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Remote ischemic preconditioning delays fatigue development during handgrip exercise

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Ischemic preconditioning (IPC) of one or two limbs improves performance of exercise that recruits the same limb(s). However, it is unclear whether IPC application to another limb than that in exercise is also effective and which mechanisms are involved. We investigated the effect of remote IPC (RIPC) on muscle fatigue, time to task failure, forearm hemodynamics, and deoxygenation during handgrip exercise. Thirteen men underwent RIPC in the lower limbs or a control intervention (CON), in random order, and then performed a constant load rhythmic handgrip protocol until task failure. Rates of contraction and relaxation (ΔForce/ΔTime) were used as indices of fatigue. Brachial artery blood flow and conductance, besides forearm microvascular deoxygenation, were assessed during exercise. RIPC attenuated the slowing of contraction and relaxation throughout exercise ($P < 0.05$ vs CON) and increased time to task failure by 11.2% (95% confidence interval: 0.7–21.7%, $P < 0.05$ vs CON). There was no significant difference in blood flow, conductance, and deoxygenation between conditions throughout exercise ($P > 0.05$). In conclusion, RIPC applied to the lower limbs delayed the development of fatigue during handgrip exercise, prolonged time to task failure, but was not accompanied by changes in forearm hemodynamics and deoxygenation.

Exposure of a tissue to brief periods of ischemia, a procedure known as ischemic preconditioning (IPC), leads to resistance against cell injury caused by subsequent prolonged ischemia and the stress caused by reperfusion (Murry et al., 1986). In addition to this effect, it has been recently shown that IPC improves exercise performance. IPC applied to the lower limbs increased maximal workload during an incremental cycling test (de Groot et al., 2010; Crisafulli et al., 2011) and decreased the time to complete a 5-km running trial (Bailey et al., 2012a,b). When applied to the upper limbs, IPC reduced the time in 100- and 200-m swimming trials (Jean-St-Michel et al., 2011), increased underwater swimming distance (Kjeld et al., 2014), and decreased the time to complete a 1000-m rowing trial (Kjeld et al., 2014). In these studies (de Groot et al., 2010; Crisafulli et al., 2011; Jean-St-Michel et al., 2011; Bailey et al., 2012a,b; Kjeld et al., 2014), IPC was applied in the limbs directly involved on the exercise modality, which is known as local IPC (LIPC). On the contrary, it has been consistently described that protective effects against ischemia-reperfusion injury can also be obtained by IPC applied at distance (Przyklenk et al., 1993), in a process called remote IPC (RIPC). A potential remote effect of IPC on exercise performance has been suggested by a previous study (Kjeld et al., 2014). However, it remains unclear whether RIPC applied to a different limb than the one in exercise improves exercise performance.

It is plausible that if the RIPC improves exercise performance, it delays the development of fatigue, which, in turn, usually courses with slowing of muscle contraction (De Ruiter et al., 1999) and relaxation (Edwards et al., 1972), and culminates with reduction in muscle force (Bigland-Ritchie et al., 1983). One of the multiple factors involved in the development of fatigue is the perfusion of skeletal muscles (Amann & Calbet, 2008), which can limit the convective delivery of O_2 (Lee et al., 2000; Van Beekvelt et al., 2001; Mortensen et al., 2008) and/or the removal of metabolites (Luu & Fitzpatrick, 2013) during both small muscle and whole body exercise. In this sense, RIPC increases blood flow in skeletal muscle (Wang et al., 2004), liver (Kanoria et al., 2006), and heart (Zhou et al., 2007) during reperfusion after prolonged ischemia, which is partially mediated by increase in endothelial nitric oxide synthase phosphorylation, and consequently, nitric oxide production (Li et al., 2012). Thus, among many potential mechanisms (e.g., cellular, neural, and humoral mechanisms), it is possible that the effect of RIPC on exercise performance

could be partially mediated by improvement in skeletal muscle perfusion during exercise.

Based on this background, we hypothesized that RIPC might delay the development of fatigue and prolong the execution of high-intensity exercise, which might be accompanied by improvement in skeletal muscle blood flow. Therefore, the development of fatigue was evaluated by measurement of contraction and relaxation rates throughout a constant workload rhythmic handgrip exercise, and performance was evaluated by the time of failure to maintain the target force level during the protocol (i.e., time to task failure). Blood flow was evaluated in the macrocirculation via Doppler ultrasound, whereas the balance between O_2 utilization and delivery was evaluated in the microcirculation via near-infrared spectroscopy.

Methods

Subjects

Subjects were healthy men, physically active, non-smokers, and were not taking any medication. Nine participated in Protocol 1 (27 ± 5 years; 22.8 ± 3.1 kg/m^2; 1752 ± 1405 MET × minutes/week) and 13 participated in Protocol 2 (25 ± 4 years; 22.1 ± 2.7 kg/m^2; 1455 ± 270 MET × minutes/week). Six of them participated in both protocols. After being informed of all procedures and potential risks of participation in the study, each subject signed a written consent form as approved by the Faculty of Medicine Ethical Committee for Research at Fluminense Federal University (CAAE: 00932712.0.0000.5243) and conformed to the Declaration of Helsinki.

