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Ischemic Preconditioning Improves Maximal Accumulated Oxygen Deficit in NCAA Division I Middle-Distance Runners

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ISCHEMIC PRECONDITIONING IMPROVES MAXIMAL ACCUMULATED OXYGEN DEFICIT IN NCAA DIVISION I MIDDLE-DISTANCE RUNNERS

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science in Nutrition and Exercise Science and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABBREVIATIONS

aO_2D	Accumulated oxygen deficit
BP	Blood pressure
BMI	Body mass index
DBP	Diastolic blood pressure
ICP	Ischemic preconditioning
Kcals	Kilocalories
RER	Respiratory exchange ratio
RHR	Resting heart rate
SBP	Systolic blood pressure
V_E	Minute ventilation
VO_2max	Maximal oxygen uptake
VO_2	Oxygen uptake

ABSTRACT

ISCHEMIC PRECONDITIONING IMPROVES MAXIMAL ACCUMULATED OXYGEN DEFICIT IN NCAA DIVISION I MIDDLE-DISTANCE RUNNERS

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Purpose: There is an ongoing debate concerning whether ischemic preconditioning elicits consistent and meaningful exercise performance benefits. We have previously demonstrated no performance benefits of ischemic preconditioning at submaximal aerobic exercise intensities. It is likely that the beneficial effects of ischemic preconditioning on performance only involve supramaximal anaerobic exercise bouts, which elicit greater metabolic and neuromuscular stress. The aim of the study was to test the hypothesis that ischemic preconditioning improves maximal accumulated oxygen deficit (aO_2D), an indicator of anaerobic capacity, in NCAA Division I middle-distance runners. Methods: A randomized sham-controlled crossover study was employed in which 10 NCAA Division I middle-distance (800 to 1600 meter) track athletes (age: 21±1 yr; VO2 max: 65±7 mlO2·kg·-1·min-1) completed three supramaximal treadmill running trials (110% VO2max; ~12.6 mph @ 5% grade) to volitional exhaustion coupled with indirect calorimetry to assess maximal aO2D at baseline, after a sham control trial (mock preconditioning), and with limb-based ischemic preconditioning (4×5 min cycles of brachial artery ischemia/reperfusion). Maximal aO_2D (mlO2·kg-1) for each trial was determined by first calculating the theoretical oxygen demand required for the supramaximal running bout (linear regression extrapolated from 9×5 min submaximal running stages). The actual oxygen demand measured during the supramaximal bout was

then subtracted from the theoretical value to obtain the aO_2D. Statistical Analysis: A three-way repeated-measures ANOVA with adjustment for multiple comparisons was used for within-group differences (i.e., baseline vs sham vs ischemic preconditioning) in aO2D. Results: Ischemic preconditioning (122±38 sec) increased (P=0.0001) supramaximal time to exhaustion by 22% compared with both baseline (99±23 sec, 95% CI: 4.8-40.6, P=0.014) and sham (101±30 sec 95% CI: 6.7-34.2, P=0.001). Effect size for these trial differences as estimated by the Partial Eta2 (0.58) were large. Furthermore, the aO2D was considerably greater (P=0.009) with ischemic preconditioning. During their supramaximal run in the presence of ischemic preconditioning, aO2D was 46±35 mlO2·kg-1, a substantial (Partial Eta2 = 0.43) increase compared with baseline (35±27 mlO2·kg-1, P=0.025, 95% CI:1.3-19.2) and sham (38±32 mlO2·kg-1, P=0.046, 95% CI: 0.13-14.0). There were no statistical differences in time to exhaustion and maximal aO2D between baseline and sham. Conclusions: Limb-based ischemic preconditioning considerably improves time to exhaustion and anaerobic capacity as measured by the maximal aO_2D in NCAA Division I middle-distance track athletes. Additional work is needed to confirm whether these laboratory-based performance benefits can be translated to better outcomes in real track meets in elite athletes seeking a competitive advantage.

Keywords

Ischemia, reperfusion, anaerobic performance, oxygen uptake, accumulated oxygen deficit

Introduction

Ischemic preconditioning (IPC) was originally established as a protective mechanism in cardiac cells against a subsequent ischemic event. This process includes brief cycles of non-lethal reactive hyperemia, or periods of circulatory occlusion and reperfusion, causing a decreased formation of muscle damage [1]. The clinical applications protect the cardiac cells by triggering an up-regulation of energetics of the tissues to prevent cellular damage through a reduced formation of proinflammatory responses [2] and metabolic stress [3]. Some of these responses include an increase of blood flow [4,5] that initiates a release of vasoactive triggers [6]. As a result of the vascular responses caused by preconditioning, this method was applied to explore changes in exercise performance. The first study by de Groot et al. found that IPC improved exercise performance and maximal oxygen uptake in cyclists [7]. Additionally, other studies have shown that ischemic preconditioning elicits improvements in aerobic exercise in running [8], cycling [9], and swimming [10]. While research has shown an improved ability in aerobic exercise bouts, Jean-St-Michel et al. suggests that the aerobic energy transaction contributes to the anaerobic events, where the oxidative system is fully taxed [11]. Patterson et al. reported enhanced peak anaerobic power during twelve all-out 6 sec cycling sprints but no influence on fatigue or EMG activity of the vastus lateralis [12]. A recent study by Cruz and colleagues [13] showed that ischemic preconditioning resulted in greater oxygen consumption and quadriceps muscle activation during 45 min of active recovery following a 60 sec maximal cycling sprint, while showing improvements in performance by 2.1%. These findings imply that the rate of fatigue during subsequent short bouts of anaerobic cycling

