Abstract

Purpose: Brief consecutive periods of limb ischemia and reperfusion induced by a blood pressure cuff, known as ischemic preconditioning (IPC), have been reported to increase maximal power output (MPO) and maximal oxygen consumption (VO_{2max}) during maximal incremental cycle ergometer tests. However, the underlying mechanisms are still unclear. Therefore, the purpose of the study was to investigate the effects of IPC on MPO, VO_{2max}, RPE, and underlying performance related parameters.

Methods: A double-blinded, randomized crossover study design was utilized to investigate the effects of IPC, consisting of four five-minutes cycles of ischemia interspersed with five minutes of reperfusion, on cycle ergometer performance, underlying physiological parameters, and rating of perceived exertion (RPE). Fourteen young, healthy men reported to the laboratory three times; one familiarization session and two intervention sessions, with two and seven days of rest and washout in between, respectively. After the familiarization test, in which the maximal incremental cycle ergometer test was completed, the participants were stratified and randomized into two different conditions (IPC and sham). During the two intervention sessions, in order to counterbalance, participants initially received either the IPC (250 mmHg) or sham (20 mmHg) treatment. Subsequently, the participants performed a step-transition test and a maximal incremental cycle ergometer test. During the second intervention session, the participants received the remaining treatment to complete the crossover. The researchers responsible for the cycling tests were blinded to the intervention, and the IPC and sham responsible researcher was absent while participants cycled. During both cycling tests, MPO, VO_{2max}, submaximal VO_2, rating of perceived exertion (RPE) on a Borg 6-20 scale, heart rate (HR), blood lactate concentration (BL) as well as NIRS-derived muscle oxygenation (tissue saturation index, TSI), ▲deoxygenated hemoglobin (▲HHb) and ▲oxygenated hemoglobin (▲O_2Hb) were measured.

Results: MPO, VO_{2max}, HR_{max}, and maximal deoxygenation (minimum TSI) did not significantly change with IPC compared to sham (all *P*-values > 0.13). Furthermore, IPC had no significant effect on VO_2, HR, TSI, ▲HHb, and ▲O_2Hb during the submaximal workloads of the incremental cycling test (all *P*-values > 0.18). However, IPC did significantly attenuate RPE during cycling at 245 W ($P = 0.007$) and 280 W ($P = 0.011$), but not at 105 W ($P = 0.145$), 140 W ($P = 0.034$), or 175 W ($P = 0.020$). The *P*-values at 140 W and 175 W were non-significant due to the Holm-Bonferroni correction of the significance level. Furthermore, IPC had no significant effect on VO_2, HR, BL, and RPE at 50 W and at 60% of ventilatory threshold in the step-transition test (all *P*-values > 0.11).

Conclusion: The present study demonstrated that IPC did not improve MPO and VO_{2max}, or affect any of the measured underlying physiological parameters, for young, healthy males during a maximal incremental cycle ergometer test and a step-transition test. However, IPC significantly attenuated the RPE of the participants during cycling at 245 W and 280 W.

Ischemic preconditioning does not improve maximal power output or maximal oxygen consumption but attenuates rating of perceived exertion – a crossover study.

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Introduction

Ischemic preconditioning (IPC) is a therapeutic phenomenon in which repeated periods of vascular occlusion are applied to a body part, originally to reduce infarct size from subsequent prolonged ischemia followed by reperfusion (Hausenloy & Lim, 2012). This was first demonstrated by Murry et al. (1986) in the canine heart, where infarct size in the heart following 40 minutes of ischemia was reduced by 75% after IPC compared with a control group.

More recently, IPC has been investigated as a method of enhancing performance (De Groot et al., 2010; Barbosa et al., 2011). De Groot et al. (2010) were the first to document the performance enhancing effects of IPC. They found that IPC increased maximal power output (MPO) and maximal oxygen consumption (VO_{2max}) in trained cyclists during a maximal incremental cycle ergometer test (De Groot et al., 2010). Since then, the effects of IPC on performance have been investigated for several different exercise modalities, such as strength-based exercise (Marocolo et al., 2016a), sprint-based exercise (Patterson et al., 2015), and time-trial exercise (Tocco et al., 2015). However, IPC is most frequently reported to increase performance in aerobic-based tasks compared to anaerobic- as well as sprint- and power-based tasks (Cocking et al., 2019; Salvador et al., 2016). Furthermore, the performance improvement with IPC is greater for aerobic performance compared to anaerobic as well as power and sprint performance.

Although the mechanisms for improved performance are unclear, several theories have been suggested. Hopper et al. (2000) demonstrated that adenosine and ATP-sensitive potassium (K_{ATP}) channels are, at least in part, responsible for the infarct-reducing effects of IPC. This was evident as an adenosine blocker reduced the protective effect of IPC (Hopper et al., 2000). Adenosine and K_{ATP} are also involved in the vasodilation and matching of oxygen (O_2) delivery to metabolic demands during exercise (Joyner & Proctor, 1999; Saltin et al., 1998). Therefore, De Groot et al. (2010) hypothesized that the increase in MPO and VO_{2max} in their study was due to an increase in adenosine, which has been shown to occur after IPC. They believed that increased adenosine levels would be able to improve O_2 delivery and matching, and thus increase aerobic exercise performance and VO_{2max} (De Groot et al., 2010). However, these biomarkers were not measured in the study. Nonetheless, other studies have also found aerobic performance increases following IPC with no increases in VO_{2max} (Crisafulli et al., 2011; Bailey et al., 2012). Because evidence has shown that exhaustion is regulated by the central nervous system (CNS) while an unused reserve of muscle fibers still remains (Noakes, 2011), it was hypothesized by Crisafulli et al. (2011) that IPC is able to desensitize the afferent nerves responsible for sending fatigue signals to the CNS. More specifically, it was suggested that IPC may reduce sensitivity of type III

and IV afferent nerves to fatigue signals, and hence increase the possible amount of active motor units before exhaustion occurs, resulting in greater force production (Crisafulli et al., 2011). Furthermore, it is suggested that the afferent nerve desensitization should result in reduced fatigue perception, thus attenuate ratings of perceived exertion (RPE) and hence delay exhaustion (Cruz et al., 2015).

Studies have shown reduced RPE during the fourth minute of constant load cycling at 100% of MPO to failure (Cruz et al., 2015) and during the first kilometer (km) of a five km run (Bailey et al., 2012) following IPC. Notwithstanding, other studies have reported that IPC had no effect on RPE during maximal incremental cycling (Jacobs et al., 2015) and 12 x 6 second (s) cycle sprints (Patterson et al., 2015). Therefore, the effects of IPC on RPE remain inconclusive (Incognito et al., 2016a).

However, it is suggested that IPC may be able to increase muscle contraction efficiency and lower ATP costs, hence decreasing oxygen consumption for a given workload (Crisafulli et al., 2011). The first exercise study to, in part, support this proposition demonstrated reduced blood lactate (BL) accumulation for the IPC condition compared to the sham condition in a crossover study when participants reached a running speed of 14 km/hour (h) during the submaximal part of a maximal incremental treadmill running test (Bailey et al., 2012). However, whether this was a result of reduced lactate production or increased lactate clearance rate remains unsolved (Bailey et al., 2012). Nonetheless, evidence of reduced aerobic metabolism has been found in pig skeletal muscle following IPC (Pang et al., 1995) and later in human skeletal muscle (Ambrozic et al., 2013). These studies provide evidence that muscle metabolism during vascular occlusion following IPC was lower, as Pang et al. (1995) demonstrated using radioactive microsphere technique (Pang et al., 1995), and Ambrozic et al. (2013) reported using near-infrared spectroscopy (NIRS), demonstrating that muscle tissue deoxygenation rate was reduced compared to control.

Another study showed that exercise endurance was increased in a work-to-work step-transition test on cycle ergometer which was concluded with cycling at severe intensity, at 70% of the difference between the ventilatory threshold (VT) and peak oxygen consumption (VO_{2peak}), until exhaustion (Kido et al., 2015). The researchers utilized NIRS to investigate the muscle deoxygenation dynamics during the step-transition test. The deoxygenated hemoglobin/myoglobin (HHb) was used as a measure for relative changes in muscle deoxygenation (Kido et al., 2015). The study demonstrated that the amplitude of the HHb was lower during moderate and severe exercise following IPC, indicating a lower O_2 consumption (VO_2) (Kido et al., 2015). Furthermore, the study showed that VO_2 was unchanged with IPC (Kido et al., 2015).

Similarly, Patterson et al. (2015) have used NIRS to assess tissue saturation index (TSI) during 12 x 6 s cycle sprints. The study demonstrated a greater maintenance in TSI during exercise following IPC compared to a sham procedure (Patterson et al., 2015). According to Patterson et al. (2015), this is an indication of improved O_2 delivery following IPC.

Provided the evidence above, it was hypothesized that IPC would increase MPO by reducing oxygen requirements during fixed workloads and/or increasing VO_{2max}, combined with attenuations of RPE.

