Effect of Ischemic Preconditioning on Lactate Accumulation and Running Performance

TOM G. BAILEY[1], HELEN JONES[1], WARREN GREGSON[1], GREG ATKINSON[1,2], NIGEL TIMOTHY CABLE[1], and DICK H. J. THIJSSEN[1,3]

[1]*Research Institute for Sport and Exercise Science, Liverpool John Moores University, Liverpool, England, UNITED KINGDOM;* [2]*Health and Social Care Institute, School of Health and Social Care, Teesside University, Middlesbrough, England, UNITED KINGDOM; and* [3]*Department of Physiology, Radboud University Nijmegen Medical Centre, Nijmegen, THE NETHERLANDS*

BASIC SCIENCES

ABSTRACT

BAILEY, T. G., H. JONES, W. GREGSON, G. ATKINSON, N. T. CABLE, and D. H. J. THIJSSEN. Effect of Ischemic Preconditioning on Lactate Accumulation and Running Performance. *Med. Sci. Sports Exerc.*, Vol. 44, No. 11, pp. 2084–2089, 2012. **Purpose**: Repeated bouts of ischemia followed by reperfusion (i.e., ischemic preconditioning (IPC)) protect against damage after a myocardial infarction. Recent observational data indicate that IPC improves exercise performance. However, no previous study has examined potential underlying mechanisms for this effect of IPC. Therefore, we examined the potential of IPC to improve 5-km running time trial performance and reduce lactate accumulation during an incremental exercise test. **Methods**: In a randomized, crossover study, 13 healthy men performed running exercise, which was preceded by IPC (4 × 5-min 220 mm Hg bilateral leg occlusion) or a control intervention (C) (4 × 5-min 20 mm Hg bilateral leg occlusion). Participants performed a graded maximal treadmill running test, starting with five 3-min submaximal stages (10–14 $km \cdot h^{-1}$), followed by increments of 1 $km \cdot h^{-1}$ every 2 min to 16 $km \cdot h^{-1}$, followed by an incline of the treadmill of 2% every 2 min. Blood lactate was examined at each 3-min stage. After 45 min of rest in the supine position, subjects then performed a 5-km running time trial. **Results**: We found similar submaximal gas parameters during running exercise with both interventions. The overall increase in blood lactate during the submaximal stages was 1.07 ± 0.11 $mmol \cdot L^{-1}$ lower when exercise was preceded with IPC versus C (P = 0.023). The 5-km running time trial was completed in a time that was 34 s faster after IPC versus C (95% confidence interval, 5–64 s; P = 0.027). **Conclusion**: IPC improves 5-km time trial performance in healthy male individuals. Moreover, we found that IPC is associated with an attenuated rise in blood lactate concentration at submaximal level during an incremental running test. This could indicate that IPC allows for higher work rates and thus improves time trial performance.
Key Words: ISCHEMIC PRECONDITIONING, EXERCISE PERFORMANCE, ONSET OF LACTATE ACCUMULATION, TIME TRIAL, EXERCISE TRAINING

Repeated bouts of ischemia followed by reperfusion, commonly called ischemic preconditioning (IPC), are established intervention to protect against damage to the myocardium induced by a myocardial infarction, and these also protect other organs (12,27). In addition to the clinical relevance of IPC, recent observations revealed that IPC improves cycling (10,11) and swimming performance (18) in moderately and highly trained athletes. Despite these marked effects on exercise performance, no previous researcher examined potential mechanisms that may contribute to these remarkable findings.

Data from animal studies have indicated that IPC improves muscle blood flow via increases in intramuscular adenosine triphosphate (ATP)-sensitive potassium channels and adenosine levels (31). This increased blood flow improves oxygen delivery and may contribute to an increased removal of lactate (9,21), including the potential up-regulation of intra- and extracellular lactate shuttles during exercise (7,15,31). In addition, IPC also improves muscle contraction efficiency, possibly by enhancing muscle force and contractility (22) and/or via increased efficiency of excitation–contraction coupling (29). A more efficient muscle contraction augments mitochondrial capacity, subsequently improving the balance between lactate production and removal (18). Therefore, IPC may alter lactate metabolism in humans, consequently contributing to an improved exercise performance. Therefore, the aim of the present study was to examine the potential of IPC to improve running performance in healthy men and to examine blood lactate accumulation during incremental exercise after IPC compared with a sham treatment. We hypothesized that IPC

