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Neuromuscular fatigue in repeated cycling sprints with different levels of hypoxia and blood flow restriction

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Abstract:

Introduction: In supramaximal exercises such as repeated sprinting (RSA), neuromuscular fatigue can lead to reduced power output even though the task may be sustained. It is known that fatigue can be related to central (neural) or peripheral (muscle) alterations depending upon the task. However, fatigue may appear prematurely in hostile environments such as hypoxia or under restricted blood flow (BFR) (Amann et al., 2006). The induced ischemia during BFR creates a local hypoxic environment, which affects intramuscular function and motor unit recruitment thus exacerbating fatigue (Scott et al., 2014). To the best of our knowledge, no previous research has investigated the effect of BFR and hypoxia on neuromuscular fatigue during repeated sprints, which was therefore the aim of the current study.

Methods: Eleven athletes (6 men; 5 women) (26.7±4.2 yrs; 68.0±14.0 kg; 172±12 cm) participated in the study including one familiarization session followed by nine experimental trials (0%, 45%, 60%BFR; and 400m, 2000m, 3800m simulated altitude, respectively). Subjects were familiarized with neuromuscular stimulation and maximal voluntary contraction (MVC). Each test session included RSA until exhaustion with the assessment of MVC, central activation (twitch interpolation technique), as well as electrical evoked force at rest (twitch) and doublet at frequencies of 10Hz (P10) and 100Hz (P100) pre- and post-RSA. Power output was obtained during RSA. Two way repeated measures ANOVA were performed to assess differences pre- to post- (condition x time) and between conditions (hypoxia x occlusion) with Bonferroni post-hoc test ($p<0.05$).

Results: Voluntary activation level (VAL) decreased pre- to post- at 60%BFR independent of altitude (by 15.6, 17.2, and 16.2 % at 400m, 2000m, and 3800m, respectively, $P<0.001$). Additionally, a 7.1% decrease ($P<0.05$) was observed in 45%BFR-3800m. The normalization of RMS by the M-wave also decreased ($P<0.001$) at post in 60%BFR independent of altitude (by 36.2%, 43.4%, and 41.5%). The P10, P100, P10/P100, and twitch decreased pre- to post- ($P<0.001$) across all conditions. Specifically, there was a difference with increased hypoxia for P10 ($P<0.05$), P100 ($P<0.01$) and twitch ($P<0.05$). In addition, the difference with increased occlusion was demonstrated for P100 ($P<0.01$) and twitch ($P<0.05$). Power output decreased throughout all conditions with an effect of hypoxia and occlusion ($P<0.001$).

Discussion: Indeed, the RSA-induced central and peripheral fatigue parameters were different across conditions. Previous research has suggested that peripheral fatigue is closely controlled during exercise, meaning that central motor drive and thus performance (power output) may be self-regulated to prevent muscle fatigue from rising above a tolerated level (Gandevia, 2001). Accordingly, in the current study, the peripheral factors (P10, P100, and twitch) were affected in all conditions, while the central factors (VAL and RMS/M) were affected solely by 60%BFR conditions independent of altitude. Thus, central drive seems to be more affected by higher levels of occlusion than hypoxia, even when peripheral fatigue occurs.

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Index of abbreviations

AOP: Arterial occlusion pressure

ATP: Adenosine triphosphate

BFR: Blood flow restriction

Ca^{2+}: Calcium

CNS: Central nervous system

CO: Cardiac output

EMG: Electromyography

EPR: Exercise pressor reflex

FI: Fatigue index

FiO_2: Fraction inspired of oxygen

H^+: Hydrogen ion

HFF: High frequency fatigue

LFF: Low frequency fatigue

MAP: Mean arterial pressure

MVC: Maximal voluntary contraction

N_2: Nitrogen

P10: 10Hz stimulation

P100: 100Hz frequency stimulation

PCr: Phosphocreatine

RF: Rectus femoris

RMS: Root mean square

RSA: Repeated sprint ability

RSH: Repeated sprint in hypoxia

TIT: Twitch interpolation technique

VAL: Voluntary activation level

VL: Vastus lateralis

VM: Vastus medialis

VO_{2max}: maximal oxygen uptake

1. INTRODUCTION

1.1 The neuromuscular fatigue

Muscle fatigue is a multifactorial, complex, and reversible phenomenon that can be defined as an exercise-induced decrease in maximal force production or an inability to sustain further exercise at a required force (Gandevia, 2001). Regarding this definition, one may interpret that fatigue is delayed and appears only after a protracted period of exercise. However, modifications occur as soon as the effort begins, even if the physiological mechanisms underlying fatigue may not always be detected at the onset of exercise (Bigland-Ritchie and Woods, 1984). Apparition of fatigue is common in all types of exercise and thus occurs in both low intensity as well as high intensity exercise. Likewise, different origins of fatigue may occur according to these modalities, which are also dependent on the duration of exercise (Millet and Lepers, 2004).

Many models have been proposed (physiological, biochemical, psychological, biomechanical, and neurological) in order to explain fatigue, whereas some others have characterized fatigue as an interaction of central and peripheral processes (Allen et al., 2008; Gandevia, 2001). Hence, fatigue does not only occur within the muscles. Degradation of performance may be attributed to the failure of both muscle and neural components and therefore to the neuromuscular system. The latter, as represented in Figure 1 below, shows the different sites where alterations can occur that can affect fatigue.

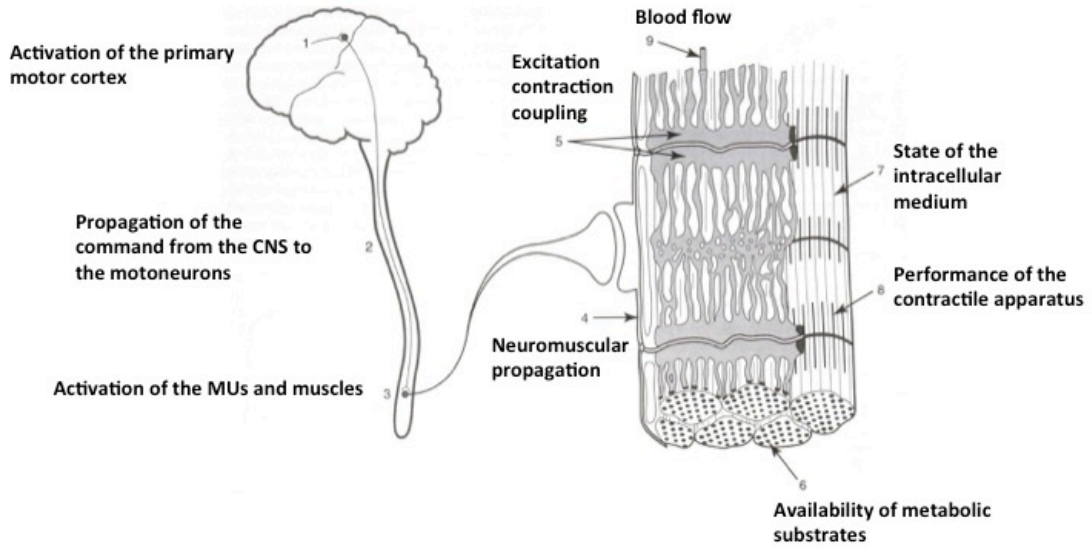


Figure 1. The neuromuscular system (Bigland-Ritchie, 1985)

The neuromuscular system distinguishes central and peripheral mechanisms underlying fatigue with the neuromuscular junction as the communication site (synapse) between the nerve (motor neuron) and the muscle. Peripheral fatigue is defined as fatigue produced by changes at or distal to the neuromuscular junction, whereas central fatigue is a progressive reduction in voluntary activation of muscle during exercise (Gandevia, 2001) due to failure of the central nervous system (CNS) to excite or drive motoneurons adequately (Goodall et al., 2012). Despite the complexity of this system, the determination of the origin of fatigue (central versus peripheral) is possible. Moreover, spinal or supraspinal origination of central fatigue may be distinguished depending on the technique of investigation used. Specifically, the assessment of the peripheral component is usually distinguished by stimulating the motor nerve (neurostimulation) in a relaxed muscle state (Millet et al., 2011), although muscle stimulation (myostimulation) and magnetic stimulation are also widely used techniques. Comparisons pre- and post-exercise in parameters such as peak twitch (mechanical response to a stimulation), M-wave (electrical response to the stimulation) and force evoked at different frequencies (usually 10 Hz and 100 Hz) can reflect perturbations downstream of the neuromuscular junction. On the other side, central fatigue can be examined by assessing two parameters: the voluntary activation level (VAL) and ratio RMS/M (root mean square (RMS) of the amplitude of muscle activation/M-wave (M)), in order to provide information about neural alterations upstream to the neuromuscular junction. The gold standard to assess VAL is the twitch interpolation technique (TIT) from Merton (Merton, 1954) that consists in superimposing a twitch or high frequency stimulation (generally 80-100 Hz) during a maximal voluntary (isometric) contraction (MVC), and comparing the superimposed response to the same potentiated response evoked on the relaxed muscle (Allen et al., 1995). The use of high frequency potentiated stimulations is now usually recommended (Duchateau, 2009; Place et al., 2007), although the level of discomfort and pain is greater with this type of stimulation (Bampouras et al., 2012). Furthermore, the ratio RMS/M corresponds to the root mean square of the maximal response in the amplitude of muscle activation via electromyography (EMG) during a MVC and normalized by the amplitude of the M-wave. Although larger variations of measurement have been found for this ratio (Place et al., 2007), its use allows individual assessment of muscles or muscle groups, in opposite of TIT. However, these two central parameters do not allow insight to determine if the distinction of fatigue is from a spinal or supraspinal origin. Figure 2 displays the different anatomical locations linked with the potential mechanisms of fatigue and their assessment techniques.

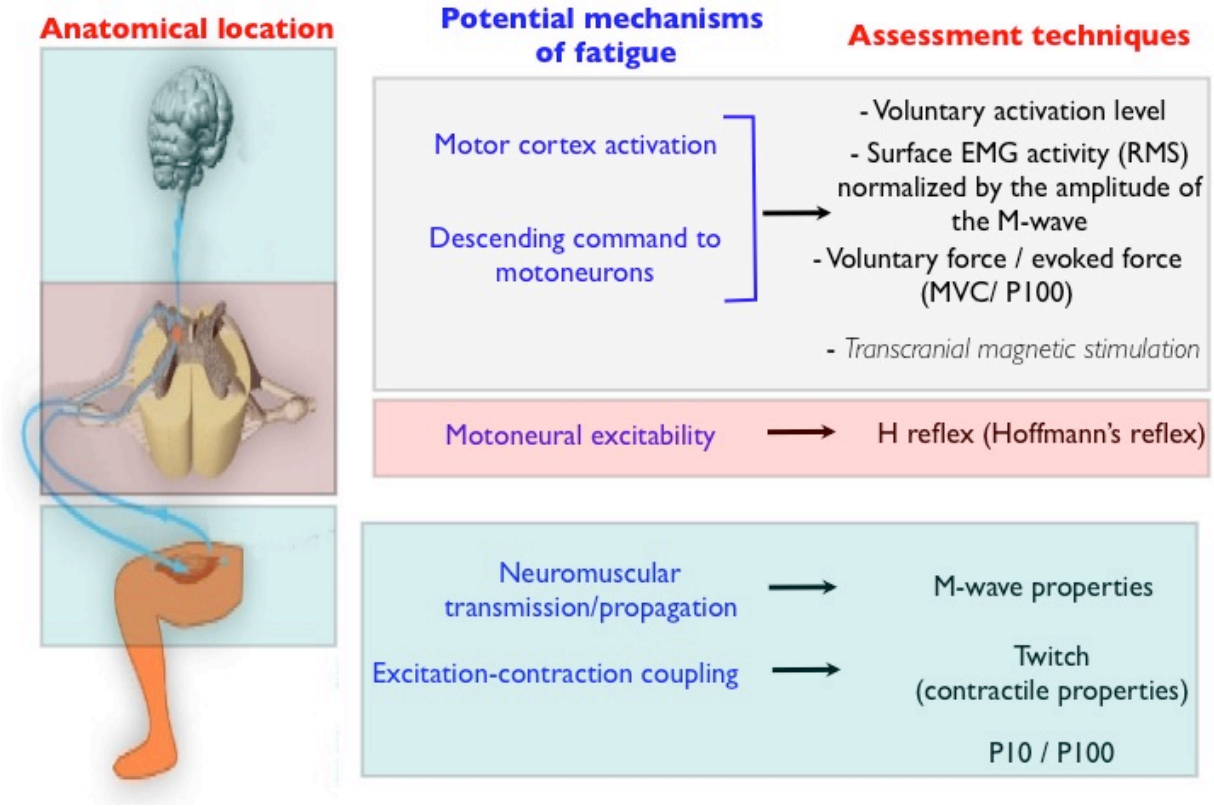


Figure 2. Potential mechanisms of fatigue linked to their anatomical location and their assessment techniques (adapted from Girard & Millet, 2008)

The apparition and degree of fatigue are closely related to the task (Enoka, 1995) and alterations of the neuromuscular system are thus specific to exercise and its modalities.

In team and intermittent sports (i.e, tennis, soccer, hockey, etc.), athletes are required to produce repeated short bouts of exercise (<30sec) at high intensities interspersed with brief recovery periods (<60sec) over an extended period of time (1-4 hours) (Bangsbo et al., 1991; Bishop et al., 2001; Manrique & González-Badillo, 2003; Faude et al., 2007; Girard, 2011; Girard & Millet, 2008; Glaister, 2005; Spencer et al., 2005). Although sprinting activity represents a relatively short duration of a total game (1-3% of effective playing time) (Spencer et al., 2005; Spencer et al., 2004), it is estimated that intense periods of sprinting activity may determine the outcome of a game, and thus influence the ability to win possession of the ball or to concede goals (Trapattoni, 1999). Furthermore, reductions in sprinting speed and high speed running actions, which can potentially affect the game, have been observed during elite soccer matches in men and women (Krustrup et al., 2005; Mohr et al., 2003). The improvement of repeated sprint ability (RSA) may therefore be effective to improve performance during a game.

1.2 Fatigue in repeated sprints

In repeated sprints, fatigue manifests as a decline in the maximal sprinting speed (running) or a reduction in peak power (cycling) or total work over sprint repetitions (Bishop, 2012). Fatigue can be evaluated through the comparison of "Pre and Post exercise" status, during the task with the collection of the EMG muscle activation as well as via fatigue index (FI) or percentage decrement score (S_{dec}). This indicates the ability to resist fatigue during repeated sprints (Girard et al., 2011). Finally, other indices such as mechanical work and sprint time can be useful in combination with the previous indices to assess RSA performance and fatigue.

Muscle excitability, limitation of energy supply as well as metabolites accumulation have been listed as limiting muscular factors in RSA (Girard et al., 2011). With regard to muscle excitability, ionic disturbances have been observed following intense dynamic contractions and linked to decreases in sodium (Na+)/potassium (K+)-adenosine triphosphatase (ATPase) activity (Clausen et al., 1998). Indeed Juel et al. (2000) showed that during one leg knee extensor exercise, concentration of potassium (K^+) outside of muscle cells was exacerbated (at least doubled), potentially due to a failure of the sodium/potassium pump (Na^+/K^+ pump). These modifications, including an accumulation of extracellular K^+, impair cell membrane excitability and diminish the force development. It is, however, necessary to add that most of the studies investigated muscle excitability in *in vitro* conditions and it is therefore still unclear if RSA is affected by ionic disturbances. Additionally, alterations of muscle excitability in RSA, which can be evaluated by changes in amplitude of the M-wave, have led to contradictory results. Some researchers (Racinais et al., 2007) have shown an increase of the M-wave, whereas some others have reported a steady level (Billaut et al., 2013; Girard et al., 2013; Hureau et al., 2015) or a decrease (Perrey et al., 2010) after RSA. Further, performance decrement in RSA has been associated with a metabolic accumulation, which presents as a muscular acidosis and an accumulation of inorganic phosphate (Pi) in the muscle tissue. The decrease in blood and muscle pH (Ratel et al., 2006; Spencer et al., 2008) via the accumulation of hydrogen ions (H^+), resulting from RSA, could have detrimental effects on the contractile apparatus, ATP production (derived from glycolysis) and thus on performance. In addition, the accumulation of Pi could potentially affect the calcium release from the sarcoplasmic reticulum and/or affect the myofibrillar calcium sensitivity, inducing a lower number and/or force of the cross-bridges, as suggested *in vitro* studies (Dutka and Lamb, 2004; Westerblad et al., 2002). Finally, the depletion as well as the limitation of energy supply resynthesis (phosphocreatine (PCr)) have been pointed out to

limit RSA. Although different metabolisms (ATP-PCr, anaerobic glycolysis, aerobic metabolism) supply energy, the resynthesis of PCr has been established as a potentially major determinant of RSA (Mendez-Villanueva et al., 2012).

Besides these muscular limiting factors, neural alterations (reduction in neural drive and muscle activation) have been shown in several studies (Kinugasa et al., 2004; Racinais et al., 2007) while others reported a steady level of neural activation (Billaut et al., 2005; Hautier et al., 2000) during RSA. To illustrate this point, Racinais et al. (2007) reported in addition to the peak power output decrement, a decrease of 16.5% in maximal voluntary contraction (MVC) following 10 cycling sprints of 6 seconds interspersed with 30 seconds of recovery. Furthermore, and despite an increment of the M-wave amplitude (+13.7%), researchers noted a decrease in the peak twitch force production (-9%), a decrease of 3% of the voluntary activation level (VAL) assessed with TIT, and a decrement of 14.5% of the RMS/M-wave ratio. These results show alterations at the peripheral as well as at the central level following 10 x 6s cycling sprints. In another study, Mendez-Villanueva et al. (2008) reported a decrease of 14.6% (±6.3%) in the amplitude of the surface EMG activity of the *vastus lateralis* in a set of 10 cycling sprints (6 seconds sprint/30 seconds recovery). Authors related this reduction in EMG activity to the progressive inhibition of motor units and/or decrease in motor units firing rate and also to peripheral alterations, though specifying the impossibility to distinguish the precise mechanism underlying the results due to the use of surface EMG. In addition, Hureau et al. (2015) recently investigated the development of peripheral and central fatigue during cycling sprints. Twelve males performed on different days the following tests: 1, 4, 6, 8, 10 x 10s sprints with 30s of passive recovery as well as 8 x 10s sprints with 10s passive recovery. Researchers demonstrated a gradual reduction in power output (-25 ± 7%) as well as in peripheral and central indices (Twitch = -47±11%; VAL = -11±6%) from the first to the sixth sprint. While also reporting no further reduction in the different indices when subsequent sprints were performed. Moreover, the 10s recovery protocol led to a significant reduction in power output without additional effects of peripheral and central fatigue. Finally, the decrement in mechanical output during repeated sprinting exercise has been explained by a combination of both types of fatigue. Authors have suggested, as in other studies (Amann et al., 2009; Amann and Dempsey, 2008; Gagnon et al., 2012), that central motor drive was altered to limit the development of peripheral fatigue.

As previously mentioned, the apparition and the degree of fatigue can be affected by several factors. Firstly, the exercise mode (running versus cycling) has been reported to induce different levels of fatigue. Thus, decrement scores are generally greater in repeated cycling sprints (10-25%) when compared to repeated running sprints (5-15%) (Rampinini et al., 2014) implying a task dependency of fatigue (Enoka, 1995). The time of the day has also been investigated (Giacomoni et al., 2006; Racinais et al., 2010, 2005), such that afternoon performance has been reported to induce sharper decreases in performance. This was suggested to be related to an improved muscle power in the afternoon compared to the morning with an enhancement of the first sprint and thus a greater fatigue index. Anthropometric factors (sex, age and training status) also have an impact on fatigue, as well as distribution of work/recovery periods and the environment in which exercise is performed. Regarding the latter, fatigue may appear prematurely in hostile environments such as in hypoxia or under restricted blood flow (BFR) (Amann et al., 2006).

1.3 Fatigue in hypoxia

Training in altitude has increased in popularity over the years and the development of new training methods has emerged (Figure 3). Even if the use of altitude training was predominantly targeting endurance sports (live high-train high/low) in the beginning, the development of new innovative training methods such as repeated sprint in hypoxia (RSH) makes hypoxic training beneficial for several sports, including team and intermittent sports. It is important to noticed however, that the use of artificial altitude (normobaric hypoxia), which can be obtained by reducing the fraction of oxygen via addition of nitrogen (N_2) or extraction of oxygen molecules without modifying the barometric pressure, has been reported to induce different physiological responses (Millet et al., 2012) compared to hypobaric hypoxia (real altitude). Hypobaric hypoxia consists of a same fraction of oxygen in the air but with a reduction of the barometric pressure.

Specifically, the benefits of RSH compared to the same training in normoxia have been previously demonstrated by several studies (Brocherie et al., 2015; Faiss et al., 2013; Galvin et al., 2013; Puype et al., 2013; Faiss et al., 2015), while another did not (Goods et al., 2015). Faiss et al. (2013) reported that performance in RSA cycling was improved after RSH training (increased number of sprints before failure) although no difference in peak power between the groups was observed. According to these authors, RSH led to specific molecular adaptations as well as increased variations of blood perfusion, therefore inducing a better clearance of waste metabolites and thus delaying fatigue during a RSA test to ultimately

allow more sprints to be performed.

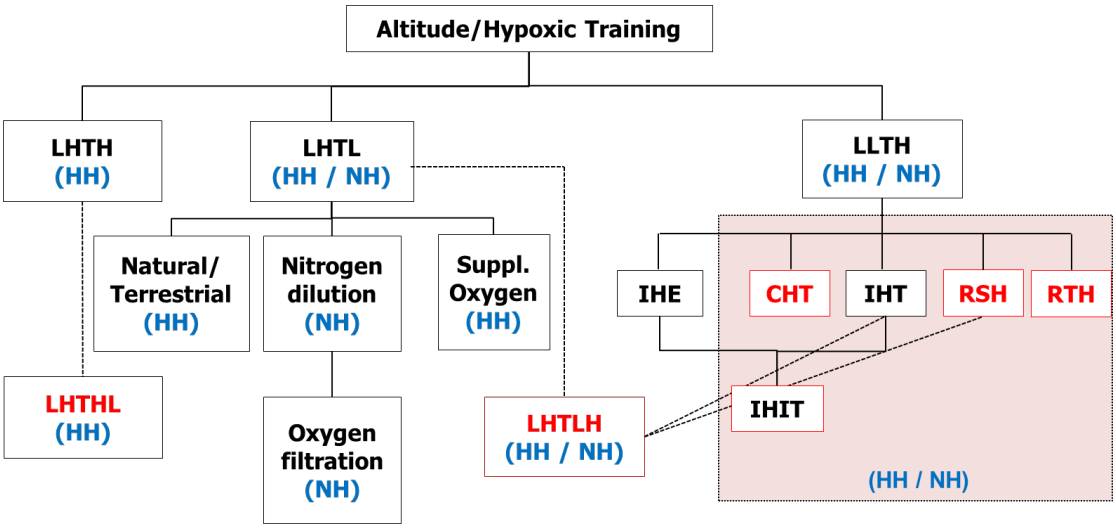


Figure 3. Panorama of the actual altitude/hypoxic training methods (LHTH : Live High Train High; LHTL : Live High Train Low ; LLTH : Live Low Train High. IHE : Intermittent Hypoxic Exposure ; CHT : Continuous Hypoxic Training; IHT :Intermittent Hypoxic Training; RSH : Repeated Sprint Training in Hypoxia ; RTH: Resistance Training in Hypoxia. IHIT : Intermittent Hypoxia Interval-Training; LHTLH : Live High Train Low and High; LHTHL: Live High Train High and Low. HH : Hypobaric Hypoxia ; NH : Normobaric Hypoxia). From Millet et al., 2015.