Therefore, NIRS, VO_2, heart rate (HR), and BL measurements were utilized to investigate the effects of IPC on oxygen uptake dynamics, and more specifically on oxygen delivery and oxygen utilization during a maximal incremental exercise and a work-to-work step-transition test on a cycle ergometer. The latter was included to provide supportive information regarding VO_2 kinetics that may help explain any changes in MPO and VO_{2max}. Furthermore, a Borg 6-20 scale was used to investigate the effects of IPC on RPE.

Materials and methods

Participants

Fifteen young, healthy men participated in the present study. However, one participant broke the pre-study restrictions. Therefore, 14 participants' data were included in the study. Participant characteristics are summarized in Table 1. Furthermore, only non-smoking, non-diabetic and uninjured participants, free of cardiovascular illnesses, were included in the study. The study was approved by the Research Ethics Committee of North Denmark, Denmark (N-20170081), and all participants gave their informed, written consent. Vigorous training of any sort and blood flow restriction training were prohibited for 48 hours and seven days prior to any test sessions, respectively. Furthermore, consumption of caffeine, alcohol and analgesic medication were not allowed for 24 hours prior to any of the tests.

Table 1: Participant characteristics ($n = 14$)
SD = standard deviation.

Characteristic	Mean (± SD)
Age (yr)	24.9 (± 2.1)
Height (cm)	186.0 (± 5.0)
Weight (kg)	85.9 (± 12.2)
Cardiovascular fitness rating (ml $O_2/kg/min$)	45.3 (± 5.6)
Physical activity (h/wk)	7.1 (± 3.7)

Study design

A double-blinded, randomized crossover study design was utilized to investigate the effects of IPC on MPO, underlying physiological parameters, and RPE. The study consisted of three individual testing sessions for each participant comprising one familiarization session and two intervention sessions (IPC and sham intervention). A minimum of 48 hours of rest and seven days of washout were prescribed after the familiarization test and the first intervention session, respectively (see Figure 1).

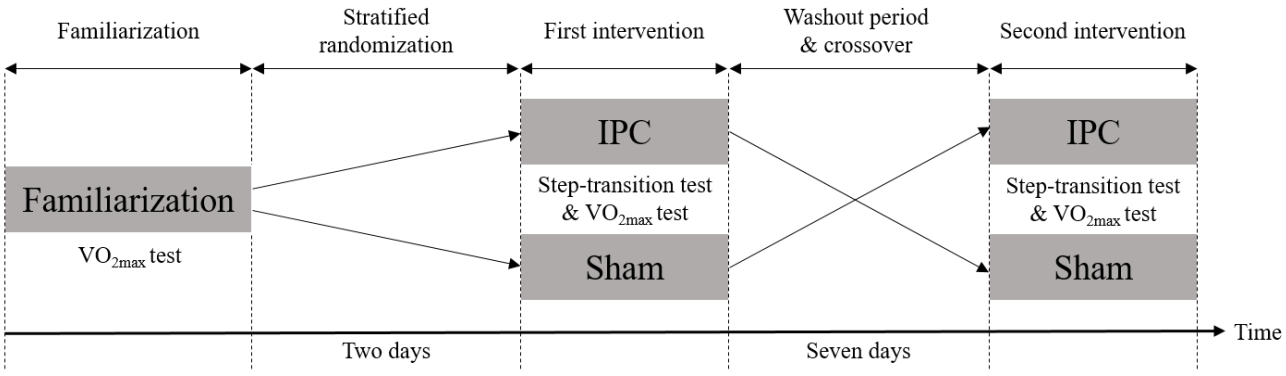

Figure 1. Schematic overview of the study design. IPC = Ischemic preconditioning. Randomization was stratified with respect to age. VO_{2max} test = Maximal incremental cycling test.
Both cycle ergometer tests were performed after receiving IPC/sham treatment.

During the familiarization session, participants were familiarized with the research equipment and the pressure of the cuffs during IPC for one minute and performed the maximal incremental cycle ergometer test. Furthermore, the participants were informed about the two following sessions. In an attempt to blind the participants, the participants were informed that the purpose of the study was to investigate the different effects of two cuff pressures on cycling performance. The researchers were blinded by assigning one researcher to manage the IPC and sham treatments, while this researcher was not present during the cycle tests and had no influence on the outcomes thereof. The two following sessions, the participants received either the IPC or sham treatment in randomized and counterbalanced order stratified by age. Subsequently, the participants completed the step-transition test, followed by a standardized five-minute break before commencing the maximal incremental cycle ergometer test (see Figure 2).

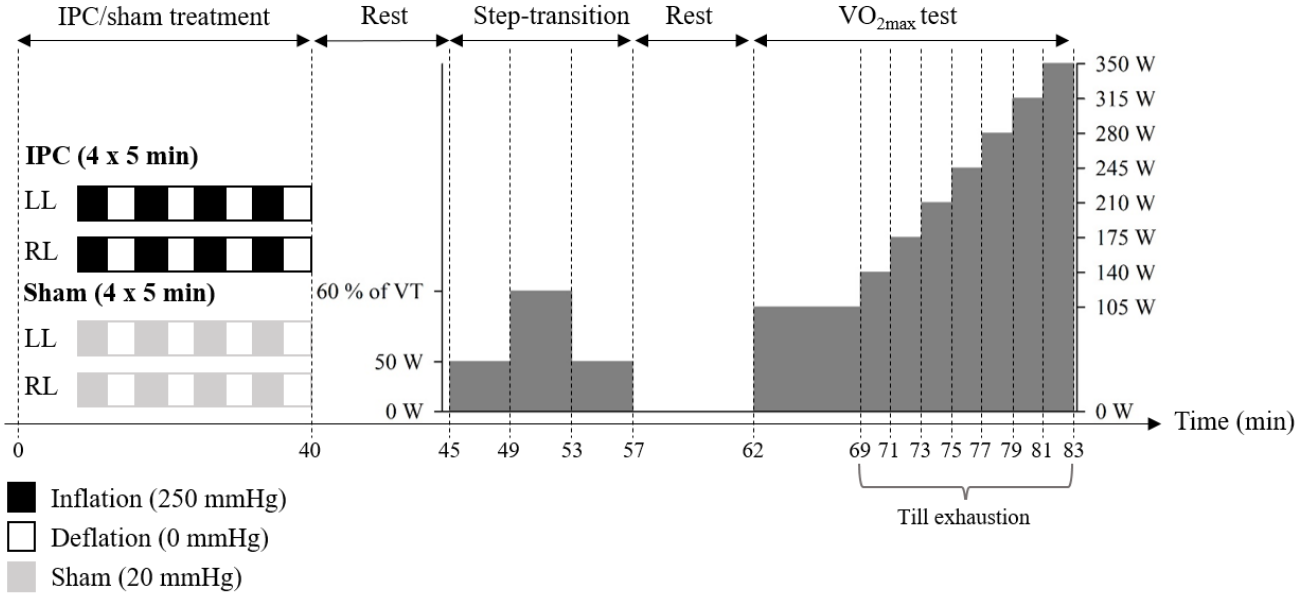

Figure 2. Schematic overview of an intervention session. IPC = ischemic preconditioning. LL = left leg. RL = right leg. Step-transition = step-transition test. VO_{2max} = maximal incremental cycle ergometer test. The maximal incremental cycling test continued until exhaustion. During both rest periods participants were passive and seated. NIRS data were collected continuously throughout the entire session.

Near-infrared spectroscopy (NIRS)

The NIRS optode was placed on the right vastus lateralis (VL) muscle belly of the participant for the full duration of each session, and NIRS data were collected continuously throughout the test sessions with a sampling frequency of 2 Hz. The optode placement was standardized using SENIAM's recommendations for surface electromyography electrode placement[1] (see Figure 3A and 3B). Before fixating the NIRS optode, the

[1] http://Seniam.org

skin area was shaved and cleaned with alcohol swabs. Furthermore, the optode cable was fixed to the participant's right hip before the cycling tests to minimize signal noise due to cable movement (Artinis Medical Systems, 2012). At the first intervention session, the optode borders were marked with a marker, and the participants were instructed to maintain the marks until the second intervention for identical placement of the optode. NIRS measurements were collected using the Oxymon Mk III (Artinis Medical Systems, PW, Elst, The Netherlands), which was powered by OxySoft (OxySoft/DAQ version 2.1.6, Artinis Medical Systems, PW Elst, The Netherlands).