Address for correspondence: Dick H. J. Thijssen, PhD, Research Institute for Sport and Exercise Science, Liverpool John Moores University, Tom Reilly Building, Byrom Street, Liverpool L3 3 AF, England, United Kingdom; E-mail: D.Thijssen@ljmu.ac.uk.
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improves exercise performance and leads to an attenuated accumulation of lactate during submaximal exercise.

METHODS

Participants

In a randomized, single-blind (the participant was naive regarding the potential effect of IPC on performance) crossover study, 13 healthy moderately trained males (age, 25 ± 6 yr; height, 176 ± 4 cm; weight, 77 ± 7 kg) volunteered to participate. On the basis of their medical history, participants were free of health problems and did not use any medication. Before testing, all participants were informed of the methods of the study but remained naive of the study rationale to prevent any placebo effect of IPC. All subjects provided written informed consent before participation. The study was approved by the institutional ethics committee and adhered to the Declaration of Helsinki (2000).

Experimental Design

All participants refrained from alcohol, caffeine, and additional nutritional training supplements 24 h before all exercise testing. Participants reported twice to the laboratory to perform the same testing procedure, either preceded by four cycles of 5-min bilateral cuff inflation to 220 mm Hg (i.e., IPC) or cuff inflation to 20 mm Hg (i.e., control). In a randomized, counterbalanced, single-blind, crossover study, participants performed incremental submaximal running to assess blood lactate accumulation. Subsequently, this exercise test was then completed to voluntary maximal exhaustion. After a 45-min rest in the supine position, subjects performed a 5-km running time trial on a treadmill (after being familiarized with this time trial three to four times before performance of this test).

Measurements

IPC. IPC was performed in the supine position using bilateral arterial occlusion (11). Automated occlusion cuffs were placed proximally around the upper thigh and inflated to 220 mm Hg to block arterial inflow for 5 min. The ischemic procedure was repeated four times bilaterally, with each ischemic episode separated by 5 min of rest. On another occasion, participants followed an identical protocol, but instead, the cuff was inflated to 20 mm Hg (which did not alter the arterial inflow). The latter procedure was used as the control intervention (C), and the order of the conditions was counterbalanced.

Blood lactate accumulation. A discontinuous incremental test was used to assess accumulation of blood lactate. The test commenced after a 5-min warm-up ranging between 6 and 10 $km \cdot h^{-1}$. (This was standardized for all tests.) This test was performed on a motorized treadmill (Pulsar 4.0; h/p/cosmos, Willich, Germany). Five cycles of 3-min submaximal stages (10–14 $km \cdot h^{-1}$, i.e., 1 $km \cdot h^{-1}$ increment per stage) were performed, interspersed with 30 s of passive recovery to obtain lactate measurements (5). During the test, breath-by-breath expired gases were continuously monitored (Oxycon IV; Viasys, Jaeger, Germany) for oxygen consumption ($\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$)), ventilation ($\dot{V}_E$ ($L \cdot min^{-1}$)), and RER and were averaged over the last 15 s of each stage. Heart rate was measured continuously with a chest strap and monitor (RS800; Polar, Kempele, Finland), although RPE was measured at the end of each stage using Borg 6–20 scale. A 2.5-mL venous blood sample was collected via a forearm cannula at rest and at each 30-s period between each submaximal stage. Upon collection, each sample was immediately placed on ice and spun in a refrigerated centrifuge. Plasma was stored at −80°C and later analyzed for lactate concentration (Randox Daytona, Crumlin Co Antrim, UK) Blood lactate concentration ($mmol^{-1}$) was plotted against workload (intensity) during the incremental running test. The absolute increase in blood lactate was plotted against time and compared between both conditions. In addition, the onset of blood lactate accumulation (OBLA) was analyzed as the point ($km \cdot h^{-1}$) that was associated with a lactate level that first exceeded the 4 $mmol^{-1}$ threshold (19). OBLA represents a marker of endurance capacity, which is frequently used to compare and predict endurance ability (28) and time trial performance (4). The reproducibility of the OBLA at a given intensity has been reported as high (r = 0.88) and is able to detect meaningful changes because a change in lactate threshold of 1.62 $km \cdot h^{-1}$ is necessary for a change in training status to be recognized (14).