Although interest around RSH has grown, few studies have focused on neuromuscular fatigue in RSH. A general fatigue can be observed indirectly through greater decrement scores in hypoxia than normoxia or with a similar comparison in total work. Morrison et al. (2015) compared the performance of ten amateur team-sport athletes during a repeated sprint protocol (4 sets of 4, 4-s running sprints) in normobaric normoxia (FiO_2=20.9%) versus normobaric hypoxia (FiO_2=14.0%). Researchers reported a lower peak speed and distance covered (total work) when performing in hypoxia and suggested that these results could be related to a reduced arterial oxygenation saturation which could contribute to fatigue during RSH, given that PCr (Haseler et al., 1999) and H^+ removal (Tomlin and Wenger, 2001) are oxygen dependent processes. In addition, Billaut et al. (2013) found a reduction in mechanical work (-8.3%) in fifteen 5-second cycling sprints interspersed with 25 seconds recovery in hypoxia (FiO_2=13.8%) when compared with normoxia. Furthermore, researchers observed a 13.7% decrease in the quadriceps electromyography (RMS_{sum}) across sprints in the hypoxic condition. The main finding was that cerebral oxygenation, quadriceps activation (RMS/M; VAL), and cycling performance were lower in hypoxia than normoxia. Thus, suggesting a regulation of the locomotor muscle fatigue development from the central nervous system (CNS).

1.4 Fatigue under blood flow restriction

A hypoxic stimulus can also be created with the use of a local instead of a systemic approach. Mainly used in resistance training to improve muscle strength and hypertrophy, blood flow restriction (BFR) (also known as "Kaatsu training") consists of limiting the arterial blood flow in the muscles by applying a wrapping device or inflatable cuff. The induced pressure restricts the arterial blood flow while simultaneously preventing the venous return. The induced ischemia during BFR creates a localized hypoxic environment, which affects intramuscular function and classical motor unit recruitment patterns (Scott et al., 2014). In order to maintain the same level of strength, subjects recruit more motor units with low loads, which can be noticed through greater muscle activation (Yasuda et al., 2009). Moreover, it has been reported (Takarada et al., 2000b; Yasuda et al., 2005) that with a low intensity training (20% of 1RM) and use of moderate vascular restriction (100 mmHg), the effects on hypertrophy and strength were similar in both athletes and patients.

The exact underlying mechanisms remain elusive but gains in strength and hypertrophy following BFR have been associated to a greater accumulation of metabolites as well as an increase in intramuscular signaling, anabolic hormone concentrations, intracellular swelling, and motor unit recruitment (Scott et al., 2014).

One of the main parameters when using BFR is the induced pressure. However, the latter may vary a lot according to many parameters and thus, restriction pressure should be made relative to the individual and to the specific cuffs used (Jessee et al., 2016). It has been reported that the size and width of cuffs (Loenneke et al., 2012a) have highly influenced the level of pressure to occlude the arterial blood flow. Thus, wider cuffs lead to lower arterial occlusion pressure (AOP) than narrow ones. Likewise, these authors also reported effects of sex and race on the level of AOP, which highlight the need to individualize these measurements.

The number of studies focusing on fatigue in repeated sprints under BFR is void. However, Cook et al. (2007) compared the effect of nine protocols on muscle fatigue (decrement in MVC) on 21 subjects which consisted of three sets of knee extensions performed until failure with 90s recovery between sets. In that study, eight protocols included BFR with various intensities (20 or 40% MVC), pressure (~160 mmHg or 300 mmHg), and occlusion duration (off during the rest between sets or continuously applied). The ninth condition was high-load exercise (80% MVC) without BFR. The results of the previous study indicated that exercise performed under BFR induced equal or greater fatigue than exercise with high load without BFR. Indeed, isometric force decreased in post measurements by 19% (high load condition)

and between 24-33% (depending BFR condition). Other authors (Häkkinen and Pakarinen, 1993) have demonstrated similar or higher values (Pierce et al., 2006) of decrement scores that can be explained by the larger volume of the protocol (5 versus 3 sets). In addition, Yasuda et al. (2009) found that complete occlusion led to higher declines in MVC than moderate BFR (39-48% vs 16-19%, respectively), whereas MVC in the control group (without BFR) did not change. Researchers also reported an increase of muscle activation (via EMG) in moderate and complete occlusion compared with the control. More recently, Fatela et al. (2016) investigated the acute effects of different levels of BFR (40%, 60%, 80% of absolute vascular occlusion) on muscle activation and fatigue in low intensity unilateral knee extensions (20% of 1-repetition maximum) composed of 4 sets (30+15+15+15 repetitions). Researchers showed a 5.2% decrement in MVC at 80%BFR, as well as a decrease in median frequency of the VM and RF in all conditions (except at 40%BFR for the VM), which was majored at higher levels of BFR. In addition, an increment in RMS within all sets in both muscles with higher values at 80%BFR was reported. According to these findings, researchers suggested that pressure should be individualized, as the neuromuscular fatigue level varies with the relative BFR intensity.

As observed previously, there is a lack of studies focusing on BFR combined with high intensity exercise. BFR has been used mainly in resistance training in order to develop strength and hypertrophy as well as in low intensity exercise (cycling or walking) (Abe et al., 2010, 2009, 2006). To the best of our knowledge, no study has combined and investigated BFR and/or hypoxia during repeated sprinting, which was therefore the aim of the current study.

The present study aims to compare the neuromuscular fatigue in various levels of hypoxia and vascular occlusion during a repeated sprint protocol performed to exhaustion.

We hypothesized that:

- Peripheral and central fatigue will be induced and observed in all conditions, since exercise is performed until exhaustion.
- The level of peripheral fatigue is independent of the level of occlusion and also the level of hypoxia, contrary to central fatigue.
- BFR leads to stronger detrimental effect on central fatigue than hypoxia.

2. MATERIALS AND METHODS

2.1 Participants

Six men and five women took part in the study (26.7 ± 4.2 years old; 68.0 ± 14.0 kg; 172 ± 12cm). Volunteers needed to be healthy, actively trained and aged between 18 and 40 years old. Participants were required to train at least 4 hours per week in endurance activity in the legs (i.e. cycling, running, rowing, skiing, skating, etc.) and be accustomed to maximal intensity exercise in the legs as well as no skeletal or muscular injury in the last 3 months, pain, or other medical condition that could affect the outcome of the study.

The eleven participants completed 10 sessions including one familiarization. Before the start of the study, participants were informed about the procedure and risks, signed a consent form (Appendix 1), and answered a health questionnaire (Appendix 2).

The study protocol was accepted by the local ethical committee (*Commission cantonale vaudoise d'éthique de la recherche sur l'être humain, CER-VD 138/15*) on April 21th 2015.

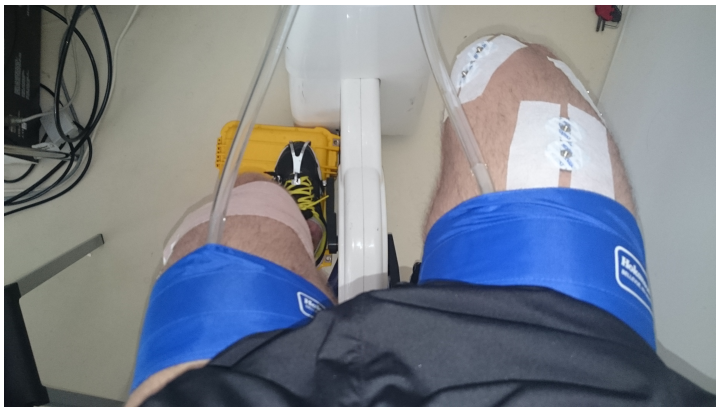
2.2 Experimental design

Each testing session (n=9) took place at the "*Centre Sport et Santé"* in the normobaric hypoxic chamber (*ATS Altitude Training, Sydney, Australia*) of the Institute of Sport Science, University of Lausanne (ISSUL), Switzerland. The chamber (2.4m x 5m x 2.5m) allows, via a filter and compressor system, to extract oxygen molecules and to reduce the FiO_2 without modification of the barometric pressure.

Participants completed the trials with minimum 48h rest between each session in order to limit an accumulation of fatigue and each trial was executed at the same time of day (± 1 hour).

During the first session, anthropometrics data (height, weight, skin folds) were collected and participants filled out and signed the consent and health forms.

The AOP (pressure to obstruct the arterial inflow) was determined with 11x85cm cuffs



Picture 1. Bilateral cuffs applied on proximal thigh during RSA.

(*SC10D Rapid Version Cuff, D.E. Hokanson Inc., Bellevue, WA, USA*) that were applied proximal to the thigh and which were used for all testing sessions. The amount of pressure of the cuffs was progressively increased with an inflation system (*E20/AG101 Rapid Cuff Inflation System, D.E. Hokanson Inc., Bellevue, WA, USA*). Vascular occlusion was assessed on the femoral artery using a Doppler ultrasound probe (*L 12-5L60N, ClarUs EXT, Telemed Medical Systems, Milano, Italy*) and AOP was determined when no more arterial blood flow was detected via Doppler ultrasound (*EchoWave II 3.4.4, Telemed Medical Systems, Telemed Ltd. Lithuania, Milano, Italy)*.

Participants were then familiarized with the assessment of neuromuscular fatigue including maximal voluntary contraction (MVC) as well as the twitch and stimulations at different frequencies (100 Hz, 10 Hz, twitch) at rest and during MVC (superimposed 100 Hz doublet). Finally, participants were familiarized to the repeated sprint protocol to exhaustion.

The following nine testing trials were performed in a random order and included exercise in 3 levels of normobaric hypoxia (400m, 2000m, 3800m; FiO_2: 20.4%, 16.4%, 12.8% respectively) and 3 levels of BFR based on the AOP (0%BFR, 45%BFR, 60%BFR).

Figure 4 represents the entire study design.

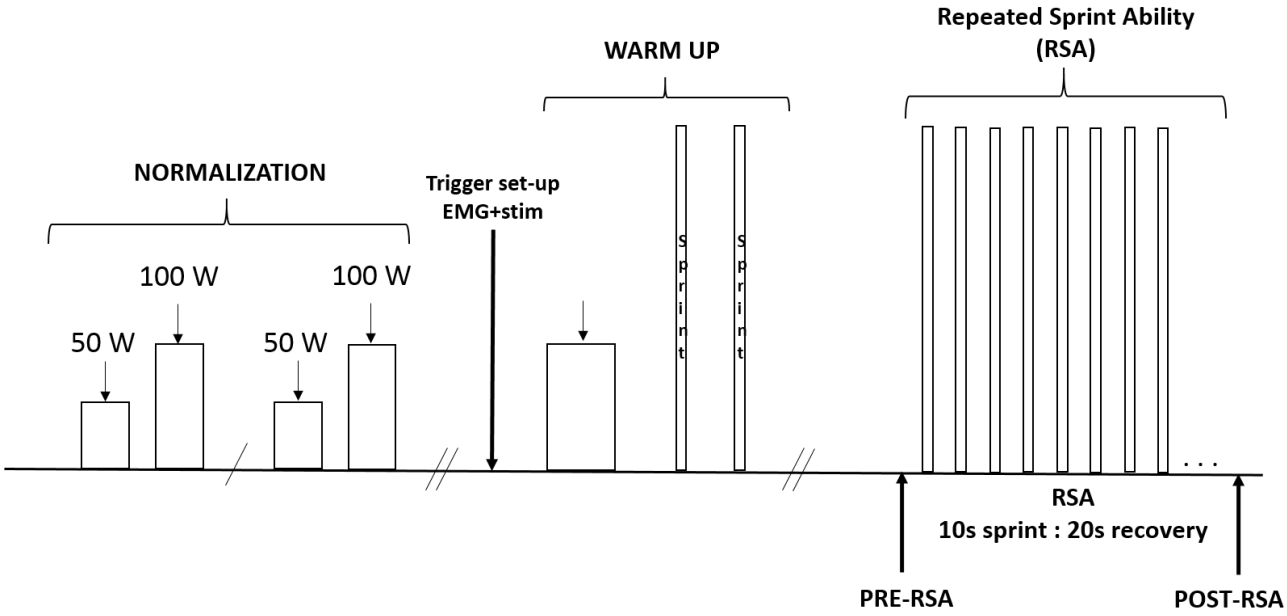


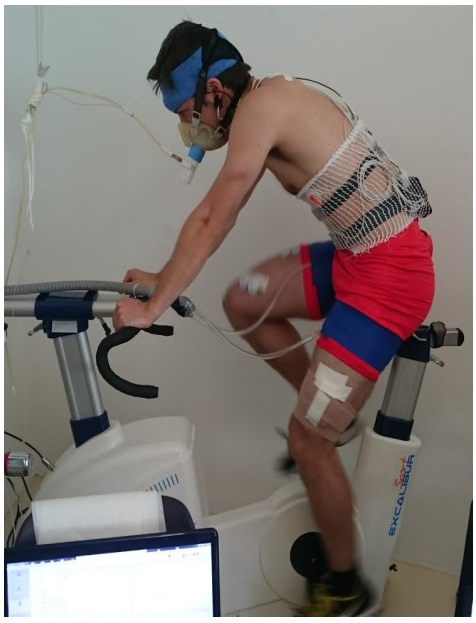
Figure 4. Study design. Two normalization cycling stages (3 minutes at 50W and 3 minutes at 100W) were done twice (outside and inside the hypoxic chamber) and were followed by the determination of the optimal intensity of stimulation. A 6-minutes submaximal stage was performed prior to two 10s sprints interspersed with 3 minutes recovery. Assessment of fatigue was performed PRE and POST repeated sprints (RSA), as indicated by the arrows.

As shown above, the current work takes place in a larger study but only repeated sprints and neuromuscular fatigue will be reported here. Each session included a normalization phase

consisting of 3 minutes of cycling at 50 W followed by 3 minutes at 100 W outside of the hypoxic chamber. The same normalization was then done inside the chamber and was followed by the neuromuscular set-up (determination of the optimal intensity of stimulation). After a 6-minutes submaximal stage, a warm up including 2 maximal 10s sprints was performed and a recovery period of 5 minutes followed. Assessment of fatigue was then performed Pre- and Post-RSA to exhaustion. In addition, blood lactate samples were also collected Pre-and Post-RSA at the end of the fatigue assessment with a Lactate Scout (*SensLab GmbH, Leipzig, Germany*).

This study was single-blinded and therefore, participants were not aware of any condition or any feedback of performance in order to avoid confounding factors and any pacing strategy. Furthermore, participants were asked not to practice any strenuous activity, not to consume any alcohol in the 24 hours prior the testing session and not to consume caffeine 3 hours prior the session.

2.3 Repeated Sprint Test



Picture 2. Equipment on subject during RSA

Participants performed a repeated sprints test to volitional exhaustion in the nine conditions listed above. The bike was set up and adjusted for each subject and for each testing session for reproducibility. Two warm-up sprints with 3 minutes of recovery in between were executed in the condition of the day prior the test. Peak power of the best warm-up sprint was then used as peak power reference for the RSA test. Subjects needed to perform the first sprint at least at 95% of the peak power of the warm-up sprint. The test consisted of 10 seconds maximal all out cycling sprint (with a torque factor of 0.8Nm/kg) and 20 seconds recovery with 20W resistance on a magnetic ergometer (*Lode Excalibur Sport, Lode, Groningen, The Netherlands*). One minute of easy cycling (20W, 85 RPM) was executed before starting the first sprint. When it was a BFR condition, cuffs were put bilaterally proximal on the tights and a slight amount of pressure was applied in order to hold the cuffs and limit their movements during that first minute. The amount of pressure was then adjusted to the required level three seconds

before the first sprint and was maintained until the end of the post-neuromuscular assessment. Participants were asked to maintain a cadence of 85 RPM prior each sprint and were strongly verbally encouraged during the whole test to perform as many sprint as possible. The test was stopped when subjects reached exhaustion or could not maintain a cadence higher than 70 RPM or maintain 50% of the mean power. During the entire test, subjects donned several other measuring devices (muscular and cerebral oxygenation, *PortaMon and PortaLite, Artinis, Zetten, The Netherlands*; hemodynamic parameters, *Physio Flow®, Manatec type PF05L1, Paris, France*; heart rate monitor, *Polar RS400, Kempele, Finland*; oxygen saturation, *8000Q2 1m, Nonin Medical Inc., Amsterdam, The Netherlands*) and were equipped with a mask for the measurements of the gas exchange (*Medgraphics CPX, Loma Linda, USA*). Finally, all bike data (number of sprints, total work (J), power average (W), peak power (W) and maximal average power (W)) were directly recorded by the ergocycle.

2.4 Neuromuscular fatigue assessment and analysis

2.4.1 Materials

Participants were prepared and equipped prior each testing session. In order to collect the EMG activity, nine electrodes (Ag/AgCl) of 10mm surface (*Kendall, Covidien, REF 31118733, Mansfield, MA, USA*) were spread on the vastus lateralis (VL), vastus medialis (VM) and rectus femoris (RF) of the right thigh that had previously been shaved and wiped with sandpapers and alcohol. The placement was marked after the first session and participants were asked to keep the marks with a permanent pen during the whole study.

Picture 3. Neuromuscular assessment

5 x 10 cm stimulation electrodes (*Compex, Ecublens, Switzerland*) were placed on the right femoral nerve (inguinal triangle) and at the equivalent level on the mid-gluteus. Subjects were seated on a custom-made chair ergometer that was equipped with a force gauge at the ankle level. The chair was adjusted for each participant and for each trial in order to obtain a 90° leg bending.

The stimulation electrodes were connected to a Digitimer (*model DS7AH, Hertfordshire, UK*) and the EMG electrodes were connected to an acquisition system Biopac MP150 (*MP150, BIOPAC, Goleta, CA*).

Data were recorded with a frequency of acquisition of 2000 Hz for the EMG signal and 1000 Hz for the force signal. The data were collected and analyzed with the software Acqknowledge (*AcqKnowledge, BIOPAC, Goleta, CA*).

Amplitude (peak to peak) was collected for MVC, evoked forces (superimposed doublet at 100 Hz, 100 Hz stimulation at rest (P100), 10 Hz stimulation at rest (P10), twitch), and M-wave.

The ratio of evoked force at low and high frequency was calculated as "amplitude of P10/amplitude of P100".

Root mean square of the raw signal was assessed with a 250ms interval on either side of the peak force during MVC and was normalized by the amplitude of the M-wave of the VL to obtain the ratio RMS/M.

The VAL was calculated with the following formula allowing a correction (Place et al., 2007) if the superimposed doublet was not exactly at the peak force moment of MVC:

VAL = {1 – (superimposed doublet amplitude x voluntary torque just before the superimposed doublet/maximal voluntary torque)/potentiated doublet amplitude} x 100

2.4.2 Sequence of stimulation

The optimal intensity of stimulation (i.e., which recruited all knee-extensor motor units) was determined at the beginning of each testing session (as explained in the previous "experimental design" section). The intensity was gradually increased by 20 mA until the amplitude of the twitch and M-wave reached a plateau. In some subjects, electrode positions were adjusted to obtain a better M-wave shape and amplitude. To ensure all quadriceps motor units recruitment, the intensity was increased by 20% of the pre-determined value.

The assessment of fatigue was achieved PRE and POST repeated sprints (approximately 3 minutes after the end of RSA) and is illustrated in Figure 5.

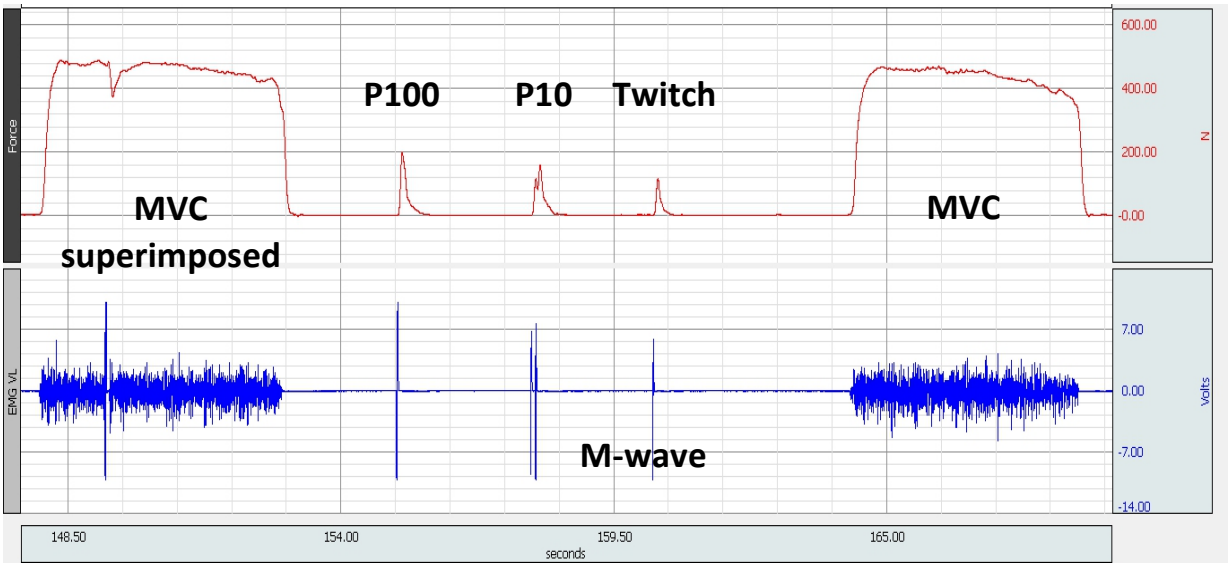


Figure 5. Neuromuscular assessment Pre- and Post-RSA with MVC with superimposed 100Hz doublet, P100, P10, Twitch, MVC. Force is in red (top channel) and EMG of the vastus lateralis in blue (bottom channel)

Subjects began with a MVC with a superimposed doublet at 100 Hz, followed by 100 Hz stimulation at rest, 10 Hz stimulation at rest, and a twitch at rest. All stimulations were separated by ± 2 seconds. A last MVC was performed 3 seconds after the stimulation sequence.

