Effects of ischemic preconditioning on maximal constant-load cycling performance

Rogério Santos de Oliveira Cruz, Rafael Alves de Aguiar, Tiago Turnes, Kayo Leonardo Pereira, and Fabrizio Caputo

Human Performance Research Group, College of Health and Sport Science, Santa Catarina State University, Florianópolis, Santa Catarina, Brazil

Submitted 15 June 2015; accepted in final form 3 September 2015

Cruz RSO, de Aguiar RA, Turnes T, Pereira KL, Caputo F. Effects of ischemic preconditioning on maximal constant-load cycling performance. J Appl Physiol 119: 961–967, 2015. First published September 10, 2015; doi:10.1152/japplphysiol.00498.2015.—This study investigated the effects of ischemic preconditioning (IPC) on the ratings of perceived exertion (RPE), surface electromyography, and pulmonary oxygen uptake (VO₂) onset kinetics during cycling until exhaustion at the peak power output attained during an incremental test. A group of 12 recreationally trained cyclists volunteered for this study. After determination of peak power output during an incremental test, they were randomly subjected on different days to a performance protocol preceded by intermittent bilateral cuff pressure inflation to 220 mmHg (IPC) or 20 mmHg (control). To increase data reliability, the performance visits were replicated, also in a random manner. There was an 8.0% improvement in performance after IPC (control: 303 s, IPC 327 s, factor SDs of ×/+ 1.13, P = 0.01). This change was followed by a 2.9% increase in peak VO₂ (control: 3.95 l/min, IPC: 4.06 l/min, factor SDs of ×/+ 1.15, P = 0.04), owing to a higher amplitude of the slow component of the VO₂ kinetics (control: 0.45 l/min, IPC: 0.63 l/min, factor SDs of ×/+ 2.21, P = 0.05). There was also an attenuation in the rate of increase in RPE (P = 0.01) and a progressive increase in the myoelectrical activity of the vastus lateralis muscle (P = 0.04). Furthermore, the changes in peak VO₂ (r = 0.73, P = 0.007) and the amplitude of the slow component (r = 0.79, P = 0.002) largely correlated with performance improvement. These findings provide a link between improved aerobic metabolism and enhanced severe-intensity cycling performance after IPC. Furthermore, the delayed exhaustion after IPC under lower RPE and higher skeletal muscle activation suggest they have a role on the ergogenic effects of IPC on endurance performance.

endurance exercise; aerobic metabolism; surface electromyography; ratings of perceived exertion; pulmonary oxygen uptake kinetics

Originally developed with the aim of protecting cardiac muscle fibers (51), local acute ischemic preconditioning (IPC) consists of a potent endogenous mechanism that has been shown to protect various tissues against ischemia-reperfusion injury (27). Particularly, the benefits of IPC on noncontracting skeletal muscles during prolonged periods of blood flow occlusion have been described in detail (1, 31, 55, 60). Hence, it has not taken long for the procedure to be translated to improve exercise performance in humans (16, 17, 32).

In the last few years, IPC has been shown to enhance endurance performance during cycling (16, 17, 37), running (6), rowing (39), and more recently in a handgrip exercise (7). However, there are also studies reporting unchanged performance after the procedure (14, 23, 61). Of note, while the benefits of IPC on endurance are currently being investigated, the mechanisms underlying these improvements are still unclear. Research has identified a greater ability for aerobic energy transduction after IPC (7, 17, 37). For example, IPC accelerated muscle deoxygenation kinetics in a moderate-intensity cycling exercise (37) and increased oxygen uptake (VO₂) peak during a maximal ramp exercise test (17). Conversely, other studies reported that IPC could increase endurance performance without affecting the VO₂ responses (5, 6, 14, 16). On the other hand, an increase in neural drive to the active muscles has also been speculated (16). In the only relevant study, surface electromyographic (EMG) potentials and maximal isometric force were increased in skeletal muscle of animals following IPC (57), suggesting increased motor unit recruitment. However, the effects of IPC on EMG amplitude has not yet been investigated in exercising humans. Finally, it has also been suggested that IPC lowers the sensitivity of the body to fatigue signals (16). If so, this should result in lower ratings of conscious perception of effort [rating of perceived exertion (RPE)] during a constant-load exercise, as it is derived from sensory input arriving from many different biological systems, including the cardiovascular, respiratory, and musculoskeletal systems (15).