could be reduced with preconditioning. Additionally, the aforementioned studies indicate potential improvement in anaerobic performance. One approach that has not been directly applied to determine the effects of preconditioning on anaerobic performance is the accumulated oxygen deficit (aO_2D) method.

Accumulated oxygen deficit is a method to indirectly quantify anaerobic capacity of skeletal muscle during exercise [14]. It is a method based on the oxygen deficit concept, as the larger the oxygen deficit for a given bout of exercise, the greater must be the ATP contribution from non-mitochondrial sources (anaerobic). If an exercise bout can be performed that maximally taxes the muscles' abilities to regenerate ATP from non-mitochondrial sources, it would provide a means to determine the aO_2D. The measurement of aO_2D is done by calculating the difference between the estimated oxygen demand and the actual value that is obtained for oxygen uptake during an all-out supramaximal test. This is done by working out maximum oxygen consumption first, followed by a series of submaximal performance values for oxygen uptake. The submaximal performance values are used to draw a line of regression so that estimated oxygen uptake can be computed. Once this is done and the athlete performs an aO_2D test at a supramaximal effort to exhaust the anaerobic system, the actual oxygen uptake is measured and taken away from the estimated value to get the oxygen deficit.

The direct investigation on the effects of preconditioning on maximal anaerobic performance determined by aO_2D is very limited. Accumulated oxygen deficit data elicits the determination of the maximum oxidative response to measure maximum anaerobic capacity. The aim of this study is to determine the effect of remote ischemic preconditioning as it improves anaerobic capacity as assessed by the accumulated oxygen

deficit in NCAA Division I middle-distance track athletes. We hypothesize that the effects of preconditioning will improve the aO_2D and performance in the runners.

Methods

Subjects

Ten healthy, NCAA Division I Track middle-distance runners (age 21±1 yr) were studied: 6 men and 4 women. Track athlete event groups included those who competed in mid-distance events ranging from 800 meters to the mile (1609m). The subjects training distance was chosen due to the energy system in which they trained to grant accurate data collection for the type of supramaximal exercise induced by the aO2D tests. Subjects train all 12- months of the year and are in competition 9- months of the year. Subjects typically take one day off every 2-3 weeks. Each subject trained at a minimum of 30 miles per week and as high as 90 miles per week. Male and female runners trained at an average speed of 14.8 km/hr and 13.4 km/hr, respectively. This study was conducted during the competition season, therefore, subjects continued training and competing throughout the study. The subjects completed the tests for this study on non-workout or high intensity days, i.e. rest days.

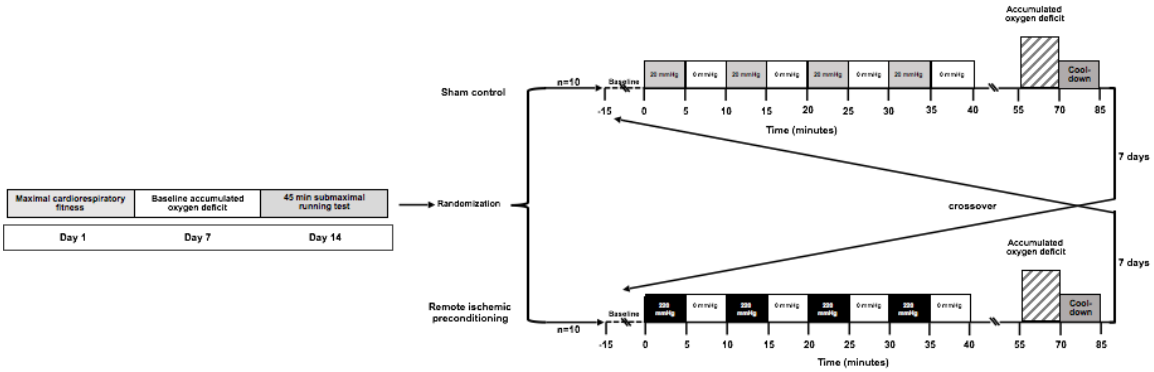
Upon enrollment, participants underwent a complete medical history and basic health assessment. Information concerning the use of medications, lifestyle and physical activity habits, and history of cardiovascular, pulmonary, or metabolic disease were documented. None of the subjects smoked, consumed tobacco-containing products, or were taking medications, including vitamins and performance-enhancing supplements. Participants were excluded from the study if they presented with a history of disease or

injury that precluded exercise participation. Before participation, informed consent was obtained from all individual participants included in the study. However, to lessen expectation bias, subjects were not informed of any potential exercise performance application of ischemic preconditioning. Subjects were instructed to continue normal training during the study. This study was conducted according to the Declaration of Helsinki and approved by the Institutional Review Board for the Protection of Human Subjects at South Dakota State University.