All this sequence was performed in the condition of the day except the last MVC that was always executed without BFR (the cuffs pressure were released just after the twitch).

2.5 Statistical analysis

Data are presented as mean ± standard error (SE). Two-way repeated measures ANOVA were performed to assess differences pre to post (condition x time) and between conditions (hypoxia x occlusion) with Bonferroni post-hoc test. Correlations were made with the Pearson product-moment correlation coefficient. Null hypothesis was rejected at $p<0.05$.

Data were first collected in an excel file (*Microsoft Excel, Microsoft Corporation, Redmond, WA, USA*). All subsequent analyzes were made using the software SigmaStat 3.5 (*Systat Software, San Jose, California, USA*).

3.RESULTS

Subject (n) Pressure (mmHg)	1	2	3	4	5	6	7	8	9	10	11
100% AOP	171	163	210	192	137	208	207	242	239	165	167
60% AOP	103	98	126	115	82	125	124	145	143	99	100
45% AOP	77	73	95	86	62	94	93	109	108	74	75

Table 1. Arterial occlusion pressure (AOP) in mmHg determined for each subject via Doppler ultrasound and the corresponding percentage (60% and 45%) used in the study.

First of all, Table 1 illustrates the AOP (in mmHg) determined via Doppler ultrasound for each subject during the familiarization. The equivalent pressure (in mmHg) that was used in the study corresponds to the percentage (60% or 45%) based on AOP.

As shown above, AOP differs between subjects ranging from 137 mmHg to 242 mmHg (representing a 43.4% variation). Mean AOP is 191 mmHg (±10.1).

3.1 Performance and global fatigue

Performance in RSA is firstly presented in Figure 6 with the comparison of total work performed between the nine conditions. This figure shows the work performed until the exhaustion or until task failure and attests the severity of the task.

As observed, there was almost a linear decrease in total work across condition, with five times less total work in condition 3800-60% compared to condition 400-0%. Significant decreases were observed in all conditions except at 2000-45%. Total work was only affected by hypoxia in conditions without BFR with a decrease of 34.5% (2000-0%) and 37.4% (3800-0%) compared to condition 400-0%.

Compared to condition 400-0%, there was 52.3% (400-45%) and 68.5% (400-60%) less total work. At 2000m, a decrease of 29% (2000-45%) and 60.2% (2000-60%) was reported compared to 2000-0%. Finally and compared to 3800-0%, there was 51.2% (3800-45%) and 68.2% (3800-60%) less total work.

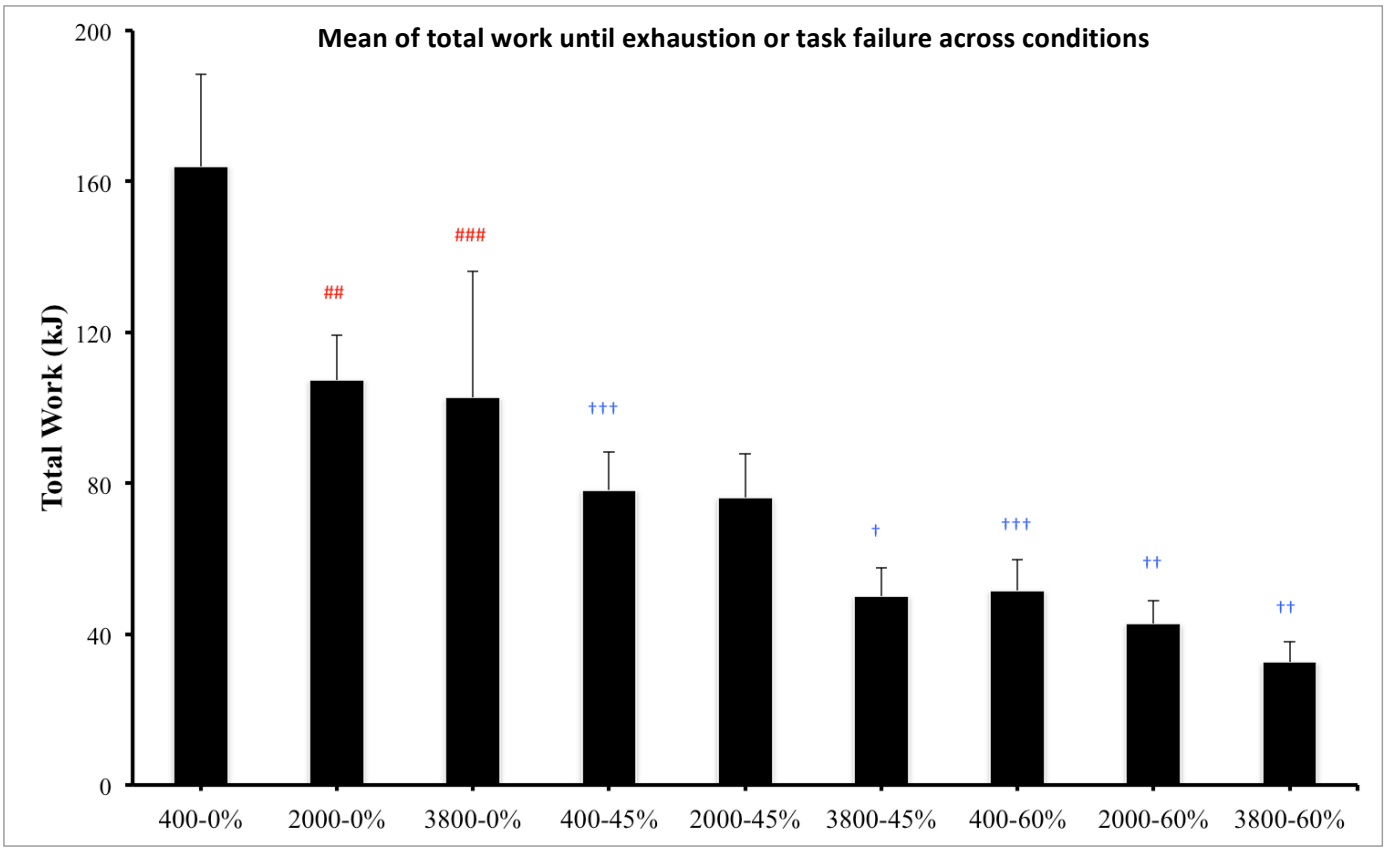


Figure 6. Total work performed (kJ) across conditions. 400, 2000, 3800 indicate the level of hypoxia (400m, 2000m and 3800m, respectively); 0%, 45%, 60% indicate the level of BFR (percentage of AOP). ## < 0.01 ### < 0.001 for differences with 400m; † < 0.05 †† < 0.01 ††† < 0.001 for differences with 0%

Secondly, Figure 7 represents the MVC performance pre- and post-RSA. There was a significant decrease ($p<0.001$) pre to post in all conditions at 60%BFR, independently of the level of hypoxia. Moreover, differences between 60%BFR and 0%BFR were observed ($p<0.001$) for the three levels of hypoxia as well as between 60%BFR and 45%BFR ($p<0.05$) at 400m and at 3800m ($p<0.001$). Significant changes were observed in the first two conditions at 45%BFR (400m and 2000m), whereas no significant changes occurred in the other remaining conditions (0%BFR and 3800-45%). The only effect of hypoxia appeared in condition 3800-0% and showed a difference ($p<0.05$) with 2000m.

The decrease pre to post in MVC was mainly affected by BFR. At 0% BFR, the change pre to post was 7.5%, 12.6% and 6.1% for 400m, 2000m, 3800m, respectively.

At 45% BFR, a decrease of 23.9%, 28.4%, and 12.7% was observed at 400m, 2000m, 3800m, respectively.

Finally, at 60% BFR, MVC decreased by 45.1%, 49.6% and 52.6% at 400m, 2000m, 3800m, respectively.

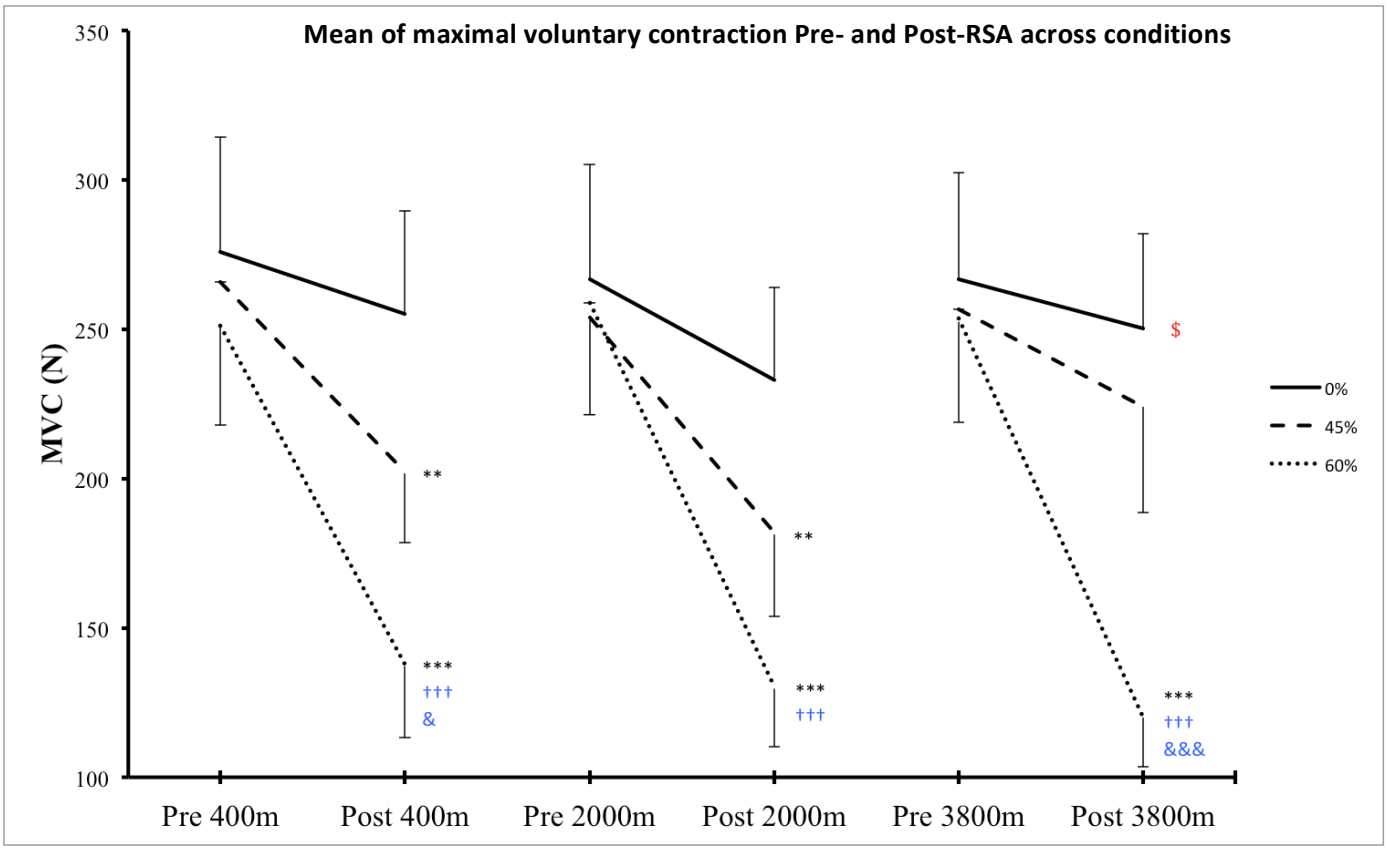


Figure 7. Performance in maximal voluntary contraction (MVC) pre and post repeated sprints (RSA) across conditions. 400m, 2000m, 3800m indicate the three levels of hypoxia; Pre and Post correspond to the assessment Pre- and Post-RSA; full lines represent conditions at 0%BFR; dashed lines represent conditions at 45%BFR, dotted lines represent conditions at 60%BFR. ** < 0.01 ***<0.001 for differences with pre; $ < 0.05 for differences with 2000m; ††† < 0.001 for differences with 0%; & < 0.05 for differences with 45%; &&& < 0.001 for differences with 45%

Thirdly, Figure 8 shows the percentage change pre to post in MVC (% loss of force) correlated to the total work. It appears a significant ($p<0.05$) correlation between these two variables ($r = -0.78$). Thus, the largest decrement in MVC was associated with the smallest amount of total work (conditions at 400/2000/3800-60%). The conditions without BFR were the ones where the highest level of total work is produced. However, those conditions also had the smallest decrease in MVC pre to post.

Contrarily, conditions at 60%BFR had the largest decrement in MVC (between 45.1% and 52.6%) but with five times less total work compared to conditions without BFR. Interestingly, the percentage of decrement of MVC increased massively with the amount of BFR with no effect of altitude.

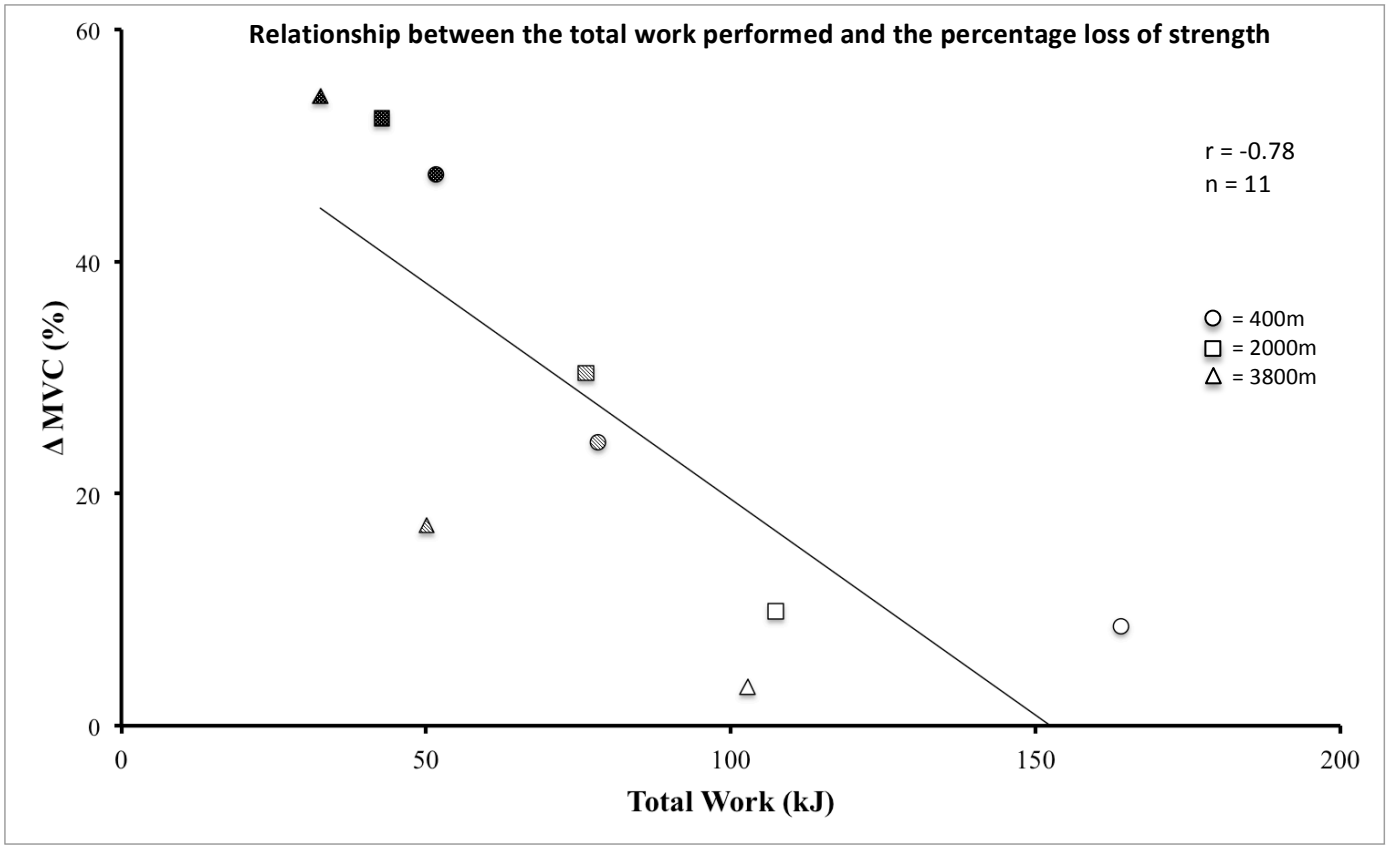


Figure 8. Relationship between the Total Work (kJ) performed and the relative pre-to-post change in maximal voluntary contraction (MVC) (%). Circles indicate conditions at 400m; squares indicate conditions at 2000m; triangles indicate conditions at 3800m; white shapes represent condition at 0%BFR; grey shapes represent condition at 45%BFR; black shapes represent conditions at 60%BFR; r: correlation coefficient; $p<0.05$; n: number of subjects

3.2 Central fatigue

As explained previously, central fatigue was assessed pre- and post-RSA using the twitch interpolation technique. The voluntary activation level (VAL) is presented in Figure 9 below with mean values (±SE).

There was a large effect ($p<0.001$) of BFR at 60%, independently of the level of hypoxia. In these conditions and post RSA, VAL decreased by 13.4%, 14.4% and 13.8% at 400m, 2000m, and 3800m, respectively. Furthermore, there was a significant difference between 60%BFR and 0%BFR ($p<0.001$) for the 3 levels of hypoxia as well as a difference ($p<0.01$) between 60%BFR and 45%BFR at 2000m. The highest level of hypoxia (3800m) combined with the mid-level of BFR (45%) also altered VAL with a decrease pre to post of 5.8% ($p<0.05$) and a significant difference compared to 0%BFR ($p<0.01$).

Finally, VAL decreased by 4.7% (400-45%) and 4.1% (2000-45%) although changes were not significant. Finally, a slight increase (non-significant) in VAL post-RSA is noticed at 0%BFR with differences pre to post of 1.9% (400-0%), 2.5% (2000-0%) and 1.7% (3800-0%).

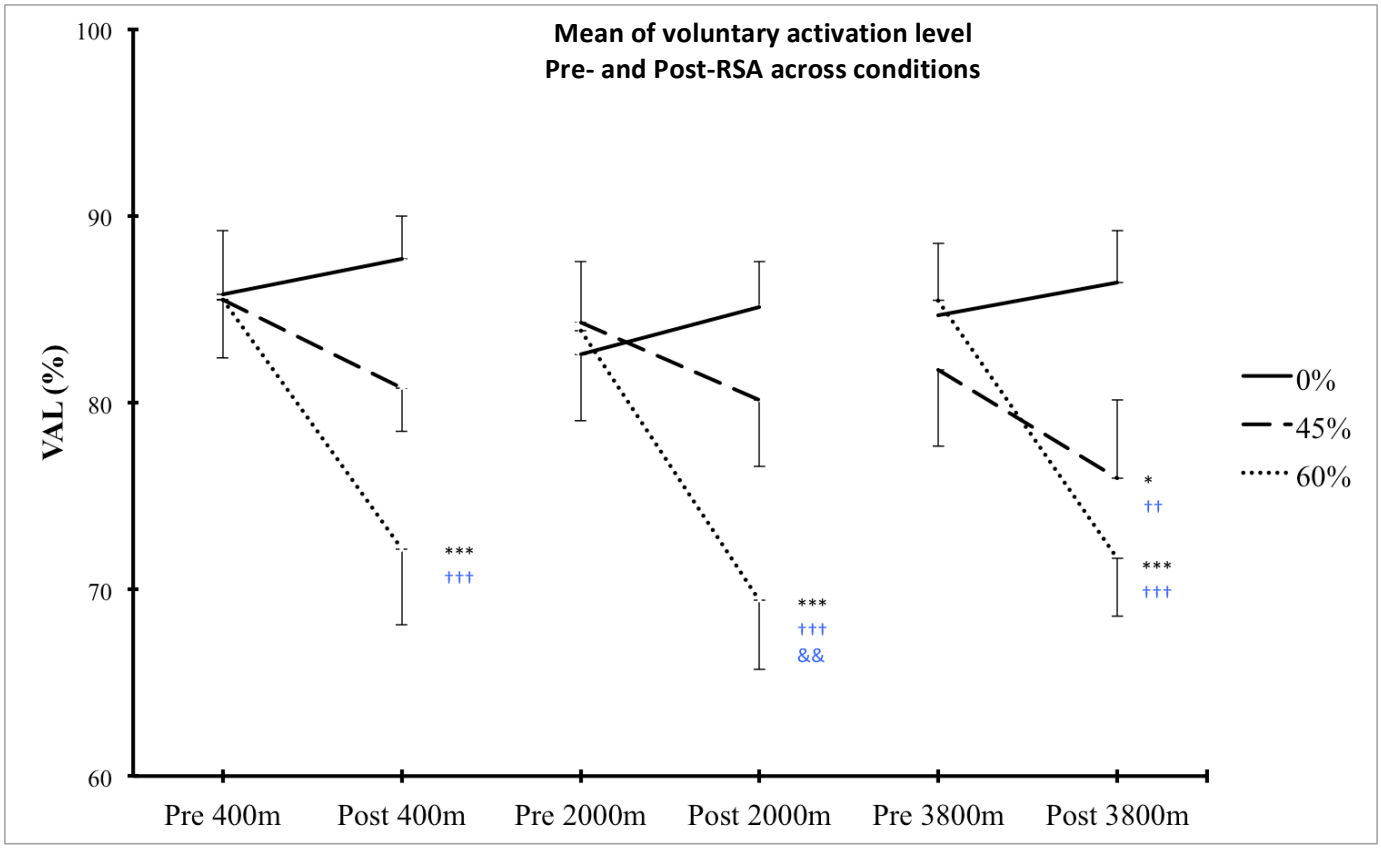


Figure 9. Changes pre to post in voluntary activation level (VAL) in percentage across conditions. 400m, 2000m, 3800m indicate the three levels of hypoxia; Pre and Post correspond to the assessment Pre- and Post-RSA; full lines represent conditions at 0%BFR; dashed lines represent conditions at 45%BFR, dotted lines represent conditions at 60%BFR. * < 0.05 ***<0.001 for differences with pre; †† < 0.01 ††† < 0.001 for differences with 0%; && < 0.01 for differences with 45%