While time trials are the preferred choice for studies simulating the actual nature of a sporting event, they are not optimal for tracking eventual changes induced by IPC on VO₂ kinetics or the rates of increase in RPE and EMG amplitude. On the other hand, time-to-exhaustion (Tlim) tests have a similar sensitivity to that of time trials to changes in endurance (3), with an advantage in that they do not permit fluctuations of exercise intensity that can add “noise” to the measurement of physiological markers (45). Therefore, we have examined the hypothesis that IPC could improve the exercise tolerance during a constant-load cycling exercise performed at the peak power output attained during an incremental test (PPO). The choice of the test could be relevant to athlete performance, as it closely resembles the requirements of a track 4-km pursuit event. Additionally, we investigated the hypotheses that the ergogenic effects of IPC on endurance performance could be related to 1) an improved aerobic metabolism, 2) an increase in neural drive to the active muscles, and/or 3) a reduction in the perception of effort. Therefore, the primary aim of this study was to evaluate the effect of IPC of the lower limbs on endurance performance. Our secondary aim was to investigate the IPC effects on VO₂ kinetics, neuromuscular function, and perceptual responses.
METHODS

Ethical approval. This study was carried out in accordance with the guidelines contained in the Declaration of Helsinki and was approved by the Ethics Committee in Human Research of the Santa Catarina State University. The subjects were fully informed of any risks and discomforts associated with the experiments before giving their written, informed consent to participate.

Subjects. A group of 12 recreationally trained cyclists (18), ranging in age from 20 to 36 yr, with an average height of 177 cm (range: 171–188 cm) and an average body mass of 79 kg (range: 68–96 kg), volunteered for this study. None was receiving any pharmacological or specific dietetic treatment. All participants attended properly fed and hydrated and were instructed not to perform strenuous exercise and to abstain from alcohol on the day before each session. They were also asked to maintain the same dietary pattern throughout the experiment and to refrain from consuming caffeine for at least 2 h before each trial.

Study design. Subjects were required to report to the laboratory on five occasions over a 15-day period (±4 days), and all tests were interspersed with ~48 h of recovery. After an incremental test to determine PPO, subjects were then randomly submitted in sessions to a performance protocol preceded by either intermittent bilateral cuff inflation to 220 mmHg (IPC) or to 20 mmHg (control). To increase data reliability, visits 2 and 3 were replicated in visits 4 and 5, also in a random manner. The inclusion of an additional round of exercise in each condition reduces the typical error by a factor of 0.7 (i.e., 1/√n, where n is the number of transitions), and thus improved the signal-to-noise ratio by a factor of 1.4 (29). Each subject was always tested at the same time of day (±2 h) to minimize the effects of diurnal biological variation in a temperature-controlled laboratory (21 ± 1°C). All cycle tests were performed on an electro-dynamically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands). The ergometer seat and handlebar were adjusted for comfort, and the settings were replicated for subsequent tests. During the tests, subjects were instructed to maintain their preferred cadence for as long as possible until volitional exhaustion. They were verbally encouraged to give their best effort and were blinded to the time elapsed during exercise. Breath-by-breath VO2 and EMG responses were continuously registered, and capillary blood samples were taken at specific time points throughout the tests. During performance trials, the RPE were measured every minute by using Borg’s 6–20 scale (8), but subjects were uninformed about this frequency of collection.