Protocols

Protocol 1 assessed between-day reliability of contraction and relaxation rates and time to task failure during constant load rhythmic handgrip exercise. Protocol 2 assessed the effect of RIPC on contraction and relaxation rates, time to task failure, forearm hemodynamics, and deoxygenation during the same handgrip task (Fig. 1). Both protocols included one or two familiarization visits, followed by two experimental visits. Prior to the experimental visits, subjects refrained from exercise practice for 5 days, abstained from caffeine and alcohol for 24 h, and fasted overnight when tests were carried out in the morning, or fasted for 6 h before tests in the afternoon. Experimental visits were separated by a minimum of 5 days and repeated at the same time of the day for each subject, in a room with temperature between 22 and 24 °C.

Maximal voluntary contraction (MVC)

Subjects performed MVCs in the supine position, with the dominant hand. The shoulder was at 90 ° abduction, elbow fully extended, and the forearm in supination. Subjects squeezed a dynamometer connected to a data acquisition system (MP150, Biopac Systems Inc., Goleta, California, USA) to a maximal force within 1–2 s and maintained this level for 2–3 s (Hunter et al., 2009). At least three trials were performed, with 1-min interval. The MVC was the greatest force achieved among trials that ranged within 5% from each other.

Rhythmic handgrip

Subjects performed rapid handgrip contractions, in a rhythm of 60 contraction-relaxation cycles/min, dictated by a metronome, in the same position described for the MVC protocol. The target force was 45% of the MVC, which was displayed in the ceiling by a projector. The exercise intensity was chosen based on pilot experiments, in which subjects' time to task failure ranged from 2 to 10 min, consistent with a workload corresponding to the severe exercise domain (i.e., above the critical power intensity) (Hill, 1993). This rhythmic handgrip protocol, at this exercise intensity, was used because exercise tolerance is not limited by central hemodynamics (Stratton et al., 1983), but is partially limited by forearm blood flow (Lee et al., 2000; Van Beekvelt et al., 2001). Contractions were performed until task failure, which was determined when at least three consecutive contractions were lower than 40% MVC (Fig. 2), despite strong verbal encouragement. Time to task failure was the time from the onset of the first contraction to the end of the last valid contraction. Subjects and the researcher who gave the verbal encouragement were blinded to the exercise timing during the protocol and the exercise duration from previous visits. In addition, subjects were not informed about the criterion of task failure and the 40% cut-off level. Rate of force development during contraction [ratio between force and time ($\Delta F/\Delta t$)] and relaxation ($-\Delta F/\Delta t$) were calculated to assess the fatigue development throughout the exercise protocol (Fig. 2).

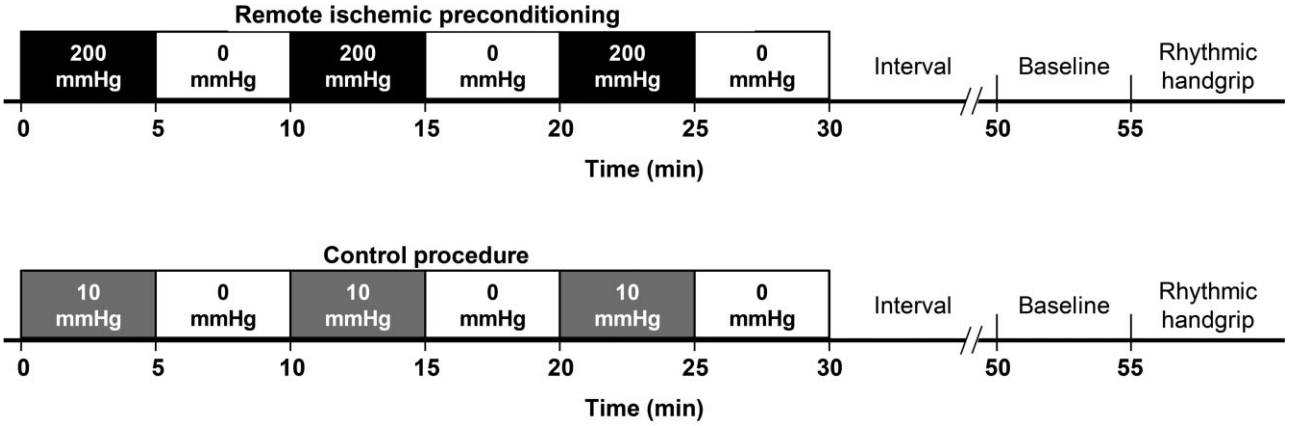

Fig. 1. Design of Protocol 2. The order of remote ischemic preconditioning and control trials was randomized and visits were separated by at least 5 days. Black blocks represent inflation of thigh cuffs to 200 mmHg during remote ischemic preconditioning, gray blocks represent inflation of thigh cuffs to 10 mmHg during control procedure, and white blocks represent deflation of cuffs.