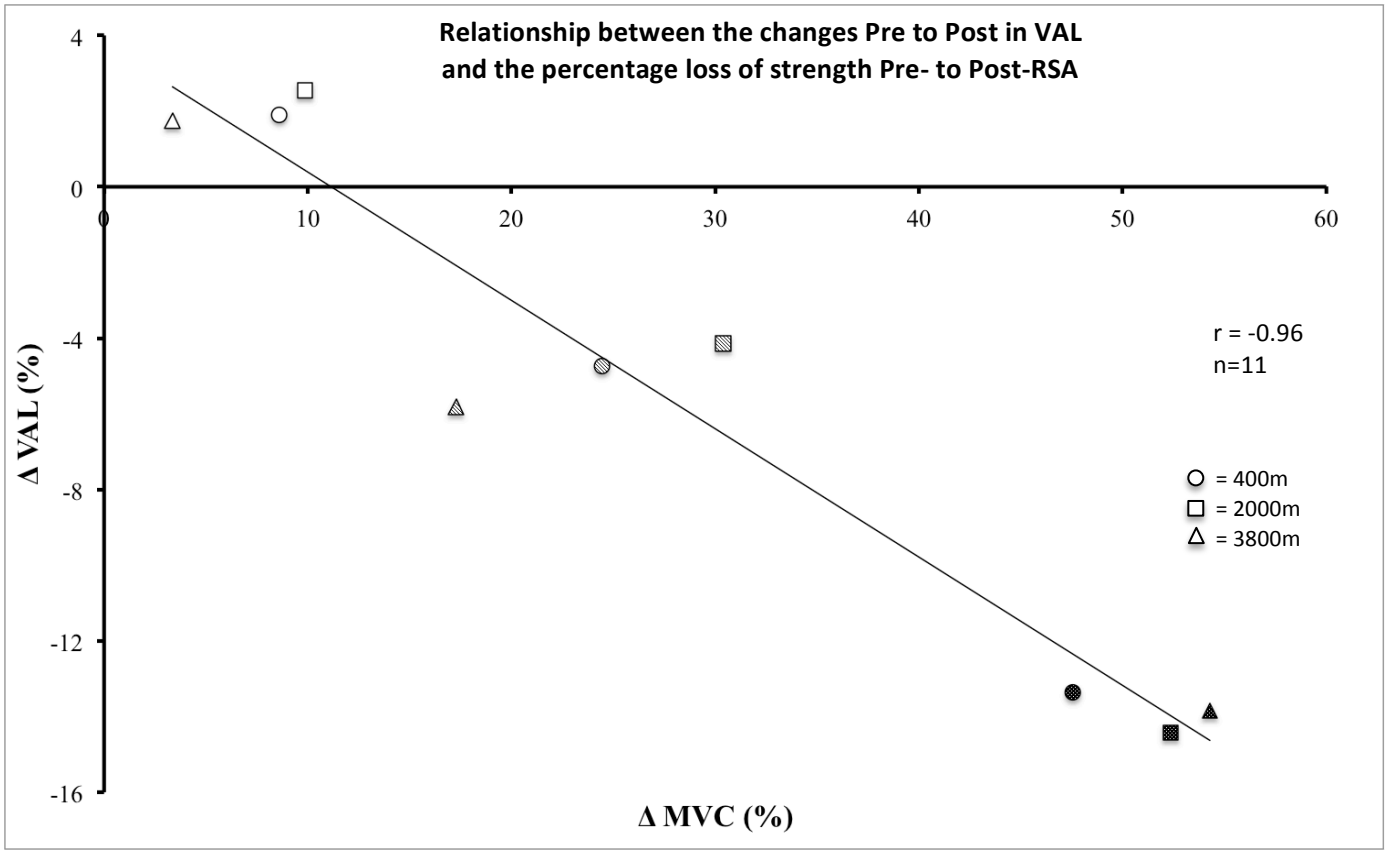


Figure 10. Relationship between changes (in percentage) pre to post in voluntary activation level (VAL) and changes in maximal voluntary contraction (MVC) across conditions. Circles indicate conditions at 400m; squares indicate conditions at 2000m; triangles indicate conditions at 3800m; white shapes represent condition at 0%BFR; grey shapes represent condition at 45%BFR; black shapes represent conditions at 60%BFR; r: correlation coefficient; p<0.001; n: number of subjects

A strong significant ($p<0.001$) correlation exists ($r = -0.96$) between the decrease in VAL and the decrease in MVC and is illustrated in Figure 10 above.

In this way, the highest decrement in MVC was associated with the largest reduction in VAL. Furthermore, a large effect of BFR was observed on these two variables while a slight impact of hypoxia was noted. The conditions at 60%BFR (black shapes) led to the greatest decrement in VAL as well as MVC, whereas 45% BFR (3 grey shapes) and 0%BFR (3 white shapes) induced smaller decrements on these two variables. Interestingly, 3 distinct levels are observed on the graph above corresponding to the 3 levels of BFR.

The second central fatigue marker is represented in the Figure 11 below with the ratio RMS/M.

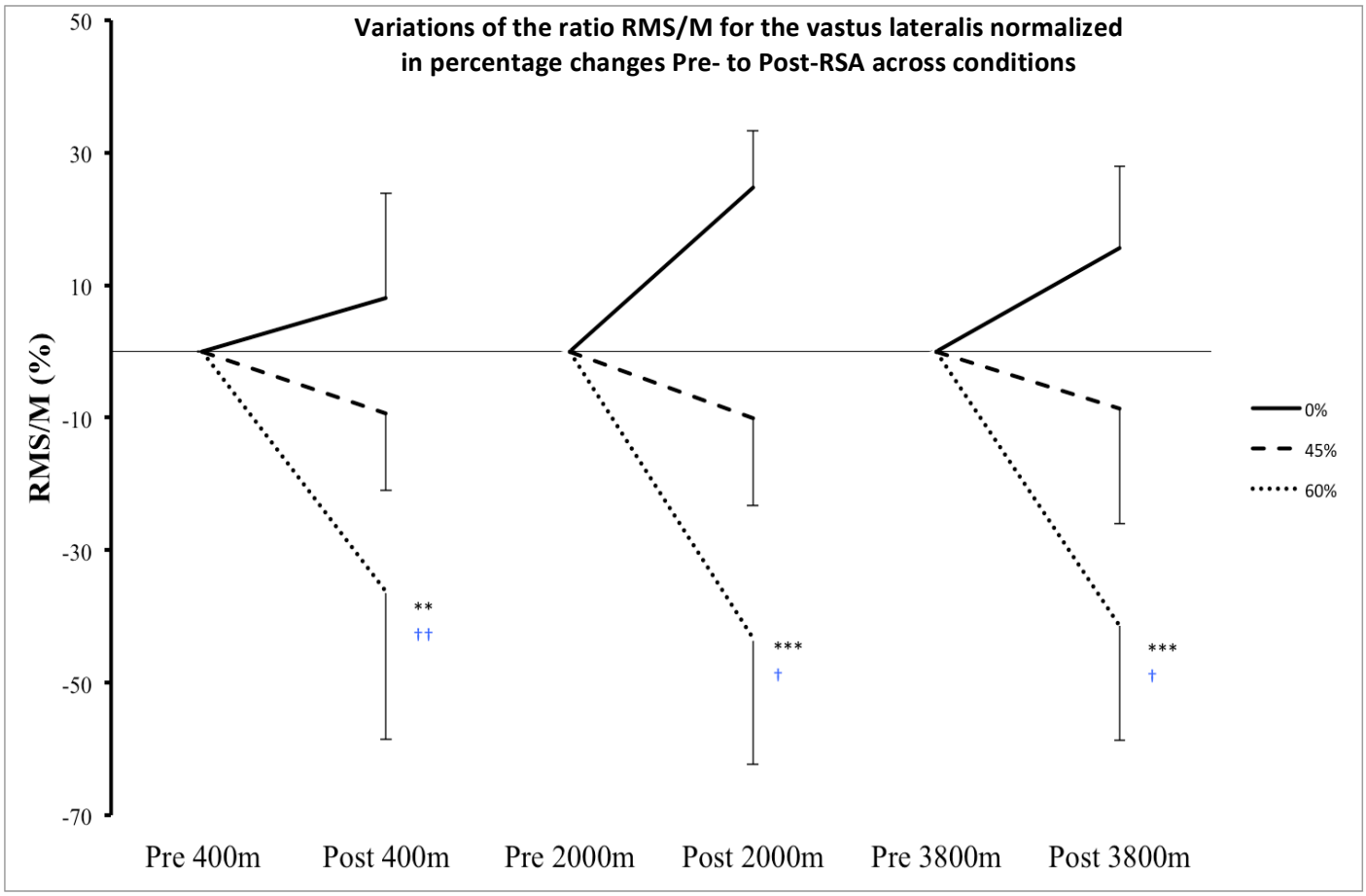


Figure 11. Variations of the ratio RMS/M for the vastus lateralis normalized in percentage changes pre to post (%). 400m, 2000m, 3800m indicate the three levels of hypoxia; Pre and Post correspond to the assessment Pre- and Post-RSA; Full lines represent conditions at 0%BFR; dashed lines represent conditions at 45%BFR, dotted lines represent conditions at 60%BFR. ** < 0.01 ***<0.001 for differences with pre; † < 0.05 †† < 0.01 for differences with 0%

The only significant changes occurred at 60% BFR with a decrease from pre to post of 37% ($p<0.01$), 38.9% ($p<0.001$), and 38% ($p<0.001$) at 400m, 2000m, and 3800m, respectively.
This larger decrease of RMS/M at 60%BFR was associated with a significant difference with condition at 0%BFR ($p<0.01$ at 400m; $p<0.05$ at 2000m and 3800m).
At 45% BFR, a non-significant decrease of -9.3%, -10.1%, -8.6% was observed at 400m, 2000, 3800m, respectively.
Lastly, an increase in RMS/M was noticed in the conditions at 0%BFR with changes pre to post of 8% (400-0%), 24.7% (2000-0%) and 15.6% (3800-0%). However, these variations were not significant.

3.3 Peripheral fatigue

The Figure 12 underneath represents changes pre to post in the amplitude of the M-wave of the VL. Neither BFR nor hypoxia affected the M-wave in any condition.

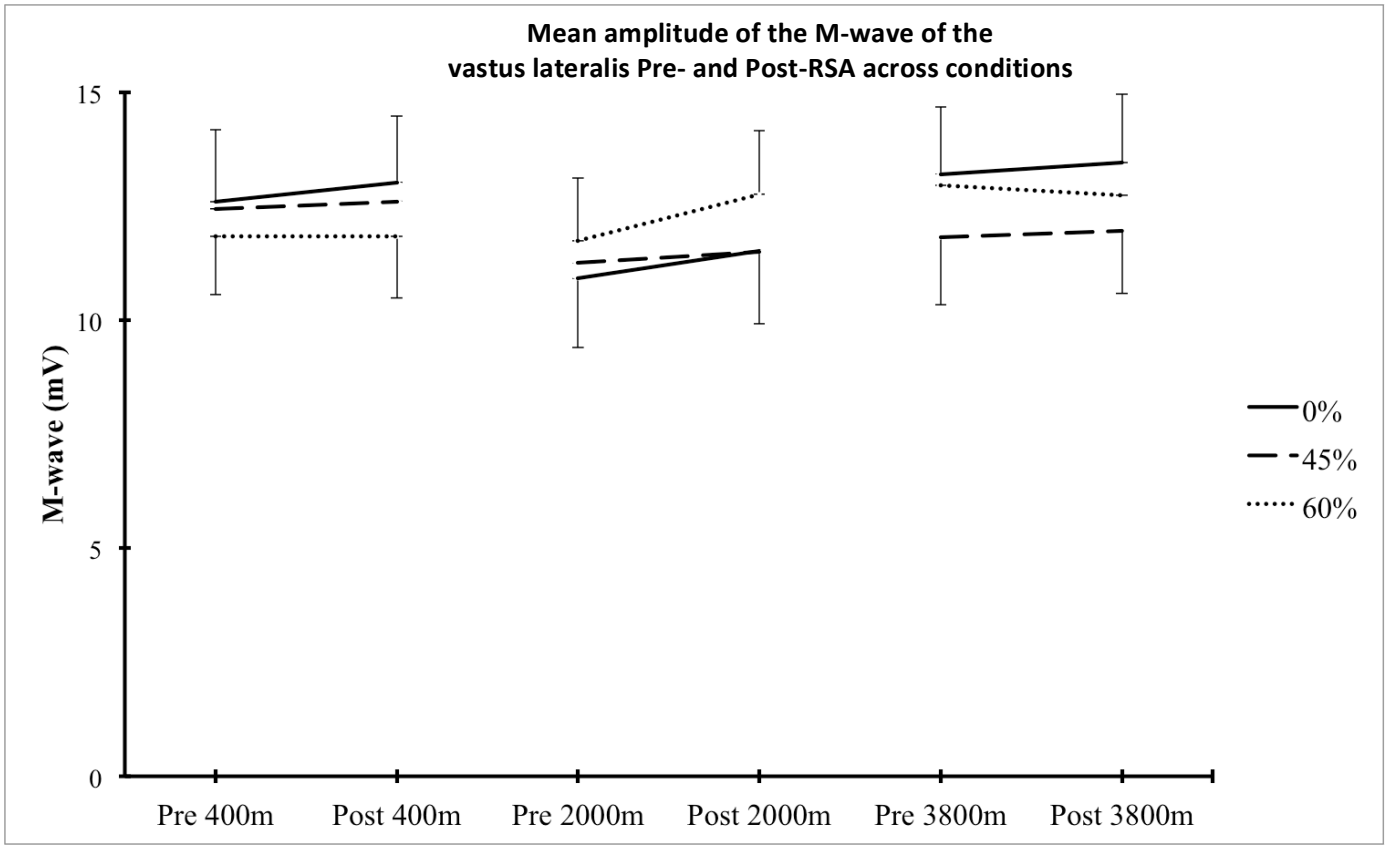


Figure 12. Mean amplitude (mV) of the M-wave of the vastus lateralis Pre- and Post-RSA. 400m, 2000m, 3800m indicate the three levels of hypoxia; Pre and Post correspond to the assessment Pre- and Post-RSA; full lines represent conditions at 0%BFR; dashed lines represent conditions at 45%BFR, dotted lines represent conditions at 60%BFR.

Figure 13 below points out the variations pre to post of evoked forces by high frequency stimulations (P100).

Significant changes were found in all conditions except at 3800-0%. P100 decreased considerably at 60%BFR ($p<0.001$). In these conditions, the amplitude of P100 was 30.6% (400-60%), 27.3% (2000-60%), and 26.1% (3800-60%) lower post-RSA compared to pre-RSA. Moreover, a difference in post between 400-60% and 400-0% ($p<0.01$) was reported as well as between 3800-60% and 3800-0% ($p<0.05$). Conditions at 45%BFR led to a decrease of 22.8% ($p<0.001$), 25.1% ($p<0.001$), and 16.1% ($p<0.05$) at 400m, 2000m, and 3800m, respectively without any significant effect of BFR or hypoxia. Finally, P100 decreased by 19.3% ($p<0.01$), 21.1% ($p<0.01$), and 10.6% ($p>0.05$) in condition at 0%BFR and with an effect of hypoxia at 2000m ($p<0.05$) and 3800m ($p<0.05$) compared to 400m and 2000m, respectively.

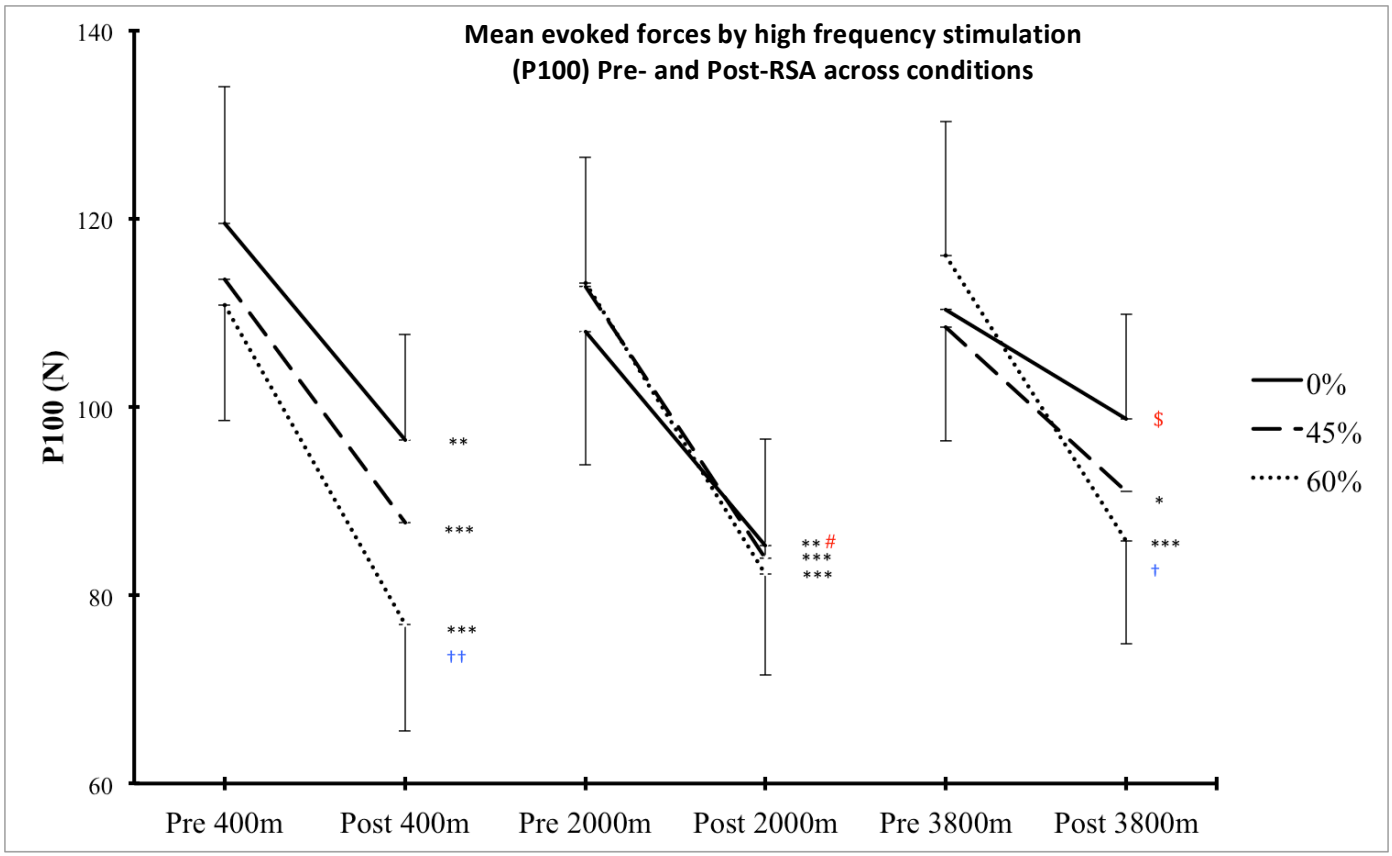


Figure 13. Changes pre- to post-RSA in low frequency stimulation (P10) across conditions. 400m, 2000m, 3800m indicate the three levels of hypoxia; Pre and Post correspond to the assessment Pre- and Post-RSA; full lines represent conditions at 0%BFR; dashed lines represent conditions at 45%BFR, dotted lines represent conditions at 60%BFR. * < 0.05 ** < 0.01 ***<0.001 for differences with pre; # < 0.05 for differences with 400m; $ < 0.05 for differences with 2000m; † < 0.05 †† < 0.01 for differences with 0%

Low frequency fatigue is reflected by the changes in evoked forces at rest by low frequency stimulations (10 Hz). As demonstrated in Figure 14, all conditions induced significant decrements in P10 ($p<0.001$), independently of BFR and hypoxia, except in condition 3800m-0% where a difference ($p<0.01$) occurred when compared to condition 2000-0%.

Hence, P10 decreased by 42.4%, 43%, and 31.4% at 0%BFR and at 400m, 2000m, and 3800m, respectively; by 40.6%, 40.9%, and 35.8% at 45% and at 400m, 2000m, and 3800m, respectively; and finally by 48.2%, 46.2%, and 42% at 60%BFR and at 400m, 2000m, and 3800m, respectively.

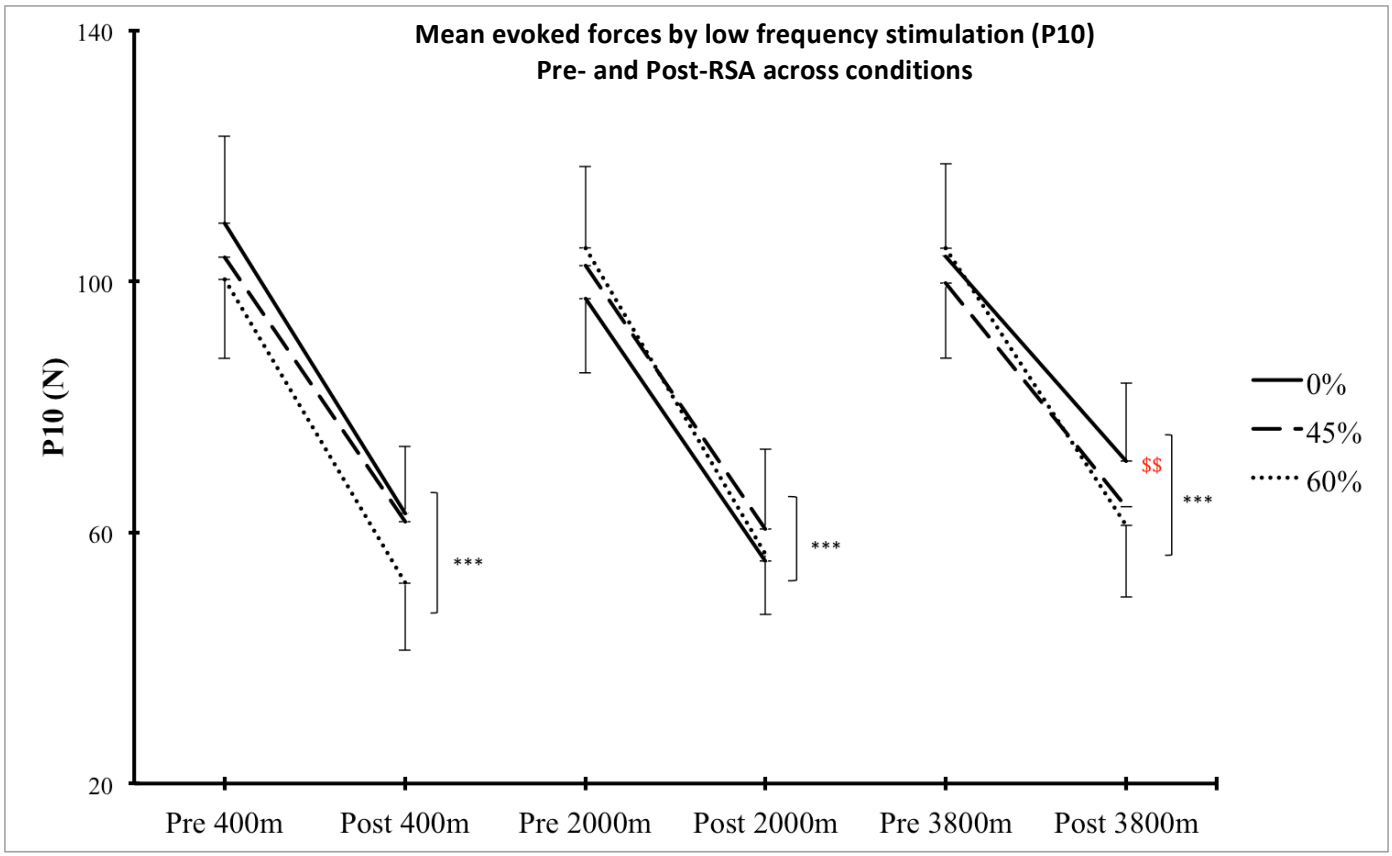


Figure 14. Changes pre- to post-RSA in low frequency stimulation (P10) across conditions. 400m, 2000m, 3800m indicate the three levels of hypoxia; Pre and Post correspond to the assessment Pre- and Post-RSA; full lines represent conditions at 0%BFR; dashed lines represent conditions at 45%BFR, dotted lines represent conditions at 60%BFR. ***<0.001 for differences with pre; $$ < 0.01 for differences with 2000m

The last marker of peripheral fatigue, the ratio P10/P100, is represented on Figure 15 below. P10/P100 was decreasing significantly ($p<0.001$) in all conditions without any effect of hypoxia or BFR. At 400m, reductions of 30.4%, 28.2%, and 25.6% were observed at 0%BFR, 45%BFR, and 60%BFR, respectively. At 2000m, post values were 25.7%, 21.5%, and 27% lower than pre at 0%BFR, 45%BFR, and 60%BFR, respectively. Finally, the ratio decreased pre

to post by 29.9%, 28.7%, and 24.8% at 3800m and at 0%BFR, 45%BFR, and 60%BFR, respectively.

