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Ischemic preconditioning of the muscle improves maximal exercise performance but not maximal oxygen uptake in humans

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Crisafulli A, Tangianu F, Tocco F, Concu A, Mameli O, Mulliri G, Caria MA. Ischemic preconditioning of the muscle improves maximal exercise performance but not maximal oxygen uptake in humans. *J Appl Physiol* 111: 530–536, 2011. First published May 26, 2011; doi:10.1152/japplphysiol.00266.2011.—Brief episodes of nonlethal ischemia, commonly known as "ischemic preconditioning" (IP), are protective against cell injury induced by infarction. Moreover, muscle IP has been found capable of improving exercise performance. The aim of the study was the comparison of standard exercise performances carried out in normal conditions with those carried out following IP, achieved by brief muscle ischemia at rest (RIP) and after exercise (EIP). Seventeen physically active, healthy male subjects performed three incremental, randomly assigned maximal exercise tests on a cycle ergometer up to exhaustion. One was the reference (REF) test, whereas the others were performed after the RIP and EIP sessions. Total exercise time (TET), total work (TW), and maximal power output (W_{max}), oxygen uptake (VO_{2max}), and pulmonary ventilation (VE_{max}) were assessed. Furthermore, impedance cardiography was used to measure maximal heart rate (HR_{max}), stroke volume (SV_{max}), and cardiac output (CO_{max}). A subgroup of volunteers ($n = 10$) performed all-out tests to assess their anaerobic capacity. We found that both RIP and EIP protocols increased in a similar fashion TET, TW, W_{max}, VE_{max}, and HR_{max} with respect to the REF test. In particular, W_{max} increased by ~4% in both preconditioning procedures. However, preconditioning sessions failed to increase traditionally measured variables such as VO_{2max}, SV_{max}, CO_{max}, and anaerobic capacity. It was concluded that muscle IP improves performance without any difference between RIP and EIP procedures. The mechanism of this effect could be related to changes in fatigue perception.

stroke volume; power output; reperfusion; muscle contraction

IT HAS BEEN DEMONSTRATED THAT brief episodes of nonlethal ischemia render the myocardium more resistant to subsequent ischemic insults. This phenomenon, known as ischemic preconditioning (IP) (24), is one of the most effective, protective stimuli currently known against cardiac cell death caused by infarction, as well as damage caused by ischemia-reperfusion, such as arrhythmias and stunning (2, 5a, 6, 8, 28–30). Moreover, cardiac IP has been found capable of improving cardiac performance and stroke volume (SV) in patients with stable angina (9, 22). From the studies cited, it appears that the positive effects exerted by IP on the myocardium are well demonstrated and characterized.

Along with the actions on the myocardium, emerging evidence supports the notion that IP may be beneficial, virtually for every tissue of the body. Indeed, IP has been described for kidney, brain, liver, and skeletal muscle (15, 19–21, 23, 27, 33). However, in contrast to what has been reported for the myocardium, studies dealing with skeletal muscle preconditioning are still lacking, and the phenomenon needs to be investigated further. A recent study has found that skeletal muscle IP improves its function and increases maximal oxygen uptake (VO_{2max}) and power output (W_{max}) during maximal cycling performance in healthy and physically fit subjects (15). In this investigation, the authors found only a slight (1.6%) but significant increase in subjects' performances following the preconditioning maneuver. In another very recent investigation, it has been demonstrated that skeletal muscle IP improves maximal performance in highly trained swimmers. In detail, in this study, an average improvement of 0.70 s in a 100-m performance has been shown (18).

However, it must be pointed out that in these studies, IP was induced at rest and therefore, in a condition that is not expected to cause a substantial metabolite production and accumulation. Since it has been reported that to induce IP in the heart, a certain amount of metabolite accumulation (such as adenosine, bradykinin, reactive oxygen species, and opioids) is necessary—i.e., a threshold for metabolite concentration has to be reached to trigger the biochemical cascade that leads to IP (5a)—it can be speculated that in these studies, the threshold was barely reached, thus explaining the limited enhancement reported.

We wondered whether ischemia induced after exercise (EIP), which certainly correlates to a larger metabolite accumulation than when induced at rest (RIP), is able to cause a more intense preconditioning effect. Based on these suppositions, we designed a study to compare the consequences on exercise performance of muscle IP obtained by means of brief episodes of ischemia induced at rest and after exercise. We hypothesized that EIP would lead to a larger amount of metabolite accumulation and consequently, to a more-evident preconditioning effect than RIP, thus allowing the achievement to a higher work rate.