Maximal running test. On completion of the submaximal stages, continuous 2-min stages were performed (speed increase by 1 $km \cdot h^{-1}$ per 2 min to a maximal running speed of 16 $km \cdot h^{-1}$, then followed by a grade increase of the treadmill of 2% every 2 min) until volitional exhaustion. Breath-by-breath expired gases were continuously monitored (see previous data) and were averaged over the last 15 s upon maximal exhaustion. Heart rate was measured continuously with a chest strap and monitor (RS800, Polar). Blood lactate was determined 3 min after cessation of the test.

Five-kilometer time trial. Upon completion of the incremental maximal running test (standardized for all participants), a 45-min rest period in the supine position followed, then a 5-km running time trial was performed on a motorized treadmill (Pulsar 4.0, h/p/cosmos). Participants were instructed to run 5 km as quickly as possible, although running time and running speed were blinded to the participant. The speed of the treadmill was set at 8 $km \cdot h^{-1}$, and once the participant was ready, the time trial was started. Throughout the time trial, participants were allowed to alter running speed but were kept blinded for running speed and running time. The only information available to the participants during each time trial was total distance covered (m) as to adjust work output to pace toward the known end point (1). No further information or encouragements were provided. Heart rate was monitored continuously, with RPE recorded at the end of each 1000 m. All trials were

TABLE 1. Exercise characteristics during the incremental running test in healthy subjects (n = 13).

	10 km·h^{-1}	11 km·h^{-1}	12 km·h^{-1}	13 km·h^{-1}	14 km·h^{-1}	*P* values
$\dot{V}O_2$ (mL O_2·kg^{-1}·min^{-1})						
Control	34.10 ± 2.48	36.53 ± 2.21	39.44 ± 2.48	42.08 ± 2.44	45.37 ± 2.84	Time: <0.001
IPC	34.36 ± 1.22	36.64 ± 1.17	39.46 ± 1.82	41.90 ± 2.28	44.96 ± 2.81	IPC: 0.971
						Time × IPC: 0.796
$\dot{V}_E$ (L·min^{-1})						
Control	65.46 ± 9.17	71.61 ± 10.94	81.38 ± 15.03	94.08 ± 17.52	106.54 ± 19.07	Time: <0.001
IPC	64.73 ± 8.8	72.69 ± 10.41	81 ± 12.77	94.46 ± 18.76	105.85 ± 17.9	IPC: 0.881
						Time × IPC: 0.630
RER						
Control	0.84 ± 0.03	0.88 ± 10.4	0.91 ± 0.03	0.94 ± 0.05	0.99 ± 0.04	Time: <0.001
IPC	0.83 ± 0.03	0.86 ± 0.04	0.91 ± 0.03	0.94 ± 0.07	0.98 ± 0.03	IPC: 0.378
						Time × IPC: 0.521
Heart rate (beats·min^{-1})						
Control	136 ± 12	152 ± 14	166 ± 8	175 ± 7	181 ± 12	Time: <0.001
IPC	136 ± 13	150 ± 12	166 ± 11	176 ± 5	182 ± 14	IPC: 0.761
						Time × IPC: 0.540
RPE						
Control	10 ± 2	12 ± 2	13 ± 2	14 ± 1	16 ± 1	Time: <0.001
IPC	10 ± 2	12 ± 2	13 ± 2	14 ± 2	16 ± 2	IPC: 0.357
						Time × IPC: 0.841

performed with a fan placed 0.5 m in front of the treadmill to provide air circulation and cooling to the participant to match field conditions. Before the beginning of the experimental trials, participants received at least three supervised familiarization trials, with the 5-km time trial revealing a coefficient of variation (CV) of 2.2% after familiarization.

