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Effects of ischemic and hypoxic preconditioning on VO_2 and muscle oxygenation kinetics

Master Thesis in Sport Sciences, Training and Performance

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Abstract

Purpose: Oxygen uptake kinetics (VO_2) is a key determinant of endurance performance. Ischemic preconditioning (IPC) has been shown to potentially enhance endurance performance. The aim of the present study was to investigate the effects of ischemic preconditioning on VO_2 and muscle oxygenation kinetics during sub-maximal cycling exercise and to compare with hypoxic (HPC) or hypoxic-ischemic (HIPC) preconditioning.
Methods: In a single-blind, crossover randomized study, fourteen active men and women (age [mean ± SD standard deviation]) = 25.4 ± 2 years, body mass = 71.8 ± 10.7 kg, body height = 179.6 ± 11.2 cm) performed 3 x 6 minutes sub-maximal cycling exercise, with 6 minutes recovery. Those sub-maximal bouts were performed after 40 min recovery after a preconditioning phase of 4 x 5 min periods of cycling at 1.5 W/kg at 85 rpm either with bilateral leg occlusion at 30 mmHg in normoxia (Control/CON) in normobaric hypoxia (HPC, F_IO_2 13.6%) at 60% of relative total occlusion pressure in normoxia (IPC), or in hypoxia and 60% of the total occlusion pressure (HIPC, F_IO_2 13.6%) with 5-min periods of rest.
Results: None of the three PC conditions induced a beneficial change in the VO_2 or muscle oxygenation kinetics. Further, HPC altered the VO_2 kinetics, while slowing the time constant of the VO_2 kinetics. When added to ischemia (HIPC), hypoxia blunted the effect of the ischemia on the muscle oxygenation. IPC, HPC and HIPC had no influence on the respiratory efficiency or the work efficiency. Further, IPC has a hyperemic effect on the sub-maximal performance and seemed to induce a greater muscle perfusion.
Conclusion: This study investigated four preconditioning conditions (control, IPC, HPC and HIPC), in which HPC slows the VO_2 kinetics and IPC has a hyperemic effect on the sub-maximal performance. No other parameters have a potentially ergogenic effect on the sub-maximal performance.

Acknowledgements

First of all I would like to thank all the subjects who took part in this study. They took a lot of their own time to give me the opportunity to collect some important data for my master thesis. Without them nothing could have been possible.

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Index of abbreviations

Δ : Delta

[O_2Hb]: Oxyhemoglobin concentration

[HHb]: Desoxyhemoglobin concentration

[tHb]: Total hemoglobin concentration

TSI: Tissue saturation index (%)

P_IO_2: Inspired pressure of oxygen (mmHg)

F_IO_2: Fraction inspired of oxygen (mmHg)

PC: Preconditioning

$\dot{V}O_2$: Pulmonary oxygen uptake

$\dot{V}O_{2\,max}$: Maximal oxygen uptake

$\dot{V}O_{2\,peak}$: Peak oxygen uptake

VL: Vastus lateralis

C: Control preconditioning

IPC: Ischemic preconditioning

HPC: Hypoxic preconditioning

HIPC: Hypoxic-ischemic preconditioning

T_{occ}: Total occlusion pressure (mmHg)

O_2 : Oxygen

P_aO_2 : Arterial oxygen pressure

CT1: Time constant (seconds) of the kinetics

TD1: Time delay (seconds) of the kinetics

GE: Gross efficiency (%)

EE: Energy expenditure (J/S)

RER: Respiratory exchange ratio

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1. Introduction

Traditionally, athletes have achieved peak performance goals through long-term structured training schedules. Investigations have observed a variety of methods for optimizing training protocols from increasing strength to improving aerobic endurance (Jones, 2004). However, other kind of training methods performed just before the competitions have been shown to improve the performance. On competition days, sports scientists and coaches exploit a variety of strategies to improve athletes' performances, such as warm-up exercises, supplementation with stimulants, passive heat maintenance, and post-activation potentiation (Carr Aj, 2011) (Smith Mike, 2004) (Kilduff Liam P, 2013). The influence of the warm-up on subsequent aerobic exercise performance has been investigated since 1930 (Simonson E, 1936). The warm-up has been associated with acute increases in peripheral and central circulation and elevated core and muscle temperature (Simonson E, 1936). A study found that performance in both aerobic and anaerobic criterion tasks was significantly improved when preceded by warm-up of 15-minute intermittent treadmill at 60% of maximal oxygen consumption, compared with no warm-up (Simonson E, 1936). This result demonstrates the importance of the warm-up methods to enhance performance.

Scientists are using a new, non-invasive method "preconditioning" to further understand potential benefits of warm-ups. The American Heart Association defines this method as a "process where the exposition of an organism, an organ or a cell to a moderate stress, protect him or her in the next day from a bigger stress ". There are two methods of creating the preconditioning stimulus condition, the ischemic preconditioning (IPC) and the hypoxic preconditioning (HPC).

Originally introduced by Murry Charles E (1986), " IPC by brief episodes of ischemia and reperfusion in the organs, especially the heart provides protection from tissue damage such as myocardial injury". Sharma and colleagues stated that "IPC is a phenomenon in which transient episodes of ischemia and reperfusion administered to an organ attenuate the lethal cellular injury sustained from a subsequent, prolonged ischemic insult of the same organ" (Sharma Vikram , 2015). Since 1986, IPC has been demonstrated to protect many organs, including the myocardium (Cho Y Pang, 1995), the liver (Murry Charles E, 1986) and skeletal muscle (De Groot Patricia, 2010) from the damage caused by a subsequent prolonged

ischemic event. Research has shown that IPC can also decrease the chance of myocardial infarction and protect the endothelium (Alves Lintz José, 2013). In the recent years, IPC has been shown to enhance performance during endurance exercise (Przyklenk K, 1993) however the mechanisms underlying these improvements are still unclear.

Exercise performance is determined by many factors however; oxidative metabolism may be considered the most important (Patterson S D, 2014.). The dynamics of pulmonary oxygen uptake (VO_2) and muscle deoxygenation during exercise are frequently used for assessing oxygen (O_2) supply and extraction (Patterson S D, 2014.). These systemic and local O_2 dynamics may be useful for understanding the beneficial effects of IPC on exercise performance (Kido Kohei, 2015). IPC is believed to release circulating protective factors into the bloodstream during repeated cycles of inflation and deflation of a standard blood pressure cuff on a limb, producing significant benefits with respects to hypoxic-ischemic tissue injury (Liu Jing, 2005). Across a range of various exercise modes, performance has been enhanced by 1-8 % (Przyklenk K, 1993) (Liu Jing, 2005) (De Groot Patricia, 2010) (Kjeld Thomas, 2014) (Bailey Stephen J, 2010) which makes it potentially beneficial for athletic events which small margins are the difference between winning or losing. Research to date has primarily focused on events of an endurance nature and has identified improvements in maximal oxygen uptake (VO_{2max}) (Bailey Stephen J, 2010), power output at VO_{2max} (Jean-St-Michel E, 2011), running time trial performance (De Groot Patricia, 2010), and 1000m rowing performance (Kido Kohei, 2015). Relatively little research has focused on performance during shorter durations. Some studies have found that IPC accelerated muscle deoxygenation kinetics in a moderate intensity cycling exercise (Liu Jing, 2005) and increased peak oxygen uptake (VO_{2peak}) during a maximal ramp exercise test (Bailey TG, 2012). The VO_{2peak} is strongly affected by the cardiovascular system in addition to peripheral systems, which suggests that IPC induced improvements in exercise performance may result from peripheral adaptations, including those in the skeletal muscle rather than cardiovascular adaptation (Sharp Frank, 2004). Contrarily, researchers have also shown that IPC could increase endurance performance without affecting VO_2 responses (Gürke L, 1996). IPC has resulted in improved metabolic efficiency by attenuating ATP depletion (Enko K, 2011), glycogen depletion (Phillips D J, 1997), and lactate production (Enko K, 2011) during prolonged ischemia. In addition, IPC may improve skeletal muscle blood flow by inducing conduit artery vasodilatation (Crewe H, 2008), enhancing functional sympatholysis (Crewe H, 2008), and preserving endothelial and microvascular function during stress. In another study, an

increase in neural drive to the actives muscles has also been speculated, as the surface electromyography (EMG) potentials and maximal isometric force were increased in skeletal muscle of animals following IPC (Åstrand Per-Olof, 1964), suggesting increased motor unit recruitment. Unfortunately, the effect of IPC on EMG amplitude has not yet been investigated in exercising humans. It has also been suggested that IPC lowers the sensitivity of the body to fatigue signals. This should result in lower ratings of conscious perception of effort (RPE) during a constant load exercise, as it is derived from sensory input arriving from many different biological systems, including the cardiovascular, respiratory, and musculoskeletal systems (Åstrand Per-Olof, 1964). IPC can also ameliorate the O_2 response during ischemia potentially by increasing nitric oxide (NO) production. An increase of NO levels has enhanced exercise endurance and had modified systemic and local O_2 responses during exercise (Larsen Filip J, 2001). One potential mechanism underlying IPC induced effects may be the acceleration of systemic and local O_2 dynamics due to NO – dependent mechanisms (Larsen Filip J, 2001).

The second type of preconditioning stimulus is hypoxic preconditioning (HPC). Hypoxia is defined as a decrease in tissue or ambient tissue oxygen concentration below normal (Sharp Frank R, 2004). HPC has been studied in the brain, heart, retina, and other tissues (Sharp Frank R, 2004). The initial preconditioning stimulus is believed to trigger a cascade of endogenous adaptive mechanisms resulting in the development of tolerance (Englander EW, 1999). Depending on the nature of the preconditioning stimulus, delayed tolerance is initiated for several hours and can persist for several days (Bergerson, 2001). HPC has been shown to protect the brain and the heart against ischemia, as well as protect the brain from several types of injury including ischemia seizures and edema (Emerson MR, 1999). HPC protects many types of ischemia including focal and global ischemia in adult and neonatal brain with and without reperfusion (Sasaki H, 2001). Many of the molecules implicated in various other types of preconditioning are also induced by hypoxia or hypoxia induced tolerance (Sasaki H, 2001). These molecules induced by hypoxia are known to be protective similar to erythropoietin (EPO) or vascular endothelial growth factor (VEGF). In addition, ischemia, thrombin, hemorrhage, and possibly hypoglycemia all induce hypoxia inducible factor (HIF) and HIF target genes (Englander EW, 1999). Little is known about the mechanisms that occur in the time period between hypoxic preconditioning and the development of tolerance to brain ischemia. Hypoxic tissue induces a broad range of adaptive responses including electrophysiological and biochemical modification (Haddad GG, 1997). In addition, hypoxia

stimulates the expression of many genes, some of which are involved in erythropoiesis, angiogenesis, vasodilatation, glucose transport, and stimulation of anaerobic glycolysis (Simon, 1999). Finally, mild to moderate hypoxia (> 8% oxygen) does not produce cell death in the brain if hypoxia does not produce cardiac arrhythmias and hypotension (Simon, 1999). However, recent studies show that hypoxia can damage both nuclear and mitochondrial DNA in brain cells and that this stimulates DNA repair (Englander EW, 1999). Therefore, the studies inducing hypoxia have to be controlled very carefully.

In a human live, there are many different metabolic rates required to perform various tasks, for example, get up from a chair, climb stairs, run to catch a bus, or engage in activities requiring physical labor or professional sports. Due to very limited non-oxidative muscle energy stores, at the transition from rest to exercise, there must be simultaneous pulmonary, cardiovascular, and muscular system responses that rapidly increase the flux of O_2 from the atmosphere to the muscles, specifically to the mitochondria allowing for ATP production (Grassi B, 2011). These transitory phases, prior to achievement of any steady state responses suggests that a finite metabolic capacitance may have evolved as a crucial feature of the energy transfer pathways. At present, the kinetic response of VO_2 following the onset of exercise is recognized as a sentinel parameter of aerobic function and its measurement is becoming standard in laboratories around the World (Poole David C, 2012). In view of the relationship between physical performance and VO_2 kinetics, the development of strategies aimed at enhancing VO_2 kinetics and reducing the size and/or the progression of the VO_2 slow component offer hope for improving exercise tolerance (Gaesser, 1996).

The VO_2 kinetics can be split into three phases. The first phase is the cardiodynamic adaptation, in which the rapidity of the phase response is attributed to the almost instantaneous cardiac output increase as initiated by vagal withdrawal and the mechanical pumping action of the contracting muscles (Poole David C, 2012). The second phase is the main phase, where the rate of VO_2 increase is quantified by the time constant (CT). The time constant of the VO_2 kinetics translates the capacity of an athlete to regulate the VO_2 steady state, τ denoting the time to reach 63% of the primary phase. τVO_2 is a fundamental parameter of aerobic performance and differences in τVO_2 (speed of the VO_2 kinetics) may help explain the broad range of physical capabilities and exercise tolerance across populations (Kido Kohei, 2015). The faster this second phase can be achieved the better, in part, because this incurs a smaller O_2 deficit for any given increase in VO_2, and intracellular perturbations

are minimized (Grassi B, 2011). The third phase is the steady state / slow component phase. During a moderate intensity exercise, an athlete reaches steady state, however, with heavy or severs intensities, a slow component is observed. The slow component of O_2 uptake kinetics in long term constant rate exercise can be described as an increase in the energy expenditure above that predicted from the sub-maximal VO_2 work rate relationship, leading to a reduced work efficiency (Whipp BJ, 1970).

An important parameter to mesure the perfromance is the efficiency. Indeed efficiency has been suggested to be an important factor in relation to obesity (Sidossis L.S, 1992), weight loss (Lammert O, 1982), exercise performance (Olds T, 1995) and hence it is important to know the reproducibility of this measurement. For example, cyclists with very similar physiology and using similar equipment may display large differences in exercise performance as a result of small differences in efficiency (Jeukendrup A.E, 2000). The gross efficiency (GE) is an important parameter of the total efficiency. The basic definition of gross efficiency (Sidossis L.S, 1992) is the ratio of work done during the specific activity to the total energy expended and expressed as a percentage. Gaesser and Brooks (Gaesser G.A, 1975) suggested that GE distorts the essentially linear relationship between work rate and energy expenditure to make it appear that efficiency increases with work rate. This distortion occurs due to the proportion of energy expenditure that is used to maintain homeostasis becoming smaller as total energy expenditure increases.

The respiratory exchange rate (RER) is also an important parameter to evaluate the efficiency of the work. The (RER) indirectly shows the muscle's oxidative capacity to get energy (Ramos-Jiménez Arnulfo, 2008). The RER (CO2 production/O2 uptake) increase with the exercise intensity and measured under steady state conditions is commonly used to indirectly determine the relative contribution of carbohydrate and lipids to overall energy expenditure (Simonson DC, 1990). A high RER indicates that carbohydrates are being predominantly used, whereas a low RER suggests lipid oxidation (Simonson DC, 1990). Moseley and al (2001) founded that once the RER rose consistently above 1.00 for an entire workload, the measures of energy expenditure were no longer valid due to the contribution of unmeasured anaerobic work.

In this context, a recent study by Kido et al. (2015) examined the effects of IPC of the lower limb on the VO_2 kinetics and muscle deoxygenation during square wave transitions from low to moderate intensity, as well as from moderate to severe intensity cycling exercise (Kido Kohei, 2015). They found that IPC increased the time to exhaustion, which supports an increase in exercise performance. Furthermore, this group concluded that IPC did not change the pulmonary VO_2 kinetics but changed the kinetics of muscle deoxygenation. Specifically, IPC accelerated the kinetics in the transition from low to moderate intensity exercise (the amplitude of the deoxyhemoglobin (HHb) /Myoglobin (Mb) response was reduced), but no change of the kinetics was demonstrated in moderate to severe intensity exercise. Other studies have investigated the IPC effect on the balance between O_2 utilization and delivery in the microcirculation via near-infrared spectroscopy (NIRS). Indeed, Barbosa et al (2015) found an improvement of exercise tolerance accompanied by higher HHB/ Mb at peak exercise compared to the control (Barbosa T.C, 2015). Furthermore, Patterson et al. (2014) reported greater tissue oxygenation in leg cycling when sprints were preceded by IPC (Patterson S D, 2014.). These two opposite results elude that the effect on IPC on muscle deoxygenation and on other mechanisms during exercise still needs clarification.

It is known that different exercise intensities alter the VO_2 kinetics. Moderate intensity is below the gas exchange threshold (GET), heavy intensity is above the GET but under the critical power (CP), severe intensity is above the CP, which leads to VO_{2max}, and extreme intensity is such that fatigue ensures before VO_{2max} is achieved (Poole David C, 2012). The response to the preconditioning depends also of the intensity (Kido Kohei, 2015).

The purpose of the current study is to investigate the effect of the ischemic and hypoxic preconditioning on the VO_2 and muscle oxygenation kinetics during a sub-maximal exercise. The intensity corresponds to a heavy exercise as described above. We have examined the physiological responses, VO_2 and muscle oxygenation kinetics and tested three hypotheses:

i. The ischemic and hypoxic preconditioning have a potentially ergogenic effect on the sub-maximal performance compared with the control condition.
ii. The ischemic and/or hypoxic preconditioning will enhance the VO_2 kinetics by enhancing the time constant.

iii. The combination of the ischemic and the hypoxic condition during the preconditioning has no supplementary effect on the sub-maximal performance than the hypoxic / ischemic preconditioning alone.

2. Methods

2.1 Participants

In a randomized, single blind, crossover study, 14 healthy male and female subjects (age [mean ± SD (standard deviation)] = 25 ± 3 years, body mass = 71.8 ± 10.7 kg, height = 179.7 ± 11.8 cm) accepted to participate in the current study. All subjects approved being active (minimum two training sessions per week). None of the subjects were informed about the effect of ischemic or hypoxic preconditioning. All the subjects were able to complete the entire study, thus 14 subjects were taken for the data's analysis. This study was approved by the ethics committee (Commission Cantonale d'Ethique de la Recherche sur l'être Humain number 138/15, approved on 21.04.2015).

2.2 Experimental procedure

Subjects (n=14) completed five visits in the hypoxic chamber of ISSUL (University of Lausanne Sport Science Institute) located at Dorigny in the sport center "CSS" (Centre Sport Santé). All visits were performed in a randomized order, at the same time of day, and separated at least by 48 hours to avoid fatigue accumulation. The day of the tests, the subjects were asked not to consume caffeine.