Ischemic preconditioning. IPC was performed in the supine position using bilateral arterial occlusion. The occlusion cuffs were placed proximally around the upper thighs and inflated to 220 mmHg to restrict arterial inflow for 5 min. The ischemic procedure was repeated four times, each separated by 5 min of reperfusion. This protocol (i.e., four 5-min cycles of limb ischemia interspersed with 5 min of reperfusion) was chosen for two main reasons: 1) it has been successfully applied in previous studies investigating the ergogenic effects of IPC (23, 32, 39, 56); and 2) clinical studies have shown that at least three ischemia-reperfusion cycles are necessary to protect against skeletal muscle necrosis (i.e., infarction or tissue death) and endothelial dysfunction after prolonged periods of imposed ischemia (47, 55). Limb IPC comprising three to five cycles of 5-min inflations was found to be safe and well tolerated in both patients and healthy volunteers (25, 40, 46). Of interest, no objective signs of neurovascular injury were observed, with no subjects experiencing skin breakdown, prolonged discoloration, or temperature or pulse disparities in the treated limb. There were no cases of deep vein thrombosis or injury to the preconditioned limb, and no protocol was prematurely terminated due to subject discomfort. Furthermore, 5 min of blood flow restriction is far from depleting the muscle energy charge potential (55). On control condition, participants followed an identical protocol, but, instead, the cuff was inflated only to 20 mmHg.

Incremental exercise test. In the first visit, subjects underwent an incremental test to determine the maximum VO2 (VO2max) and PPO. The test consisted of 3 min of unloaded baseline pedaling (16 ± 4 W, equivalent to the lowest workload provided by the cycle ergometer), followed by a step increase in power output of 0.5 W/kg body mass every 3 min. PPO was defined as the power output attained at exhaustion if the test was terminated at the end of a 3-min stage. If the test was terminated before the last stage had been completed, PPO was calculated as the power of the previous stage plus the power increment times the duration (s) of exercise at the final stage divided by 180 s (43).

Tlim tests. Before the Tlim protocol, subjects were submitted to a standard warm-up protocol, followed by 5 min of passive recovery on the bike. The performance test consisted of 3 min of unloaded baseline pedaling, followed by a sudden increase to the required power output (i.e., 100% of PPO), which should be kept until volitional exhaustion, or participants could no longer maintain the chosen cadence minus 5 rpm for >5 s, despite strong verbal encouragement.

Gas exchange measurements. Throughout each testing protocol, cyclists wore a facemask, and respiratory gas exchange was measured breath by breath using an automated open-circuit gas analysis system (Quark CPET, Cosmed, Rome, Italy). Gas analyzers were always previously calibrated using ambient air, and gases contained 16% oxygen and 5% carbon dioxide. The turbine flow meter used for the determination of minute ventilation (Ve) was calibrated with a 3-liter calibration syringe. During the incremental test, the pulmonary gas exchange was measured with the aid of a 7-liter mixing chamber (52). The pulmonary VO2 data from each test were initially examined to exclude occasional errant breaths caused by coughing, swallowing, sighing, etc., which were considered not to be reflective of the underlying kinetics; i.e., only values greater than 3 SDs from the local mean were omitted. During the incremental exercise, the pulmonary VO2max attained was taken as the highest 15-breath rolling average value attained. An identical procedure was employed on the Tlim tests for the determination of peak VO2 and its associated heart rate (HR) and Ve. To avoid being influenced by the amount of data used in the comparison between the control and IPC conditions, the VO2 onset kinetics were analyzed by fixing the time window to the shortest Tlim recorded for each subject (i.e., isotime). The breath-by-breath data were first linearly interpolated to provide second-by-second values, and, for each individual, identical repetitions were time-aligned to the start of each exercise, and the ensemble was averaged. A biexponential model was then applied to characterize the VO2 kinetics in its fast and slow components:

\[ V\dot{O}_2(t) = V\dot{O}_{2b} + A1(1 - e^{-t/TD1e1}) + A2(1 - e^{-t/TD2e2}) \]

where \( V\dot{O}_2(t) \) represents the absolute VO2 at a given time \( t \); \( V\dot{O}_{2b} \) represents the mean VO2 in the final 30 s of the baseline period; \( A1 \), TD1, and \( t1 \) represent the amplitude, time delay, and time constant, respectively, describing the fundamental increase in VO2 above baseline; and \( A2 \), TD2, and \( t2 \) represent the amplitude, time delay before the onset of, and time constant describing the development of, the VO2 slow component (\( V\dot{O}_{2SC} \)), respectively. The initial phase was not modeled, and, therefore, the first 20 s on-transient were omitted to obviate any distorting influence of phase I on the subsequent kinetics.