Statistics

Statistical analyses were performed using SPSS 17.0 (SPSS, Chicago, IL) software. All data are reported as means ± SD, and statistical significance was assumed at $P < 0.05$. A two-factor (trial × time) repeated-measures general linear model with 95% confidence intervals (CIs) was used to assess differences in parameters (blood lactate levels, oxygen consumption, and heart rate) during the five stages of the incremental test to examine our primary hypothesis. A two-factor repeated-measures general linear model (trial × time) was also used to examine differences in parameters during the 5-km time trial (RPE and heart rate). A paired *t*-test was used to compare OBLA, maximal gas parameters, and 5-km time trial performance and speed between IPC and C. For all analyses, trial order (IPC or C first) was entered into the statistical model as a between-subjects factor, partitioning out this source of variability. Mean speed data were logarithmically transformed for analysis as data were not normally distributed. According to previous advice, the least significant difference test was used for pairwise multiple comparisons (30). Our primary outcome was 5-km time trial performance, and our primary comparison was between IPC and control conditions. Time trial protocols have been found to be highly reliable with reported test–retest CVs of 1%–2% (2). Using the nomogram presented in the study of Batterham and Atkinson (3) and the nQUERY statistical power software (Statistical Solutions, Cork, Ireland), we estimated that a sample size of 13 would allow detection of a mean difference in performance of 1.8% assuming that the test–retest CV is 2.2%, the use of a two-tailed paired *t*-test, alpha = 0.05, and beta = 0.20.

RESULTS

Blood lactate accumulation. Heart rate, oxygen consumption, ventilation, and RPE increased across the five incremental stages, but these increases were of similar magnitude in both conditions (Table 1). Resting blood lactate levels were similar between both tests (Fig. 1). Blood lactate concentration increased over time in both conditions ($P < 0.001$). Interestingly, the difference in blood lactate accumulation from rest was 1.07 ± 0.11 mmol^{-1} lower (difference from rest to 14 km·h^{-1}: IPC, 3.14 ± 0.35 and C, 4.21 ± 0.46 mmol^{-1}) when exercise was preceded with IPC compared with control, resulting in a significantly lower blood lactate concentration at 14 km·h^{-1} (Fig. 1). A later OBLA was evident when exercise was preceded with IPC, but this did not reach statistical significance (13.1 ± 1.9 and 14.6 ± 1.4 km·h^{-1}; mean difference of 1.5 km·h^{-1}; 95% CI, −0.18 to 3.87; $P = 0.079$).

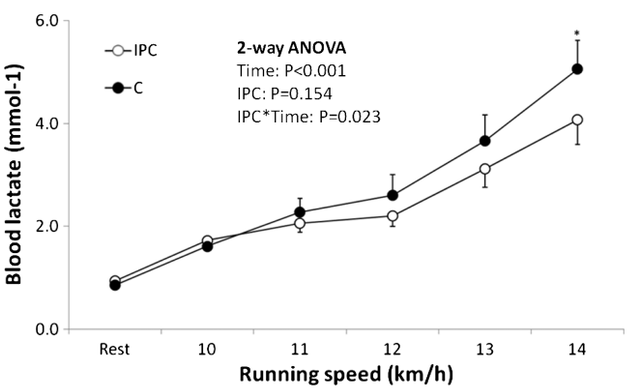

FIGURE 1—Blood lactate levels at rest at all five submaximal stages during the incremental running test with exercise being preceded by IPC (*solid circles*) or C (*open circles*). *Error bars* represent standard error (SE). **Post hoc* significantly different between C and IPC.

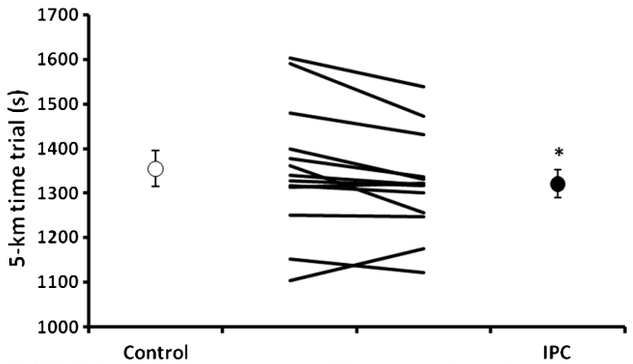

FIGURE 2—Individual and mean (SE) data on 5-km time trial performance after IPC and C in healthy young men (n = 13). *Denotes a significant treatment effect of IPC (P = 0.027).