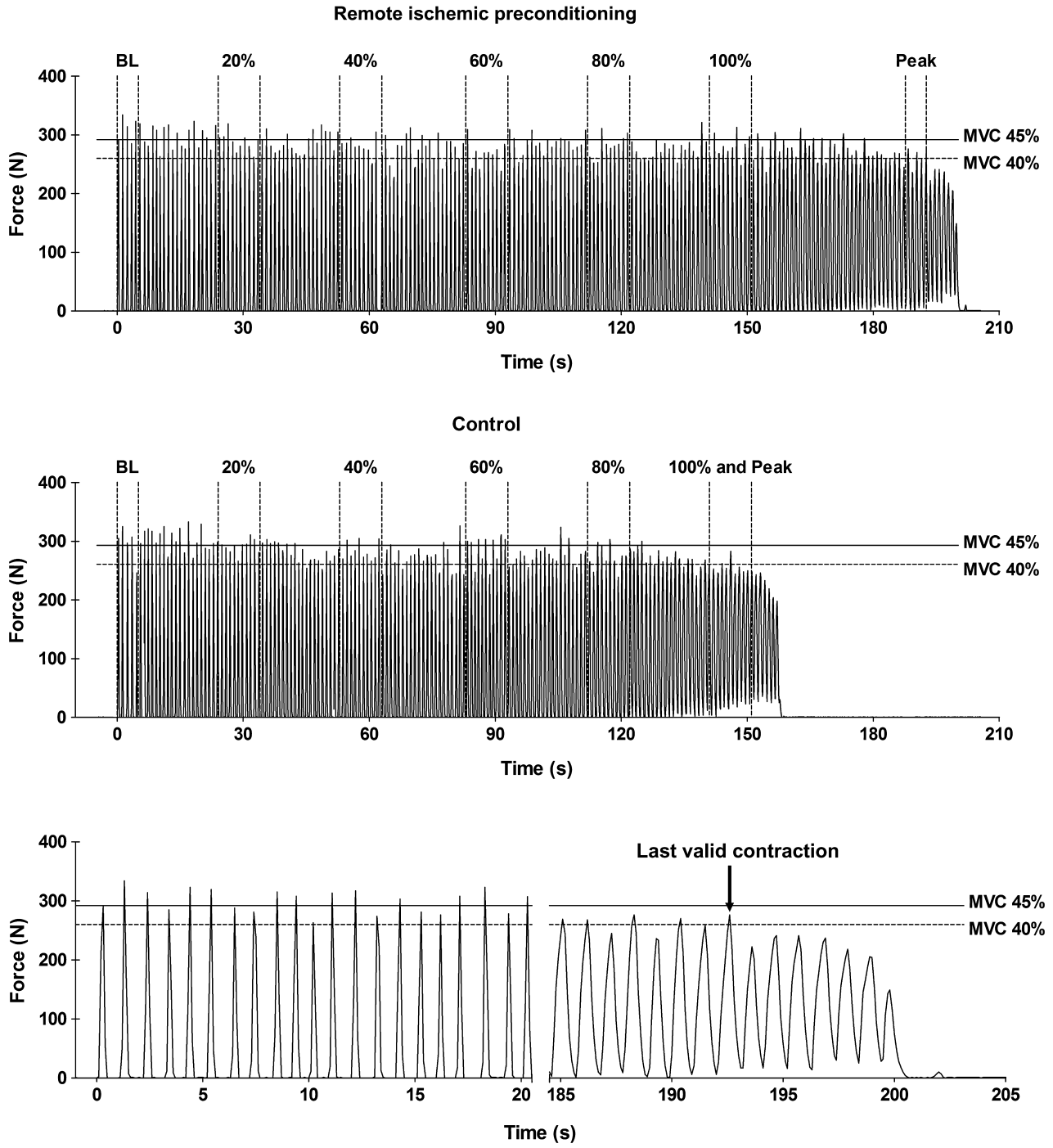

Fig. 2. Force tracing of one subject during the constant load rhythmic handgrip trial. Top: Trial preceded by remote ischemic preconditioning. Middle: Trial preceded by a control procedure. Vertical lines indicate the limits of the first 5 s (baseline [BL]), isotime points (20%, 40%, 60%, 80%, and 100%), and peak exercise. One hundred percent isotime represents the time to task failure of the shortest trial. Bottom: Tracings were short and tapered at the beginning and became large and flattened toward the end of exercise, as a result of the slowing of contraction and relaxation. Task failure occurred when three consecutive contractions were below 40% MVC.

Remote ischemic preconditioning

In Protocol 2, handgrip exercise was preceded, in a random order, by RIPC or a control procedure (CON). RIPC consisted of occlusion cuffs (SC10D, Hokanson Inc., Bellevue, Washington, USA) placed proximally around each thigh that were simultaneously and rapidly inflated to 200 mmHg for 5 min (E20 Rapid Cuff Inflator and AG101 Air Source, Hokanson Inc.) and deflated for 5 min of reperfusion. This procedure was repeated three times (de Groot et al., 2010). The inflation pressure occluded femoral arteries and led to lower limb ischemia, which was confirmed by imaging the popliteal artery by a Doppler ultrasound (Vivid 7, GE Medical Systems, Horten, Vestfold, Norway). CON was similar to RIPC, but cuffs were inflated to only 10 mmHg, which did not cause arterial or venous occlusion and ischemia. The investigator who gave the encouragement during exercise was blinded to the procedure applied in each session, and subjects were unaware of the

purpose of each procedure. Rhythmic handgrip started 25 min after the last cycle of reperfusion (Fig. 1).

