Thigh Ischemia-Reperfusion Model Does Not Accelerate Pulmonary VO$_2$ Kinetics at High Intensity Cycling Exercise

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Background: We aimed to investigate the effect of a priming ischemia-reperfusion (IR) model on the kinetics of pulmonary oxygen uptake (VO$_2$) and cardiopulmonary parameters after high-intensity exercise. Our primary outcome was the overall VO$_2$ kinetics and secondary outcomes were heart rate (HR) and O$_2$ pulse kinetics. We hypothesized that the IR model would accelerate VO$_2$ and cardiopulmonary kinetics during the exercise.

Methods: 10 recreationally active men (25.7 ± 4.7 years; 79.3 ± 10.8 kg; 177 ± 5 cm; 44.5 ± 6.2 mL kg$^{-1}$ min$^{-1}$) performed a maximal incremental ramp test and four constant load sessions at the midpoint between ventilatory threshold and VO$_2$ max on separate days: two without IR (CON) and two with IR (IR). The IR model consisted of a thigh bi-lateral occlusion for 15 min at a pressure of 250 mmHg, followed by 3 min off, before high-intensity exercise bouts.

Results: There were no significant differences for any VO$_2$ kinetics parameters (VO$_2$ base 1.08 ± 0.08 vs. 1.12 ± 0.06 L min$^{-1}$; $P = 0.30$; $\tau = 50.1 ± 7.0$ vs. 47.9 ± 6.4 s; $P = 0.47$), as well as for HR (MRT$_{180s}$ 67.3 ± 6.0 vs. 71.3 ± 6.1 s; $P = 0.54$) and O$_2$ pulse kinetics (MRT$_{180s}$ 40.9 ± 3.9 vs. 48.2 ± 5.6 s; $P = 0.31$) between IR and CON conditions, respectively.

Conclusion: We concluded that the priming IR model used in this study had no influence on VO$_2$, HR, and O$_2$ pulse kinetics during high-intensity cycling exercise.

Keywords: oxygen uptake kinetics, ischemia-reperfusion, cardiopulmonary test, physical exercise, exercise physiology

INTRODUCTION

Pulmonary oxygen uptake (VO$_2$) kinetics plays a role in the oxidative metabolism during constant load exercise (Poole et al., 2008). Considering that the area above the curve is accepted as a representative surrogate of the required additional energy supplying – i.e., anaerobic energy sources (Margaria et al., 1963), any acute or chronic adjustments that could reduce the time to attain a steady state of oxygen uptake and improves the energy imbalance becomes relevant for aerobic exercise performance (Gastin, 2001) or health related outcomes (Poole and Jones, 2012).
Several methods have been used to acutely challenge the VO₂ control system and to enhance VO₂ kinetics, such as breathing hyperoxic gas (MacDonald et al., 1998, 2000); prior exercise (Gerbino et al., 1996; Burnley et al., 2001, 2002) a reactive hyperemia induced by ischemia-reperfusion (IR) (Walsh et al., 2002; Faisal et al., 2010) – Which have resulted in controversial findings. The IR model involves blocking arterial to the target site (usually through a cuff pressure device), for a given period (continuously or intermittently). Upon cuff release, blood flow is augmented well above resting values (Kooijman et al., 2008). The brief exposure of muscle tissue to a blood flow absence causes an immediate reduction of the local O₂ saturation, followed by a reduced adenosine triphosphate (ATP) and phosphocreatine (PCr) intramuscular concentrations, and an increase in adenosine diphosphate (ADP) and inorganic phosphate (Pi) intramuscular concentrations (Boushel et al., 1998). On the very end, an increase in vascular dilation (Enko et al., 2011), a potential increase in local blood flow, O₂ muscle saturation and cardiac function (Daly and Bondurant, 1962; Faisal et al., 2010) is expected, which may accelerate VO₂ kinetics by improving O₂ delivery and availability. Nonetheless, the blood flow restriction (e.g., increases in ADP, Pi, etc.) may affect the VO₂ time course through spin-off manifestations, likely improvements in the mitochondrial oxidation activity through the increases in ADP and Pi concentrations (Boushel et al., 1998; Walsh et al., 2002). Despite the physiological rationale, the IR model has received little attention to study VO₂ kinetics at the onset of exercise.