Experimental design

A simplified overview of the experimental protocol is shown in Figure 1. A randomized, double-blind, sham-controlled crossover design was used to investigate the influence of the acute remote ischemic preconditioning on the primary outcome in the difference of accumulated oxygen deficit (i.e., ischemic preconditioning or sham) separated by ~1 week between trials. All subjects completed a series of pre-screening measurements including each subject's VO_2max for the first session. The following week the subject completed a 45-minute sub maximal treadmill running test. Following the submaximal test, subjects completed the first assessment of baseline aO_2D test, followed by a randomized aO_2D test with control or remote preconditioning for the proceeding week. Randomization was computer generated by a list and was kept blind to the primary researcher. After at least 7 days of washout, subjects crossed over to repeat the third aO_2D test (remote preconditioning or control).

Figure 1. Experimental study design



Hemodynamic measurements

Non-dominant arm auscultatory resting systolic and diastolic blood pressure were measured using a stethoscope and sphygmomanometer (Diagnostic 700 Series; American Diagnostic Corporation, Hauppauge, NY, USA) following 5 min of seated quiet rest using standard procedures. Resting blood pressure measurements were performed twice separated by 3 min, and averaged. Resting heart rate was measured using a 60 sec radial pulse count.

Anthropometric measurements

Standing height was measured using a Detecto medical beam balance (Cardinal Scale Manufacturing Co, Webb City, MO, USA). Body mass was measured with a digital scale (Seca 876 digital scale, Seca Corporation, Hamburg, Germany). Body mass index was calculated as weight (kilograms) divided by height (meters) squared. Percent body fat was estimated by air displacement plethysmography (BODPOD, COSMED USA Inc., Illinois).

Maximal Oxygen Uptake

Maximal oxygen uptake (VO_2max) was determined using open-circuit spirometry combined with indirect calorimetry (Parvo Medics TrueOne® 2400, Salt Lake City, UT) in response to incremental treadmill running (Woodway Pro, Woodway USA, Waukesha, WI). Flow and gas calibration was performed prior to each test using standard operating procedures provide by the manufacturer. Subjects were equipped with a mouthpiece and nose clip, and a heart rate monitor affixed to the chest with receiver integrated with the metabolic cart (Polar Electro Inc., Lake Success, NY, USA). Resting expired gases were collected for 2 min. Thereafter, a 15 min warm-up was performed at 12.8 km/h and 13.7 km/h for female and males, respectively. Velocity was increased or consistent (preferred pace) during the warm-up phase until a self-selected running pace was identified between 12.8-14.3 km/h. The pace of the test was determined using each subject's running tempo pace, based off of the individual's 5k race pace. After a brief rest period, the first stage of the VO_2max protocol was initiated and subjects ran at the predetermined velocity, based off of the subject's race pace, at 0% incline for 2 min. Thereafter, the workload was increased by raising the velocity of the treadmill every 2 minutes until the third stage, or 6 minutes. During the following stages, the incline of the treadmill increased by 2% as velocity increased every 2 min until volitional fatigue. The treadmill did not surpass a grade of 6% or below 4% at the subjects VO_2max. Rating of perceived exertion was obtained at the end of each 2 min stage. Subjects were provided verbal encouragement throughout the test until exhaustion. Oxygen uptake (VO_2), minute ventilation, heart rate, and kcals/min were recorded. VO_2 data were smoothed with a 10-second moving average with VO_2max denoted as the highest 10-second moving average obtained during the last

minute of exercise [15] with no further increase in VO_2 (<150 mlO_2/min) despite increased workload. All tests were terminated by volitional exhaustion.

Submaximal Oxygen Uptake (45-minute running test)

Submaximal oxygen uptake was determined using open-circuit spirometry combined with indirect calorimetry (Parvo Medics TrueOne® 2400, Salt Lake City, UT) in response to incremental treadmill running (Woodway Pro, Woodway USA, Waukesha, WI) at a 0% grade for 45 minutes. The protocol consisted of nine, 5-minute running stages of increasing intensity. The test initiated as ~50% of the VO2max of each subject and reached 80% but did not surpass. This test is designed for the subjects to reach steady-state exercise. Buck and McNaughton [16] studied the submaximal exercise bout which elicits a steady-state response to be achieved in running at 30-90% of the subjects VO2max. At fixed submaximal workloads below ventilatory threshold, steady-state conditions are usually reached within minutes after the onset of exercise. This protocol was used to determine the theoretical cost of oxygen at the intensity of the aO_2D test, otherwise known as aO_2D *theoretical* (see equations in aO_2D section below). Subjects were equipped with a mouthpiece and nose clip, with a heart rate monitor affixed to the chest with the receiver integrated with the metabolic cart (Polar Electro Inc., Lake Success, NY, USA). Flow and gas calibration was performed prior to each test using standard operating procedures provided by the manufacturer. Resting expired gas was collected for 2 minutes prior to the test. Oxygen uptake, minute ventilation, and heart rate was recorded during the test. At the end of the 45 minute test, a 5 minute cool down was initiated immediately.