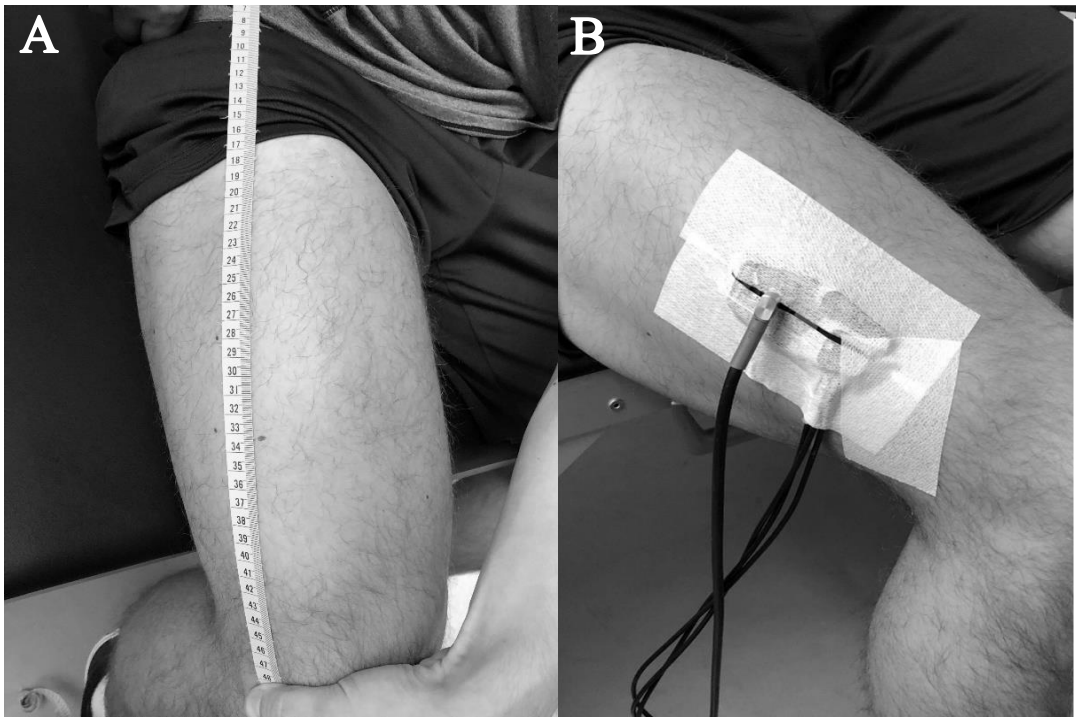

Figure 3. A. shows measurement of location for placement of near-infrared spectroscopy (NIRS) optode on vastus lateralis using SENIAM guidelines.
B. illustrates mounted NIRS optode.

Different types of NIRS measurements were obtained and used in the study: HHb, Oxygenated hemoglobin[2] (O_2Hb), and TSI. HHb can be interpreted as a measure of O_2 extraction (Jones et al., 2016). O_2Hb data were included as a supportive measure for interpretation of TSI. HHb and O_2Hb are expressed as ▲HHb and ▲O_2Hb respectively, since both are defined as a relative change from resting baseline values. Baseline was set as a mean of 120 seconds (i.e. the last 240 data points) while the participants were placed in a resting supine position before receiving IPC/sham treatment. TSI is an absolute percentage of oxygenated Hb relative to total hemoglobin (THb) (Artinis Medical Systems, 2012). The data for ▲HHb, ▲O_2Hb and TSI at submaximal workloads were calculated as means of the last 15 seconds (i.e. the last 30 data points) of measurement of each workload, and the value for TSI at exhaustion was calculated as the last 15 seconds before the test was terminated.

[2] NIRS does not distinguish between hemo- and myoglobin. Therefore Hb denotes both hemo- and myoglobin in the present study.

Ischemic preconditioning and sham protocol

The IPC and sham procedures consisted of four five-minute cycles of cuff inflation and deflation, resulting in a total duration of 40 minutes. The IPC and sham procedures were executed using a sphygmomanometer (WelchAllyn Durashock Silver, DS-5601-300, New York City, NY, USA), and a tourniquet cuff (Tourniquet cuff, VBM, Sulz am Neckar, Germany) placed proximally on both thighs with participants in supine position (see Figure 4). The applied pressure was 250 mmHg for the IPC procedure and 20 mmHg for the sham procedure. Furthermore, the NIRS system was used to confirm arterial occlusion during the IPC treatment, which was interpreted from a steady state in total hemoglobin.

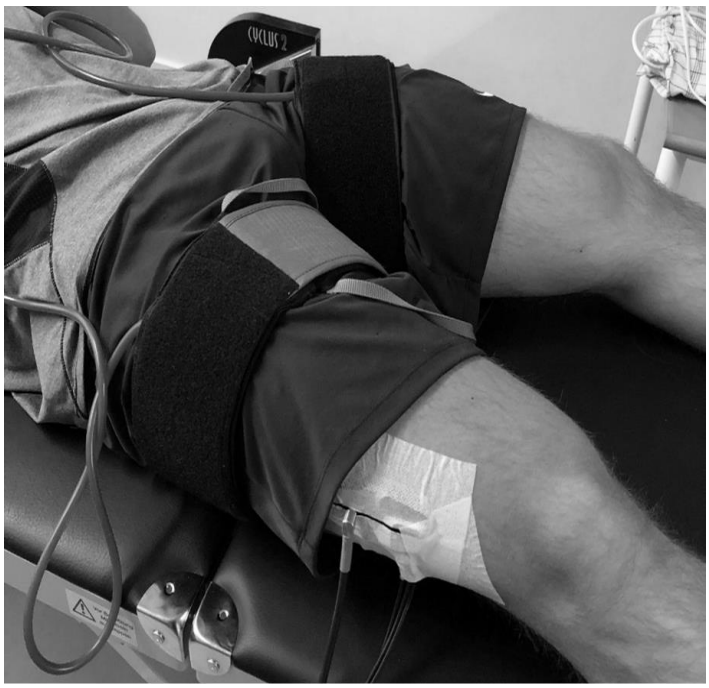

Figure 4. Illustrates the setup for treatment with ischemic preconditioning and sham.

Maximal incremental cycle ergometer test

The VO_{2max} test was performed on a mechanically braked cycle ergometer (Monark Ergomedic 894 E, Monark Exercise AB, Vansbro, Sweden). For the setup of the cycle ergometer tests, see Figure 5. The test protocol was inspired by Andersen (1995). The test started with seven minutes of warm-up at 105 W, followed by an increment of 35 W after the warm-up as well as every following two minutes (see Figure 2). The test was cycled with 70 revolutions per minute (RPM), which was managed using a metronome set at 70 beats per minute (BPM). The participants were blinded to the power output and the exercise time during the maximal incremental test. Verbal encouragement was given to ensure that VO_{2max} was achieved and that the participants cycled until exhaustion. During the early stages, verbal encouragement was given at least every minute, and as cycling became more challenging for the participant, the frequency of verbal encouragement was increased. When exhaustion was near,

Figure 5. Illustrates the setup for the step-transition test and the maximal incremental cycle ergometer test.

which was evaluated through RPE, HR and general visual inspection of the participant, verbal encouragement was given continuously. When the participant could not sustain a cadence of 70 RPM, which was evaluated by the researcher, the test was terminated.

Fifteen seconds before each increment, RPE on a Borg 6-20 scale (Borg, 1982) was collected. Furthermore, HR was noted at, and averaged for, 15, 10 and 5 seconds before each increment using a HR watch and belt (Suunto Ambit3 Run, Suunto, Vantaa, Finland). After exhaustion, participants were asked to keep pedaling with the ergometer unloaded, and BL concentration was measured one minute after exhaustion. BL was sampled using finger prick safety lancets (Vivomed, Downpatrick, United Kingdom), Accutrend lactate test strips (Accutrend, Roche, Rotkreuz, Switzerland), and a portable lactate measuring device (Accutrend Plus, Roche, Rotkreuz, Switzerland). Furthermore, MPO was calculated as the power output of the last completed workload plus 35 W times the percentage cycled of the following interval.

For the determination of VO_{2max}, VO_2 data were averaged in 30 second intervals, and the highest VO_2 was located. As suggested by Duncan et al. (1997), the following criteria were used to validate the attainment of VO_{2max}: 1. a plateau in VO_2; 2. a maximal heart rate (HR_{max}) of maximum 10 BPM below the estimated HR_{max}, calculated as 208 - 0.7 x age (Tanaka et al., 2001); and 3. BL concentration above 8 mmol/l. Therefore, VO_{2max} was considered achieved once two of the three above criteria were fulfilled.

Furthermore, for submaximal VO_2 values, data were exported in 5-second intervals and averaged from 30 to 5 seconds prior to each increment to avoid that data from the next interval were included.

Gas exchange was measured using an automated gas analysis system (Jaeger Vyntus, CareFusion, Hoechberg, Germany) powered by SentrySuite software (Version 2.19.2, SentrySuite, CareFusion, Hoechberg, Germany). Prior to each cycle test, the system was both gas and flow calibrated. The gas analyzers were calibrated using a gas tank with a known concentration of O_2 (15%) and CO_2 (5%). The flow analysis was an automatic two point flow calibration.

Furthermore, the same gas tube was used for each participant every time. The gas analysis system was mounted and recorded for the full duration of both cycle ergometer tests.