Few studies were conducted with contradictory and inconclusive findings, like an impairment (Faisal et al., 2010), no effect (Kido et al., 2015) or an improvement (Walsh et al., 2002) in the pulmonary VO₂ kinetics. At high intensity exercise, VO₂ kinetics appears to be sensitive to the fundamental component amplitude (Ap) (Walsh et al., 2002; Wilkerson et al., 2006), slow component amplitude (As) (MacDonald et al., 1998; Burnley et al., 2002; Walsh et al., 2002), time-constant of fundamental component (τp) (MacDonald et al., 1998) and the "overall" kinetics (Walsh et al., 2002; Tordi et al., 2003; Nascimento et al., 2015). Also, VO₂ kinetics is dependent on systemic and peripheral mechanisms, such as increased activity of oxidative enzymes, muscle blood flow and improved O₂ distribution in the exercise muscles (Burnley et al., 2002; Wilkerson et al., 2006; Faisal et al., 2010; Poole and Jones, 2012). Notwithstanding, there is a question that still provokes further investigation for the physiologists: is the VO₂ kinetics at the onset of exercise limited by muscle O₂ delivery or by an oxidative enzyme inertia? In that scenario the reactive hyperemia by occluding target members, then augmenting muscle blood flow for several minutes prior to a subsequent exercise bout (Faisal et al., 2010), with less pronounced changes in the metabolic environment (i.e., peripheral mechanisms) seems an innovative approach.

Finally, it has been suggested that O₂ delivery does not limit the phase II of VO₂ kinetics in healthy subjects during a high intensity exercise (Poole et al., 2008), at least in some instances. However, at some “tipping point,” an O₂ limitation could occur and VO₂ kinetics can be modified through alterations in O₂ availability. Specifically, high-intensity approaches the tipping point, where VO₂ kinetics is O₂-delivery dependent and may potentially be more susceptible to the influence of blood-muscle O₂ influx (Poole and Jones, 2012). Thus, this study aims to investigate the effect of a priming IR protocol prior to high-intensity exercise on pulmonary VO₂ kinetics and cardiac parameters (heart rate and O₂ pulse). We hypothesized that a prior IR stimulus would accelerate VO₂ and cardiac related kinetics at the onset of high-intensity cycling.

**MATERIALS AND METHODS**

**Participants**

Ten recreationally active men (age 25 ± 5 years; body mass 79.3 ± 11 kg; height 177 ± 5 cm; VO₂ max 44.5 ± 6.2 mL kg⁻¹ min⁻¹) volunteered to participate in this study. Participants were classified as physically active by the American College of Sports Medicine (ACSM) criteria (cardiorespiratory exercise training for ≥30 min-days⁻¹ on ≥5 days-week⁻¹ for a total of ≥150 min-week⁻¹) (Garber et al., 2011). Some of them were involved in further recreational sport activities but not in any systematic physical exercise program. Participants were informed about risks and possible discomfort related to the protocol study. After that, a written informed consent was given before starting the study.

The subjects were instructed to avoid any intake of caffeine for 3 h, or alcohol and strenuous exercise in the 24 h preceding the test sessions. They were also instructed to arrive at the laboratory in a rested and fully hydrated state, and to eat the last meal up to 2 h prior to the experiment. All tests were conducted at the same time of day in controlled environmental laboratory conditions (19–22°C; 50–60% RH), to minimize the effects of diurnal biological variation on the results. All procedures were performed in accordance to the Declaration of Helsinki statement and were reviewed and approved by the Institutional Research Board (Ethics Committee for Research in Human Beings, Universidade Federal de Santa Catarina, IRB number 51045315.7.0000.0121).

**Experimental Design**

The subjects visited the laboratory on five occasions. In the first visit, participants underwent a maximal incremental cycling test to determine intensity parameters for the experimental protocol (Visit 1); the other four experimental visits were randomly assigned, and separated by at least 48 h.