VO_2 data was smoothed out with a 15 second moving average. The 15 second moving average was selected because it induces minimal data loss with little data and trend distortion as recommended by Robergs et al. [15]. Steady-state exercise data during the last two minutes of each 5-minute submaximal running stage was calculated from the 15 second moving averages. This was obtained by averaging the final four data points for oxygen uptake, heart rate, minute ventilation, respiratory exchange ratio, and kcals/min. Due to the workload at a 0% grade, the work performed on the treadmill for the aO2D calculation was determined using the horizontal work (kg m/min), calculated by the speed (mph), multiplied by the subjects mass, adding that to the vertical work (kg m/s^2) done by multiplying mass by gravity by subject's height, as percent grade. The work performed on the treadmill during the submaximal test was used to calculate aO_2D *theoretical* by plotting the steady-state oxygen uptake values against the workload performed. A linear relationship was determined for each subject by calculating the regression of the steady-state oxygen uptake on exercise intensity, or work, thus identifying the oxygen demand for all intensities. Table 3 shows the heart rate, oxygen uptake, and work performed at each stage. Heart rate, VO_2, and RER were recorded to determine that each subject reached steady-state without exceeding for the purpose of keeping the test below maximum efforts, see Table 3 for values. This technique confirms the submaximal nature of the test, allowing accurate extrapolation of the linear data to represent the actual oxygen consumed.

Supramaximal (aO_2D) Test

The Accumulated Oxygen Deficit (aO_2D) test was used to determine the difference in oxygen demand and actual oxygen uptake. The test was designed as a supramaximal test at each of the subjects calculated 110% VO2max workload. The subjects performed the series of three aO_2D tests separated each by one week; baseline, followed by either a state of remote preconditioning or sham, randomly. Prior to administering the aO_2D trials, subjects were instructed to refrain from any exercise for 24 hours and to abstain from ingesting caffeine and any supplement or ergogenic aid that may enhance exercise performance. Subject began with a 15-minute warm-up at 12.8 km/h and 13.7 km/h for female and males, respectively. Subjects were allowed a 3-min break before an extended 3-min warm-up performed at ~80% of their calculated treadmill running velocity of the test. This was followed by three-15 sec running sprints at their individual grade of 4%-6% with up to 90% of the velocity of the respective test. The warm-up for each of the three tests was identical for each subject. Subjects were equipped with a mouthpiece and nose clip, with a heart rate monitor affixed to the chest with the receiver integrated with the metabolic cart (Polar Electro Inc., Lake Success, NY, USA). Flow and gas calibration was performed prior to each test using standard operating procedures provided by the manufacturer. Resting expired gas was collected for 2 minutes prior to the test. The subjects were instructed to run each test the same, by running until absolute volitional exhaustion. Subjects began the test at rest and advanced onto the moving belt at the selected speed and grade then exercised at the respective supramaximal intensity until exhaustion. Subjects were provided verbal encouragement throughout the test until exhaustion. The individual relationship between the time to exhaustion and oxygen

uptake (VO2) determined the corresponding oxygen demand in each test. The accumulated oxygen deficit was calculated as the difference between the accumulated oxygen demand and the actual accumulated oxygen uptake between tests:

a. aO_2D theoretical (ml/kg) = Oxygen demand theoretical (ml/kg/min) x duration (min) of aO_2D test

b. aO_2D uptake actual (ml/kg) = Actual oxygen uptake (ml/kg/min) x duration (min) aO_2D test

VO2 data was smoothed out to breath-by-breath averaging using a 4-breath average to produce an accurate representation of the VO2 response for the short duration, high intensity test. Using the individual's calculated 110% VO2max, the test was designed to cause volitional exhaustion within 3 minutes from initiation of the test. Due to the length of the test, the 4-breath averaging best suites the data for accurate interpretation of the VO2 data. VO2 data, heart rate, and respiratory exchange ratio was recorded for analysis and confirmation for true achievement of maximal oxygen uptake. Time to exhaustion for each test was collected and used to calculate aO_2D for remote preconditioning and sham tests in comparison to baseline aO_2D.

