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# Fatigue neuromusculaire induite par répétition de sprints : Etiologie et impact de l'hypoxie

Arthur Peyrard

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## **Fatigue neuromusculaire induite par répétition de sprints – Etiologie et impact de l'hypoxie.**

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## List of abbreviations

<b>ADP</b>	Adenosine diphosphate
<b>AMP</b>	Adenosine monophosphate
<b>Ampli<sub>FIRST</sub></b>	Amplitude of the first part of the M-wave
<b>Ampli<sub>SECOND</sub></b>	Amplitude of the second part of the M-wave
<b>AMT</b>	Active motor threshold
<b>ANOVA</b>	Analysis of variance
<b>AOD</b>	Accumulated oxygen deficit
<b>AOD</b>	Accumulated oxygen deficit
<b>AP</b>	Action potential
<b>aRSA</b>	Arm-cycling repeated sprint ability
<b>ATP</b>	Adenosine triphosphate
<b>ATP</b>	Adenosine triphosphate
<b>ATP<sub>ANA</sub></b>	Rate of ATP synthesis through oxidative phosphorylation
<b>ATP<sub>OX</sub></b>	Rate of ATP synthesis through oxidative phosphorylation
<b>BCAA</b>	Branched-chain amino acids
<b>BFR</b>	Blood flow restriction
<b>Ca<sup>2+</sup></b>	Calcium
<b>Ca<sup>2+</sup></b>	Calcium
<b>CBF</b>	Cerebral blood flow
<b>CHO</b>	Carbohydrate
<b>CHT</b>	Continuous hypoxic training
<b>CK</b>	Creatine kinase
<b>Cl<sup>-</sup></b>	Chlorure
<b>ClC-1</b>	Sarcolemmal chlorure channels
<b>CMEP</b>	Cervicomedullary motor evoked potentials
<b>CNS</b>	Central nervous system
<b>CSA</b>	Cross sectional area
<b>CV</b>	Coefficients of variation
<b>DA</b>	Dopamine
<b>Db10</b>	Force response obtained with paired stimuli at 10 Hz
<b>Db100</b>	Force response obtained with paired stimuli at 100 Hz

<b>DHPR</b>	Dihydropyridine receptors
<b>DL</b>	Diffusing capacity of the lung
<b>D<sub>M</sub></b>	Diffusion process
<b>DOMS</b>	Delayed onset muscle soreness
<b>Dur<sub>FIRST</sub></b>	Duration of the first part of the M-wave
<b>Dur<sub>SECOND</sub></b>	Duration of the second part of the M-wave
<b>EC</b>	Excitation-contraction
<b>EEG</b>	Electroencephalography
<b>EMG</b>	Electromyography
<b>EMS</b>	Electrical muscle stimulation
<b>ENS</b>	Electrical nerve stimulation
<b>ERT</b>	Estimated resting twitch
<b>FI</b>	Fatigue index
<b>GPX</b>	Glutathione peroxidase
<b>GR</b>	Glutathione reductase
<b>GSH</b>	Glutathione
<b>GSSG</b>	Oxidized glutathione
<b>H<sub>2</sub>O<sub>2</sub></b>	Hydrogen peroxide
<b>HFF</b>	High-frequency fatigue
<b>HH</b>	Hypobaric hypoxia
<b>HIF-1<math>\alpha</math></b>	Hypoxia-inducible factor 1-alpha
<b>HL</b>	High-load
<b>HR</b>	Heart rate
<b>HRT</b>	Half relaxation time
<b>HVR</b>	Hypoxic ventilation response
<b>ICC</b>	Intra-class correlation coefficient
<b>IHE</b>	Intermittent hypoxic exposure
<b>IHIT</b>	IHE during interval training
<b>IHT</b>	Interval hypoxic training
<b>IMP</b>	Inosine monophosphate
<b>IPC</b>	Ischemic pre-conditioning
<b>ITT</b>	Interpolated twitch technique

<b>K+</b>	Potassium
<b>KAATSU</b>	Blood flow restriction
<b>KATP</b>	ATP- sensitive K <sup>+</sup> channels
<b>La</b>	Blood lactate concentration
<b>LDH</b>	Lactate dehydrogenase
<b>LF / HF</b>	Low frequency fatigue ratio
<b>LFF</b>	Low-frequency fatigue
<b>LHTH</b>	Live high-train high
<b>LHTHL</b>	Live high-train high and low
<b>LHTL</b>	Live high-train low
<b>LHTLH</b>	Live high-train low and high
<b>LL</b>	Low-load
<b>LLTH</b>	Live low-train high
<b>LLTL</b>	Live low-train low
<b>LT4</b>	Lactic Threshold
<b>MCT-4</b>	Monocarboxylate transporter-4
<b>MEP</b>	Motor evoked potential
<b>MHC</b>	Myosin heavy chain
<b>Mmax</b>	Amplitude of the M-wave
<b>MNS</b>	Magnetic nerve stimulation
<b>MRI</b>	Magnetic resonance imagery
<b>MU</b>	Motor unit
<b>MUAP</b>	Simulated motor unit action potential
<b>MVC</b>	Maximal voluntary contraction
<b>NA</b>	Noradrenaline
<b>Na+</b>	Sodium
<b>NAC</b>	N-acetyl cysteine
<b>NH</b>	Normobaric hypoxia
<b>NH</b>	Normobaric hypoxia
<b>NIRS</b>	Near-infrared spectroscopy
<b>NME</b>	Neuromuscular function evaluation
<b>NMF</b>	Neuromuscular function

<b>NO•</b>	Nitric oxide
<b>NOX2</b>	NADPH-oxydase 2
<b>O<sub>2</sub><sup>•-</sup></b>	Superoxide anions
<b>OBLA</b>	Onset of blood lactate accumulation
<b>OCC</b>	Pressure used induced total occlusion
<b>OH•</b>	Hydrogen radical
<b>ONOO<sup>-</sup></b>	Peroxynitrite
<b>PAP</b>	Post activation potentiation
<b>Pcr</b>	Phosphocreatine
<b>Pcr</b>	Phosphocreatine
<b>PGC-1α</b>	Peroxisome proliferator-activated receptor gamma coactivator 1α
<b>Pmax</b>	Maximal Power Output
<b>Pmean</b>	Mean Power Output
<b>PO</b>	Power Output
<b>Pt</b>	Pic twitch
<b>RF</b>	Rectus femoris
<b>RI</b>	Reuptake inhibitor
<b>RMS</b>	Root mean square
<b>RNS</b>	Reactive nitrogen species
<b>ROS</b>	Reactive oxygen species
<b>RPE</b>	Rate of perceived exhaustion
<b>RSA</b>	Repeated sprint ability
<b>RSE</b>	Repeated sprint exercise
<b>RSH</b>	Repeated sprint training in hypoxia
<b>RSH</b>	Repeated sprint training in hypoxia
<b>RSN</b>	Repeated Sprint Training in Normoxia
<b>RST</b>	Repeated sprint test
<b>RTH</b>	Resistance training in hypoxia
<b>Sbest</b>	Best sprint performance
<b>SD</b>	Standard deviation
<b>Sdec</b>	Decrement score
<b>SEM</b>	Standar error of mean

<b>SERCA</b>	Sarcoplasmic reticulum calcium pumps
<b>SFAP</b>	Single fiber action potential
<b>SICI</b>	Intracortical inhibition
<b>SIH</b>	Sprint Interval Training in hypoxia
<b>SIT</b>	Superimposed twitch
<b>SOD</b>	Superoxide dismutase
<b>SP</b>	Silent period
<b>SR</b>	Sarcoplasmic reticulum
<b>SSC</b>	Stretch-shortening cycle
<b>Sworst</b>	Worst sprint performance
<b>T-tubules</b>	Transverse tubules
<b>T0</b>	Theoretical torque produced at null velocity
<b>TMS</b>	Transcranial magnetic stimulation
<b>TPT</b>	Time to reach Pic Twitch force
<b>TRP</b>	Tryptophan
<b>TYR</b>	Tyrosine
<b>V0</b>	Maximal theoretical angular velocity produced under zero load
<b>VA</b>	Voluntary activation
<b>VA<sub>TMS</sub></b>	Voluntary activation calculated with TMS
<b>VHL</b>	Voluntary hypoventilation
<b>VHL</b>	Voluntary hypoventilation at low lung volume
<b>VL</b>	Vastus lateralis
<b>VM</b>	Vastus medialis
<b>VO<sub>2</sub></b>	Oxygen uptake
<b>VO<sub>2max</sub></b>	Maximal oxygen uptake
<b>Yo-yo IR</b>	Yo-Yo IR test
<b>θ V<sub>c</sub></b>	Time required for the O <sub>2</sub> to combine with hemoglobin
<b>[tHb]</b>	Total hemoglobin/myoglobin
<b>2,3-BPG</b>	2,3-Bisphosphoglyceric acid
<b><sup>31</sup>P-MRS</b>	Phosphorous magnetic resonance spectroscopy
<b>5-HT</b>	Serotonin

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# **General introduction**

There is no need for any scientific background to acknowledge the existence of fatigue. Who hasn't experienced tiredness, exhaustion or the sensation of weakness associated with any task that has been too long, too intense or too strenuous?

Fatigue can rely on so many different features that one of the most challenging aspects when trying to characterize it, is probably to provide an adequate definition. The field of sports sciences has always been driven by the desire to assess human performance and to understand its limits and this is probably why fatigue has been part of its early development and its definition often relies on the quantification of a force, or power decrement. Pioneers work (Mosso 1891; Hill and Kupalov 1929) have been focusing their interest on physiological changes within the muscle associated with exercise-induced fatigue which led the scientific community to believe that performance was mainly limited through peripheral phenomenon linked to lactic acid concentration. For years, the study of fatigue was limited to muscle compartment and performed on isolated muscle fibers. However, voluntary contractions don't start from the neuromuscular junction but originate from the brain and any failure in the chain of command from the cortex to the muscle is likely to induce a diminution in the force or power produced by the muscle itself. Merton (1954) proposed the use of superimposed electrical stimulation to assess the ability to fully recruit a considered muscle and quantify a lack of motor drive from the central nervous system, typically named central fatigue.

It is now rather established that fatigue originates concomitantly from both peripheral and central compartment (Bigland-Ritchie et al. 1984). Furthermore, the progressive development of new methodological approach (e.g. electromyographic recordings, muscle biopsies, magnetic stimulation, magnetic resonance imaging...) have widened our understanding of neuromuscular function and fatigue associated with exercise in healthy but also in clinical populations. Therefore, peripheral fatigue is no longer associated with the lactic acid concentration but rather to an impairment of the muscle membrane excitability and the excitation-contraction coupling due to the accumulation of byproducts of the muscle contraction. Metabolic changes within the muscle have also been linked to central fatigue via muscle afferents feedback impacting the central nervous system at the spinal and supraspinal levels (Gandevia 2001). This confirms the close link between peripheral and central development of fatigue and reaffirms the complexity of its development.

The methodology currently used to assess neuromuscular function is conceptually the same as the one proposed by Merton (1954) using isometric contractions. This probably contributes to explain why most of the literature investigating neuromuscular fatigue in humans employs isometric contraction to induce fatigue. This is rather problematic as it appears that fatigue origin depends on the type of activities performed, often referred as the task dependency principle, and many parameters have been shown to influence fatigue etiology such as intensity, duration, and mode of exercise (Enoka and Stuart 1992). It is therefore complicated to transpose findings on fatigue etiology from small muscle mass involved in isometric contraction to whole-body exercise using a multitude of synergist muscles working together.

Repeated sprint exercise has been shown to be a good index of performance in a variety of intermittent sports and a multitude of different approaches have been proposed to improve athlete's capacity to maintain a high level of power production through the repetition of efforts. This type of exercise is very strenuous for the neuromuscular function and is known to induce marked peripheral fatigue in the very early stages with the progressive development of the central compartment.

Recently, a new method involving the use of hypoxia added to repeated sprint training session has been developed and provides interesting results regarding performance improvement. In parallel, hypoxia is responsible for a cascade of physiological adaptation leading to an impairment of cerebral and muscle oxygenation and impact the development of neuromuscular fatigue exacerbating its central aspect, thus, a potential beneficial feature of repeated sprint training in hypoxia could arise from adaptations through the central nervous system.

Therefore, this thesis will focus on the fatigue associated with repeated sprint exercise, and its impact on the neuromuscular function when performed in a deprived-oxygen environment.

Three main chapters articulate this manuscript.

Firstly, an overview of the literature is presented, defining the main concepts on which this work is based and offering scientific evidence on the actual knowledge regarding repeated sprint exercise, the manifestation of fatigue and the impact of environmental factors on its etiology.

In a second chapter, the experimental work accomplished during the last years is compartmented into two main studies aiming to provide answers to a lack of experimental data or methodological flaws in the current literature.

Finally, the results obtained are synthesized, discussed and put into perspectives in the last chapter of this manuscript.

# **Chapter 1 - Background**

# 1 Repeated sprint exercise, definition and factors limiting performance

A lot of intermittent activities (soccer, rugby, and hockey among others) require participants to produce repeatedly maximal or near maximal efforts of short duration with a brief incomplete recovery period. Therefore, the athlete ability to repeat such effort is a strong aspect of physical performance during competitions (Spencer et al. 2005). This has been named repeated sprint ability (RSA) and a variety of protocols have been developed to determine it (Dawson et al. 1993; Aziz et al. 2000; Spencer et al. 2005).

The next section will try to give more insight and provide scientific backward from the literature regarding the definition of RSA, the differences between RSA modalities and an introduction to the fatigue induced by such test and how it can be measured.

## 1.1 Definition

While the term “sprint” is often used to characterized short “supramaximal” exercise, it sometimes refers to exercise lasting ~30 s (Bogdanis et al. 1996; Ball et al. 1999). For the purpose of this thesis, a sprint will be defined as a brief exercise where maximal workout (power/speed) can be sustained, or nearly sustained until the end of exercise (figure 1.A; Girard, Mendez-Villanueva, and Bishop 2011). Furthermore, when talking about repetitive sprints, it is important to differentiate two modalities, repeated, and intermittent. Intermittent sprints are interspaced with a period of recovery long enough (60 to 300 s) to allow near complete restoration of performance between each sprint whereas repeated sprints are characterized by a shorter period of incomplete recovery (<60 s) inducing inevitable diminution in performance over the repetitions (figure 1B).

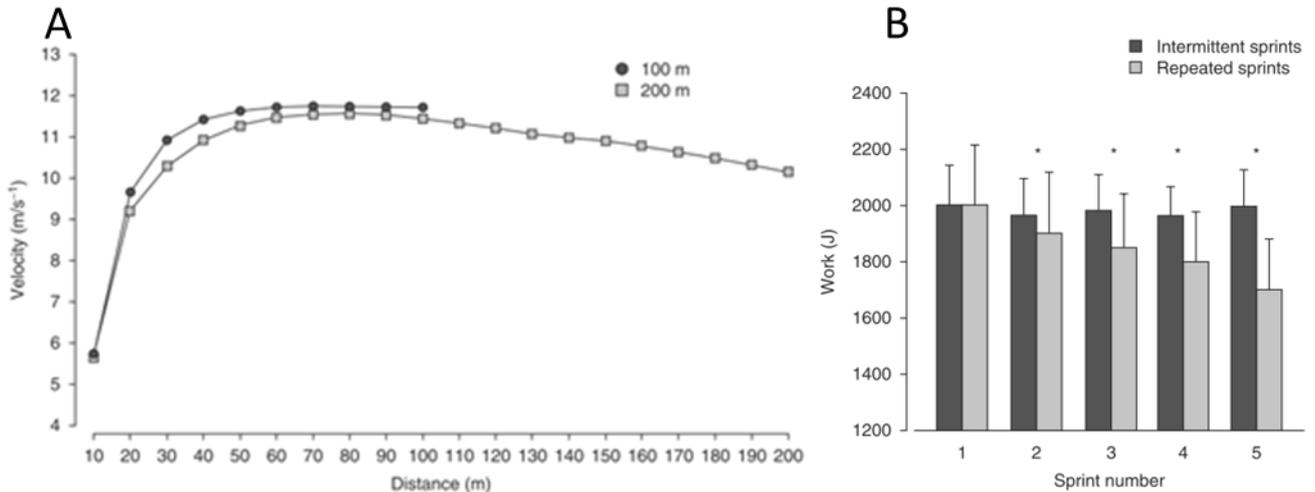


Figure 1. Panel A: speed profiles of the 100 m and 200 m world record for men. In this example, the 100 m would be considered as a sprint exercise whereas the 200 m would be an all-out exercise. Panel B: Illustration of the impact of recovery on 4 s cycling sprint performance. Intermittent sprints were interspaced with 120 s of recovery whereas repeated sprints were performed every 30 s. \* show significant diminution compare to the first sprint ( $P < 0.05$ ). Adapted from Girard et al, (2011).

Fatigue is a complex phenomenon involving several processes and section 2.2 will try to provide an overview of it, but before that, it can already be mentioned that during repeated-sprint exercise (RSE), fatigue will rapidly develop as soon as the first sprint (figure 1.B shows a significant diminution in work already during the 2<sup>nd</sup> sprint). This fatigue can be induced by numerous factors as an inappropriate motor command from the brain to the accumulation of byproducts of the contraction inside the muscle.

Overall, a great diversity of tools exists to quantify fatigue during RSE and the next part will present some of them as well as some of its major limiting factors.

## 1.2 The manifestation of fatigue and limiting factors

### *Fatigue Indices*

Depending on the exercise modalities (i.e. cycling or running), fatigue will manifest as a reduction in power output, speed or an increase in time. To quantify the RSA researchers proposed different indices. First, the best sprint performance (i.e. it has to be obtained from the first sprints, otherwise it probably implies that the participants didn't give is best), then the

average sprint performance (considering the whole RSE) and lastly, a fatigue score index that can be calculated in various ways. Two different methods of calculating this index appear to prevail in the literature. The first is named Fatigue index (FI) and represents the drop of performance from the best sprint to the worst during the RSE (equation 1).

$$FI = \frac{100 \times (S_{best} - S_{worst})}{S_{best}}$$

*Equation (1)*

Where  $S_{best}$  is the best sprint performance and  $S_{worst}$ , the worst sprint performance.

The second indicator is named decrement score (Sdec) and compares the overall sprint performance to an “ideal performance” (i.e. as if the participant were able to reproduce his best sprint during the whole session) as shown in equation 2.

$$Sdec = \left(1 - \frac{S_{mean}}{S_{best}}\right) \times 100$$

*Equation (2)*

Where  $S_{mean}$  is the mean performance of all sprints. The advantage of Sdec is that it considers the totality of the RSE whereas FI only depends on the best and worst sprint. Furthermore, Glaister, (2008) concluded that Sdec provided the best reliability to quantify fatigue over RSE. Nevertheless, it should be noted that they obtained an ICC of 0.75, which stand as good, by comparing 2 RSE with different recovery period (35 vs 65 s). FI and Sdec should always be interpreted with care as they are greatly dependent on the best (usually the first) sprint. Therefore, any pacing strategy will have drastic effects on this parameter.

Interestingly, the initial performance is relatively well correlated with the subsequent loss of power/speed over the RSE (Bishop et al. 2003; Mendez-Villanueva et al. 2008). This can be explained by the fact that the athletes producing higher power output are likely the ones with the highest energy consumption. Because the anaerobic pathway is the major supplier of ATP, the metabolites are likely to accumulate more inside the muscle leading to an exacerbated level of fatigue (see section 2.2 for further details). This is supported by the fact that athletes with lower anaerobic power reserves (i.e. less reliance on anaerobic metabolism) have a relative higher fatigue resistance.

An interesting factor having an impact on RSE is the time of the day, indeed, performing RSE in the afternoon increased the performance of the first sprint without impacting the

subsequent ones (Racinais et al. 2005). This leads to a sharper decrement and therefore is associated with an impaired RSA when calculating the Sdec or FI (i.e. higher best sprint whereas the rest of the test is identical). This further highlight the fact that those indices should be interpreted with care (always the three together, best sprint, an average of all the sprint and finally, fatigue score) and that RSE should be performed at a similar time of day if they are to be compared to each other.

### 1.2.1 Energy supply

#### *Pcr resynthesis*

Once the relatively small amount of adenosine triphosphate (ATP) available has been consumed, it is resynthesized from the stock of phosphocreatine (Pcr). The latter represent roughly  $80 \text{ mmol} \cdot \text{Kg}^{-1}$  of dry muscle and the maximal turnover rates is of approximately  $9 \text{ mmol} \cdot \text{Kg}^{-1}$  of dry muscle (Hultman and Sjöhölm 1983) and is, therefore, a very important component of RSA. For instance, Gaitanos et al. (1993) showed that after a single 6 s cycling-sprint, Pcr stores were depleted to 43% of resting values. They also showed, that after a repetition of ten 6 s sprints with 30 s recovery, Pcr store was further diminished to 16% of baseline values. Those results were confirmed in another study by Dawson et al. (1997) using six sprints and a recovery period of 24 s. The necessary time to fully replenish the Pcr content is about 5 min (Bogdanis et al. 1995), then it can be postulated that shorter recovery time will lead to performance decrement over the RSE. The Pcr diminution reported by Gaitanos et al. (1993) was obtained through biopsies and therefore doesn't differentiate the muscle fiber types (see section 2.2.1.2) and, it has been shown that type-II muscle fibers have higher Pcr depletion compare to type-I muscle fibers (Söderlund et al. 1991; Karatzaferi et al. 2001b). Therefore, during RSE, it is possible that some of the type-II fibers deplete even more their Pcr store, leading to the performance diminution (Sahlin and Ren 1989).

Because recovery periods during RSE are less than 60 s, the depletion of ATP/Pcr is likely to be one of the major factors limiting performance. This even led several authors to propose that due to the similar time course of recovery between Pcr and power output, the latter was limited by Pcr availability (Bogdanis et al. 1995). Furthermore, creatine supplementation has been associated with an increased Pcr resynthesize, less accumulation of metabolites and a

better RSA performance (Yquel et al. 2002). This would suggest that RSA performance is strongly related to Pcr resynthesize.

### *Anaerobic glycolysis*

During an isolated 6 s sprint, 40% of the total energy is supplied by the anaerobic glycolysis (figure 2.A). Even if lactate concentration gets relatively high when sprints are repeated, glycolysis contribution progressively decreased as the sprints are repeated (Gaitanos et al. 1993). Indeed, Gaitanos et al. (1993) reported an 8-fold decrease from the first to the tenth 6 s sprints (interspaced with 30 s recovery) in the ATP production from glycolysis while power output still represents 73% of the first sprint. The link between anaerobic glycolytic rate and RSA performance is however controversial. As stated earlier, subjects with the greatest power output decrement during RSE were the one having the highest glycolytic rate during the first sprint (Bishop et al. 2004) but also the highest isolated sprint performance (Bogdanis et al. 1995). This way of thinking can lead to the interpretation that training, which improves the ability to supply ATP through the anaerobic pathway, could lead to an impairment of the RSA. Thus, further researches are needed to evaluate if increasing the impact of anaerobic metabolism impact both the development of higher isolated sprint performance and the ability to repeat them.