The experimental trials consisted of 3 min of baseline cycling at 20 W plus 8 min at Δ50% intensity. Gas exchange was collected throughout exercise bouts to analyze VO₂ and O₂ pulse kinetics. Heart rate (HR) was measured during the entire exercise bouts as well. In two of the four trials, cycling exercise was preceded by 15 min bilateral thigh blood flow occlusion, whereas two trials without IR were conducted as control sessions (Figure 1).

**VO₂ max, GET and Δ50% Determination**

To determine physiological indexes for exercise sessions, we conducted the maximal incremental ramp test on a cycle ergometer (Lode Excalibur, Groningen, Netherlands). Participants exercised for 4 min at a constant load of 20 W.
to stabilize gas measurements and then the exercise intensity increased systematically by 1 W every 2 s (rate of 30 W min⁻¹) until volitional exhaustion.

Oxygen uptake was measured breath-by-breath using a gas analyzer (Quark PFT Ergo, COSMED, Rome, Italy). The gas analyzer was calibrated immediately before each test using ambient air and certificated alpha standard gases containing 16.0% of oxygen and 5.0% of carbon dioxide. The turbine flowmeter was calibrated with a syringe of three liters (Quark PFT Ergo, COSMED, Rome, Italy). HR was continuously recorded by a HR monitor integrated into the gas analyzer system. Earlobe capillary blood lactate samples (25 μL) were taken before and immediately after the incremental exercise from the ear lobe and analyzed by an electrochemical method (YSI 2700 STAT, Yellow Springs, OH, United States).

Maximal oxygen uptake was defined by two criteria, depending on the presence of a VO₂ plateau. The VO₂ plateau was defined as any 60 s period in which the VO₂ stabilized without increases higher than 150 mL min⁻¹. In the absence of a VO₂ plateau, we considered the maximum oxygen uptake as VO₂ max, by the maximal observed value in a 15 s interval (Day et al., 2003). Maximal power output (Wpeak), maximal heart rate (HRmax) and maximal lactate were considered as the highest values observed during exercise. Gas exchange threshold (GET) was calculated using the V-slope method (Beaver et al., 1986). The increase in VO₂ ventilatory equivalent (VE/VO₂) with no further increases in VCO₂ ventilatory equivalent (VE/VCO₂) was verified through visual inspection and used as a secondary criterion.

The Δ50% exercise intensity was calculated by defining GET-VO₂ and VO₂ max, and then adding 50% of the difference between them to GET-VO₂. After this, we made an interpolation using the linear regression model with the equation of a VO₂/intensity relationship (Souza et al., 2016), in order to determine the Δ50% workload.

**VO₂, HR, and O₂ Pulse Kinetics**

Each testing session consisted of a square-wave transition of 3 min at 20 W followed by 8 min at Δ50% intensity. Earlobe capillary blood lactate samples were collected 30 s before the baseline ([Lac]rest), 30 s before exercise ([Lac]pre) and immediately after the exercise ([Lac]post). O₂ pulse was determined as the fraction between VO₂ and HR (Whipp et al., 1996), both interpolated by 1 s intervals after matching the data of the two identical sessions corresponding to IR and CON conditions.

**Data Acquisition**

Oxygen uptake and HR were recorded breath-by-breath during the entire exercise sessions (Quark PFT Ergo, COSMED, Rome, Italy) and were exported as raw data for filtering and analysis (OriginPro 7.0, United States). For VO₂, signals suggesting sighs and coughs were initially removed from each test. According to Lamarra et al. (1987), occasional breath values were excluded from analysis using three standard deviations from the local mean as the criterion. Breath-by-breath data were linearly interpolated by 1 s intervals and then time-aligned to the start of exercise. After this procedure, both data series of each condition were matched and averaged in order to provide a unique profile for each exercise condition. The last filtering procedure was applied to reduce interpolated data to a 5 s stationary mean (Rossiter et al., 2005). The cardiopulmonary phase (i.e., first 20 s of exercise) was not included in the analysis (Rossiter et al., 2005).