2.3 First visit: Familiarization

During the first session, anthropologic measures of body mass and height were measured. The participants were then informed about the protocol and the potential risks. Figure 1 describes the experimental design of this familiarization visit. Then, total blood occlusion or the pulse elimination pressure was measured. Subjects were seated on a chair while a pneumatic cuff (cuff size 11x85cm, bladder size 10x41cm) was placed on the right upper leg and connected to a cuff system (E20/AG101 Rapid Cuff Inflation System, D.E. Hokansson Inc., Bellevue, WA, USA). Air pressure in the cuff was gradually increased until the point at which blood flow was no longer present in the femoral artery. Using Doppler ultrasound, it was possible to

ensure that blood flow was completely occluded, and the total occlusion was reached when no pulse or blood flow was observed. This procedure was repeated two or three times for reliability and accuracy. The average of the measures was retained as the total occlusion pressure (Tocc) for each subject. In many studies, the occlusion level has been set at 220 mmHg (Przyklenk K, 1993) (Clevidence M. W, 2012) (Gibson N, 2015) (Loenneke J. P., 2011) however, some studies have reported that the femoral artery was not completely occluded at a pressure level of 250 mmHg (Iida H, 2007) (Sharma V, 2014). The leg circumference may influence pressure level to induce total arterial blood flow blockage (Iida H, 2007). Following these measurements, subjects were given an explanation on the different variables measured during the next four testing sessions such as oxygen uptake, muscle oxygenation via near-infrared spectroscopy (NIRS), arterial oxygen saturation, blood lactate, rating of perceived exertion of the legs and the breathing with the Borg scale, and heart rate. Afterward, subjects were seated on the bike (Lode Excalibur Sport Ergometer, Lode B.V., Netherlands). The bike was set for each subject and the dimensions recorded for use during all subsequent sessions.

During this first session, the subjects were asked to perform a 3-min all-out test. This test is currently used to measure the critical power (CP). One method of estimating the heavy–severe domain boundary is to establish the CP which requires a subject to exercise to exhaustion at several constant work rates on separate days. The relationship between power output and time to exhaustion is hyperbolic and is defined by two parameters: CP, which represents the highest sustainable work rate and the curvature constant (W'), which is the maximum amount of work that can be performed (Vanhatalo A, 2007). D. Hill also defined the critical power, as "The basis of the critical power concept is that there is a hyperbolic relationship between power output and the time that the power output can be sustained. The intensities above CP is often referred to as anaerobic work capacity (Hill D.W, 2002). Vanhatalo have previously demonstrated that a 3-min all-out cycling test results in a highly reproducible power profile that levels out during the final 30–60 s at a power output approximately halfway between the lactate threshold (estimated by gas exchange indices) and the peak work rate attained in a ramp test. That is close to the power output at which the heavy–severe domain boundary would be expected to occur (Vanhatalo A, 2007). As stated previously , the 3-min test duration was chosen to provide a protocol that was long enough to yield a stable power output at the end of the test, but not so long that subjects would fail to complete the test (Vanhatalo A, 2007).

In the current study, the 85% of the CP was chosen for the sub-maximal test intensity. The subjects began with a warm up of five minutes at 50 W and five minutes at 100 W. During this warm up an explanation of the 3-min all-out test was given. The protocol of the 3-min all-out test was the same as the study of Vanhatalo A, (2007). The test began with 3 minutes of unloaded baseline pedaling at each subject`s preferred cadence, followed by a maximal 3-min effort. Subjects were asked to increase the cadence during the last 5 s of the baseline period. The resistance on the pedals during the 3-min effort was set using the linear mode of the ergometer so that the subjects would attain the power output halfway between $VO_{2\ peak}$ and the GET on reaching their preferred cadence (linear factor = power/ cadence squared). Strong verbal encouragement was provided throughout the test, although the subjects were not informed of the elapsed time, to prevent pacing. To ensure an all-out effort, subjects were instructed to maintain their cadence as high as possible at all times throughout the test (Vanhatalo A, 2007). At the end of the 3-min all-out test, the critical power (CP) was calculated as the mean power of the last 30 seconds. During the 3-min all-out test subjects wore a mask to measure the gas exchange (see section 2.5.4 oxygen uptake).

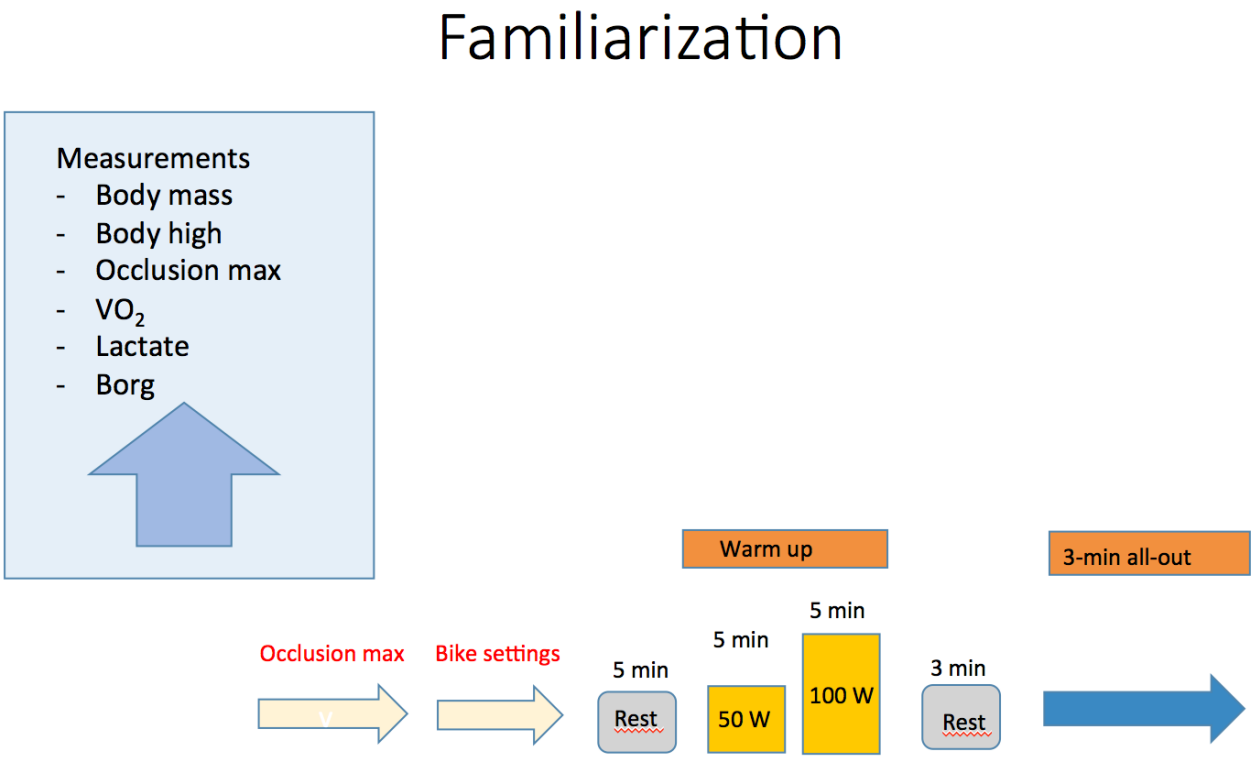

Figure 1: Experimental design: Familiarization.

2.4 Protocol

2.4.1 Warm-up

Each test visit had the same structure, as illustrated by Figure 2. Subjects were equipped with all the materials (NIRS, HR monitor, oximeter), and began with a two-minute normalization resting period while sitting stationary on the bike. Following which subjects warmed up for five minutes at 50 W and five minutes at 100 W in order to provide a baseline for normalization of the NIRS. Subsequently, the mask to simulate the altitude (Everest Summit II Generator, Hypoxico Inc, New York, NY, USA) and the automatic cuffs were placed on the proximal most part of both legs to begin the preconditioning exercise.

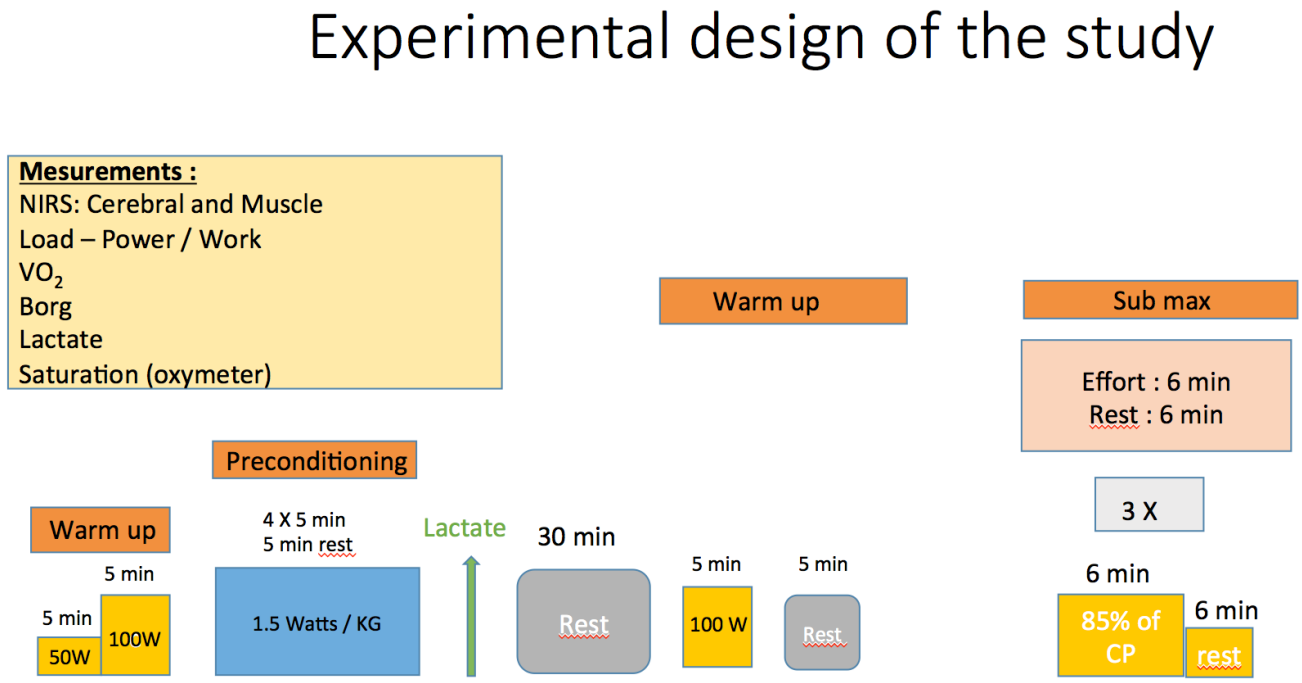

Figure 2: Experimental design: Test visits.

2.4.2 Preconditioning: Four conditions

Four conditions were tested:

1. Control (C): Normoxia (400 m F_IO_2 20.9%) without occlusion (cuffs inflated at 30 mmHg).
2. Ischemic preconditioning (IPC): Normoxia with 60% of total occlusion.

3. Hypoxic preconditioning (HPC): At a simulated altitude (F_IO_2 13.6%) without occlusion (cuffs inflated at 30 mmHg).
4. Hypoxic and Ischemic preconditioning (HIPC): At a simulated altitude (F_IO_2 13.6%) with 60% of total occlusion.

2.4.3 Exercise period of the preconditioning

The optimal number and length of occlusion-reperfusion cycles is unknown (Vanhatalo A, 2007). Most of the studies investigating IPC followed the same model during the preconditioning session (Sharma Vikram , 2015) (Kjeld Thomas, 2014) (Przyklenk K, 1993) (Hausenloy D. J, 2010). The same procedure was thus replicated in the current study. Preconditioning consisted of four cycles of five minutes of cycling (with one of the four conditions as indicated above) alternated by five minutes of rest/reperfusion (passive seated rest on the bike). The intensity of cycling was the same in all stages and conditions, corresponding to a resistance of 1.5 W/kg. After each stage, participants indicated the rating of perceived exertion (RPE, 6 – 20; Borg scale) to represent the feeling in the legs and of the breathing. Studies on the IPC preconditioning recommended a cadence around 85 rpm during all the preconditioning (Moseley Luke, 2001). In the current study the subjects were also asked to pedal at 85 rpm during all the preconditioning conditions.

2.4.4 Resting period between the preconditioning and the sub-maximal test

As known from previous research, IPC induces an early (1-2h) and a late (12-72h) phase of effectiveness on ischemia-reperfusion injury (Incognito A. V, 2015). The time lag between the end of preconditioning and start of exercise performance ranged in general between 5-105 minutes, however, the optimal time lag has not been thoroughly investigated (Faiss R, 2013). In the present study, the preconditioning session was followed by a thirty-minute break. The participants could rest and drink water while seated on a chair. A forty-minute period was taken between the end of preconditioning and the start of the sub maximal test. During this break, the gas exchange system (Quark CPET, Cosmed, Rome, Italy) was calibrated. Air volume was calibrated with a 3L calibration syringe and gases concentration with a standard air carboy (5.03% CO2, 15.06% O2 calibration gas, PanGas AG, Dagmersellen, Switzerland).

2.4.5 Sub-maximal test

The sub-maximal test consisted of three trials of six minutes of cycling at 85% of the CP alternated by six minutes of rest. In previous research, authors used one exercise transition to calculate the pulmonary VO_2 kinetics (Barbosa T.C, 2015). Nevertheless, repetitions of exercise transitions are crucial to increase the signal-to-noise ratio (Barbosa T.C, 2015). Authors found that four to eight exercise bouts are used for moderate-intensity transitions and two to four exercise bouts are used for severe-intensity transitions (Poole David C, 2012). In the current study, with three bouts of exercise at 85% of CP, the subjects performed heavy intensity exercise. Subjects performed a standardized warm-up consisting of five minutes cycling at 100 W. After the warm-up, a break of 5 minutes was needed to set up the gas analyzer mask and to prepare the subjects for the trials. Subjects were asked to pedal with a cadence of 85 rpm during all trials. During the last 30s of each trial, the oxygen saturation was recorded from the fingertip. After each trial the RPE of the legs and the breathing were asked, and blood lactate sample from the earlobe measured. During the resting period, the subjects could pedal for two minutes with no resistance and then rest on the bike for four minutes to complete the recovery period. The gas analyzer mask was removed between trial two and three to let the subjects drink water ad libidum. During the exercise periods of the preconditioning and the sub-maximal test, the subjects were asked to remain in the same seated position on the bike and were not allowed to stand on the pedals. During the entire sub-maximal test, NIRS, oxygen saturation, heart rate and gas exchanges were recorded. The heart rate HR was taken directly by the Quark CPET of Cosmed with a Garmin heart rate monitor during the sub-maximal exercise (HRM Run Heart Rate Strap, Garmin, Kansas, USA). The variables measured were:

- Lactate measured in [$mmol^{-1}$]
- Heart rate (bpm)
- Finger and earlobe oxygen saturation (%)
- RPE [6-20]: (leg and breathing)
- VO_2
- Muscle oxygenation with near-infrared spectroscopy (NIRS)

2.5 Analysis

2.5.1 Heart rate

Heart Rate was monitored at 5 Hz by telemetry during the preconditioning with a heart rate monitor (Polar RS400, Kempele, Finland). The data were then downloaded on *Polar Pro Trainer 5* software. Files from *Polar Pro Trainer 5* were then extracted and saved as excel documents. Illustrated in Figure 3, mean of the last minute of each cycle (4 cycles) was calculated. The mean of each end cycle value also was calculated as an average for the condition. During the three trials of the sub-max test, the HR was monitored at 1 Hz by telemetry with a heart rate monitor (Garmin) directly monitored by the gas exchange system (Cosmed, as described above). As illustrated in Figure 4, the mean of the heart rate of each trial (3 trials) was calculated. The mean of each end cycle value was also calculated.

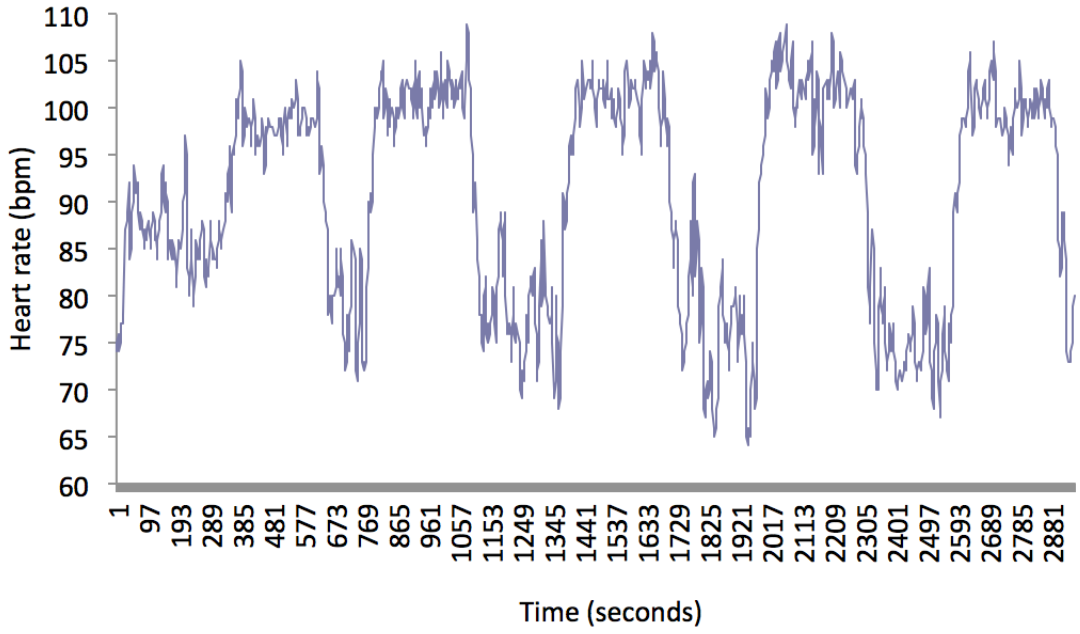

Figure 3: Illustration of heart rate (bpm) during warm-up and the preconditioning.

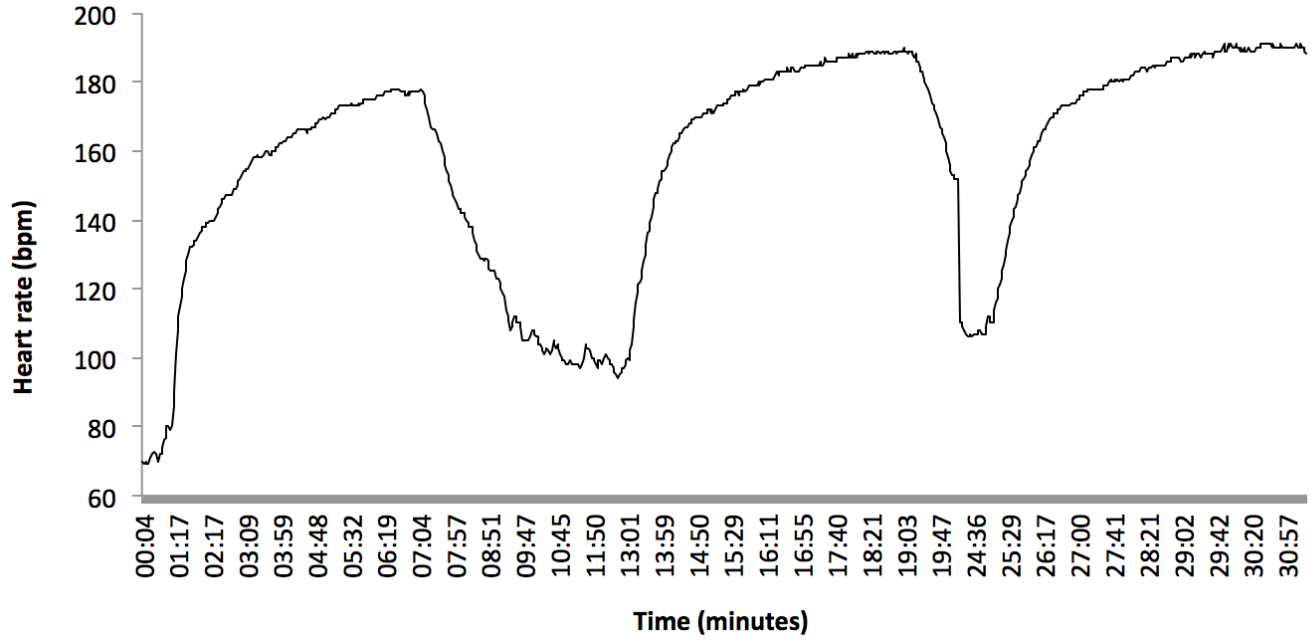

Figure 4: Illustration of heart rate (bpm) during the three trials of the sub-maximal test.