Ischemic Preconditioning

At least one week after completing the baseline aO_2D measurement, subjects were randomized to complete either the ischemic preconditioning or the sham control trial first, each immediately followed by a aO_2D test. All measurements were performed in a temperature-controlled room after a 4 hr fast. To induce ischemic preconditioning,

subjects rested in the supine position with high-pressure cuffs placed unilaterally on the upper right arm muscle and inflated to 220 mmHg (EC20 rapid cuff inflator, DE Hokanson, Inc., Bellevue, WA) for 5 min to occlude the brachial artery, followed by 5 min of deflation (reperfusion). This procedure was repeated four additional times and was similar to the ischemic conditioning protocols employed by other exercise performance studies [7, 17]. Heart rate and blood pressure were monitored during the last 2 minutes of each 5 minute ischemic episode. The sham control trial consisted of the same procedure described above for the ischemic preconditioning trial except the pressure cuff were inflated to 20 mmHg to avoid ischemia and reperfusion. The total time to perform these trials was 40 min (i.e., 20 min of intermittent ischemia/sham and 20 min of intermittent reperfusion). Within 15 min following the ischemic preconditioning and sham trials, subjects were prepared to complete a supramaximal aO_2D test. The rationale for electing to begin the aO_2D trials within 15 minutes of preconditioning is based on the notion that the early window of cytoprotection is invoked within minutes after the preconditioning ischemia, disappearing 1-2 hours later [18]. However, it is important to note that the cytoprotective mechanisms have not been linked directly to improvements in exercise performance. To avoid experimenter bias, aO_2D were completed by the same investigators blinded to the conditioning trial of each subject. All subjects wore the same athletic shoes for the three trials.

Blood Lactate

A small sample (0.7 µl) of capillary whole blood was obtained via finger prick from the index finger at the baseline and last two minutes of each 5-minute reperfusion stage

during preconditioning and sham control trials while lying in a supine position (Lactate Plus, Nova Biomedical, UK). A total of five samples were collected during each of the two trials. The Lactate Plus analyzer has been shown to provide valid and reliable (r=0.99) measurements of blood lactate concentration compared with bench top grade analyzers [19]. The analyzer was calibrated with low (1.0–1.6 mmol/l) and high (4.0–5.4 mmol/l) quality control solutions prior to measurements. Lactate was obtained within one minute while the subject laid in a supine position during the trials. The same investigator measured blood lactate during each trial.

Statistical Analysis

Measures of central tendency were used to calculate baseline demographic data. Data were checked for normality and spread and were normally distributed with the exception of blood lactate concentrations. A three-way repeated-measures ANOVA with adjustment for multiple comparisons was used for within-group differences (i.e., baseline vs sham vs ischemic preconditioning) in aO2D. Linear regression was used to determine differences in the slope of the linear increase in oxygen uptake (mlO2/kg/min) relative to treadmill work (kgm) among aO2D trials. A 2×5 (trial × time) repeated measures ANOVA was used to determine differences in resting heart rate and blood pressure at the end of each 5 min ischemic/sham episode for the IPC and sham trials. Area under the lactate curve across IPC and sham trials was determined using a trapezoidal model. Differences in lactate between sham and IPC were determined using the non-parametric Wilcoxon sign rank test for within subjects' designs. Statistical significance was set at $P<0.05$. Data are presented as means±SD and analyzed with SPSS version 20 (IBM, Inc.,

Armonk, NY).

Results

Subject characteristics are shown in Table 1. Subjects were considered normal weight based on BMI, normotensive, and presented with VO_2max values at or above the 90th percentile for age and sex [20]. Thirteen middle-distance athletes volunteered to participate in this study, three subjects dropped out each due to injury during training. Thereby, data was collected and analyzed for 10 subjects (6 men, 4 women). Each subject participated in five data collection sessions. On average, each session was at least one week apart. The maximum time between IPC and sham trials during the study was 2 weeks; the average was 1 week apart for the subjects. Each subject continued normal track training during the study; the average weekly mileage was 50 miles per week in which the athlete trained. The subjects are all considered elite athletes in their field, where the mean VO_2max was 65.0±7.3 ml/kg/min. Mean systolic blood pressure was 128±13 mmHg and mean diastolic blood pressure was 72±7 mmHg.

Table 1. Subject characteristics

Variable	Total Group (N=10)
Sex, men/women	6 / 4
Age, year	20 ± 1
Body mass, kg	63.8 ± 8.4
BMI, kg/m^2	21.0 ± 1.4
Percent Fat, %	10.1 ± 4.7
Height, m	1.74 ± 0.1
Systolic BP, mmHg	128 ± 13
Diastolic BP, mmHg	72 ± 7
RHR, bpm	52 ± 7
VO_2max, ml/kg/min	65.0 ± 7.3

Values are mean ± SD. BMI: body mass index; BP: blood pressure; RHR, resting heart rate; VO_2max, maximal oxygen consumption.

Table 2 shows the mean heart rate and blood pressure changes before and during the 5-min bouts of sham and preconditioning.

Table 2. Heart rate and blood pressure changes before and during four, 5 min bouts of unilateral sham and ischemic preconditioning of the arm.

	Heart rate, bpm				
	Baseline	5 min	10 min	15 min	20 min
Sham	55±9	56±10	55±8	55±8	56±8
Ischemic preconditioning	53±12	53±15	52±7	51±8	52±6
	Systolic/Diastolic Blood pressure, mmHg				
	Baseline	5 min	10 min	15 min	20 min
Sham	118/62±10/5	118/60±9/5	115/60±11/5	116/61±12/6	116/61±12/6
Ischemic preconditioning	120/64±8/6	118/65±9/6	117/63±9/5	116/63±8/7	118/61±9/7

Values are means±SD.