For modeling the VO₂ profile, a non-linear regression technique was applied (Rossiter et al., 2002), where phase II and a slow component were fitted separately. The phase II was modeled from the end of the cardio-dynamic phase to the beginning of the slow component phase. The slow component phase was determined by four criteria: (1) the narrowest confidence interval for t; (2) breakpoint and the systematic rising of phase II amplitude and time-constant with a decrease in TD; (3) breakpoint and a systematic rising of fitting $R^2$; (4) visual inspection of residual plot, considering the end-phase by the farthest point from zero. The model was constrained in the VO₂ baseline (Barstow et al., 1996) to identify key parameters according to the Eq. 1:

**Equation 1. Mono-exponential model for VO₂ kinetics.**

$VO₂(t) = VO₂\ base + Ap \left[1 - e^{-\frac{(t-\tau)p}{TDp}}\right]$  \hspace{1cm} (1)

where VO₂ (t) represents VO₂ values for a given time t; VO₂ base is the average VO₂ of the last minute of 20 W load period; Ap is the amplitude of phase II; $\tau_p$ is the phase II time-constant; and TD is the phase II time delay.

The slow component amplitude was determined by the difference between the VO₂ end and the sum of the VO₂ base and Ap, according to Eq. 2:

**Equation 2. VO₂ slow component calculation.**

$A_s = VO₂\ end - (VO₂\ base + Ap)$  \hspace{1cm} (2)

Where, As is the slow component amplitude; VO₂ end is the average VO₂ value over the last 15 s; and Ap is the phase II amplitude.

The mean response time (MRT), which describes an overall pattern of VO₂ kinetics for the exercise bout, was calculated by a mono-exponential model from the beginning to the end of the exercise, excluding the time delay (Barstow and Molé, 1991), according to Eq. 3. The same equation was used to fit HR and O₂ pulse kinetics at the onset of the exercise (Wilkerson et al., 2006), with the end-point fixed at 180 s in order to augment fitting quality (i.e., better $R^2$), since the bi-exponential pattern of HR and O₂ pulse were not detected for all of the subjects:

**Equation 3. Mean-response time (overall kinetics) for VO₂, HR and O₂ pulse.**

$VO₂(t) = VO₂\ base + Ap \left[1 - e^{-\frac{t}{\tau_d}}\right]$  \hspace{1cm} (3)

**Ischemia-Reperfusion Protocol**

For IR, a 15 min bilateral thigh occlusion was introduced by the application of 250 mmHg pressure simultaneously in each thigh of the participants while they rested in a supine position (Ger-Ar, São Paulo, Brazil). Occlusion cuffs, chosen to be 20% wider than the upper leg diameter, were placed at the inguinal leg site. After 15 min of inflated cuffs, they were released, and the exercise load...
started following 3 min of 20 W cycling. Capillary blood lactate was taken from the ear lobe at rest and every 5 min until the end of the occlusion period ([Lac]rest, [Lac]5, [Lac]10, and [Lac]15). The occlusion time, mode and the time between cuff releasing and the beginning of exercise were determined based on a time-window where the blood flow remained augmented against the baseline conditions (Hampson and Piantadosi, 1988; Paganelli et al., 1989; Faisal et al., 2010). The period of time between the subject’s displacement from the bed to the cycle ergometer, as well as the data acquisition procedures (cuff displacement, face mask, HR strap adjustment, and sitting) were also controlled, in order to ensure trial reliability.

Statistical Analysis

Descriptive data is presented as mean ± standard deviation. Firstly, we tested the normality of the distribution by the Shapiro-Wilk test (n < 50). For variance analysis, sphericity assumptions were tested by the Mauchly’s test, and corrections were made by the Greenhouse-Geisser factor. For VO2, HR, and O2 pulse kinetics parameter comparison, the two-tailed Student t-test for paired samples was used. Log transformations were made when necessary. For lactate concentration analysis, the Shapiro-Wilk test (<0.05) for [Lac] during bilateral thigh IR and VO2 kinetics parameters.