Maximal running test. We found no differences in $\dot{V}O_{2max}$ between IPC and C (52.1 ± 4.6 and 51.5 ± 4.3 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$, respectively, P = 0.258). No differences were observed in $\dot{V}_E$ (IPC, 145 ± 19; C, 144 ± 20 $L \cdot min^{-1}$; P = 0.451), RER (IPC, 1.19 ± 0.11; C, 1.18 ± 0.08; P = 0.613), and HR (IPC, 195 ± 6; C, 195 ± 4 $beats \cdot min^{-1}$; P = 0.742) at the end of the maximal running test between both conditions. No differences in postexercise lactate levels were found between IPC and C (IPC, 10.6 ± 0.8; C, 10.1 ± 0.6; P = 0.202). No differences in time to exhaustion on the incremental exercise were found between IPC and control (IPC, 19.8 ± 2.5 min; C, 19.6 ± 2.2 min; P = 0.374).

Five-kilometer time trial. Mean time trial performance was 1355 ± 146 and 1321 ± 114 s after the control and IPC intervention, respectively (34 ± 49 s; 95% CI, 5–64 s; P = 0.027) (Fig. 2). These times correspond to a mean running speed of 13.7 ± 1.2 $km \cdot h^{-1}$ for IPC and 13.4 ± 1.5 $km \cdot h^{-1}$ for C (P = 0.038). Heart rate and RPE gradually increased during the 5-km time trial, but these increases were similar between conditions (Table 2). However, RPE was significantly lower during the first 1000 m of the 5-km time trial after IPC compared with C (0.9 ± 0.3; 95% CI, −1.5 to −0.3; P = 0.030; Table 2).

DISCUSSION

Several previous studies found the potential of IPC to enhance exercise performance during cycling and swimming exercise (10,11,18). We extend this knowledge as we found that IPC has a statistically and practically significant effect on 5-km *running* performance in healthy men. More importantly, we have found that IPC attenuates the accumulation of blood lactate during an incremental running test.

The importance of lactate metabolism for endurance performance has recently been demonstrated. Jacobs et al. (17) found increases in blood lactate concentration and in mitochondrial capacity, accounting for 68% of the variation in cycling time trial performance. Also, lower blood lactate concentrations at a given workload improves endurance exercise in various populations, including the highly trained (23,25). The reduction in blood lactate accumulation after IPC at submaximal level in the current study may relate to a positive effect on exercise performance. We observed the average running speed during the 5-km time trial to be 13.7 $km \cdot h^{-1}$, and this corresponded with the chosen running speed during the incremental test (14 $km \cdot h^{-1}$), which was associated with the largest difference in blood lactate levels (Fig. 1). Nevertheless, a direct effect of lower blood lactate levels on the time trail performance can only be inferred given that we did not perform lactate measurements during the time trial. It is also possible that IPC exerted an ergogenic effect, as evidenced by diminished subjective perception of a given exercise intensity that was evident in the first 1000 m of the 5-km time trial. Nonetheless, our findings support the applicability of IPC as a novel strategy to improve exercise performance in humans, potentially because of an attenuated blood lactate accumulation.

Recent studies, including from our laboratory, have highlighted the potential of IPC to improve cycling and swimming exercise performance (10,11,18). Our data add novel information that IPC also improves running performance. In addition, we focused on endurance exercise (i.e., 5-km time trial), whereas previous articles have examined short-term exercise performance (i.e., 100-m swimming [18]) or focused on performance during a maximal exercise test (10,11). Another important difference between studies relates to the timing of IPC in relation to the exercise bout. Previous researchers have observed beneficial effects of IPC when applied immediately before exercise. In marked contrast, the faster 5-km time trial was performed 90 min *after* IPC (90 min consists of the submaximal/incremental running test and 45-min resting period). This suggests that the benefits of IPC on exercise performance have a longer time window than initially anticipated. Finally, the benefits of IPC were apparent in the 5-km time trial, despite

TABLE 2. Exercise characteristics during the 5-km time trial in healthy subjects (n = 13).