Table 3 shows steady-state heart rate, oxygen uptake and RER during the 9 stage submaximal running trial. There were progressive increases (all $P<0.01$) in heart rate, oxygen uptake and RER, as expected across stages.

Table 3. Steady-state heart rate, oxygen uptake, and RER during 45-minute submaximal test to determine oxygen demand.

Stage 1		
	Heart rate (bpm)	135±12
	VO2	37±5
	RER	0.825±0.045
Stage 2		
	Heart rate (bpm)	142±9
	VO2	39±4
	RER	0.863±0.052
Stage 3		
	Heart rate (bpm)	150±10
	VO2	41±4
	RER	0.859±0.052
Stage 4		
	Heart rate (bpm)	155±12
	VO2	43±4
	RER	0.865±0.054
Stage 5		
	Heart rate (bpm)	159±11
	VO2	45±5
	RER	0.860±0.057
Stage 6		

	Heart rate (bpm)	162±11
	VO2	47±8
	RER	0.862±0.048
Stage 7		
	Heart rate (bpm)	167±11
	VO2	48±6
	RER	0.879±0.048
Stage 8		
	Heart rate (bpm)	172±11
	VO2	50±16
	RER	0.885±0.057
Stage 9		
	Heart rate (bpm)	176±12
	VO2	51±8
	RER	0.898±0.060

Data represent mean±SD. IPC, ischemic preconditioning; VO2, oxygen uptake; RER, respiratory exchange ratio.

Effects of Ischemic Preconditioning on Supramaximal Time to Exhaustion

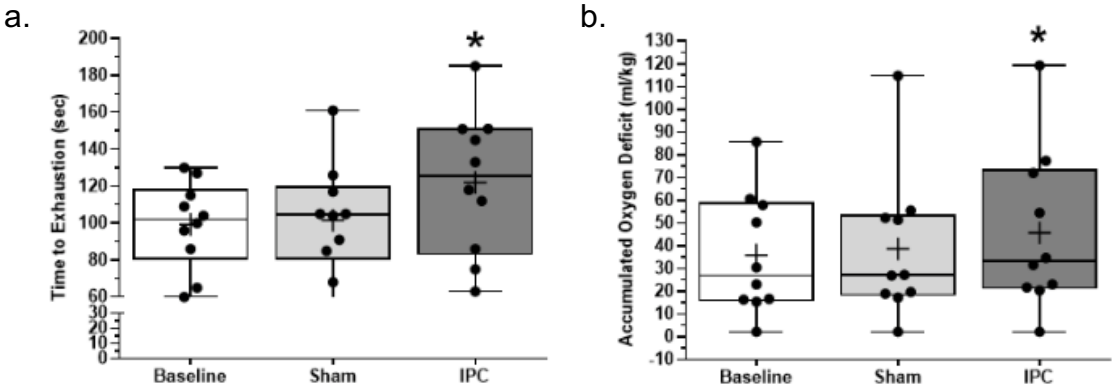
Figure 2(a) below, illustrates the time to exhaustion during the supramaximal exercise tests between groups (A) baseline, (B) sham, (C) IPC, reporting time and standard deviation. Ischemic preconditioning (122±38 sec) significantly increased (P=0.0001) time to exhaustion compared to baseline (99±23 sec, 95% CI: 4.8-40.6, P=0.014) and sham (101±30 sec, 95% CI: 6.7-34.2, P=0.001). Time to exhaustion increased by 22% in the ischemic preconditioning trial compared to baseline and sham trials. Effect size for the differences in trial as estimated by the Partial Eta^2 (0.58) were large. There were no

statistical differences in time to exhaustion between baseline and sham (P=1.0). There were also no significant differences in maximum heart rate between trials.

Effects of Ischemic Preconditioning on Accumulated Oxygen Deficit

The values reported in Table 3 were used to calculate the linear regression of work and oxygen demand to determine the oxygen uptake. Figure 2(b) below, illustrates the results showing that aO_2D increased significantly with ischemic preconditioning (P=0.009) compared to baseline and SHAM trials in the supramaximal exercise tests. The ischemic preconditioning aO_2D measured 46±35 mlO2·kg-1 (Partial Eta2 = 0.43), significantly increased from baseline (35±27 mlO2·kg-1, P=0.025, 95% CI:1.3-19.2) and sham (38±32 mlO2·kg-1, P=0.046, 95% CI: 0.13-14.0). There were no statistical differences in aO_2D between baseline and SHAM trials.