Forearm hemodynamics and deoxygenation

Brachial artery diameter and blood velocity were measured using duplex Doppler ultrasound (Vivid 7, GE Medical Systems), with a linear-array transducer at 5.0 MHz in the pulsed-wave mode. The artery was imaged longitudinally 2–5 cm above the antecubital fossa, in a position that allowed the best border visualization and velocity tracing. This site was marked and used for data acquisition on the subsequent visit. Blood velocity was measured with an insonation angle of 60°. All videos were recorded and analyzed by the same researcher (T. C. B.). Videos were analyzed offline using an edge-detection and wall tracking software (Vascular Research Tools 5; Medical Imaging Applications LLC, Coralville, Iowa, USA) to determine brachial artery diameter and blood velocity. Diameter was measured at end-diastole of each cardiac cycle and between handgrip contractions during exercise. Anterograde blood velocity was measured during the interval between handgrip contractions. Retrograde blood velocity occurred only during forearm muscle contractions and was not considered for data analysis. Blood flow was calculated as: $\pi \times r^2 \times V \times 60$, where r is the radius (mm) of brachial artery and V is blood velocity (m/s). Brachial artery conductance was the ratio between blood flow and time-aligned mean arterial pressure (MAP), recorded beat-by-beat on a finger of the non-dominant hand (Finometer Pro, Finapres Medical Systems BV, Amsterdam, the Netherlands). Heart rate (HR) was measured by an ECG (Finometer Pro, Finapres Medical Systems BV).

A probe (Standard Rigid Sensor, ISS Inc., Champaign, Illinois, USA) for near-infrared spectroscopy (NIRS) was placed medially on the volar forearm, at the widest girth of the forearm. The probe was covered with a dark cloth to avoid contamination of the signal by ambient light and secured with elastic straps to avoid motion artifacts. The NIRS probe consisted of eight light-emitting diodes at two wavelengths (690 and 830 nm) with source-detector separations of 2.0, 2.5, 3.0, and 3.5 cm. After a warm-up period of at least 30 min, the near-infrared spectrometer (Oxiplex TS, ISS Inc.) was calibrated using a calibration block (phantom). The device incorporated continuous measurements of reduced scattering coefficients, which generated reliable absolute values of oxygenated hemoglobin and myoglobin [oxy-(Hb + Mb)] concentration and deoxygenated hemoglobin and myoglobin [deoxy-(Hb + Mb)] concentration. These measurements represent the Hb and Mb quantity and status at the level of arterioles, venules, capillaries, and intracellular sites of O_2 transport and uptake (Mancini et al., 1994). However, only the deoxy-(Hb + Mb) was presented herein because (a) it has a minor influence on the skin blood flow (Mancini et al., 1994; Davis et al., 2006; Tew et al., 2010); (b) is sensible to variations in muscle microvascular blood flow (Mancini et al., 1994; Ferreira et al., 2005, 2007); and (c) reflects the muscle O_2 uptake-to-blood flow ratio [i.e., the increase in blood flow decreases deoxy-(Hb + Mb), whereas the decrease in blood flow increases deoxy-(Hb + Mb)] (Mancini et al., 1994; Ferreira et al., 2005, 2007). All of these characteristics are not observed for oxy-(Hb + Mb) and its derived variables (i.e., total hemoglobin and tissue saturation index) (Mancini et al., 1994; Ferreira et al., 2005, 2007; Davis et al., 2006).

Data analysis

Given that the duration of exercise was different between RIPC and CON, data were compared at baseline (BL), 20%, 40%, 60%, 80%, and 100%, relative to time of task failure of the shorter trial per individual (i.e., isotime points). Moreover, the 100% isotime point of the shorter trial, which represents peak exercise for this trial, was compared to the peak of the longer trial. A representative example of identification of isotime points and task failure determination is demonstrated in Fig. 2. BL values were the average for the first 5 s of exercise for contraction and relaxation rates, or the average for the last 30 s of rest prior to exercise for other measurements. Isotime points and peak exercise were averaged for 10 s.

Statistical analysis

The sample size was calculated considering $5 \pm 10\%$ [mean ± standard deviation (SD)] of the difference between RIPC and CON for primary endpoints (i.e., contraction rate, relaxation rate, and time to task failure) and a coefficient of correlation equal to 0.90 between measurements. This analysis estimated that a sample size of 10 subjects would detect differences between RIPC and CON at $P < 0.05$ with 0.80 of power. The observed power of our results was 0.96, 0.85, and 0.56 for contraction rate, relaxation rate, and time to task failure, respectively. Normality of data distribution was tested by the Shapiro-Wilk test. Analysis of the data of Protocol 1 encompassed the calculation of the coefficient of variation (CV), the intraclass correlation coefficient (ICC), and the P-value from a two-way repeated measures analysis of variance (ANOVA) [factors: visit (1st vs 2nd) and time (BL, 20%, 40%, 60%, 80%, and 100%)] or from the Student's t-test for dependent samples (peak analysis). Time to task failure from Protocol 2 was not normally distributed. Thus, it was transformed into natural logarithm for inferential analysis, which normalized the distribution. Contraction and relaxation rates, duty cycle frequency, forearm hemodynamics, and deoxygenation were compared at isotime points by two-way repeated measures ANOVA [factors: condition (RIPC vs CON) and time (BL, 20%, 40%, 60%, 80%, and 100%)], followed by the Bonferroni *post-hoc* test when significant F-values were found. Time to task failure and comparisons at peak exercise were made by the Student's t-test for dependent samples. Two subjects were excluded from time to task failure and peak analyses since they presented an increase in time to task failure above the following outlier criterion: 75th percentile + 1.5 × (75th percentile − 25th percentile). Data are reported as mean ± SD, or as otherwise described. Statistical significance was considered for $P < 0.05$ on two-tailed analyses.