Low frequency fatigue was more affected than high frequency fatigue as the ratio diminished.

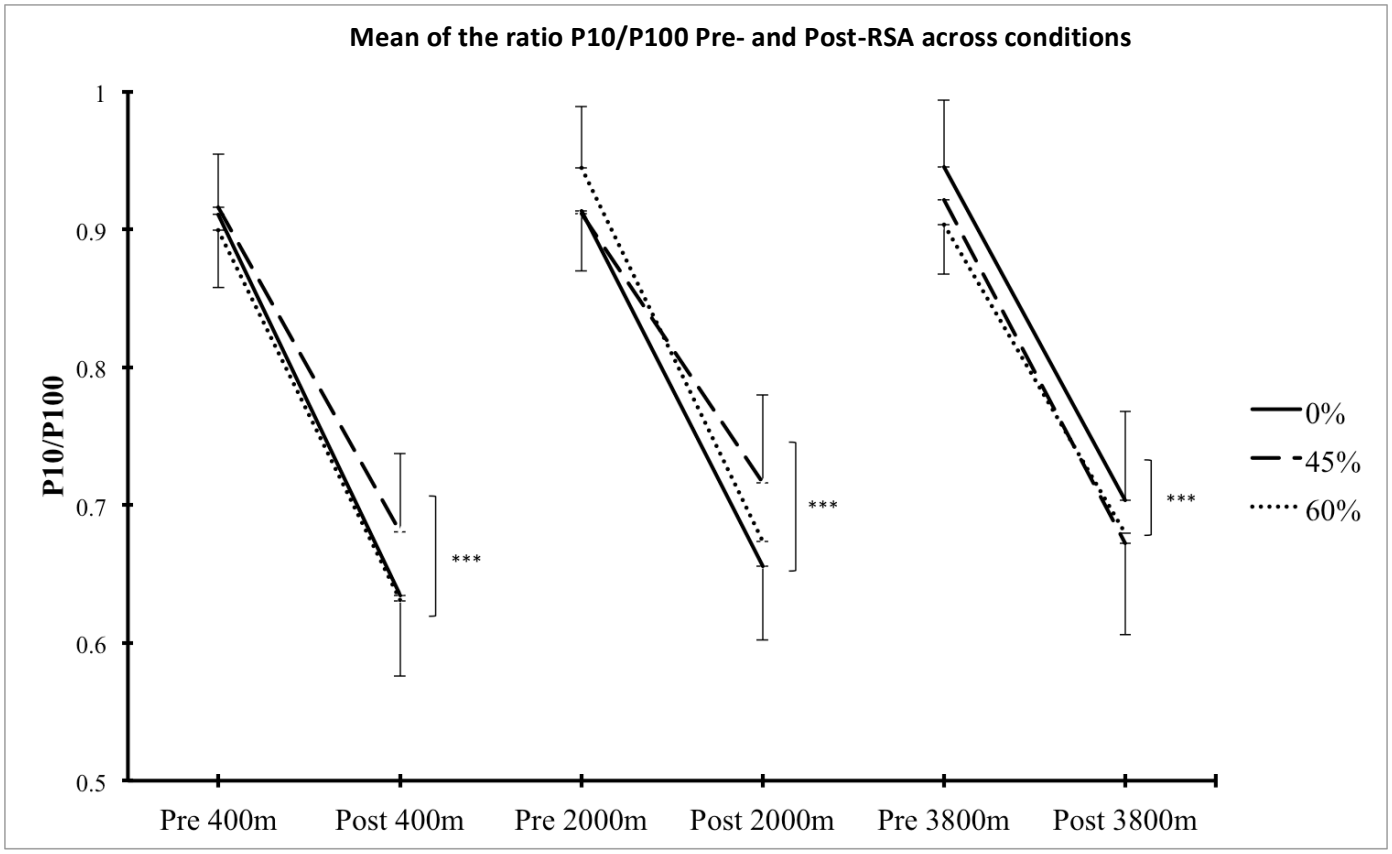


Figure 15. Variations of the ratio P10/P100 Pre- and Post-RSA across conditions. 400m, 2000m, 3800m indicate the three levels of hypoxia; Pre and Post correspond to the assessment Pre- and Post-RSA; full lines represent conditions at 0%BFR; dashed lines represent conditions at 45%BFR, dotted lines represent conditions at 60%BFR. ***<0.001 for differences with pre

Figure 16 shows the correlations between VAL and total work as well as the ratio P10/P100 and the total work.

The ratio P10/P100 was not significantly correlated ($p>0.05$) to the total work ($r = -0.29$), contrary to the relationship between VAL and the total work, which was more strongly correlated ($r = 0.85$; $p<0.01$).

Thus, the largest decrement in VAL occurred when the smallest amount of work was performed, corresponding to the conditions with a high level of BFR. Contrarily, a slight increase in VAL was observed in the conditions where the highest total work was produced, corresponding to the conditions without BFR. Regarding the ratio P10/P100, one may note that its evolution was nearly constant and follows a low slope trendline, suggesting that

P10/P100 developed at about the same level, independently of how much total work was produced. This is highlighted by the low correlation coefficient (r = -0.29), showing a poor relationship between these two factors.

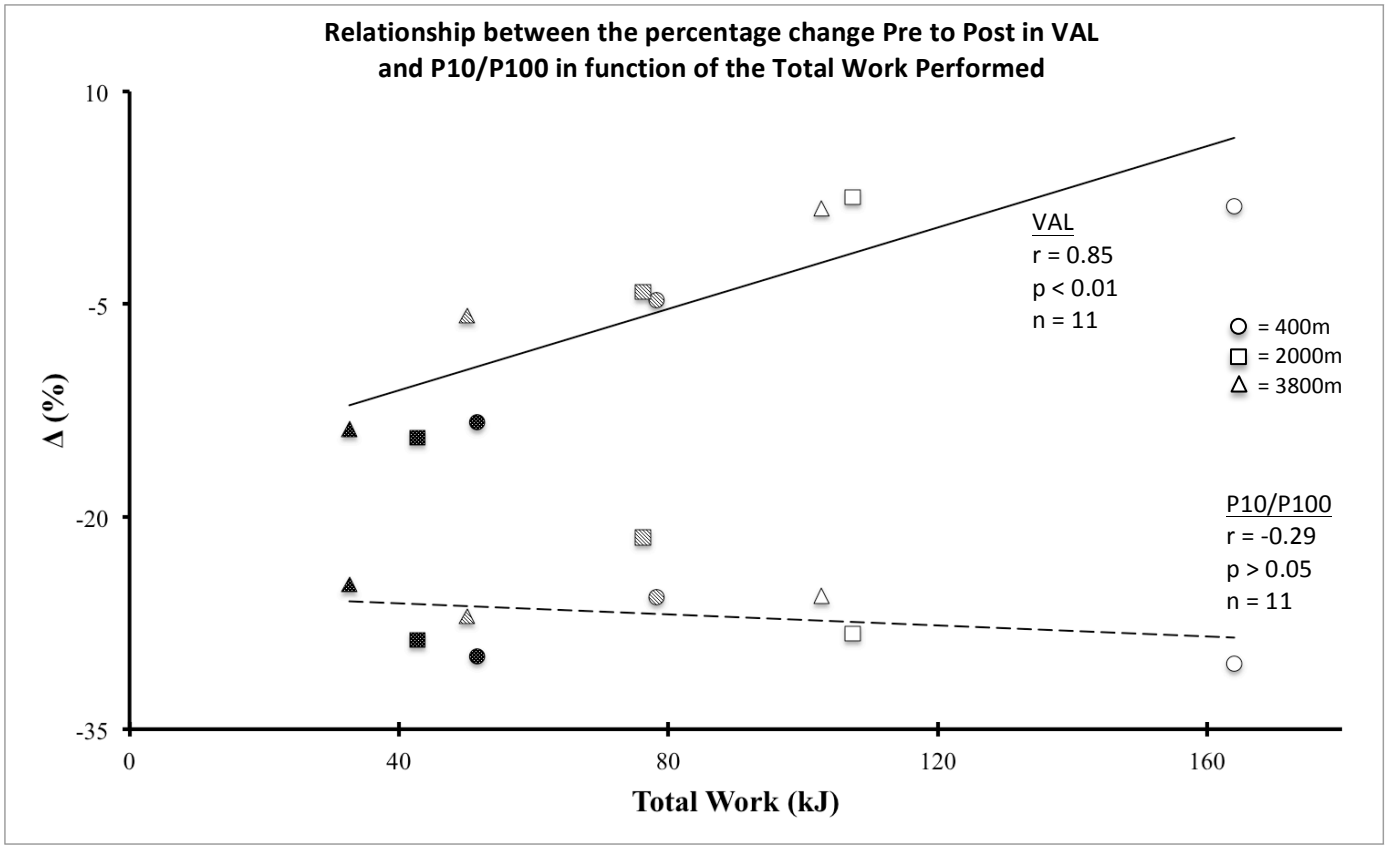


Figure 16. Relationship between the percentage changes Pre to Post in voluntary activation level (VAL), ratio low frequency/high frequency stimulations (P10/P100) in function of the Total Work performed (kJ). Circles indicate conditions at 400m; squares indicate conditions at 2000m; triangles indicate conditions at 3800m; white shapes represent condition at 0%BFR; grey shapes represent condition at 45%BFR; black shapes represent conditions at 60%BFR; Full trendline represents VAL with coefficient correlation=0.85 and p<0.01; dashed trendline represents the ratio P10/P100 with coefficient correlation=-0.29 and p>0.05; n: number of subjects.

4. DISCUSSION

The aim of this work was to investigate the neuromuscular component and assess the fatigue induced by repeated sprints to exhaustion under different levels of hypoxia and different levels of BFR. To the best of our knowledge, the present study is the first one to combine RSA, hypoxia and BFR.

The principal novel finding of this study was the large central fatigue induced in conditions at 60%BFR, independently of the level of hypoxia. Secondly, we showed that central alterations also occurred when a high level of hypoxia (3800m) was combined with a lower level of BFR (45%). Performance (total work) and global fatigue (MVC) decreased in all conditions but the decrements were greater with BFR. Finally, peripheral markers decreased in all conditions after RSA to exhaustion, with only a minor impact of the environment.

4.1 Performance and global fatigue

4.1.1 Effect of hypoxia and BFR on total work

Exhaustion or task failure was reached faster when BFR was applied as shown with the reduced amount of total work (Figure 6) and number of sprint performed (see Figure 20 in appendix) in these conditions compared to 0%BFR. Moreover, the amount of BFR impacted the total work as higher amount of pressure led to greater reduction in total work and thus faster exhaustion or task failure.

Interestingly, the only significant effect of hypoxia on total work occurred at 0%BFR. As soon as BFR was applied and independently of the percentage, hypoxia affected neither the total work nor MVC, suggesting already a stronger effect of BFR on fatigue than hypoxia.

Bowtell et al. (2014) reported significant difference in total energy expenditure during 10x6s all out sprint (30s recovery) at various levels of hypoxia. Hence, total work (kJ) was 595±60 at 12% FiO_2, 632±63 at 13% FiO_2, 638±57 at 14% FiO_2, 668±60 at 15% FiO_2 and 695±79 at 21% FiO_2. Compared to condition in normoxia (FiO_2 = 21%), the decrease of total work was 4% at 15% FiO_2 and of 9% at 13% FiO_2. Smith and Billaut (2010) also reported a mechanical work altered by hypoxia (FiO_2=0.13) compared to normoxia (-7.6%) in ten 10s sprints interspersed by 30s recovery. This is consistent with our results, as a lower level of FiO_2 reduced the total work. However, the present study results have shown larger decrement in total work at about the same level of hypoxia (FiO_2=16.4% and 12.8%). Indeed, total work in the current study was decreasing by 34.5%, and 37.4% at 2000m and 3800m compared to

condition in normoxia. The difference of protocols (fixed number of sprints versus to exhaustion) may explain the largest decrease in total work at altitude in the current study.

Secondly, the sprint duration (10s) and work/recovery ratio (1:2) used may also have affected the time to exhaustion. Glaister et al. (2005) pointed out the differences in physiological response and performance when the recovery time was shortened. The 25 participants of that study performed 2 protocols consisting of 20x5s cycling sprints with differing recovery periods (10s or 30s). The longer recovery period (30s) resulted in lower measurements of fatigue (performance decrement), heart rate, oxygen uptake, and respiratory exchange ratio (RER). Likewise, researchers noted an increase in the rate of perceived exertion (RPE) and blood lactate in both protocols with significant lower values for the longer recovery period (30s). The choice of the duration of the sprints and of the ratio influences considerably the development of fatigue. The work period was longer in the current study (10s vs 5s) compared to the one of Glaister et al. (but with the same ratio 1:2) and that may explain the greater decrement in total work and faster exhaustion reaching compared to other studies where smaller work periods were performed (Bowtell et al., 2014) interspersed with longer recovery periods (Smith and Billaut, 2010), thus allowing a more fully recovery.

It is known that the contribution of aerobic processes is limited (<10%) when performing a single sprint of short duration (6 sec) (McGawley and Bishop, 2008). Indeed, the energy supply for such effort is mainly related to phosphocreatine (PCr) (46%) and anaerobic glycolysis (40%). However, the contribution of aerobic processes tends to increase when sprints are repeated and when the effort extends. It has been estimated that aerobic metabolism could contribute up to 40% of the total energy supply during the final repetitions of a RSA (Girard et al., 2011). Several authors reported PCr metabolism and resynthesis as a major determinant during RSA, as PCr represents the most immediate reserve for the re-phosphorylation of adenosine triphosphate (ATP) (Girard et al., 2011). Indeed, depletion of PCr stocks occurs rapidly, especially in high intensity exercise and sprints where lots of energy is required from the onset of exercise. The resynthesis has been shown to be an oxygen dependent process following an exponential curve including a fast and a slow recovery component. The specificity of the task and more specially the ratio used during RSA will consequently affect the depletion of PCr according to the duration of work/recovery period. Moreover, the rate of PCr resynthesis may vary between subjects and according to the initial rate of PCr recovery, maximum aerobic capacity and metabolic conditions at the end of the exercise.

In the current study, the number of sprint performed varied between 30 (400-0%) and 8 (3800-60%). In fact, the present protocol consisted of a progressive reduction in oxygen availability through a systemic and/or localized hypoxia. Therefore, it is possible to infer a variable contribution of oxidative metabolism, which is more limited in some conditions than others and related to environmental conditions.

It has been reported that athletes may reach 80-100% of the VO_{2max} after consecutive sprints (Dupont & Al, 2005; Buchheit, 2010), and therefore, that VO_{2max} may be a limiting factor of RSA (Girard et al., 2011). However, it is possible to never reach such level of VO_2 according to the ratio used, as too short periods of work or too long periods of recovery may limit the development of VO_2, inhibiting the possibility to reach VO_{2max}.

Furthermore, strong correlations exist between VO_{2max} and the decrement score in RSA, proving the necessity to have a good aerobic fitness in order to resist fatigue (Hamilton et al. 1991; McMahon and Wenger 1998; Bishop et al. 2004). The VO_2 kinetic has also been studied in RSA (Dupont et al., 2005) and it has been proposed that faster kinetics (on-phase and off-phase) permits reduction in the oxygen deficit (at the onset of exercise) and faster re-oxygenation post sprint of muscle O_2 and PCr, thus improving performance in RSA (Bogdanis et al., 1996). These last authors also showed that the same level of PCr was reached between subjects at the end of a first 30s sprint. However, researchers demonstrated a slower PCr resynthesis in subjects who had the highest peak power output (PPO) and mean power output (MPO) in the first sprint, which led to an interesting inverted correlation ($r = -0.81$) between PPO and the percentage PCr resynthesis as well as another one linking endurance fitness (VO_{2max}) to PCr resynthesis. These previous findings are interesting, as it has often been reported that subjects with the higher PPO in the first sprints are the ones with the highest decrement score during RSA (Girard, 2011), which could also explain the variations of performance between subjects in the current study.

The VO_{2max} or VO_2 kinetic was not measured in the present study but it is worth noting that the total work performed and the variations according to the standard error were greater in these first three conditions without BFR compared to the subsequent ones. It is possible that the difference in fitness, maximal oxygen uptake, and oxygen uptake kinetics between subjects made a greater difference of performance in these conditions without BFR, as they allow a higher use of the aerobic component without developing too much neuromuscular fatigue (attests by the low decrement in MVC and low central fatigue parameters). Although the 20s recovery between each sprint does not permit the full recovery and the full PCr resynthesis, the difference in aerobic capacity between subjects may lead to a faster PCr

replenishment. Therefore explaining the greater variations in the condition without BFR. Likewise, the difference in the distribution of muscle fibers between subjects, influenced by many factors (gender, age, training status)(Miller et al., 1993), may lead to substantial differences in performance in the sense that a higher type II fiber distribution would be reflected by a higher capacity to produce power but a faster fatigue. While contrarily, a greater distribution of type I fiber would be efficient to sustain a prolonged effort but with limitation of the capacity to develop high power.

As stated previously, total work was only altered by hypoxia in conditions without BFR. As the level of hypoxia rises, aerobic performance and VO_{2max} are altered due to the reduced FiO_2. Indeed, altitude is well known to affect VO_{2max} (Buskirk et al., 1967; Daniels and Oldridge, 1970; Dill and Adams, 1971; Faulkner et al., 1968). It has been estimated that VO_{2max} is decreased by 7-9% each additional 1000 meter and that it continues decreasing in a linear way as the level of hypoxia increases (Wehrlin and Hallén, 2006).

Although the performance in the first sprint is principally determined by neuromuscular system and by intramuscular storage and the influence of environment is then negligible (Billaut and Bishop, 2009), hypoxia can affect the energy metabolism when effort is sustained or sprints are repeated, since the VO_{2max} is reduced by altitude and also that PCr resynthesis is an oxygen-dependent process.

Additionally, Hogan et al. (1999) reported a significant reduction in workload and time to exhaustion as FiO_2 was reduced during incremental plantar flexion in human. Researchers showed that intramuscular metabolic state at exhaustion was similar among the different FiO_2 but the time to exhaustion was significantly shorter when FiO_2 was reduced. Moreover, the study reported that muscle PCr and pH were significantly reduced when FiO_2 decreased and suggested that the higher rate of fatigue in hypoxia could also be the result of a faster accumulation of inorganic phosphate and a slower waste removal.

As in other studies, significant decreases in total work were observed in the current study when hypoxia was added and thus faster exhaustion or task failure. This could be firstly related to the lower FiO_2 that would slow the on-transient VO_2 and increases the O_2 deficit during each sprint. Thus, utilizing the anaerobic system to a greater extent in order to provide ATP provision (Girard et al., 2011). Secondly, Haseler et al. showed that PCr recovery is oxygen dependent and suggested that hypoxia extends time to recovery while hyperoxia shortens the latter, compared to normoxia. Indeed, the kinetics of PCr have also been shown to be prolonged by hypoxia compared to normoxia with a Tau (time to reach 63% of the curves amplitude) of 57.5s in hypoxia (FiO_2 = 0.10) compared to 37.6s in normoxia (FiO_2 =

0.21) (Haseler et al., 2007). Finally, Mendez-Villanueva et al. (2012) indicated a significant correlation (r=0.67) between the recovery of PCr and the recovery of repeated sprint performance, suggesting that the inability to produce power during repeated sprints is related to PCr metabolism.

Taken together, the effect of the ratio and the brief period of recovery (20s) in the current study, as well as the addition of systemic hypoxia have probably led to an incapacity to restore PCr over time as well as an accumulation of Pi and metabolite waste, leading to a faster exhaustion.

However, there was not a significant difference in the decrement of total work between the mid (2000m) and high (3800m) level of hypoxia without BFR. This can be explained by the fact that one subject performed 80 sprints in the condition at 3800m. Without that performance, the number of sprints decreases to 15.1 (instead of 21) and total work to 70.2 kJ (instead of 102.7 kJ). Considering this last value of total work, it would make all conditions at 3800m with a quasi-similar total work compared to the following one in normoxia but with BFR added. This may have some interesting practical applications, as less fatigue was observed when performing at a high level of hypoxia but without or with less BFR.

The total work as well as the number of sprints was then considerably reduced when BFR was applied. Moreover, the amount of pressure affected the total work and a higher percentage of BFR led to a faster exhaustion or task failure, without any effect of hypoxia. Knowing the oxygen-dependent process of PCr resynthesis and its important contribution during RSA, recovery period in BFR conditions were probably altered due to the limitation of the incoming blood flow, which varied according to the amount of pressure. Several authors reported no recovery of PCr (Colliander et al., 1988; Harris et al., 1976; Sahlin et al., 1979) when blood flow is totally occluded. In addition, Meyer et al. (2008) pointed out a slower PCr recovery during exercise under BFR (~120 mmHg) compared to free-flow conditions. In this way, the accumulation of metabolites within the muscles and the impossibility to restore the stock of PCr may be limiting factors in these conditions but the degradation of total work and the fastest exhaustion or task failure may also have been exacerbated by the manifestation of central fatigue, which will be discussed later.