Step-transition test

A 12-minute step-transition test on the bicycle ergometer was implemented to investigate steady state exercise economy. The test consisted of three intervals lasting four minutes each: 1. A steady state warm-up phase at 50 W with 50 RPM, 2. an on-transition phase at 60% of VT with an RPM of 70, 3. and an off-transition phase at 50W with 50 RPM. The VT was identified before the first intervention through the gas exchange data from the first maximal incremental cycle test, which was performed at the familiarization test. For the determination of VT, gas exchange data were exported in 30 second intervals. The VT was identified using methods described by Amann et al. (2004): 1. the point at which VCO_2 (plotted as a function of time) increased disproportionately

to VO_2 (see Figure 6a). 2. The point at which the ventilatory equivalent of CO_2 (VE/CO_2) increased disproportionately to the ventilatory equivalent of O_2 (VE/VO_2) (see Figure 6b). The workload at VT was determined as the power output of the last completed workload plus 35 W times the percentage cycled of the interval in which VT occurred.

The average workload (± standard deviation (SD)) at 60% of VT was 121 W (± 17.7). During the test, RPE and BL measurements were collected three minutes into each phase, which according to Burnley & Jones (2007) is the time for achievement of steady state when transitioning to moderate intensity. Three HR measurements were collected, and averaged, after steady state at each interval, i.e. at 3:00, 3:05, and 3:10, and 7:00, 7:05, and 7:10 as well as at 11:00, 11:05, 11:10 of the off-transition phase.

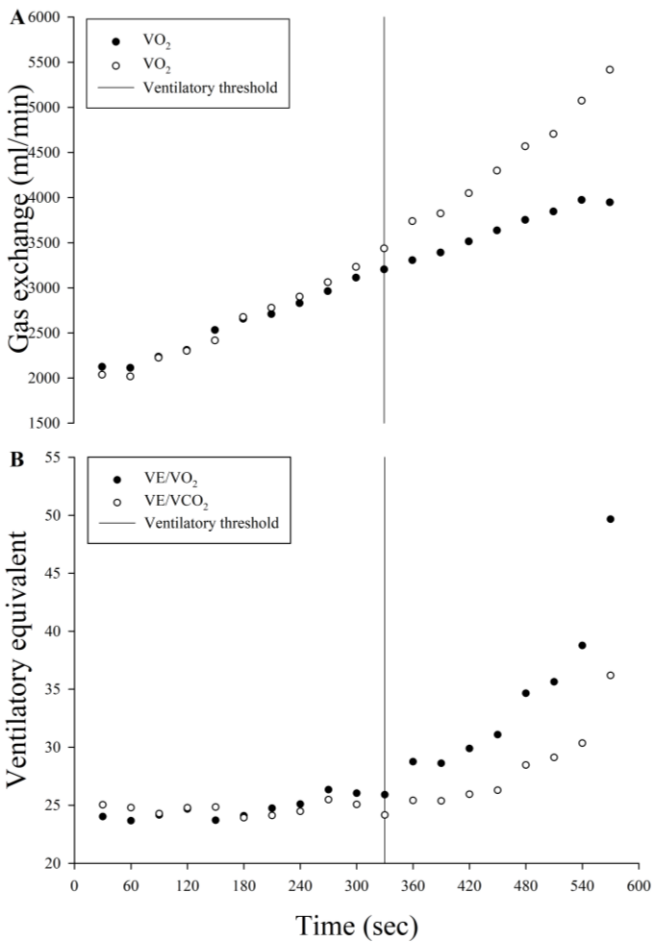

Figure 6. Shows respiratory data for a participant during a maximal incremental cycling test. The vertical line denotes the ventilatory threshold of this particular participant. VE = Ventilation. **A**. Illustrates the V-slope method. **B**. Illustrates the ventilatory equivalent method. Ventilatory equivalent of oxygen (VE/O2) and carbon dioxide (VE/CO2). (Graph A: n = 14; graph B, C, and D: n = 13).

Statistical analysis

Data were analyzed using SPSS (IBM CORP, version 25.0, Armonk, NY). The statistical significance level was set to an alpha-value of 0.05. Parametric data (i.e. all data except RPE) for all participants are presented as mean values ± SD. For the parametric data, the Shapiro-Wilk test was used to check for normality. All data, except ▲HHb at 105 W, were normally distributed. In the maximal incremental cycling test, all results for parameters at exhaustion (i.e., MPO, HR_{max}, TSI, and BL) and VO_{2max} data were analysed using two-tailed paired *t*-tests. Data for submaximal workloads (i.e. VO_2, HR, TSI, RPE, ▲HHb, and ▲O2Hb) were analyzed using a two-way repeated measures analysis of variance (ANOVA) to calculate the main effects of condition (IPC, sham), workload (105 W, 140 W, 175 W, 210 W, 245 W) and to check for interaction (condition [2] x workload [5]). For the post-hoc analysis, Bonferroni corrections were applied to counteract the problem of multiple comparisons. For analysis of the step-transition data, a two-way repeated measure ANOVA was used to check for interaction (condition [2] x workload [2]) and to calculate the main effect of condition (IPC, sham) and workload (50 W, 60 % of VT). In all tests, if the assumption of sphericity was not fulfilled, Greenhouse-Geisser corrected degrees of freedom were applied.

For the non-parametric data (i.e. RPE) of both the maximal incremental cycling test and the step-transition test, each of the five and two workloads, respectively, were compared between IPC and sham conditions using the Wilcoxon signed rank test. The Holm-Bonferroni correction was applied to counteract the multiple comparison error, and thus the alpha-values were set according to the Holm-Bonferroni calculations and the number of comparisons (Holm, 1979). RPE data are presented as median values with interquartile range.

Results

Results for parameters at exhaustion and VO_{2max}

For all results regarding VO_{2max} as well as TSI, MPO and HR at exhaustion, see Figure 6. RPE at exhaustion was not included, because an RPE score of 20 was one of the criteria for the achievement of VO_{2max}, hence all participants ended with an RPE score of 20.

Maximal Power Output (MPO). MPO did not significantly differ between IPC and sham condition $t(13) = -1.163$, $P = 0.266$ (see Figure 7a and Table 2).

Maximal Oxygen Consumption (VO2max). VO_2 data for one participant were excluded due to a miscalibration (therefore, $n = 13$). The paired *t*-test showed that VO_{2max} did not significantly differ between conditions $t(13) = 1.070$, $P = 0.304$ (see Figure 7b and Table 2).

Maximal Heart Rate (HRmax). HR measurements for one participant were not obtained due to a technical malfunction. The paired *t*-test showed that HR_{max} did not significantly differ between conditions $t(12) = 1.223$, $P = 0.245$ (see Figure 7c and Table 2).

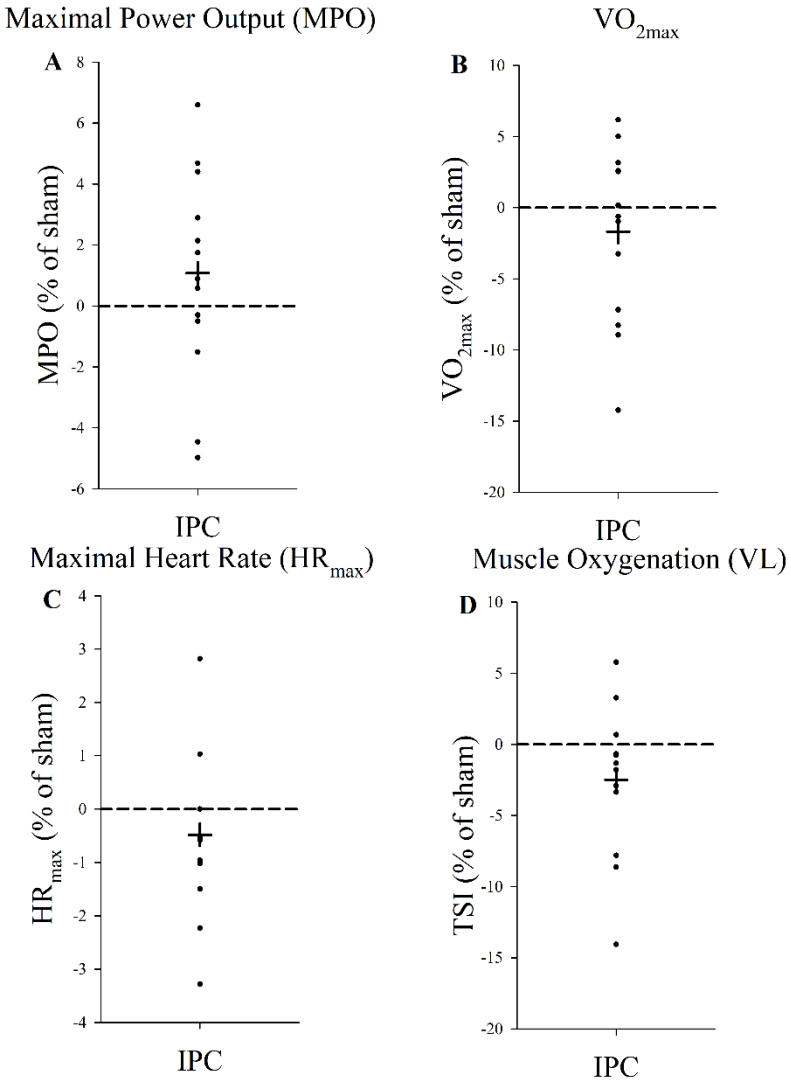

Figure 7. Shows individual data for all participants at exhaustion in the maximal incremental cycle ergometer test. Results are presented as the IPC condition normalized to the sham condition, meaning that a negative value denotes a decrease in performance compared to sham, and a positive value denotes an increase. The dashed line at 0 on the y-axis is the value from the sham condition. + Represents mean value. VL = Vastus Lateralis. TSI = Tissue saturation index.