Results

Protocol 1 – between-day reliability

Contraction and relaxation rates at peak exercise presented CVs of 7.6% and 8.1% and ICCs of 0.92 ($P < 0.05$) and 0.76 ($P < 0.05$), respectively. Time to task failure had a CV of 9.3% and ICC of 0.93 ($P < 0.05$). Student's t-tests showed no systematic biases between measurements ($P > 0.05$). Duty cycle frequency was constant throughout exercise (time effect: $P > 0.05$). Contraction and relaxation rates became gradually slower from baseline to peak exercise (time effect: $P < 0.05$; Fig. 3). There was no difference between visits for duty cycle frequency, contraction, and relaxation rates at isotime points (condition effect: $P > 0.05$, interaction effect: $P > 0.05$; Fig. 3) and peak exercise ($P > 0.05$; Fig. 3).

Protocol 2: remote ischemic preconditioning

Duty cycle frequency was constant during exercise (time effect: $P > 0.05$) and similar between RIPC and CON (condition effect: $P > 0.05$, interaction effect: $P > 0.05$).

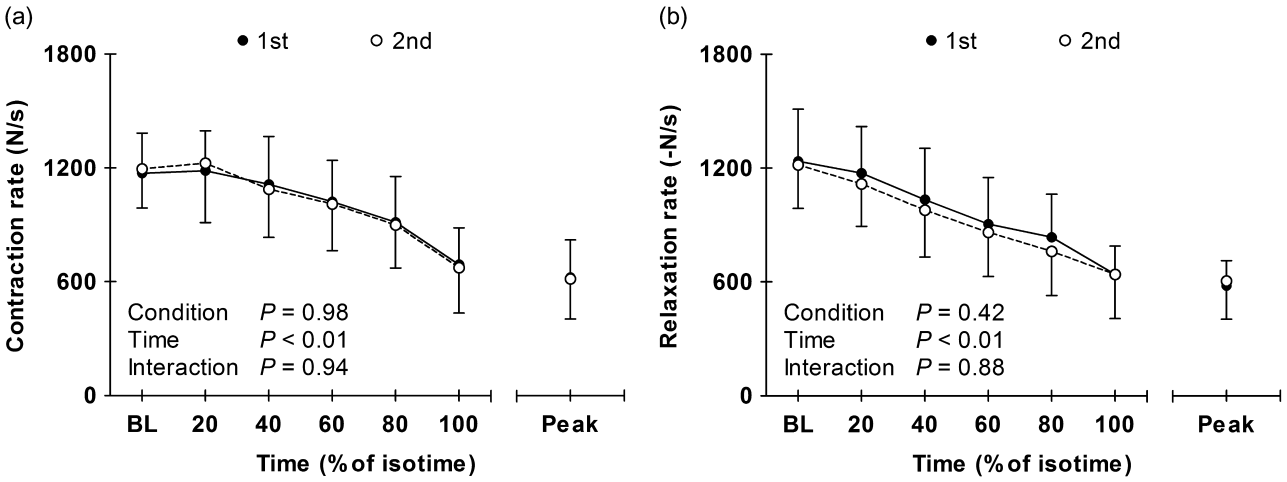

Fig. 3. Contraction (a) and relaxation (b) rates at the first 5 s (baseline [BL]), 20%, 40%, 60%, 80%, 100% isotime, and peak exercise in Protocol 1 – between-day reliability. Data are mean ± SD.

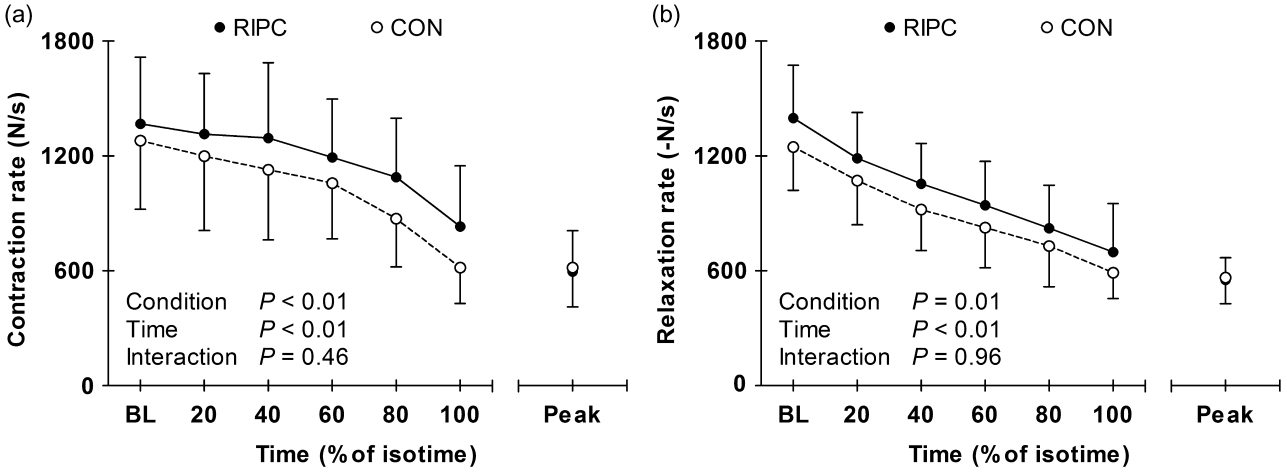

Fig. 4. Contraction (a) and relaxation (b) rates at the first 5 s (baseline [BL]), 20%, 40%, 60%, 80%, 100% isotime, and peak exercise in Protocol 2 – remote ischemic preconditioning. RIPC, remote ischemic preconditioning; CON, control. Data are mean ± SD.