Blood lactate measurements. At the end of each stage during the incremental test, capillary blood samples (25 μl) were taken from the earlobe and analyzed for blood lactate concentration. For the performance trials, samples were collected at the end of the 3 min of unloaded baseline pedaling and at exhaustion. All blood samples were stored in Eppendorf tubes containing 50 μl of 1% NaF in a −20°C environment. Later, samples were analyzed by enzyme electrode technology (YSI 1500 SPORT, Yellow Springs, OH). Calibration was made using a standard solution of 5 mmol/l.
**RESULTS**

**Incremental exercise test.** Subjects attained a PPO of 297 W \((\times / 1.11)\), and the \(\dot{V}O_2\)max was 4.05 l/min \((51 \text{ ml kg}^{-1} \text{ min}^{-1})\), with a factor SD of \(\times / 1.15\).

**Tlim tests.** The typical variation of Tlim measured at PPO was 9.9% (factor limits of \(\times / 1.3\)). After the application of IPC, performance was improved by 8.0% (confidence limits of \(\pm 5.7\%\, P = 0.01\)). The back-transformed means (i.e., the mean of the log-transformed variable converted back into raw units) for control and IPC conditions were 303 and 327 s, respectively, with a factor SD of \(\times / 1.13\).

**RPE.** There was an attenuation in the rate of increase in RPE during performance (Fig. 1), with the overall RPE being lower after IPC \((P = 0.01)\). At the end of the 4th minute of exercise, the average RPE was 0.8 units lower in the IPC condition (confidence limits of \(\pm 0.5\) units, \(P = 0.003\)). No changes were observed between conditions in RPE in the last minute before exhaustion \((P = 0.55)\).

**EMG.** The typical error of controlled EMG measurements was 3.8% (factor limits of \(\times / 1.2\)). The neuromuscular activity increased from the 1st minute of exercise to voluntary exhaustion in both the control and IPC conditions \((P < 0.001, \text{ Fig. 2})\), being uniformly higher during the IPC trial (overall effect of 2.3%, confidence limits of \(\pm 2.1\%\, P = 0.04)\).

**Physiological and metabolic responses.** The effects of IPC on cardiorespiratory and metabolic responses are presented in Table 1. At the end of 3 min of unloaded baseline pedaling, the blood lactate concentration and the pulmonary \(\dot{V}O_2\) were identical in both conditions. During performance, the peak \(\dot{V}O_2\) attained was significantly higher in the IPC condition, despite similar HR and \(\dot{V}E\). As there were no important changes in phase II of \(\dot{V}O_2\) onset kinetics, this increase in peak \(\dot{V}O_2\) resulted in a higher amplitude of the \(\dot{V}O_2\)SC, as seen in Fig. 3.

**Correlations.** The residuals of peak \(\dot{V}O_2\) largely correlated with 1) the residuals of the accumulated \(\dot{V}O_2\) at isotone \((r = 0.65, P = 0.02)\); and 2) those of performance improvement \((r = 0.73, P = 0.007)\). The performance improvement was also subject and treatment (i.e., individual responses to the IPC procedure) had been taken into account. The Pearson product moment correlation coefficient \((r)\) was used to examine the relationship between residuals. The uncertainties in the effects were expressed as 95% confidence limits, and all tests were analyzed at an \(\alpha\)-level of 0.05.
related to the changes in the slow component phenomenon \((r = 0.79, P = 0.002)\).

**DISCUSSION**

The major original findings of this investigation include the following. 1) Preconditioning of the lower limbs increased TIm by 8% during maximal constant-load performance in recreational cyclists, which was closely related to a significantly higher \(V_{O2\, SC}\) (and hence peak \(V_O2\)), rather than an acceleration in phase II kinetics. Such increased aerobic energy provision might have possibly reduced the rate of utilization of the finite anaerobic energy stores, delaying exhaustion (19, 34, 62). 2) These changes on endurance performance and aerobic metabolism were accompanied by an attenuation in the rate of increase in RPE and a progressive increase in the myoelectrical activity of the vastus lateralis throughout exercise. Together, these results suggest that a lower sensitivity of the body to fatigue signals and increases in central motor drive/output may possibly play a part in the beneficial effects of IPC on endurance performance, as previously speculated by Crisafulli et al. (16).