2.5.2 Finger oxygen saturation (%)

At the end of each preconditioning cycle (during the last 30 s of each stage), finger oxygen saturation (%) (8000SM Sensor, Nonin Medical Inc., Amsterdam, The Netherlands) was measured on the forefinger of the right hand, as well during the sub-maximal trials.

2.5.3: Blood lactate

Blood lactate concentration ($mmol^{-1}$) was measured with an automated system (Lactate Scout, SensLab, GmbH, Leipzig, Germany). The right earlobe was cleaned and pricked, thus taking a small droplet into the strip for analysis. Lactate was measured at 1 minute after the termination of the preconditioning session. The procedure was repeated at the end of each trial of the sub-maximal test.

2.5.4: Oxygen uptake

Oxygen uptake consumption was recorded during the sub-maximal test using a gas analyzer (Quark CPET, Cosmed, Rome, Italy). The signals were recoded with the "Breath by breath"

mode in the software. Subjects were fit with a mask and cables were carefully suspended to avoid excess movement during sprints. Concerning the analysis, the values were exported in Excel, and an average was calculated during the last minute of each sub-maximal trial. Figure 5 below shows an example of the $\dot{V}O2$ evolution during the sub maximal test. Data were transferred in excel files to run a macro. The macro allowed investigation of the amplitude, time delay, and time constant of the O_2 kinetics. Several models have been proposed to describe the $\dot{V}O2$ kinetics. For primary analysis, we used the exponential model.

$$\dot{V}O2\,(t) = \dot{V}O2_b + A_1\,[1 - e^{-[(t - td1)/\,\tau_1]}]\,U_1 \text{ Phase 2 (primary component)} + A_2[1 - e^{-[(t - td2)/\,\tau_2]}]\,U_2 \text{ Phase 3 (slow component)}$$

where $U_1 = 0$ fort < td_1 and $U_1 = 1$ for $t \geq td_1$, and $U_2 = 0$ for $t < td_2$ and $U_2 = 1$ for $t \geq td_2$. $\dot{V}O2_b$ is the $\dot{V}O2$ at rest; A_1 and A_2 are the asymptotic amplitudes for the second and third exponential, respectively; τ_1 and τ_2 are the time constants of each exponential; and td_1 and td_2 represent the time delays of each equation.

The amplitude of slow component was assigned the value

$$A'_2 = A_2\,[1 - e^{-[(te - td2)/\,\tau_2]}]$$

where t_e is the time at end of exercise.

As pointed out by Linnarsson (1974), when the second exponential component has a time constant that is substantially longer than the duration of the data collection, it is indistinguishable from a linear "drift." If this case appears, the linear model proposed by Paterson and Whipp and colleagues (Paterson DH, 1991) (Whipp BJ, 1970) would be used.

The parameters of the model were determined with an iterative procedure by minimizing the sum of the mean squares of the differences between the estimated $\dot{V}O2$ based on the model and the measured $\dot{V}O2$. Values of the measured $\dot{V}O2$ that were greater than three standard deviations from the $\dot{V}O2$ of the model were considered outliers and were removed. These outlier values were assumed to be due to abnormal breaths during exercise, such as shallow breathing or breath holding. These values represented <1% of the total data collected. Iterations continued until successive repetitions reduced both the sum of residuals by $<10^8$ and

the correlation coefficient of the relationship between residuals and time by $<10^6$. The bootstrap method was used to test the confidence interval of the model parameters. This method estimates the potential error in the determination of model parameters using repeated samples from the original data set.

During the current study, the gross efficiency (GE), work efficiency (WE), net efficiency (NE), energy cost (EC) and Energy expendure (EE) were mesured. GE, WE and NE were calculated from measures of energy expended, VO_2 and work rate. Energy expended (EE) was calculated from the measures of VO_2 and VCO_2 obtained from the gaz alalyser and analyzed using the formula of Brouwer.

Energy Expenditure ($J.s^{-1}$)

$$[(3.869 \times V \cdot O2) + (1.195 \times V \cdot CO2)] \times (4.186/60) \times 1000$$

GE was calculated as the mean of all data collected in the last 2 min of every work rate over and including 95 W and until the respiratory exchange ratio exceeded 1.00

$$GE(\%) = (\text{Work Rate(W)})/\text{Energy Expended } (J \cdot s^{-1}) \times 100\%$$

EC was calculated as the power output divided by the rate of oxygen consumption and expressed as $kJ \cdot L^{-1}$.

The RER was directly calculated by the gas analyzer system. The system gave us the direct values.

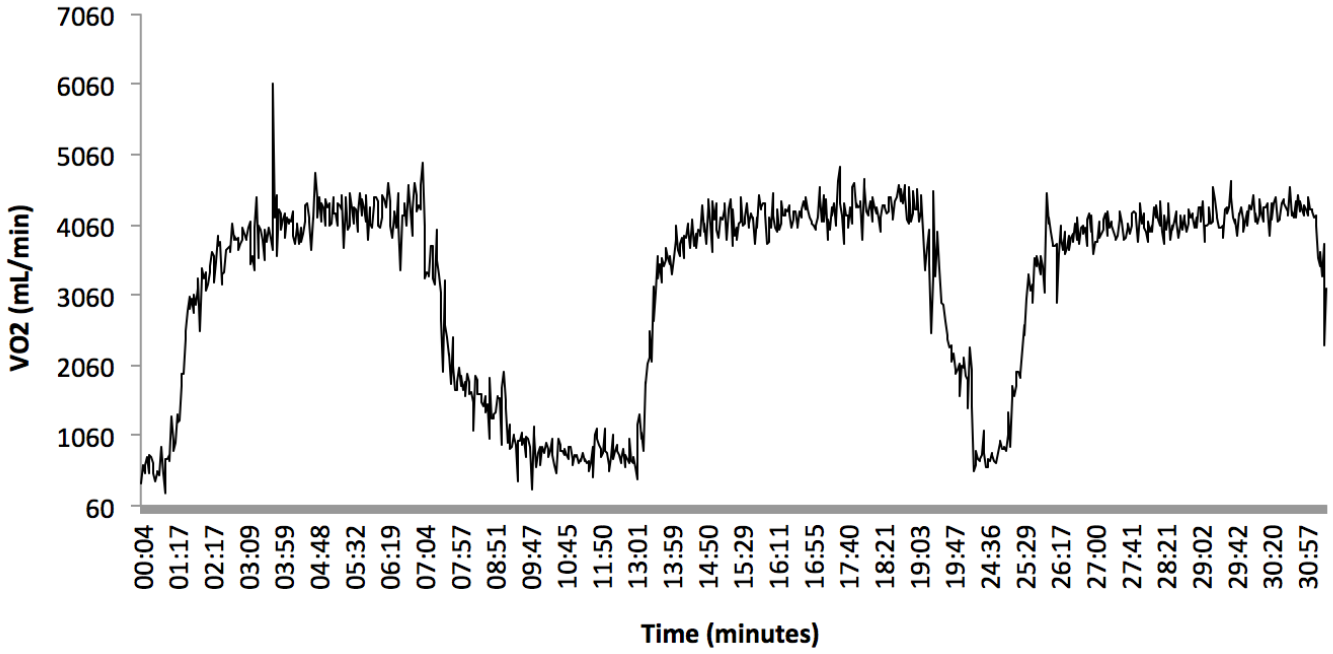

Figure 5: VO2 (ml/min) during the three trials of the sub-maximal test.

2.5.5: Muscle oxygenation

Muscle oxygenation was measured using the near infrared spectroscopy (NIRS) technique explained by Boushel R, (2000). Near-infrared spectrometry (NIRS) is a non-invasive technique permitting to calculate tissue concentrations in oxyhemoglobin ([O_2Hb]), desoxyhemoglobin ([HHb]), total hemoglobin ([tHb]), and tissue saturation index (TSI %). This technology uses light that is transmitted through body's skin, muscle, and adipose tissue. Light absorption is measured and calculated with a modified form of the Beer-Lambert-Law. This law gives us [O_2Hb], [HHb], [tHb] and TSI based on the amount of light that is absorbed or reflected into the device as an indication of the concentration of each variable. A previous study has reported that [O_2Hb] values were more sensitive to blood changes than [HHb] (Delpy D. T, 1997). The TSI is the oxygenation tissue index; it's an estimation of the oxygen tissue saturation (StO_2) in percentage. The StO_2 is the concentration of O_2Hb in relation with the total of hemoglobin; it's an absolute parameter (Patterson MS, 1989). The TSI reflect the dynamic balance between the O_2 income troughs the muscular circulation and the consummation of O_2 by the muscles (Delpy DT, 1997).

A PortaMon NIRS device (PortaMon, Artinis, Zetten, The Netherlands) was placed on the right vastus lateralis (VL) to measure muscle oxygenation. The PortaMon device was carefully covered in a transparent plastic wrap to avoid humidity and maintain a waterproof barrier for the device's function. The PortaMon was attached to the leg with a standard bandage with a bit of tension to allow as little movement as possible during contractions and taped to the skin to assure its good stability and avoid any movement during cycling. This bandage also created a dark environment, which improved the quality of the NIRS recording. A NIRS PortaMon was recording during the entire duration of each test. Using a permanent pen, probe placement was precisely marked along all test visits to assure same position placement during each visit, and subjects were asked to keep the mark throughout the duration of the study.

All signals were recorded with a sampling frequency of 50 Hz (Oxysoft 3.0.53, Artinis, The Netherlands). Data were down sampled to 10 Hz during the exportation and at 10Hz during the analysis using excel macros. For the analysis, the macro indicated the delta of each of the three variables normalized to the warm-up. The purpose to normalize with the warm-up values was to allow a comparison of the change in values, as it is an arbitrary concentration. It also allows greater constituency and variation as a way to compare between subjects. We analyzed the PC and sub-maximal test in the same way. Each delta variable corresponds to an average value of the final 60 seconds of each PC stage or sub-maximal trial that has been normalized against the difference of the averages of the last 30 seconds of the 100W and 50W stages performed at the start of the testing visit each day. In details the normalization value is the average of the last 30 sec of the warm-up 100 W minus the average of the last 30 sec of the warm-up 50 W. Then the average of the last 60 sec of the PC or sub-maximal is normalized by this normalization value.
The equation is: (last 60sec average of PC or Sub – normalized value) / normalized value.

2.5.6 Statistical analysis

All results are presented as mean ± standard deviation (SD) on graphs. Interactions do not appear on the graphs due to lack of space. For the preconditioning phase and the sub-maximal test two kinds of statistic were performed with Sigmastat (SigmaStat 3.5, Systat Software, San Jose, California, USA). One-way repeated measures ANOVA [condition (C, IPC, HPC and HIPC)] was applied on the mean of each end cycle value of every parameter. During

preconditioning and the sub-maximal test, a two-way repeated measures ANOVA was also applied on the values of each end cycle of every parameter. During the sub-maximal test, a one way repeated measures ANOVA was applied on the mean of the last minute of the first trial. Due to some missing data, statistical analysis was performed with SPSS for preconditioning NIRS values, using a mixed model developed by Fabienne Crettaz Von Roten. All statistical results were rejected under a significant value < 0.05. Strong significance was set < 0.001.

3. Results

3.1 Familiarization

Table 1 shows the different characteristic parameters of initial measurements.

ID subject	Body mass	Preconditioning intensity	Body height	Total occlusion	60% occlusion
	kg	W	cm	mmHg	mmHg
1	55.5	83	159	222	133
2	71.2	107	180	187	112
3	72.8	109	185.5	182	109
4	60.7	91	168.6	166	100
5	57.7	87	170.9	204	122
6	81.9	123	193.2	188	113
7	71.6	107	181	223	134
8	90.7	136	200	195	117
9	63.7	96	170.5	175	105
10	77.7	117	183.5	194	116
11	76.6	115	187.5	198	119
12	70.6	106	177.8	239	143
13	66.4	100	168.3	168	101
14	88.6	133	188	223	134
Mean	71.84	108	179.56	197.43	118
SD	11	16	11	22	13

Table 1: Subjects information's

Mean ± SD: Mean of all subjects

Table 2 shows the results of the three-min all-out test. This data were used to set the intensity of the sub-maximal test.

	Critical power 30 last seconds	Submaximal intensity	Average power 3 min all out	Peak power 3 min all-out
	W	W	W	W
Mean	250	212	343	1157
SD	74	63	79	231

Table 2: Subjects results for the three minutes all-out test.

Mean ± SD: Mean of all subjects

Figure 6 represents the evolution of the power during the 3-min all-out test.

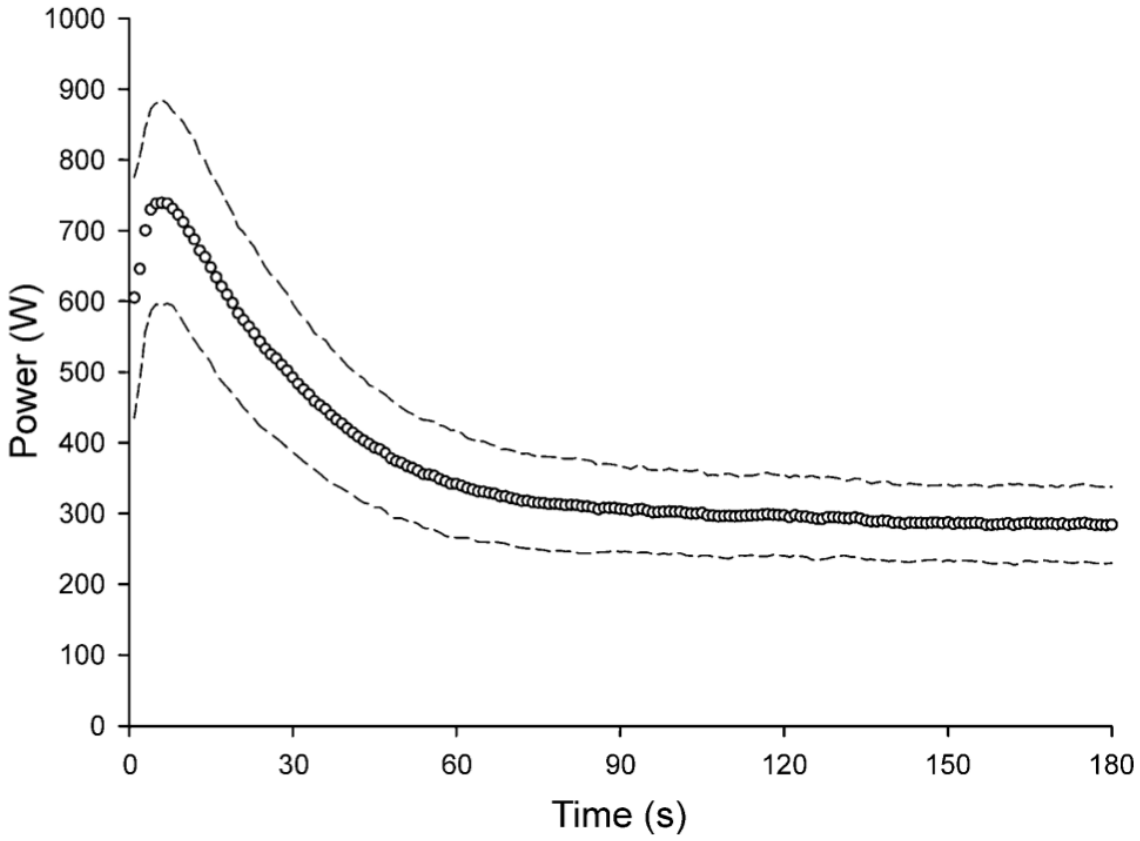

Figure 6: Evolution of the power (W) during the 3-min all-out test.

3.2 Preconditioning

3.2.1 Physiological responses

During the preconditioning and the sub-maximal test the data of the oxygen saturation from the earlobe was not analyzable. It was impossible to interpret this data.

Figure 7 shows heart rate (HR) during preconditioning cycles. HR was lower in control condition than in IPC (P = 0.006), HPC and HIPC (P <0.001). HPC and IPC condition showed lower HR than HIPC (P <0.001). There was no difference in HR between conditions IPC and HPC.

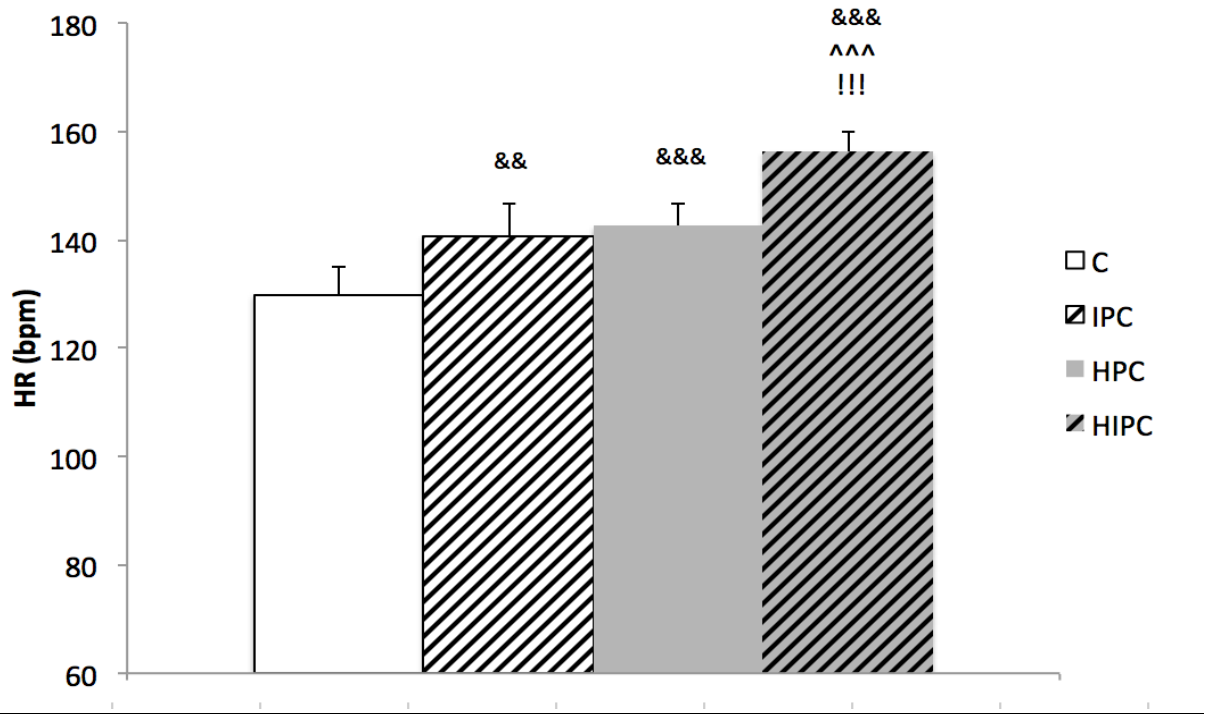

Figure 7: Heart rate (HR) during preconditioning.