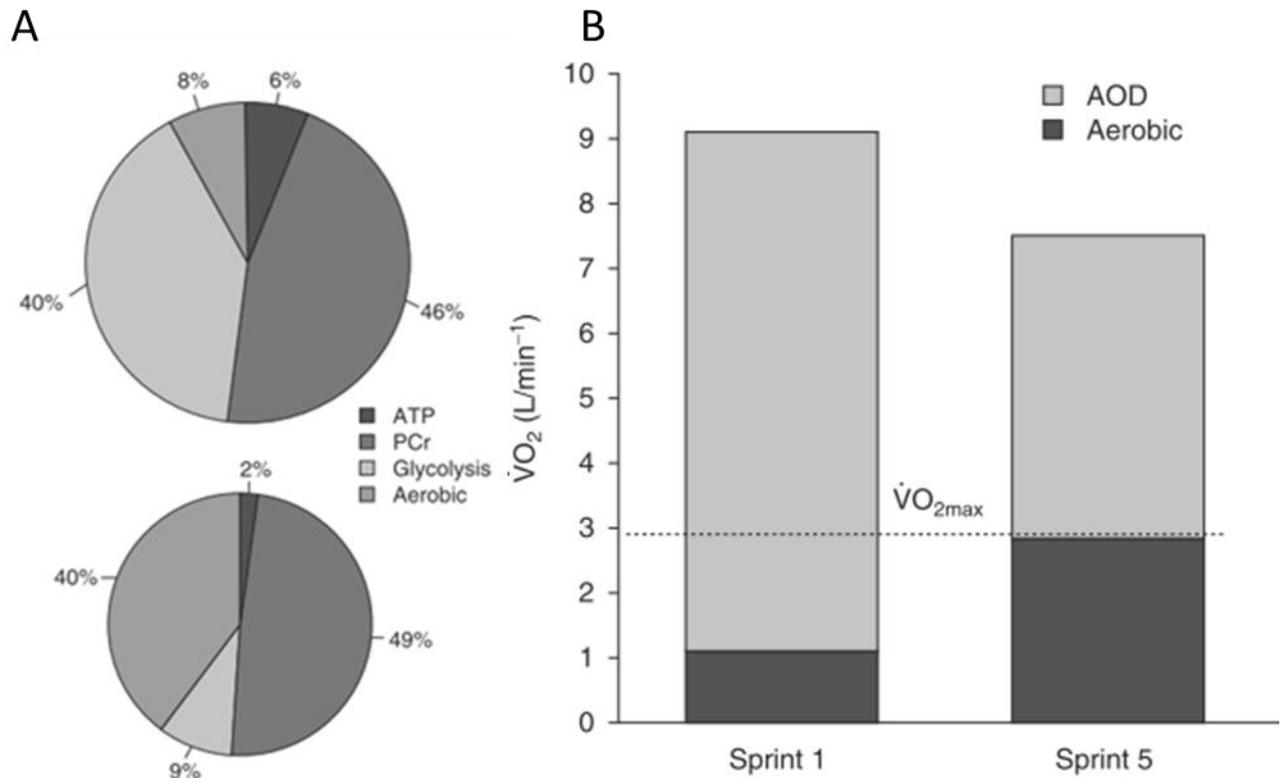


Figure 2. Panel A shows the changes in metabolism during the first (upper) and the last (lower) sprint of repeated sprint exercise (RSE). Note that the area of each circle represents the total absolute energy used during each sprint. ATP, Adenosine triphosphate; Pcr phosphocreatine. Panel B represents the increase in the aerobic contribution through RSE. The dashed line represents the maximal oxygen uptake ( $\dot{V}O_{2max}$ ). AOD, accumulated oxygen deficit;  $\dot{V}O_2$ , oxygen uptake. Adapted from Girard et al. (2011b).

### *Oxidative metabolism*

During an isolated sprint, oxidative phosphorylation accounts for less than 10% of the total amount of energy required, however when sprints are repeated, this amount progressively goes up to 40% during the last sprints. Furthermore, athletes with moderate to high value of  $\dot{V}O_{2max}$  may reach it during the latter sprints (McGawley and Bishop 2008). This suggests that  $\dot{V}O_{2max}$  could limit the aerobic contribution during RSE and that appropriate training could enhance the aerobic component during the last sprint of the RSE, thus diminishing fatigue and improving RSA performance. This could explain the significant correlation ( $r = -0.45$  to  $-0.75$ ) observed between RSA performance indices (Sdec, FI) and  $\dot{V}O_{2max}$  (Bishop and Spencer 2004; Bishop and Edge 2006; Rampinini et al. 2009). However, those results are to take with care as other studies reported a non-significant correlation between those two

parameters (Aziz et al. 2007; Castagna et al. 2017). The discrepancy in the results could arise from differences in the aerobic fitness of the tested population, a relatively homogenous population within the study could mask a potential correlation. This also brings back the relationship between the power performed during the first sprint and the overall RSA performance and one could argue that because endurance-trained participants have a relatively lower maximal power production capacity, this could lead to a better RSA performance when considering Sdec. However, this is speculative and further studies should try to dissociate those 2 parameters. Another possibility to explain the lack of correlation between Sdec and  $VO_{2max}$  is the fact that the latter is more limited by the capacity of the cardiovascular system to deliver oxygen to the muscle (Bassett and Howley 2000). But during maximal exercise, aerobic capacity seems to be limited by the oxygen utilization through the mitochondria rather than its delivery per se, this has been shown by Calbet et al. (2015) using 30 s all-out exercise. Therefore, further studies should investigate this parameter during RSE but this argument is supported by an inverse relation between FI and the maximal ADP-stimulated mitochondrial respiration directly measured in the muscle fiber (Thomas et al. 2004).

Interestingly, participants who had the highest decrease in systemic oxygen saturation during RSE (20 × 5 s-sprints) were also the ones with the greatest score decrement and the lowest  $VO_{2max}$  (Billaut and Smith 2010). Other indirect markers of oxidative capacities have been correlated to RSA performance such as  $VO_2$  kinetics (Dupont et al. 2005, 2010) or the speed associated to the onset of blood lactate accumulation (da Silva et al. 2010). For example, Dupont et al. (2005) reported that participants with faster  $VO_2$  kinetics during submaximal exercise (the necessary time for oxygen consumption to reach a steady state) were also the ones with the lowest performance decrement during 15 × 40 m-sprint with 25s recovery.

Recently, the technological development of near-infrared spectroscopy (NIRS) techniques allows the investigation of localized tissue oxygenation in a noninvasive manner during exercise. Authors reported that the amplitude of the deoxygenation level associated with sprint repetitions remained constant through the test [10 × 6 s-sprint (Racinais et al. 2007), or 10 × 10 s-sprint (Billaut and Smith 2010) with 30 s of recovery]. This indicates that the oxygen extraction would not be impacted during RSE. Study with recovery times long enough between sprints reported that the muscular oxygenation was maintained through the test (Smith and Billaut 2010; Billaut and Buchheit 2013) whereas, with a shorter recovery period, Racinais et al. (2007) reported a progressive augmentation of the concentration of deoxyhemoglobin. After an 8-weeks training protocol, Buchheit and Ufland (2011) reported

an increase in the rate of muscle re-oxygenation strongly correlated to the increase in RSA performance. This is further supported by Billaut and Buchheit (2013) who showed that the impaired re-oxygenation during RSE induced by hypoxia was associated with a diminution of total work performed compare to normoxia. Finally, Brocherie et al. (2015) showed that the RSA performance was directly correlated to re-oxygenation kinetics during RSE (6 × 35 m-sprint with 10 s recovery). This suggests that muscle re-oxygenation could also be a limiting factor of RSA.

To summarize at the beginning of the RSE, Pcr and glycolysis mainly contribute to supply the high energy demand of the task. Rapidly, Pcr stores are depleted because of the too short period of recovery and glycolysis is progressively inhibited. Consequently, the relative amount of energy provided by the aerobic metabolism increases whereas the total amount of energy and the associated power production decreases through the RSE.

### **1.2.2 Mechanical factors**

Changes in mechanical behavior of the muscle (mainly due to stiffness regulation) may directly impact RSA performance, especially when considering running exercise (Ross et al. 2001). It is generally accepted that a stiffer system allows a better energy transfer during the concentric phase of the movement (Farley et al. 1991; Chelly and Denis 2001) reported a close relationship between sprint running performance and stiffness and it has therefore been postulated that its alteration could be an important factor regarding the RSA performance. Morin et al. (2006) previously showed that the impairment of the spring-mass model properties (used to characterized lower limb properties in runners) was associated to a diminution in performance during repeated all-out exercise (i.e. 4 × 100 m with 2 min recovery). Girard et al. (2011b) also reported alteration in leg-sprint behavior during 12 × 40 m-sprints interspaced with 30 s of recovery. It seems that the lower stride frequency and the concomitant diminution in RSA performance would result from an increased vertical displacement of the center of mass (decreased vertical stiffness) rather than a change in leg stiffness. Morin et al. (2011a) also showed that during RSE consisting of 4 sets of 5 × 6 s-sprints, the ability to properly orient the force (i.e. horizontally and not vertically) was reduced. Therefore, a technical aspect of sprinting could also be impacted during RSE and further studies investigating the impact of biomechanical factors on performance are required.

### 1.2.3 Omitted factors

Two really important factors limiting performance have been omitted in this section because they will be later discussed in a more detailed manner. The development of muscular fatigue through the accumulation of byproducts within the muscle or because of inappropriate motor command from the brain will be presented in section 2.

The other factor concerns the environmental component. Indeed, when RSE is performed under added stress such as hot or hypoxic condition, the performance will be impacted. A full section will consider the physiological effect of hypoxia and the associated change when combined with RSE. The impact of temperature is outside the scope of this thesis but one could refer to the review from Girard, Brocherie, and Bishop (2015)

### 1.2.4 Task dependency

Inevitably, the fatigue experienced during RSE will be impacted by the exercise modalities in which the test will be performed, this is referred as task-dependency and is a well-known component of fatigue-based studies (Enoka and Stuart 1992).

#### *Cycling vs Running*

For example, differences in fatigue origin have been reported when comparing cycling vs running RSE (Rampinini et al. 2014; Tomazin et al. 2017). However, there is some discrepancy in the literature as some studies reported higher decrement in performance in cycling modalities compare to running (Girard et al. 2011a; Rampinini et al. 2014) Whereas other reported higher decrement in running compare to cycling (Tomazin et al. 2017). Tomazin et al. (2017) reported a decrease from the first to the last sprint on normalized power produced during both activities (i.e. watt per kilogram). Their RSE consisted in 4 sets of 5 x 6 s-sprint with 24 s-recovery with 3 min recovery between sets and they showed that running

sprints led to a diminution of ~29% whereas cycling sprint led to a decrease of ~17% of peak power output (which is in line with previous studies). In their study Rampinini et al. (2014), had participants performed a 5 × 6 s-sprint 30 s-recovery RSE. They calculated the decrement score from the mean power output (cycling) and the mean sprint time (running) and showed that Sdec running was 4.5% and Sdec cycling was 14%. Those differences might arise from the different mode of calculation (Sdec on mean power vs FI on peak power) as both FI and peak power values are less reliable compared to Sdec and mean power. However, it appears more logical to compare normalized power in both activities rather than sprint time vs power output. Moreover, the huge disparity between both results is unlikely to be explained only by the differences in calculation mode and the RSE modality potentially played a role as some participants in the Tomazin et al. (2017) study were unable to complete the running protocol. Therefore, further studies are needed in order to answer this question. Both studies also compared fatigue etiology and once again, their findings are not the same. Whereas Tomazin et al. (2017) reported the same amount of peripheral fatigue but central fatigue only in the running condition (see section 2.2 for details); (Rampinini et al. 2014) reported exacerbated peripheral fatigue and rate of perceived exhaustion (RPE) with cycling-RSE despite similar physiological responses (i.e. same concentration of blood lactate, and H<sup>+</sup>).

Taken together these results highlights the need for further researches to establish the differences between cycling and running sprints.

### *Arm-cycling vs Leg-cycling*

More recently, researchers have started to investigate the impact of RSE on upper limbs, probably for different reasons. First, RSA of the arm is of interest in many sports; Sandbakk et al. (2015) et Kamandulis et al. (2018) recently reported that a 4 weeks training period, with 3 sessions a week including three rounds of 14 × 3 s of all-out punches increased punching forces and maintenance of punching frequency of competitive boxers. And secondly, the use of upper limbs also allows researchers to investigate fatigue etiology with specific tools (i.e. cervicomedullary motor evoked potentials) easier to apply on the arm due to methodological considerations (Pearcey et al. 2016). Recently, Halperin et al. (2018) compared the upper and lower body responses to intermittent (i.e. 180 s recovery) and repeated (i.e. 30 s of recovery) cycling sprint consisting of 10 × 10 s-sprint, the next paragraph will only focus on

the comparison between upper and lower limb and the impact of recovery period will be discussed later. Raw power was inevitably higher for the legs compared to the arms, but interestingly, this wasn't associated with differences in heart rate (HR) or RPE. When normalized (i.e. as the first sprint being 100%) no differences were found between both sprint modalities (i.e. same HR, RPE and power decrement) except for the maximal voluntary contraction force (MVC) developed pre- and post- RSE which showed less impairment in the arms compared to the legs. Those results are quite surprising considering the differences usually observed between arm and legs in muscle typology (Koppo and Bouckaert 2001), force generation capacity (Harbo et al. 2012) and smaller muscle mass (Janssen et al. 2000). Indeed, when comparing upper and lower body exercise, the literature usually reports higher HR associated with the leg, however, exercise modalities are often of longer duration and lower intensity (Schneider et al. 2002; DeJong et al. 2009; Salwa B et al. 2014). Therefore, it is possible that the short exercise duration associated with a work-to-rest ratio of 1:3 prevented differences in systemic responses (i.e. HR) between upper and lower limb. The differences regarding MVC are interesting, however, because authors didn't associate any other measurement to it, it is quite hard to analyze them. One integrative way of interpreting those results would be to consider that arm-cycling and leg-cycling sprint probably don't rely respectively on elbow flexors and knee extensors in the same proportion (i.e. maybe leg-cycling are more reliant on knee extensors than arm-cycling is on elbow flexors...). But other parameters should be considered as neuromuscular fatigue has been shown to rely on different mechanisms depending on the considered limb. For instance, Gruet et al. (2013), reported that upper limbs were more sensible to spinal adaptation. J. A.L. Calbet et al. (2015) showed that upper vs lower limbs exercise elicited different hemodynamics adaptation during continuous cycling that could impact differently fatigue etiology. Lastly, when comparing neuromuscular fatigue etiology between muscle groups, conflicting results are reported. Neyroud et al. (2013) showed similar fatigue in different muscle group (i.e. plantar flexors, thumb adductors, elbow flexors, and knee extensors) after a sustained contraction at 50% of MVC. Senefeld et al. (2013) reported that the loss of MVC was 16% higher in the elbow flexors compare to the knee extensors after 90 submaximal isotonic contractions performed at maximal shortening velocity. And lastly, Vernillo et al. (2018) reported a higher decrease in MVC from the knee extensors compare to the elbow flexors after a 2 min-sustained MVC with the leg vs arm but the peripheral fatigue was higher in the elbow flexors. Taken together these results suggest that fatigue etiology can hardly be compared if exercise modalities are

not the same and therefore further studies comparing directly the effect of arm vs. leg cycling sprints are required to draw any solid conclusion.

### *Number of sprints, recovery duration and modality*

Another factor of prime importance is obviously the number of repetitions performed during the RSE, the duration of the sprint and the associated recovery pattern (i.e. duration, nature). For example, Balsom et al. (1992) compared the impact of three different recovery time (30,60 and 120 s) on RSE consisting of 15 × 40 m. They showed that the mean running speed was impaired from the sprint number 11 with 120 s recovery whereas it occurred after the 7<sup>th</sup> sprint with 60 s, and after the 3<sup>rd</sup> with 30 s recovery. The acceleration time was only impacted in the 30 s-recovery condition. Blood lactate was similar during the RSE but increased more in the 30 s-recovery condition at post 4 min. VO<sub>2</sub> was stable through the whole RSE with 60 and 120 s recovery whereas it increased from sprint 1 to sprint 7 and was higher with 30 s-recovery. Overall, those results have been confirmed several times and it can be affirmed that recovery duration associated with RSE is one of the major factors impacting performance.

To characterize RSE, the authors proposed to use the work-to-rest ratio, a ratio of 1:4, indicates that the recovery time is 4 times longer than the sprint time. But one can wonder if it is enough to fully illustrate the RSE demand. Little and Williams (2007) compared 2 different distances with 2 work to rest ratio (15 m-sprint with a 1:4 ratio; 15 m-sprint with a 1:6 ratio; 40 m-sprint with a 1:4 ratio, and 40 m-sprint with a 1:6 ratio,). They reported a significant main effect of the work-to-rest ratio on sprint speed and blood lactate with respectively an exacerbated decrease and increase with the lowest ratio (i.e. greater speed diminution and lactate accumulation with shorter recovery). They also reported a main effect of sprint distance on both blood lactate and decrement in sprint time (i.e. Increased concentration of blood lactate with longer sprint and exacerbated decrease in sprint time with shorter sprints). These results suggest that the work-to-rest ratio paradigm is a good indicator but is not enough to illustrate the demand of RSE as the same ratio with different sprint duration/distance is likely to produce distinct outcomes.

The last point is about the nature of recovery, a recent review from Madueno et al. (2018) came to the conclusion that passive recovery reduced both physiological and perceptual demands of RSE and the subsequent performance decrement compare to active recovery.

Therefore, the use of active recovery can be a useful training overload to induce further physiological adaptations.

Regarding what's been said about task specificity, it is important to admit that most of the scientifically based knowledge concerning fatigue development during RSE comes from cycle-based studies in a laboratory environment. While it is appropriate to control and isolate specific variables, it is nonetheless quite far from what is happening on the ground. Whereas It is necessary to go further in that direction to fully understand the exact underlying mechanisms of RSE, it is also necessary to provide new tools and new reliable methods closer to real exercise modalities.

Next, section 2 will try to provide an overview of what is fatigue and how it is possible to characterize it depending on its potential origin. Then it will present how RSE can impact the etiology of fatigue development.

After that, section 3 and 4 will present environmental factors such as hypoxia and blood flow restriction that can influence RSA performance and its associated fatigue via potential physiological mechanisms.

Finally, section 5 will present a new methodological approach to improve RSA via the use of top-up stimulus from environmental factors, repeated sprint training in hypoxia.

## 2 Physiological Manifestation of fatigue

Fatigue is a universal symptom that is experienced through many different tasks or physical activities of everyday living and repeated sprint exercise is no exception. Many attempts have been trying to provide a precise definition of this phenomenon, often referring to a decline in performance (Allen and Westerblad 2001), changes in the EMG activity (Kallenberg et al. 2007) or the endpoint of sustained activity (Enoka and Duchateau 2016). Taken together, those three parameters represent the most widely used tools to detect apparition of fatigue. It is important to note that fatigue and exercise failure are two different concepts. Indeed, the later point out to the inability to sustain a demanded task and therefore only appears at a specific time point whereas fatigue refers to “a response that is less than the expected or anticipated contractile response, for a given stimulation” (Macintosh and Rassier 2002). This definition has the advantage to allow detection of progressive changes in contractile function through any type of stimulation.

When considering voluntary contraction, muscles are activated via complex pathways starting in the brain and involving information coming from different parts of it (e.g. body position from the parietal lobe, task planning from the frontal lobe...) to the motor cortex. The information will then go down through the spinal cord and reach lower motoneurons that will carry the action potential (AP) to the muscle. Although motor performance is in fine limited by the muscle ability to produce force and/or velocity resulting in a decrement of power production of the active muscle, fatigue is most of the time characterized by a decrease in maximal voluntary contraction force (MVC) and can have for origin any site involved in the programming and execution of the required muscle contraction. Figure 3 represents some of the major potential mechanisms involved in neuromuscular function (taken from Hunter 2018). Fatigue appears to be therefore a multi-factorial mechanism and is going to be impacted differently depending on the type of activity involved. This refers to the concept called “task dependency” which state that the specificity of the required task will be determinant in the relative contribution of the different mechanisms to fatigue (Enoka and Stuart 1992). Intensity, duration of exercise, mode and speed of contraction are among the variables that influence fatigue etiology. For instance, a big part of the literature used repetitions of single-joint submaximal isometric contractions (Bigland-Ritchie et al. 1984) or sustained isometric MVC (Taylor et al. 1996) as a model to investigate the different aspects of NMF. While being practical, these methods have inherent limits since they're far from

ecological activities where dynamic whole-body exercise are usually involved, resulting in greater muscle mass recruitment and cardiorespiratory demands compare to single-joint exercise (Sidhu et al. 2013). These differences are likely to exacerbate the importance of respiratory muscle work, core and brain temperature, cerebral and muscle oxygenation and therefore impacting fatigue etiology (Nybo and Nielsen 2001; Secher et al. 2008; Rasmussen et al. 2010). More specifically, (Sidhu et al. 2012) showed that corticospinal responses after submaximal sustained cycling exercise are different from single-joint exercise, again emphasizing the importance of the task dependency. However, the use of whole-body exercise to investigate fatigue also implies limitation as most of the time, a delay is necessary to adjust the experimental setup for the NMF evaluation allowing quick recovery of some parameters (Taylor et al. 2000) leading to probable underestimation misinterpretation of fatigue etiology. Among all those parameters used to characterized neuromuscular fatigue, MVC is probably the most widely spread and provides an easy way of quantification. However, it must be interpreted as a global index and can't differentiate the origin of fatigue. Before going further, the next parts will focus on defining and describing the potential sites at which can occur muscle fatigue and for clarity, the concepts of peripheral and central fatigue will be presented even if some authors prefer avoiding those terms (Enoka and Duchateau 2016). From now on, peripheral fatigue will refer to any changes acting at, or distal to the neuromuscular junction whereas central fatigue will be characterized by a decline in voluntary activation (VA) or neural drive from the central nervous system (Goodall et al. 2012). In the upcoming section, the different compartment susceptible to be impacted during fatiguing exercise will be presented. Firstly, peripheral mechanisms including membrane excitability, calcium release and sensitivity as well as force production by the actin-myosin cross-bridges will be discussed and non-invasive methods allowing their investigation will be exposed. Secondly, the impact of physical activity on central adaptation will be exposed after providing some noninvasive tools allowing its investigation. Central adaptation will be separated in spinal and supraspinal changes. Finally, the impact of repeated sprint ability test on neuromuscular function will be discussed.

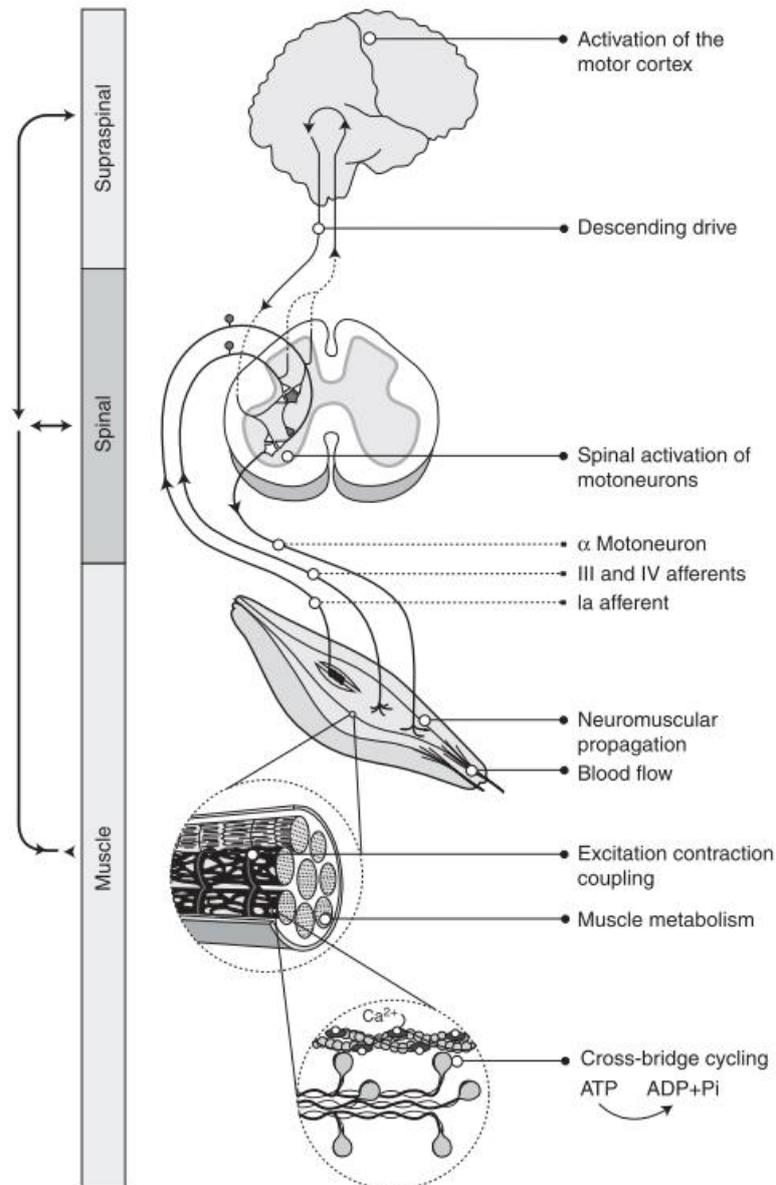


Figure 3: Schematic view of the sites and processes that lead to force generation at the muscle and can contribute to fatigue. Shown within the spinal cord schematic are selected excitatory and inhibitory pathways to the spinal cord that have been found to modulate the output of the motoneuron pool during fatiguing contractions. Taken from (Hunter 2018)

## 2.1 Peripheral mechanisms

The fatigue process induced by intense or prolonged exercise involves a direct decline of the contractile function of skeletal muscle fibers. It is generally associated with increased energy demands to fulfill the ATP resynthesize necessary to match ATP consumption by the different

compartment of the muscle. Among those consummators of ATP, the first coming in mind are of course the actin-myosin cross-bridge but other mechanisms explained below also required ATP consumption such as the sarcoplasmic reticulum calcium pumps (SERCA) or to a lesser extent the sodium-potassium ( $\text{Na}^+\text{-K}^+$ ) pumps. Inadequate energy furniture to those proteins would have dramatic consequences to homeostasis and muscle function like noncycling cross-bridges leading to unsynchronized movement or even rigor development of the muscle. Fortunately, mechanisms exist to prevent those disastrous effects through effective systems to resynthesize ATP and to decline ATP consumption. The latter mechanism obviously leads to a decline in force/powder production and is called fatigue. This section will address the cellular mechanisms inducing fatigue at or distal to the neuromuscular junction. First, the impairment in muscle membrane excitability will be discussed followed by the excitation-contraction coupling (E-C coupling) failure. In this second part, the accent will be made on decreased myofibrillar  $\text{Ca}^{2+}$  sensitivity, decreased  $\text{Ca}^{2+}$  release, and reduced force per cross bridge.

