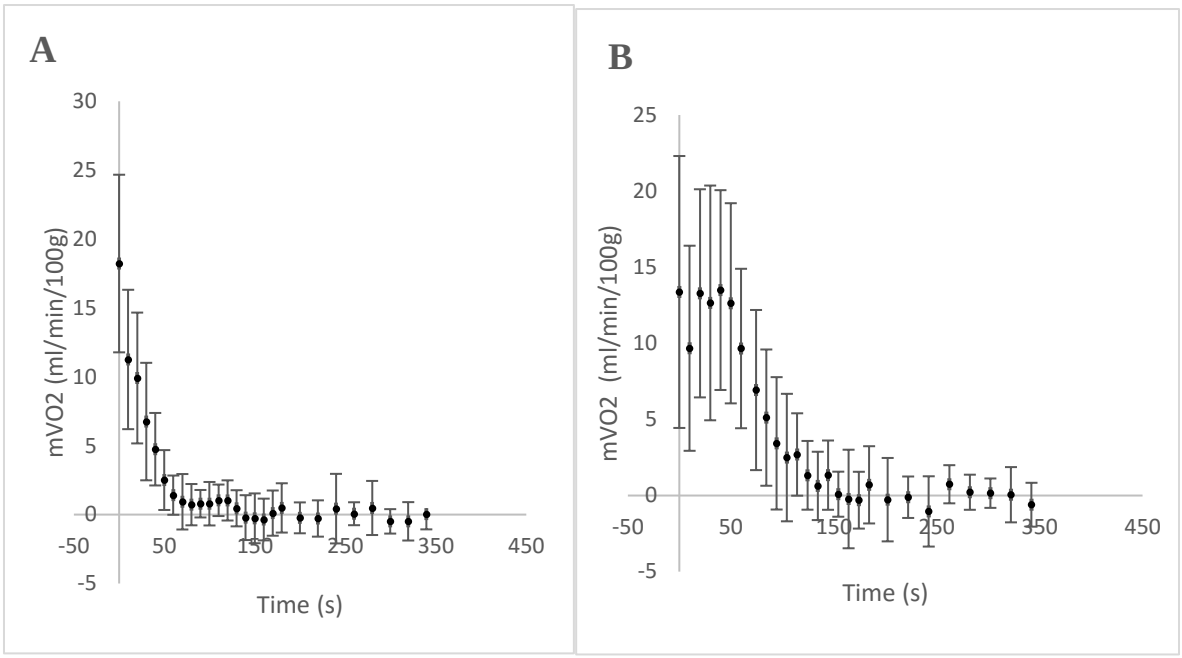


Figure 7. Group mean (SD) of mVO2-values during RRO after seven minute cycling on LI (A) and HI (B)

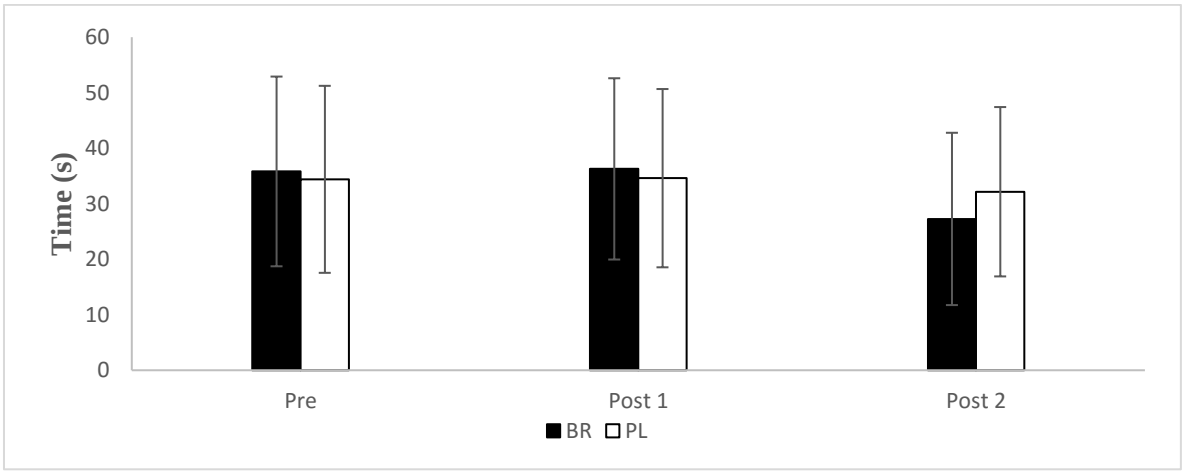


Figure 8. Group mean (SD) of TC from RRO after seven minute cycling on LI. Pre: pre-test; Post 1: Acute post-test (2h); Post 2: Chronic post-test (6d).

Lactate

Group mean (SD) values of blood lactate after seven minute cycling are shown in table 2. Group mean (SD) for resting blood lactate from all tests was 1.21 ± 0.32 mmol. Two way ANOVA for repeated measures found no main effect of supplementation on lactate after cycling on LI ($F(1, 8) = 0.821$, $p = 0.391$), no main effect of test ($F(2,16) = 0.687$, $p = 0.517$) and no interaction effect between supplementation x test ($F(2, 16) = 1.088$, $p = 0.361$) . This concludes that BR did not affect blood lactate in this study.