	1000 m	2000 m	3000 m	4000 m	5000 m	*P* values
Heart rate ($beats \cdot min^{-1}$)						
Control	167 ± 8	173 ± 7	176 ± 5	177 ± 5	185 ± 6	Time: <0.001
IPC	166 ± 11	172 ± 6	177 ± 8	179 ± 5	189 ± 7	IPC: 0.159
						Time × IPC: 0.180
RPE						
Control	14 ± 3	15 ± 2	16 ± 1	17 ± 1	19 ± 1	Time: <0.001
IPC	13 ± 3*	15 ± 2	16 ± 1	17 ± 1	19 ± 1	IPC: 0.136
						Time × IPC: 0.030

* Significantly different between IPC and C at $P < 0.05$.

performance of a running test immediately after IPC and before the time trial. This suggests that the effects of IPC remain, even after an initial exercise bout. Practically, these observations are highly important, because application of IPC during athletic competition will likely be performed before the warm-up stage, which is usually some time before the actual performance event.

The positive effects of IPC during exercise performance raise questions regarding potential underlying mechanisms. Our data clearly demonstrate that the magnitude of lactate accumulation during incremental stages of running exercise was significantly attenuated when exercise was preceded by IPC, a finding that is supported by the trend for a later OBLA after IPC. The importance of this finding is that submaximal blood lactate concentration may contribute to the variation in cycling time trial performance (17), with lower blood lactate concentrations at a given workload being related to an improved endurance capacity (23,25). Our findings of an attenuated accumulation in blood lactate after IPC suggest that the involvement of psychological factors as a primary working mechanism for IPC during exercise performance is negligible. Therefore, this study is the first to identify a reduced blood lactate during incremental running exercise after IPC.

It is important to note that the attenuated accumulation of lactate during the exercise bout preceded by IPC is not explained by differences in exercise intensity level (Table 1), which were similar between both tests. Moreover, because of our randomization procedure and statistical analysis, it is also unlikely that familiarization or order effects explain our findings. A potential explanation for the altered lactate levels in our study may relate to an increased clearance or decreased production of lactate during incremental exercise, or both. For example, IPC may work through improvements in vascular function, which regulate blood flow to remove and transport lactate for uptake and use. Interestingly, IPC improves muscle blood flow via increases in intramuscular ATP-sensitive potassium channels and adenosine levels (31), whereas IPC is also able to protect the vascular wall against potentially harmful stimuli (20,24). In addition, an up-regulation of the mitochondrial permeability transition pore both locally and systemically during exercise after IPC may have enhanced mitochondrial lactate influx and subsequent oxidation in the working muscle (15) and, systemically, that is, heart for oxidation (13). An alternative but not mutually exclusive explanation could relate to a reduction in muscle lactate production after IPC. Animal studies have shown previously that IPC can enhance muscle efficiency in ATP usage via ATP sparing, augmented mitochondrial flux, or increased efficiency in the excitation–contraction coupling (18,22,29). Nevertheless, within the bounds of our current data, we can only speculate about the mechanisms underlying our findings, upon which future studies should focus.

Despite the positive effects of IPC on exercise performance, we found no effects of IPC on oxygen consumption or any other parameter during the maximal running test. This finding is in agreement with one previous study (10) but in contrast with another (11). A potential explanation for the disparity in findings may relate to the mode of exercise used for the maximal exercise tests (e.g., treadmill or cycle ergometer). Peak values and oxygen uptake kinetics differ between a cycling ergometer and treadmill exercise test (16,26). Nonetheless, examining parameters at the submaximal exercise level, our data are in agreement with a previous study that used a cycling ergometer and found no differences between the control and IPC condition (10).