### **2.1.1 Muscle membrane excitability and extracellular $\text{K}^+$ accumulation**

To obtain muscle contraction, AP must be generated at the neuromuscular junction and propagate in both directions of the muscle fiber surface (sarcolemma). This is made possible by the opening of voltage-gated  $\text{Na}^+$  channels, through a signal from the motoneuron, leading to  $\text{Na}^+$  influx that will cause the inside of the membrane to be more positively charged (membrane depolarization). Once the inside of the membrane reached its maximal potential,  $\text{Na}^+$  channels close and  $\text{K}^+$  channels open, leading to the efflux of  $\text{K}^+$  and repolarization of the membrane. In fine, the AP will reach the transverse tubules (T-tubules) to activate the voltage sensors channels and trigger  $\text{Ca}^{2+}$  release (these steps will be described in the latter part). The  $\text{Na}^+\text{-K}^+$  pumps will restore the concentration gradient of  $\text{Na}^+$  and  $\text{K}^+$  using ATP energy so the membrane is ready to propagate a new action potential.

*Impact of fatigue on muscle membrane excitability*

Despite the action of the Na<sup>+</sup>-K<sup>+</sup> pumps, marked changes in the concentration of these ions occur during intense exercise (McKenna et al. 2008). For example, extracellular K<sup>+</sup> concentration can increase from approx. 4mM at rest to above 10 mM during high-intensity exercise (Mohr et al. 2004; Juel et al. 2017). Using continuous 60-Hz stimulation of rat fast-twitch muscles in vitro, Clausen et al. (2004) reported considerable K<sup>+</sup> efflux from the muscles associated with a huge (approx. 80%) diminution in force and M-wave in 30 s. Other experiments have shown that imposing continuous high-frequency stimulation at 80Hz on human muscles in vivo caused both force and M-wave to decline rapidly and recover substantially when the rate of stimulation was reduced to 20Hz (Bigland-Ritchie et al. 1979). These findings suggest that in some situations with imposed stimulation regimes, changes in ion concentrations, particularly K<sup>+</sup>, are not adequately compensated by the activity of the Na<sup>+</sup>-K<sup>+</sup> pump or other means and could lead to reductions in muscle excitability and force. However, during exercise under physiological condition, protecting mechanisms have been shown to exist to prevent this decrease in membrane excitability (See Allen et al. 2008 for review). For example, Nielsen et al. (2001) showed that the impaired excitability and severely (~75%) decreased force production induced by exposing isolated rat muscles to increased K<sup>+</sup> (11 mM) were almost completely reversed by acidification. This protective effect arises from the inhibition of sarcolemmal Cl<sup>-</sup> (ClC-1) channels (Pedersen et al. 2005, 2016a) facilitating the Na<sup>+</sup> induced depolarization and protecting against AP failure (Pedersen et al. 2016b). During the early stage of fatigue, acidosis inhibits ClC-1 channels which counteracts the reduced excitability caused by the K<sup>+</sup> efflux over the sarcolemma (Pedersen et al. 2009a, b; de Paoli et al. 2013). However, in the late stage of fatigue, the passive sarcolemma conductance has been found to increase in type II fibers only, promoting AP failure (Pedersen et al. 2009b) due to parallel activation of ClC-1 channels and ATP- sensitive K<sup>+</sup> (KATP) channels (Pedersen et al. 2016a). Those KATP channels stay closed if [ATP] is above 2mM (Spruce et al. 1985) therefore they only open at some advanced level of fatigue. Taken together, these results suggest that sarcolemma excitability could be impaired during the late stage of fatigue because of the concomitant reduced K<sup>+</sup> gradient and the opening of ClC-1 and KATP channels. However, further researches are required in order to be able to determine how these mechanisms interact with each other and their relatives' implications in different types of fatigue.

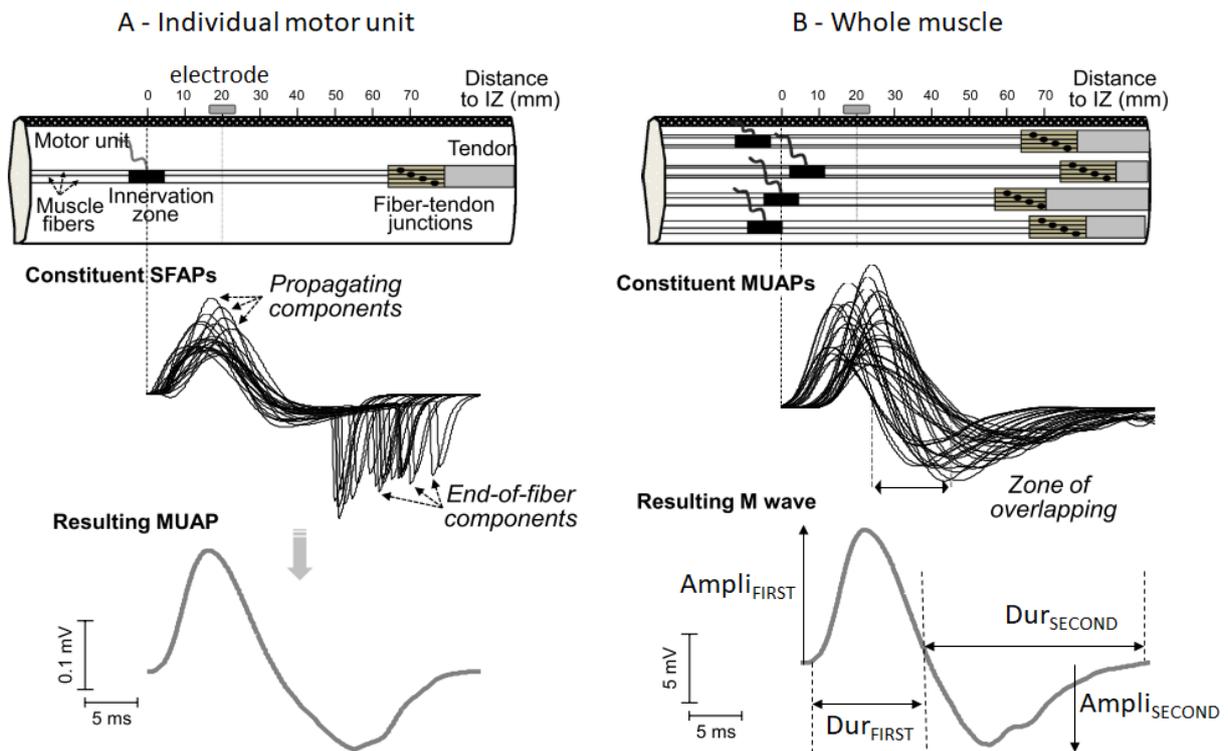


Figure 4: Schematic representation of (A) a motor unit and example of a simulated motor unit action potential (MUAP) obtained as the sum of the temporally dispersed single fiber action potential (SFAP) and (B) a muscle and example of a simulated compound muscle action potential (M-wave) obtained as the sum of the temporally dispersed motor unit action potentials (MUAPs).  $Ampli_{FIRST}$  is the amplitude of the first part of the M-wave;  $Ampli_{SECOND}$  is the amplitude of the second part of the M-wave;  $Dur_{FIRST}$  is the duration of the first part of the M-wave and  $Dur_{SECOND}$  is the duration of the second part of the M-wave. Adapted from (Rodriguez-Falces and Place 2018).

#### *Noninvasive methods for measuring muscle membrane excitability: The M-wave*

Muscle membrane excitability refers to the ability of the sarcolemma to generate and propagate this AP. In studies involving human participants, by applying supramaximal electrical or magnetic stimuli to the nerve trunk (respectively electrical nerve stimulation, ENS or magnetic nerve stimulation, MNS), the impulse will travel through the motor axons to the neuromuscular junction. With electrodes placed over the skin of the desired muscle, an EMG response can be recorded and is called the compound muscle action potentials (M-wave, see figure 4). The M-wave represents the sum of the individual motor unit action potential (MUAP) recruited by the electrical stimulus. whereas the MUAP is the sum of the temporally dispersed single fiber action potential (SFAP). It is, therefore, a useful noninvasive tool to

investigate muscle excitability and its potential alterations during fatiguing contractions (Rodriguez-Falces and Place 2018). The resultant question is then to determine whether M-wave is impaired and how can it be detected when considering fatigue induced by voluntary contraction instead of electrically induced ones.

Current literature assumes that impaired neuromuscular propagation results in a decrease in M-wave size. In a recent review, (Rodriguez-Falces and Place 2018) reported discrepancies from a wide range of studies that investigated M-wave changes during exercise. Their result is quite surprising as it is still unknown if exercise-induced fatigue results in an M-wave decrement or increment. For the authors, the controversy partly arises because M-wave is always analyzed as a whole (i.e., peak to peak amplitude and total area) instead of analyzing separately its first and second phases. Indeed, it appears that during a sustained MVC, the amplitude of the first phase of the M-wave increases progressively before reaching a plateau whereas its second phase amplitude would first increase before decreasing due to mechanical changes making its interpretation inconsistent (Rodriguez-Falces and Place 2017). Authors go further and proposed that impaired sarcolemma excitability would be manifested by an augmentation (and not a depression) of the first part of the M-wave. They explain that the increase extracellular  $K^+$  would have two distinct effects on the individual transmembrane action potentials (figure. 5a), 1) reduce its amplitude (Horowicz et al. 1959; Juel 1988) and 2) enlargement of the main spike (Metzger and Fitts 1986; Lännergren et al. 1987). These two changes have a competing effect on the resulting M-wave, the reduced amplitude of the individual transmembrane action potentials will also reduce the M-wave but the enlarged intracellular potential will sum up more effectively resulting in greater M-wave. (Dimitrova and Dimitrov 2002). Therefore, while being smaller in amplitude, a lengthened transmembrane action potential would lead to an enlargement of the M wave (figure. 5b).

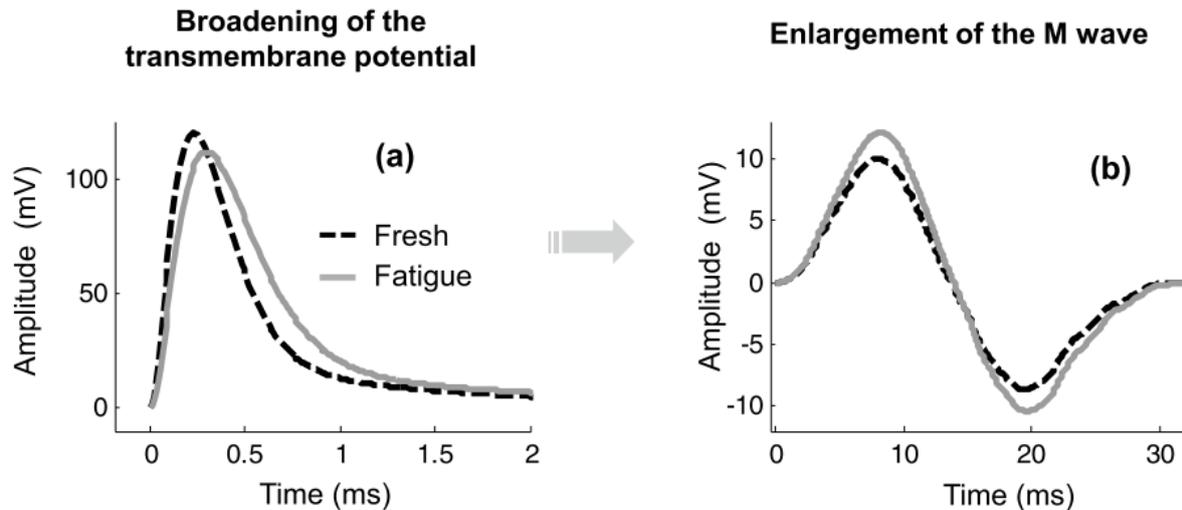


Figure 5: Simulation showing the effect of fatigue on (a) the transmembrane action potential and (b) the resulting M-wave. Taken from from (Rodriguez-Falces and Place 2018).

Based on those considerations regarding M-wave properties, (Rodriguez-Falces and Place 2018) provide some recommendations to assess changes in muscle membrane excitability using the M-wave technique. Among them, they recommend the use of monopolar electrodes that should be placed above or near the neuromuscular junction to ensure that the M-wave first phase isn't too much influenced by end-of-fibers signal and to minimize cross talk. They also strongly advised to perform separate analysis of the first and second M-wave phases (as illustrate on figure. 4B) and that only the first phase (and preferentially its amplitude) should be interpreted as changes in sarcolemma excitability.

However, even when following those criteria's, some limits inherent to the technique might persist as the electrodes are fixed on the skin, therefore only allowing investigation of superficial muscles. During dynamic contractions, it is important to keep in mind that length of the muscle is going to change and so is the relative position of the electrodes from the muscle, impacting the recorded signal. Lastly, environmental parameters (temperature, sweat, movement...) may deteriorate both the EMG signal and the stimulus used. It is then important to deliver a stimulus at supra-maximal intensity (120 to 150% of optimal response) to take into consideration possible alteration of the experimental setup.

### 2.1.2 Excitation-Contraction coupling and metabolic change

Peripheral fatigue is often associated with excitation-contraction (EC) coupling failure. The events involved in EC coupling are quite well described and starts with an AP arriving at the presynaptic terminal of an  $\alpha$ -motor neuron, which activates voltage-gated channel allowing  $\text{Ca}^{2+}$  to enter and bind on synaptic vesicles, triggering exocytose and the release of acetylcholine in the synaptic cleft. The binding of acetylcholine to a receptor on the muscle fiber will allow  $\text{Na}^+$  to enter the membrane and create depolarization and propagation of the AP through the sarcolemma and into the interior of the muscle down the T-tubules, activating the T-tubular voltage sensors called the dihydropyridine receptors (DHPRs). DHPR then activates the sarcoplasmic reticulum (SR)  $\text{Ca}^{2+}$  release channels (ryanodine receptors type 1, RyR1), releasing the  $\text{Ca}^{2+}$  into the cytosol where it binds to the troponin-tropomyosin complex and frees the actin filament. The myosin heads can now reach the actin, which enables the ATP-dependent cross-bridge cycling and the muscle fiber to contract, resulting in force production. Because  $\text{Ca}^{2+}$  is constantly pumped back by the ATP-consuming SR  $\text{Ca}^{2+}$  pump (SERCA), its intracellular concentration  $[\text{Ca}^{2+}]_i$  goes down rapidly when  $\alpha$ -motor neuron stop firing and the muscle fiber relaxes.

#### *Different type of muscle fibers*

Muscle fibers rapidly lose their ability to produce force during intense activity when energy demand is higher than the aerobic capacity and the required energy must come from the anaerobic metabolism. The current classification of skeletal muscle fiber is based on the expression of myosin heavy chain (MHC) which determines the rate of cross-bridge cycling and therefore the maximal shortening velocity of a muscle cell, type I being the slowest, type IIa intermediate and type IIx the fastest (Bottinelli and Reggiani 2000). It should be noted that two muscle fibers having the same MHC expression (i.e., two type IIa fibers) can still have different maximal shortening velocities showing that other factors are also contributing to muscle fibers capabilities (Bottinelli and Reggiani 2000; Bottinelli 2001). However, quite often there is a gene co-expression so that the slow MHC type I is co-expressed with "slow" isoform of other proteins. For example, in type II fibers, the density of both DHPR and RyR1 is higher compared to type I fibers (Lamb 1992; Delbono and Meissner 1996) leading to a higher rate

and amount of  $Ca^{2+}$  release per AP (Baylor and Hollingworth 2003). In order to pump back this  $Ca^{2+}$ , type II fibers also have a different expression of the SERCA pump (named SERCA1a) and approximately a six-fold number of pumps compare to type I fibers.

In fine, there are large differences between muscle fibers regarding their speed of contraction, intracellular  $Ca^{2+}$  handling, glycolytic vs. oxidative capacity, etc. all resulting in differences in fatiguability. A reductive but widely accepted approach is to consider that type I muscle fibers consume ATP slowly, have a high aerobic capacity and so are more fatigue resistant compared to type II even if it's mainly due to other factors than their MHC isoform (Allen et al. 2008).

#### *Potential sites of EC coupling failure*

As stated, fatigue appears faster for a higher rate of ATP production, especially when the anaerobic pathway is required (i.e., the more intense is the exercise, the quicker is the apparition of fatigue). In a recent review, (Cheng et al. 2017) identified three components responsible for the decrease in force production during fatigue. The first two are, a diminution of the ability of the actin-myosin cross-bridges to generate force and a reduced myofibrillar  $Ca^{2+}$  sensitivity, they characterized an impairment in the myofibrillar function and occur in the early stage of the fatiguing exercise. The third one, a decrease in SR  $Ca^{2+}$  release, occurs in the later stage of fatigue. A visual representation of the 3 types of failure in the E-C coupling is provided in figure 6.

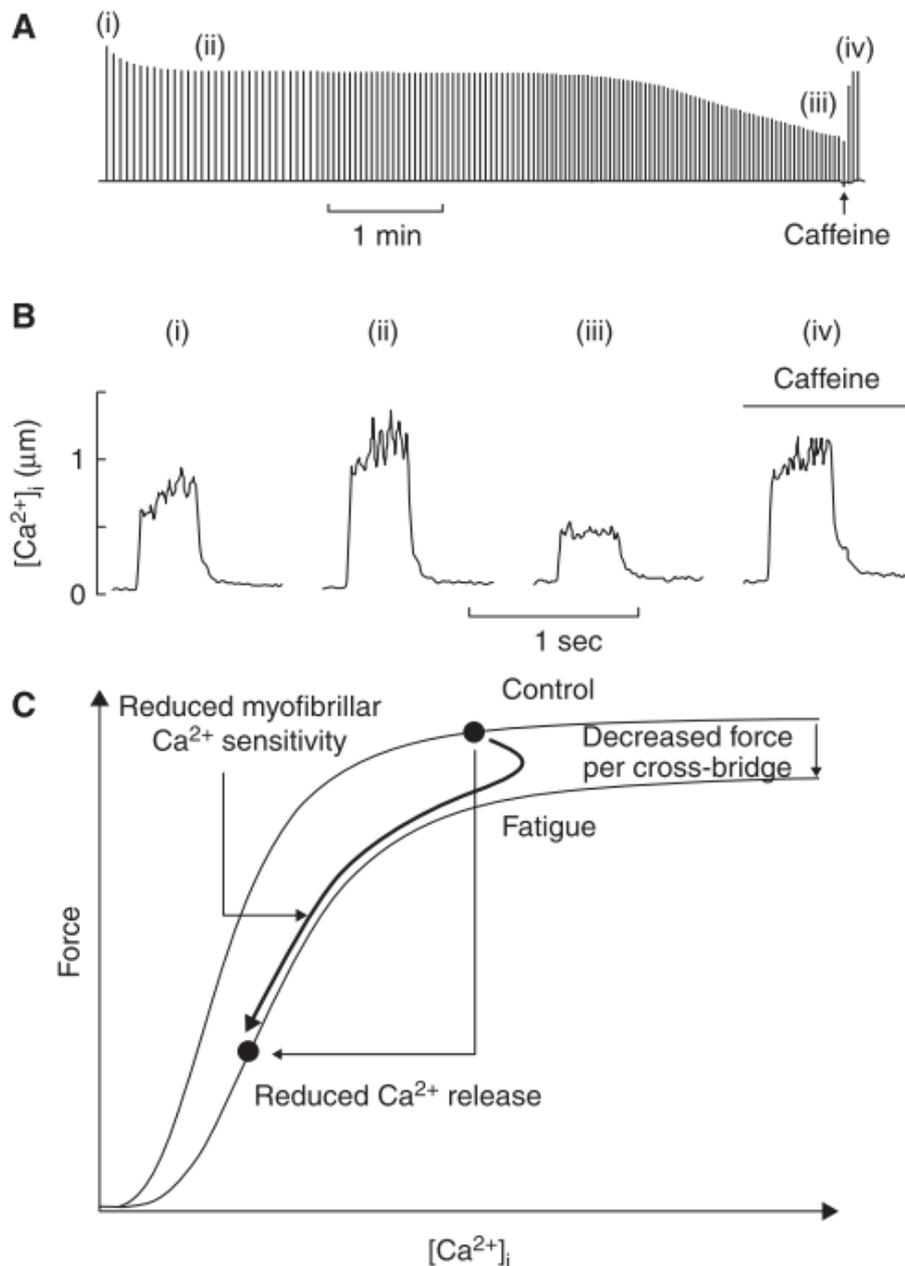


Figure 6: Three mechanisms underlying the force decrease during fatigue induced by repeated tetanic stimulation. Typical pattern of force decline (A), and corresponding  $[Ca^{2+}]_i$  records (B). Tetanic force initially decreases while tetanic  $[Ca^{2+}]_i$  increases (i to ii); hence, the initial force decline is caused by impaired myofibrillar function. The final force decrease is caused by reduced sarcoplasmic reticulum (SR)  $Ca^{2+}$  release (ii to iii). Application of caffeine facilitates SR  $Ca^{2+}$  release and the resulting increase in tetanic  $[Ca^{2+}]_i$  leads to a marked force increase (iv). (C) The relation between force and  $[Ca^{2+}]_i$  during fatigue induced by repeated tetanic stimulation (thick line) plotted together with the force- $[Ca^{2+}]_i$  relationship in the unfatigued state and during fatigue (thin lines). This assessment shows that acute fatigue involves initial decreases in force per cross-bridge and myofibrillar  $Ca^{2+}$  sensitivity, which are followed by decreased SR  $Ca^{2+}$  release.  $[Ca^{2+}]_i$  intracellular calcium concentration. Taken from (Cheng et al. 2017).

*Metabolic changes and their effect on myofibrillar function*

During intense exercise, glycogen breakdown through the anaerobic pathway results in the production of lactate ions and hydrogen ions ( $H^+$ ) whereas phosphocreatine (PCr) breakdown results in creatin and inorganic phosphate ion ( $P_i$ ) accumulation through the creatine kinase (CK) reaction ( $PCr + ADP \leftrightarrow Cr + ATP$ ) (Westerblad et al. 2010). An increase in  $[H^+]$  reduces the pH (i.e., acidosis) and for a long time, the “lactic acid” (i.e., the lactate ion and its associated  $H^+$ ) was own responsible for muscle function failure. (Posterino et al. 2001) showed that lactate has no major effects on the contractile function and researchers suggest that it can be a substrate for the aerobic glycolysis (Brooks 2018) but there is less consensus in the literature regarding its associated  $H^+$ . Many studies found a correlation between acidosis and the decrease in muscle function (Cady et al. 1989; Kent-Braun 1999) but evidence suggests that this correlation might not be causative. In their review, (Allen et al. 2008) concluded that the  $[H^+]_i$  by itself has quite small effects on muscle fatigue and is not part of the main factors affecting muscle function. The same goes with creatine ions in the muscle that have been reported to have no major impacts on muscle function (Murphy et al. 2004). Thus, increased  $[P_i]$  in the muscle has been proposed to be the dominant cause of force decrement during fatigue. First by a direct action on cross-bridge function, preventing the myosin head to bind strongly to the actin, that could contribute to approx. 10% of the force decline in physiological exercise (Dahlstedt et al. 2001; Allen et al. 2008; Cheng et al. 2017). And secondly, because  $P_i$  accumulation reduces the myofibrillar sensitivity to  $Ca^{2+}$  (Debold et al. 2006) which may have a large impact on force production (figure 6.C). Nonetheless, the role of acidosis has been brought back recently as new findings suggest that associated to  $P_i$  accumulation, elevated of  $[H^+]$  could exacerbate the observed diminution of force (Fitts 2016) but again, this doesn't seem to be consensual (Westerblad; 2016).