METHODS

Subjects. Seventeen healthy male individuals, whose age, height, and mass were 35.2 ± 9.1 yr, 176 ± 5.2 cm, and 73.5 ± 8.4 kg, respectively, volunteered to participate in this investigation. All were physically active, and none had any history of cardiac or respiratory disease or was taking any medication at the time of the study. None of them showed health problems at the time of physical examination and on the electrocardiogram at rest. The study was carried out according to the Declaration of Helsinki and was approved by a local ethics committee. All subjects gave written, informed consent and were unaware of the nature of the study.

Experimental design. Each subject underwent the following protocol, randomly assigned to eliminate any order effect:

1) reference incremental maximal test (REF test): subjects performed a bicycle incremental test on an electromagnetically braked cycle ergometer (Cardioline STS400, S. Pedrino di Vignate, Italy) to assess the maximum workload achievable (W_{max}). This test consisted of a linear increase of workload of 25 W/min, starting from 25 W at a pedaling frequency of 60 rpm up to exhaustion (i.e., the point when the subject was unable to maintain a pedaling rate of at least 50 rpm); the effort was preceded by 3 min of resting, during which baseline data were gathered.

2) incremental maximal test after RIP test: subjects performed the same test as described for the REF session, but it was preceded by the IP obtained in subjects lying supine by occluding leg circulation with two pneumatic cuffs positioned around the thighs and inflated 50 mmHg above each subject's systolic blood pressure (SBP). Circulatory occlusion lasted 5 min and was performed three times, each separated by 5 min of reperfusion. The exercise test started 5 min after the ischemic maneuver. This protocol was chosen, since it had already been used in a similar study (15).

3) incremental maximal test following EIP test: subjects performed the same test as in the REF and RIP sessions. In this experimental setup, IP was obtained by occluding leg circulation after an exercise that lasted 5 min and was performed at a constant workload that corresponded to 70% of the subject's anaerobic threshold (AT) assessed previously. This protocol, i.e., submaximal exercise-circulatory occlusion, was chosen, since it had been found in previous studies to lead to sufficient metabolite accumulation for muscle metaboreflex activation (11, 13). Just at the end of the exercise, two pneumatic cuffs, previously placed around the thighs, were rapidly inflated to 50 mmHg above the subject's SBP to induce ischemia in the exercised legs. Cuffs were kept inflated for 3 min, and then, 5 min later, the subjects performed the incremental test.

Tests were set apart by at least 1 wk, and the day before tests were scheduled, subjects were requested to avoid caffeine, alcohol ingestion, or consumption of any drug. Tests were performed between 09.00 and 14.00 h in a temperature-controlled room (room temperature set at 25°C; relative humidity at 50%).

Assessment of respiratory variables and hemodynamics. Values of oxygen uptake (VO_2), carbon dioxide production (VCO_2), and pulmonary ventilation (VE) were obtained by means of a portable metabolic system (MedGraphics VO2000, St. Paul, MN), which provides a three-breath average of variables through telemetric transmission. This system has been shown to be reliable and to have good agreement with standard metabolic devices for laboratory use (4, 26). Prior to testing, the VO2000 was calibrated according to the manufacturer's instructions. The AT was determined using the V-slope method, which detects AT by using computerized regression analysis of the slopes of the VCO_2 vs. VO_2 plot during exercise (3). Achievement of VO_{2max} was considered as the attainment of at least two of the following criteria: *1*) a plateau in VO_2, despite increasing speed (<80 mL·min^{-1}); *2*) a respiratory exchange ratio above 1.10; and *3*) a heart rate (HR) ± 10 beats·min^{-1} of predicted maximal HR (HR_{max}), calculated as 220 − age (17). Hemodynamic parameters throughout tests were assessed with an impedance cardiograph (PhysioFlow, Manatec Biomedical, Petit Ebersviller, France), which allows measurement of the following parameters: SV, HR, and cardiac output (CO) (5). Subjects were also connected to a manual sphygmomanometer to measure SBP and diastolic blood pressure (DBP), which was taken in the left arm, given by the same physician throughout all sessions. Mean blood pressure (MBP) was calculated as SBP + (SBP − DBP)/3. Systemic vascular resistance (SVR) was obtained by multiplying by 80 the MBP/CO ratio, where 80 is a conversion factor to change units to standard resistance units.

Calculations and data analysis. VO_{2max} and HR_{max} and maximal values of VCO_2 (VCO_{2max}), VE (VE_{max}), SV (SV_{max}), CO (CO_{max}), mean arterial pressure (MAP_{max}), and SVR (SVR_{max}) were calculated as the average of the last 15 s of exercise. By dividing VO_{2max} values by CO_{max}, the maximum artero-venous oxygen difference (A-V O_2D_{max}) was obtained. We also calculated the total exercise time (TET) and the total work (TW) during each test.