4.1.2 Effect of BFR and hypoxia on MVC

As it has been shown in the results section, the loss of force was mainly influenced by the percentage of BFR applied. The highest level of BFR led to the greatest loss of force and decrement in total work, independently of the level of hypoxia. However, the pre-MVC

measurements were not affected by BFR, independently of the percentage, showing that BFR has no impact on brief (>5sec) and maximal effort such as MVC.

Several studies reported a loss of strength following repeated sprints. To exemplify, Billaut et al. (2006) as well as Billaut and Basset (2007) reported a decrease in MVC of 13% and 10.9%. Racinais et al. (2007) showed a decrease of 16.5% while Giacomoni et al. (2006) reported a decrease of 15% and 13% (morning versus evening, respectively). Each of these protocols consisted in 10 sprints of 6s with 30s recovery. In the present study, lower loss of strength in conditions without BFR (7.5%, 12.6% and 6.1% at 400m, 2000m and 3800m) were noted. These values are a bit surprising, and one could have expected higher decrement of force considering that our protocol is to exhaustion and according to the smaller work/recovery ratio compared to the previous studies. It may be explained by the short period of recovery between the end of RSA and the assessment of MVC. As a matter of fact, a measurement of the blood flow was firstly done once subjects reached exhaustion and before they moved to the chair ergometer for the fatigue assessment. Blood flow measurement was taken between 1.30 and 1.45 minutes after the end of RSA and assessment of fatigue often started around 3 minutes after the end of exercise. Sahlin & Seger showed a rapid partial reversal of MVC after a cycling task to exhaustion at 75% VO_{2max} and suggested two phases of recovery involving a rapid initial phase with a half recovery time ($t_{1/2}$) of about 2 minutes. Moreover, $t_{1/2}$ for the PCr resynthesis has been calculated (Prampero and Margaria, 1969) and corresponds approximately to 30-40s. According to these previous findings, it is possible that post MVC values were underestimated due to the recovery of the subjects between the end of exercise and the assessment of MVC. However, larger decrements in MVC were observed when BFR was applied (23.9%, 28.4%, 12.7% at 45%BFR and 400m, 2000m, 3800m, respectively), which reached almost half the voluntary strength at 60% BFR (45.1%, 49.6% and 52.6% at 400m, 2000m and 3800m, respectively). These values were potentially also underestimated because of the recovery phase, as explained previously. Nevertheless, the pressure of the cuffs was maintained and only released at the end of the assessment of fatigue. It is probable thus, that the recovery allowed during a few minutes was altered compared to the conditions without BFR. Total occlusion has been proved to block the recovery of PCr (Colliander et al., 1988; Harris et al., 1976; Sahlin, et al., 1979), whereas submaximal occlusion tends to slow down PCr recovery compared to free flow conditions (Meyer et al., 2008). In addition, Yasuda et al. (2009, 2008) observed that only one minute of free-flow recovery led to differences in MVC values compared to a first one directly performed at the end of an arm BFR exercise. Therefore, the

assessment of MVC in the current study, even though the time was the same for all conditions (approximately 3 minutes after the end of RSE), was probably not performed in the same recovery conditions, as the percentage of BFR surely exacerbated the kinetic of PCr recovery and waste removal.

Compared to the literature, few cycling studies have reported such level of force decrement. More precisely, no high intensity cycling exercise reported such loss of strength, probably due to the limited number of sprint performed in these studies. To illustrate, although the modalities of exercise are not the same as in the present study, Sahlin & Seger (1995) reported 34% loss of force in knee extensor MVC following a constant cycling exercise to exhaustion at 75% VO_{2max} (total average time to exhaustion = 1h25). Likewise Booth et al. (1997) reported a decrement of 28% after 1h12 (mean time to exhaustion) cycling at 75% VO_{2max}. Girard and Racinais (2014) showed that MVC was decreasing following a cycling exercise at constant load to exhaustion but with no further effects of hypoxia (FiO_2) or temperature compared to control condition, although time to exhaustion was reduced in heat or in hypoxia.

Use of BFR has been shown to decrease MVC but the few studies that investigated BFR in cycling did not report the loss of force. As examples, Wernbom et al. (2012) showed a reduction of 62% in MVC following 5 sets of knee extension to concentric torque failure and Loenneke et al. (2012) found similar decrease after 4 sets (30-15-15-15) of knee extension.

According to the previous illustrations, it seems that BFR leads to high decrement of force although direct comparisons are delicate due to the difference of task and muscle contractions.

The rating of perceived exertion (RPE), which permits to describe the level of exertion during physical activity from 6 (no exertion) to 20 (maximal exertion) (Borg, 1982), points out the various discomforts across conditions, and shows interestingly a decrease in RPE-breathing when BFR increases (see table 2 p.69). In addition, the RPE-legs rises with the percentage of BFR applied whereas the levels of hypoxia do not seem to affect the perceived exertion nor for the breathing, nor for the legs. In this way, it looks like the limiting factors in RSA (according to the RPE) were more related to the breathing in conditions without BFR and to the legs in vascular occlusion's conditions, independently of the amount of pressure. One can justify by the fact that BFR is associated with a lower number of sprints and therefore a lower fraction of oxygen uptake, compared to without BFR, where subjects have time to reach higher levels of VO_2. Subsequently, the highest percentage of BFR led to the smallest number of sprints and thus a lower time spent in sprinting, which manifests by less time to

reach or remain at a high level of VO_2 and therefore less breathing discomfort perceived. Moreover, BFR acted on the RPE as attest the almost maximal values quantified by subjects (mean of 19.6±0.09 at 60%BFR), proving the maximal effort of the legs, which, moreover, is obtained more quickly compared to conditions without BFR where RPE is lower (17.9 (±0.4)) and after a much more extended effort.

According to the correlation between total work and strength loss (Δ MVC), an inverse relationship has been shown pointing out that the greatest loss of force is associated to the smallest amount of work. In their review, Millet & Lepers (2004) mentioned that fatigue is increasing in a non-linear way over time until no further decrease in knee extensors strength after extended running exercises (more than 2 hours).

The current results go in an opposite way. As a matter of fact, the greater losses of strength were observed in the conditions where the smaller amount of work was performed (as shown in Figure 8), and were principally due to the large central fatigue induced in these conditions, whereas long trials elicited only slight decrease in strength. Likewise, the level of fatigue (loss of strength) was directly related to the percentage of BFR applied as three different levels are observed, corresponding to the three percentage of BFR (Figure 7). Fatela et al., (2016) recently reported that neuromuscular fatigue varies in function of the relative level of BFR. The present findings, as indicate the general fatigue markers (total work and MVC), are in accordance with the previous statement as we observe an increase of fatigue as the BFR level grows. However, this current study shows that this increase of fatigue is independent of the levels of hypoxia.

Finally, a point of interest is that the condition at high altitude and medium BFR (3800-45%) induced a larger decrease in total work as well as mean power (appendix p.68) compared to the following condition in normoxia and 60%BFR. However, a larger decrement in MVC was observed at 400-60% compared to 3800-45%. Hence, the condition at 3800-45% manifests a smaller performance compared to 400-60% but at the same time, the reduced performance is associated to less fatigue.

4.2 Central fatigue

4.1.1 Effect of BFR and hypoxia on central fatigue

Many studies have been focusing on fatigue in different exercise mode and development of central fatigue has mainly been related to prolonged exercise (Millet et al., 2003, 2002;

Nybo, 2008). Millet & Lepers (2004) showed that prolonged exercise is associated with a larger reduction in the percentage of VAL, especially with running.

In opposite, the results of the current study show that central alterations occurred when the less total work was performed. Indeed, the conditions at 60%BFR were the ones with the smallest amount of work but with the greatest amount of central fatigue (largest decrease in VAL and RMS/M). Moreover, this largest decrease in VAL at 60%BFR helps to explain the important loss of strength in MVC. There exists a strong correlation ($r = -0.96$; $p<0.001$) between these two variables that have been shown in Figure 10 and it appears that the alteration of the central component and loss of strength are closely linked to the percentage of BFR. Indeed, three distinct levels of decrement in VAL and MVC were observed and each level corresponded to the different percentage of BFR while hypoxia had no direct impact on both of the variables. According to our results, central alterations occurred only when a certain percentage of vascular occlusion (60%) was applied or when high altitude (3800m) was combined with the medium level of BFR (45%).

The conditions at 0%BFR and the first two at 45%BFR (400m and 2000m) did not lead to any central alterations in opposite of others studies that reported decrement in VAL already after six 10s cycling sprints (Hureau et al., 2015) or two 30m sprints (Goodall et al., 2015). The current results are a bit surprising, as one could expect an emphasized decrease in VAL due to the protocol to exhaustion. More unexpected, VAL was increasing from pre to post (between +1.75 to +2.55%) in conditions at 0%BFR, even if these changes were non-significant. This is mainly explained by the low performance of a few subjects that did not perform a true maximal contraction, probably due to the discomfort of the 100 Hz doublet nerve stimulations. For example, one of the 11 subjects reached a VAL of 55.2%, 64.9% and 52.4% (at 400m, 2000m, 3800m, respectively and 0%BFR) pre-RSA and improved his performance during the post-RSA assessment. Without taking in account these performances, a decrement in VAL of about 1% in these first three conditions is observed. This may also explain the relative low values of VAL pre-RSA (between 81.78% and 85.79%) compared to other studies. Secondly, the brief recovery period (±3min) at the end of RSA (due to the measurement of the blood flow and the set-up on the chair) probably affected VAL, which depends (for the calculation) on the performance in MVC as well as rest stimulations (doublet 100 Hz). It has been shown that peripheral (Froyd et al., 2013) and central fatigue (Bigland-Ritchie et al., 1986; Gandevia et al., 1996) recovers quickly at the end of an exercise. Indeed, Froyd et al. demonstrated that peak torque responses to electrical stimulation recovered rapidly and were already significant after 1 min (twitch and

P10) and 2 min (P100, P10/P100) post exercise, as well as MVC, which recovered significantly within the first 2 min after the end of exercise. In addition, evidences from previous research suggest that recovery of central fatigue can occur very quickly (within 2 min). Therefore, the absence of significant reduction in VAL in the current study does not necessarily reflect an absence of central fatigue during or at the end of RSA. This could be particularly true for the conditions without BFR, as significantly central alterations were ascertained in 4 on 6 conditions under BFR. Although the recovery time before the post fatigue assessment was the same in all condition, the conditions at 60%BFR led to important VAL and RMS/M decrements, which potentially suggests, besides the effects of a high percentage occlusion on central fatigue, that BFR may slow the recovery of both types of fatigue and that recovery could depend on the percentage of BFR. Findings from different studies (Bigland-Ritchie et al., 1986; Gandevia et al., 1996) have reported that complete vascular occlusion prevents recovery of both MVC and VAL. Although one may consider this short recovery period as a limit leading to an underestimation of the post-VAL and others fatigue markers values, high decrements in central markers were observed in some of the conditions.

The lower performance in conditions at 60%BFR has been previously explained by the possible more accentuated limitation of PCr resynthesis and waste removal due to the limited incoming arterial blood flow induced by the amount of pressure of the cuffs. Another main point to explain the reduced total work and number of sprint performed is the premature appearance of fatigue in these conditions. In order to produce a high level of power during an all out exercise such as RSA, high levels of muscle activation and neural drive are required. The central markers (decrement in VAL and RMS/M) at 60%BFR attest of a decline in the ability to activate maximally the muscles although it is not possible to know if it is an inability to recruit all motor-units or if the ones recruited are not able to discharge at their maximal frequency (Belanger and McComas, 1981). This limited activation has been observed in RSA and it has been proposed that central motor drive and thus performance (power output) may be self-regulated to prevent peripheral muscle fatigue from rising above a tolerated level (Amann and Dempsey, 2008; Gandevia, 2001; Hureau et al., 2014).

4.2.2 Arc reflexes

Training under BFR has been associated to an accelerated accumulation of metabolites (increase in Pi, H^+, blood lactate, and decrease in pH) (Scott et al., 2014; Suga et al., 2012, 2009; Takada et al., 2012), certainly linked to the enhanced recruitment of type II motor

units to sustain the necessary force level (Moore et al., 2004; Moritani et al., 1992), and that are trapped in the occluded muscle due to the cuffs pressure preventing the venous return.

Many studies have focused on the different feedback mechanisms occurring during exercise to regulate and adjust the physiological responses. The role of muscle afferents III and IV, which represents more than 50% of the total muscle afferents (Laurin et al., 2015), has often been linked to the development of central fatigue (Amann, 2012, 2011; Gandevia, 2001; Kaufman and Forster, 2010). These thinly myelinated (afferent III) and unmyelinated (afferent IV) nerve fibers are projecting to different sites within the central nervous system and are stimulated by contraction-induced mechanical and chemical stimuli. The mechanical (type III) and chemical (Type IV) sensitive nature of these nerve fibers, although many of them are poly-modal, plays also a twofold role. One of them is related to the development of the exercise pressor reflex (EPR), which includes the muscle metaboreflex (Boushel, 2010; Mitchell et al., 1983) and the muscle mechanoreflex (Victor et al., 1989), while the other one is an inhibiting role facilitating central fatigue (Amann et al., 2015; Gandevia, 2001). It has however been reported (Kaufman et al., 1984a; McCloskey and Mitchell, 1972) that the metaboreflex is independent of the central motor command, even if they are both mediated via afferents III/IV. Hence, EPR turns out to mediate autonomic adjustment of the cardiovascular response during an effort by enhancing sympathetic activity and reducing parasympathetic activity (Kaufman and Hayes, 2002). It depicts by an increase in cardiac output (CO) (increase in heart rate and stroke volume) generating an increase in mean arterial pressure (MAP), which could rise according to the intensity of exercise. Augustyniak et al. (2001) suggested that the elevation of MAP during mild or moderate exercise is primarily due to an increase in CO. However, in the case of severe exercise where CO is near or at its maximal and could not rise anymore, the metaboreflex would induce a peripheral vasoconstriction through sympathetic outflow in order to redistribute as much blood flow as possible from the periphery toward the skeletal muscle. Interestingly, it has been studied that the metaboreflex is more related to a reduced oxygen delivery than to a reduction in blood flow (Sheriff et al., 1987).

As second component of the EPR and with the same finality as the metaboreflex (i.e., to regulate the autonomic cardiovascular response via increase in sympathetic activity and blood pressure), the mechanoreflex is triggered by tissue compression during skeletal muscle contraction (Kaufman et al., 1984b) via muscle afferents III. Moreover, its response is in direct proportion to exercise intensity as reported Adreani et al. (1997) and Spranger et al. (2015).

Taken together and in the context of the current study, one may speculate the development of central fatigue in conditions at 60%BFR as follows. The rapid accumulation of metabolites that were trapped within the muscle due to BFR as well as the tissue contractions due to the high intensity sprint activity and the external cuffs pressure stimulated the afferents III and IV in order to adjust the cardiovascular response. Through the increase in discharge of these thin fiber muscle afferents inducing EPF (metaboreflex and mechanoreflex), an increase in sympathetic outflow led to an increase in CO in order to elevate the blood flow delivery to the ischemic skeletal muscles. In opposite to light or medium exercise intensity where an increase in MAP is principally due to the augmentation of CO, the maximal nature of the sprints did not permit to increase CO sufficiently, as its level was already maximal or near maximal. Therefore and as suggested Augustyniak et al. (2001), a compensated peripheral vasoconstriction occurred, so as to redistribute the peripheral blood flow to the occluded muscles with an increase in MAP as consequence. These authors also observed an alteration of the metaboreflex during severe exercise, which could be related to the regulating effect of the previous increased in MAP by the baroreflex (Kim et al., 2005). The latter, via the modulation of CO and peripheral vasoconstriction, turns out to be the first short-term regulator of systemic blood pressure in order to maintain a stable MAP (Olivier and Stephenson, 1993). Sheriff et al. (1990) showed that arterial baroreflex denervation doubled the pressor responses during mild exercise compared to when baroreflex was intact and where pressure was normally regulated. Moreover, Kim et al. completed the discussion by suggesting that the major mechanism by which the arterial baroreflex buffers the muscle metaboreflex is through an inhibition of the induced peripheral vasoconstriction. In any case, the effect of the baroreflex against the metaboreflex should lead to a progressive decrement in MAP, with as consequences a reduction of CO and therefore reductions in blood flow. Freezing afferents III and IV have been shown to reduce oxygen delivery through a lower blood flow as well as a faster peripheral fatigue development (Amann et al., 2011, 2010). Moreover, responses to exercise from afferents III and IV have been proved to be larger in absence of the baroreflex (Kim et al., 2005; Sheriff et al., 1990). Reflecting the previous arguments and referring to Amann et al. (2015), the development of peripheral fatigue could be exacerbated and linked to the manifestation of the baroreflex. However, other findings could contradict the previous arguments as it has been shown in cats that the baroreflex modulates the EPR response without changing muscle blood flow during muscular contraction (Waldrop and Mitchell, 1985). It could be therefore argued, depending on the validity of this model in human, that the activity of baroreflex does not affect the

development of peripheral fatigue, as the blood flow remains unaffected. In this context, it is interesting to report that maximal heart rate (Figure 22 in appendix) in the current study was always lower in conditions under BFR. Moreover, the highest percentage of BFR was associated with the lowest heart rate. Although it could be due to the reduced total work and thus the lower time spent exercising at high intensity, an effect of the baroreflex inducing a decrease in MAP and CO may also be speculated. Subsequently, it is possible that the response of EPR, even if limited or countered by the baroreflex, was still insufficient to re-oxygenate the ischemic skeletal muscle due to BFR, which limited the incoming blood flow. Therefore and in accordance with the concept that central fatigue aims at limiting the development of peripheral fatigue, muscle afferents III and IV completed their second role they have been associated to, which is to reduce central command via their inhibitory effect on the output from spinal motoneurons, having thus as consequences a reduction in muscle activation and leading to task failure (Amann et al., 2013, 2009, 2008; Bigland-Ritchie et al., 1986; Gandevia et al., 1996).

Figure 17 resumes the mechanisms of the metaboreflex leading to an increase of blood pressure.

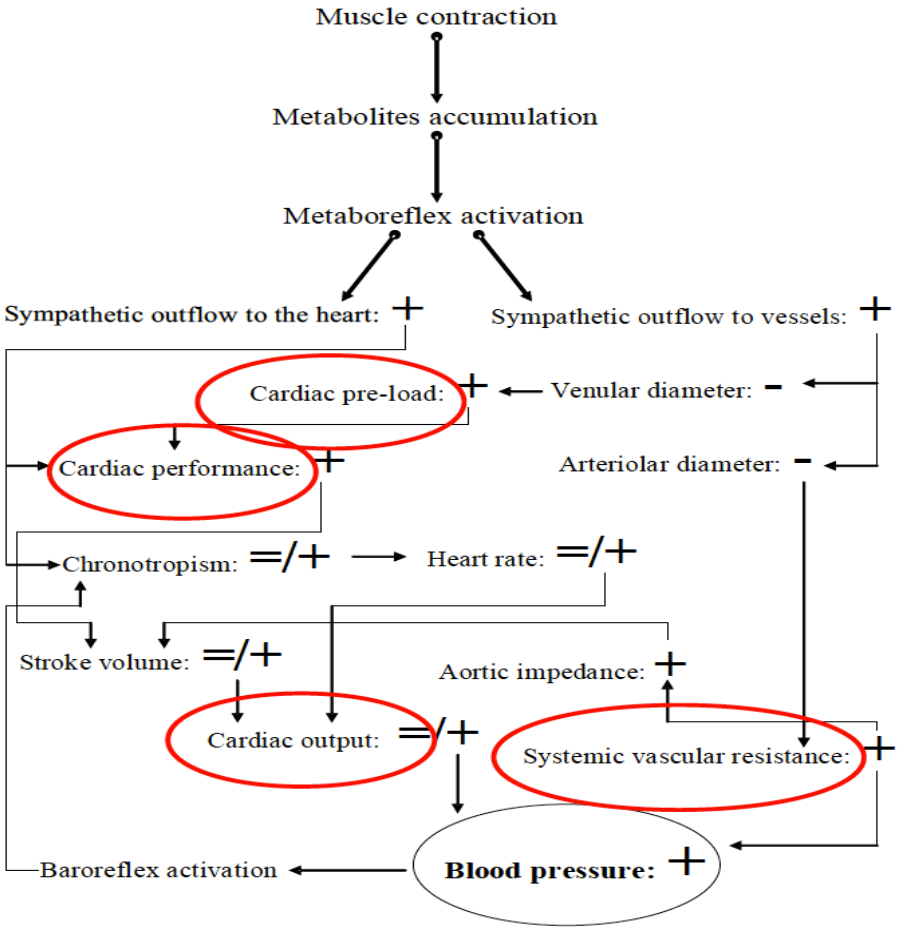


Figure 17. Schematic representation of putative mechanisms by which the metaboreflex operates to increase blood pressure. Piepoli, Dimopulos, Crisafulli Int. J.Cardiol. 2008, 130: 3-10.

The Figure 18 below is a speculated model leading to reduction in central drive in conditions at 60%BFR and 3800-45%.