Limitations. A potential limitation is that we cannot extrapolate our findings to elite athletes and/or different types of exercise performance. However, previous studies have already revealed the potential for IPC to improve performance in highly trained athletes, although benefits of IPC are described during different types of exercise (11,18). Another potential limitation is that we applied IPC to the lower limbs only and therefore are unable to comment on the effect of local (i.e., in the legs only) or systemic (i.e., in the upper limbs as well) effects of IPC. This is of unique importance because IPC has well-established remote effects when applied clinically, for example, by protecting cardiac tissue when being applied to the lower limbs before (cardiac) surgery (6,8). Whether such remote effects of IPC are also present for exercise performance is currently unknown. Also, because of the study design, it is unknown whether the promoting effects of IPC on 5-km time trial performance are only evident after a bout of incremental exercise to exhaustion. However, the performance-enhancing effects of IPC currently available in the literature are evident after no prior high-intensity exercise. For this reason, it appears an intervention of IPC could possibly be used for acute enhancements in performance and/or for events that require consecutive bouts or rounds within a short time frame. This however is hypothetical and is beyond the scope of the study. A final limitation is that we cannot exclude the possibility that improvement of our outcome parameters after IPC actually relates to a decrease in these parameters after the sham treatment. However, we believe this option unlikely because 20 mm Hg has no significant physiological effects.

In conclusion, we confirmed the potential of IPC to improve running exercise performance in healthy men. More importantly, we found that IPC attenuated blood lactate accumulation during submaximal running exercise, which may contribute to the beneficial effect of IPC on exercise performance. Our findings support the applicability of IPC as a novel, inexpensive, easily applied, and noninvasive strategy to improve exercise performance in humans. However, future studies should focus on elucidating the mechanism(s) responsible for the observed decrease in blood lactate concentration during incremental exercise.

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None of the authors have declared conflict of interest.