Mean ± SEM: Mean of all subjects by condition.

Conditions: Control (C), Ischemic preconditioning (IPC), Hypoxic preconditioning (HPC), Hypoxic and ischemic preconditioning (HIPC). && <0.01, &&& <0.001 for difference with C, ^^^ <0.001 for difference with IPC,!!! <0.001 for difference with HPC.

Figure 8 illustrates the peripheral oxygen saturation (SpO_2) during preconditioning. There was no difference between the IPC and the control condition. The control condition and IPC show better oxygen saturation than HPC and HIPC condition (P <0.001). The percentage of oxygen saturation of HIPC was also higher than HPC (P= 0.008).

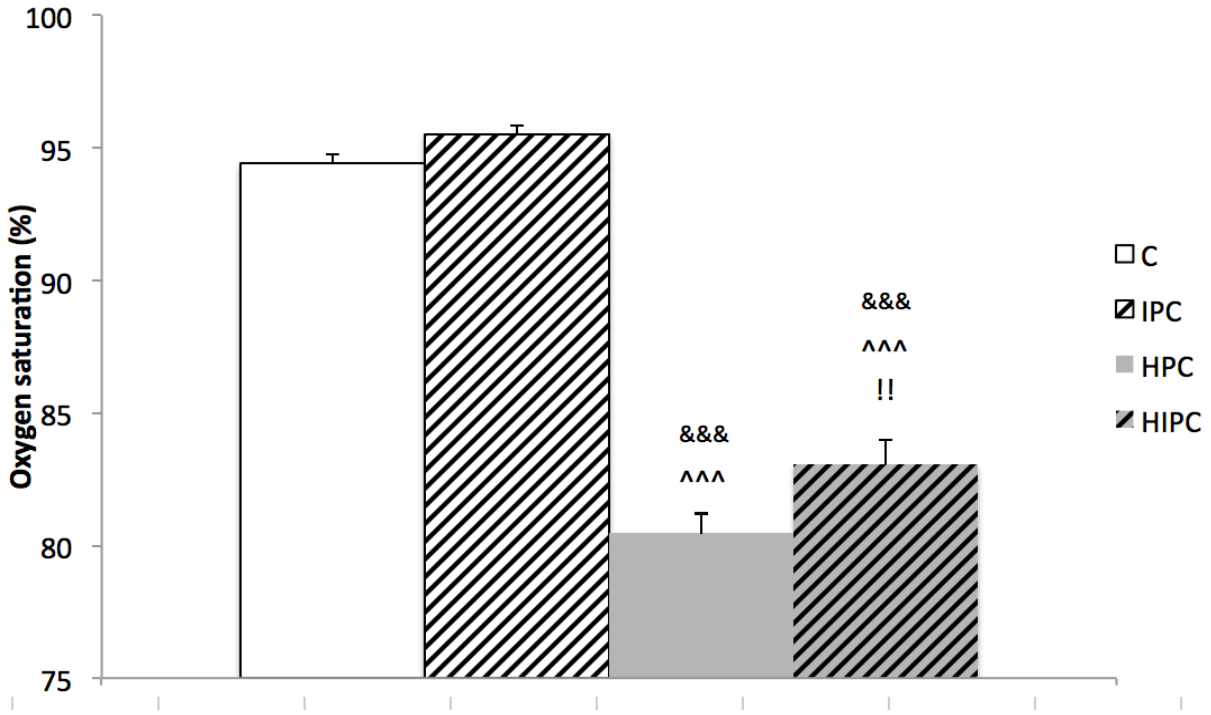

Figure 8: Oxygen saturation (SpO_2) during preconditioning.

Mean ± SEM: Mean of all subjects by condition.

Conditions: Control (C), Ischemic preconditioning (IPC), Hypoxic preconditioning (HPC), Hypoxic and ischemic preconditioning (HIPC). &&& <0.001 for difference with C, ^^^ <0.001 for difference with IPC, !! <0.01 for difference with HPC.

Figure 9 shows the rate of perceived exertion (RPE) of the legs during preconditioning. The occlusions conditions (IPC and HIPC) increase the RPE of the leg in comparison with the control and HPC conditions (P <0.001). There was no difference between the control condition and HPC, as well as no difference between the IPC and HIPC.

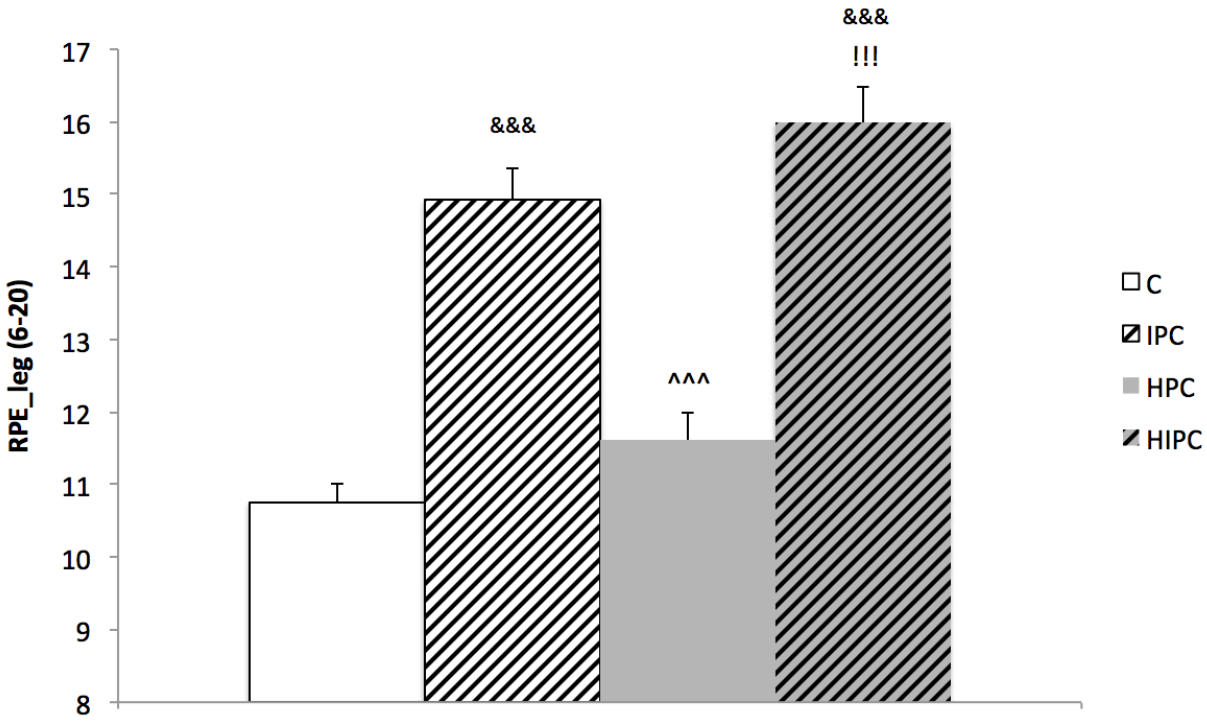

Figure 9: Rate of perceived exertion of the legs during preconditioning.

Mean ± SEM: Mean of all subjects by condition.

Conditions: Control (C), Ischemic preconditioning (IPC), Hypoxic preconditioning (HPC), Hypoxic and ischemic preconditioning (HIPC). &&& <0.001 for difference with C, ^^^ <0.001 for difference with IPC, !!! <0.001 for difference with HPC.

Figure 10 shows rated perceived exertion of the breathing. There were no differences between the control, IPC, and HPC conditions. HIPC increased the RPE of the breathing in comparison with all others conditions (P <0.001).

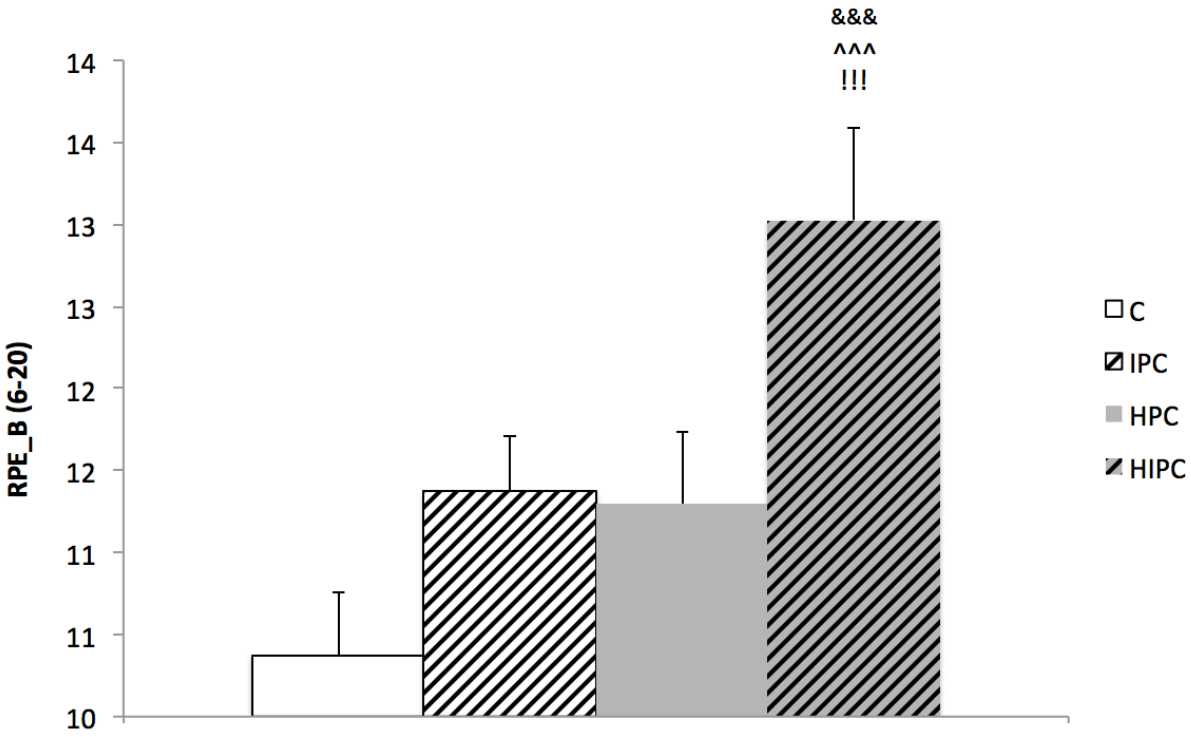

Figure 10: Rated perceived exertion of the breathing during preconditioning.

Mean ± SEM: Mean of all subjects by condition.

Conditions: Control (C), Ischemic preconditioning (IPC), Hypoxic preconditioning (HPC), Hypoxic and ischemic preconditioning (HIPC). &&& <0.001 for difference with C, ^^^ <0.001 for difference with IPC, !!! <0.001 for difference with HPC.

Figure 11 shows the blood lactate concentration (Lac) immediately following the last stage of the preconditioning. There was no difference between the control condition and HPC, or between IPC and HIPC. The control condition was lower than the IPC and HIPC conditions ($P = 0.005$ and $P < 0.001$). Lactate in HPC was also lower than in HIPC ($P = 0.018$).

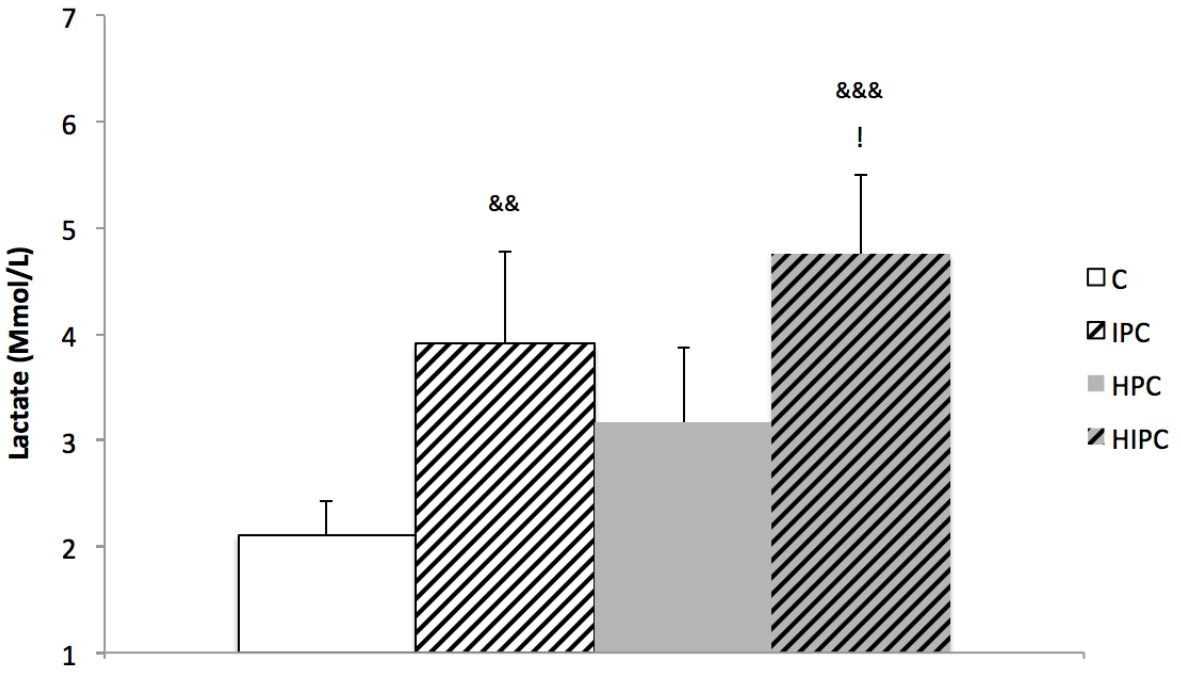

Figure 11: Lactate (Lac) during preconditioning.

Mean ± SEM: Mean of all subjects by condition.

Conditions: Control (C), Ischemic preconditioning (IPC), Hypoxic preconditioning (HPC), Hypoxic and ischemic preconditioning (HIPC). && <0.01, &&& <0.001 for difference with C, ! <0.05 for difference with HPC.

3.2.2 Muscle oxygenation

For all the four parameters delta desoxyhemoglobin (ΔHHb), delta oxyhemoglobin (ΔO_2Hb), delta total hemoglobin (ΔtHb) and delta tissue saturation index (ΔTSI (%)), there was no difference between the conditions with a one-way ANOVA. To continue to analyze the data, a two-way repeated measures ANOVA with condition x stage was used.

Figure 12 shows the ΔHHb during preconditioning. There was no significant difference between the conditions and the stages.

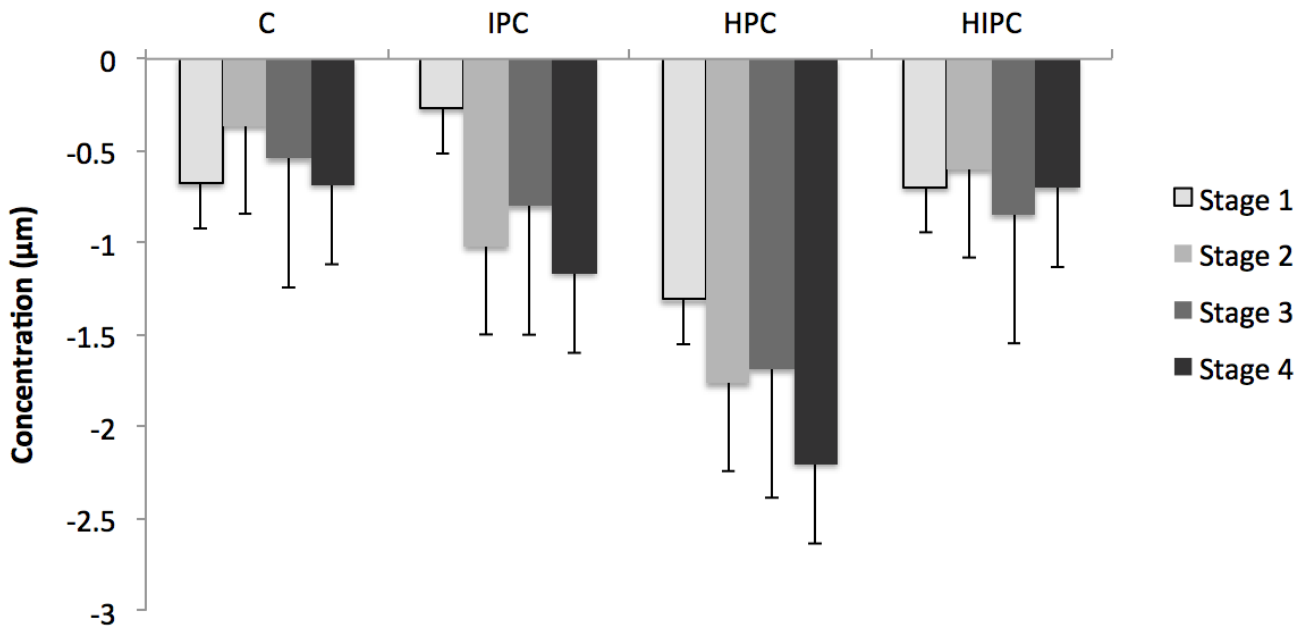

Figure 12: ΔHHb normalized during preconditioning.

Mean ± SEM: Mean of all subjects by condition and stage.

Conditions: Control (C), Ischemic preconditioning (IPC), Hypoxic preconditioning (HPC), Hypoxic and ischemic preconditioning (HIPC).

Stages: Stage 1, Stage 2, Stage 3, and Stage 4.

Figure 13 shows ΔO_2Hb during preconditioning. IPC condition had a lower concentration of O_2Hb than the control (P= 0.02), HPC (P <0.001), and HIPC (P <0.001) conditions. There was no difference between control, HPC, and HIPC conditions. In addition, there was no difference between the stages in any condition.