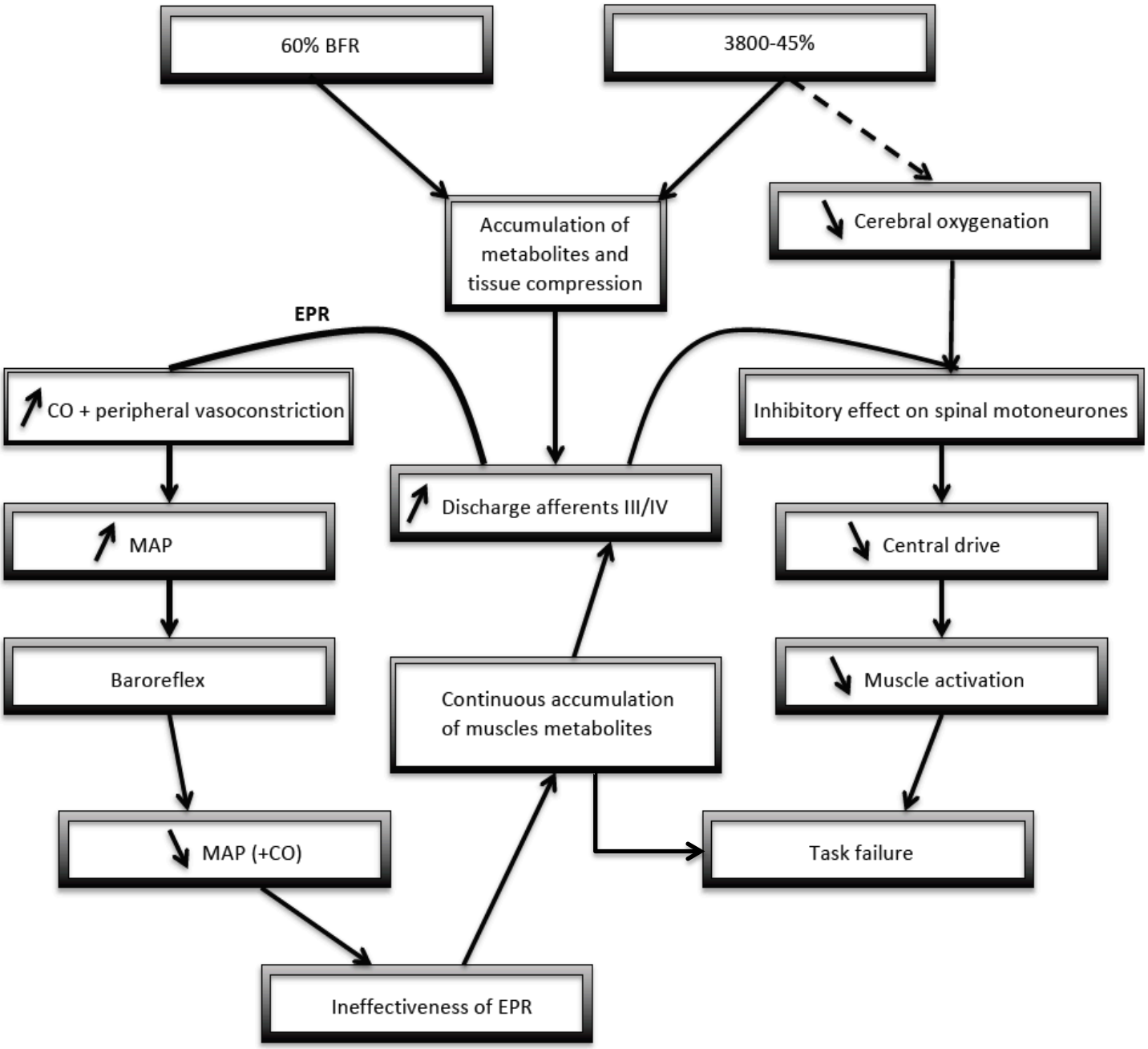


Figure 18. Potential mechanism of central fatigue leading to exhaustion or task failure in condition at 60%BFR and 3800-45%. EPR: exercise pressor reflex; CO: cardiac output; MAP: mean arterial pressure

In a first instance, the EPR reflex occurred in order to increase the blood flow and oxygen delivery into the ischemic muscle, via the response of muscle afferents III and IV. However, BFR (by restricting the incoming blood flow) shunted the effect of the EPR, which led to a

necessary reduction in the central command in order to limit the development of peripheral fatigue.

As stated earlier, the condition at high altitude and mid BFR (3800-45%) also led to a significant ($P<0.05$) decrease in VAL but not in RMS/M. It is interesting because a cumulative effect of a high level of hypoxia and medium level of occlusion is observed, as other conditions at this percentage of BFR did not affect VAL. According to the speculated previous model and to other researches, the metaboreflex has been shown to be more triggered by a reduced oxygen delivery than by a reduction in blood flow (Sheriff et al., 1987). The finality is notwithstanding the same, as a reduction in blood flow through BFR is associated to a decrease in oxygen delivery. However, the supposed cumulative effect of a high level of hypoxia and mid-BFR may manifest as an amplified muscle deoxygenation through systemic hypoxia and localized ischemia. One may consequently hypothesize that the autonomic regulations, via reflex arcs, did not achieve to deliver sufficient oxygen supply to the ischemic muscle due to the lowest FiO_2, which is in addition limited by the effect of BFR (even if the pressure is smaller). In view of the progressive accumulation of metabolites accordingly to the hypoxic environment within the ischemic muscle, afferents III and IV (via their inhibitory effect on the spinal motoneurons) reduce the central command in order to limit the development of peripheral fatigue.

To briefly resume this part, the supposed mechanisms are that arc reflexes try to regulate and adjust the cardiovascular response in order to increase the blood flow and the oxygen delivery to the muscle but the shunting effect of BFR does not permit a sufficient increase of blood flow, leading to a decrease in central drive to protect muscles from an irreversible development of peripheral fatigue. Although the assessment of fatigue was realized 3 minutes after the end of RSA, allowing subjects to recover, large decrements in VAL in 4 on 9 conditions were observed. This is potentially because of spinal motoneurons output and muscle activation remain low until muscles afferents III and IV recovered, which depends amongst others on the restoration of the blood circulation (Amann et al., 2015; Gandevia et al., 1996; Taylor et al., 2000).

In the current study, central fatigue was principally linked to muscles afferents III and IV but it is important to report also that alterations of central motor command can be triggered from other sites and independently of somatosensory feedback (Amann et al., 2007; Gandevia, 2001). Evidences suggest that low O_2 availability in the brain might lead to central fatigue. There are indeed several central oxygen-sensitive sites that adjust and regulate

sympathetic and respiratory activity and which are situated in the thalamus, hypothalamus, pons, and medulla (Neubauer and Sunderram, 2004). In this way, decrease in cerebral oxygenation has been showed to induce central alterations (Goodall et al., 2012; Nybo and Rasmussen, 2007), even though several homeostatic mechanisms are implemented to avoid low brain oxygenation (Miyamoto and Auer, 2000). Hence, the brain is protected against hypoxia at rest because of an increase in cerebral blood flow (CBF) when arterial tension becomes low. However, hyperventilation induced by strenuous exercise can restrain an increase in CBF due to the high-induced arterial carbon dioxide pressure (PCO_2), which can lead to a reduced cerebral perfusion that contributes to the development of central fatigue (Nybo and Rasmussen, 2007). Smith and Billaut (2010) reported critical changes in cerebral oxygenation in hypoxia (FiO_2 = 0.13) during 10 sprints of 10s with 30s recovery. They also observed that these changes did not occur in normoxic condition (FiO_2 = 0.21) and suggested that hypoxia influences prefrontal cortex (but not muscle) oxygenation during RSA, which could explain the lower mechanical work they observed in hypoxia. Finally, a potential combination of different mechanisms is also practicable. A lower brain oxygenation can potentially lead to central fatigue but this phenomenon could be majored by the stimulation of afferents III and IV due to the accumulation of metabolites (as proposed in the model above).

However, our results did not show any effect of hypoxia in any markers of central fatigue. Firstly, one can debate if the level of hypoxia was high enough to elicit any central alterations. Goodall et al. (2012) used in their study the same altitude level (3800m) but with different exercise modalities (constant-load cycling exercise) and they noticed a significant manifestation of central drive with a supraspinal origin of fatigue. Researchers observed a decline in cortical activation in parallel with reductions in cerebral oxygen delivery and oxygenation and explained the decrease in exercise performance in hypoxia by a potential suboptimal output from the motor cortex as consequences of reduced oxygen availability in the brain.

Secondly, the delay between the end of the task and the assessment of fatigue may have been too long to assess central fatigue. As stated several times, the recovery elapses quickly at the end of the task (1-3 minutes) and we potentially evaluated the fatigue at a point where central alterations already recovered, especially in conditions without BFR. Thirdly, the impact of BFR may have potentially blunted the effect of hypoxia, according to his strong effect on the fatigue development.

Hence, we showed that central fatigue occurred in 4 on 9 conditions. These four conditions included the highest level of BFR and the highest level of hypoxia combined with medium BFR. Although the period between the end of RSA and the fatigue assessment was long enough to elicit recovery, the latter was limited in some conditions probably because of the continuous presence of BFR during this lapse of time. Consequently, we cannot totally refute the potential existence of a central fatigue induced in other conditions (without BFR particularly), which may have manifested from other origins than the model we speculated previously.

4.3 Peripheral fatigue

The peripheral fatigue was ascertained with the different markers (twitch, P10, P100, P10/P100, M-wave) and no particular variations between conditions were observed. Interestingly, the current results showed that almost the same level of peripheral fatigue was reached across conditions.

4.3.1 Muscle excitability

First of all, the only peripheral index that did not change in any condition was the M-wave. Indeed, we have shown that nor hypoxia or BFR, whatever their levels, impacted the M-wave, demonstrating no alterations in membrane excitability after RSA to exhaustion. Alteration of the M-wave can be the reflect of changes of the nervous conduction, synaptic transmission or related to the conduction at the sarcolemma level (Duchateau and Hainaut, 1985). It suggests consequently that alterations leading to peripheral fatigue occurred beyond the sarcolemma. Researches on RSA focusing on neuromuscular activity have mentioned varied variations on the M-wave. Whereas some of them (Racinais et al., 2007) showed an increase in M-wave, some others reported a steady level (Billaut et al., 2013; Girard, Bishop & Racinais, 2013; Hureau et al., 2015) or a decrease (Perrey et al., 2010) after RSA.

4.3.2 Evoked forces and performance of the contractile apparatus

In the current study, the twitch evoked by nerve stimulation was reduced after exercise from 26% to 47% in function of the conditions, while no significant changes of the contraction time (CT) and half relaxation time (HRT) were observed. In the studies cited

previously (Perrey et al.; Racinais et al.), researchers noted alterations of the twitch after RSA of 9% to 15%, while Girard et al. showed that twitch was reduced by more than 40%. Referring to the previous authors, these variations may have been provoked by differences in subjects, in protocol (nature of exercise, timing of the assessment, muscle groups), as well as in stimulation characteristics. As for other measurements, the biggest decrements were observed in conditions at 60%BFR but the difference between conditions were minor. To illustrate, the amplitude of the twitch decreased by 47.5%, 45.2%, 46.1% at 400m, 2000m, and 3800m respectively compared to 38.4%, 42.6%, 26.4% without BFR and at 400m, 2000m, and 3800m, while condition at 45%BFR lies in between these values. The reduction in the amplitude of the twitch is caused, referring to Westerblad & Allen, by a combination of reduced calcium (Ca^{2+}) sensitivity of the myofilaments (myosin and actin), reduced maximum tensing-generating capacity, and reduced Ca^{2+} release from the sarcoplasmic reticulum.

Stimulations of different frequencies (100Hz and 10Hz) were used in order to assess the origin of the peripheral fatigue. High frequency fatigue (HFF) is determined by a decrease in torque elicits by high frequency stimulations, whereas low frequency fatigue (LFF) consist in a force decrement elicits by low frequency stimulations. Both types of stimulation decreased post-RSA and in all the conditions as attests the ratio P10/P100. Moreover, these markers were altered by RSA but with minor influences of BFR and hypoxia. While the ratio P10/P100 was absolutely not affected by any of the conditions, P10 underwent only the effect of hypoxia in one condition, whereas P100 fluctuated a bit more across conditions. Thus, the evolution of the ratio P10/P100 implies a more pronounced decrement of LFF (P10) than HFF after RSA to exhaustion, independently of BFR and hypoxia. This result is in concordance with other studies (Hureau et al., 2015; Rampinini et al., 2014) that reported more intense degradation of LFF compared to HFF after RSA, although LFF has often been a characteristic of eccentric contractions and stretch shortening cycle exercises (Martin et al., 2004). Edwards et al. (1977) pointed out the main features of LFF and noted that this type of fatigue persists for a much longer period of time (up to 24h after exercise) compared to HFF that recovers in a couple hours (1-2), implying consequently different origins of peripheral fatigue. Hence, HFF has been associated to an extra-cellular accumulation of K^+ while LFF is a failure in the excitation-contraction coupling due to a reduction of Ca^{++} release (Jones, 1996). It has been suggested, referring to Girard et al. that the accumulation of Pi could alter the calcium release from the sarcoplasmic reticulum and/or affect the myofibrillar calcium

sensitivity. These potential phenomenon proved *in vitro* could limit the force of the cross-bridges or limit their number (Dutka and Lamb, 2004; Westerblad et al., 2002).

4.3.3 Effect of BFR and hypoxia on peripheral fatigue

The results of the present study have shown alterations at the peripheral level and more specifically beyond the sarcolemma and are linked to a failure in the excitation contraction coupling potentially due to a reduction in Ca^{2+} release.

It is interesting to report that peripheral fatigue has almost never been affected by BFR or hypoxia, as about the same level of peripheral fatigue was reached across conditions.

However, BFR and hypoxia seems to have a time effect as the same level of peripheral fatigue was reached but with five times less total work in some conditions. The correlation graph in the result section illustrates the relationship between VAL, P10/P100 and total work. Interestingly, the central marker of fatigue was highly correlated to the total work (diminution of VAL with the reduction of total work), whereas the ratio P10/P100 followed almost a linear flat relationship (close to a zero slope). Furthermore, the ratio P10/P100 was not significantly correlated to the total work, showing that peripheral fatigue develops at about the same level in all conditions but with the feature that the rate of fatigue development is accelerated when the total work is reduced. In other words, BFR or hypoxia do not exacerbate the peripheral fatigue but affect its rate of development.

Metabolites accumulation

According to our results, the rate of development (but not the level) of peripheral fatigue was principally fastened with BFR. Although the specific mechanisms underlying BFR are not yet clear, benefits resulting from training under vascular occlusion are believed to be related to an increase of the accumulation of metabolites, anabolic hormone concentrations, intramuscular signaling and swelling as well as motor unit recruitment (Loenneke et al., 2012; Takano et al., 2005; Takarada et al., 2000a). Indeed, a greater metabolic stress have been observed in resistance training under BFR, which manifests with an increase in Pi, PCr depletion and pH reduction (Suga et al., 2009), as well as an increase in lactate production (Pierce et al., 2006). In brief, it is thought that training with blood flow restriction makes the metabolism more anaerobic and similar to high intensity exercise. Hence, in the case of maximal intensity exercise such as repeated sprints, the accumulation of metabolites (Pi, H^+) can occur even when performed in normoxia or without BFR. We could then speculate that

an accumulation is exacerbated due to the blocked venous return that limits the metabolites waste removal. Indeed and oppositely to RSA in normoxia where aerobic contribution increases over sprint repetitions, the hypoxemia induced by BFR and the progressive muscle deoxygenation potentially limits the contribution of the aerobic metabolism. In addition, the contribution of type II fibers, mainly recruited in high intensity effort, may decrease over sprint repetitions, as they are also more susceptible to fatigue. In that case, performance would be more determined by the type I fiber, which is more resistant to fatigue but can produce less force. This change in metabolism has been observed by Karatzaferi et al. (2001) and could be limited in conditions with BFR, where the unavailability of oxygen restricts the contribution of oxidative fiber, increasing the reliance on anaerobic metabolism to produce energy with, in counterpart, a production and an accumulation of metabolites. The systemic hypoxia could potentially have the same effect with the main difference that the venous return is not occluded, which allows a better waste removal compared to BFR conditions.

Although studies have reported increases in blood lactate post BFR exercise, our results (see figure 21 p.69 in appendix) have shown the opposite. As a matter of fact, conditions at 60%BFR elicited the smallest blood lactate values (8.6, 7.9, 6.9 for 400m, 2000m and 3800m, respectively) while 0%BFR elicited the highest ones (9.5, 11.6, 10.7 for 400m, 2000m and 3800m, respectively) and with 45%BFR in between (7.2, 9.3, 9.0 for 400m, 2000m and 3800m, respectively). The lactate values have been reported (Dassonville et al., 1998) to vary in function of the sampling sites. According to the fact that the blood lactate samples were collected at the earlobe, our results may not be representative of the lactate concentration within the working muscles.

Limitation of energy supply

This topic has been approached in the first part of the discussion with in particular the importance of PCr resynthesis, which is probably limited in BFR (and hypoxia) conditions because of its oxygen dependent nature.

The limitation of energy supply has been pointed out in RSA and could be aggravated by BFR and hypoxia. The potential role of BFR and hypoxia on energy supply could to be linked to the re-oxygenation rate during the recovery intervals.

Different studies on RSA have availed the NIRS (Near Infrared Spectroscopy) to measure the oxygen availability in the working muscle. Hence, a steady level of deoxygenation (increase in deoxyhemoglobin) during 10 seconds sprints (30 seconds recovery) has been reported by

Smith and Billaut (2010), showing that the capacity to use oxygen was preserved during 10 sprints. However, Racinais et al. (2007) demonstrated a progressive muscle deoxygenation in the same modalities (10x6s sprints and 30s recovery) of exercise, although they reported also that oxygen extraction was not impaired. According to the current study, it is possible that the smaller ratio used led to a progressive muscle deoxygenation. Additionally, hypoxia as well as BFR may have affected the kinetic of re-oxygenation by restricting the delivery of oxygen. This would be supported by the study of Billaut and Buchheit (2013) who showed a decline in the muscular re-oxygenation in hypoxic conditions (FiO_2 = 0.13) during RSA.

Hence, this limitation of the muscle re-oxygenation under BFR and/or in hypoxia may emphasize the reliance on anaerobic metabolism as well as limit the resynthesis of PCr, which is dependent on oxygen.

To conclude, many studies proposed that development of peripheral fatigue is closely controlled not to exceed a certain level and could be limited by the apparition of central fatigue (Amann et al., 2013, 2009; Amann and Dempsey, 2008; Hureau et al., 2015, 2014). Although central fatigue was not observed in all condition, possibly due to methodological issues, the almost same level of peripheral fatigue was reached at task failure or exhaustion in all conditions, reinforcing the idea that development of peripheral fatigue is closely controlled by central mechanisms.

As a reminder, our hypotheses were that i) peripheral and central fatigue is induced in all conditions; ii) the level of peripheral fatigue is independent on the level of occlusion and hypoxia, contrary to central fatigue; iii) BFR leads to stronger detrimental effect on central fatigue than hypoxia.

According to our results, we did not observe central fatigue in all conditions and our first hypothesis needs therefore to be shaded. We can nevertheless validate our 2nd hypothesis, as we show a steady level of peripheral fatigue across conditions, whereas central alterations occurred in specific situations only (conditions at 60%BFR and 3800-45). We also observed that BFR leads to stronger effect on central fatigue in accordance with our 3rd hypothesis.

5. PRACTICAL RECOMMENDATIONS

First of all, this study is only a first step on the topic BFR, hypoxia and repeated sprints and other studies are therefore needed to evaluate the best modalities of exercise. However, the results obtained in the current study have already a particular interest for practical applications.

One of the first point, and several authors already highlighted it, is to prescribe an individual pressure based on the arterial blood flow when using BFR, as the physiological responses are highly fluctuating in function of the percentage applied but also according to inter-individual variations. Our results show that a too high level of BFR is not recommendable because of the high central fatigue induced. In addition, our results suggest that central alterations can also manifest when combining a high level of hypoxia with a lower level of BFR.

Therefore, it seems to be more rational to use a lower percentage of BFR (such as 45%), where less central fatigue is observed and where the effort can be sustained longer.

Then, it is important to take into consideration that repeated sprints with BFR particularly, can cause important fatigue state, even when few sprints are performed. As we have shown, the level of peripheral fatigue was about the same but with five times less total work between condition in normoxia without BFR and high hypoxia combined with 60%BFR. Therefore, it should be avoided prescribing RSA with BFR based on RSA studies in normoxia and without BFR. Other studies should be first engaged to determine the best modalities of training using BFR in RSA.

For now, the condition in mid hypoxia (2000m) and mid BFR (45%) seems to be a good compromise between the level of fatigue reached and the performance in repeated sprints.

Other characteristics are also to take into consideration (cardiovascular, respiratory components) in order to design a training combining RSA and BFR.

6. STRENGTHS AND LIMITATIONS

Some of the limits have already been discussed previously. The main one is that the assessment of fatigue was not executed directly at the end of RSA and that this brief recovery period may surely have led us to an underestimation of our results.

Another point is that only one assessment of fatigue was executed Pre and Post. As exposed in the discussion, some measurements of the MVC “pre”, which was also used for the calculation of VAL and RMS/M, were sometimes not a truly maximal contraction. As the protocol was long and tough, we were compelled to make the assessment only once pre and post, which may have affected our results.

Then, the method used in order to assess central fatigue (twitch interpolation technique) did not allow do determine the exact sites inducing central fatigue. It would be interesting to replicate this study, using TMS in parallel of neurostimulation, in order to explore the possible different origins of fatigue between BFR and hypoxia.

Finally, we could only speculate on the accumulation of metabolites, as we did not collect blood samples, which could be another aim of a future study.

However, this study is a new innovating step in the field of RSA and high intensity interval training and we are the first to investigate this topic. The study was massive and included 110 visits at the laboratory in order to compare the different physiological responses during the trials and was realized in trained women and men. Each session, lasting for about 3 hours, was employing high technology devices, which allowed us to investigate a lot of physiological components accurately (neuromuscular fatigue, cardiorespiratory component, muscular and brain oxygenation as well as local and systemic blood flow) in RSA as well as in submaximal exercise. We were, moreover, able to individualize BFR for each subject instead of working with fixed amount of pressure, giving in this way more consistency to the physiological responses.

Although it is only a first step in the topic, the combination of these environmental stimuli (hypoxia and BFR), which have proved their benefits individually, is definitely an innovating and challenging project that will need future researches.

7. CONCLUSION

The current aimed to assess the neuromuscular fatigue in performing cycling repeated sprints to exhaustion with various levels of hypoxia and blood flow restriction.

According to the results, we showed that performance was decreased when hypoxia and BFR was added, with a particular effect of vascular occlusion. Exhaustion or task failure was therefore reached much faster in conditions under BFR (with a detrimental effect of a higher level of BFR) and hypoxia to a lesser extent. Results indicated that a high level of BFR allows the performance of a limited number of sprints, principally due to the manifestation of central fatigue, which has been proposed to limit the development of peripheral alterations. In these conditions, no differences were observed when higher levels of hypoxia were added, and one can therefore assume that a high percentage of BFR blunts the effect of hypoxia. The current study also indicated that a high level of hypoxia combined with a lower percentage of occlusion led to high decrements in performance, as was shown with a link to central drive alterations. Additionally, the same level of peripheral fatigue was reached in all conditions and low frequency fatigue was much more affected than high frequency fatigue.

To conclude, the results of the current study were in agreement with arguments proposing a regulation of exercise by central mechanisms in order to limit the development of a too high and irreversible level of peripheral fatigue.

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9. APPENDIX

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Formulaire de consentement

Titre de l'étude :
Effets de l'hypoxie et de l'occlusion vasculaire sur l'oxygénation et l'activité musculaire lors de sprints répétés

Le soussigné :

- certifie avoir été informé sur les objectifs et le déroulement de l'étude.
- affirme avoir lu attentivement et compris les informations écrites fournies en annexe, informations à propos desquelles il a pu poser toutes les questions qu'il souhaitait.
- certifie avoir été informé des avantages et des risques éventuels qui sont associés à cette étude, et des contraintes qu'impliquait sa participation à l'étude.
- Certifie ne pas être diminué physiquement par quelque blessure que ce soit au moment de l'étude.
- certifie avoir été informé de la couverture via autoassurance en responsabilité civile de l'Etat de Vaud.
- atteste qu'un temps de réflexion suffisant lui a été accordé.
- certifie avoir été informé qu'il pouvait interrompre à tout instant sa participation à cette étude sans préjudice d'aucune sorte.
- a été informé que les données recueillies pendant l'étude sont traitées de façon confidentielle et anonyme.
- s'engage à informer l'investigateur responsable de tout phénomène inattendu pouvant survenir pendant cette étude et à se conformer aux recommandations de l'investigateur responsable de l'étude.
- accepte de participer à cette étude.