VO2 kinetics and O2 pulse parameters were obtained during the 8 min square wave exercise with or without previous limb IR protocol. In Figure 2, the overall exercise VO2 pattern is illustrated by averaging all the subject’s 5 s data points. VO2 measured at the end of square-wave trials were 3.38 ± 0.11 L min⁻¹ and 3.41 ± 0.09 L min⁻¹ (P = 0.39) for CON and IR conditions, respectively, overestimating predicted VO2 about 22.9% ± 0.3% and 24.1 ± 0.2%. There were no differences between the conditions for any VO2 kinetics parameters (VO2 base, Ap, As, VO2 end, τp, TDp e MRT). In addition, there were no interactional effects between the condition and time for the capillary blood lactate concentrations (P = 0.16), as well as no effect of conditions (CON vs. IR) (P = 0.20) for [Lac]rest (0.86 ± 0.32 and 1.16 ± 0.38 mmol L⁻¹), [Lac]pre (1.04 ± 0.35 and 1.16 ± 0.44 mmol L⁻¹) and [Lac]final (10.26 ± 1.62 and 9.43 ± 1.46 mmol L⁻¹); and for [Lac] during bilateral thigh blood flow occlusion (P = 0.64; Figure 3).

HR and O2 Pulse Kinetics

Table 3 shows HR and O2 pulse kinetics results. MRT and the amplitude values are related to the time-fixed first 180 s of the exercise. There were no differences for any analyzed variables.

DISCUSSION

Main Findings

The main aim of this study was to investigate the impact of a prior bilateral thigh IR model on pulmonary VO2 kinetics during a cycling exercise at 50% intensity. The secondary aim was to investigate central control of the O2 pathway via the
high-intensity exercise, neither HR and O\textsubscript{2} pulse, rejecting our hypothesis that a previous blood flow occlusion protocol would accelerate pulmonary VO\textsubscript{2} kinetics in a high intensity exercise. Our data also suggest that the IR model did not accelerate any central variables kinetics at the onset of the exercise. To the best of our knowledge, this is the first study to report the effects of IR on HR and O\textsubscript{2} pulse kinetics during a high intensity cycling exercise.

In light of the previous studies in the literature related to IR model, the absence of effects is in accordance to the study of Kido et al. (2015), that did not find any acceleration of pulmonary VO\textsubscript{2} kinetics during a severe intensity exercise when preceded by a similar protocol (ischemic preconditioning - 3 × 5 min bilateral occlusion, with 300 mmHg of pressure, ending within 5 min before exercise), especially at the onset of the exercise (\(\tau_p\)) and also for “overall” kinetics (MRT). Faisal et al. (2010) found a diminished muscle VO\textsubscript{2} when the exercise began following 3 min of recovery after 15 min of forearm ischemia during an upper arm heavy exercise model, although an enhancement of forearm blood flow was observed. On the other hand, the findings of Walsh et al. (2002) suggests that “overall” pulmonary VO\textsubscript{2} kinetics could be accelerated by previous thigh IR lasting 5 to 10 min using about 250 mmHg of pressure. However, the major difference between the Walsh et al. (2002) study to ours/others was the timing between the end of a blood flow occlusion and the beginning of exercise.

Whilst it has been suggested that the local muscular hyperemia could be accessed by up to 2–3 min after the cuff release (Mullen...
et al., 2001; Faisal et al., 2010), we consider this potential as a feature to explain possible differences. In our study, we chose a 3 min time frame until the beginning of the exercise based on a study of Faisal et al. (2010). In addition, the baseline exercise before the Δ50% exercise, which is recommended and often used for pulmonary VO\textsubscript{2} kinetics experimental assessments (Rossiter et al., 2002; Poole and Jones, 2012), may have influenced muscle blood flow levels before Δ50% exercise as well.