Tissue Saturation Index (TSI). One measurement for TSI was excluded since the difference between the two conditions was >80 %, which was deemed invalid (therefore, n = 13). TSI did not significantly differ between sham and IPC condition $t(12) = 1.598$, $P = 0.136$ (see Figure 7d and Table 2).

Blood lactate. Due to large deviations from trial to trial, it was decided that any deviations greater than 50% were removed from the dataset (therefore, n = 9). However, IPC had no significant impact on BL accumulation following the maximal incremental test $t(13) = 0.980$, $P = 0.467$ (see Table 2).

Table 2: Mean values (± standard deviation) at exhaustion.

	Sham	IPC	P-value
Maximal power output (W) ($n = 14$)	293.0 (± 28.2)	296.2 (± 29.5)	$P = 0.266$
Tissue saturation index (%) ($n = 13$)	57.9 (± 10.1)	56.6 (± 10.9)	$P = 0.136$
Maximal oxygen consumption (ml/min) ($n = 13$)	3806.4 (± 336.0)	3740.7 (± 385.0)	$P = 0.304$
Maximal heart rate (BPM) ($n = 13$)	187.5 (± 10.2)	186.6 (± 10.0)	$P = 0.245$
Blood lactate (mmol/l) ($n = 9$)	14.3 (± 3.2)	13.5 (± 3.3)	$P = 0.467$

Results for submaximal workloads

Data were included from the five first workloads of the maximal incremental cycling test (i.e 140 W, 175 W, 210 W, 245 W, and 280 W). Including data for all completed intervals would reduce the data to three participants, and including data from all participants would result in only four intervals for the analyses. Therefore, it was decided that a compromise between the number of participants and intervals included was the optimal solution (therefore, n = 10). Data for one representative participant can be seen in Figure 8.

Oxygen consumption (VO_2). There was no significant interaction between condition and workload $F(4,36) = 1.978$, $P = 0.119$. Furthermore, there was no significant difference betweens conditions $F(1,9) = 2.095$, $P = 0.182$. Moreover, VO_2 data showed a significant main effect of workload $F(4,36) = 548.908$, $P < 0.001$. The post-hoc analysis showed significant differences between each interval (all P-values < 0.001). For submaximal VO_2 results, see figure 9A.

Heart rate (HR). There was no significant interaction between condition and workload $F(4,32) = 1.170$, $P = 0.343$. Furthermore, there was no significant main effect of condition $F(1,8) = 4.336$, $P = 0.071$.The submaximal HR showed a significant main effect of workload $F(4,32) = 184.478$, $P < 0.001$. The post-hoc analysis showed significant differences between all intervals (all P-values < 0.001). For submaximal HR results, see Figure 9b.

Tissue Saturation Index (TSI). There was no significant interaction between condition and workload $F(1.878,16.898) = 1.210$, $P = 0.320$. Furthermore, there was no significant main effect of condition $F(1,9) = 0.011$, $P = 0.918$. However, there was a significant main effect of workload for TSI $F(2.013,18.118) = 55.885$, $P < 0.001$. The post-hoc analysis showed that the differences between all workloads were significant (P-values < 0.014) except between 245 W and 280 W ($P = 0.055$). For submaximal TSI results, see Figure 9C.

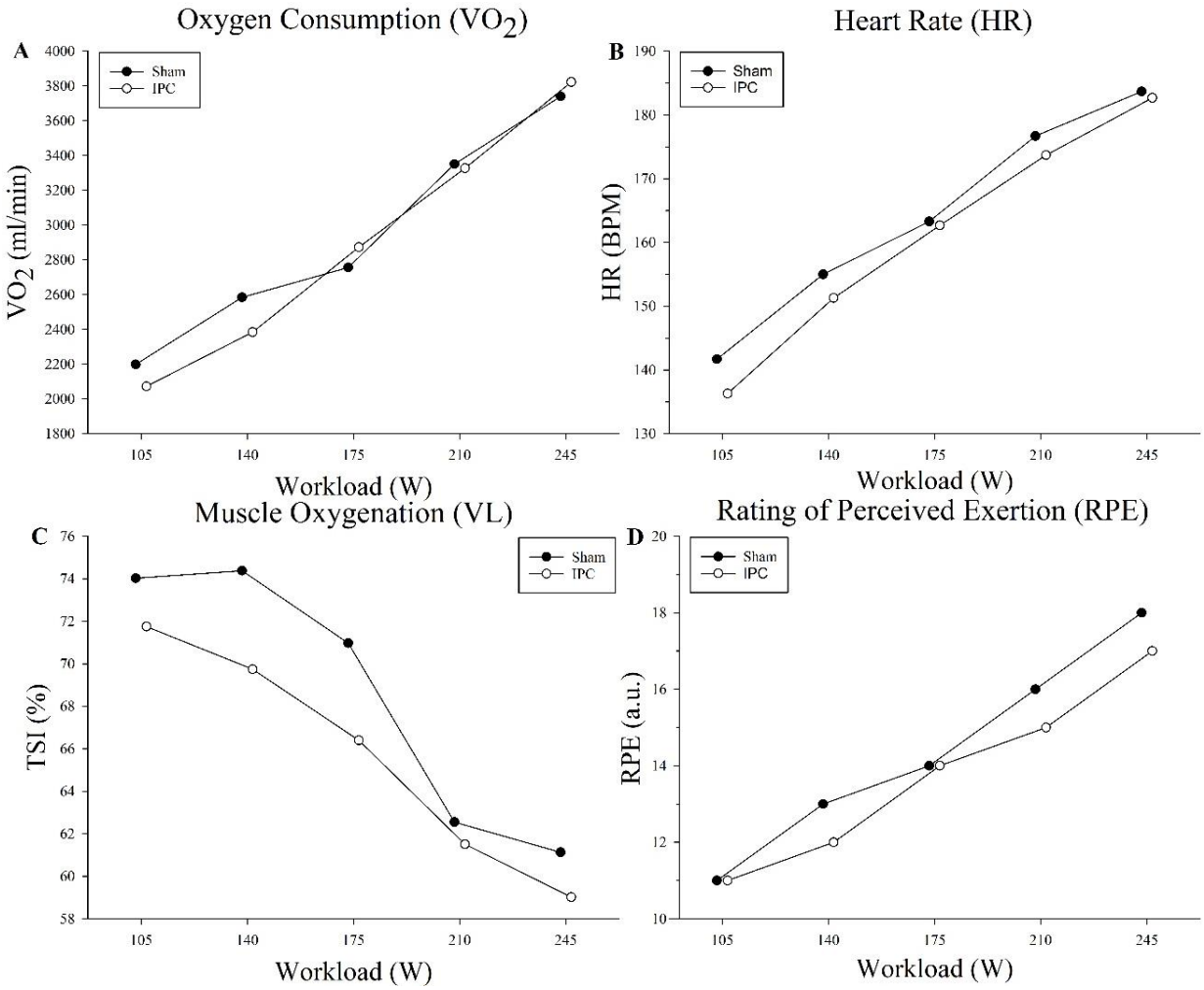

Figure 8. Illustrates data for one representative participant from the maximal incremental cycle ergometer test. VL = Vastus Lateralis. TSI = Tissue saturation index. A.u. = arbitrary units (Borg 6-20 scale).

Rating of Perceived Exertion (RPE). The Wilcoxon signed rank tests of RPE showed that participants reported significantly lower RPE at 245 W (P = 0.007) and 280 W (P = 0.011) in the maximal incremental cycling test after IPC compared to the sham condition. However, no significant differences were found between RPE values of the two conditions at 105 W (P = 0.145), 140 W (P = 0.034), or 175 W (P = 0.020) in part due to the Holm-Bonferroni correction of the significance level. For RPE results, see Figure 9D.

▲Deoxygenated hemoglobin (▲HHb). Data for ▲HHb showed no significant interaction between condition and workload $F(4,36) = 1.641$, $P = 0.185$. Furthermore, was there no significant main effect of condition $F(1,9) = 1.593$, $P = 0.239$. However, there was a significant main effect of workload $F(1.251,11.263) = 49.592$, $P < 0.001$. The post-hoc analysis showed that all workloads differed significantly from each other (All P-values $\leq$ 0.011). For ▲HHb results, see 10A.