During most type of exercise, ATP resynthesizes matches ATP consumption through the action of both anaerobic sources and mitochondrial oxidative phosphorylation (Bangsbo et al. 1996; Westerblad et al. 2010). But when a very demanding task is performed  $[ATP]$  can decrease by up to 80% in single type II muscle fibers (approx. 0.7 to 1.7 mM) whereas in slow fibers, on the other hand, there is probably little decline (Karatzaferi et al. 2001a). And because the CK and adenylate kinase ( $2ADP \leftrightarrow AMP + ATP$ ) reaction are close to equilibrium, this ATP breakdown leads to the accumulation of ADP,  $P_i$ , AMP, and Cr. AMP will subsequently be broken down by AMP deaminase to inosine monophosphate (IMP) and ammonia ( $NH^3$ ). Lastly,  $Mg^{2+}$  mostly bind with ATP and have a much lower affinity with ADP,

AMP et IMP, when [ATP] decrease, free  $[Mg^{2+}]$  in the cytosol rises. Because some steps of the EC coupling involve ATP consumption, its breakdown could impact the contractile function. During the cross-bridge cycle, ATP is required to detach the myosin head from the actin filament after the power stroke, but a physiological decrease in [ATP] doesn't impact force production. It is only when [ATP] fall below 0.5mM that contractile velocity might be impacted (Dutka and Lamb 2004). ADP acts as a weak competitor of ATP and an increase in [ADP] increase isometric force while slowing the contraction (Cooke 2007). Finally, intracellular  $[Mg^{2+}]$  reached during fatigue is not enough to impact cross-bridge properties (Swenson et al. 2014).

In summary, ATP and associated ADP, AMP, IMP et  $Mg^{2+}$  changes have only small effects on actin-myosin cross-bridge properties and the myofibrillar function is mostly impacted by Pi accumulation resulting in both a decrease in force produced by active cross-bridges and myofibrillar  $Ca^{2+}$  sensitivity.

#### *Metabolic changes and their impact on SR $Ca^{2+}$ release*

As mentioned above, fatigue mechanisms are here to prevent disastrous effects of complete [ATP] breakdown on muscle function. Probably one of the best ways to avoid that is by limiting the SR  $Ca^{2+}$  release which will concomitantly reduce the number of cross-bridges cycling and the  $Ca^{2+}$  reuptake by SERCA, both ATP consuming. But this process will lead inevitably to impairments of muscle performance.

Experiments on different muscle fibers (frog, mouse, and rat) all showed similar evolution pattern of  $[Ca^{2+}]_i$  during fatigue induced by electrical brief repeated tetani (Allen et al. 1989; Westerblad and Allen 1991; Lunde et al. 2001) presenting an initial increase followed by a plateau (more or less long depending on the fiber type) and a final decrease (as represented in figure 6.B). In addition to its effect on myofibrillar function, Pi, again, act as an important limiting factor of SR  $Ca^{2+}$  changes. Indeed authors showed using either pharmacological interventions or genetically modified mice (with no CK expression) that, reduced [Pi] blunted the initial increase of  $[Ca^{2+}]_i$  and delayed its secondary decrease (Dahlstedt et al. 2000; Dahlstedt and Westerblad 2001). However, after CK injection in the genetically modified mice, the evolution pattern of  $[Ca^{2+}]_i$  went back to normal (Dahlstedt et al. 2003). The first effect of Pi on the blunted increase of  $[Ca^{2+}]_i$  can be explained by the fact that Pi may inhibit or even reverse SR  $Ca^{2+}$  pumping (Duke and Steele 2000; Stienen et al. 2017) which in the

short term could increase  $[Ca^{2+}]_i$ . Different processes could act concomitantly, but they remain unclear and further researches are needed to fully understand the mechanisms (Allen et al. 2008). Regarding the late decline of  $[Ca^{2+}]_i$ , two mechanisms have been reported and mainly rely on the impact of  $[Pi]$  on the SR  $Ca^{2+}$  release. The first one is a  $Pi$  induced inhibition of the RyR1 receptors as shown on the skinned muscle fiber by Duke and Steele (Duke and Steele 2000). Furthermore, Westerblad and Allen (1992) showed that the inhibition effect of  $Pi$  was markedly larger when combined to intracellular  $[Mg^{2+}]$  corresponding to those observed in the fatigued muscle (approx. 1.6mM) compare to rested muscle (0.8mM). The second effect is a  $Ca^{2+}$ - $Pi$  precipitation that could occur in the SR, causing a reduction of the amount of free  $Ca^{2+}$  available for release. Because of the fatigue-induced increases of  $[Pi]$  in the myoplasm, some  $Pi$  could enter the SR and when the  $Ca^{2+}$ - $Pi$  solubility product is exceeded, precipitation occurs. Even if the theory behind this phenomenon is appealing, the actual occurrence of SR  $Ca^{2+}$ - $Pi$  precipitation has never been shown directly and while several experimental results support it (Westerblad and Allen 1991; Kabbara and Allen 1999), others are difficult to reconcile with this mechanism (Stephenson 2006). Therefore, although it is quite clear that  $Pi$  causes a decrease in  $[Ca^{2+}]_i$  in late fatigue, the exact processes still need investigation and it is likely an accumulation of several inhibitory actions that lead in fine to the decreased SR  $Ca^{2+}$  release. Indeed, RyR1 opening is strongly stimulated by ATP binding, with its breakdown products (ADP, AMP) acting as weak competitive agonists. To observe a ~20% decrease of SR  $Ca^{2+}$  release, cytoplasmic  $[ATP]$  has to be reduced to 0.5mM and an increase of  $[Mg^{2+}]_i$  to 3mM is necessary to reduce the SR  $Ca^{2+}$  release by ~40% (Dutka and Lamb 2004). Even if those values aren't physiological, when combined altogether, low  $[ATP]$ , raised  $[Mg^{2+}]$  and, presence of ADP, AMP or adenosine, the impairment of SR  $Ca^{2+}$  release is exacerbated (Blazev and Lamb 1999; Dutka and Lamb 2004). However, it can be noted that again, pH doesn't seem to be implicated in the impairment of  $Ca^{2+}$  release (Lamb and Stephenson 1994; Balog and Fitts 2001).

To conclude, during an intense exercise, several mechanisms act together to diminish  $Ca^{2+}$  release in acute fatigue to avoid complete exhaustion and consequent complications. This will reduce the cross-bridge cycling and the  $Ca^{2+}$  reuptake by SERCA pumps which are the two main sources of ATP hydrolysis. This is mainly done by i) RyR1 inhibition induced by concomitant increased of  $[Pi]$ ,  $[Mg^{2+}]_i$ , and/or alteration in  $[ATP]$  and its byproducts; ii) probable  $Ca^{2+}$ - $Pi$  precipitation in the SR and iii) decreased membrane excitability because of a reduced  $K^+$  gradient over the sarcolemma and the opening of  $ClC-1$  and  $KATP$  channels (c.f., part 2.2.1.1). Further researches are required to understand the precise role of all those

mechanisms in different types of fatigue. Nevertheless, the current literature shows that skeletal muscle fibers can decrease SR  $\text{Ca}^{2+}$  release to prevent catastrophic metabolic changes and it comes at the price of a reduced muscle power output.

### *Glycogen depletion*

When considering prolonged exercise, glycogen store and depletion becomes an important part of fatigue development. Using the biopsies technique, Bergström et al. (1967) showed a depletion of muscle glycogen after prolonged cycling (~2 hours) and noted a correlation between the time to exhaustion and the pre-exercise store of glycogen. Since then, many studies have confirmed the relation between muscle glycogen reserve and exercise capacities (Ørtenblad et al. 2013). Studies using electron microscopy distinguished different zone in the muscle where the glycogen is preferentially stored. It can be found either in the subsarcolemmal, intermyofibrillar or intramyofibrillar compartments and it appears that during exercise, the intramyofibrillar compartments is used first (Ørtenblad et al. 2011; Nielsen et al. 2011). More recently, Nielsen et al. (2014) found a correlation between reduced SR  $\text{Ca}^{2+}$  release and depletion in intramyofibrillar glycogen after repeated tetanic stimulation of intact single mouse muscle fibers. Thus, intramyofibrillar glycogen is preferentially reduced during fatigue and this is associated with decreased SR  $\text{Ca}^{2+}$  release and, hence, reduced force production.

Those results confirmed the ones from Ørtenblad et al. (2011) with human where they took muscles biopsies before, immediately after and 4 hours after a 1-h Nordic ski race. During the first 4 h of recovery, participants received either water or carbohydrate-enriched food. Those who received carbohydrates showed a noticeable recovery of glycogen and SR  $\text{Ca}^{2+}$  release as opposed to the subjects who received only water. Taken together, these results suggest that glycogen depletion during exercise contributes to fatigue by lowering  $\text{Ca}^{2+}$  release. The exact mechanism(s) by which depletion of intramyofibrillar glycogen leads to decreased SR  $\text{Ca}^{2+}$  remains to be established. Future studies are also needed to elucidate links between the above-discussed mechanisms of reduced SR  $\text{Ca}^{2+}$  release in acute fatigue and localized glycogen depletion.

### *Reactive oxygen/nitrogen species*

It is quite well established that most types of physical activities increase reactive oxygen species (ROS) and reactive nitrogen species (RNS) production (Powers and Jackson 2010). It has also been shown that the use of ROS scavengers exogenously added in muscle fibers reduce the rate of fatigue (Reid et al. 1992; Moopanar and Allen 2005) suggesting that ROS probably play a role in fatigue development. Figure 7 provides a quick overview of the ROS/RNS origins and pathways. The first produced ROS are the superoxide anions ( $O_2^{\bullet-}$ ) and because they have very short lifetimes, they will quickly breakdown in various ROS, the principals being hydrogen peroxide ( $H_2O_2$ ), and hydroxide radicals ( $OH^{\bullet}$ ). ( $O_2^{\bullet-}$ ) can also interact with nitric oxide ( $NO^{\bullet}$ ), which is the primary RNS in cells, to form peroxynitrite ( $ONOO^-$ ). The production mechanisms of ROS and RNS are quite complex and doesn't rely on a single source. The production of  $NO^{\bullet}$  in the skeletal muscle appears to be mainly triggered by increased neuronal nitric oxide synthase activity (Hirschfield et al. 2017). For some time, it was believed that ( $O_2^{\bullet-}$ ) comes in majority from the mitochondria and while this origin has been confirmed (Wei et al. 2011; Cheng et al. 2015) other studies proposed NADPH-oxidase 2 (NOX2) as the major ROS source in active skeletal muscle (Michaelson et al. 2010; Sakellariou et al. 2013; Pal et al. 2013). It should be acknowledged that other sources of ROS have been shown such as the t-tubular membrane (Hidalgo et al. 2006).

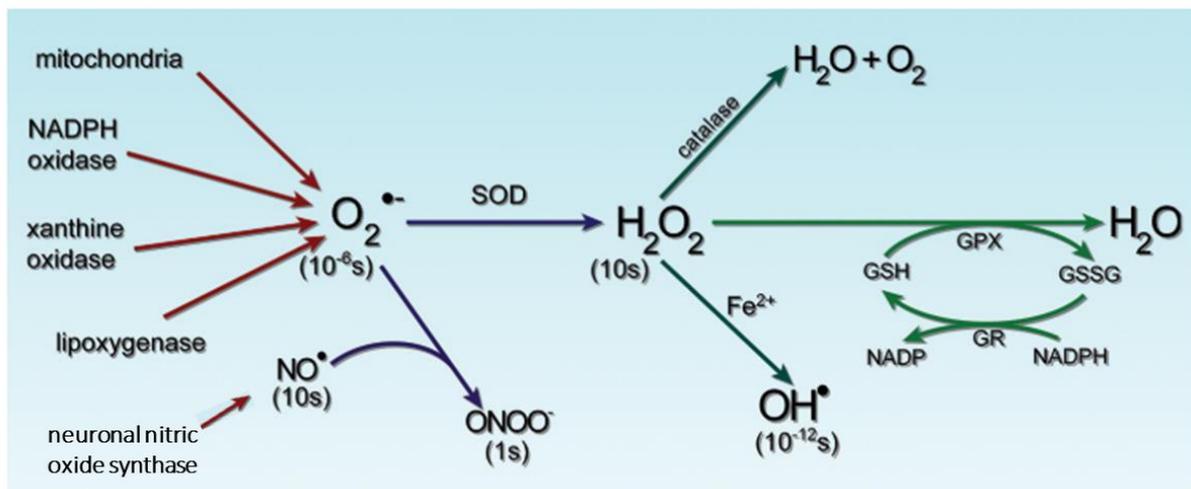


Figure 7: The major reactive oxygen/nitrogen species in muscle. Numbers in brackets indicate approximate lifetimes of various species.  $O_2^{\bullet-}$ : superoxide anions;  $NO^{\bullet}$ : nitric oxide;  $ONOO^-$ : peroxynitrite; SOD: superoxide dismutase;  $H_2O_2$ : Hydrogen peroxide;  $OH^{\bullet}$ : hydrogen radical; GSH: glutathione; GPX, glutathione peroxidase; GSSG: oxidized glutathione; GR, glutathione reductase. Adapted from Allen et al, 2008

It is still unclear if all these different sites contribute equally to the increased ROS activities observed during muscle contractions or if one site predominates. It is probable that different sites are active in different situations and the associated effect of ROS are localized. For example, ROS generation by the sarcoplasmic reticulum or t-tubule systems may be of more importance in the regulation of SR  $Ca^{2+}$  handling than ROS generated by mitochondria or extracellular oxidases (Powers and Jackson 2010). Therefore, the impact of ROS on exercise-induced fatigue likely depends on exercise modalities. Decreased ROS/RNS have clearly been associated with improved endurance performance during studies investigating submaximal contraction (Reid et al. 1992; Powers et al. 2011) whereas no effects were detected with high-intensity exercise or near maximal contractions (Matuszczak et al. 2005; Powers et al. 2011). These differences could be explained because during high-intensity exercises, the above-mentioned mechanisms (e.g., Pi and  $Mg^{2+}$  accumulation) probably dominate (Allen et al. 2008) whereas during submaximal repeated contractions, the relation between force and  $Ca^{2+}$  release and sensitivity is steeper (figure 6C), therefore, small changes induced by ROS/RNS could have quite a substantial effect on force production (Lamb and Westerblad 2011; Cheng et al. 2016). Furthermore, depending on the considered ROS injected, some studies found an increase in performance. For example, exogenous injection of  $H_2O_2$  results in an increase myofibrillar  $Ca^{2+}$  sensitivity (Andrade et al. 1998; Cheng et al. 2015) and some skinned fiber experiments show increased rather than decreased myofibrillar  $Ca^{2+}$  sensitivity after fatiguing contractions (Watanabe et al. 2015). McKenna et al. (2006) also showed that the antioxidant N-acetyl cysteine (NAC, which that support the glutathione resynthesize and so the elimination of  $H_2O_2$ ) minimized the reduction in  $Na^+-K^+$  pump activity and the  $K^+$  accumulation that usually occur during exercise. Because the  $Na^+-K^+$  pump is redox sensitive (Glynn 1963) authors propose that the ROS scavenger NAC can reduce the fatigue associated with ionic changes induced by the  $Na^+-K^+$  pump.

To sum up, it is quite clear that active muscle increases ROS/RNS production which contributes to fatigue but the mechanisms behind their production and their specific impact on the muscular function are still uncertain. Overall, experimental data tend to show that SR  $Ca^{2+}$  is more impacted by  $O_2^{\bullet-}$  or  $ONOO^-$  due to associated perturbation of RyR1 (Bellinger et al. 2008; Andersson et al. 2011) whereas  $H_2O_2$  and  $OH^{\bullet}$  seem to decrease myofibrillar sensitivity to  $Ca^{2+}$  (Cheng et al. 2016). The membrane excitability also seems to be impacted through the effect of ROS on the  $Na^+-K^+$  pump.

Accessorially, it can be mentioned that RyR1 modification trigger mitochondrial biogenesis and other beneficial adaptations (Bruton et al. 2010; Place et al. 2015). Because ROS/RNS

induce modifications of RyR1 structure and function, this could explain why treatment with antioxidants reduce the beneficial effects of endurance training (Gomez-Cabrera et al. 2008; Merry and Ristow 2016).

### *Others potential mechanisms*

As explained, the contractile function of the muscle fiber relies on the SR  $Ca^{2+}$  release and even if the absolute majority of  $Ca^{2+}$  is being pumped back by the SERCA, a small amount of the released  $Ca^{2+}$  will end up leaving the cell essentially by  $Na^+$ - $Ca^{2+}$  exchanger and plasma membrane  $Ca^{2+}$ -ATPase (Hidalgo et al. 1986; Balnave and Allen 1998). A depletion of SR  $Ca^{2+}$  could then be responsible for the decreased muscle function with fatigue. But to avoid  $Ca^{2+}$  depletion during physical activities, the rate of  $Ca^{2+}$  influx is upregulated during prolonged contraction and in fine, if any changes are to be detected, the total muscle  $Ca^{2+}$  content increases (Everts et al. 1993). Therefore  $Ca^{2+}$  flux over the sarcolemma doesn't appear to be part of the responsible mechanisms inducing fatigue.

Lastly, the mitochondria's are located close to the SR and the two structures are bind via small linkages (Boncompagni et al. 2009) allowing  $Ca^{2+}$  to go back and forth from the SR and the mitochondria. Increased mitochondrial  $[Ca^{2+}]$  is known to stimulate mitochondrial respiration and ATP production (Brookes et al. 2004; Glancy et al. 2013). However excessive  $Ca^{2+}$  can result in cell death (Duchen 2000). Therefore mitochondrial  $[Ca^{2+}]$  needs to be tightly controlled to maximize beneficial effects without inducing the deleterious ones, suggesting that important mechanisms probably occur between SR and mitochondria, but those specific mechanisms remain poorly understood. Regarding their potential impacts on the development of muscular fatigue, authors showed the very limited impacts of mitochondria  $Ca^{2+}$  uptake on intracellular  $[Ca^{2+}]$  under physiological condition. Even if more data appears necessary, there are little chances for mitochondrial  $Ca^{2+}$  uptake to be a limiting factor in fatigue development.

### *General conclusions on peripheral muscle fatigue*

Acute muscle fatigue develops when energy production depends on anaerobic metabolism. First, impairments in myofibrillar function will occur through a decrease in cross-bridges force production and reduced  $\text{Ca}^{2+}$  sensitivity, then Impairment of the SR  $\text{Ca}^{2+}$  release will become predominant in the latter stages of fatigue to protect the muscle integrity from the consequences of a complete depletion of ATP. Several mechanisms act together to decrease SR  $\text{Ca}^{2+}$  release; among them: i) an increase in  $[\text{Pi}]$ ,  $[\text{Mg}^{2+}]$  and a decrease in  $[\text{ATP}]$  that will inhibit RyR1 opening; ii) an entrance of Pi in the SR that will induce precipitation with  $\text{Ca}^{2+}$ , reducing the free  $\text{Ca}^{2+}$  available for release; and iii) an extracellular  $\text{K}^+$  accumulation as well as an increased opening of  $\text{ClC-1}$  and  $\text{KATP}$  channels reducing sarcolemma excitability. While it is quite clear that physical increases ROS/RNS production, their impact on muscle fatigue is not well understood. Preliminary results suggest that they could act on SR  $\text{Ca}^{2+}$  release and/or  $\text{Ca}^{2+}$  sensitivity. When considering more prolonged exercise at a lower intensity, it is likely that the reduction in SR  $\text{Ca}^{2+}$  release could be related to glycogen depletion.

#### **2.1.3 Noninvasive tools for the evaluation of EC coupling during physiological exercise**

While most of the results presented in the precedent sections are obtained from isolated muscle or muscle fibers that involve the dissection from animals, some noninvasive tools exist to evaluate the neuromuscular function (NMF). As the M-wave for the membrane excitability (presented above), EC coupling can be indirectly studied by using electrical or magnetic stimuli applied to the nerve trunk (respectively electrical nerve stimulation, ENS or magnetic nerve stimulation, MNS) or in some cases, directly over the muscle motor point (electrical muscle stimulation, EMS). Those stimulations will initiate a force response, thus bypassing the supraspinal and spinal compartment and, based on the stimulation characteristic (i.e., it's frequency) and the associated motor response, physiological interpretations, and extrapolations regarding the induced fatigue by a specific exercise can be made to identify potential sites of EC coupling failure. Of course, the information's obtained using such techniques are indirect and therefore based on some concept relying on the

different mechanisms exposed earlier. In the upcoming section, two modes of evaluation will be discussed.

### *The Peak twitch*

The peak twitch (Pt) is the motor evoked response associated with the M-wave described previously. When a single supramaximal stimulus is delivered through the nerve (either using ENS or MNS), it will recruit some motor unit resulting in both an M-wave and a force response (see figure 8). With fatigue development, the shape of the force response is likely to be impacted negatively (reduced and/or broadened), as presented earlier and, because spinal and supraspinal responses are bypassed, the force diminution comes either from impairments of the membrane excitability, SR  $\text{Ca}^{2+}$  release,  $\text{Ca}^{2+}$  sensitivity or force produced by active cross-bridges. However, if the M-wave doesn't change, therefore the fatigue contribution comes from myofibrillar function (i.e.,  $\text{Ca}^{2+}$  sensitivity or force produced by active cross-bridges) or SR  $\text{Ca}^{2+}$  release. Other parameters can be determined from the Pt such as the time to reach peak twitch force (TPT) which can be interpreted as a global index of muscle typology (More type-II oriented muscle will display a faster TPT) and an indicator of  $\text{Ca}^{2+}$  release or the half relaxation time (HRT), that represent the ability of the muscle to relax. A faster HRT could be an indirect factor of the SERCA pumping back the  $\text{Ca}^{2+}$  into the SR as discussed by (Behm et al. 1996).

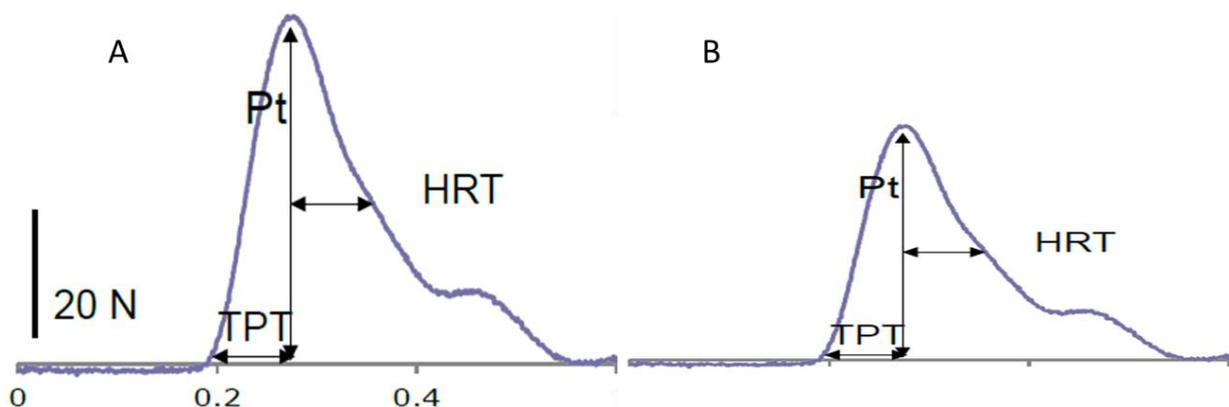


Figure 8. Example of peak twitch response in an unfatigued (A) and fatigued (B) state. Pt represents the force amplitude of the peak twitch, TPT represents the time to reach Pt and HRT is the half relaxation time. Adapted from a presentation given by N.Place (2017).

It is important to take some methodological consideration when investigating the neuromuscular function using the Pt. Indeed, after a contractile conditioning activity, the Pt response to the exact same stimulus will be higher, this is known as the post-activation potentiation (PAP). PAP principal mechanism is an increase in  $\text{Ca}^{2+}$  sensitivity and has its strongest effect when intracellular  $[\text{Ca}^{2+}]_i$  is low (i.e., the steep part of the force- $\text{Ca}^{2+}$  relationship, figure 6.C), therefore, PAP is greater when considering single or low-frequency stimuli because with high-frequency, the intracellular  $[\text{Ca}^{2+}]_i$  is rapidly saturated (Sale 2002). Fatigue and PAP often coexist (Rassier and MacIntosh 2000), it is then important to record peak twitch in a saturated intracellular  $[\text{Ca}^{2+}]_i$  induced by a conditioning contraction (e.g., brief MVC), both pre- and post- exercise if the purpose is to identify the effect on the fatiguing task on the contractile properties of the muscle involved. Others methodological constraints arise from the fact that not all motor nerves are accessible for stimulation (i.e., sciatic nerve) or that both agonist and antagonist muscles are innervate by spatially close nerve (i.e., Biceps and triceps brachii). To counteract this limitation, stimulation of the motor point of the muscle can be used to obtain force response. With the use of MNS, it is sometimes not possible to deliver supramaximal stimulation notably with obese population.