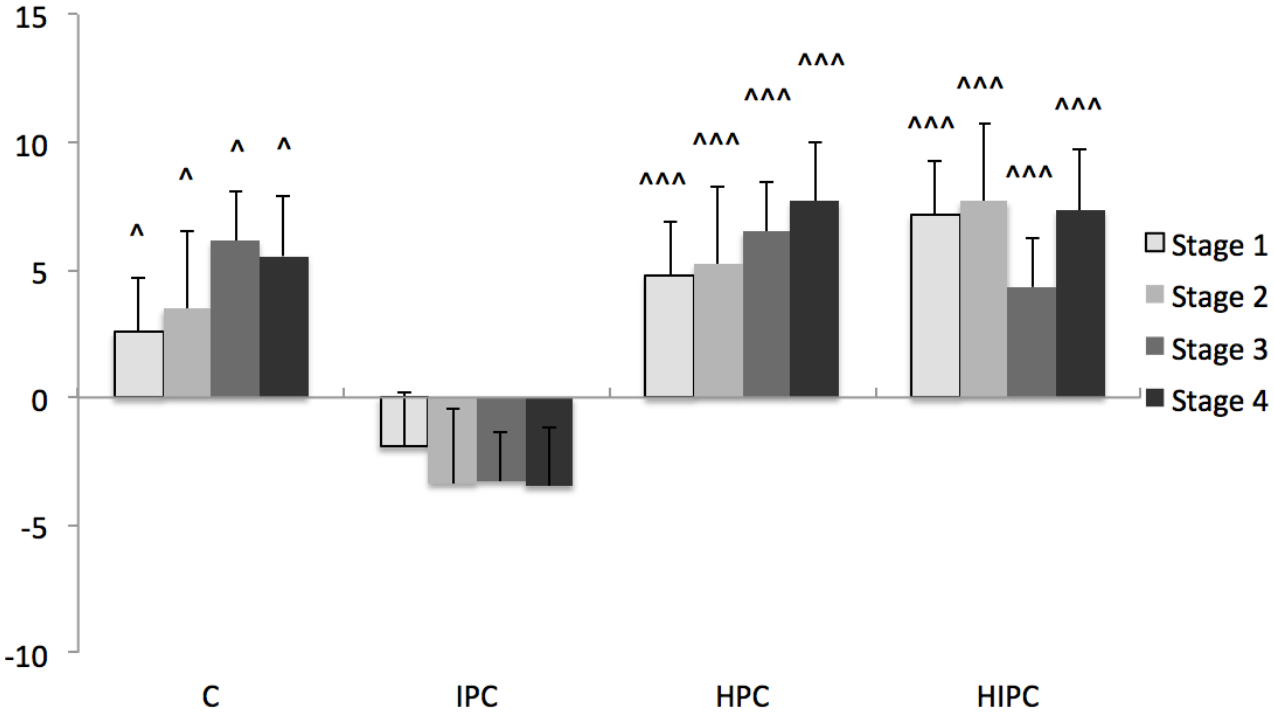

Figure 13: ΔO_2Hb normalized during preconditioning.

Mean ± SEM: Mean of all subjects by condition and by stage.

Conditions: Control (C), Ischemic preconditioning (IPC), Hypoxic preconditioning (HPC), Hypoxic and ischemic preconditioning (HIPC). ^ <0.05, ^^^<0.001 for difference with IPC

Stages: Stage 1, Stage 2, Stage 3, and Stage 4.

Figure 14 shows ΔtHb during preconditioning. The HIPC condition had a lower concentration of ΔtHb than the IPC condition (P = 0.018). There was no difference between the other conditions. In all conditions stages 3 and 4 had higher concentration of ΔtHb than stage 1 (P <0.001). There was no difference between the first and second stage.

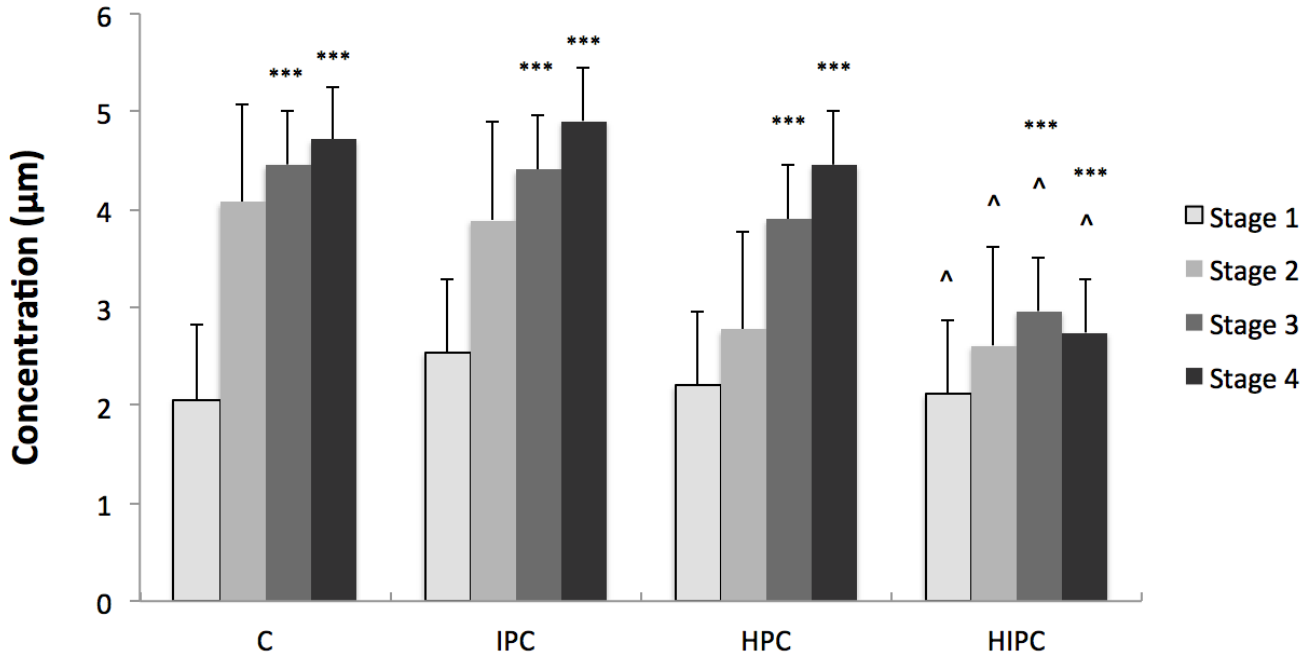

Figure 14: Δ tHb normalized during preconditioning.

Mean ± SEM: Mean of all subjects by condition and by stage.

Conditions: Control (C), Ischemic preconditioning (IPC), Hypoxic preconditioning (HPC), Hypoxic and ischemic preconditioning (HIPC). ^ <0.05 for difference with IPC.

Stages: Stage 1, Stage 2, Stage 3, and Stage 4. *** <0.001 for difference with stage 1.

Figure 15 shows the Δ TSI (%) during the preconditioning. All stages had a lower percentage of Δ TSI in IPC than in the control condition (P= 0.028). There was no difference between the other conditions. In all conditions, stages 3 and 4 had higher percentages of Δ TSI than stage 1 (P <0.001). There was no difference between the other stages.

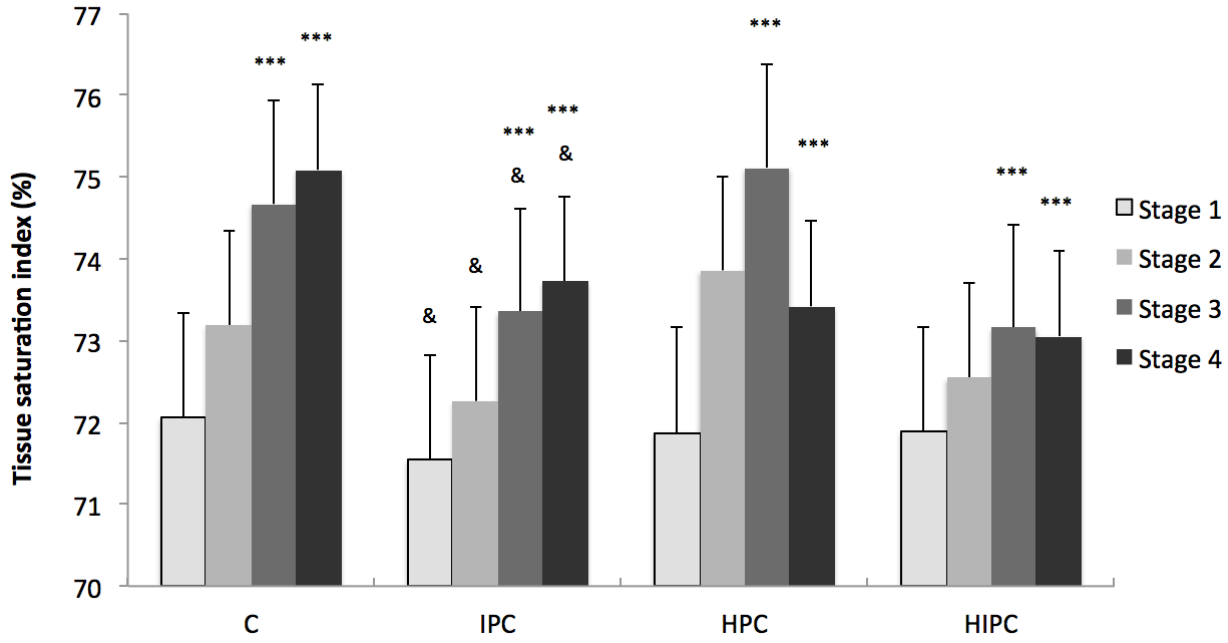

Figure 15: TSI normalized during preconditioning.

Mean ± SEM: Mean of all subjects by condition and by stage.

Conditions: Control (C), Ischemic preconditioning (IPC), Hypoxic preconditioning (HPC), Hypoxic and ischemic preconditioning (HIPC). & <0.05 for difference with C.

Stages: Stage 1, Stage 2, Stage 3, and Stage 4. *** <0.001 for difference with stage.

3.3: Sub maximal performance

During the sub-maximal test, the HR, SpO2, RPE_L, RPE_B, blood lactate, VO_2, and NIRS were measured.

3.3.1: Physiological responses

Figure 16 shows the HR during the three trials of the sub maximal performance. HR was higher with HPC than with IPC (P = 0.067). There was no difference between the other conditions.

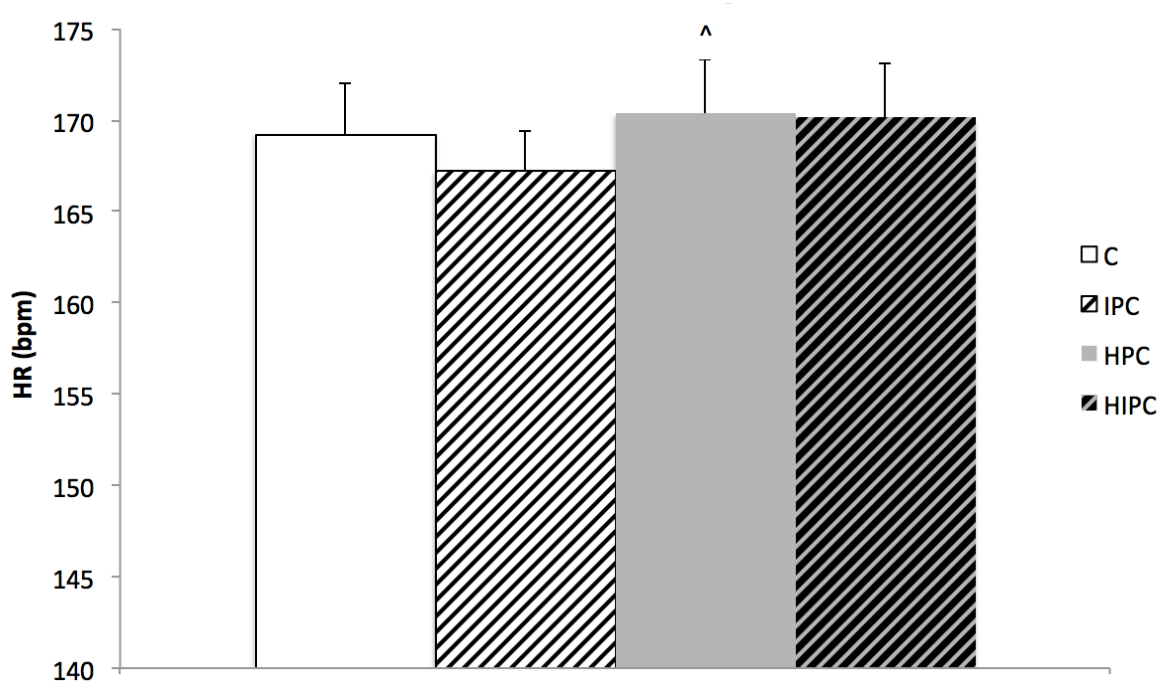

Figure 16: Hart rate by condition (HR) during sub-maximal test.

Mean ± SEM: Mean of all subjects by condition.

Conditions: Control (C), Ischemic preconditioning (IPC), Hypoxic preconditioning (HPC), Hypoxic and ischemic preconditioning (HIPC). ^ < 0.05 for difference with IPC.

Figure 17 shows the RPE of breathing during the three trials of the sub-maximal performance. The HIPC condition was lower than HPC (P = 0.037). There was no difference between the other conditions.

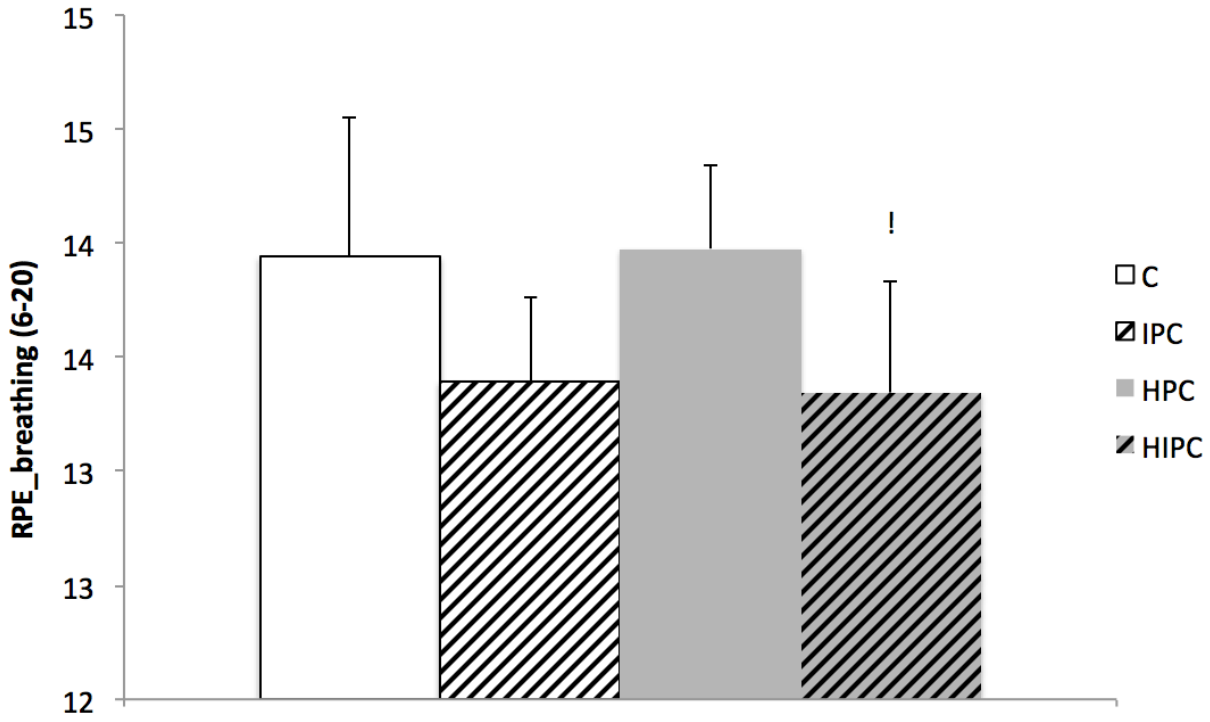

Figure 17: Rated perceived exertion of the breathing (RPE_B) during sub-maximal test.

Mean ± SEM: Mean of all subjects by condition.

Conditions: Control (C), Ischemic preconditioning (IPC), Hypoxic preconditioning (HPC), Hypoxic and ischemic preconditioning (HIPC). ! < 0.05 for difference with HPC.

Table 3 represents the results of the gross efficiency (GE), the energy cost (EC), the energy expenditure (EE) and the respiratory exchange ratio (RER).

	Condition 1	Condition 2	Condition 3	Condition 4	Significance
GE (%)	19.4% ± 1%	19.5% ± 1%	19.3% ± 2%	19.4% ± 1%	NO
EC (kj/L)	4.12 ± 0.3	4.14 ± 0.3	4.1 ± 0.35	4.12 ± 0.3	NO
EE (J/s)	1086 ± 270	1077 ± 269	1091 ± 268	1085 ± 278	NO
RER	1.01 ± 0.05	1.01 ± 0.05	1.01 ± 0.05	1 ± 0.07	NO

Table 3: Gross efficiency, energy cost, energy expenditure and respiratory exchange ratio.

Mean ± SD : Mean of all subjects by condition

Conditions: Control (C), Ischemic preconditioning (IPC), Hypoxic preconditioning (HPC), Hypoxic and ischemic preconditioning (HIPC).

3.3.2: VO_2 kinetics

Figure 18 shows the time constant of the VO_2 kinetics. CT1 was slower with HPC condition than with the control condition (P = 0.047). There was no difference between the other conditions or trials.

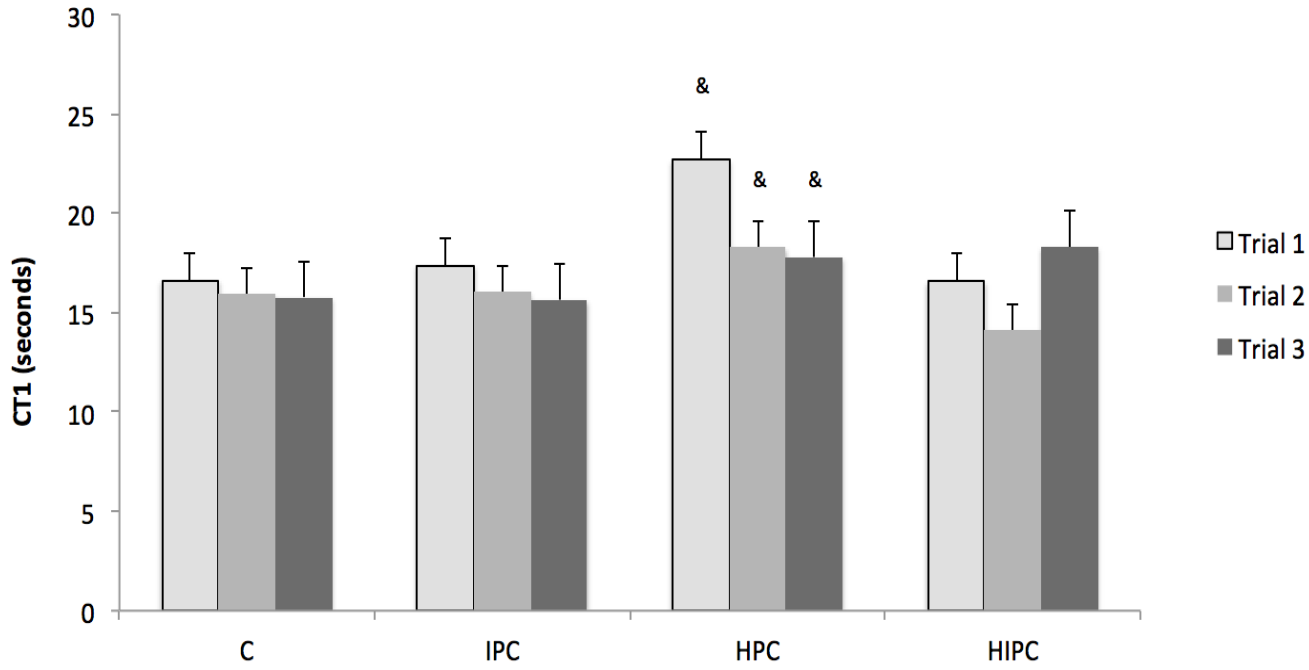

Figure 18: Time constant of the VO_2 kinetics during the sub-maximal performance.