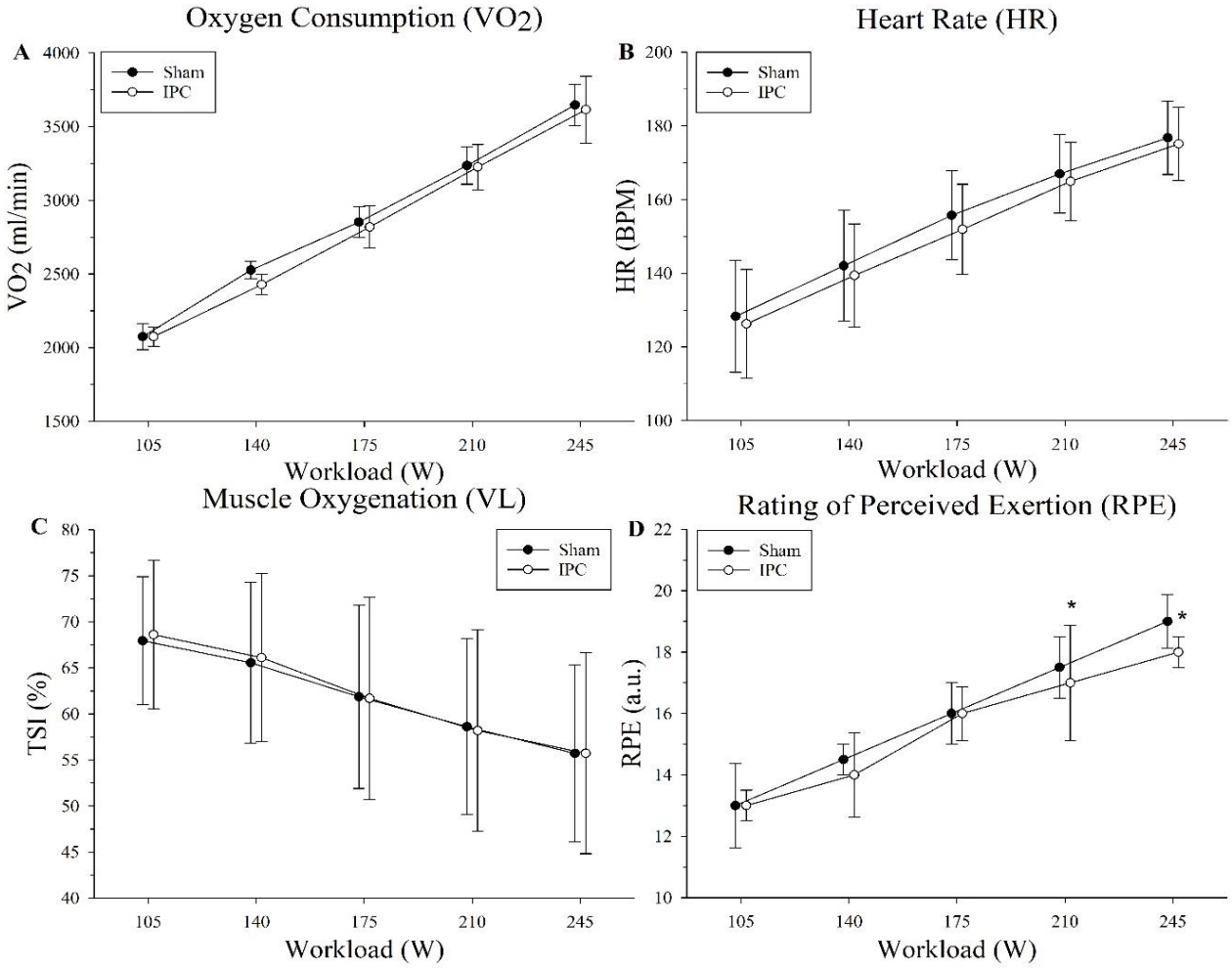

Figure 9. Mean results for all participants (graph A and C: n = 10, graph B: n = 9, graph D: interval 1-4: n = 14 and interval 5: n = 10). Graph A, B, and C are presented as mean values, and error bars represent standard. Graph D presents values as medians, and error bars are interquartile range. VL = Vastus Lateralis. TSI = tissue saturation index. A.u. = arbitrary units (Borg 6-20 scale). *Significant difference between conditions (P-value < 0.05)

▲Oxygenated hemoglobin (▲O2Hb). Data for O_2Hb showed no significant interaction between condition and workload $F(1.972,17.747) = 2.925$, $P = 0.080$. Nor was there a significant main effect of condition $F(1,9) = 0.203$, $P = 0.663$. However, there was a significant main effect of workload $F(1.587,14.283) = 28.900$, $P < 0.001$. The post-hoc analysis showed that values differed significantly between all workloads (all P-values ≤ 0.019), except between 105 W and 140 W, as well as between 105 W and 175 (both P-values > 0.05). For ▲O_2Hb, see Figure 10B.

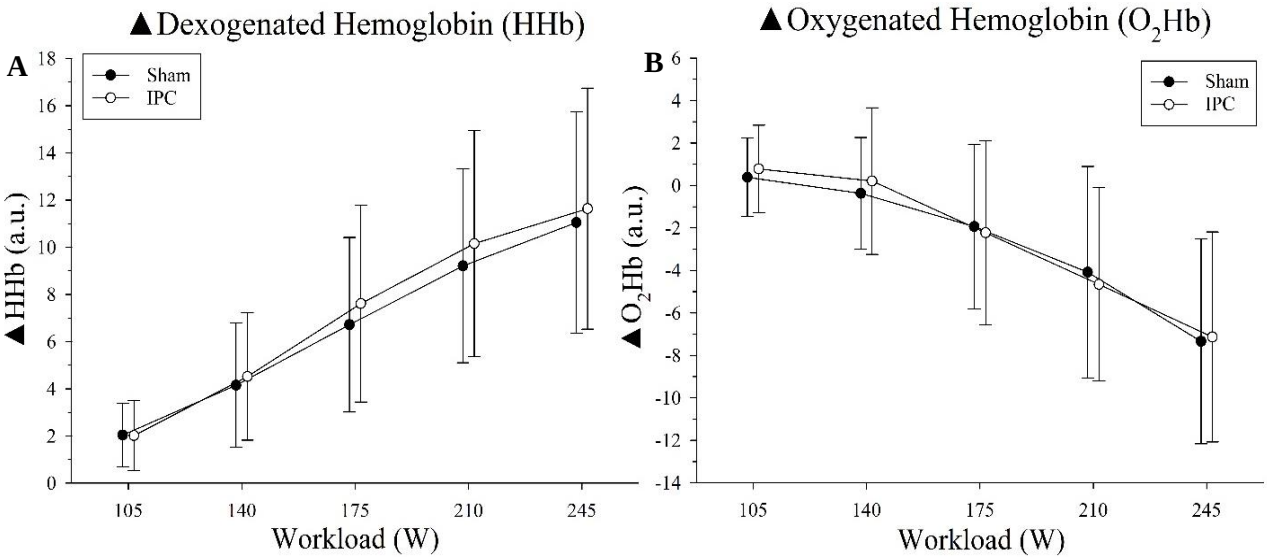

Figure 10. The graph displays mean oxygenation results for vastus lateralis during the maximal incremental cycling test for all participants (n = 10). Both graphs are presented as mean values, and error bars represent standard deviations. ▲ denotes a difference from resting baseline.

Step-transition test

Oxygen Consumption (VO_2). There was no significant interaction between condition and workload $F(1,12) = 0.008$, $P = 0.928$. Furthermore, there was no significant main effect of condition $F(1,12) = 0.001$, $P = 0.992$. However, there was a significant main effect of workload $F(1,12) = 149.915$, $P < 0.001$ meaning that the VO_2 increased during the on-transition for both conditions. Results regarding VO_2 from the step-transition test can be seen in Figure 11A.

Heart Rate (HR). There was no significant interaction between condition and workload $F(1,11) = 0.528$, $P = 0.483$. Furthermore, there was no significant main effect of condition $F(1,11) = 2{,}938$ $P = 0.114$. However, there was a significant main effect of HR $F(1,11) = 161.075$, $P < 0.001$, meaning tht HR increased significantly for both conditions during the on-transition. For results regarding HR in the step-transition test, see Figure 11B.

Blood Lactate (BL). The results showed no significant interaction between condition and workload $F(1,8) = 0.610$, $P = 0.457$. Furthermore, there was no significant main effect of condition $F(1,8) = 0.657$, $P = 0.441$. Moreover, there was no significant main effect of workload $F(1,8) = 4.172$, $P = 0.075$. For BL results from step-transition test, see Figure 11C.

Rating of perceived exertion (RPE). The Wilcoxon signed rank tests showed no difference between conditions for RPE data at 50 W ($P = 0.492$) or 60 % of VT ($P = 0.417$). For RPE results from the step-transition, see Figure 11D.