#### *Low- to high-frequency ratio*

To investigate the peripheral origin of fatigue, repeated stimuli can be delivered at different frequencies to have insight on different mechanisms behind force impairment.

(Chin and Allen 1996) showed that after a fatiguing protocol (repeated 0.35 s tetani at 100Hz interspaced with 3-4 s of recovery) on isolated mice muscle fiber, there was a proportionally greater loss of force at low-frequencies (30-50 Hz) stimulations compared with high frequencies (100Hz). In the same study, this phenomenon called low-frequency fatigue (LFF) also appears in an experimental condition with elevated  $[\text{Ca}^{2+}]_i$  but without fatigue (through the concomitant use of caffeine + tetani stimulations) and authors reported unaltered  $\text{Ca}^{2+}$  sensitivity (figure 9) or myofibrillar force production capacity. They concluded that LFF arises from an increase  $[\text{Ca}^{2+}]_i$  during fatigue that will trigger different processes leading to EC coupling failure and impaired  $\text{Ca}^{2+}$  release and that it doesn't depend on changes in myofibrillar function. Because during low-frequency recruitment of the muscle, the Force- $[\text{Ca}^{2+}]_i$  relationship is steep, only small changes in  $\text{Ca}^{2+}$  release can lead to drastic force diminution (figure 9).

It is important to note that this fatigue phenomenon is not necessarily caused by exercise involving low-frequency recruitment and furthermore, the force impairment recovery can take hours or even days to recover (Jones 1996; Keeton and Binder-macleod 2006). Initially, LFF was characterized by the ratio between the force response to low- and high-frequency tetani (see figure 10.A), but this method is associated with a high level of discomfort/pain when used with human participants. Verges et al. (2008) showed a high correlation between the force ratio obtained with tetani stimulation and paired stimulations at 10 and 100Hz. Therefore, LFF is now characterized as follow:

$$\text{LFF} = \text{Db}10 / \text{Db}100$$

With Db10 and Db100, the force response obtained with paired stimuli at respectively 10 and 100 Hz (cf. figure 10.B).

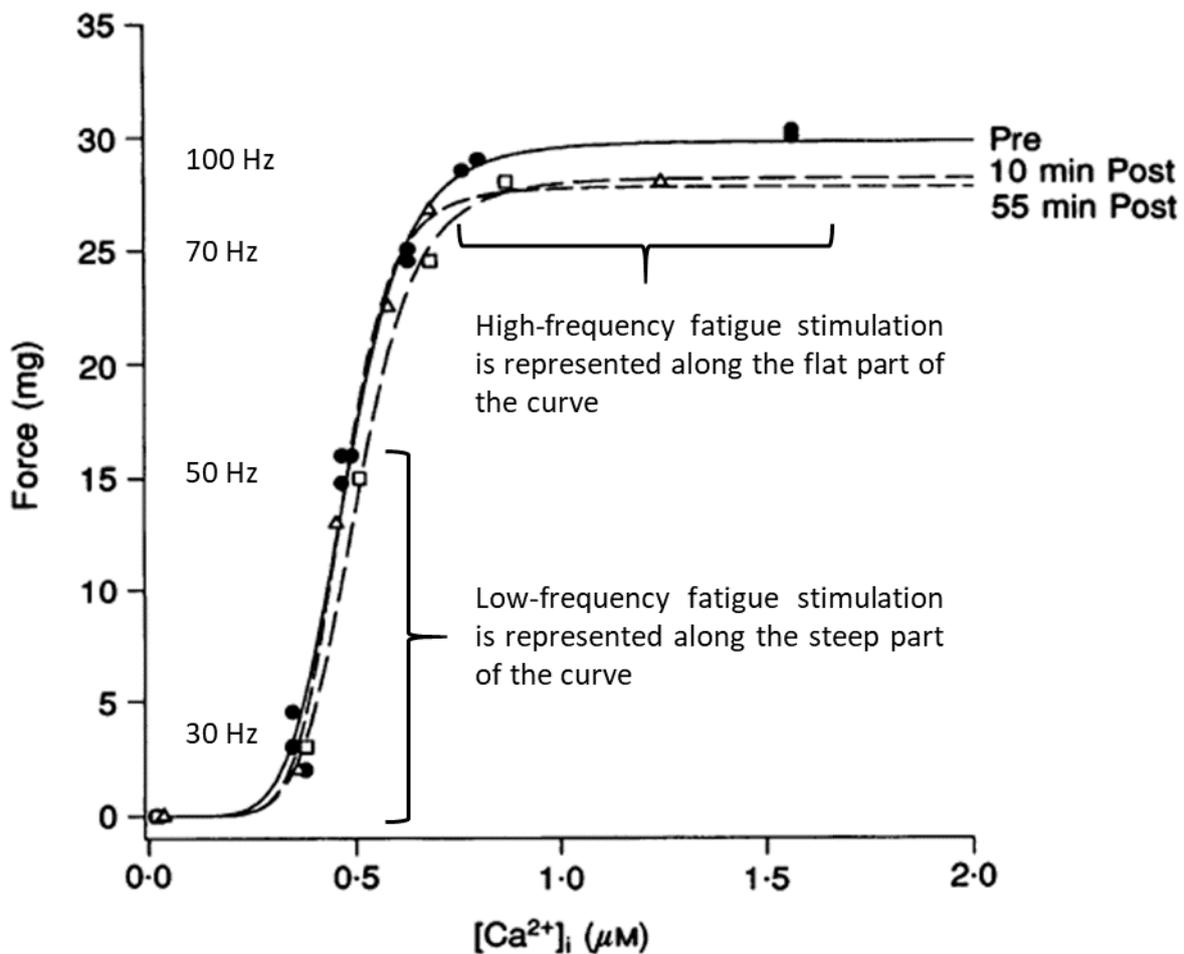


Figure 9. Force- $[Ca^{2+}]_i$  relationship before, 10 min and 55 min after an experimental condition inducing elevated  $[Ca^{2+}]_i$  but without fatigue (through the concomitant use of caffeine + tetani stimulations). Data for force and

$[Ca^{2+}]_i$  were obtained at 30, 50, 70 and 100 Hz. Because  $[Ca^{2+}]_i$  at high frequencies is on the horizontal part of the curve, moderate falls in  $[Ca^{2+}]_i$  have no effect on muscle force. At low frequencies,  $[Ca^{2+}]_i$  is on the steep part of the curve, so falls in  $[Ca^{2+}]_i$  produce large changes in force. Adapted from Chin and Allen (1996) and Keeton and Binder-macleod (2006).

On the other hand, high-frequency fatigue (HFF) is characterized by a loss of force when the muscle is stimulated at high frequencies. This type of fatigue is often associated with slower propagation and shorter amplitude of muscle AP and is exacerbated if extracellular  $[Na^+]$  is decreased or  $[K^+]$  is increased. This type of fatigue is characterized by a diminution of Db100 and appears to recover quickly (Jones 1996) which is in adequation with its physiological explanation as impairment in the ionic balance is rapidly counteracted. Db100 is also a useful tool to investigate the central compartment of fatigue as it will be discussed in the next section.

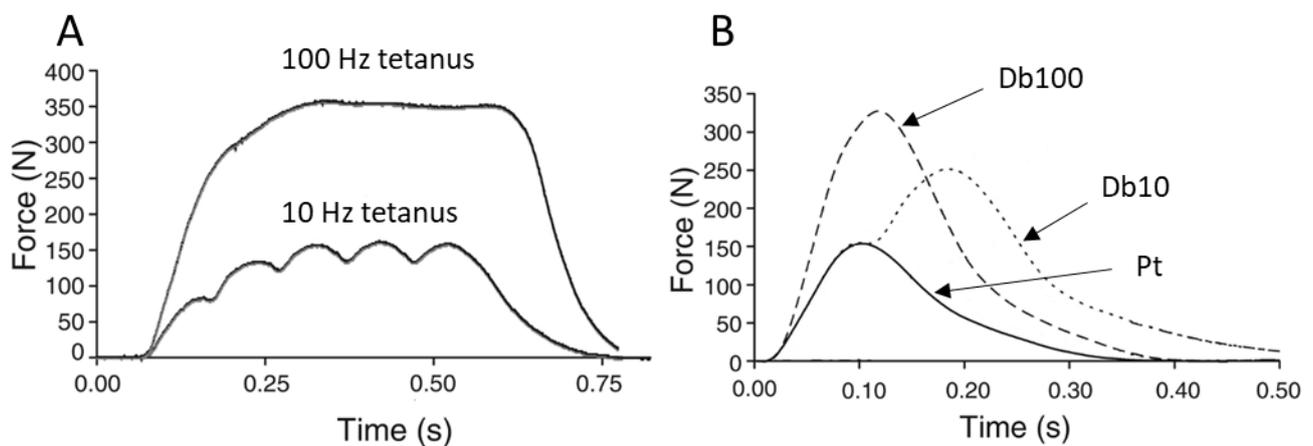


Figure 10. Quadriceps force recordings from a representative participant. Panel A shows his response to 10 and 100Hz tetani stimulation via electrical muscle stimulation. Panel B shows his response to a single (Pt) and paired pulses at 10 Hz (Db10) and 100 Hz (Db100) stimulation of the femoral nerve.

## 2.2 Central mechanisms

Repeated muscle contractions will inevitably lead to peripheral adaptation as explained in the above section. However, in the 1980s, studies started to investigate the impact of the brain in fatigue development because as much as fatigue can be characterized by a reduction in

contractile function, a reduced stimulus from the central nervous system (CNS) will inevitably result in a less powerful contraction, creating a central aspect of fatigue (Davis et al. 2000). Indeed, to initiate a voluntary contraction, the motor cortex is stimulated by others area of the brain and generates APs that will travel to the spinal cord along the corticospinal tract to finally activate the  $\alpha$ -motoneuron (see figure 11). Any failure along this chain of action will result in the so-called central fatigue that can be further divided into spinal and supraspinal fatigue. The latter refers to a suboptimal drive from the motor cortex whereas spinal fatigue is related to a failure at the lower motoneuron level (Gandevia 2001). Quite recently, the use of the transcranial magnetic stimulation (TMS) permitted new understandings underpinning mechanisms behind both supraspinal and spinal compartments of fatigue. This section will first present different methods of investigation of central fatigue and will then debate potential changes either at the supraspinal and spinal level before finishing by presenting the role of muscle afferents feedback on the whole system.

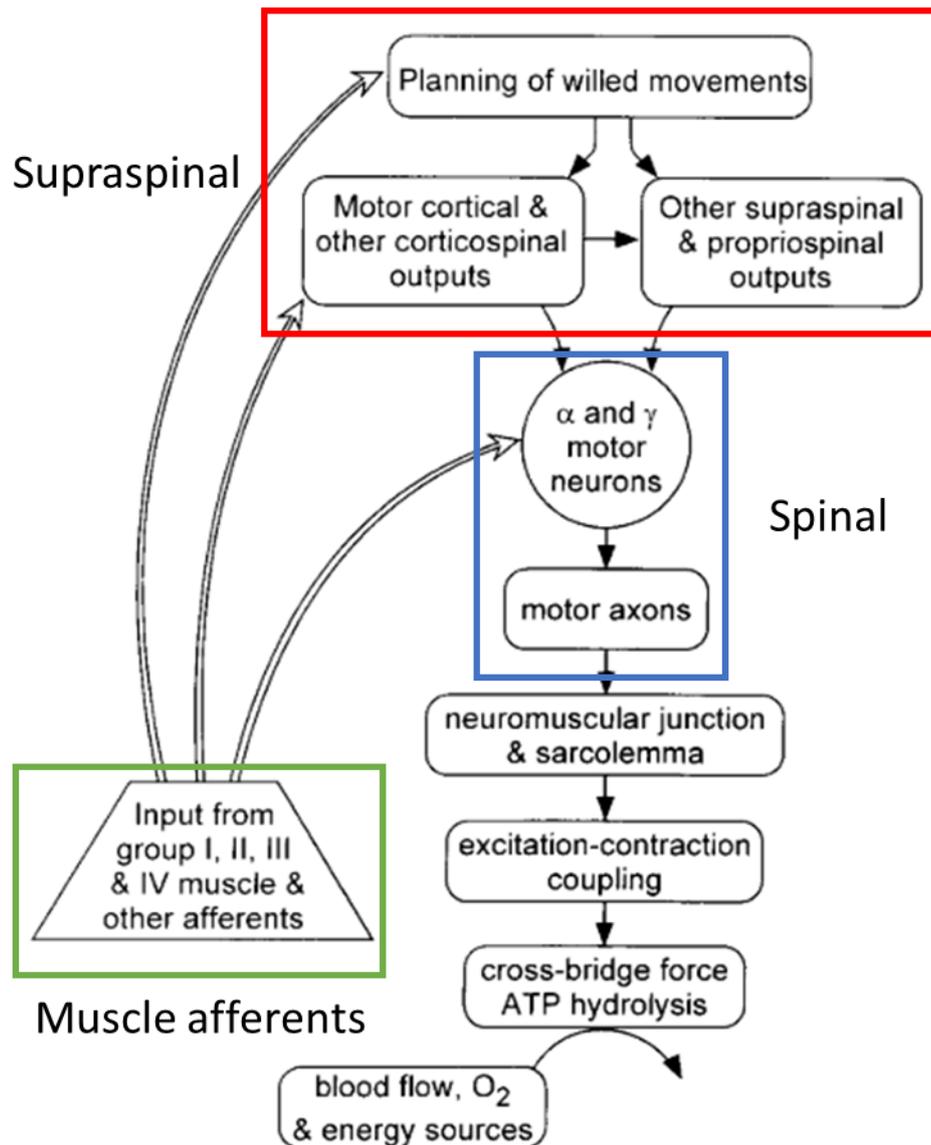


Figure. 11 Different steps involved in voluntary force production with the major associated feedbacks from the muscle acting at three distinct levels. Adapted from Gandevia, (2001).

### 2.2.1 Investigation of the central compartment of fatigue

Several noninvasive techniques have been developed to investigate the central compartment of the neuromuscular function during exercise in humans. In this section, a brief overview of two of the techniques used during this thesis will be presented.

### *The interpolated twitch technique*

For a review of the interpolated twitch technique (ITT) method, one can refer to Shield and Zhou, (2004). ITT is commonly used to assess the extensiveness of muscle activation during MVC. (Merton 1954) reported a negative linear regression between superimposed and voluntary force (i.e. the higher is the voluntary force production, the less will be the evoked force), thus, by quantifying the force induced by the superimposed twitch (SIT) relatively to the one obtained in the relaxed muscle (see figure 12), it is possible to determine the voluntary activation (VA) using the equation:

$$VA (\%) = \left( 1 - \left( \frac{SIT}{\text{resting twitch}} \right) \right) \times 100$$

*Equation (3)*

Where the resting twitch is the force obtained at rest using the same stimulation modalities as the one used for the SIT.

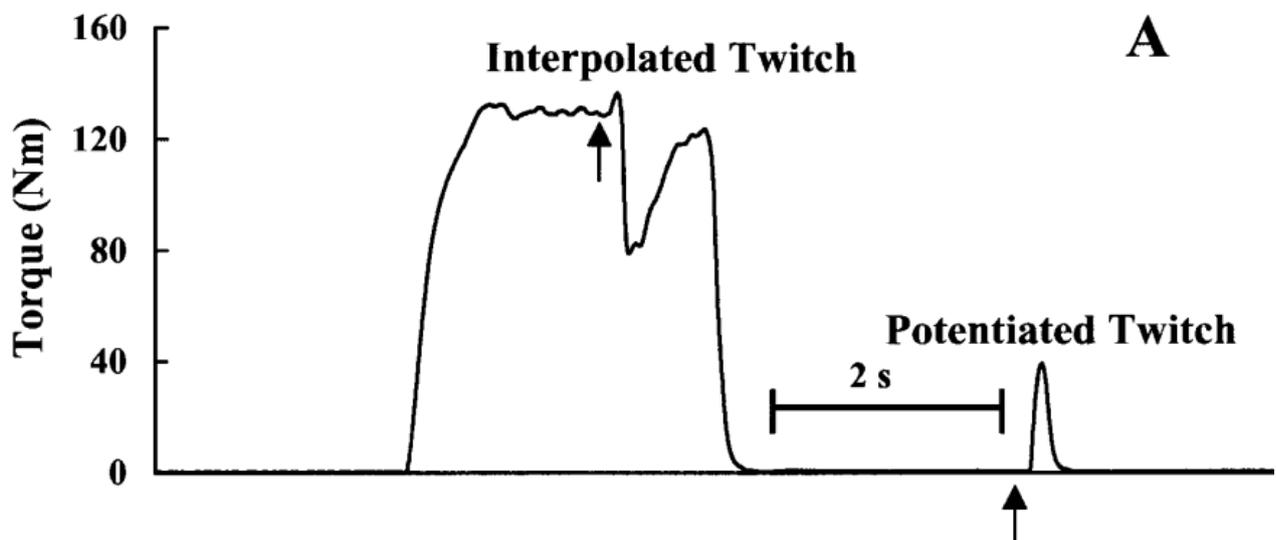


Figure 12: Typical example of measurement using the twitch interpolated technique. A paired-pulse stimulus was delivered (arrow) at 100Hz to the posterior tibial nerve during a maximal voluntary contraction (interpolated twitch) and again in the subsequent recovery period (potentiated twitch. Taken from Shima et al. (2002).

To minimize the limit associated with the post-activation potentiation (see section 2.2.1.3) it is recommended to deliver the control twitch right after the MVC (~1.5 to 5 s after; (Allen et al. 1995). To maximize the validity and reproducibility of the method, a few guidelines are to

be respected. First, the site of stimulation should only recruit the agonist's muscle and the intensity of stimulation should be supramaximal, especially when investigating fatigue because of changes in motoneurons thresholds. Depending on the considered muscle, it is recommended to use 120 to 150% to the optimal intensity (i.e. the smallest intensity for which the evoked response is maximal). It is also recommended to use doublets at high frequency (Suter and Herzog 2001) because they provoke higher and less variable force increasing the signal to noise ratio. Furthermore, for the study investigating fatigue, the higher frequencies will be less impacted by the impairment in EC coupling (Bigland-Ritchie et al. 1986). Thus, the literature recommends the use of paired-pulse at 100 Hz like the one used to investigate the low-frequency ratio. Lastly, it is important to have the participant familiarized to the technique as it can be associated with some discomfort potentially preventing the reach of the true MVC due to the anticipation of the stimulus. Despite that, it is possible for the participant (or the experimenter...) to perform poorly, each MVC should be therefore examined during the test and bad trials rejected if the force doesn't present a plateau at the time of the stimulation or if two MVC exhibit too much variability. When investigating fatigue, the delay between the end of exercise and the measure is of prime interest and therefore it is not always possible to performed multiple trials. A modified formula for VA has been proposed by (Strojnik and Komi 1998) to limit the impact of a missed stimulation (i.e. not delivered during the plateau):

$$VA (\%) = \left( 100 - \frac{SIT \times \frac{F_{init}}{F_{max}}}{\text{resting twitch}} \right) \times 100$$

*Equation (4)*

Where  $F_{init}$  is the force at the moment of the stimulus and  $F_{max}$  is the true maximal force reach during the MVC. Despite the use of this formula, researchers should always aim to deliver the SIT during the plateau.

VA has been extensively used to characterized fatigue etiology during exercise and it appears that the capacity to activate skeletal muscle decrease as fatigue increase (Gandevia et al. 1996). Subsequently, a diminution of VA will induce a diminution of the MVC produced by the participant and is interpreted as a reduced motor output from the CNS creating a central aspect of fatigue. However, the determination of VA with the ITT alone doesn't allow to determine whether this diminution is due to cortical or spinal factors and this will be discussed in the forthcoming section

### *Transcranial magnetic stimulation*

Transcranial magnetic stimulation (TMS) is used in exercise physiology mainly to explore the plasticity of the motor cortex before and after a fatiguing task for example. For a recent review of the technique, one can refer to (Todd et al. 2016). Technically (and briefly) speaking, a short high-current magnetic pulse is induced in the magnetic coil placed on the top of the head (Hallett 2000). As shown in figure 13, a magnetic field arise perpendicularly to the plan of the coil and a subsequent electric field perpendicular to the magnetic field is produced below the coil. This allows the stimulation of specific brain area without the discomfort associated with electrical stimulation.

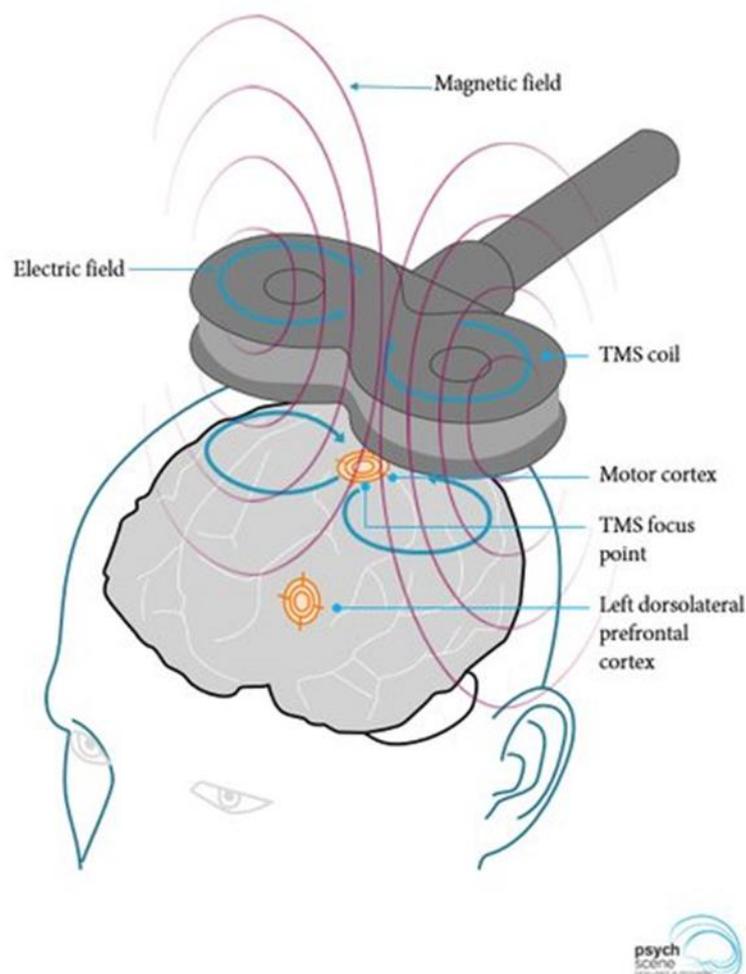


Figure 13. Overview of the TMS induced current. Taken from <http://psychscenehub.com>

When the coil is placed over the motor cortex and a TMS delivered, the generated electrical field will depolarize neurons, thus leading to a muscle contraction. This response characterized by Taylor and (Gandevia 2001) as a mainly monosynaptic corticomotoneuronal excitation can be recorded with EMG placed over the considered muscle and is called a motor evoked potential (MEP, see fig. 14). For the authors, MEP depends 'on transmission through relevant excitatory and inhibitory interneurons and on the excitability of the motoneurons pool' but because it is measured at the muscle level (EMG), it also depends on the muscle membrane excitability, which can be characterized by the M-wave. In order to isolate the spinal and supraspinal part of the response, it is necessary to normalize the MEP to the M-wave obtained through peripheral nerve stimulation.

When TMS is superimposed to a voluntary contraction, a period of near silence in EMG is seen right after the MEP (typically lasting ~100-300 ms) and is called silent period (SP, see figure 14). While this SP has long been assumed to reflect intracortical inhibition (Säisänen et al. 2008), it has been suggested that spinal mechanisms probably have much more impact on the SP than previously thought Yacyshyn et al. (2016). The exact mechanisms are still debated, and the reader can refer to the recent work from (Škarabot et al. 2018) for more information. But briefly, the first ~80 ms of the SP are believed to arise from spinal adaptation whereas, after 100 ms, intracortical inhibition seems to be mainly responsible, acting through GABA<sub>B</sub> receptors.