Additional experiments to assess anaerobic capacity. Since we found that both of the preconditioning maneuvers caused a significant improvement in W_{max} without increasing VO_{2max} (see RESULTS), we hypothesized that the enhancement of the performance was the result of an improvement in the subjects' anaerobic capacity. To verify this hypothesis, in a subgroup of the enrolled subjects (*n* = 10, age 34.1 ± 7.3 yr, height 177.2 ± 4.8 cm, and mass 77.6 ± 7.2 kg), additional experiments, consisting of three supramaximal all-out tests (AOTs), randomly assigned and set apart by at least 1 wk from each other, were performed. We chose this kind of exercise bout, because it is supposed to massively recruit the anaerobic energy sources and to lead to a high level of blood lactate (BLa) accumulation (14, 16). The tests were carried out according to the following protocol: the subject rested for 3 min in an upright, seated position on the cycle ergometer to obtain baseline data; this period was followed by a warm-up period of 5 min pedaling at 50 W; the subject then underwent the supramaximal test, which consisted of pedaling at the maximum voluntary speed in the upright position against a resistance equivalent to 130% of the W_{max} reached during the REF test (i.e., 342.5 ± 54.9 W) until exhaustion. Recovery was monitored for 5 min (12, 14).

Two of the three AOTs were preceded by the same preconditioning maneuvers described previously for the incremental tests: RIP and EIP, whereas the REF test was not preceded by any preconditioning maneuver.

The same parameters described for the incremental test were monitored, with the addition of BLa measurement. Blood samples were obtained with a finger prick at rest and at the 1st, 3rd, and 5th min of recovery time; BLa concentration was measured by a portable lactate analyzer (Lactate Pro, Arkray, Kyoto, Japan). Peak BLa was considered as the highest BLa concentration among all of the measures performed during the recovery time.

Data are presented as mean ± SD. The normality assumption was checked using the Kolmogorov-Smirnov test. The α-level was set at $P < 0.05$. Comparisons between tests were performed using the repeated measures ANOVA, followed by Neuman-Keuls post hoc when appropriated. Significance was set at a *P* value of <0.05. Statistics were calculated using commercially available software (GraphPad Prism, GraphPad Software, San Diego, CA, USA).

RESULTS

All subjects completed the protocol, and none complained of unbearable pain or discomfort during the periods of leg circulatory occlusion. All subjects fulfilled the selected criteria for VO_{2max} achievement in all tests performed. Table 1 shows the baseline level of variables during the resting periods preceding the REF, RIP, and EIP tests. No significant differences in variables at rest were observed before the three conditions.

No differences were observed in any of the studied variables between the RIP and EIP tests in response to preconditioning sessions. Fig. 1 shows that W_{max} (top panel) was increased by the RIP and EIP tests with respect to the REF test. In particular, during the REF test, W_{max} averaged 277.9 ± 44 W, whereas during the RIP and EIP tests, it averaged 288.2 ± 47.6 and 289.7 ± 47.6 W, respectively. Hence, both preconditioning maneuvers induced a similar W_{max} increment compared with the REF test, which was, expressed as percentage, +3.7% and +4.2%, respectively. During the RIP and EIP tests, subjects exercised longer than during the REF test, as testified by the fact that the TET was ~40 s longer after the preconditioning sessions than during the REF test (Fig. 1, middle panel).

Table 1. *Variable mean values ± SD at rest preceding REF, RIP, and EIP protocol sessions*

	REF test	RIP test	EIP test	P value
VO_2 (ml · min^{-1})	258.3 ± 25.6	264.2 ± 20.9	247.6 ± 31.4	NS
VCO_2 (ml · min^{-1})	231.7 ± 46.8	243.2 ± 35.8	237.5 ± 35.6	NS
VE (l · min^{-1})	11.9 ± 3.3	13.2 ± 4.2	11.2 ± 4.6	NS
HR (bpm)	72.5 ± 6.2	76.4 ± 7.6	70.8 ± 6.9	NS
SV (ml)	73.8 ± 10.1	73.4 ± 11.7	76.4 ± 10.8	NS
CO (l · min^{-1})	5.3 ± 0.7	5.6 ± 0.9	5.4 ± 0.8	NS
A-V O_2D (ml · l^{-1})	48.6 ± 12.4	47.1 ± 15.2	45.7 ± 14	NS
MBP (mmHg)	86.6 ± 11.4	87.1 ± 12.5	85.7 ± 13.4	NS
SVR (dynes · s · cm^{-5})	1,310.2 ± 211.4	1,254.1 ± 282.7	1,272.8 ± 234.6	NS

REF, reference test; RIP, rest-induced ischemic preconditioning; EIP, exercise-induced ischemic preconditioning; VO_2, oxygen uptake; VCO_2, carbon dioxide production; VE, pulmonary ventilation; HR, heart rate; SV, stroke volume; CO, cardiac output; A-V O_2D, artero-venous oxygen difference; MBP, mean blood pressure; SVR, systemic vascular resistance; NS, not significant.