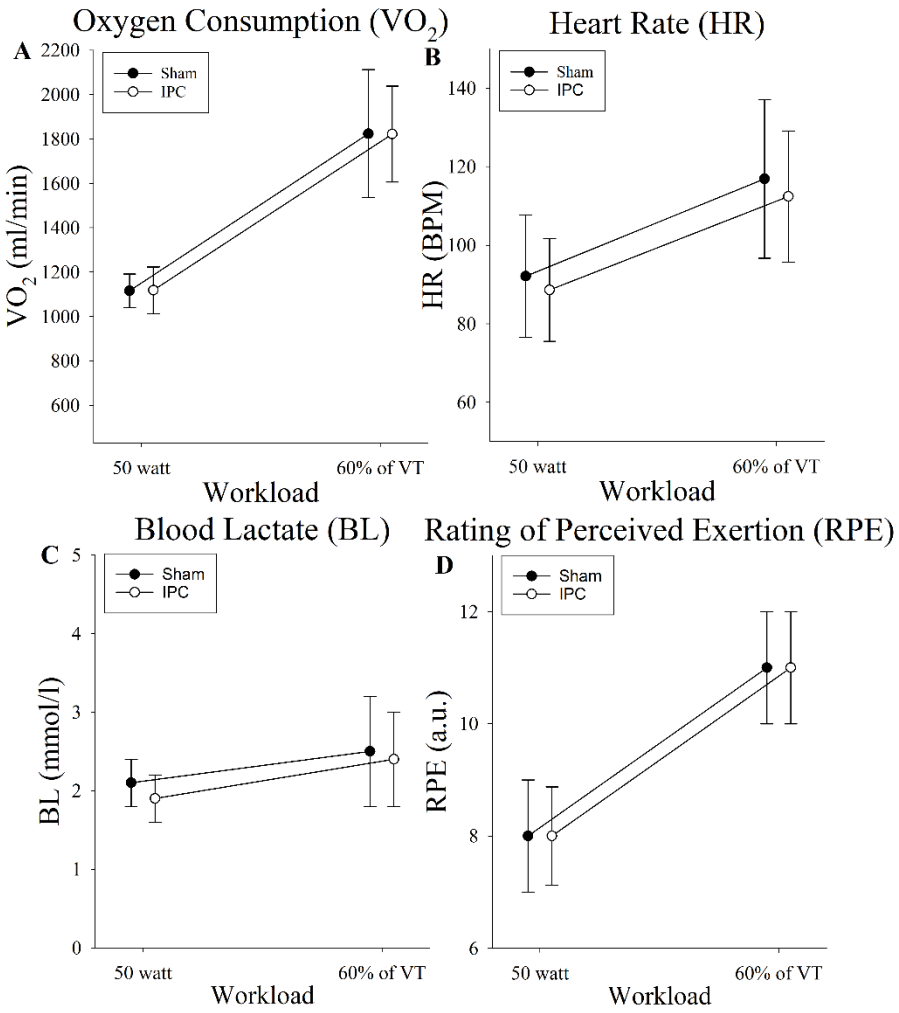

Figure 11. Illustrates results from step-transition test for all participants (A, B, and D *n* =13, C *n* = 9). All data are presented as mean values, and error bars represent standard deviations. VT = Ventilatory threshold.

Discussion

The purpose of the present study was to investigate the effects of IPC primarily on MPO, VO_{2max}, and RPE in a maximal incremental cycle ergometer test. The study also includes secondary supportive measurements of HR, BL, and TSI at exhaustion, as well as as VO_2, RPE, HR, BL, TSI, ▲HHb, and ▲O_2Hb at submaximal workloads during both the incremental test and step-transition test to elucidate the possible performance increasing effects of IPC. In this study, IPC did not have an effect on MPO, VO_{2max} or any of the secondary physiological parameters. However, IPC did significantly attenuate RPE during the incremental test at 210 W and 245 W.

Maximal power output (MPO)

The present study found that a single session of IPC did not improve MPO during a maximal incremental cycling test for young, healthy men. This is in contradiction to previous studies (De Groot et al., 2010; Crisafulli et al., 2011). Nonetheless, the present study is not the only study to report no significant performance increases during incremental cycling (Clevidence et al. 2012).

However, differences in study design may be able to explain the different outcomes of this study. For example, neither participants nor the researchers in the study by De Groot et al. (2010) or Crisafulli et al. (2011) were blinded to the intervention. A sham intervention was not included in any of the two studies. To elaborate, participants in both studies completed an incremental cycle ergometer test twice, once with no preceding IPC treatment, as a control condition, and once with preceding IPC treatment. However, the conditions were ascribed in a counterbalanced order. Nevertheless, the lack of a sham condition in the studies is problematic as it does not account for the risk of a placebo effect occurring, which has been shown be able to improve performance (Beedie & Foad, 2009). In the case of IPC, considerations regarding placebo may be especially relevant, since similar performance improving effects on a swimming time trial have been demonstrated for the IPC and sham conditions compared to a control condition, in a randomized crossover study (Marocolo et al., 2015a). Furthermore, a review by Marocolo et al. (2016b) shows that among the studies demonstrating beneficial effects of IPC, most did not include a sham condition. Furthermore, among these studies, that showed performance increases with IPC, no significant changes in underlying physiological variables were seen (Marocolo et al., 2016b). Therefore, Marocolo et al. (2016b) argue that the performance enhancing effects of IPC can be ascribed to placebo.

This emphasizes the importance of blinding the participants to the intervention through a sham condition. However, although completely blinding participants in IPC studies is difficult since the pressures typically used for IPC and sham feel very different (Lalonde et al., 2015; Cruz et al., 2015), the perception of whether the discomfort that may accompany IPC is detrimental or beneficial is unknown (Incognito et al., 2016b). Nonetheless, the researchers in IPC studies do know the possible effects of IPC, or at least may have certain expectations. Therefore, it is important that the participants are unaffected by the researchers' potential bias, which can be done by blinding the researchers (Karnicolas et al., 2010). Thus, blinding of the researchers in the present study may have ensured that any potential bias of the researchers did not affect the participants' perception of IPC's effect on cycling performance, the data collection, or the outcome of the tests (Karnicolas et al., 2010). This is a general issue with IPC studies, as, to the knowledge of the researchers, no studies have reported blinding of researchers. Therefore, it is possible that the unblinded researchers, as seen in the studies by de Groot et al. (2010) and Crisafulli et al. (2011), may have subconsciously affected the performances of the participants. Affecting the participants could have happened through differential co-interventions, meaning that participants are treated differently in the two conditions (Karnicolas et al., 2010). Therefore, a strength of

the present study is that both participants and researchers were blinded to the intervention, meaning that influencing the results, e.g. through differences in verbal encouragement, or biased assessment of outcomes was less likely (Karnicolas et al., 2010).

A significant improvement in MPO in the present study would have been surprising, since there are no significant improvements in any of the underlying physiological parameters which could explain an improvement. Nonetheless, an increase in MPO would have been expected to be a result of one or a combination of the following parameters, as seen in other studies with IPC: 1. An increase in VO_{2max}, as seen by De Groot et al. (2010); 2. an improved exercise economy as indicated by Bailey et al. (2012) and Kido et al. (2015); or 3. an attenuation of RPE, which Cruz et al. (2015) and Bailey et al. (2012) demonstrated. However, VO_{2max} as well as exercise economy were unaffected by IPC, and the attenuation in RPE was apparently not great enough to increase exercise tolerance at maximal effort, and hence increase MPO. Therefore, this may explain that no performance improvements were found for the participants included in the present study.

Maximal oxygen consumption (VO2max)

The present study found that IPC did not increase VO_{2max} for young, healthy men during a maximal incremental cycling test. This is in contradiction with previous findings (De Groot et al., 2010). Nevertheless, several studies have also shown no increases in VO_{2max} during maximal exercise following IPC treatment (Crisafulli et al., 2011; Bailey et al., 2012; Clevidence et al., 2012).

De Groot et al. (2010) speculated that the vasodilating effects of IPC may be responsible for the increased VO_{2max}. It has been reported that adenosine (Hopper et al., 2000), and nitric oxide (NO) (Kimura et al., 2007) play an important role in the protective effect of IPC. Common to these two substances is their vasodilating effects (Kimura et al., 2007; Hopper et al., 2000). De Groot et al. (2010) proposed that this vasodilation could contribute to increased oxygen consumption. Although this may have occurred, it is widely believed that VO_{2max} is primarily affected by cardiac output (Basset & Howley, 2000). However, in another study it was found that IPC did not affect HR or stroke volume (Crisafulli et al., 2011). Therefore, the fact that IPC has been reported to increase VO_{2max} does not align with current conceptions of VO_{2max}.