Mean ± SEM: Mean of all subjects by condition and by trial.

Conditions: Control (C), Ischemic preconditioning (IPC), Hypoxic preconditioning (HPC), Hypoxic and ischemic preconditioning (HIPC). & <0.05 for difference with C.

Trials: Trial 1, Trial 2, and Trial 3.

Figure 19 shows the time constant of the VO_2 kinetics during the first trial of the sub-maximal performance. The time constant with HPC was slower than with the control condition (P = 0.014). There was also a difference between HPC and HIPC conditions. The HPC condition was also slower (P = 0.014) than HIPC. There was no difference between the other conditions.

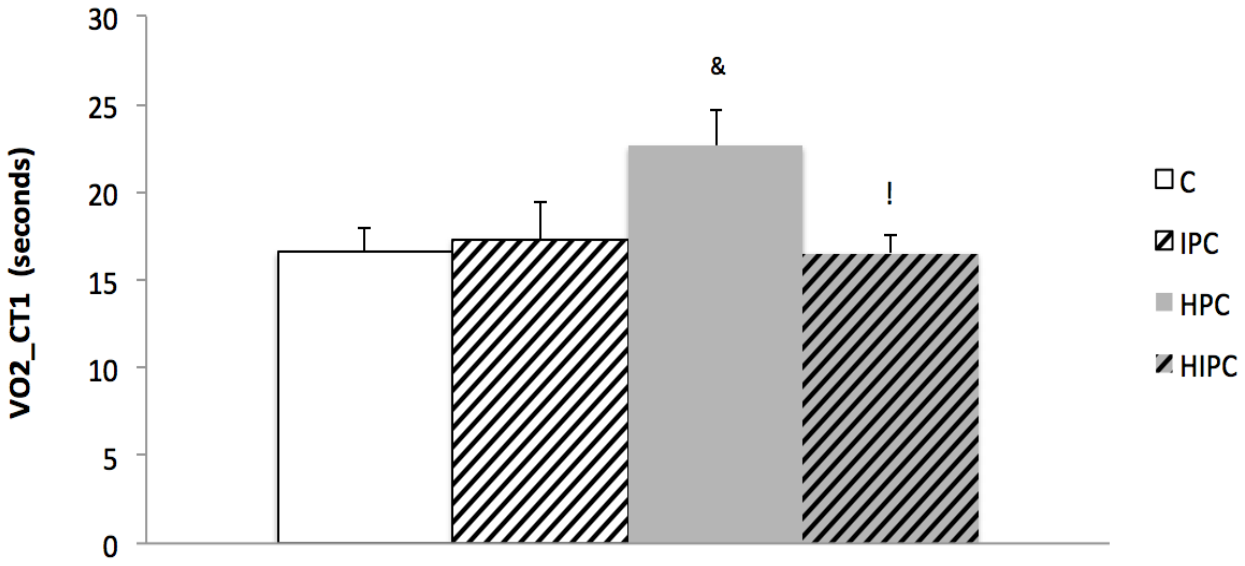

Figure 19: Time constant of the VO_2 kinetics during the first trial of sub-maximal performance.

Mean ± SEM: Mean of all subjects by condition.

Conditions: Control (C), Ischemic preconditioning (IPC), Hypoxic preconditioning (HPC), Hypoxic and ischemic preconditioning (HIPC). & <0.05 for difference with C, ! < 0.05 for difference with HPC.

3.3.3: Muscle oxygenation kinetics

Figure 20 shows the time constant of the O_2Hb kinetics. CT1 of the O_2Hb was faster with the HIPC condition than with the IPC condition (P = 0.039). There was no difference between the other conditions or trials.

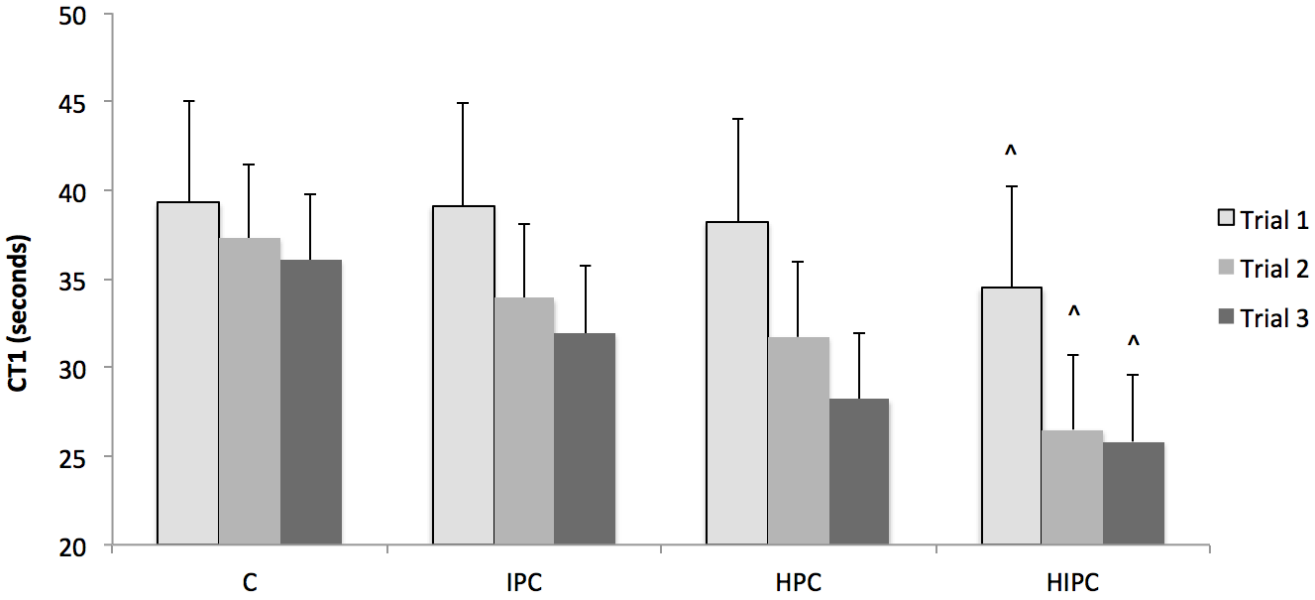

Figure 20: Time constant of the O_2Hb kinetics during the sub-maximal test.

Mean ± SEM: Mean of all subjects by condition and by trial.

Conditions: Control (C), Ischemic preconditioning (IPC), Hypoxic preconditioning (HPC), Hypoxic and ischemic preconditioning (HIPC). ^ <0.05 for difference with IPC.

Trials: Trial 1, Trial 2, and Trial 3.

Figure 21 shows the time constant of the oxyhemoglobin (O_2Hb) kinetics of the first trial of the sub-maximal exercise. CT1 was faster with the HIPC condition than with the IPC condition (P = 0.024). There was no difference between the other conditions.

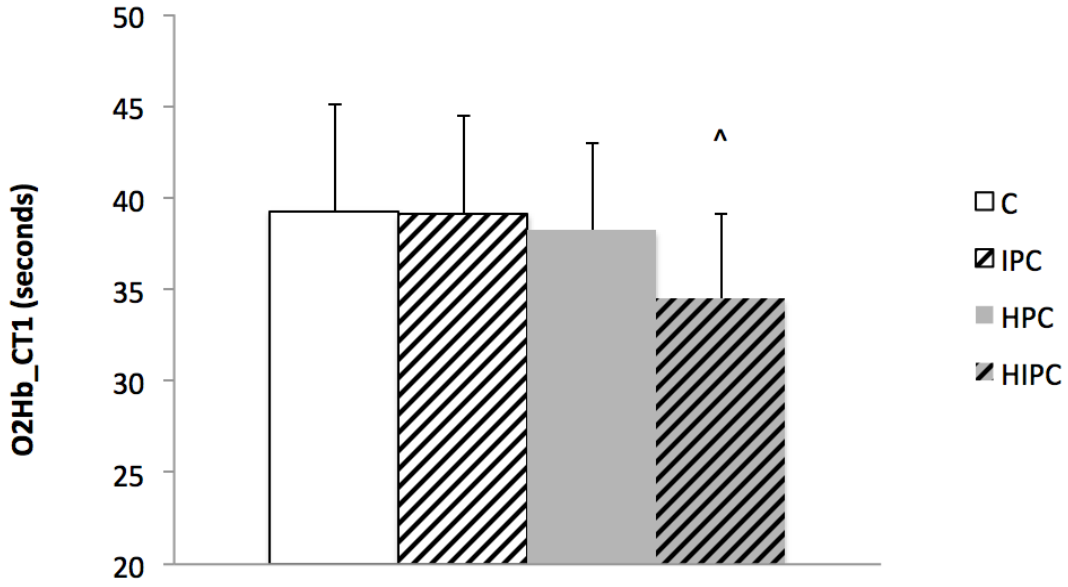

Figure 21: Time constant of the O_2Hb kinetics during the first trial of the sub-maximal test.

Mean ± SEM: Mean of all subjects by condition.

Conditions: Control (C), Ischemic preconditioning (IPC), Hypoxic preconditioning (HPC), Hypoxic and ischemic preconditioning (HIPC). ^ <0.05 for difference with IPC.

Figure 21 shows the time constant of the desoxyhemoglobin (HHb) kinetics during the first trial of the sub-maximal exercise. There was a difference between the HPC and HIPC conditions. The HPC time constant is faster than HIPC (P = 0.039). There were no differences between the other conditions.

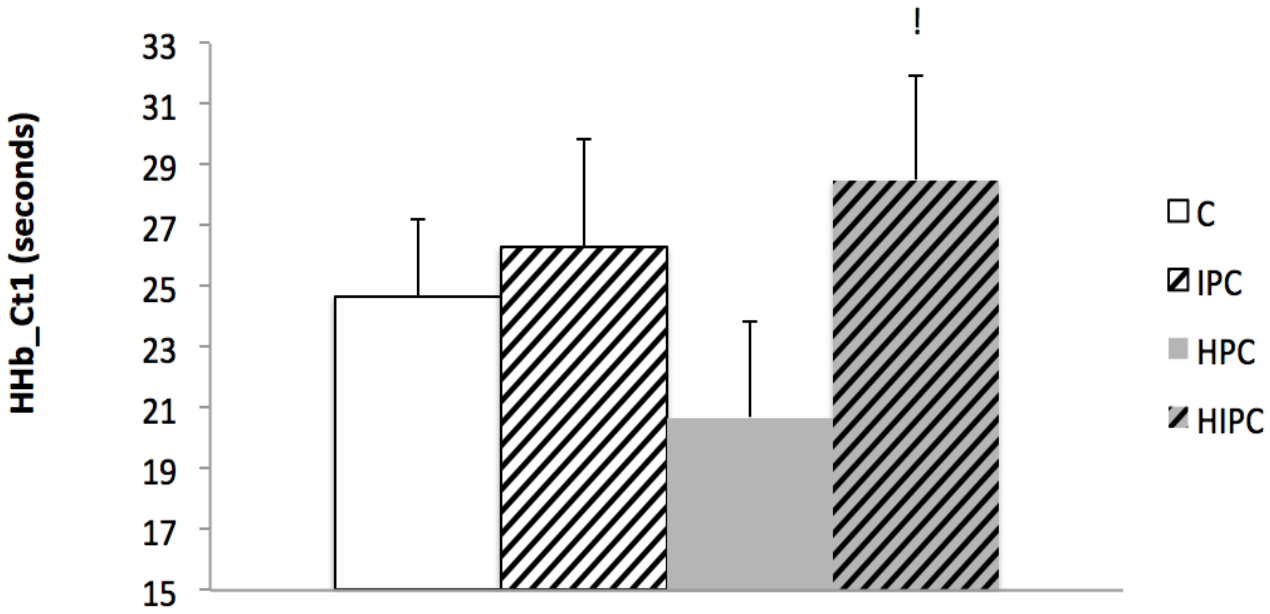

Figure 21: Time constant of the HHb kinetics during the first trial of the first sub-maximal exercise.

Mean ± SEM: Mean of all subjects by condition.

Conditions: Control (C), Ischemic preconditioning (IPC), Hypoxic preconditioning (HPC), Hypoxic and ischemic preconditioning (HIPC). ! <0.05 for difference with HPC.

3.3.4: Muscle oxygenation end trials

The concentration of delta desoxyhemoglobin (ΔHHb) during the last minute of each trial of the sub-maximal test is represented in figure 22. Control and HIPC conditions had lower concentrations of delta HHb than with HPC (P <0.001), and IPC also had lower ΔHHb than HPC (P = 0.003). There was no difference between the trials.

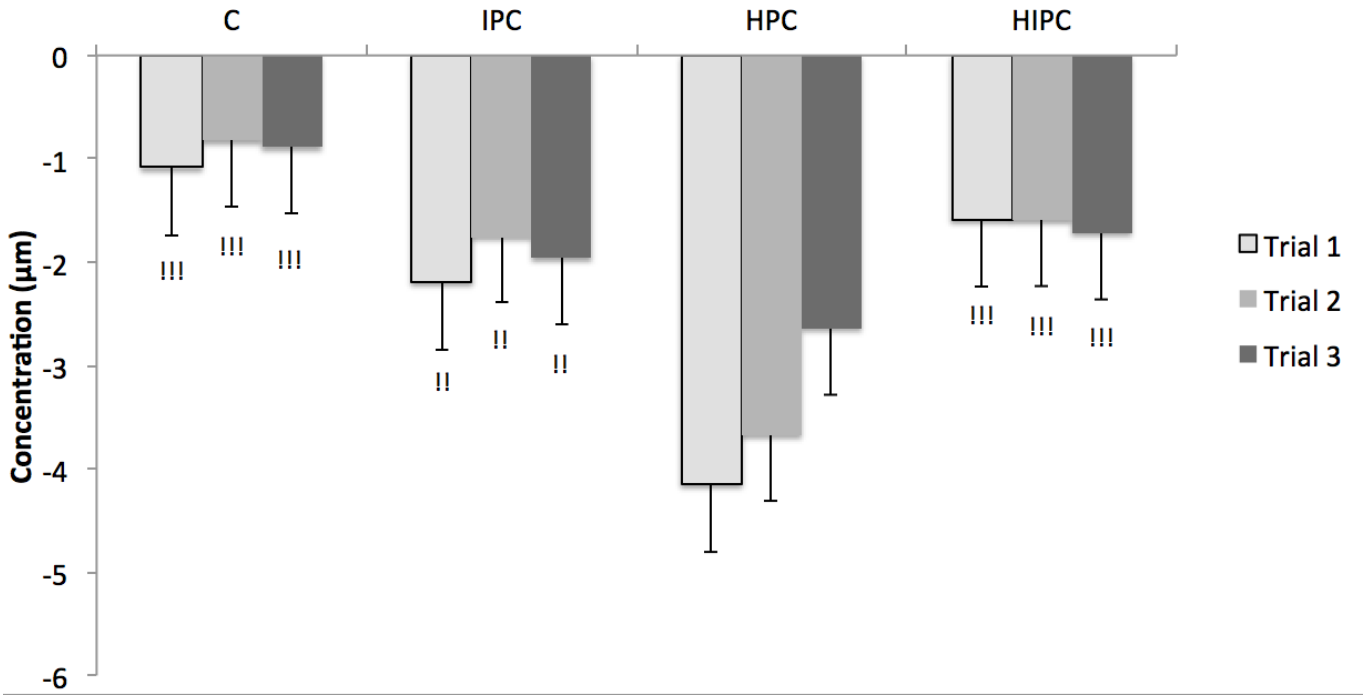

Figure 22: The concentration of ΔHHb during the last minutes of each trial of the sub-maximal exercise.

Mean ± SEM: Mean of all subjects by condition and trial.

Conditions: Control (C), Ischemic preconditioning (IPC), Hypoxic preconditioning (HPC), Hypoxic and ischemic preconditioning (HIPC). !! <0.01,!!! <0.01 for difference with HPC.

Trials: Trial 1, Trial 2, and Trial 3.

Figure 23 shows the concentration of delta oxyhemoglobin in the last minute of each trial of the sub-maximal exercise. The ΔO_2Hb with IPC was lower than with the control, HPC, and HIPC conditions (P = 0.01, P = 0.03, P <0.001). There was less ΔO_2Hb with the IPC condition at the end of each trial.

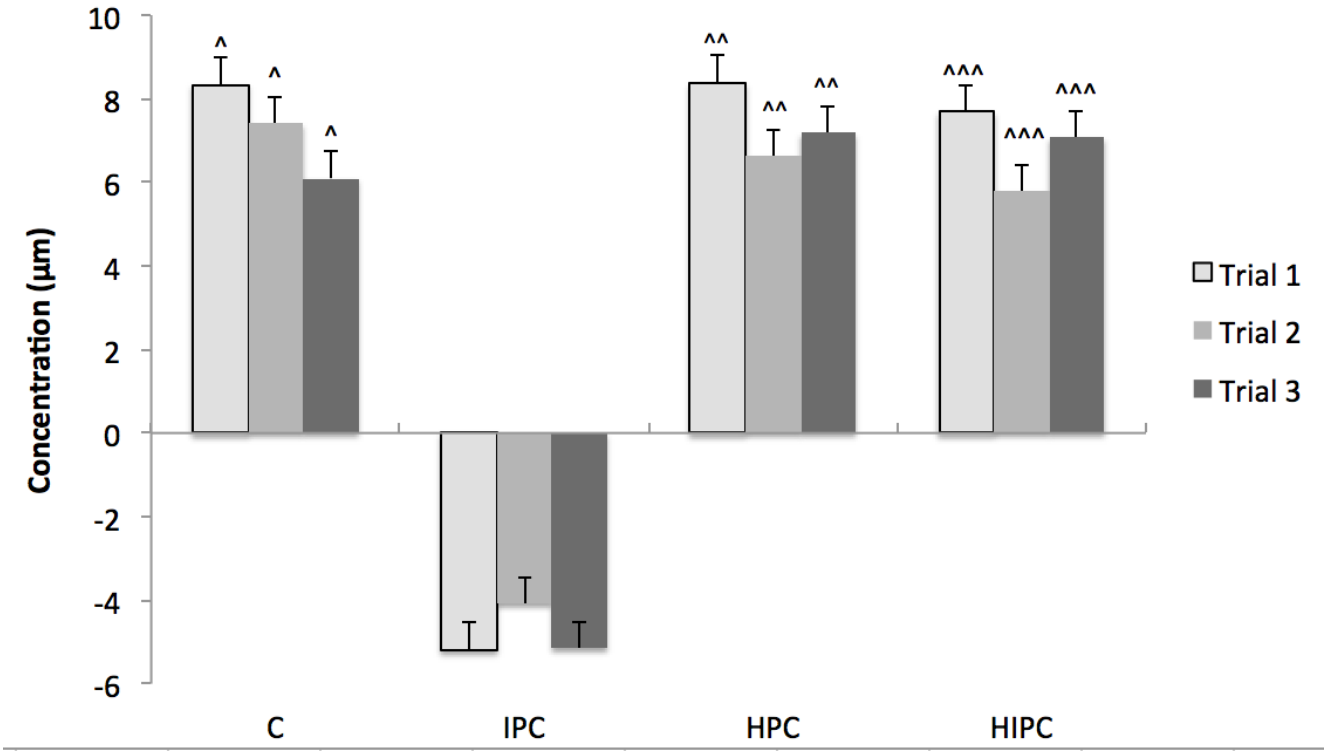

Figure 23: The concentration of ΔO_2Hb during the last minutes of each trial of the sub-maximal exercise.