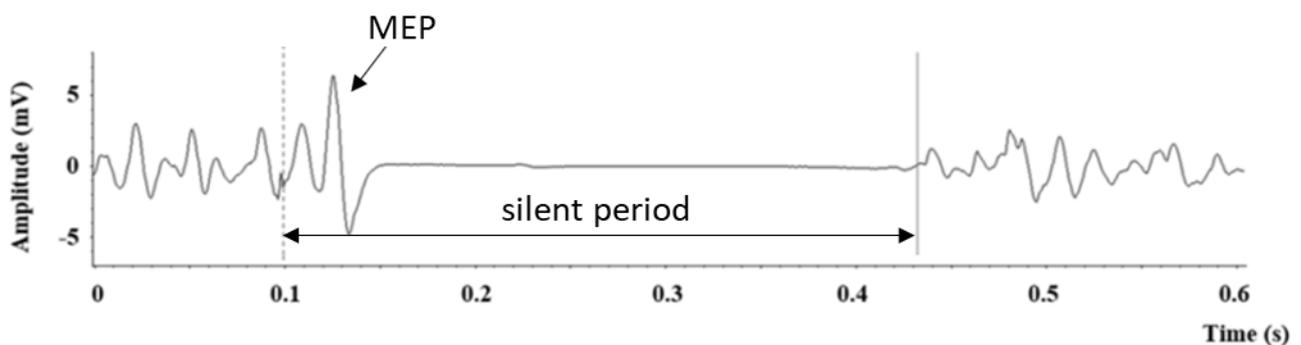


Figure 14. Typical EMG response from the vastus lateralis muscle during a submaximal contraction with a superimposed TMS. The dotted line represents the delivery of TMS and the solid line represent the end of the silent period. MEP, motor evoked potential.

When TMS is delivered during a voluntary contraction, it produces a SIT as the one obtained with peripheral nerve stimulation thus, allowing to calculate voluntary activation ( $VA_{TMS}$ ). However, some methodological differences are to be considered, for example, corticospinal

excitability is improved during a voluntary contraction so the same stimulation will elicit greater force output during contraction than at rest (Lee et al. 2008). It is therefore not possible to normalize the SIT obtained during a voluntary contraction to the one obtained at rest. An alternative method based on the fact that the relation between voluntary force and their corresponding evoked SIT is linear above 50% of MVC has been proposed by (Todd et al. 2003). Therefore, using three contractions (one brief MVC plus often 75% and 50% of the considered MVC, see figure 15), it is possible to determine the y-intercept of the regression and to obtain the theoretical SIT at rest called the estimated resting twitch (ERT). This method has been validated for the upper limb (Todd et al. 2003) and the knee extensor muscles (Goodall et al. 2009). Once the ERT is calculated, its value replaces the resting twitch value in equation (3), giving equation (5).

$$VA (\%) = \left( 1 - \left( \frac{SIT}{ERT} \right) \right) \times 100$$

*Equation (5)*

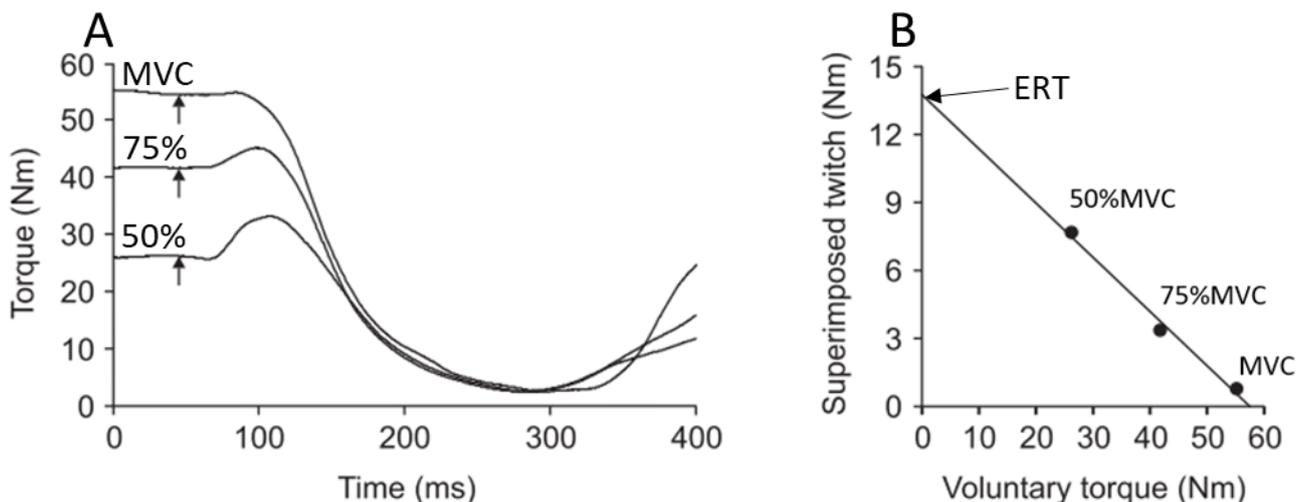


Figure 15. Procedure to calculate voluntary activation using TMS. Panel A shows torque traces obtained at maximal voluntary contraction (MVC), 75% of MVC and 50% of MVC and the size of the associated superimposed twitch. Panel B shows the linear regression obtained between the voluntary torque and the superimposed twitch allowing the determination of the estimated resting twitch (ERT). Adapted from (Todd et al. 2016).

Assessing VA using TMS presents some limitations and the most important is probably the concomitant recruitment of both agonist and antagonist muscles. To overwhelm this problem,

it is recommended to check the ratio between MEP and M-wave in both the agonist and the antagonist's muscle during VA measurement. The agonist ratio will give an indication on the capacity of the TMS pulse to recruit a large portion of the motoneurons, thus any increment in torque is likely to be better for a higher ratio [a ratio of 80% of the superimposed M-wave (Mmax) is considered large, (Todd et al. 2016)]. Concomitantly, the antagonist ratio must be the lowest possible to ensure that the motor evoked response elicited by the TMS is not contaminated by an antagonist co-contraction. To obtain a valid measurement, Todd et al. (2016) recommend having concomitant agonist MEP superior at 50% Mmax and antagonist MEP inferior at 20% Mmax, therefore the intensity of the stimulation should be adapted to meet those criteria. Unfortunately, M-wave can't be obtained in all muscle making complicated to use this procedure to determine the optimal TMS intensity. In this case, a more classical approach with a recruitment curve similar to the one used with peripheral nerve stimulation is used and the intensity kept for the VA assessment is the lowest one eliciting the highest MEP during a contraction at 20% MVC.

Furthermore, when assessing neuromuscular function with TMS during exercise, it might be difficult to ensure that the stimulation is given at the same spot. To limit this issue without restricting the recruitment to bald participants, a swim cap can be used and taped to the participant's head so the optimal coil location can be drawn on it.

The use of TMS for neuromuscular function evaluation allows having more insight compare to the ITT as it gives access to more direct indicators of the central compartment such as the MEP and the SP. However, it is still not possible to distinguish the supraspinal from the spinal impact on those indicators.

### **2.2.2 Supraspinal changes**

#### *Descending Drive*

When fatigue develops during intermittent or sustained MVC, the associated descending drive from the motor cortex is also reduced as shown by a reduction of the EMG activity (Taylor and Gandevia 2007). Conversely, (Decorte et al. 2012) found an increase in the same EMG activity during cycling correlated with the apparition of peripheral fatigue and the same results occur when sustaining submaximal isometric forces. This suggests that the

descending drive is increased to compensate for the development of peripheral fatigue and reduced spinal excitability (Søgaard et al. 2006; Klass et al. 2008) and may come from the recruitment of additional motor units (Carpentier A et al. 2001; Johnson et al. 2004). However, this ability to increase central drive is limited and will eventually decrease with fatigue in both maximal and sub-maximal exercise (Taylor et al. 2016b). From the early '90s, TMS has been used to investigate the central nervous system (CNS) and its methodology has been described above, but quickly, an extra force evoked by TMS during a MVC indicates that the motor command to the muscle was not maximal, thus suggesting that at least some of the central deficit is supraspinal or spinal (Gandevia et al. 1996).

The use of brain imaging techniques during exercise allows a much more precise investigation of which areas of the brain are recruited and can bring insight into the understanding of fatigue development. Therefore, a submaximal contraction of the hand muscles induced concomitant increase in the activity of EMG, contralateral and ipsilateral sensorimotor cortex, supplementary motor area, and cingulate motor area (Liu et al. 2003). Interestingly during a sustained MVC, force and associated EMG response decrease whereas the activation of the cortical areas increase but not enough to maintain the neural drive (Post et al. 2009).

It is difficult to draw conclusions from those studies as it seems that during both submaximal and maximal contraction supraspinal structures increase their activity whereas different outputs responses at the muscle level are observed. Indeed, during submaximal contractions, the concomitant increase in EMG and cortical activity suggest improved central drive while, during maximal contractions, augmentation of the cortical activity doesn't necessarily result in an increased motor output to the agonist's muscle. It can be then hypothesized that the increased brain activity is due to the recruitment of motor units from other synergist muscles (Gandevia 2001) or muscle not critical to the task (Post et al. 2009). In addition to TMS and imaging, fatigue-induced changes at the brain levels can be investigated through the use of pharmacological intervention to look at the specific function of some brain neurotransmitters.

### *Changes in brain neurotransmitters*

The communication between neurons in the brain is dependent of chemical compounds that act as messengers, they are named neurotransmitters and are released into the synaptic

cleft to bind receptor sites at the postsynaptic neuron to trigger excitatory or inhibitory responses. Different types of neurotransmitters exist (e.g., amino acids, peptides, and monoamines) and the one sports scientists are the most familiar with is probably the acetylcholine because of its presence at the neuromuscular junction. While at first, serotonin was suggested to play a major role in the process of central fatigue, recent research proposes that, when considering physiological temperature, dopamine and noradrenaline are more likely to have an important role (Meeusen and Roelands 2018). The upcoming paragraph will try to offer a brief overview of the impact of exercise on brain neurotransmission in humans but for more accuracy, the reader is invited to refer to Meeusen and Roelands (2018).

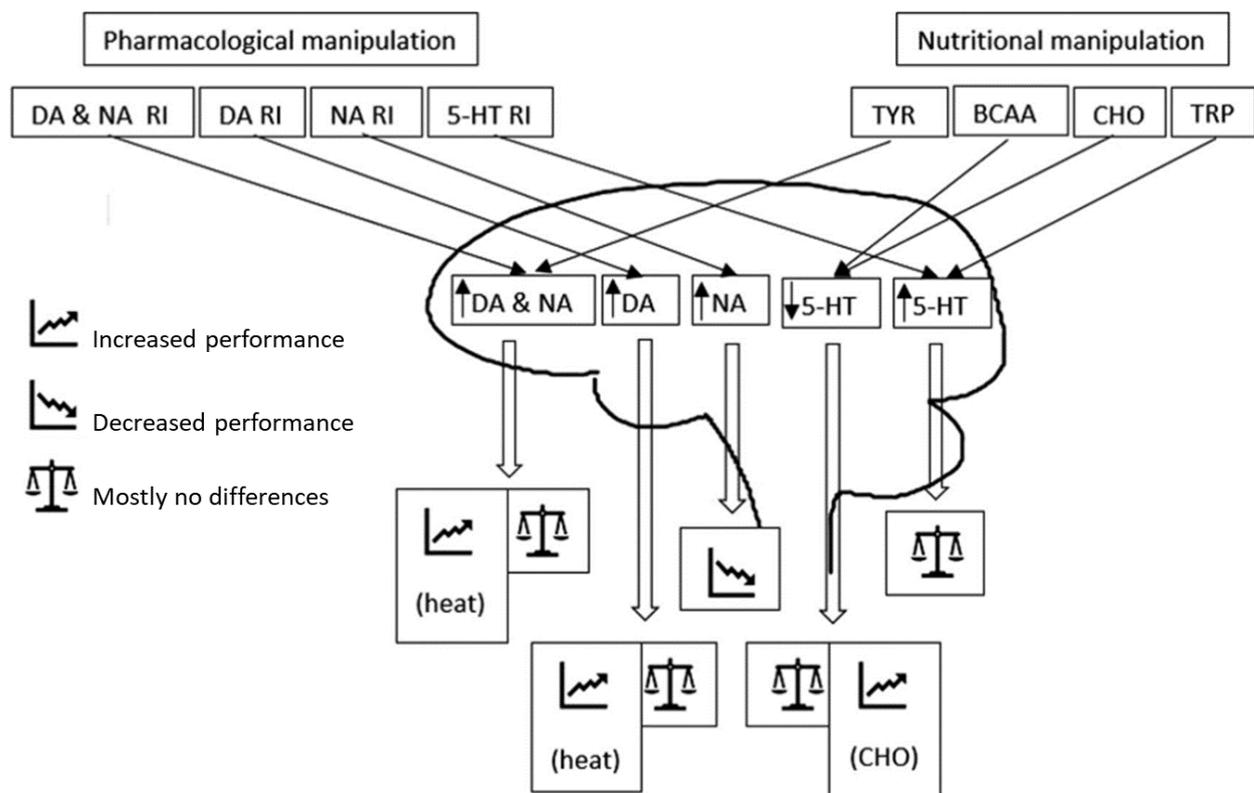


Figure 16. Overview of the effects of some pharmacological and nutritional manipulation on exercise performance through brain neurotransmitter adaptations. DA, dopamine; RI, reuptake inhibitor; NA, noradrenaline; 5-HT, serotonin; TYR, tyrosine; BCAA, branched-chain amino acids; CHO, carbohydrate and TRP, tryptophan. Taken from Meeusen and Roelands (2018).

The central fatigue hypothesis (Newsholm 1987), assumes that changes in brain neurochemistry and more specifically, a rise in serotonin level induced by exercise would augment lethargy and negatively impact mood and drive, leading to fatigue. Ten years later, Davis and Bailey (1997) added the dopaminergic activity as a potential target influencing the

onset of fatigue saying that “an increase in brain dopaminergic activity may delay fatigue by inhibiting brain serotonin synthesis and by directly activating motor pathways” (Davis and Bailey 1997). This was the starting point for exploring the use of nutritional and pharmacological interventions modifying brain neurotransmitters synthesis and their impact on fatigue (see figure 16). An interesting example is provided using carbohydrate supplementation which obviously acts as a substrate for muscle metabolism at the peripheral level, but also impact brain function as illustrated using the “mouth rinsing” paradigm. It consists of oral exposure to glucose without ingesting it, therefore suppressing its “direct” effects on muscular function. (Chambers et al. 2009) performed functional magnetic resonance imaging and showed that mouth rinsing improved performance through activation of specific brain regions involved in the motor control and reward processes. Those results have been confirmed by other studies (Turner et al. 2014; De Pauw et al. 2015) reinforcing the close link between brain chemistry and performance.

But despite these promising results and the additional insights from animal work, human studies investigating serotonergic pathways don't provide straightforward results (Meeusen and Roelands 2010), probably due to the complexity of the serotonergic system (Taylor et al. 2016b) but also maybe because serotonin might not be the key factor. Before going further, it is important to understand that neurotransmitters are also responsible for thermoregulatory responses and therefore, depending on the environmental condition and the considered neurotransmitters, responses to pharmacological intervention are susceptible to change. For example, methylphenidate (a dopaminergic reuptake inhibitor, therefore supposed to increase dopamine levels (figure 16), didn't impact endurance performance at ambient temperature but improve performance by 16% compared to placebo on a self-paced time trial (ROELANDS et al. 2008). Interestingly, in the same study, participants reach higher core temperature in the experimental condition not associated with a higher perception of it and neither their perception of effort was impacted despite the better performance. In another study, also using methylphenidate, Swart et al. (2009) found similar results on performance (they didn't mention the temperature of exercise...) and this was associated with greater metabolic and cardio-respiratory stress in the methylphenidate condition (higher oxygen consumption and heart rate) without changes in their perception of the associated effort. From those results, it appears that increased dopaminergic activity in hot environment allows participants to push harder and becoming warmer without any change in their perception of effort.

Another widely used molecule is the reboxetine (this time, a noradrenergic reuptake inhibitor, which increase noradrenaline (figure 16) which has been shown to decrease endurance performance in ambient temperature and in hot conditions (respectively -10% at 18°C and -20% at 30°C; (Roelands et al. 2008). In a recent study (KLASS et al. 2016) exposed that the diminution in endurance performance related to the use of reboxetine was also associated to an increased rate of central fatigue (using  $VA_{TMS}$ ) without changes of muscular or corticospinal excitability (M-wave and MEP). From those results, the authors concluded that the performance impairment in the reboxetine condition was due to a decreased output from the motor cortex. Interestingly, mental fatigue has also been recently linked to changes in brain neurofunction, Pageaux et al. (2015) hypothesized that the neural activity associated with cognitive tasks would exacerbate the extracellular adenosine concentration (known for its inhibitory action on the dopaminergic pathway) in certain parts of the brain subsequently increasing perceived exertion and leading to a decrease in endurance performance. This is supported by (Azevedo et al. 2016) who showed that caffeine (an adenosine receptor antagonist) counteracted the effect of mental fatigue on physical performance. However, further researches are required to determine if such a hypothesis can be experimentally verified. From those brief examples and the extended literature on human studies, Meeusen and Roelands (2018) concluded that in normal ambient temperature, the noradrenergic system had a deeply negative impact on performance fatiguability whereas in hot environment dopaminergic reuptake inhibitor alone or combined with noradrenergic reuptake inhibitor seems to increase performance.

As a general conclusion, brain neurochemistry appears to be without any doubts part of the complex mechanisms responsible for fatigue even if further researches are needed to fully understand its impact. Nevertheless, it probably acts together with other central changes such as the neural drive and/or spinal changes that will be detailed below.

### **2.2.3 Spinal changes**

Each  $\alpha$ -motoneuron have their cell bodies in the ventral horn of the spinal cord and innervate several muscle fibers through their axon which constitutes the smallest contractile unity called a motor unit (MU). MU size can vary greatly from a muscle to another and the ratio between the number of motoneurons and the number of muscle fiber is an indicator of how accurately

this muscle can be controlled (the fewer fibers per motoneuron, the more accurate will be the muscle). MU also differ depending on the type of fibers they innervate. Small motoneuron will constitute slow MU (S) innervating small type I muscle fibers, on the other hand, large motoneuron innervate type IIx muscle fibers and constitute the so-called fast fatigable MU (FF) and somewhere between those two lasts the fast fatigue resistant MU (FR) of intermediate size (figure 17). Furthermore, when the muscle is solicited, the smaller MU will be recruited first. This is known as the Henneman's size principle (Henneman et al. 1965).

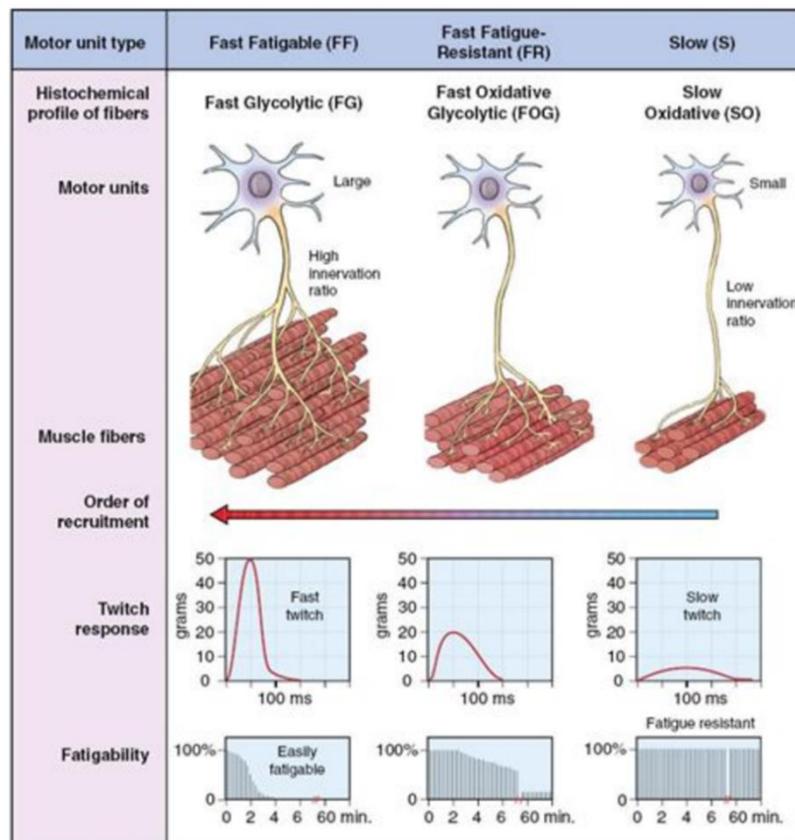


Figure 17 Overview of the characteristics and classification of different motor units. Taken from Kinesiology of the Musculoskeletal System, 2<sup>nd</sup> ed.

Muscle properties are divided into three relations (Partridge and Benton 2011), the force-frequency (F-f), the force-length (F-l) and the force-velocity (F-v). From the F-f relationship depends the conversion between the motoneuron firing rate into isometric force, this is the direct link between the neural drive and the motor response. The force resulting from the neural drive during dynamic exercise will depend on the three relationships, thus, the same descending drive will generate different force output depending on the length of the muscle and the velocity of the contraction. Therefore, most of the study focus on isometric strength,

so it is easier to isolate the F-f component. Thus, during an isometric contraction, a progressive increase in force development will require a progressive increase in the frequency of the descending drive to the muscle. This increased motor drive will have two concomitant consequences, i) increasing the force produced by the already recruited MU by increasing their discharge rate and ii) recruiting new MU. During the beginning of the gradual muscle contraction, the increase of force will be mainly due to the recruitment of new MU (see example in figure 18.A, at ~20% MVC already 50% of the MU have been recruited) and the first recruited MU (i.e., S unit) might reach a plateau (see figure 18.B). This phenomenon is referred as “saturation discharge rate” and correspond to a plateau of the discharge rate of a motoneuron despite increased excitatory signal coming to it and is due to intrinsic mechanisms of the considered motor unit (Fuglevand et al. 2015). However, it should be noted that not all studies report such saturation (Duchateau and Hainaut 1990). When approaching MVC, the force will keep increasing because of an increase in force produced by each MU through an increased discharge rate rather than because of the recruitment of new MU. Interestingly, (Kernell 2006) reported that when motoneurons are recruited and starts to fire action potentials, the resulting force is ~10% of its maximal force.

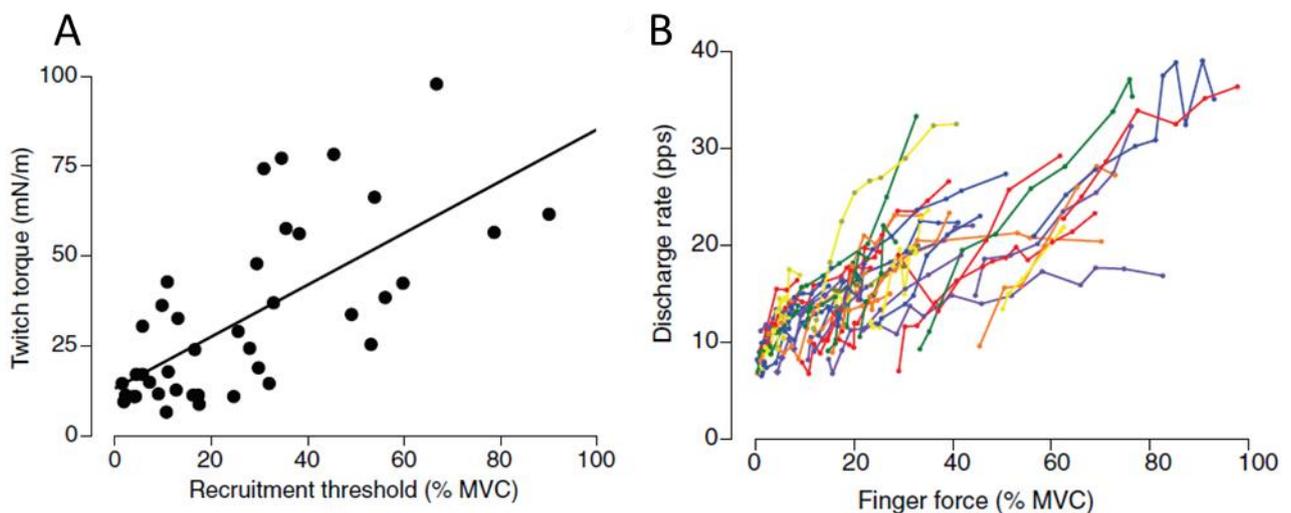


Figure 18. Panel A: the relation between twitch torque and recruitment threshold for 40 motor units (MU) from a single individual. The linear regression was statistically significant  $P < 0.001$ . Panel B: activity of 38 MU of the first dorsal interosseus during brief isometric contraction at different target forces. Each line represents a different MU, for which ten contractions at different targeted forces have been performed. The lower limit represents the minimal target forces at which the motor unit discharged action potential repetitively. 12 of the 38 MU display a clear plateau in discharge rate. Adapted from (Heckman and Enoka 2012)

During a sustained isometric contraction at an intensity superior to the upper limit of MU recruitment, the modulation of the discharge rate differs among muscle. For example, sustaining high force contraction with the tibialis anterior or the forearm extensors muscle result in a decrease of the MU discharge rate (Rubinstein and Kamen 2005) whereas no changes are observed in extensor hallucis longus (Macefield et al. 2000). This decline in discharge rate contribute to the decline observed in MVC (Fuglevand and Keen 2003). When considering a lower level of force, the firstly recruited MU display a decrease in their discharge rate whereas the freshly recruited MU will first increase, then decreased their discharge rate (Conwit et al. 2000; Carpentier A et al. 2001; Calder et al. 2008). The fact that new MUs are being recruited while others present a decline in the discharge rate has been interpreted as an increased in central drive superimposed to intrinsic adjustments of the motoneuron properties (i.e., adaptation and changes in afferent feedback) (Carpentier et al. 2001; Garland and Gossen 2002; Johnson et al. 2004).