A possible explanation could be that it may have influenced the VO_{2max} values that De Groot et al. (2010) averaged intervals of one minute for VO_2, which could mean that the VO_{2max} may not be representative of the actual VO_{2max} (Crisafulli et al., 2011). Thus, steady state was likely not achieved before this one-minute interval and sustained throughout the full minute. Therefore, the VO_{2max} would have been less affected by averaging intervals of shorter durations (Crisafulli et al., 2011). Therefore, one could assume that by increasing the duration of the exercise, the fact that the VO_{2max} was maintained for longer would have affected the one-minute average, thus increasing the VO_{2max} for one intervention. Yet, whether this is the case cannot be deducted from the data available from De Groot et al. (2010).

In the present study, BL was included to elucidate whether a possible increase on MPO was due to an increase in anaerobic metabolism (Goodwin et al., 2007). However, there was no significant increase in BL following IPC compared to the sham intervention.

Submaximal oxygen consumption (VO2). To address the potential improved exercise economy following IPC, VO_2 was measured during five fixed workloads of the maximal incremental test and in the step-transition test. If the VO_2 was lower following IPC during the same workloads, this could have been an indication of lower O_2 cost due to either increased phosphate/oxygen (P/O) ratio (i.e. lower O_2 cost of adenosine triphosphate (ATP) resynthesis) or a reduction in ATP cost of force production (Bailey et al., 2010).

It has been shown that microvascular deoxygenation rate during vascular occlusion at rest following IPC was lowered (Ambrozic et al., 2013), which may indicate that mitochondrial P/O ratio was improved rather than reduced ATP cost of force production. Yet, the reason behind the lowered deoxygenation rate cannot be explained through the use of NIRS, and therefore, the underlying mechanism remains unclear (Ambrozic et al., 2013).

Whether the reduction in deoxygenation rate seen following IPC during rest is evident during exercise is less clear. However, one study provides evidence that IPC increases exercise economy, as the amplitude of the deoxygenation is lower during moderate intensity exercise following IPC (Kido et al., 2015). It is suggested that this may be a result of a mitochondrial adaptation, since the O_2 extraction, where the mitochondria are the locust, is accelerated (Kido et al., 2015).

Nevertheless, as mentioned, the results of the present study do not indicate that IPC reduces oxygen consumption at fixed workloads during cycling.

HR was included as supportive data to the VO_2 data. However, in accordance with the fact that there were no changes in VO_2 during the same workloads of the incremental test following IPC, there were also no changes in HR. Furthermore, as for VO_2, there were no changes in HR in any phases of the step-transition test. Similarly, there were no changes in BL, indicating that there were no changes in anaerobic or aerobic metabolism during the step-transition test as a result of IPC (Goodwin et al., 2007). The BL measurements were included to elucidate whether a potential decrease in VO_2, was merely a result of an increase in anaerobic metabolism, rather than an increased exercise economy (Bailey et al., 2012).

Rating of Perceived exertion (RPE)

In the present study, participants reported significantly lower RPE after IPC at 210 W and 245 W compared with the sham condition. This shows that IPC might attenuate RPE during exercise performed subsequently to IPC. Furthermore, the lack of effect on RPE at lower workloads (i.e. 105 W, 140 W, and 175 W) might indicate that the effect of IPC on RPE is conditional on workload. Since IPC is a therapeutic method of enhancing ischemic tolerance and reducing tissue infarct from prolonged ischemia and reperfusion, it has been speculated that IPC is merely effective on performance, when there is an ischemic threat (Clevidence et al., 2012), which is only the case in severe intensity (Dempsey & Wagner, 1999).

In other studies, IPC has been found to elicit mixed results on RPE. Lalonde & Curnier (2015) reported a significant decrease in RPE (1-10 Borg scale) during a Wingate test compared to a sham condition. Similarly, Bailey et al. (2012) showed a significant decrease in RPE after IPC on the first km of a 5-km treadmill time trial. Furthermore, Cruz et al. (2015) reported a significant decrease in RPE (Borg 6-20 scale) in the fourth minute of cycling at 100% MPO to failure. However, other studies show no effect of IPC on RPE in neither intermittent running (Marocolo et al., 2017), submaximal bilateral knee extension (Marocolo et al., 2016a), graded maximal cycling (Jacobs et al., 2015), 375 kj time trial (Cocking et al., 2018), or repeated 6 s cycle sprints (Gibson et al. 2015; Patterson et al., 2015).

The effect of IPC on RPE could be a result of a desensitization of type III and IV afferent nerves, which would result in a higher firing threshold and therefore lower discharge rate of these muscle afferents (Cruz et al., 2015; Cruz et al., 2017). Theses muscle afferents are believed to transmit fatigue signals to the CNS and thus modulate endurance performance by lowering the central motor drive from the CNS (Amann et al., 2011). The muscle afferents are known to be opioid-mediated (Cruz et al., 2015), and it has been shown that IPC results in an increase of circulating endogenous opioid peptides (Dragasis et al., 2013). Therefore, it is possible that the increased amount of circulating endogenous opioid peptides following IPC treatment is the mediator of the attenuated RPE compared to sham.

It has been proposed that the afferent fatigue signals are a defense mechanism which terminates the effort while the muscle metabolic homeostasis is sufficiently intact (Gibson & Noakes, 2004; Tucker et al., 2006). Thus, evidence has been found that even during exhaustive exercise, there is a lack of complete skeletal muscle recruitment, meaning that there could be a functional skeletal muscle recruitment reserve (Noakes, 2011). An inhibition of the fatigue signal is thought to result in a overshoot in central motor drive, which enables recruitment of a larger fraction of the muscle fibres (Amann et al., 2009; Noakes, 2011), which could increase MPO.

Therefore, Crisafulli et al. (2011) hypothesized that IPC could increase endurance through desensitization of afferent nerves with concurrent decreases in RPE, resulting in an increase in the number of recruited motor units before exhaustion occurs.

Though IPC did not improve performance in the present study, the attenuated RPE might suggest that IPC is able to inhibit central fatigue, which could elicit performance benefits under different circumstances than in a maximal incremental cycling test. For example, as seen in Cruz et al. (2015) both reduced RPE and performance improvement occurred in cycling at 100% MPO to failure. However, should IPC be able to attenuate RPE, it is possible that it could elicit beneficial results in a time trial, in which workload is self-paced, since it has been suggested that RPE plays an important role i the regulation of effort during self-paced exercise (Tucker, 2009). Incognito et al. (2016a) also emphasizes that the most consistent evidence on performance enhancement following IPC is found in time-trial performance..

Nonetheless, the role of afferent feedback on RPE is a disputed matter, which is illustrated by the many different viewpoints appearing in the commentary by Angius et al. (2017). For instance, it is argued that blocking small motor afferents has been shown not to provide any ergogenic effect during exercise (Amann et al., 2011; Angius et al., 2017) or attenuate RPE during exercise (Marcora, 2009). Thus, whether a desensitization of small muscle afferents may be able to explain the performance increasing effect of IPC, remains inconclusive (Angius et al., 2017).

However, the purpose of the present study was not to investigate the mechanisms by which IPC attenuates RPE, and therefore nothing can be concluded regarding the mediators of IPC with the methods included. Therefore, further studies must be made on the matter in order to identify the underlying physiological mechanisms.

Step-transition test

No differences were found between conditions for any parameters in the step-transition test. The step-transition test was included in the design with the primary purpose of enabling the examination of exercise economy during steady state cycling. This is not possible with the two-minute intervals in the maximal incremental cycling test, as steady state is not achieved until approximately three minutes (Burnley & Jones, 2007).

The workload of 50 W during the steady state warm-up phase was chosen for practical reasons since this was the lowest workload deemed possible for the participants to maintain. However, both 30 W (Kido et al., 2015) and 20 W (DeLorey et al., 2003) have previously been set as the initial workloads in tests similar to the one performed in the current study.

The workload in the on-transition phase was set at 60 % of VT, which is a relatively low workload compared to Kido et al. (2015) and DeLorey et al. (2003), who use 90 % of VT and 80 % of lactate threshold, respectively. The workload was chosen in order to ensure that it was the aerobic energy system which was predominantly active. This was due to the fact that a review by Incognito et al. (2016a) states that the effect of IPC is largest on aerobic exercise.

IPC did not affect the exercise economy during the step-transition test compared to the sham condition. However, IPC only decreased RPE at 210 W and 245 W compared to sham, which could indicate that a certain workload is necessary for the effect of IPC to arise.

In conclusion, an ischemic preconditioning protocol, consisting of four cycles of five-min bilateral, arterial occlusion and reperfusion of the legs, in young, healthy males did not increase maximal power output or maximal oxygen consumption for young, healthy men during a maximal incremental cycle ergometer test. Furthermore, muscle oxygenation, and blood lactate concentration following the maximal incremental test were unchanged by ischemic preconditioning. Moreover, no changes occurred in oxygen consumption, blood lactate concentration, heart rate, tissue saturation index, ▲oxygenated hemoglobin, or ▲deoxygenated hemoglobin during submaximal workloads. However, ischemic preconditioning attenuated rating of perceived exertion at 210 W and 245 W during the maximal incremental test.

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