Similarly, the TW (bottom panel) was higher during the RIP and EIP tests compared with the REF test.

VO_{2max} and VCO_{2max} were instead unaffected by both preconditioning maneuvers (Fig. 2, top and middle panels, respectively), whereas VE_{max} reached higher values during the RIP and EIP tests compared with the REF test (bottom panel).

As for hemodynamic parameters, Fig. 3 shows that HR_{max} was increased by the RIP and the EIP tests, whereas SV_{max} and CO_{max} were unaffected. In particular, despite the HR_{max} increment after both of the preconditioning procedures, CO_{max} did
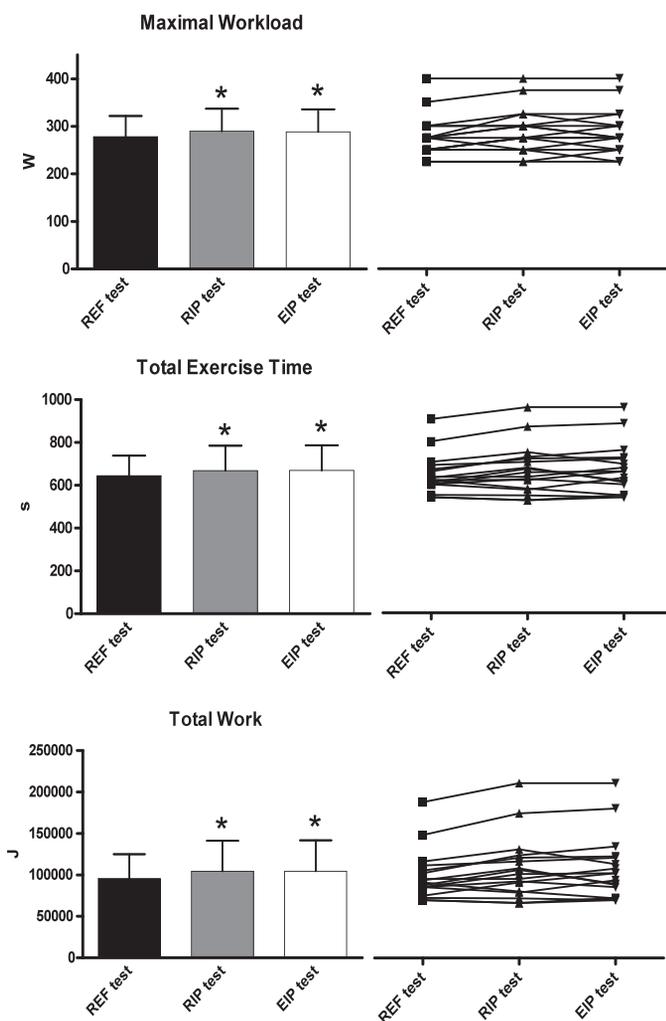

Fig. 1. Values of maximal workload, total exercise time, and total work reached by subjects during reference (REF), rest-induced ischemic preconditioning (RIP), and exercise-induced IP (EIP) protocol sessions. Values are mean ± SD. *$P < 0.05$ vs. REF test. Figure also shows individual data.
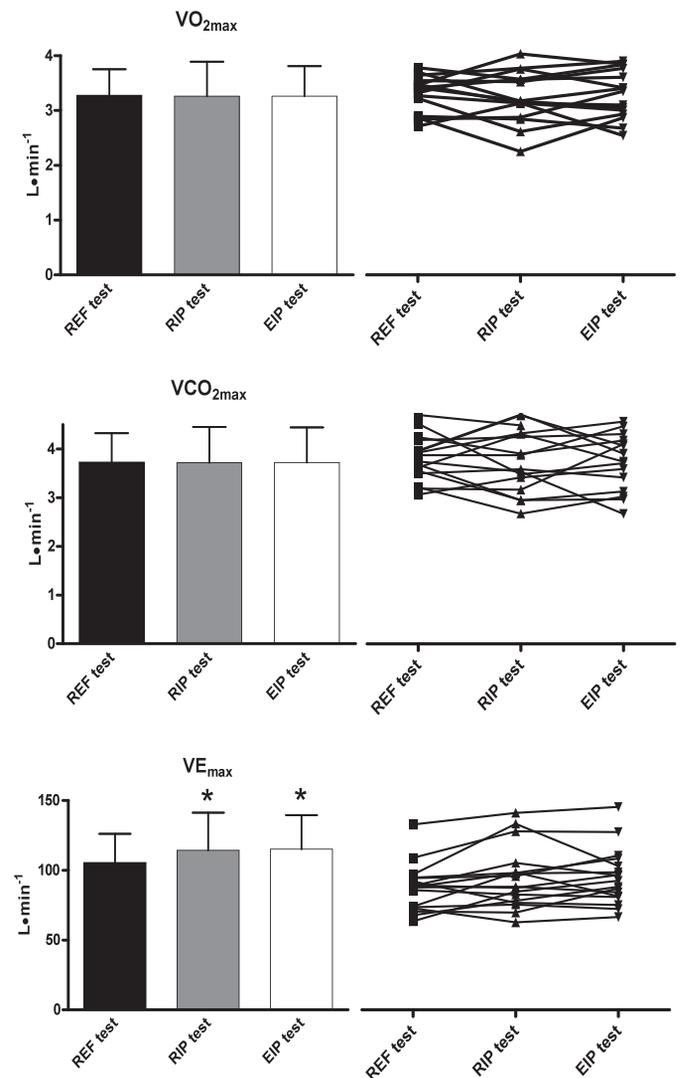