Despite this increased central drive, some MU eventually stop discharging AP and this de-recruitment is matched by the recruitment of a new MU, this refers to MU substitution, however, if the previously de-recruited MU is activated again, this is named MU rotation (Heckman and Enoka 2012). During submaximal contractions, studies have shown that this MU rotation process occurs among MU with identical recruitment thresholds (Bawa and Murnaghan 2009). Those adjustments have been attributed mainly to changes in the balance between the central drive and inactivation of voltage-gated channels and are not considered as CNS-controlled strategy recruitment (Westad et al. 2003; Bawa and Murnaghan 2009). It is important to note that the lower is the intensity of the sustained contraction, the more variable are the observed changes (Harwood et al. 2012).

Dynamic contractions have been less studied probably because of the incapacity to well control the associated changes in F-v and F-L relationships but preliminary results suggest that the discharge rate decline in sub-maximal (Harwood et al. 2012) and high-intensity exercise (Cowling et al. 2016), with the greater decline observed in the latter.

### *The decrease in spinal motoneuron excitability and intrinsic properties*

A motoneuron act as the interface between the motor command arriving from the brain and the effector of the movement which is the muscle. It is innervated by various excitatory and inhibitory afferents (figure 19) and the treatment of all those signals, including the supraspinal

(already discussed) and the muscle afferents (discussed below), will result in the production of the motor drive arriving at the muscle. Therefore, any changes in its excitability or intrinsic properties could reduce the command arriving at the muscle, therefore impacting contractile function.

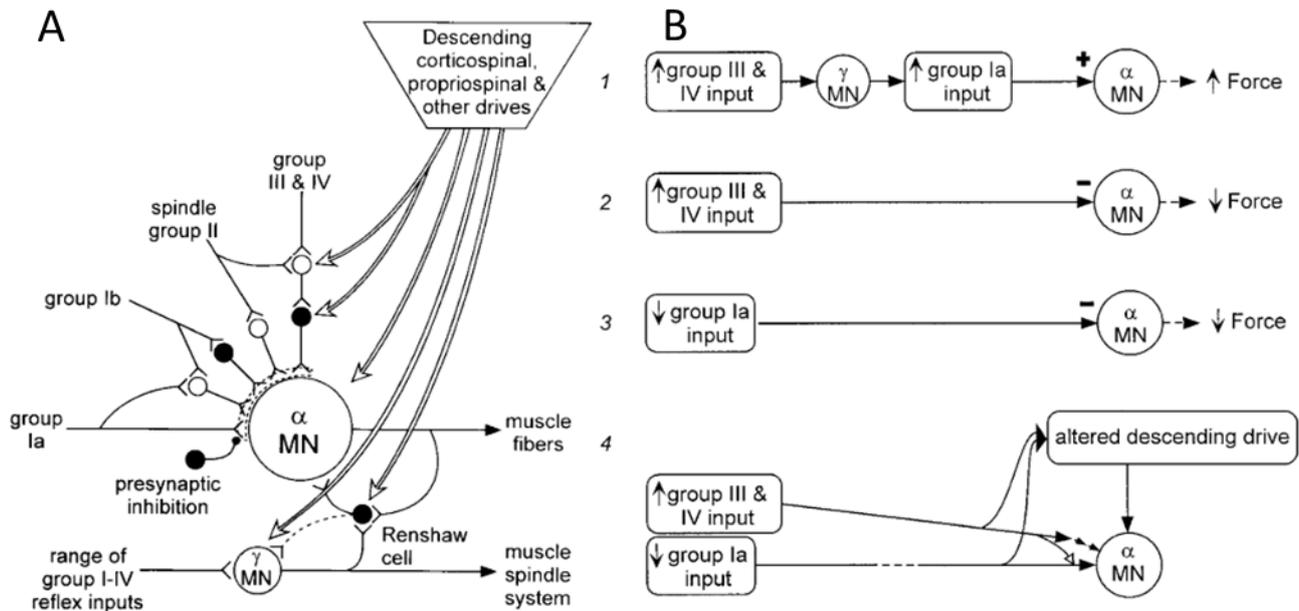


Figure 19. Panel A: summary of inputs to  $\alpha$ - and  $\gamma$ -motoneuron for an agonist's muscle. Cells with solid circles are inhibitory. The dotted curved region at pre-motoneuronal terminals denotes presynaptic inhibition acting selectively on the afferent paths to the motoneurons. Panel B: Summary of possible routes for afferents feedback to change the firing rate of motoneuron during fatigue. Adapted from (Gandevia 2001)

Some evidence suggests that repetitive activation of a motoneuron induces a diminution of its excitability or how it responds to excitatory input (Brownstone 2006). Johnson et al. (2004) showed that to maintain a steady firing rate over time, additional MU were recruited, implying that the targeted motoneuron needed progressively more descending drive to maintain a similar output. Additionally, EMG response to CMEP is reduced during sustained MVC (Taylor and Gandevia 2007) suggesting again a diminution of motoneuron excitability. Furthermore, if CMEP is delivered during the silent period following TMS delivery while the subject is performing a sustained submaximal voluntary contraction, CMEP is also reduced (McNeil et al. 2011). This diminution of motoneuron excitability is believed to arise from changes of the intrinsic properties of the muscle (Taylor et al. 2016b) However the specific mechanisms occurring are unknown.

As mentioned above, neurochemistry in the brain affect the neuronal pathway and it seems also to be the case when considering motoneurons from the spinal cord. Descending serotonergic neurons from the brainstem innervate the  $\alpha$ -motoneuron both at the dendrites and soma levels where serotonin act as an excitatory transmitter, increasing motoneuron excitability through persistent inward current (Heckman et al. 2008). However, Cotel et al. (2013) using an animal model showed that descending serotonergic neurons would be responsible for a decrease in spinal motoneurons excitability through spillover of serotonin. This might be explained by the presence of inhibitory serotonin receptors at the beginning of the axon, therefore, a too high level of descending drive from the serotonergic neurons would induce an accumulation of serotonin near the axon limiting motoneurons firing rate. Thus, the descending serotonergic system could act on motoneuron excitability in different manners, i) by increasing its excitability via a stimulation of the excitatory receptor at the dendrites and soma level; ii) reducing its excitability via a diminution of the descending drive and iii) reducing its excitability because of too much descending drive, leading to an accumulation of serotonin at the axon level. While the given example focusses on the serotonergic pathway, many other neuromodulators are likely to influence motoneuron excitability such as norepinephrine (Johnson and Heckman 2014). Researchers barely scratched the surface regarding the potential impact of neuromodulation on motoneuron excitability and much further work is required in that direction.

Besides changes in intrinsic properties and neuromodulation impacting its excitability, motoneurons are also influenced by the modulated muscle afferents feedbacks (as shown in figure 19; Enoka et al. 2011). Therefore, those feedbacks can be either inhibitory (group Ib, III/IV and Renshaw cell) or excitatory (descending drive and muscle spindle afferents). While some of them are reduced during fatigue, like the group Ib and Renshaw cells (Gandevia 2001) others, like firing rate of the III/IV muscle afferents are increased and lead to a decrease in motoneuron excitability (review from Taylor et al. 2016b). Even though relative mechanisms are still under debate, they likely involve direct inhibition of the motoneuron pool, presynaptic inhibition of Ia afferents and a reduced descending drive from the supraspinal compartment (review from Taylor et al. 2016b). Those effects will be discussed in the next part focusing on muscle afferent feedbacks. To conclude on spinal adaptations, it is likely that a multitude of factors acting together can explain the progressive changes in motoneuron excitability during exercise. Thus, the final response (increased or decreased in excitability) will depend on the magnitude of the different adaptation and are probably not the same regarding the task specificity.

## 2.2.4 Muscle afferent feedback

Muscle afferents are numerous and can have either inhibitory or excitatory effects at the spinal or supraspinal compartment. Figure 19 provides an overview of the muscle afferent at the motoneuron level. They can be categorized by different parameters and have been classified as group I - IV. The figure 19.B present potentials pathway from which muscle afferents could impact motoneuron excitability including a possible altered descending drive. Among the excitatory afferents, muscle spindles (group Ia and II) can directly modulate the sensitivity of the motoneuron pool through transmissions via the Ia pathway. Using microneurography, (Macefield et al. 1991) showed that a sustained submaximal voluntary contraction was associated to a decreased in firing rate of muscle spindles afferents and concluded that this was probably part of the process resulting in a decreased MU discharge rate during sustained submaximal contraction as previously discussed. The effect of muscle spindle on motoneuron pool can also be attributed to modulation via presynaptic inhibitory mechanisms and can be studied through changes in reflexes evoked by weak electrical stimulation (called H-reflex). During sustained MVC (Duchateau and Hainaut 1993) or submaximal contractions (Duchateau et al. 2002), the amplitude of this H-reflex progressively decrease, and authors concluded that not only muscle spindle firing rate decreased, but also the efficacy of the Ia-induced facilitation diminished. H-reflex has also been shown to decrease in the antagonist's muscle, for example, during a sustained submaximal contraction of the elbow flexors, H-reflex of the triceps brachii decreased whereas CMEP (that is not dependent of presynaptic inhibition) increased (Lévénez et al. 2008). These results suggest an adaptation of the co-activation during contraction via the muscle spindle afferents on both motoneurons of the agonist and antagonist muscles.

The small diameter muscle afferents from the group III and IV have been closely related to peripheral and neural changes during both single-joint and whole-body exercise. Group III are mechanically sensitive and respond to muscle contraction or stretch while group IV are sensitive to intramuscular metabolites and metabolic changes.

### *Role of afferents in peripheral fatigue*

Group III/IV muscle afferents play a role in the development of peripheral fatigue by being involved in the regulation of the cardiovascular, hemodynamic, and ventilatory response to exercise (figure 20). Indeed, feedback from the working muscle (Kaufman and Forster 2011) and central command (Waldrop et al. 2011) allows a rapid increase in ventilation, cardiac output and limb blood flow to promote optimal oxygen delivery to the working muscle. Both blood flow and oxygen delivery are considered as key compartment impacting the development of peripheral fatigue during both single-joint and whole-body exercise (Barclay 1986).

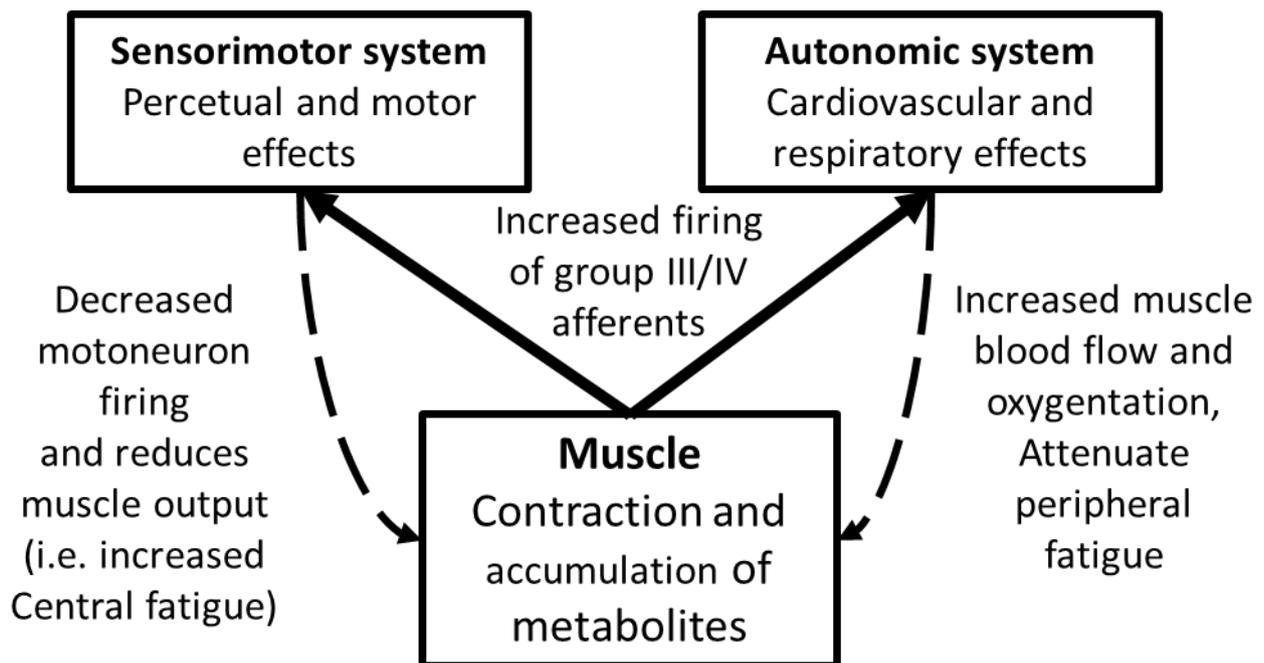


Figure 20. Group III and IV muscle afferents increase central fatigue but attenuate peripheral fatigue. Firing of group III and IV muscle afferents increases during fatiguing contractions. During exercise, these afferents produce reflex increases in heart rate, blood pressure, and respiration to improve muscle blood flow and oxygenation. This slows the development of fatigue of the muscle itself (peripheral fatigue). At the same time, the afferent firing also leads to a reduction in voluntary neural drive to the muscle. That contributes to central fatigue. The afferents evoke sensations of muscle discomfort and fatigue, increase supraspinal fatigue, pre-synaptically inhibit Ia input to motoneurons, and have different actions on different motoneuron pools. Adapted from Taylor et al, (2016).

*Role of muscle afferents in central fatigue*

As presented in figure 19, group III and IV muscle afferents impact directly or not the spinal motoneurons during fatiguing exercise resulting in an impairment of the voluntary activation and thus, exercise performance (Bigland-Ritchie et al. 1986; Amann et al. 2009). This has been shown by using a sustained maximal isometric contraction to induce fatigue (as reported by a decrease in VA) and to study its recovery time course. When classical recovery is performed, VA goes back to baseline values within minutes whereas if a blood pressure cuff is inflated around the limb to trap the metabolites inside the muscle, therefore maintaining the firing of the group III and IV, VA stays depressed until cuff deflation (Gandevia et al. 1996). This result has been confirmed in whole-body exercises using pharmacological intervention to block afferent feedback from locomotor muscle (using fentanyl) during high-intensity cycling exercise. Authors reported that when III and IV muscle afferents were blocked, the output from spinal motoneurons was less restricted compare to the control condition with intact muscle feedbacks.

To conclude, fatiguing exercises are related to many changes within the human body, some associated to the peripheral compartment, some associated with the central nervous system; some limiting performance (power output, time to exhaustion...) others allowing to go further down the limit. Despite the diverging theory behind the concept of fatigue and which compartment prevails (i.e. central vs. peripheral governor), the actual knowledge doesn't allow researchers to conclude if one is more important than the other and, in the end, one could probably not exist without the other.

## 2.3 Neuromuscular function and repeated sprint exercise

As mentioned in a previous section (2.1.2) repeated-sprint exercise (RSE) induces a diminution in performance through the test that can be measured using different indices [e.g. mean power output ( $P_{\text{mean}}$ ), decrement score ( $S_{\text{dec}}$ )...]. Such exercise is extremely strenuous for the neuromuscular function (NMF) and therefore is associated with the development of both central and peripheral fatigue (Collins et al. 2018). This section will try to synthesize the current knowledge in the literature regarding the development of both central and peripheral fatigue during acute RSE and the potential impacting variables. In the second section, some methodological consideration will be discussed.

### 2.3.1 Impact of RSE on neuromuscular function

From the first sprint of RSE, performance is impacted regarding of the exercise modalities (Mendez-Villanueva et al. 2008; Girard et al. 2011a); however, its diminution doesn't appear to be linear. Indeed, authors often report a greater loss of performance from the first sprints compared to the latest (Racinais et al. 2007; Mendez-Villanueva et al. 2012) and this has led the community to hypothesize that maybe this could be linked to differences in fatigue etiology. To investigate NMF, researchers often used isometric contraction pre- and post-RSE to identify and interpret potential changes in fatigue indices (e.g. MVC, VA, M-wave...). And until recently, not many studies had investigated the time course and etiology of fatigue during RSE and how it was impacted by the recovery period (i.e. Repeated vs intermittent sprint, see section 2.1.1). Because of the longer period of rest between sprint in the intermittent modality, one could argue that it would facilitate the intracellular process of recovery, therefore, impacting peripheral and central aspect of fatigue (Spencer et al. 2005; Twomey et al. 2017). Pearcey et al. (2015), had participants performed an intermittent-RSE consisting of ten 10 s leg-cycling sprint with 180 s of recovery between sprint and measured NMF before, during (at sprint 5) and after exercise. The 180 s of recovery wasn't enough to mask the development of fatigue and the diminution of performance; thus, they obtained the same pattern with two slopes observed with RSE (higher decline in performance during the first 5 sprints vs. the last 5). They also found that following the first 5 sprints, fatigue was peripheral whereas after, a combination of both peripheral and central fatigue occurred.

Hureau et al. (2016) investigated NMF after RSE consisting of either 1, 4, 6, 8 or 10 sprints with 30 s of recovery and an additional session of 8 sprints with only 10 s recovery. This original design allowed them to obtain kinetics of NMF changes induced during RSE. They found that already after the first sprint, peripheral fatigue occurred and remained so during all the subsequent sprints whereas central fatigue happened only after sprint 6. Interestingly, the decrease in power output was more pronounced when recovery was fastened to 10 s compared to 30 s whereas neuromuscular fatigue was not altered (i.e. same impairment with both recovery time, figure 21). Those results suggest that whatever the recovery period between sprints (i.e. 10, 30 or 180 s) the relative kinetics of fatigue appears to be similar with a rapid onset of peripheral fatigue and progressive development of central fatigue. This really questions the impact of recovery time on NMF during RSE.

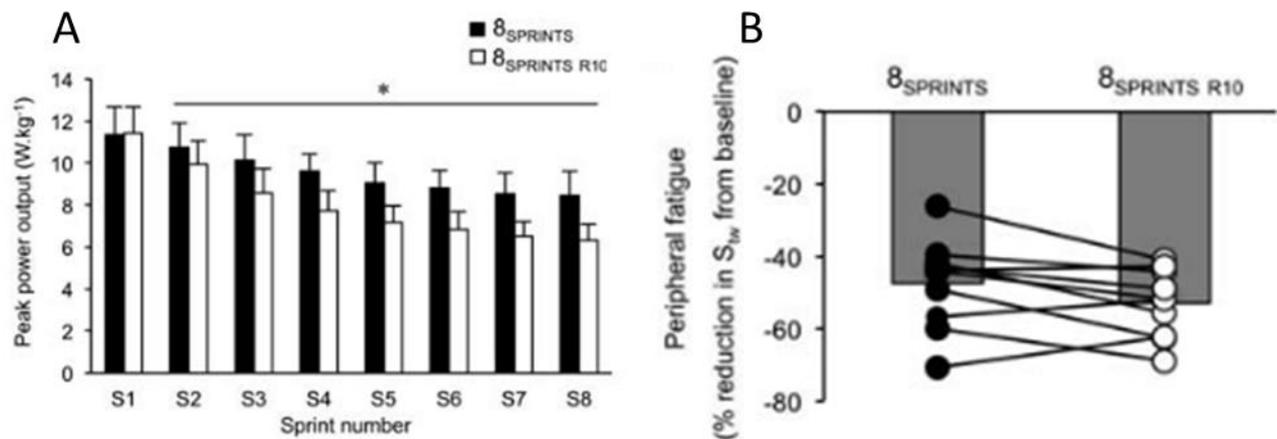


Figure 21. Influence of the recovery period on exercise performance and peripheral fatigue. Panel A shows the evolution of peak power output during each sprint and panel B shows the associated development of peripheral fatigue in both conditions. 8<sub>SPRINTS</sub>, repeated sprint exercise consisting of 8 sprints of 10 s with 30 s recovery; 8<sub>SPRINTS</sub> R10, repeated sprint exercise consisting of 8 sprints of 10 s with 10 s recovery; S<sub>tw</sub>, single twitch force response. Adapted from (Hureau et al. 2016).

### *RSE and recovery time*

A practical way of limiting the decline in performance associated with the development of neuromuscular fatigue during RSE would be to increase the recovery time between sprints. Overall, the preservation of repeated sprint performance with increased recovery time has already been shown (Glaister et al. 2005; Billaut and Basset 2007). Thus, it has been hypothesized that it may be due to greater resyntheses of Pcr and ATP stores (Bishop et

al. 2004; Bishop and Claudius 2005; Mendez-Villanueva et al. 2012) and improved evacuation of muscle metabolites (Glaister 2005). However, the results mentioned earlier from Hureau et al. (2016) doesn't report any differences in fatigue associated with RSE with either 10 or 30 s recovery between sprints (figure 21). Neuromuscular fatigue is also known to recover relatively quickly depending on its etiology and, two minutes after a sprint protocol, authors showed that it was minimal (Perrey et al. 2010; Girard et al. 2013; Hureau et al. 2014). Then, a possibility is that it takes a longer duration than 30 s for the neuromuscular function to recover. Monks et al. (2017) performed a similar study like the one exposed earlier (Hureau et al. 2016), however, they compared a recovery period of 30 s and 180 s. Their findings were similar with improved performance during RSE (i.e. less power decrement with 180 s recovery) but no differences regarding neuromuscular fatigue development. This suggests that recovery time doesn't impact performance and fatigue etiology to the same extent (i.e. the longer period of recovery improves RSE performance without changing fatigue development). This is rather surprising, and a possible hypothesis is that the use of isometric contraction is not an effective modality to evaluate fatigue induced by whole-body exercise involving alternative high-speed muscle contractions. However, further data are needed to support those findings.

From those results, it seems that peripheral fatigue appears from the very early stage and persists through the RSE and may be the major factor limiting performance (Perrey et al. 2010; Goodall et al. 2014a). On the other hand, central fatigue appears later (Goodall et al. 2014a; Pearcey et al. 2015) and some authors suggest that it could play a role by limiting the central drive as a protective mechanism to avoid further peripheral fatigue preventing muscle damage. The next section will discuss the potential underlying physiological mechanisms behind fatigue.

### **2.3.2 RSE and the underlying physiology of neuromuscular fatigue**

Neuromuscular fatigue results in a combination of both peripheral and central mechanisms.

#### *Peripheral fatigue*

The first aspect to look at when considering the development of peripheral fatigue is to investigate whether the membrane excitability is impaired or not. An indirect measure can be obtained through the M-wave assessment and despite the conflicting results obtained with various types of exercises and the methodological issues associated regarding its analysis (see section 2.2.1.1) it seems that in a majority of studies, M-wave isn't impacted by RSE during cycling (Hureau et al. 2016; Tomazin et al. 2017; Monks et al. 2017) with some exception (increase of M-wave reported by Racinais et al. 2007). Results are a bit more confusing when considering running RSE as authors reported no changes (Goodall et al. 2014a; Tomazin et al. 2017) or an impairment (Perrey et al. 2010) of M-wave. Overall, these findings suggest that the neuromuscular transmission is maintained during RSE and indicate that the origin of fatigue is located beyond the sarcolemma. Indeed, concomitantly to the M-wave measurement, the same authors also reported a diminution in the twitch force assessed via peripheral nerve stimulation translating an impairment of the EC coupling. Among the several factors impacting EC coupling (see section 2.2.1.2) most of them are also impacted by RSE.