The benefit of IPC on aerobic metabolism has been suggested to be mediated by peripheral mechanisms (59), although possible effects on central hemodynamics cannot yet be fully ruled out. The active skeletal muscle is the predominant site of the \(V_{O2\, SC}\) phenomenon (58), which is consistent with the progressive increase in the EMG signal observed herein. Furthermore, the similar HR and \(V_e\) at peak \(V_O2\) suggests no excessive energetic requirements of cardiorespiratory work contributing to the differences (20, 38). The present results are thus in accordance with the recent observations of Barbosa et al. (7). The authors investigated the effects of remote IPC on forearm hemodynamics and deoxygenation during a constant-load rhythmic handgrip protocol by means of Doppler ultrasound and near-infrared spectroscopy. At the time of task failure, brachial artery blood flow was unaffected, but the deoxygenated hemoglobin and myoglobin concentration in the forearm was higher, indicating higher fractional \(O_2\) extraction. Although speculative, this lower blood flow/\(V_O2\) could be due to the recruitment of additional fibers, i.e., a baseline effect as seen for fast- vs. slow-twitch muscles (22, 49). Alternatively, an increase in maximal mitochondrial \(O_2\) flux capacity after IPC remains to be investigated, despite the fact that, during high-intensity cycling, in vivo maximal mitochondrial function seems to be limited by convective \(O_2\) delivery rather than an intrinsic mitochondrial limitation (10, 11).
It has been speculated that activation of ATP-sensitive potassium channels and/or elevated adenosine levels may play a role in the effects of IPC on aerobic metabolism and endurance performance (17). These suggestions find wide support in animal studies investigating the IPC-induced protection against ischemia-reperfusion injury on noncontracting muscles (31, 54, 60). An important consequence of both mechanisms would be an enhanced vasodilatory response following limb occlusion (50). This reactive hyperemia could possibly be improving the distribution of the regional skeletal muscle blood flow to the most active fibers, matching O₂ demand with O₂ delivery, or increasing mean capillary transit time, allowing a higher O₂ extraction per unit of blood flowing through the fully activated muscle fibers. Indeed, there are studies showing that regular IPC episodes can cause improvements in endothelial function in healthy individuals (35, 36). However, to the best of our knowledge, there is no evidence indicating improvements in skeletal muscle blood flow or vascular conductance after acute IPC under exercise conditions (7). Meanwhile, in the present study, as well as in a recent report (37), the phase II of VO₂ kinetics during severe-intensity cycling was unchanged, which could have been sensitive to an improved matching of local muscle O₂ availability to O₂ utilization. Furthermore, it may not be possible to increase peak leg VO₂ by inducing vasodilation during maximal cycling exercises, likely due to an alteration in the perfusion/VO₂ relationship (12).

Traditionally, the VO₂SC is at least partly associated with additional motor unit recruitment (26, 33, 41, 42), as exercise proceeds and the initially recruited fibers become fatigued. On the other hand, it has also been postulated that a progressive recruitment of muscle fibers is not obligatory for the development of the VO₂SC, and a reduction in the efficiency of contractions of fatigued fibers has been implicated (64). This interpretation, originating from an experiment involving isolated dog gastrocnemius muscle in situ, has recently received a significant support from studies involving humans (13, 63). The fact that endurance performance was improved after IPC suggest that, at least in this particular situation, the increase in VO₂SC (i.e., relative to control, not the entire VO₂max) may have been driven by the former, which has been considered the unique solution to maintain the same power output, even if there is a decrease in fiber efficiency (9). If IPC would make the muscle fibers less efficient owing to a lower level of metabolic stability (e.g., decreases in the Gibbs free energy of ATP hydrolysis and [phosphocreatine], increases in pH, [H⁺], [ADP], [P], [IMP], [NH₃], etc., where brackets denote concentration), the relationship between power output and VO₂ at submaximal intensities should have also been affected. This was proved not to be the case in a range of submaximal intensities reported by previously published literature (14, 17) and would be somewhat inconsistent with the ATP-sparing effect observed after IPC in noncontracting muscles during prolonged periods of ischemia (1, 55), although it is unknown whether the latter would stand during normal muscular activity with normal blood supply.