A classic study of Daly and Bondurant (1962) provided accurate measurements of HR, cardiac output and stroke volume after 15 min of a 250 mmHg forearm blood flow occlusion, suggesting a sudden rise of those variables in the first 15 s of cuff releasing. Thus, the central parameter results, such as HR and stroke volume (O\textsubscript{2} pulse), need to be considered when evaluating VO\textsubscript{2} kinetics. Also, it is noteworthy to highlight that we did not find any effects of IR model on [Lac] during the occlusion period, neither any differences in [Lac] rest values and [Lac]pre. Blood lactate has been suggested as a properly mediator of O\textsubscript{2} transport enhancement by vascular tissue dilation (Krustrup et al., 2001; Tordi et al., 2003), which could partially explain the VO\textsubscript{2} kinetics acceleration after priming exercise conditions (Wilkerson et al., 2004; Nascimento et al., 2016). We also observed a concomitant absence of change in [Lac] and central hemodynamic parameters following the IT intervention. This may have occurred due to a higher than expected O\textsubscript{2} delivery and availability during thigh occlusion, as well as a better mitochondrial oxidation caused by higher levels of ADP, Pi, and Cr, which would attenuate any changes in [Lac].

**Limitations and Future Directions**

We considered that our findings need to be interpreted in light of its limitations. Here we point out some future directions for research that investigators could further consider.

When defining our protocol, we considered three major factors: (1) the pressure cuff level; (2) the time period for occlusion; (3) and the time between the end of occlusion and the beginning of the exercise. In regard to the first, a 250 mmHg level was chosen because of its efficiency to completely occlude the femoral artery blood flow (Sharma et al., 2014) and because it has been successfully by others (Salvador et al., 2015). For the same reason, the occlusion time was chosen – i.e., 15 min of bilateral thigh IR, as it had been shown to trigger reactive hyperemia (Kooijman et al., 2008) and necessary metabolic disturbances to maximize (Sharma et al., 2014). Finally, the time between the end of the IR model and the beginning of the exercise was selected for two main reasons: firstly, a physiological rationale to augment the local blood flow, supported by previous experimental results (Faisal et al., 2010); and secondly a logistic reason that is related to the necessary time to strictly adhere to the entire necessary methodological procedures for the VO\textsubscript{2} kinetics assessments in cycle ergometry, such as the participants transposition from the supine to the upright position, moving to the ergometer, cuff deflation and equipment removal.

Our results reinforce the absence of the central limitation in the onset-exercise pulmonary VO\textsubscript{2} kinetics, and, strengthen the evidence on this side of the discussion. Therefore, we recommend that further research limited to this aspect is unlikely to change the direction of the evidence by its robustness. However, investigators still interested in using IR to study the onset-exercise pulmonary or muscular VO\textsubscript{2} kinetics, and should consider a reduction in the time to the beginning of the exercise and to control the confounding factors necessary to reduce muscle inertia and rest muscle tension (Hughson and Morrissey, 1983) such as the 3 min of a baseline exercise.

**CONCLUSION**

We found that VO\textsubscript{2}, HR, and O\textsubscript{2} pulse kinetics during a high-intensity cycling exercise was not accelerated when preceded by a 15 min bilateral thigh blood flow occlusion and 3 min off. These results have important implications in possible strategies to accelerate VO\textsubscript{2} kinetics and improve metabolic efficiency. Considering there were no effects of IR when preceded by the off-period between the blood flow release and the exercise starting, further research should use other methods to investigate whether VO\textsubscript{2} kinetics are limited by oxygen delivery or oxidative enzyme inertia.

**ETHICS STATEMENT**

IRB: Comite de Ética em Pesquisa com Seres Humanos – Universidade Federal de Santa Catarina Procedures: we first submitted to IRB’s appreciation our project with rationale, methods, risks, and benefits. Together, the Consent Form was approved and, before any enrollment with this study, the subjects needed to assign it. In the consent form, information regarding risk, benefits, study’s rationale and relevance and their wrights to drop out of the study whenever they want was made available to them. All signed forms are still stored in the project’s folder.

**AUTHOR CONTRIBUTIONS**

All authors designed the study and interpreted the data. LH and PS collected and analyzed the data. LH wrote the first draft. PS, RdL, and LG wrote the final draft.

**FUNDING**

LH and PS received Ph.D. funding support by the CAPES Foundation. LG is granted by the CNPq Council.

**ACKNOWLEDGMENTS**

We want to thank the Exercise Physiology Laboratory staff (LAEF/UFSC) for supporting data collection, Dr. Eurico Wilhelm for supporting the reporting development.
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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.