Fig. 2. Values of maximal oxygen uptake (VO_{2max}), maximal carbon dioxide production (VCO_{2max}), and maximal pulmonary ventilation (VE_{max}) reached by subjects during REF, RIP, and EIP protocol sessions. Values are mean ± SD. *$P < 0.05$ vs. REF test. Figure also shows individual data.

Fig. 3. Values of maximal heart rate (HR_{max}), maximal stroke volume (SV_{max}), and maximal cardiac output (CO_{max}) reached by subjects during REF, RIP, and EIP protocol sessions. Values are mean $\pm$ SD. *$P < 0.05$ vs. REF test. Figure also shows individual data.
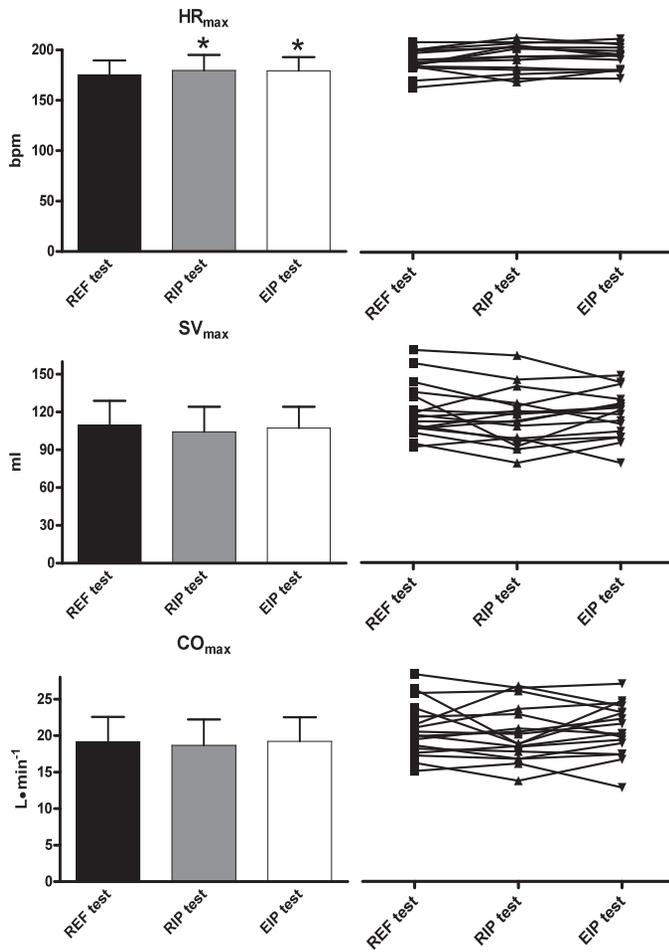
not increase with respect to the REF test, since a slight, statistically nonsignificant SV_{max} decrement took place in these settings, thereby keeping CO at the same level as in the REF test.

Finally, Fig. 4 illustrates that there was no significant difference in A-V O_2D_{max}, MAP_{max}, and SVR_{max} among the various protocols and sessions.

Table 2 shows results of the session of additional experiments to assess anaerobic capacity. No significant differences were found in any of the variables considered in all of the tests performed, and therefore, the preconditioning maneuvers did not affect the subject's anaerobic performance.

DISCUSSION

The present study demonstrates that IP improves maximal cycling performance in healthy and physically active subjects. We, in particular, found that IP was able to induce a significant, although slight ($\sim$4%), enhancement of W_{max}, TET, and TW. This result is in accordance with recent reports that demonstrated a beneficial effect of IP upon exercise performance (15, 18).