Signature du participant : ……..
Signature de l'investigateur : ……

Lieu et date : ……

Appendix 1. Consent Form

Questionnaire sur l'aptitude
à l'activité physique - Q-AAP
(version révisée en 2002)

Q-AAP et VOUS

(Un questionnaire pour les gens de 15 à 69 ans)

L'exercice physique pratiqué d'une façon régulière constitue une occupation de loisir saine et agréable. D'ailleurs, de plus en plus de gens pratiquent une activité physique de façon régulière. Règle générale, augmenter la pratique sportive n'entraîne pas de risques de santé majeurs. Dans certains cas, il est cependant conseillé de passer un examen médical avant d'entreprendre un programme régulier d'activités physiques. Le Q-AAP (questionnaire sur l'aptitude à l'activité physique) vise à mieux cerner les personnes pour qui un examen médical est recommandé.

Si vous prévoyez modifier vos habitudes de vie pour devenir un peu plus actif(ve), commencez par répondre aux 7 questions qui suivent. Si vous êtes agé(e) de 15 à 69 ans, le Q-AAP vous indiquera si vous devez ou non consulter un médecin avant d'entreprendre votre nouveau programme d'activités. Si vous avez plus de 69 ans et ne participez pas d'une façon régulière à des activités physiques exigeantes, vous devriez consulter votre médecin avant d'entreprendre ces activités.

Lisez attentivement et répondez honnêtement à chacune des questions suivantes. Le simple bon sens sera votre meilleur guide pour répondre correctement à ces questions. Cochez OUI ou NON.

OUI	NON		
☐	☐	1.	**Votre médecin vous a-t-il déjà dit que vous souffriez d'un problème cardiaque et que vous ne deviez participer qu'aux activités physiques prescrites et approuvées par un médecin?**
☐	☐	2.	**Ressentez-vous une douleur à la poitrine lorsque vous faites de l'activité physique?**
☐	☐	3.	**Au cours du dernier mois, avez-vous ressenti des douleurs à la poitrine lors de périodes autres que celles où vous participiez à une activité physique?**
☐	☐	4.	**Éprouvez-vous des problèmes d'équilibre reliés à un étourdissement ou vous arrive-t-il de perdre connaissance?**
☐	☐	5.	**Avez-vous des problèmes osseux ou articulaires (par exemple, au dos, au genou ou à la hanche) qui pourraient s'aggraver par une modification de votre niveau de participation à une activité physique?**
☐	☐	6.	**Des médicaments vous sont-ils actuellement prescrits pour contrôler votre tension artérielle ou un problème cardiaque (par exemple, des diurétiques)?**
☐	☐	7.	**Connaissez-vous une autre raison pour laquelle vous ne devriez pas faire de l'activité physique?**

Si vous avez répondu

OUI à une ou plusieurs questions

Consultez votre médecin AVANT d'augmenter votre niveau de participation à une activité physique et AVANT de faire évaluer votre condition physique. Dites à votre médecin que vous avez complété le questionnaire sur l'aptitude à l'activité physique et expliquez-lui précisément à quelles questions vous avez répondu «OUI».

- Il se peut que vous n'ayez aucune contre-indication à l'activité physique dans la mesure où vous y allez lentement et progressivement. Par ailleurs, il est possible que vous ne puissiez faire que certains types d'efforts adaptés à votre état de santé. Indiquez à votre médecin le type d'activité physique que vous comptiez faire et suivez ses recommandations.
- Informez-vous quant aux programmes d'activités spécialisés les mieux adaptés à vos besoins, offerts dans votre localité.

NON à toutes ces questions

Si, en toute honnêteté, vous avez répondu «NON» à toutes les questions du Q-AAP, vous êtes dans une certaine mesure, assuré(e) que:

- vous pouvez augmenter votre pratique régulière d'activités physiques en commençant lentement et en augmentant progressivement l'intensité des activités pratiquées. C'est le moyen le plus simple et le plus sécuritaire d'y arriver.
- vous pouvez faire évaluer votre condition physique. C'est le meilleur moyen de connaître votre niveau de condition physique de base afin de mieux planifier votre participation à un programme d'activités physiques.

REMETTRE À PLUS TARD L'AUGMENTATION DE VOTRE PARTICIPATION ACTIVE :

- si vous souffrez présentement de fièvre, d'une grippe ou d'une autre affection passagère, attendez d'être remis(e); ou
- si vous êtes enceinte ou croyez l'être, consultez votre médecin avant de modifier votre niveau de pratique sportive régulière.

Veuillez noter que si votre état de santé se trouve modifié de sorte que vous deviez répondre «OUI» à l'une ou l'autre des questions précédentes, consultez un professionnel de la santé ou de la condition physique, afin de déterminer s'il vous faut modifier votre programme d'activités.

Formule de consentement du Q-AAP: La Société canadienne de physiologie de l'exercice, Santé Canada et ses représentants n'assument aucune responsabilité vis-à-vis des accidents qui pourraient survenir lors de l'activité physique. Si, après avoir complété le questionnaire ci-dessus, un doute persiste quant à votre aptitude à faire une activité physique, consultez votre médecin avant de vous y engager.

Toute modification est interdite. Nous vous encourageons à copier le Q-AAP dans sa totalité.

Dans le mesure où le Q-AAP est administré avant que la personne ne s'engage dans un programme d'activités ou qu'elle fasse évaluer sa condition physique, la section suivante constitue un document ayant une valeur légale et administrative.

«Je sous-signé(e) affirme avoir lu, compris et complété le questionnaire et avoir reçu une réponse satisfaisante à chacune de mes questions.»

NOM _____

SIGNATURE _____ DATE _____

SIGNATURE D'UN PARENT or TUTEUR (pour les mineurs) _____ TÉMOIN _____

N.B.– Cette autorisation de faire de l'activité physique est valide pour une période maximale de 12 mois à compter du moment où le questionnaire est rempli. Elle n'est plus valide si votre état de santé change de telle sorte que vous répondez «OUI» à l'une des sept questions.

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Appendix 2. Health Form

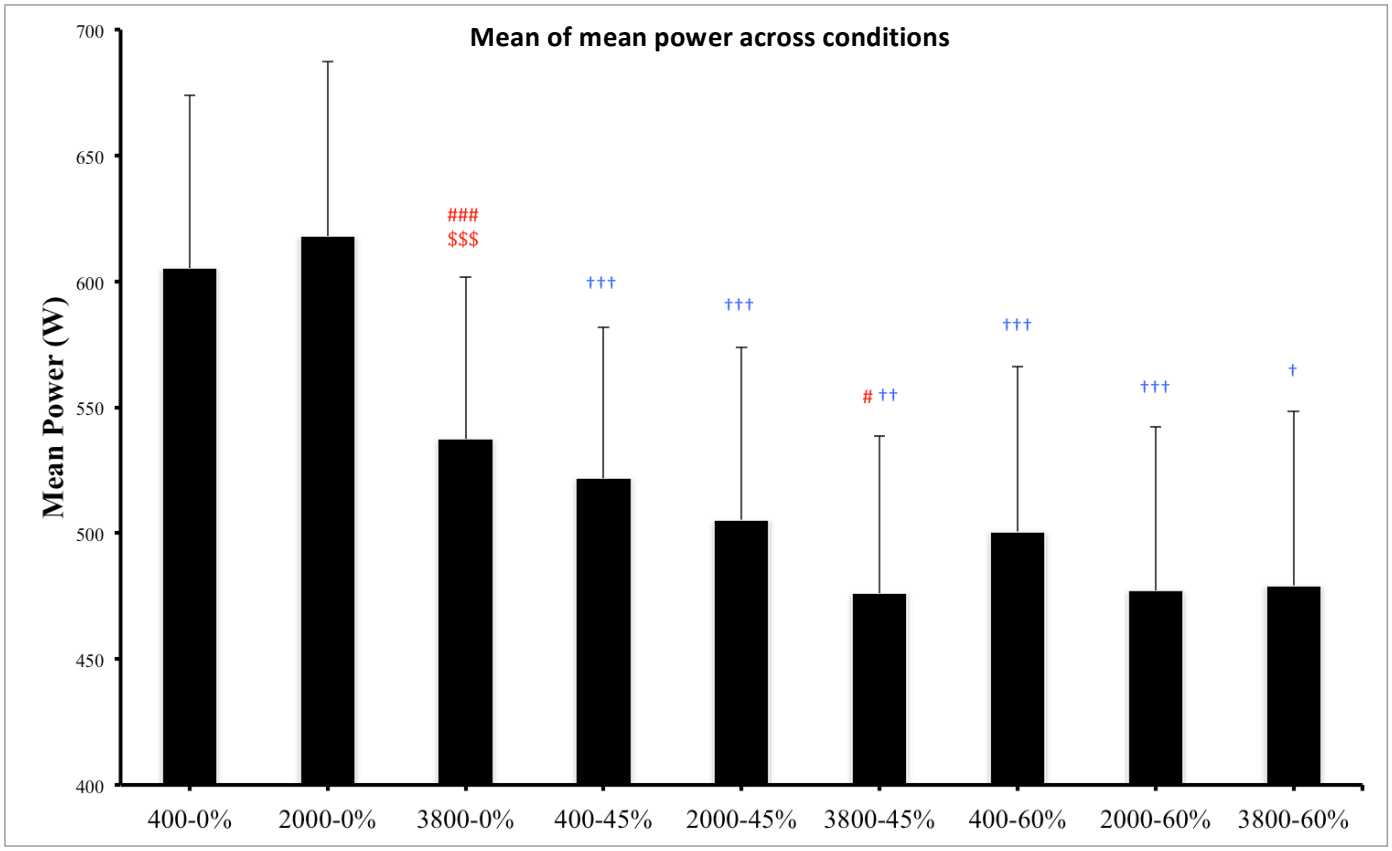

Figure 19. Mean of mean power performed across conditions. 400, 2000, 3800 indicate the level of hypoxia (400m, 2000m and 3800m, respectively); 0%, 45%, 60% indicate the level of BFR (percentage of AOP). ## < 0.01 ### < 0.001 for differences with 400m; † < 0.05 †† < 0.01 ††† < 0.001 for differences with 0%

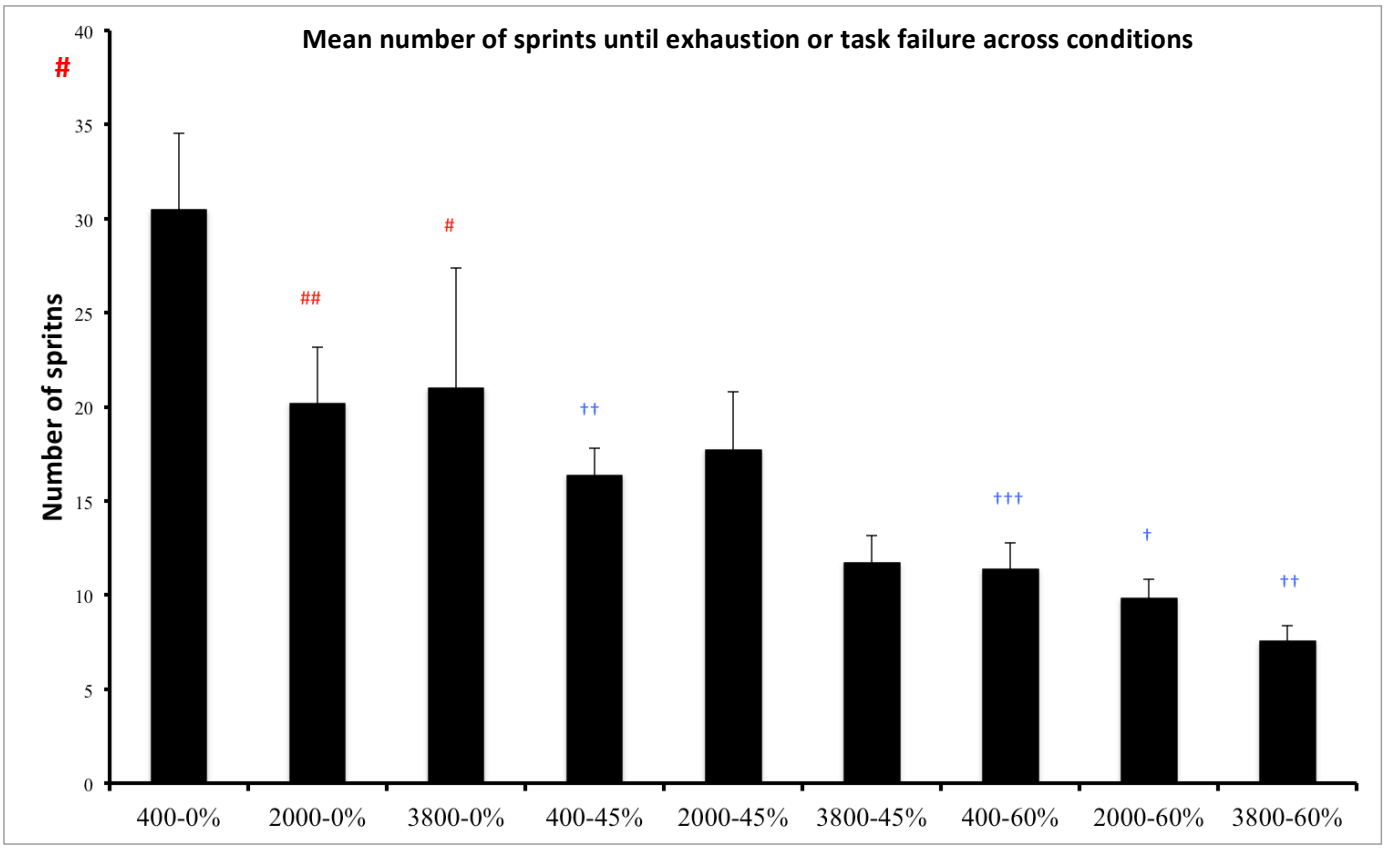

Figure 20. Mean number of sprints performed until exhaustion or task failure across conditions. 400, 2000, 3800 indicate the level of hypoxia (400m, 2000m and 3800m, respectively); 0%, 45%, 60% indicate the level of BFR (percentage of AOP). # < 0.05 ## < 0.01 for differences with 400m; † < 0.05 †† < 0.01 ††† < 0.001 for differences with 0%

RPE-breathing	400m	2000m	3800m
0%	18.3	19.2	18.3
45%	17.7	17.1	16.9
60%	15.5	15.8	16.1
RPE-legs			
0%	17.7	18.5	17.6
45%	19.5	19.5	19.2
60%	19.5	19.7	19.7

Table 2. Mean values of the rating of perceived exertion (RPE) post RSA. Scale is from 6 (no exertion) to 20 (maximal exertion). From Borg, 1982.

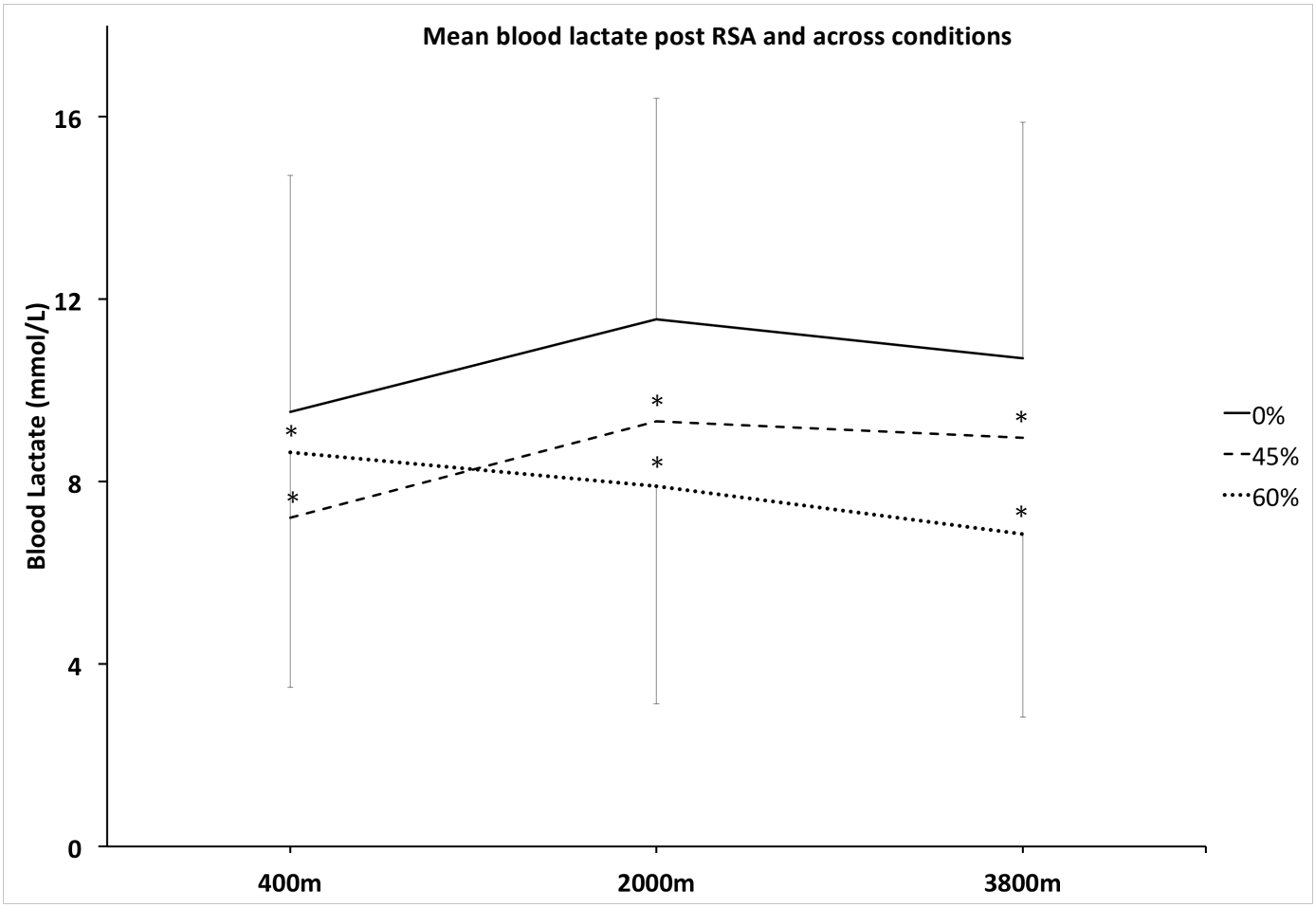

Figure 21. Mean (±SD) blood lactate concentration post RSA across condition. 400m, 2000m, 3800m indicate the three levels of hypoxia; full lines represent conditions at 0%BFR; dashed lines represent conditions at 45%BFR, dotted lines represent conditions at 60%BFR; * < 0.05 with 0%BFR.

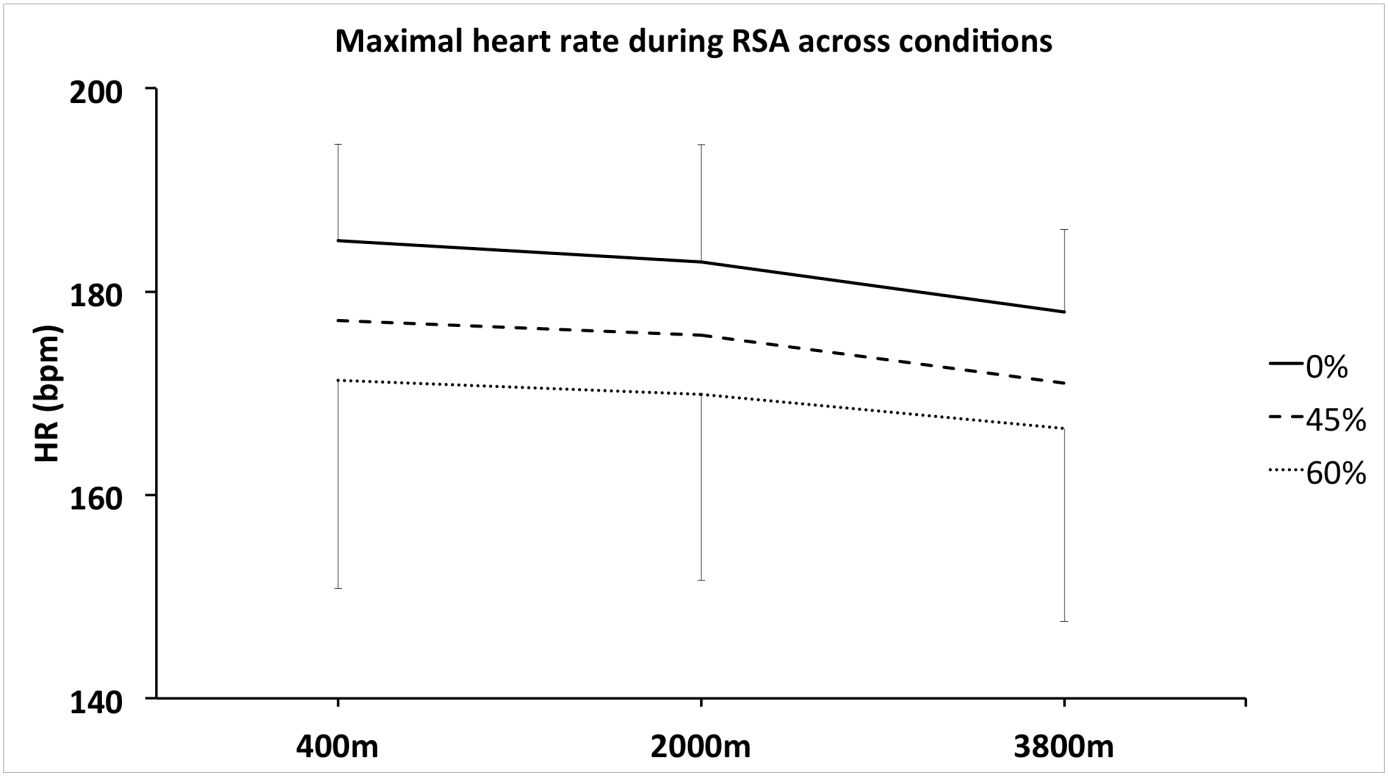

Figure 22. Mean maximal heart rate (in beat per minute) during RSA across conditions. 400m, 2000m, 3800m indicate the three levels of hypoxia; full lines represent conditions at 0%BFR; dashed lines represent conditions at 45%BFR, dotted lines represent conditions at 60%BFR.