Contraction and relaxation rates decreased throughout isotime points with both RIPC and CON (time effect: $P < 0.05$; Fig. 4). However, these rates were higher with RIPC compared to CON (contraction, condition effect: $P < 0.05$, interaction effect: $P > 0.05$; relaxation, condition effect: $P < 0.05$, interaction effect: $P > 0.05$). At peak exercise, contraction and relaxation rates were similar between conditions ($P > 0.05$). Time to task failure increased after RIPC compared to CON (RIPC: 198 ± 70 vs. CON: 179 ± 66 s, $P < 0.05$; Fig. 5). The percent increment [(RIPC − CON) × 100/CON] of 11.2% (95% confidence interval: 0.7–21.7%) was above the CV of the time to task failure from Protocol 1 (i.e., 9.3%). The two subjects that were excluded from time to task failure and other peak exercise comparisons increased by 249 s (i.e., 139%) and 231 s (i.e., 69%) their time to task failure with the RIPC. However, similar to the rest of the sample, their contraction and relaxation rates were higher throughout the protocol with the RIPC, while at peak exercise, their contraction [RIPC: 637 vs CON: 587 N/s (i.e., 8.5%); RIPC: 520 vs CON: 570 N/s (i.e., −8.8%)] and relaxation [RIPC: 755 vs CON: 738 N/s (i.e., 2.2%); RIPC: 755 vs CON: 707 N/s (i.e., 6.8%)] rates tended to be alike between RIPC and CON.

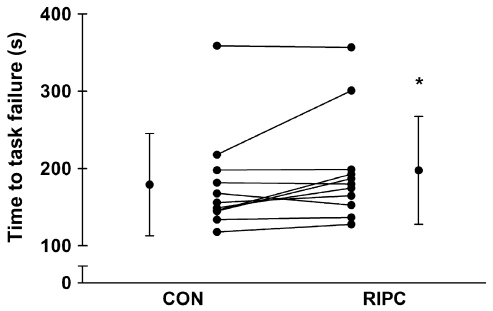

Fig. 5. Time to task failure of constant load rhythmic handgrip. Points with connecting lines are individual results. Points with whiskers represent mean ± SD for all individuals. CON, control; RIPC, remote ischemic preconditioning. *$P < 0.05$ vs CON.

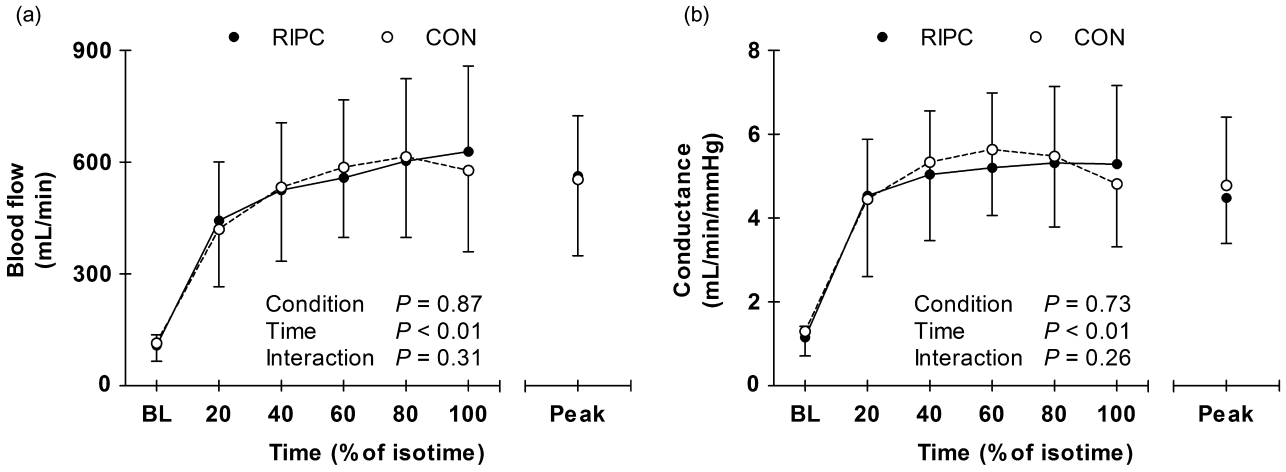

Fig. 6. Brachial artery blood flow (a) and conductance (b) at rest (baseline [BL]), 20%, 40%, 60%, 80%, 100% isotime, and peak exercise. RIPC, remote ischemic preconditioning; CON, control. Data are mean ± SD.