However, a surprising finding of the investigation is that in contrast to what we expected, there was no difference in the

capacity of enhancing the performance between the two protocols of IP used, i.e., RIP and EIP. A possible explanation of this result may be related to the fact that the protocol of IP applied at rest in this study (i.e., three cycles of 5 min of muscle ischemia, each spaced by 5 min of reperfusion) was sufficient to reach the threshold for metabolite accumulation and initiate the metabolic cascade that leads to IP, which once activated, appears to proceed independently from total metabolite blood concentration. In fact, the enhancement of the performance induced by the RIP protocol was almost identical to that induced by the EIP.

Another surprising finding was that despite the improvement in exercise capacity, both IP sessions were unable to increase VO_{2max} with respect to the REF test. This phenomenon disagrees with what was reported by de Groot and coworkers (15), who instead found an increase in VO_{2max} of $\sim$3% in a similar study. Although we cannot provide a definite explanation for such a difference, the following factors may have affected this outcome: 1) a possible different fitness level of the subjects enrolled for the study. In the study by de Groot and coworkers (15), well-trained cyclists were in fact enrolled. It is then possible to hypothesize that well-trained subjects have a dif-
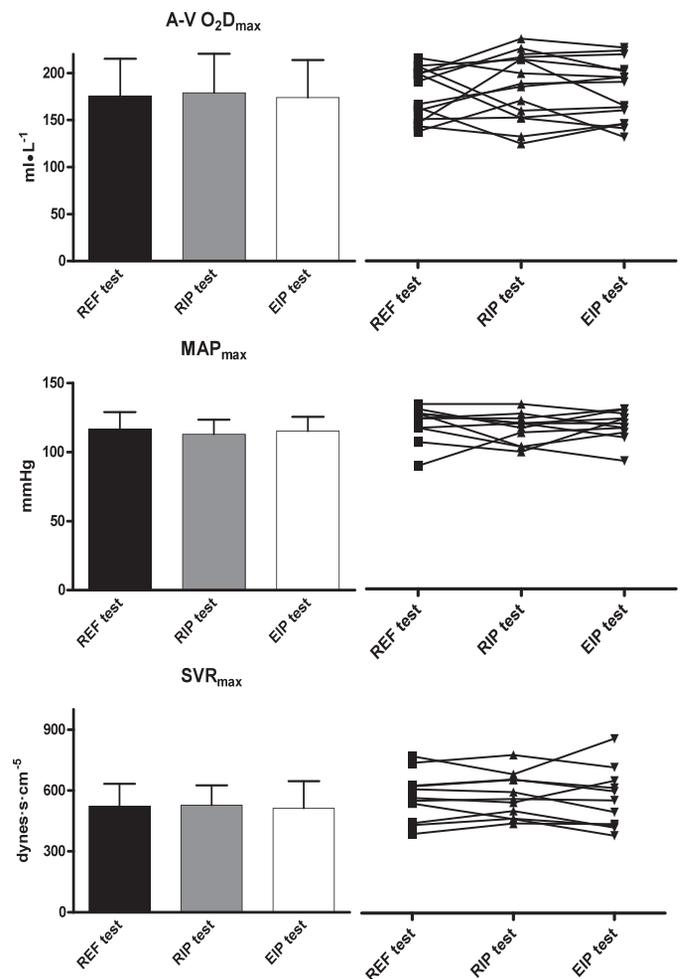

Fig. 4. Values of maximal arterial-venous oxygen difference (A-V O_2D_{max}), maximal mean arterial pressure (MAP_{max}), and maximal systemic vascular resistance (SVR_{max}) reached by subjects during REF, RIP, and EIP protocol sessions. Values are mean $\pm$ SD. Figure also shows individual data.

Table 2. *Variable mean values $\pm$ SD at peak exercise during* AOT_{REF}, AOT_{RIP}, *and* AOT_{EIP} *tests*