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REFERENCES

1. Albertus Y, Tucker R, Gibson ASC, Lambert EV, Hampson DB, Noakes TD. Effect of distance feedback on pacing strategy and perceived exertion during cycling. *Med Sci Sports Exerc*. 2005; 37(3):461–8.
2. Atkinson G, Nevill AM. Selected issues in the design and analysis of sport performance research. *J Sports Sci*. 2001;19(10):811–27.
3. Batterham AM, Atkinson G. How big does my sample need to be? A primer on the murky world of sample size estimation. *Phys Ther Sport*. 2005;6(3):153–63.
4. Bentley D, McNaughton L, Thompson D, Vleck V, Batterham A. Peak power output, the lactate threshold, and time trial performance in cyclists. *Med Sci Sports Exerc*. 2001;33(12):2077–81.
5. Bentley DJ, Newell J, Bishop D. Incremental exercise test design and analysis: implications for performance diagnostics in endurance athletes. *Sports Med*. 2007;37(7):575–86.
6. Botker HE, Kharbanda R, Schmidt MR, et al. Remote ischaemic conditioning before hospital admission, as a complement to angioplasty, and effect on myocardial salvage in patients with acute myocardial infarction: a randomised trial. *Lancet*. 2010;375(9716):727–34.
7. Brooks GA. Intra and extra cellular lactate shuttles. *Med Sci Sports Exerc*. 2000;32(4):790–9.
8. Cheung MM, Kharbanda RK, Konstantinov IE, et al. Randomized controlled trial of the effects of remote ischemic preconditioning on children undergoing cardiac surgery: first clinical application in humans. *J Am Coll Cardiol*. 2006;47(11):2277–82.
9. Cooper C, Brown G. The inhibition of mitochondrial cytochrome oxidase by the gases carbon monoxide, nitric oxide, hydrogen cyanide and hydrogen sulfide: chemical mechanism and physiological significance. *J Bioenerg Biomembr*. 2008;40(5):533–9.
10. Crisafulli A, Tangianu F, Tocco F, et al. Ischemic preconditioning of the muscle improves maximal exercise performance but not maximal oxygen uptake in humans. *J Appl Physiol*. 2011;111(2):530–6.
11. De Groot PC, Thijssen DH, Sanchez M, Ellenkamp R, Hopman MT. Ischemic preconditioning improves maximal performance in humans. *Eur J Appl Physiol*. 2010;108(1):141–6.
12. Eisen A, Fisman EZ, Rubenfire M, et al. Ischemic preconditioning: nearly two decades of research. A comprehensive review. *Atherosclerosis*. 2004;172(2):201–10.
13. Gertz EW, Wisneski JA, Stanley WC, Neese RA. Myocardial substrate utlisation during exercise in humans. *J Clin Invest*. 1988;82: 2017–25.
14. Grant SG, McMillan KM, Newell JN, et al. Reproducibility of the blood lactate threshold, 4 mmol·l(−1) marker, heart rate and ratings of perceived exertion during incremental treadmill exercise in humans. *Eur J Appl Physiol*. 2002;87(2):159–66.
15. Hashimoto T, Brooks GA. Mitochondrial lactate oxidation complex and an adaptive role for lactate production. *Med Sci Sports Exerc*. 2008;40(3):486–94.
16. Hill DW, Davey KM, Stevens EC. Maximal accumulated O2 deficit in running and cycling. *Can J Appl Physiol*. 2002;27(5):463–78.
17. Jacobs RA, Rasmussen P, Siebenmann C, et al. Determinants of time trial performance and maximal incremental exercise in highly trained endurance athletes. *J Appl Physiol*. 2011;111(5):1422–30.
18. Jean-St-Michel E, Manlhiot C, Li J, et al. Remote preconditioning improves maximal performance in highly trained athletes. *Med Sci Sports Exerc*. 2011;43(7):1280–6.
19. Jordan T, Lukaszuk J, Misic M, Umoren J. Effect of beta-alanine supplementation on the onset of blood lactate accumulation (OBLA) during treadmill running: pre/post 2 treatment experimental design. *J Int Soc Sports Nutr*. 2010;7:20.
20. Kharbanda RK, Peters M, Walton B, et al. Ischemic preconditioning prevents endothelial injury and systemic neutrophil activation during ischemia–reperfusion in humans in vivo. *Circulation*. 2001;103(12): 1624–30.
21. Kimura M, Ueda K, Goto C, et al. Repetition of ischemic preconditioning augments endothelium-dependent vasodilation in humans: role of endothelium-derived nitric oxide and endothelial progenitor cells. *Arterioscler Thromb Vasc Biol*. 2007;27(6):1403–10.
22. Lawson CS, Downey JM. Preconditioning: state of the art myocardial protection. *Cardiovasc Res*. 1993;27:542–50.
23. Lorenzo S, Minson CT, Babb TG, Halliwell JR. Lactate threshold predicting time-trial performance: impact of heat and acclimation. *J Appl Physiol*. 2011;111:221–7.
24. Loukogeorgakis SP, Panagiotidou AT, Broadhead MW, Donald A, Deanfield JE, MacAllister RJ. Remote ischemic preconditioning provides early and late protection against endothelial ischemia-reperfusion injury in humans: role of the autonomic nervous system. *J Am Coll Cardiol*. 2005;46(3):450–6.
25. Lucia H, Hoyos J, Perez M, Santalla A, Earnest CP, Chicharro JL. Which laboratory variable is related with time-trial performance time in the Tour de France? *Br J Sports Med*. 2004;38:636–40.
26. Millet GP, Vleck VE, Bentley DJ. Physiological differences between cycling and running: lessons from triathletes. *Sports Med*. 2009;39(3):179–206.
27. Murray CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation*. 1986;74:1124–36.
28. Newell J, Higgins D, Madden N, et al. Software for calculating blood lactate endurance markers. *J Sports Sci*. 2007;25(12):1403–9.
29. Pang CY, Yang RZ, Zhong A, Xu N, Boyd B, Forrest CR. Acute ischaemic preconditioning protects against skeletal muscle infarction in the pig. *Cardiovasc Res*. 1995;29(6):782–8.
30. Perneger TV. What's wrong with Bonferroni adjustments. *BMJ*. 1998;316(7139):1236–8.
31. Riksen NP, Smits P, Rongen GA. Ischaemic preconditioning: from molecular characterisation to clinical application - part 1. *Neth J Med*. 2004;62(10):353–63.