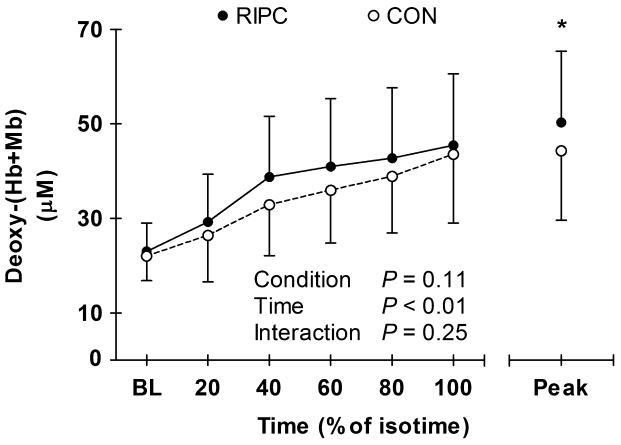

Fig. 7. Concentration of deoxy-(Hb + Mb) at rest (baseline [BL]), 20%, 40%, 60%, 80%, 100% isotime, and peak exercise. CON, control; RIPC, remote ischemic preconditioning. Data are mean ± SD. *$P < 0.05$ vs. CON.

Brachial artery diameter, blood velocity, blood flow, and vascular conductance increased from BL to exercise (time effect: $P < 0.05$; Fig. 6). There were no significant differences between RIPC and CON for these measures throughout isotime points (condition effect: $P > 0.05$, interaction effect: $P > 0.05$). Although diameter was lower in the RIPC than in CON at peak exercise (RIPC: 4.05 ± 0.36 vs CON: 4.19 ± 0.47 mm; $P < 0.05$), there were no differences for blood velocity, blood flow, and vascular conductance at this point ($P > 0.05$). The increases in HR and MAP were similar between RIPC and CON during isotime measures (time effect: $P < 0.05$, condition effect: $P > 0.05$, interaction effect: $P > 0.05$). At peak exercise, HR was similar between RIPC and CON, but MAP was higher in the RIPC (RIPC: 130 ± 17 vs CON: 119 ± 14 mmHg; $P < 0.05$). Deoxy-(Hb + Mb) increased from BL in both RIPC and CON (time effect: $P < 0.05$; Fig. 7). Throughout isotime comparisons, deoxy-(Hb + Mb) was similar between conditions (condition effect: $P > 0.05$; interaction: $P > 0.05$). However, at peak exercise, deoxy-(Hb + Mb) was higher (RIPC: 50.3 ± 15.0 vs CON: 44.3 ± 14.7 μM, $P < 0.05$) with RIPC compared with CON. Deoxy-(Hb + Mb) data were also normalized by the baseline value (i.e., baseline was considered as zero) and the results were equivalent.

Discussion

Previous studies showed that IPC improved exercise performance (de Groot et al., 2010; Crisafulli et al., 2011; Jean-St-Michel et al., 2011; Bailey et al., 2012a,b; Kjeld et al., 2014). To clarify whether this effect could be obtained by RIPC application to a different limb than the one in exercise, we applied IPC in the lower limbs and tested exercise performance in a rhythmic handgrip protocol. We found that RIPC attenuated the slowing of contraction and relaxation (i.e., delayed the development of fatigue) and prolonged the time to task failure in handgrip exercise. Contrary to our hypothesis, the improvement in exercise performance was accompanied neither by an enhancement of macrovascular blood flow nor by an improvement in the microvascular balance between O_2 utilization and delivery during exercise.

We found that contraction and relaxation rates during exercise, as well as time to task failure showed good reproducibility, which strengthens our interpretation about the RIPC effect on exercise performance. However, the impossibility to blind the subjects about the inflation pressure during RIPC and CON trials, which is a well-known weakness of all studies in this area (de Groot et al., 2010; Crisafulli et al., 2011; Jean-St-Michel et al., 2011; Bailey et al., 2012a,b; Kjeld et al., 2014), cannot rule out a placebo effect. Hence, in order to mitigate this potential bias, we blinded the subjects about the information that could influence exercise performance, such as the study hypothesis, the criterion to determine task failure, the performance on previous tests, and the exercise timing during data collection. Moreover, the researcher

responsible for verbal encouragement was blinded to the procedure applied on each visit.

Some of our findings also support the notion that the effects on exercise performance were indeed caused by RIPC and not by a placebo effect. Slowing of contraction and relaxation during exercise was attenuated by RIPC throughout isotime points, indicating less fatigability of forearm muscles. Noteworthy, this was observed since the beginning of exercise, when the influence of psychological factors on contraction and relaxation rates may be negligible. In addition, we found similar contraction and relaxation rates between RIPC and CON at peak exercise, despite longer time to task failure after RIPC, indicating that subjects exercised to their maximal voluntary capacity and reached a maximal level of fatigue at the task failure in all trials. This was also observed in the two subjects that presented an uncommon large increase in the time to task failure after RIPC. Thus, these results are in agreement with previous findings that markers of fatigue at peak exercise attain a consistent level within subjects (Clark et al., 2007), independently of the level of preexisting fatigue and/or differences in exercise performance (Amann & Dempsey, 2008).