	AOT_{REF}	AOT_{RIP}	AOT_{EIP}	*P* value
VO_{2max} (ml $\cdot$ min^{-1})	2,963.8 $\pm$ 256.6	3,068.3 $\pm$ 396.1	3,169.2 $\pm$ 382.4	NS
VCO_{2max} (ml $\cdot$ min^{-1})	3,764.5 $\pm$ 407.3	3,764.7 $\pm$ 599	3,895.6 $\pm$ 661.1	NS
VE_{max} (l $\cdot$ min^{-1})	102.1 $\pm$ 10.8	104.3 $\pm$ 14.2	106.8 $\pm$ 12.7	NS
HR_{max} (bpm)	178.8 $\pm$ 11.3	176.3 $\pm$ 13.2	172.3 $\pm$ 12.1	NS
SV_{max} (ml)	97.9 $\pm$ 6.4	101 $\pm$ 9.8	100.7 $\pm$ 15.3	NS
CO_{max} (l $\cdot$ min^{-1})	17.8 $\pm$ 1.5	17.7 $\pm$ 1.8	17.4 $\pm$ 2.9	NS
A-V O_2D_{max} (ml $\cdot$ l^{-1})	179.4 $\pm$ 26.9	176.5 $\pm$ 27.4	184.6 $\pm$ 54	NS
MBP_{max} (mmHg)	105.3 $\pm$ 17.9	107.7 $\pm$ 15.2	106.1 $\pm$ 16.3	NS
SVR_{max} (dynes $\cdot$ s $\cdot$ cm^{-5})	475.9 $\pm$ 171.8	488.5 $\pm$ 195.9	489.8 $\pm$ 186.3	NS
TET (s)	129.6 $\pm$ 67.4	116.8 $\pm$ 25.4	123.1 $\pm$ 39.1	NS
TW (J)	44,287 $\pm$ 24,613	40,241 $\pm$ 12,568	41,970 $\pm$ 14,219	NS
BLa_{peak} (mmol $\cdot$ l^{-1})	12.7 $\pm$ 3.4	11.8 $\pm$ 3.3	12 $\pm$ 3.2	NS

AOT_{REF}, REF all-out test; AOT_{RIP}, RIP AOT; AOT_{EIP}, EIP AOT; VO_{2max}, maximal VO_2; VCO_{2max}, maximal VCO_2; VE_{max}, maximal VE; HR_{max}, maximal HR; A-V O_2D_{max}, maximal arterio-venous O_2 difference; SV_{max}, maximal SV; CO_{max}, maximal CO; MBP_{max}, maximal MBP; SVR_{max}, maximal SVR; TET, total exercise time; TW, total work; BLa_{peak}, peak blood lactate.

ferent response to preconditioning than less-trained individuals because of some specific metabolic adaptation to higher training volumes. However, to the best of our knowledge, there are no studies dealing with this topic; thus our hypothesis remains speculative. Future research is needed to better clarify the point. *2*) the different time interval considered for the average of the measured variables (1 min in their study vs. 15 s in the present). This different averaging time may have affected the outcome, since during heavy strain, 15 s is less affected by the time taken by VO_2 to reach the steady state than 1-min averaging. Thus it is possible that the VO_2 values gathered in the study by de Groot et al. (15) were not representative of a situation close to steady state. *3*) that performance is not determined exclusively by an increased oxygen delivery to the exercising muscles and that mechanisms other than oxidative metabolism played a role in allowing subjects to reach a higher workload without increasing their VO_2 (see the following part of DISCUSSION).

The fact that in the tests performed after the IP sessions, the subjects did reach a higher level of physical effort compared with the REF test, notwithstanding similar VO_{2max}, is further supported by the achievement of a higher HR and VE. The HR increase was similar to what was reported for W_{max} (i.e., ~3%), whereas the VE increment was ~8%. Hence, both the cardiovascular and the respiratory drives increased their activity following the preconditioning maneuvers, suggesting that the stress imposed on control centers was enhanced in these settings, likely because an increase in skeletal muscle recruitment took place and allowed going to higher work rates.

It still remains to be explained how the subjects could perform longer without any increment in VO_2. We speculated that this result may have been the consequence of an improvement in subjects' anaerobic capacity, which provided the extra energy required to sustain the effort. To address this issue, we performed additional experiments, which consisted of three supramaximal AOTs, aimed at verifying whether IP could improve the subjects' anaerobic capacity. However, neither exercise performance nor metabolic/hemodynamic parameters showed any improvement in response to the preconditioning sessions. Since the results did not confirm our hypothesis, they are probably due to other mechanisms.

A possible explanation that relates to the cascade of metabolic effect induced by the IP maneuver is that it likely

produced a more efficient muscle contraction, thereby promoting an ATP-sparing effect, which in turn, led to a larger workload/oxygen consumed. If this improvement in muscle contraction efficiency really occurred, then the skeletal muscle did not need to rely on the anaerobic energy sources to generate the extra force needed to support the effort. This phenomenon has in fact been postulated in a pig model of IP by Pang and coworkers (27), who speculated that in the skeletal muscle, the ATP-sparing effect afforded by IP may occur because of a tightening of excitation-contraction coupling and a reduction in futile ion pumping.