Figure 2. (a) Time to Exhaustion (sec) (b) aO_2D between baseline, sham, and preconditioning (ml/kg)

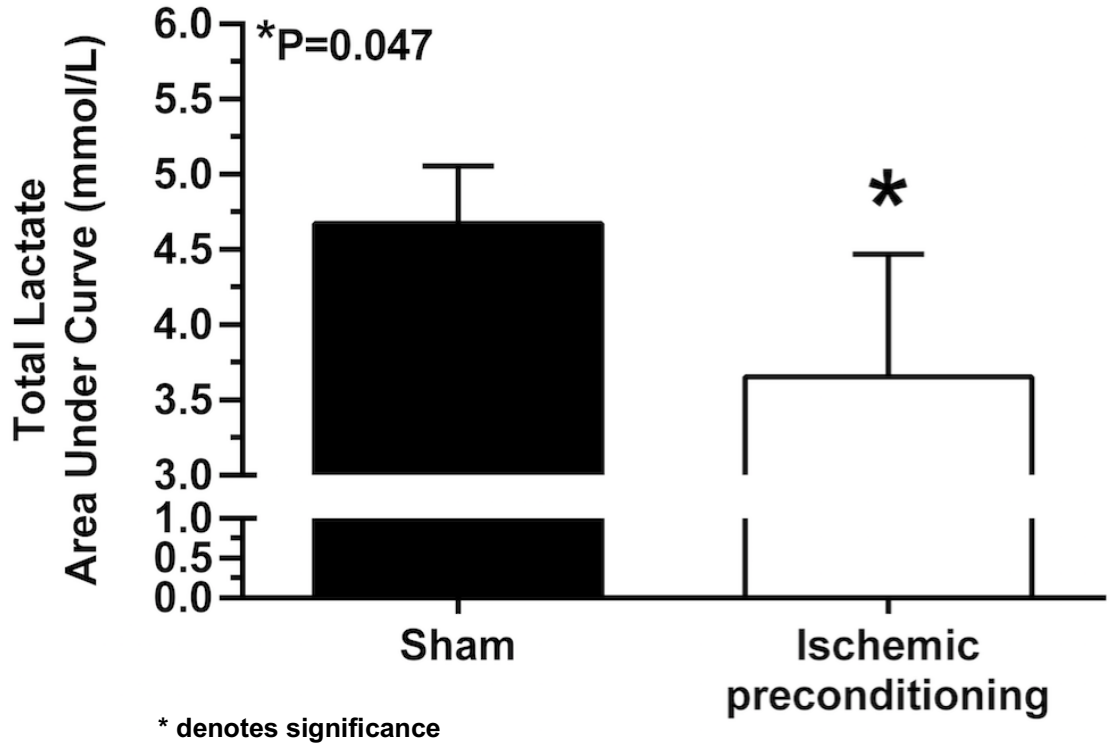


*** denotes significance**

Lactate

Figure 3 below, shows box and whisker plots of resting blood lactates for the sham and ischemic preconditioning trials. The data was analyzed using a Wilcoxon Signed Rank Test. There was a significant decrease in resting blood lactate during the ischemic preconditioning trial (median=3.5 mmol/l, Z=2.0, P=0.047) compared to sham trial. For example, median lactate decreased from 4.6 and with an IQR of 3.7 mmol/l to 5.5 mmol/l in the sham trial to 3.5 mmol/l, with the IQR of 2.8 mmol/l to 4.5 mmol/l with ischemic preconditioning.

Figure 3. Total lactate area under the curve showing the significant decrease in resting lactate (mmol/L) taken during the sham and IPC trials. There is a significant decrease (p=0.047) in resting blood lactate from sham to preconditioning trials.



Discussion

In the present study, remote preconditioning in the upper arm did significantly affect maximal accumulated oxygen deficit and anaerobic performance in elite, middle-distance runners. Relative to the baseline control, preconditioning improved time to exhaustion by 22%. This result is in agreement with previous studies of Jean-St-Michel et al. [11] and Cruz et al. [13], where they observed similar benefits of preconditioning on anaerobic performance in swimmers and cyclists, respectively. In the latter study, by Cruz et al., performance differences were identified by a greater anaerobic contribution, rather than aerobic sources. These findings are consistent with the present study, determining the improvement upon the anaerobic energy system, causing a greater capacity for anaerobic exercise and maximal intensities.

The studies investigating the effect of preconditioning on anaerobic performance have been inconsistent. Gibson et al. [21] and Clevidence et al. [17] reported inconclusive findings for anaerobic performance in a 5 by 6-sec sprint on a cycle ergometer and time to exhaustion in amateur cyclists. Additionally, Akgul et al. [22] and Lalonde et al. [23] both recently studied anaerobic power using a 30-sec Wingate Test on trained athletes, but did not find significance in mean power or power output after preconditioning. These findings suggest that preconditioning does not have an effect on peak power or maximal power output, where ATP is being recruited by the ATP-PC system and therefore, we can expect to see no significant improvements on bouts of intense, short exercise (< 30-sec). Alternatively, research by Jean-St-Michel et al. [11] suggests that energy recruited from additional pathways such as the anaerobic glycolytic as well as the aerobic oxidative system, may have more of an effect on performance in athletes. While the study shows