Table 2. Group mean (SD) values of lactate taken after seven minute cycling on LI and HI. All values are presented in mmol.

	Pre	Post 1	Post 2
LI			
PL	1.89 ± 0.71	1.74 ± 0.64	1.63 ± 0.45
BR	1.68 ± 0.52	2.06 ± 0.95	1.43 ± 0.35
HI			
PL	8.19 ± 2.08	7.31 ± 1.64	8.70 ± 2.38
BR	8.69 ± 1.78	8.47 ± 1.68	8.41 ± 1.92

Pre: pre-test; Post 1: Acute post- test (2h); Post 2: Chronic post-test (6d).

Discussion

The main purpose of this study was to investigate the acute and chronic effect of BR on mVO2-revovery following exercise. The main findings revealed no effect on mVO2-recovery following cycling on LI after both acute and chronic ingestion of BR. No acute and chronic effect of BR was found on pVO2 or HR on the last minute of LI- or HI-cycling, and no effect was found on diastolic or systolic blood pressure. BR did also not affect blood lactate. Complete analysis, of mVO2-recovery following cycling on HI, was not possible at the time of submitting this thesis. Due to an unforeseen prolonged elevation of mVO2- values at the first part of the RRO, proper fitting of a mono-exponential curve and precise calculation of a TC, could not be done.

Although mVO2-recovery was the main object in this study, we also measured changes in pVO2, HR and BP, in order to eventually back up any findings on mVO2-recovery. Surprisingly, we found no significant acute or chronic effect from BR on pVo2-results during cycling on LI and HI. Compared to several other studies, an effect on these parameters were expected. The dosage of nitrate used in this study (13 mmol, 900-1000mg nitrate) was sufficient to expect an effect. One study [31] investigated three different DNS dosages and found a significant effect on exercise performance with dosages of 8.4mmol and 16.8mmol but not with 4.2 mmol. No additional significant effect was found between 8.4 and 16.8 mmol. Another study found an effect with a dosage of only 5.2 mmol after 2.5 h, 5 days and 15 days of supplementation [29]. Analysis of the first five minutes of both pre-tests on both workloads showed no significant difference on pVO2. This proves that the subjects had a similar baseline before ingestion of either BR or PL and commencing the post-tests. This might rule out the study design as a possible reason why no effect was found in this study. The work rate for all sessions were also the same for all tests and other studies have found positive effects with similar workload [16, 31]. The subjects who participated in this present study were mainly recreationally active, and most had little experience with cycling on an ergometer bike. Activities ranged from team sports, running, sprint and strength training, but all subjects had a VO2max value below 60 ml/min/kg (49.3 ± 5.2 ml/min/kg). This was one of the exclusion criteria in this present study, as studies on subjects with a VO2max above 60, tend to find no effect from DNS [3, 5, 7, 20]. However, in some studies [5, 7], they found some subjects that individually responded to DNS. So having a VO2max above 60 might not always enough to determine if an effect from DNS is expected or not. There were also no

effect found on blood pressure in this study, even if it was expected when compared with other studies [13, 16, 29, 30]. The BP measurements in this study were done right after fitting the subjects with equipment, and within five minutes of baseline measurements. This might not have been the best procedure as the situation could be hectic, and subjects would not always be sufficient rested. Preferably, a longer resting period before commencing a BP-test would be better.

Since this present study found no effect on pVO2 or BP, it was not so surprising that also no effect was found on mVO2-recovery. Calculation of mVO2-recovery in this study were done by fitting a monoexponential curve to each mVO2-value from every RRO and then calculate a TC. This method is similar to the methods proposed by Motobe et al. (2004) and several studies by Ryan et al. [21-24]. In their studies, mVO2-recovery is used as a method to evaluate mithocondrial function. As mentioned earlier, increased mitochondrial efficiency is one of the proposed mechanisms behind the effect of DNS. Motobe et al. used NIRS and rapid occlusion following a light handgrip exercise in order to investigate if a training intervention, during an immobilized period of 21 days, would prevent a decline of oxidative function of the finger flexors. In one of the studies by Ryan et al. [21] they used NIRS, electrical stimulation and rapid occlusions in order to investigate mitochondrial function in subjects with spinal cord injury. This present study differs from earlier studies, as it is the first to investigate the effect of BR on mVO2-recovery with a dynamic whole body exercise. Earlier studies have in general used isolated exercises and light work load, and where probably less prone to motion artifacts. Greater involvement of the whole body might be a reason why this present study was unable to calculate mVO2-recovery on a higher intensity such as HI. In a study by [28], they experienced a hyperemic response after heavy hand grip exercise (75% of peak work load), which led to mVO2-values above end-exercise values. A too big oxygen deficit might be a reason why mVO2-recovery after cycling on HI was not possible to calculate. In order to calculate a more accurate TC, one suggestion might be to incorporate a delay in the curve fitting.

This present study was able to calculate mVO2-recovery following low intensity cycling (LI), but not on a higher intensity (HI). Further research is necessary to investigate the effect of more intense exercise on mVO2-recovery. Whether there is an effect of BR on mVO2-recovery is uncertain at this time.

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