Mean ± SEM: Mean of all subjects by condition and trial.

Conditions: Control (C), Ischemic preconditioning (IPC), Hypoxic preconditioning (HPC), Hypoxic and ischemic preconditioning (HIPC). ^ <0.05, ^^ <0.01, ^^^ <0.01 for difference with IPC.

Trials: Trial 1, Trial 2, and Trial 3.

Figure 24 represents the concentration of delta total hemoglobin (ΔtHb) during the last minute of each trial of the sub-maximal exercise. There was more concentration of ΔtHb with IPC than with the control (P = 0.059) and also more ΔtHb with IPC than with HIPC (P = 0.01). There were no differences between the other conditions or trials.

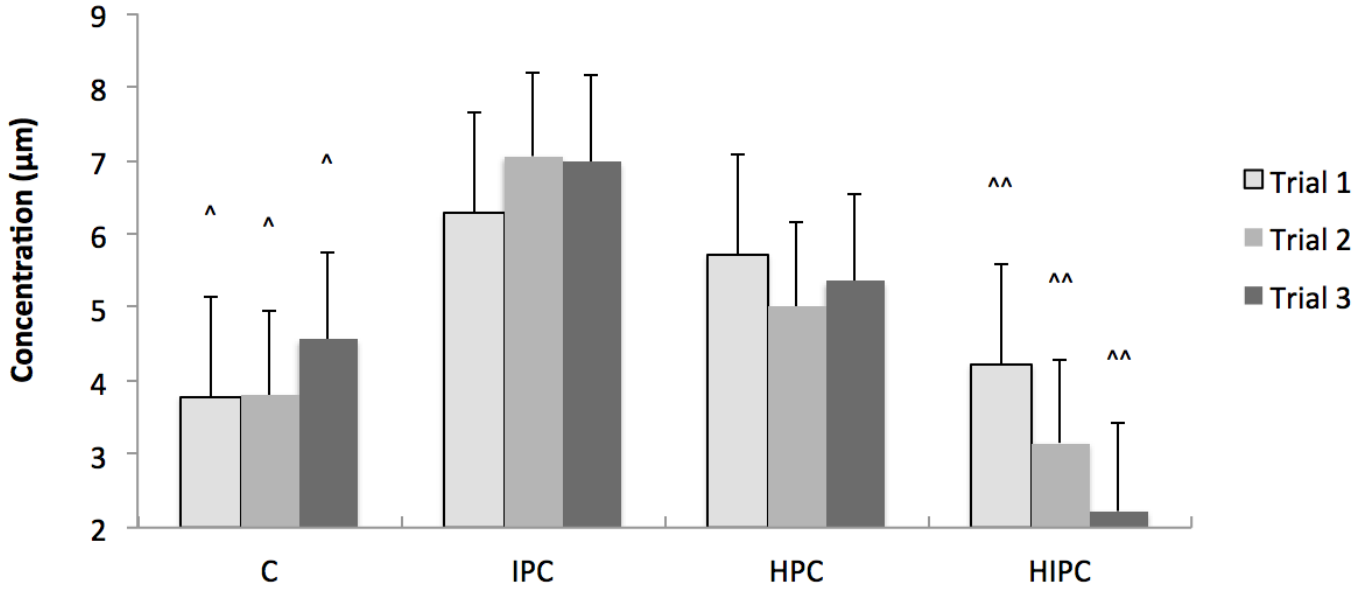

Figure 24: The concentration of ΔtHb during the last minutes of each trial of the sub-maximal exercise.

Mean ± SEM: Mean of all subjects by condition and trial.

Conditions: Control (C), Ischemic preconditioning (IPC), Hypoxic preconditioning (HPC), Hypoxic and ischemic preconditioning (HIPC). ^ <0.05, ^^ <0.01 for difference with IPC.

Trials: Trial 1, Trial 2, and Trial 3.

Figure 26 shows the delta tissue saturation index during the last minute of each trial of the sub-maximal exercise. The ΔTSI (%) was higher with HPC conditions than with IPC and HIPC (P = 0.025, P = 0.088). Trial 3 was higher than trial 1 (P <0.001) and higher than trial 2 (P = 0.045). Trial 2 was also higher than trial 1 (P = 0.051).

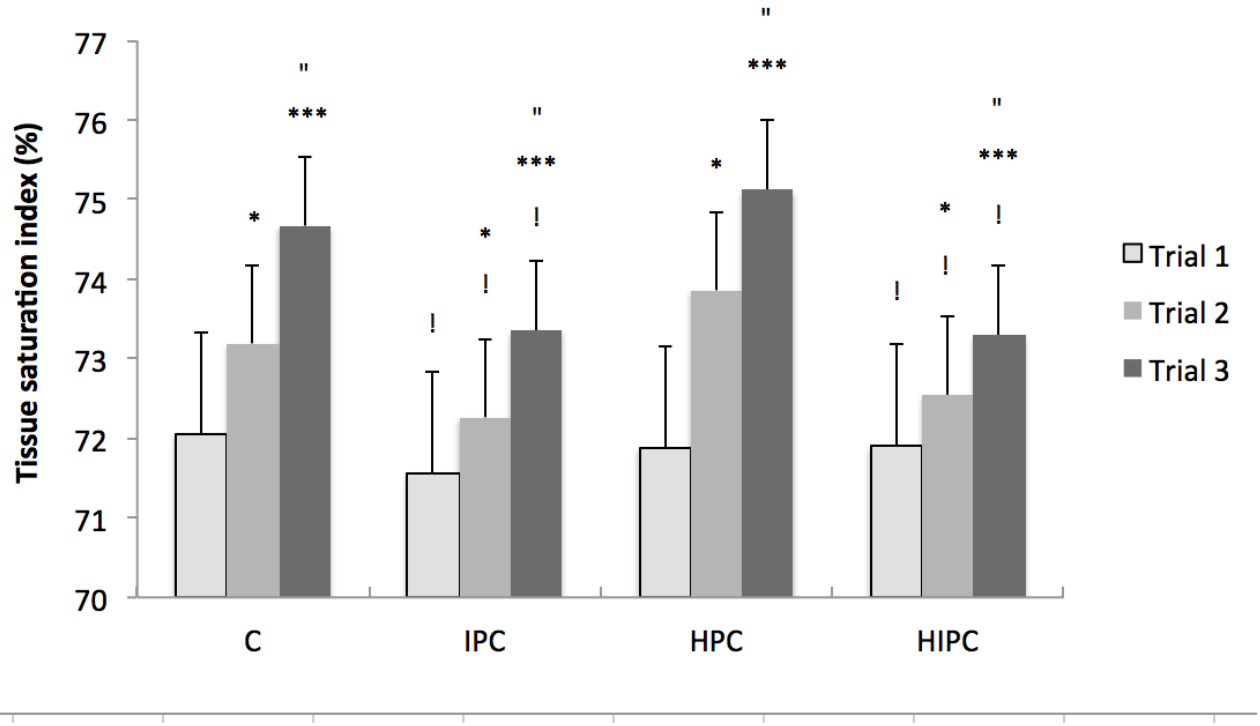

Figure 26: The ΔTSI (%) during the last minutes of each trial of the sub-maximal exercise.

Mean ± SEM: Mean of all subjects by condition and trial.

Conditions: Control (C), Ischemic preconditioning (IPC), Hypoxic preconditioning (HPC), Hypoxic and ischemic preconditioning (HIPC). ! <0.05, for difference with HPC.

Trials: Trial 1, Trial 2, and Trial 3. * <0.05, *** <0.001 for difference with 1, " <0.05 for difference with 2.

3.4 Summary of the results

Table 4 shows a summary of the different parameters of the physiological responses during the four stages of the preconditioning.

Parameter	Condition difference	Trial difference	Significance	Stages
Heart rate (bpm)	Yes	Yes	Con: <0.001 Sta <0.001	Con: C vs HIPC, IPC vs HIPC, HPC vs HIPC (P= <0.001) andC vs IPC (P= 0.006) Sta : 1 vs 3, 1 vs 4 (P= <0.001) and 1 vs 2 (P= 0.002)

O2 saturation (%)	Yes	No	<0.001	C vs HPC, C vs HIPC, PC vs HPC, IPC vs HIPC (P= <0.001) and HPC vs HIPC (P= 0.008)
RPE leg	Yes	Yes	Con: <0.001 Sta: 0.002	Con : C vs IPC, C vs HPC, C vs HIPC and IPC v HIPC (P= <0.001) Sta : 1 vs 4 (P= 0.003)and 2 vs 4 (P= 0.032)
RPE breathing	Yes	Yes	Con: <0.001 Sta: 0.002	Con: C vs IPC, C vs HPC and C vs HIPC (P= <0.001) Sta : 1 vs 3 (P= 0.009), 2 vs 4 (P= 0.026) and 1 vs 4 (P= <0.001)
Lactate (lac) Post stage 4	Yes	X	<0.001	C vs HIPC (P= <0.001), HPC vs HIPC (P= 0.018) and C vs IPC (P= 0.005)

Table 4: Summary of the physiological responses during the four stages of the preconditioning.

Mean ± SEM: Mean of all subjects by condition and stage.

Conditions: Control (C), Ischemic preconditioning (IPC), Hypoxic preconditioning (HPC), Hypoxic and ischemic preconditioning (HIPC).

Stages: Stage 1, Stage 2, Stage 3, and Stage 4.

Table 5 represents a summary of the different parameters of muscle oxygenation during the four stages of the preconditioning.

Parameter	Condition difference	Trial difference	Significance	Stages
Δ HHb last minute	No	No		

Δ O2Hb last minute	Yes	No	<0.001	IPC vs HPC, IPC vs HIPC (P= <0.001) and C vs IPC (P= 0.02)
Δ tHb last minute	Yes	No	0.018	IPC vs HIPC (P= 0.018)
Δ TSI (%) last minute	Yes	Yes	Con: 0.028 Sta: <0.001	Con: C vs IPC (P= 0.098) Sta : 1 vs 3 and 1 vs 4 (P= <0.001)

Table 5: Summary of muscle oxygenation during the four stages of the preconditioning.

Mean ± SEM: Mean of all subjects by condition and stage.

Conditions: Control (C), Ischemic preconditioning (IPC), Hypoxic preconditioning (HPC), Hypoxic and ischemic preconditioning (HIPC).

Stages: Stage 1, Stage 2, Stage 3, and Stage 4.

Table 6 shows a summary of the different parameters of the physiological responses during the tree trials of the sub-maximal exercise.

Parameter	Condition difference	Trial difference	Significance	Trial
Hart Rate (bpm)	Yes	Yes	Con: 0.044 Tri: <0.001	Con: IPC VS HPC (P= 0.067) Tri: 1 vs 2, 1 vs 3, 2 vs 3 (P= <0.001)
O2 Saturation (%)	No	Yes	0.032	1 vs 3 (P= 0.033)
RPE leg (6-20)	No	Yes	<0.001	1 vs 2 (P= 0.007) and 1 vs 3 (P= <0.001)
RPE breathing (6-20)	No	Yes	<0.011	
Lactate (Mmol/L)	No	Yes	<0.001	1 vs 2, 1vs 3 and 2 vs 3
Energy Cost (Kj/I)	No	Yes	0.008	1 vs 3
Gross constant power (W/Kg)	No	No		

Extern efficiency (%)	No	No		
Respiratory quotient	No	Yes	<0.001	1 vs 2, 1vs 3 and 2 vs 3 (0.008)
CO2 volume (ml/min)	No	Yes	<0.001	1 vs 2 and 1 vs 3
O2 volume (ml/Kg/min)	No	Yes	0.008	1 vs 3
VE (L/min)	No	Yes	<0.001	1 vs 2, 1 vs 3 and 2 vs 3 (0.009)
VO2 A1	No	Yes	0.003	1 vs 3
VO2 TD1 (sec)	No	Yes	0.043	1 vs 2
VO2 CT1 (sec)	Yes	No	0.047	C vs HPC

Table 6: Summary of the physiological responses during the tree trials of the sub-maximal exercise.

Mean ± SEM: Mean of all subjects by condition and trial.

Conditions: Control (C), Ischemic preconditioning (IPC), Hypoxic preconditioning (HPC), Hypoxic and ischemic preconditioning (HIPC).

Trials: Trial 1, Trial 2, and Trial 3.

Table 7 represents a summary of different parameter of the muscle oxygenation during the three trials of the sub-maximal exercise.

Parameter	Condition difference	Trial difference	Significance	Trials
HHb A1	No	No		
O2Hb A1	No	No		
TSI A1	No	No		
HHb TD1 (sec)	No	No		
O2Hb TD1 (sec)	No	No		
TSI TD1 (sec)	No	No		
HHb CT1 (sec)	No	Yes	0.0023	1 vs 2
O2Hb CT1 (sec)	Yes	No	0.0039	IPC vs HPC
TSI CT1 (sec)	No	No	Trend	IPC vs HPC (P =0.051)

Δ HHb last min	Yes	No	<0.001	C vs HPC, HPC vs HIPC and IPC vs HPC (P = 0.004)
Δ O2Hb last min	Yes	No	<0.001	C vs IPC (P = 0.01), IPC vs HPC (P = 0.003) and IPC vs HIPC
Δ tHb last min	Yes	No	0.002	C vs IPC (P= 0.057 and IPC vs HIPC (P = 0.01)
Δ TSI (%) last min	Yes	Yes	Con = 0.011 Tr = <0.001	Con : IPC vs HPC (P= 0.025), HPC vs HIPC (P= 0.088) Trial : 1 vs 2 (P= 0.051), 1 vs 3 (P= <0.001), 2 vs 3 (P= 0.045)

Table 7: Summary of the muscle oxygenation parameters during the tree trials of the sub-maximal exercise.

Mean ± SEM: Mean of all subjects by condition and trial.

Conditions: Control (C), Ischemic preconditioning (IPC), Hypoxic preconditioning (HPC), Hypoxic and ischemic preconditioning (HIPC).

Trials: Trial 1, Trial 2, and Trial 3.

4: Discussion

The main result of the present study was that preconditioning (PC), whether ischemic or hypoxic, did not induce a potentially ergogenic effect on the sub-maximal performance. None of the physiological responses were improved during the sub-maximal performance (HR, SpO_2, lactate, VO_2, and work efficiency) Ischemic or hypoxic PC also did not lead to an enhancement in the VO_2 kinetics. The time constant was not faster in any of the conditions in comparison with the control condition. In fact, the HPC condition was detrimental for the time constant witch was slowed. For the HIPC condition, the addition of the ischemic and the hypoxic preconditioning also had no supplementary effect on the sub-maximal performance than the hypoxic / ischemic conditions on their own. An interesting finding is that when the ischemia was added to the hypoxia during the PC, it seems to blunt the negative effect of the

hypoxia on the HHb during the sub-maximal test. Result of interest is the hyperemic effect of IPC as shown by the increase in Δ tHb during the sub-maximal bouts.

4.1: Three-minute all-out test

Subjects were asked to perform a three-minute all-out test to measure the critical power (CP). During this test, subjects performed maximally with no pacing for three minutes with strong verbal encouragement. It is never sure that subjects do not pace the test, but with the verbal encouragement, the results were as expected.

4.2: Preconditioning

4.2.1: Ischemic condition (IPC)

Researchers have studied the cardiovascular functions associated with blood flow restriction (BFR) and have found that even at walking intensity, exercise with BFR induced an increase of HR (Renzi C.P, 2010). In the present study, IPC improved the HR ($P = 0.006$) in comparison with the control condition. This finding demonstrated that the cardiovascular work is enhanced during BFR. This has also been confirmed by Neto et al (2016) who found that exercise with BFR induced an increase of cardiovascular work (Neto G. R, 2016). Another study found that oxygen saturation decreased with exercise with BFR (Abe T, 2006). On the contrary, in the current study there were no differences between the control and IPC. In addition, the BFR did not seem to have an impact on the SpO_2. In the same study (Abe T, 2006), RPE was higher during BFR exercise, which is in accordance with the present study. RPE of the legs was lower in the control condition than in IPC and HIPC ($P < 0.001$). The altered supply of intramuscular oxygen, which induces a more acidic and anabolic environment, likely affected the participants' perceived exertion (Abe T, 2006). The increase of the blood lactate concentration also demonstrates that the environment is more acidic. Indeed, the results indicated that IPC and HIPC conditions had a higher lactate level ($P = 0.005$, $P < 0.001$). In a study exploring the Kaatsu training in walking, the lactate concentrations were also higher after BFR exercise (Engelen Arielle, 1996). The improvement of the blood lactate (a lower concentration) as well as lower HR can also influence the increase leg rating of perceived exertion. During BFR exercise, systolic and diastolic blood pressure increases while stroke volume decreases due to limited venous return, this could also create discomfort and increase the RPE of the leg (Engelen Arielle, 1996).

There was no difference in the conditions for the RPE in the breathing, meaning that BFR does not seem to directly affect the ventilatory system.

For the muscle oxygenation, previous research has indicated that BFR during exercise creates a rapid depletion of O_2 stores within muscle tissue (Hampson Neil B, 1998), which provokes a greater deoxygenation. In the current study, there were no differences in ΔHHb between any the conditions. However, regarding the ΔO_2Hb, IPC was lower than C, HPC and HIPC ($P = 0.02$, $P < 0.001$). The ΔO_2Hb was lower in IPC meaning that the subjects had less O_2 to use, but there were no differences in the HHb, and thus changes on the depletion of O_2 stores within the muscle tissue. For the ΔtHb, IPC was higher than HIPC ($P = 0.018$) but there was no difference with the control condition. This means that in the current study there was only a difference in ΔO_2Hb that demonstrated the effects of an ischemic exercise. Compared to the control condition, IPC had a lower Δ percentage of tissue saturation ($P = 0.028$), which also demonstrated that during BFR exercise the O_2 availability in the muscle was diminished.