A higher voluntary neural drive/output from the spinal motoneuron pool is suggested by the increase in the response of the quadriceps on EMG throughout exercise following IPC (2). While it could be due to increases in motoneuronal firing rates, this higher EMG potential after IPC was already observed in feline hindlimbs submitted to electrical stimulation of the sciatic nerve (i.e., despite similar stimulation frequencies), along with lower decreases in maximal isometric force during prolonged ischemic periods (57). Together with the lower rate of increase in RPE throughout endurance performance, these findings could be suggesting that IPC inhibits central fatigue (24), which deserves further attention. If true, this response could possibly be associated with lower discharges from opioid-mediated muscle afferents, which are known to modulate endurance performance (2). Indeed, activation of opioid receptors in the striated muscle tissue by circulating endogenous opioid peptide(s) has been associated with the early phase of protection of either local acute or remote IPC (1, 21). Although further studies are necessary, the inhibition of spontaneous discharges from these nerve fibers to the central nervous system could be resulting in an overshoot in central motor drive (4), thereby allowing the utilization of a higher fraction of the skeletal muscle recruitment (functional) reserve (4, 16, 53). The fact that the VO₂SC have been greater after IPC (rather than delayed) would support this notion, as additional fiber recruitment is the only putative mediator of the VO₂SC that could improve performance (26, 33). This possibility has already been suggested in previous studies (16, 59). Thus, instead of representing a loss of muscle efficiency commonly demonstrated during severe-intensity cycling exercises (26), the higher VO₂SC phenomenon observed after IPC may have possibly spared an important amount of the finite anaerobic energy stores. This scenario is evidenced by the higher accumulated VO₂ at isotime and the correlations found in the present study. After isotime, the anaerobic energy spared could then be used to prolong exercise tolerance (19, 34, 62).

Perhaps the main methodological constraint of IPC studies is the inability to completely “blind” the subjects (17, 23, 44), because the sensations induced by the IPC and the low-pressure protocol are clearly distinct (44). In the present study, subjects were all deprived from any information until the end of the data collection, and they were always verbally encouraged to realize their maximum. However, a placebo effect cannot be discarded, though it is unlikely that the subjects had managed their own metabolism. Second, as we employed a time window of ~48 h between performance visits, the extent at which the less intense second window of protection lasting 48–72 h could have affected the results is unknown (48). In an attempt to mitigate this limitation, the order of trials was included as an additional fixed effect in the mixed model. Finally, while the present results might suggest an increased VO₂max after IPC, it can be noted that the peak VO₂ attained in the IPC condition was not really different from the VO₂max measured during the incremental test. Therefore, even though we have used a different apparatus during the incremental exercise (i.e., a mixing chamber system coupled to the gas analyzer), we cannot comment on the potential effects of IPC on VO₂max (14, 16, 17), which remains an open question.

In conclusion, the present findings provide a link between improved aerobic energy metabolism and enhanced severe-intensity cycling performance after IPC. Furthermore, the delayed exhaustion after IPC was accomplished under lower RPE and higher skeletal muscle activation, suggesting they have a role on the ergogenic effects of IPC on endurance performance.

ACKNOWLEDGMENTS

We thank all of the colleagues who contributed.
GRANTS
We thank Conselho Nacional de Desenvolvimento Científico e Tecnológico and Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina for financial support.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Author contributions: R.S.O.C. and F.C. conceived and designed research; R.S.O.C., R.A.A., T.T., K.L.P., and F.C. performed experiments; R.S.O.C. and F.C. interpreted results of experiments; R.S.O.C. prepared figures; R.S.O.C., R.A.A., T.T., and K.L.P. analyzed data; R.S.O.C., R.A.A., T.T., and K.L.P. drafted manuscript; R.S.O.C. and F.C. edited and revised manuscript; R.S.O.C., R.A.A., T.T., and K.L.P. approved final version of manuscript.

REFERENCES