complementary findings to our previous analysis showing no effect on submaximal exercise, Jean-St-Michel et al. found a significant association between maximal performance following preconditioning. The methods analyzed swim-time performance in 100-m and 200-m swimming test in a competitive setting suggesting additional energy recruitment. These findings may be interpreted by in vivo studies performed by Fryer et al. [24], showing that IPC causes mitochondrial ATP-sensitive K channels to open and the uncoupling of oxidative phosphorylation. As a result, it is hypothesized that this allows the mitochondria uptake acetyl-CoA as a by-product of glycolysis more quickly. This allows for the maintenance of lactic acid accumulation and use during exercise. Bailey et al. [8] suggests that IPC can elicit a greater contribution from aerobic pathways allowing for the sparing of ATP generated by glycolysis via the ability to produce and efficiently use lactate. Our study is consistent with this concept, as resting blood lactate significantly decreased with preconditioning, compared to sham ($p= 0.047$). We speculate that the decrease in resting lactate during preconditioning caused an up-regulation of lactic acid as an energy source pre-exercise causing a potential augmentation of blood lactate clearance and enhanced oxidation rates. Along with lactate, other improvements caused by IPC include vasodilation inducing increased blood flow and ATP sparing derived from anaerobic pathways [25]. Additionally, we speculate that more muscle fibers are recruited following IPC, which allow for higher muscle activation potentials [13] and the possibility of the subject to exercise beyond the threshold of fatigue. One explanation of this phenomenon is explained by Barbosa et al. [26], that suggests preconditioning delays the development of fatigue possibly due to the blocking of fatigue receptors within the central nervous system. This may allow athletes to perform at

supramaximal workloads with the delay in development of fatigue with the greater increase of sympathetic activity. As a result in our present study, this mechanism needs further investigation to determine the cellular activity after preconditioning.

Unique in this study is the method of using accumulated oxygen deficit as a measure of anaerobic capacity and performance. To date, this is the first study that uses this method as a measurement for performance in this setting. Additionally, we are the first study to use a baseline bout of exercise to determine the changes from baseline and sham trials. This strengthens the results of our study as the improvements from IPC were significant from baseline ($p=0.0001$) and sham ($p=0.001$) trials. Accumulated oxygen deficit is an accurate method of determining ATP production from anaerobic metabolism. As this method determines the anaerobic capacity by determining the energy source through oxygen demand during a supramaximal effort; it is potentially the most precise method for our population. As the running intensity of the 800m and mile run both exceed 100% VO2max, the aerobic and anaerobic energy metabolisms contribute to these races; theoretically 60% aerobic and 40% anaerobic reported by di Prampero et al. [27]. The anaerobic energy system increases oxygen uptake before and after an 800m run, which is significant for the requirement to resynthesize lactic acid during the run [28]. Therefore, it is necessary for these athletes to have a high anaerobic capacity. By using the aO2D method, we could measure the effect of preconditioning on anaerobic capacity and potentially further warrant an increase in performance in middle-distance runners.

Another distinguishing element of this study is the elite population researched. This population of elite athletes is exclusive, as other studies have only included at recreational, amateur, and sedentary individuals. Additionally, there is limited research

assessing the effects of preconditioning on anaerobic capacity in runners using land-based sprinting methods. The significance in the results can be verified due to the runner's ability to perceive true physical exhaustion, while other indicators confirmed fatigue such as an RER > 1.10 and a VO2 exceeding the subject's maximum, indicating supramaximal efforts. The significance of this study potentially gives middle-distance runners a competitive edge. Within this group of athletes, milliseconds might separate competitors in the respective events. This study may contribute to the development of a natural aid for this population, as we concluded this study with a 22% improvement in time to exhaustion from baseline at a workload similar to a racing performance. The 23-sec average increase in time was not only significant but also potentially competitively significant for runners, where a 0.4% improvement in competition performance is reported as significant [29]. With the observed improvements of our study, more research should be done to support preconditioning as a natural performance enhancer in competitive runners.

Conclusions

The results of the study suggest that upper arm ischemic preconditioning improves accumulated oxygen deficit and time to exhaustion in elite, middle-distance track athletes. Further studies should be conducted to determine the effects on performance to the track and field setting to evoke possible improvements in competition for elite athletes seeking a competitive advantage.

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Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Figure Captions

Fig 1. Simplified overview of the main experimental protocol. Subjects will first undergo an assessment of maximal cardiorespiratory fitness (Day 1). At a maximum of 1 week later (Day 7), subjects will complete the first assessment of accumulated oxygen deficit. At approximately Day 14, subjects will complete a 45 min running test. Subjects will be randomized to undergo a second accumulated oxygen deficit test with control or with remote preconditioning. After about 7 days of washout, subjects will crossover to repeat a third accumulated oxygen deficit test (i.e., with remote preconditioning or control).

Fig 2. (a) This figure illustrates a significant increase in time to exhaustion (sec) in the aO_2D test following preconditioning compared to baseline ($p=0.0001$) and sham ($p=0.001$) trials. (b) This figure illustrates a significant increase in accumulated oxygen deficit (ml/kg) in the preconditioning trial (0.009) compared to baseline and sham.

Fig 3. Total lactate area under the curve illustrates the significant decrease in resting lactate (mmol/L) measurements taken during the sham and preconditioning trials. There is a significant decrease ($p=0.047$) in resting blood lactate from sham to preconditioning trials.