4.2.2: Hypoxic condition (HPC)

In the current study, HPC presented a higher HR than the control condition. This finding is congruent with a study that also found that HR was higher in hypoxic conditions (Engelen Arielle, 1996). SpO_2 in the HPC condition was also lower than in the control condition ($P < 0.001$), as expected. The subjects were under normobaric hypoxic conditions meaning that the barometric pressure was the same as the see level but the fraction of O_2 inspired (F_IO_2) was reduced by the hypoxia training device. When subjects were exercising in hypoxia conditions there was a decrease in oxygen saturation and to counter this decrease there was an increase the blood flow (Engelen Arielle, 1996), which can be illustrated in the present study by the increase in HR. HPC also had a higher RPE in the legs than the control condition ($P < 0.001$), however, the lactate concentration was not different than in the control. In a previous study, no differences were found in blood pH (Kubota Y, 2015); therefore, the decrease of the pH cannot be an explanation for the increase of RPE_L. The decrease of the oxygen saturation and therefore the lack of O_2 in the muscle can stimulate an increase in the RPE_L. The increase of RPE_L in HPC was lower than IPC and the HIPC condition ($P < 0.001$). Interestingly, even though the SpO_2 decreases in HPC, there were no differences between the conditions for SpO_2, meaning that in hypoxia exercise at the F_IO_2 of 13.6% does not cause an increase of the pulmonary ventilation to increase RPE_B. The current study found no

differences with RPE_B during any conditions. Previous literature indicated that in order to maintain O_2 delivery during hypoxic (F_IO_2 = 11%) exposure, pulmonary ventilation increases (Hammond M.D, 1986). In the current study, the simulated altitude was maybe not enough to see some influence of increased of pulmonary ventilation to have an effect on the RPE_B. For the muscle oxygenation there were no differences between HPC and the other conditions for ΔHHb, ΔO2Hb, and ΔtHb. The hypoxia induced during the exercise did not seem to provoke a muscle deoxygenation. ΔTSI (%) is also not different in the control condition and the HPC, which confirm the previous results.

4.2.3: Ischemic and hypoxic condition (HIPC)

Studies have found that during BFR and during hypoxia exercise HR increases (Renzi C.P, 2010) (Engelen Arielle, 1996). The present study shows that HR was much higher in HIPC condition than in the control condition ($P < 0.001$). When BFR was combined with a reduction of the F_IO_2, there was a greater increase the cardiovascular work. In the current study, SpO_2 in HIPC was lower in comparison with the control condition and IPC ($P < 0.001$). This result confirms the fact that SpO_2 decreases during hypoxia exercise (Engelen Arielle, 1996). An interesting finding is that SpO_2 of HIPC was higher than HPC, meaning that the addition of the BFR to the hypoxia seems to further decrease the SpO_2. BFR seems to blunt the detrimental effect of the hypoxia on the SpO_2. The mechanism behind this phenomenon is unknown for the moment. The RPE_L was higher in HIPC than in C and HPC ($P < 0.001$) but was not different with IPC. This result demonstrated that BFR was creating the most discomfort for the legs. RPE breathing in the HIPC condition was higher than C, IPC, and HPC ($P < 0.001$), as well as no differences between C, IPC, and HPC conditions were found. The addition of the BFR and hypoxia provoked an overloading of the physiological responses of the body. In this case, the unchanging RPE_B in causing by hypoxia in HPC is in HIPC added to the BFR and thus an increase of RPE_B.

For the muscle oxygenation, there were no differences in ΔHHb between HIPC and the other conditions, but ΔO_2Hb was higher with HIPC than IPC ($P < 0.001$). This result was curious since the greater muscle deoxygenation could come from the BFR. In this situation, the hypoxia condition added to the BFR seems to blunt the effect of the BFR. This result is not clear because no other study compared the combination of BFR with hypoxia to create a HIPC condition. This was confirmed by the other result where ΔtHb was lower in HIPC than

in IPC (P = 0.018) but no difference between the other conditions. For the ΔTSI (%) there was no difference between HIPC and the other conditions.

4.3: Sub-maximal test

The main result of this study was that the preconditioning had no potentially ergogenic effect on the sub-maximal performance. Any of the preconditioning conditions has an impact on factors, which could impact the performance on a sub-maximal test. The preconditioning also had no beneficial impact on the VO_2 kinetics meaning that VO_2 kinetics was not enhanced by any of the conditions during the preconditioning. There were some impacts of the preconditioning on the muscle oxygenation kinetics but not enough to enhance the sub-maximal performance.

4.3.1: Physiological responses

De Groot Patricia et al (2010) found that cardiac performance was not affected by IPC, meaning that the hypothetic beneficial effect on exercise performance occurred independently of the cardiac responses. In the current study, HR was lower in IPC than in HPC (trend of P = 0.062), but IPC was no different than the control condition, which means that IPC had no effect on the cardiovascular system. HPC and HIPC also presented no difference with the control condition. These results confirm that preconditioning has no effect on the cardiovascular work. Regarding the RPE, a study demonstrated no difference in fatigue perception between IPC and placebo (Sharma Vikram , 2015). In the current study, there was no difference with the RPE of the legs, meaning that the preconditioning had no effect on the muscle effort perception. This does not confirm the results of Crisafulli and al (2011), which found that there was a beneficial effect of IPC on the perception of fatigue. An interesting point is that the RPE of the breathing was higher in the HPC condition than in the HIPC condition (P = 0.037). During the HPC the RPE_B was higher, indicating that the supplementary work of the respiration during the preconditioning with hypoxia and ischemia can decrease the perception of the pulmonary work during the sub-maximal performance, which is ultimately beneficial for the performance. This has no direct impact on the sub-maximal performance but the decrease of the RPE of the pulmonary work could enhance the quality of the performance. A previous study showed that IPC maintained a better tissue saturation during exercise when compared to the placebo, suggesting that improved oxygen delivery to the muscles could be a contributing factor to a better performance (Patterson S D, 2014.). In the present study, SpO_2 was not different in any condition, and thus, there was no

improvement of the oxygen delivery. Bailey T.G et al (2012) speculated that reduced ATP consumption or increased efficiency of excitation-contraction coupling during exercise caused by preceding IPC could have led to the reduction in muscle lactate production. In the current study, however, there were no differences in lactate production between any conditions. This may reflect the fact that there was no effect on the ATP consumption by IPC, HPC, or HIPC during the sub-maximal performance.

In this current study, a comparison between the physiological responses during the first trial of the sub-maximal test and the four different preconditioning was made. There were no impacts of the preconditioning on any of the factors (HR, SpO_2, RPE, or lactate) meaning that none of the preconditioning conditions had an impact on the sub-maximal performance.

4.3.2: VO_2 kinetics

The VO_2 kinetics can be very important in a sub-maximal performance. The faster the response of the VO_2 kinetics, the better, partially because of inducing a smaller O_2 deficit for any given increase in VO_2 and intracellular perturbation (Poole David C, 2012). Thus, τVO_2 is a fundamental parameter of aerobic performance (Whipp BJ, 1970) (Barbosa T.C, 2015), and differences in τVO_2 (i.e. the speed of the VO_2 kinetics) may help explain the broad range of physical/athletic capabilities and exercise tolerance across populations (Poole David C, 2012). The VO_2 measured breath-by-breath in the present study represents the metabolic response. A study examining the VO_2 kinetics confirms this hypothesis, "the faster O_2 kinetics measured at the mouth is consistent with the metabolism in the exercise muscle" (Phillips D J, 1997). The VO_2 measured at the mouth is a representation of what is happening in the muscle, the values are not exact but the evolution during the exercise will be similar. Furthermore, another study concluded that the kinetics of peripheral muscle oxygenation reflect systemic VO_2 (Kawaguchi K, 2001).

The main finding of the current study was that IPC, HPC, and HIPC have no potentially ergogenic effect on the VO_2 kinetics. The amplitude and the time constant were not enhanced with any of the preconditioning conditions. This finding agrees with other studies that found no effect of IPC on the VO_2 kinetics (Kido Kohei, 2015) (Barbosa T.C, 2015).

In addition, the present study found that CT1 was slower in HPC condition in comparison with the control condition ($P = 0.047$). This finding means that the HPC condition had a negative impact on CT1 of the VO_2 kinetics. The time constant was slowed, meaning that the VO_2 kinetics was also slowed. No other study had found that HPC was detrimental for a sub-

maximal performance but other studies had found that in acute hypoxia, the VO_2 kinetics was also slowed (Hughson RL, 1995) (Cleuziou C, 2004). Researchers found that moderate hypoxia compared to normoxia induced a similar slowing of the primary component of VO_2 kinetics during moderate and heavy cycling exercises at the same relative intensity (Cleuziou C, 2004). This result demonstrates that even after 40 minutes of recovery, the effect of the acute hypoxia during PC had an impact on the sub-maximal exercise. The underlying mechanisms of this result are not clear but the decrease of the oxygen saturation during the preconditioning can impact the reactivity of the VO_2 kinetics. Cleuziou C et al (2004) suggested that differences in the primary VO_2 time constant among our three groups might be related to differences in aspects of O_2 delivery or oxidative enzyme activity between type I and type II fibers.

The present study concluded no differences in the time delay (TD1) of the VO_2 kinetics. Poole David C et al (2012) stated that the rapidity of the TD1 (cardio-dynamic responses) is attributed to the almost instantaneous cardiac increase which is initiated by vagal withdraw and the mechanical pumping action of the contracting muscle. Thus, the current study showed that none of the preconditioning had an effect on the immediate responses of the cardio-dynamic system. This is similar to the findings of Sharma Vikram and al (2015) that suggested that the effect of IPC on exercise performance might be achieved by the adaptation of local skeletal muscle rather than the systemic cardiovascular system.

There were no differences in the amplitude (A1) of the VO_2 kinetics, meaning that no preconditioning had an impact on the amplitude of the VO_2 kinetics. Logically, if CT1 or TD1 were faster or slower together, A1 would be also different. This result shows that the preconditioning had no impact on the VO_2 kinetics.

<u>Comparison with the first trial:</u>

In the current study, an analysis of the effects of the four different preconditioning on the first trial of the sub-maximal test was performed. Like the previous results, CT1 was slower on HPC in comparison with the control situation ($P = 0.047$). This confirmed the fact that HPC has detrimental impact on the VO_2 kinetics. There were no differences between the other factors (TD1 and A1). This confirms once again that the preconditioning did not enhance the VO_2 kinetics.

4.3.3: VO_2 end trials

In this section, the mean of the last minute of each trials were measured. In the current study, expiratory volume (VE), pulmonary oxygen uptake (VO_2), pulmonary carbon dioxide uptake (VCO_2) were also measured. Kido Kohei et al, (2015) found that changes in pulmonary VO_2 throughout the work-to-work test were not significantly different between the control condition and the IPC. In this study, VO_2, VE and VCO_2 were not affected by any conditions, for all the parameters there were no differences between the conditions. These findings may support the fact that IPC, HPC, and HIPC have no impact on systemic O_2 or CO_2 responses during the sub-maximal test. The respiratory exchange ratio (RER) was also measured, which is defined as the ratio of the volume of carbon dioxide given off by the body tissues to the volume of oxygen absorbed by them, usually equal to the corresponding volumes given off and taken up by the lungs. This RER could indicate a change in the work efficiency, if subjects had a RER of 0.7 (more lipids utilization) or of 1.1 (more carbohydrates utilization). However in the current study, no change of RER was observed. There were no differences between any conditions suggesting that preconditioning did not enhance the work efficiency. To complete this result, the energy cost (EC), gross efficiency (GE), and energy expenditure (EE) were also calculated. None of the parameters had an impact on the sub-maximal performance. There were no differences in any conditions for EC, GE, or EE. This means that IPC, HPC, and HIPC had no influence on the respiratory efficiency or the work efficiency.

Comparison with the first trial:

In this section only the last minute of the first trial was measured. There were no impacts on any factors (VO2, VCO2, RER, EE, EC, and GE). These results indicate that preconditioning had no impact on the respiratory efficiency or the work efficiency. Previous studies have eluded to potential molecules which may help to understand IPC-induced improvements in exercise performance. Those molecules remain relatively unknown, however it is well known that nitric oxide (NO) is produced from vascular endothelial cells following an increase in shear stress which is induced by the raid increase in blood flow, such as that occurs with reperfusion of IPC (Kooijman, 2008). In fact, a recent study determined that IPC increase the levels of the blood NO metabolites in human (Rassaf, 2014). Moreover, it has been shown that enhanced NO levels reduce the local muscular O2 cost in addition the systemic O2 cost during exercise by improving energy efficiency in the mitochondria of the skeletal muscle

(Bailey Stephen J, 2010). In the current study the energy cost was not improved, thus the implication of NO seems to be inefficient. Another study confirmed that IPC did not affect the systemic pulmonary O2 cost during the work-to-work exercise test (Barbosa T.C, 2015).

4.3.4: Muscle oxygenation kinetics

First of all, the main result of this study was that there were no potentially ergogenic effect of the preconditioning on the sub-maximal performance. The purpose in this section was to determine if there might be some improvements in muscle oxygenation kinetics, which have no direct impact on the performance but may be important for the underlying mechanisms of preconditioning.

In this current study, there was a difference between IPC and HIPC with the O_2Hb time constant ($P = 0.039$). The time constant of the O_2Hb after IPC was slower than after the HIPC. The time constant of the O_2Hb was enhanced in HIPC when compared with IPC. The additional effect of the hypoxia situation (HIPC) had a beneficial effect on the O_2Hb. This result is quite interesting, given that HPC was not different than the IPC. Thus, the HPC may have a beneficial effect only when added to the IPC and blunt the deleterious effect of IPC. No other study found the same result, as we believe to be the first to assess HIPC and thus the mechanisms remain unclear. The different preconditioning situations had no further effect on the muscle oxygenation kinetics. Specifically, there were no effects on the HHb, tHb, or the TSI. This result is contradictory to a prior study which found that IPC accelerated muscle deoxygenation in moderate-intensity exercise and enhanced severe-intensity exercise during a work-to-work cycling exercise, which concluded that IPC could enhance submaximal endurance exercise performance (Barbosa T.C, 2015).

<u>Comparison with the first trial:</u>

There were some effects with the time constant of O_2Hb and HHb of the preconditioning. The time constant of O_2Hb was slower in IPC than in HIPC, which is the same result as seen earlier with the average of the three trials ($P = 0.024$). The time constant of HHb was faster in HPC than in HIPC ($P = 0.039$). There was a beneficial effect of HPC on the time constant of HHb, this can confirm the hypothesis that when hypoxia added to ischemia, there can be beneficial on the muscle oxygenation. However, the time constant of HHb in HIPC was slower than the other conditions, meaning that when hypoxia is added to ischemia this slowed the muscle oxygenation kinetics more than in the other conditions. An explanation can be given; part of the NIRS signals parameters indicating muscular O_2 extraction; which reflects the regional balance between O_2 utilization and O_2 availability (DeLorey, 1985). Therefore,

the HPC induced acceleration of muscle oxygenation kinetics may result from an accelerated O_2 extraction in skeletal muscle.

Previous studies have shown that prior muscle contraction can speed up muscle vascular O_2 dynamics during subsequent muscle contractions (Hogan, 2001). In the present study, the preconditioning is a prior exercise, as the subjects have been working and contracting the muscles before the sub-maximal performance. The 40-minute waiting period between the prior exercise and the sub-maximal performance may be too long and therefore annihilate the effects. The resting period between the prior exercise and the performance is in studies of six minutes (Macdonald Maureen, 1997), thus in this current study the preconditioning has no beneficial effect like the prior research indicated.

4.3.5: Muscle oxygenation end trials

After having analyzed the muscle oxygenation kinetics, the purpose of this section is to describe the assessment of the mean concentration of muscle oxygenation during the last minute at the end of the trials. The previous results show that there were no potentially ergogenic effects of the preconditioning on the performance but that there were some impacts on the muscle oxygenation.

The ΔHHb was lower in HPC than in C ($P < 0.001$), IPC ($P = 0.003$), and HIPC ($P < 0.001$). Previous results shows that the HPC had a deleterious effect on the time constant of the VO_2 kinetics and the current result shows that HPC has also a negative effect on the ΔHHb in comparison with the other conditions. The ΔtHb represents a change of blood volume in the muscle (Van Beekvelt M.C, 2001), which tended to be greater with IPC ($P = 0.059$). This means that IPC has a hyperemic effect on the sub-maximal performance and seemed to induce a greater muscle perfusion. Salvador et al. (2015) also concluded that after IPC, the ΔtHb was higher indicating higher O_2 extraction after IPC. This finding is not liked with the VO_2 kinetics because IPC does not enhance the VO_2 kinetics but this result is very interesting. In the current study, this is the only result that indicated a potentially ergogenic effect of IPC on the sub-maximal exercise. The ΔTSI (%) was higher in HPC than in IPC ($P = 0.025$), as well as higher than HIPC ($P = 0.088$). Moreover, IPC and HIPC were not different than the control condition. The ischemic condition induced a lower ΔTSI (%).

4.4: Limitations

This study has several limitations. The first one was the difficulty of the reproducibility of each test for each subject. Subjects were asked to come for each tests at the same hours to

avoid differences in the circadian cycle. However, it was impossible to control their activities during the day and the hours before the test. Subjects were asked to avoid drinking coffee or alcohol the day before the test, which was hard to control. Some of the subjects were students and had stress and lack of sleep during the exam period, which was not controlled.

Secondly, during the familiarization, the critical power was measured with a three-minute all-out test. During this test, the pacing was not permitted but it was impossible to control if the subjects really did not pace. The power during the sub-maximal test was set at 85% of the critical power to reach a heavy intensity. However, if some subjects had lower physical condition or paced during the three-minute all-out test, the level of power output used during the sub-maximal test was not the same. Some of the subjects could be in moderate-heavy intensity and other could be in heavy-severe intensity. This difference could affect our results and change the performance effects of a preconditioning stimulus.

Another limitation according to NIRS analyze data, was some missing data after the analysis. The reason of this missing data is unknown, as it could be a problem with the data collection or with the automation of results in the Excel macro. During the analysis of the muscle oxygenation kinetic and the muscle oxygenation end trials, there were some missing data, which can reduce the quality of the statistical analysis.

In the literature on the preconditioning topic, the first limitation is often the lack of bouts exercises performed. In the current study, we performed four bouts during the preconditioning and three during the sub-maximal exercise. This adds quality to our study.

To conclude, this study followed the same protocol as Groot and al (2009) consisting of four cycles of 5 min occlusion alternated by 5 min of reperfusion. No study has been performed to assess the number of preconditioning cycles and the efficiency (Sharma Vikram , 2015), as well as the optimal time lag between the last cuff inflation and the exercise performance (Incognito A. V, 2015).

5: Conclusion and outlook

The purpose of the study was to investigate the effects of different preconditioning conditions on the VO_2 and muscle oxygenation kinetics during a sub-maximal exercise. The main result of this study was there were no potentially ergogenic effects of any of the preconditioning on the sub-maximal performance. Further, the preconditioning did not enhance the VO_2 kinetics and the muscle oxygenation kinetics. The physiological responses during the preconditioning and the sub-maximal test were also measured, however resulted in no beneficial effects for

performance. This study was innovative, as until now, no study has investigated the HPC and HIPC on a sub-maximal performance. To conclude, there were two main results of the study, which are very interesting and promising for further research. One of the main finding current is that with HPC, the VO_2 kinetics was slowed during the sub-maximal exercise. This was translated with a slowing of the time constant of the VO_2 kinetics. Others studies had also found this result during acute hypoxia witch the first component of the VO_2 kinetics were slowed. This means that the preconditioning had an impact on the sub-maximal exercise. The second main result is that with IPC there was a higher perfusion during the sub-maximal exercise. This was translated by a higher Δ tHb during the sub-maximal test after IPC. Further studies should be performed to investigate the mechanisms provoked by the different preconditioning. In this study, there were some effects of the preconditioning on different parameters of the sub-maximal exercise but none was enough to have a potentially effect on the VO_2 and muscle oxygenation kinetics. More studies have to be conducted to understand the underlying mechanisms of the preconditioning.

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