Moreover, it is also possible that IP lowered the sensitivity of the body to fatigue signals, thereby allowing the subjects to exercise longer. For instance, previous findings proposed that exercise appears to be controlled by a "complex intelligent system", which to protect body homeostasis, terminates the effort while muscle metabolic homeostasis is still sufficiently intact (31, 32). There is evidence that complete skeletal muscle recruitment does not occur during exercise in humans and that the ultimate regulation of the exercise performance resides in the central nervous system (CNS), which allows the presence of a skeletal muscle recruitment reserve, even during exhaustive exercise (25). For instance, this model is able to explain how an exercise can be terminated because of fatigue, even though there is a population of fresh, unused skeletal muscle fibers. This model postulates that the CNS regulates exercise on the basis of feedback from a variety of different organs. In particular, it has been proposed that the brain senses peripheral fatigue and alterations of the intramuscular metabolic milieu, presumably via the cortical projection of small-diameter muscle afferents of groups III and IV. Thus metabolic sensory muscle afferent feedback exerts an inhibitory influence on the central motor-drive function and restricts the development of muscular fatigue to an individual critical threshold (1). Therefore, this system allows preservation of a certain degree of muscular-functional reserve, which may be recruited if the inhibitory influence of muscle afferents is reduced. It is therefore possible that IP may shift the threshold level, at which this "intelligent system" terminates the exercise by desensitizing afferent groups III and IV, and that this phenomenon, in turn, increased the neural drive and the number of motor units recruited, thereby enhancing force output. The concept that there is likely a "central governor" of fatigue, which allows the

presence of a muscular-functional reserve, is testified further by the phenomenon of the "end spurt", which refers to the fact that athletes speed up at the end of the race, running the fastest when they should be the most tired (25). It should, however, be considered that the mechanisms of fatigue are extremely complex and that this study was not designed or intended to investigate the mechanisms of fatigue, and it cannot clarify the question. Future, specifically designed studies are needed to examine this important issue.

It is important to remark that whatever the cause, an enhancement of exercise performance of ~4%, i.e., of the magnitude reported in the present study, is highly relevant in elite athletes' competitions. In fact, in top-level athletes, the difference in cycling or running times may be minimal at the end of their relative competitions, and therefore, a possible, practical implication of IP maneuvers is that its practice may spread among elite athletes and even be considered as a sort of "natural doping".

Limitations of the present study. One possible limitation of the present investigation is the lack of a control group. We chose not to enroll a control group, because we found it difficult to imagine a control situation that simulated the IP maneuvers. Even the application of a low-pressure cuff in the legs to simulate the IP could potentially cause metabolite accumulation, thus inducing the IP cascade. In fact, in the rest session, IP was achieved without exercise, thus confirming that the IP cascade may be initiated by a small amount of metabolites. Otherwise, we could simply set a study where the control situation was a sequence of maximal exercise tests with the same timing as the IP sessions. However, we have already performed similar studies (8a, 9) without detecting any improvement in exercise performance in healthy subjects. Thus we chose to randomize trials to eliminate any order effect and to avoid familiarization.

Another possible limitation is the use of impedance cardiography to measure hemodynamics during exercise. It should be considered that the "gold standard" for hemodynamic assessment at rest and during exercise (i.e., the Fick and the dye-dilution methods) is invasive. Thus their use is not advisable in investigations involving healthy subjects, such as the present one. Among noninvasive techniques, the choice is restricted to re-breathing, Doppler echocardiography, and impedance cardiography, but none of them is unanimously considered accurate and reliable. Impedance cardiography, likewise, re-breathing and Doppler echocardiography, suffer from some limitations for exercise physiology application (32a). Probably, the major source of errors in hemodynamic assessment with this technique is that hard efforts affect impedance traces by generating artefacts due to legs' and chest movements. In our lab, we developed a postacquisition data analysis procedure, which selected from stored signals impedance traces not affected by artefacts. This processing signal procedure is time consuming, but it has been demonstrated to be reliable and reproducible for assessing hemodynamics in exercising subjects (11, 14). An indirect evidence that in the present investigation, impedance cardiography provided reliable hemodynamic estimation can be obtained looking at values of A-V O_2D at peak exercise, which appear to be in the range of physiological normality and similar to those calculated with other standards. So, although the impedance method suffers from difficulties during exercise, we think that it is suitable for use in studies such as the present one, providing that impedance traces are cleared by artefacts.

In conclusion, results from the present investigation provide evidence that IP preconditioning improves maximal performance in healthy and physically active subjects. The results also demonstrated that there is no significant difference in the capacity to ameliorate the performance between RIP and EIP. Further studies are however needed to better characterize the cellular and subcellular mechanisms of the phenomenon and examine the potential impact of IP on sports activities at a competitive level.

GRANTS

DISCLOSURES

The authors have no conflicts of interest that are directly relevant to the content of this manuscript.

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