The increase in the time to task failure following RIPC was superior to the improvement in exercise performance reported by most former studies that used LIPC (1.1–3.7%) (de Groot et al., 2010; Crisafulli et al., 2011; Jean-St-Michel et al., 2011; Bailey et al., 2012a,b). One possible explanation for such difference relies on the proportion of tissue mass exposed to IPC and exercise. In our study, exercise was performed with a small muscle mass and RIPC involved a large tissue mass, whereas most other studies had similar tissue mass in LIPC and exercise (de Groot et al., 2010; Crisafulli et al., 2011; Bailey et al., 2012a,b). Loukogeorgakis et al. (2007) showed that the larger the amount of tissue mass exposed to RIPC, the greater is the protection against ischemia-reperfusion injury. Therefore, it is possible that a similar phenomenon occurs concerning the RIPC effect on exercise performance.

Previous studies showed that RIPC enhanced blood flow in skeletal muscle (Wang et al., 2004), liver (Kanoria et al., 2006), and heart (Zhou et al., 2007) during reperfusion after prolonged ischemia. Therefore, we reasoned that RIPC would increase skeletal muscle blood flow and improve the balance between O_2 utilization and delivery, contributing to the improvement in exercise performance. We used a handgrip model to test this hypothesis, in which blood flow is predominantly determined at the periphery by the balance of metabolic vasodilatation and sympathetic vasoconstriction (Lee et al., 2000). However, despite a clear attenuation in slowing of contraction and relaxation throughout exercise after RIPC, blood flow and vascular conductance in the macrocirculation, as well as the balance between O_2 utilization and delivery in the microcirculation, were identical at baseline and isotime points between RIPC and CON. A previous study measured brachial artery diameter on one arm immediately after each cycle of ischemia/reperfusion in the contralateral arm and found a cumulative vasodilatation (Enko et al., 2011). Probably, the baseline brachial artery diameter after RIPC did not change in our study because we started artery imaging about 20 min after the last cycle of reperfusion.

Fatigue results from a complex interaction between many central (within the central nervous system) and peripheral (peripheral nerves, neuromuscular junction, skeletal muscle) mechanisms (Gandevia, 2001). At the muscular level, the slowing of contraction and relaxation has been attributed to the increase in hydrogen ions, ADP, and inorganic phosphate concentration, and its accumulation is partially dependent on limb blood flow and O_2 delivery during exercise (Amann & Calbet, 2008). Nonetheless, our results suggest that RIPC might alter other factors than blood flow. In this sense, animal studies focusing on the ischemia-reperfusion injury have shown that RIPC can enhance muscle efficiency in ATP usage via ATP sparing, augment mitochondrial flux, or increase efficiency in the excitation-contraction coupling (Pang et al., 1995; Moses et al., 2005; Mansour et al., 2012), so the effect of RIPC in skeletal muscle metabolism should be explored by future studies.

We observed greater MAP and forearm deoxy-(Hb + Mb) concentration after RIPC compared to CON at task failure. Previous studies showed that sympathetic activity increases progressively during fatiguing exercise (Saito et al., 1989; Seals & Enoka, 1989). Thus, the greater MAP is most likely a consequence of the longer duration of exercise, which probably allowed a greater increase in sympathetic activity (Saito et al., 1989; Seals & Enoka, 1989). This is in line with the reduction in brachial artery diameter at peak exercise after the RIPC, which may have occurred because sympathetic vasoconstriction surpassed local vasodilatation in the longer exercise trial (Shoemaker et al., 1997; Lee et al., 2000). The greater deoxy-(Hb + Mb) indicates that O_2 uptake was proportionally larger than O_2 delivery (i.e., greater O_2 extraction). Similarly, Kjeld et al. (2014) found lower O_2 saturation in the frontal cortex, forearm, and thigh at the end of a breath-hold after IPC of one forearm. Moreover, RIPC in rabbits' hind limb yielded lower hepatic O_2 saturation and higher hepatic deoxygenation during prolonged liver ischemia (Kanoria et al., 2012), suggesting that RIPC *per se* increased O_2 extraction during ischemia. However, we cannot exclude that the greater peak O_2 extraction could be merely a consequence of the longer time to task failure, so this issue needs further investigation.

Some limitations of our study should be taken into consideration. The external validity and applicability of our findings are limited due to the specificity of the exercise modality and the clinical status, gender, and age of our sample. Furthermore, despite the fact that we found no RIPC effect in brachial artery blood flow and forearm deoxygenation, we cannot rule out that the

distribution of blood flow between slow and fast twitch fibers did not change, which deserves further investigation.

In conclusion, RIPC applied to the lower limbs delayed the development of fatigue during handgrip exercise and prolonged the time to task failure. Nonetheless, the improvement in exercise performance following RIPC was accompanied neither by an increase in blood flow in the macrocirculation nor by an improvement in the balance between O_2 utilization and delivery in the microcirculation throughout exercise.

Perspectives

Our findings confirm the beneficial effect of RIPC on exercise performance, which can extend its applicability, since RIPC might be beneficial for muscle groups that are not accessible to undergo a non-invasive ischemic stimulus (dorsum, abdomen, respiratory muscles, etc.). Moreover, although we found that the fatigue development during exercise was clearly attenuated by RIPC, we found no effect of RIPC on macrovascular blood flow and microvascular deoxygenation. Therefore, other mechanisms are involved, such as improved metabolic efficiency due to humoral factors (Pang et al., 1995; Moses et al., 2005; Mansour et al., 2012), which deserves investigation.

Key words: Performance, ischemia, blood flow, vascular conductance, deoxyhemoglobin.

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