Indeed, energy furniture during the early part of RSE mainly relies on the anaerobic glycolysis and has therefore been associated with an accumulation of muscle metabolites and a Pcr/ATP breakdown. For instance, Gaitanos et al. (1993) showed that after a single 6-s cycling-sprint, Pcr and ATP stores were depleted respectively to 43% and 87% of resting values. They also showed, that after a repetition of ten 6-s sprints with 30-s recovery, Pcr and ATP store were further diminished respectively to 16% and 68% of baseline values. Those results were confirmed in another study by (Dawson et al. 1997) using six sprints and a recovery period of 24 s. The two main byproducts associated with glycolysis are  $H^+$  and  $Pi$  and their respective contributions to peripheral fatigue have been presented in section 2.2.1. The gold standard to assess skeletal muscle bioenergetics during exercise is the phosphorous magnetic resonance spectroscopy ( $^{31}P$ -MRS), but it has not yet been possible to perform RSE inside the magnetic resonance imagery (MRI) device. However, researchers showed some relative similar metabolic changes between a 3 min all-out cycling exercise and a 5 min all-out isometric knee extensors protocols (Burnley et al. 2010) and despite the relative differences in exercise modalities, all-out knee extensors protocol might provide a first insight of what could happen inside the muscle during RSE. In a recent study, (Broxterman et al. 2017), had 8 participants performing an all-out single leg isometric knee extensors exercise (3 s MVC and 2 s relaxation) inside a whole-body MRI and outside to perform NMF evaluation pre- and post- (on a separated visit). They showed a decrease in

VA (-12%) and Pt (-52%) similar as the one observed by Hureau et al. (2016) after ten 10 s sprints with 30 s of recovery (i.e. VA: ~11%, Pt: ~50%). And at the end of the exercise, Pcr was approximately 20% of its baseline value which also matches the decrease during repeated sprint presented above (Gaitanos et al. 1993). However, in the Broxterman et al. (2017) study, the decrease in MVC was much higher compared to Hureau et al. (2016) (respectively -58% vs. -19%) and this is probably due to the differences between the fatiguing and the evaluation task (i.e. fatigue is induced by RSE and evaluated using MVC) and the specificity of the fatigue induced by repeated isometric MVC (i.e. strong vaso-occlusion). Nevertheless, (Broxterman et al. 2017), present some interesting results regarding the potential links between peripheral fatigue and intramuscular metabolic perturbation during all-out exercise (figure 22).

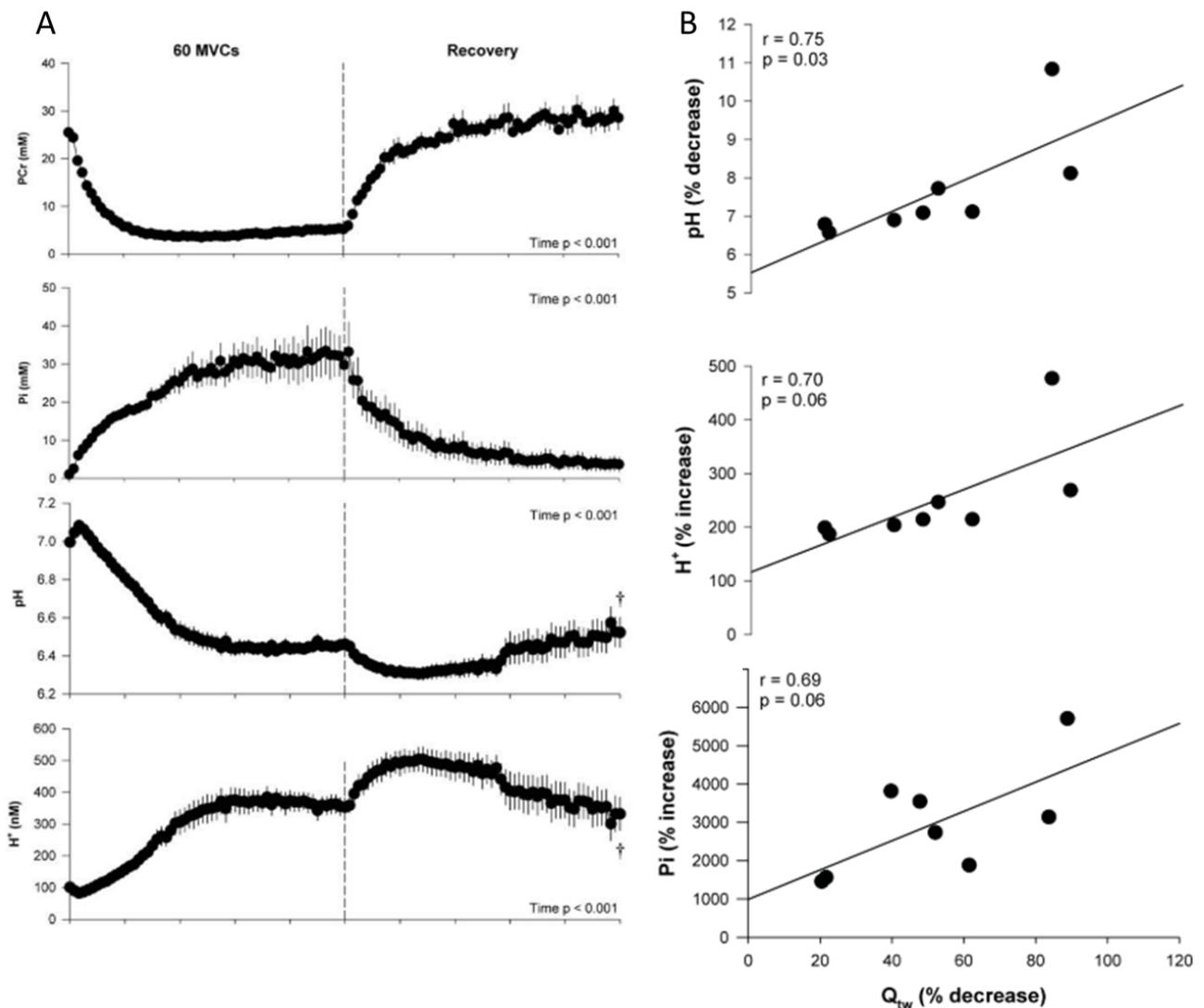


Figure 22. The link between peripheral fatigue and Intramuscular metabolic perturbation. Panel A: Intramuscular metabolite concentrations during the 5-min all-out intermittent isometric single-leg knee-extensor protocol. And panel B: the relationship between quadriceps fatigue and intramuscular metabolites (both in the percentage of change from baseline to end-exercise). Adapted from (Broxterman et al. 2017)

Indeed, the diminution in Pt was correlated with the changes in Pi, pH, and H<sup>+</sup> during exercise. Another interesting result is their bioenergetics findings of all-out exercise that appears to be not so far from the one provided in section 2.1.2 for RSE. Thus, whereas the ATP mainly originated from the anaerobic pathways at the beginning of the test, it progressively switched toward aerobic metabolism (Figure 23).

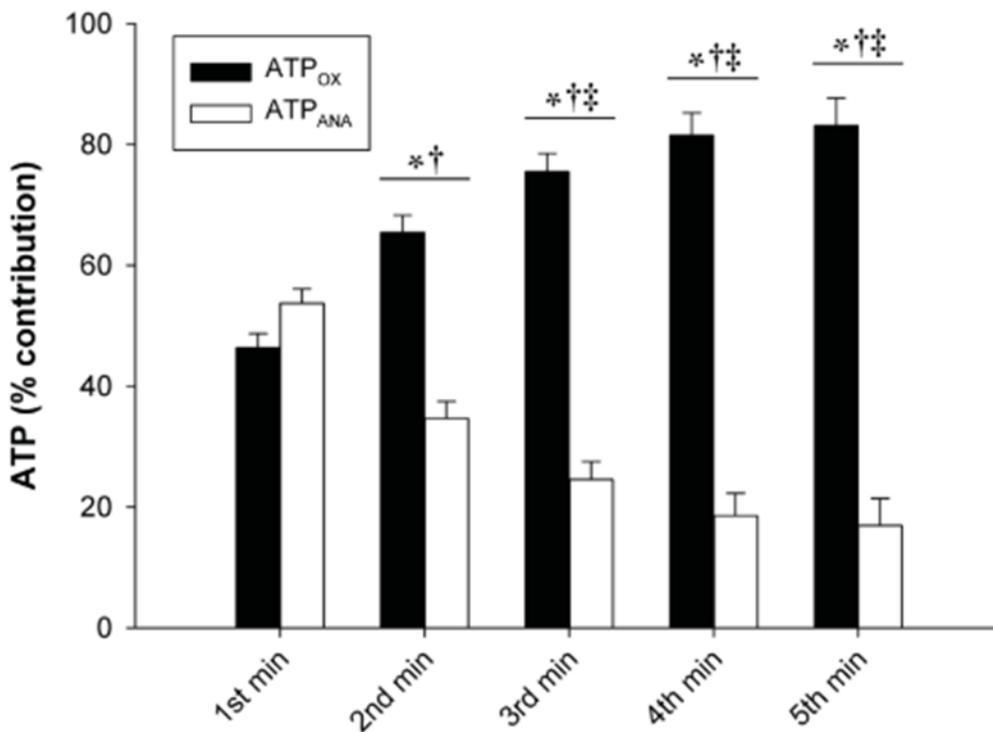


Figure 23. Contribution of anaerobic and oxidative ATP production during the 5-min all-out intermittent isometric single-leg knee-extension protocol. ATP<sub>OX</sub>, rate of ATP synthesis through oxidative phosphorylation; ATP<sub>ANA</sub>, rate of ATP synthesis through oxidative phosphorylation. Both are expressed relative to the total ATPase rate. Significantly different \* between conditions, † from 1st min, and ‡ from 2nd min ( $p < 0.05$ ). taken from (Broxterman et al. 2017)

Of course, the differences in exercise modality are to consider with care and further studies with a fatiguing protocol closer to RSE are needed to better understand intramuscular perturbation induced by the repetition of supramaximal exercises.

The last mechanism known to impact peripheral fatigue is the depletion of glycogen store. While it is often associated with prolonged exercise, the study performed by Gaitanos et al. (1993), provides some interesting information as they found a decrease in muscular glycogen (measured by biopsies) after both a single 6 s sprint (~15%) and RSE (~37%) consisting in ten 6-s sprints with 30 s recovery. Although the potential mechanisms between glycogen store and peripheral fatigue are not well understood, it is still a plausible cause that could explain performance impairment.

To conclude, it seems that because of the supramaximal nature of RSE and its dependency towards anaerobic pathways, the development of peripheral fatigue appears inevitable.

### *Central fatigue*

As discussed in section 2.2.1, different techniques exist to investigate the central component of fatigue in exercise physiology and have been used combined with RSE to try to better understand fatigue etiology.

The estimation of VA either via TMS or nerve stimulation has been reported by several authors during leg-cycling sprints (Racinais et al. 2007; Billaut et al. 2013), Arm-cycling sprints (Pearcey et al. 2016) and running sprints (Perrey et al. 2010; Goodall et al. 2014a). In a recent study, (Tomazin et al. 2017) compared fatigue etiology between running and cycling sprints and reported a similar decrease of peripheral fatigue whereas central fatigue measured 30 s after the end of the exercise was only present in the running condition. Their protocol consisted of 4 sets of five 6 s-sprints, 24 s-rest with 3 min rest between sets (i.e., 20 sprints) and their measures were after the first, second and final sets. A potential explanation from the differences observed could arise from a higher metabolic demand during running sprints (i.e. more accumulation of byproducts), leading to an exacerbated drive from the group III and IV muscle afferents. However, (Rampinini et al. 2014) reported that both modality of RSE (i.e. running vs cycling) induced similar level of metabolites (blood lactate and H<sup>+</sup>). So, VA impairment should have been detected in both sprint modalities. Another explanation could be that running induces more muscle damage than cycling due to the specific mode of contraction relying on stretch-shortening cycle (SSC) that have been shown to release K<sup>+</sup> and interleukin-6 (Dousset et al. 2007), both triggering group III and IV muscle afferents (Hoheisel et al. 2005). But because the impairment in VA disappeared after a short period of rest (5 min), it is unlikely to be the only explanation. Finally, other authors did report a diminution in VA after cycling-RSE (Pearcey et al. 2015; Monks et al. 2017) but only after the tenth sprint whereas it could be detected after the second running sprints (Goodall et al.

2014a). In a recent study, (Willis et al. 2018) performed a cycling-RSE to exhaustion and reported no associated diminution in VA or RMS/M-wave, however, their measurements were performed 3 min after the end of the test. Taken together, these results suggest different central perturbation pattern depending on sprint modalities. The experimental design used by (Tomazin et al. 2017) probably played a role in the fact that they didn't detect any changes in VA during cycling-RSE (only five 6 s-sprint per block). This highlight the need for further research to confirm and provide more insight regarding the differences in fatigue etiology and the associated mechanisms in cycling- vs. running-sprints.

While most studies couldn't differentiate spinal from supraspinal origin of fatigue and reported unaltered MEP (Girard et al. 2013). (Goodall et al. 2014a) hypothesized different mechanisms at the spinal and supraspinal level. Their interpretation comes from the fact that they have changes in VA, but not in MEP or M-wave, they concluded that a compensatory mechanism must occur at the spinal level to mask the supraspinal changes. However, they didn't have any "direct" measure of the spinal component. Some authors used additional techniques such as cervicomedullary motor evoked potentials (CMEP; Pearcey et al. 2016) or H-reflex (Perrey et al. 2010; Pearcey et al. 2017) to get more insight on what happened in the CNS during RSE. The first to do so were Perrey et al. (2010) by using the H-reflex (i.e. a method allowing to detect changes in the excitability of the motoneuron pool) after twelve 40-m running sprints interspaced by 30 s of recovery. Because of methodological limitation, H-reflex is difficult to assess, and especially on certain muscle and therefore, in this study, the muscle used was the soleus. They reported significant decrease in MVC (-11%), VA (-2.7%), Pt (-13%) and the H-reflex remained constant. Those results are to take with care as the chosen muscle is known to have a high proportion of type I muscle fibers, highly resistant to fatigue development, which could explain the relatively low impact of exercise on muscle function compared to other studies. Furthermore, H-reflex is a technique presenting a high variability and takes time to complete (i.e. the total time required for the H-reflex evaluation was ~1 min and the delay between the end of exercise and the evaluation procedure isn't mentioned.) Despite the limitations, those results suggest that the impact of RSE on central fatigue, while being small, would mainly occur at the supraspinal level (i.e. decrease in VA while similar excitability of the motoneuron pool).

The other study was performed by Pearcey et al. (2016), they incorporated measurement of MEP and CMEP during an arm cycling RSE. As for H-reflex, CMEP has some further methodological limitation compared to TMS and is relatively hard to obtain for lower-limb muscles (Taylor 2006) which probably explain why authors used arm-cycling sprints.

However, when both TMS and CMEP are used together, it is possible to estimate the respective part of the spinal and supraspinal induced changes in central fatigue. Indeed, CMEP has a large monosynaptic component reflecting motoneuron excitability when normalized by the M-wave. On the other hand, the supraspinal component can be isolated by normalizing the MEP by CMEP. For a review of the CMEP technique, one can refer to (Taylor 2006). In their study, Pearcey et al. (2016) had participants performed two intermittent-RSE consisted of ten 10 s-sprints interspaced with 150 s of recovery. The first one was used to measure force, VA and Pt responses (session A) after sprints 1, 5 and 10 whereas the second (session B) was only for corticospinal excitability assessment (i.e. TMS and CMEP during a contraction at 5% MVC force). In both sessions, the authors reported that the delay between completion of the sprint and the beginning of the NMF evaluation was 5 s. They reported a decrease in power output for both sessions (session A: 13.1% and session B: 18.7%). In session A, MVC, Pt, and VA decreased respectively by 8.7%, 27.6% and 5.6% whereas M-wave didn't change after the RSE (results are summarized in figure 24). In session B, MEP/M-wave amplitude decreased from pre- to post sprint 1 (-28.7%) and post-sprint 5 (-42.1%) but increased from post-sprint 5 to post sprint 10 (+40.1%). Finally, the changes between pre- and post-sprint 10 weren't significant (-19.6%;  $P = 0.069$ ). Interestingly, CMEP amplitude didn't change between pre- and post-sprint 1,5 and 10. But significantly increased from post-sprint 1 to post-sprint 10 (+25.5%) and post-sprint 5 to post-sprint 10 (+48.5%). MEP/CMEP ratio significantly decreased from pre-sprint 1 to post-sprint 5 (-31.9%) and post-sprint 10 (-35.8%). Those results are summarized in figure 24 and provide an interesting point of view regarding the development and etiology of central fatigue associated with arm cycling RSE.

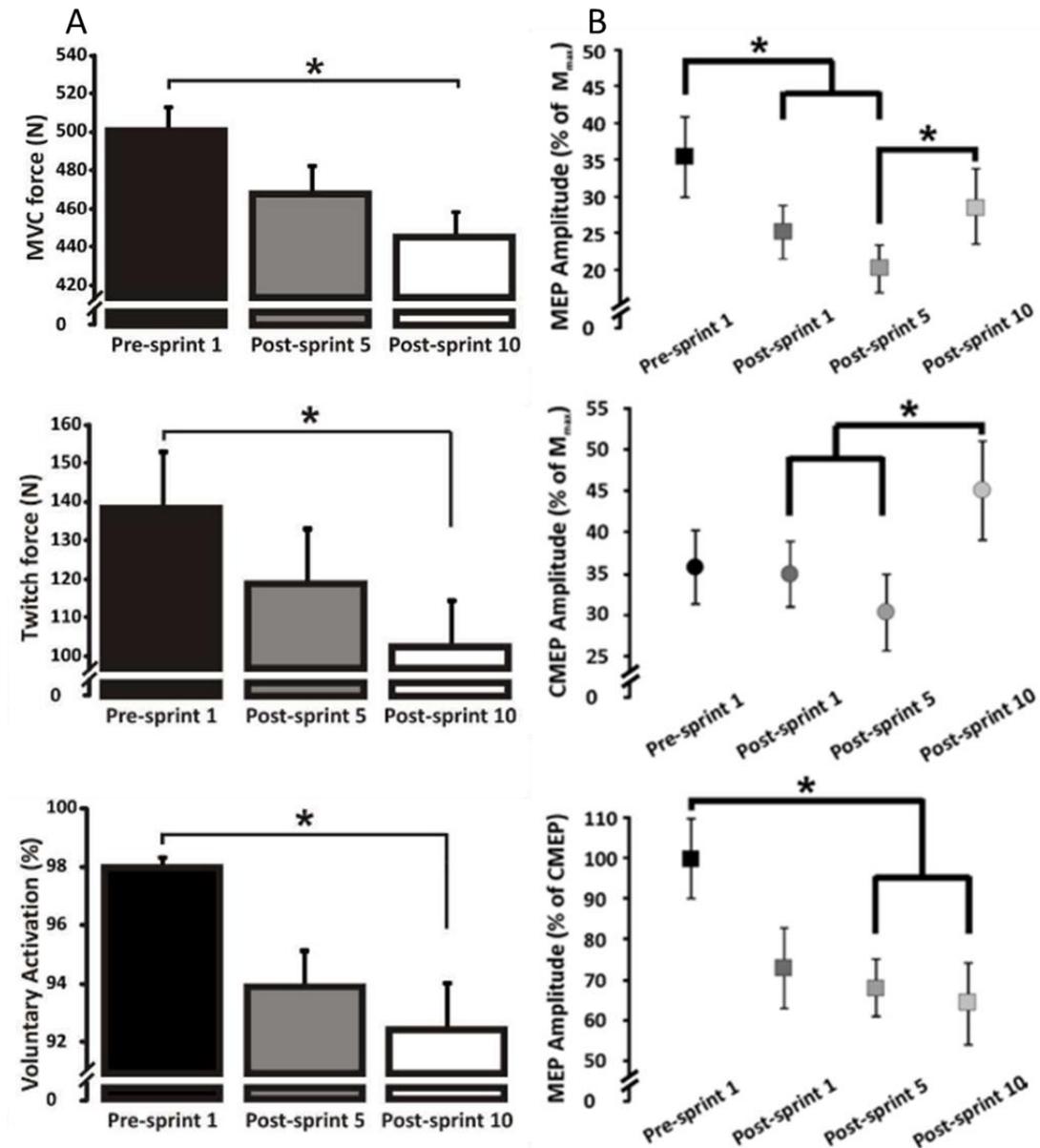


Figure 24. The effect of an arm-cycling repeated sprint ability test (ten 10 s-sprints with 150s of recovery) on the neuromuscular function of the elbow flexors muscle. Panel A shows the changes in maximal voluntary force (MVC), twitch force and voluntary activation. Panel B shows the biceps brachii changes in corticospinal excitability [as a ratio between motor evoked potential (MEP) and M-wave], spinal excitability (as a ratio between cervicomedullary motor evoked potentials (CMEP) and M-wave) and supraspinal excitability (as a ratio of MEP/CMEP). Values are presented as Mean  $\pm$  SEM. Adapted from (Pearcey et al. 2016)

Authors, therefore, reported a different pattern of evolution between spinal and supraspinal changes. It appears that during the early stage of fatigue (i.e. the first 5 sprints), MEP, representing the overall corticospinal excitability, decreased whereas, from sprint 5 to sprint 10, MEP increased to come back relatively close to its resting value. When considering separately spinal and supraspinal changes, it looks that they follow a distinguished pattern

that could explain the peculiar results obtained with MEP. Indeed, spinal excitability tends to increase whereas supraspinal excitability (characterized by MEP/CMEP) clearly decreased towards the arm cycling RSE. Because no other study looked into the effect of arm cycling RSE on NMF, the next paragraph will try to provide an overview of the potential mechanisms that could have occurred by evoking results from other exercise modalities. It should, therefore, be taken with care as it is highly speculative.

First, the increased spinal excitability can have several potential origins (as discussed in section [2.2.3](#)), such as increased excitatory afferents from the muscle spindles or a descending drive from serotonergic neurons leading to the release of serotonin at the dendrites/soma level of motoneurons and creating persistent inward currents known to induce an increase in motoneuronal excitability (Heckman et al. 2008). Previous studies have shown that the activity of the serotonergic neurons increased concomitantly to the motor output. It is then possible that due to the high motor output required during RSE, the increased activity of the serotonergic neurons leads to increased spinal excitability. However, this interpretation is only a hypothesis since it's not yet possible to evaluate directly the impact of RSE on such variables.

Supraspinal component clearly appears to be reduced during RSE and indicates a diminution of the central motor drive. This finding is in accordance with a study from (Sidhu et al. 2017) where authors reported that relatively high-intensity cycling-exercise (80% of peak power output) induced a diminution in the central motor drive (MEP/CMEP ratio) but not in spinal drive. Furthermore, when combined with a pharmacological condition blocking group III and IV muscle afferent (using Fentanyl), this reduction of central drive disappeared, leading the authors to conclude that group III and IV muscle afferents were responsible for the diminution of central drive associated to fatiguing leg-cycling exercise. Even if no studies investigated directly the impact of RSE on group III/IV muscle afferents, there are quite high chances that they play a major role in the development of fatigue as their activity has been strongly correlated to muscle metabolites accumulation (Taylor et al. 2016b).

Taken together, these results suggest that despite not being the main origin of fatigue, central components are impacted during RSE and probably even more when considering running compared to cycling exercise. The distinct pattern followed by supraspinal (i.e. a decreased) vs. spinal (i.e. increased) drive might be responsible for the overall unchanged MEP amplitude observed in some studies. Furthermore, the fact that the only study performing RSE to exhaustion (Willis et al. 2018) reported no change in VA also brings uncertainty about the potential mechanisms occurring at exhaustion. Further studies are needed to confirm the

previous results and extend the understanding of the potential mechanisms acting on the central compartment.

### *Conclusion*

Because of the huge disparity of exercise modalities used to investigate the impact of RSE on NMF (i.e. intermittent vs repeated sprint; lower- vs upper- limb; cycling vs. running), it is quite difficult to provide unbiased conclusion mainly due to the known task- and muscle-dependency of fatigue (Carroll et al. 2006a; Taylor and Gandevia 2007). However, despite all the limitation associated, the previous results regarding both peripheral and central component will be discussed and, potential mechanisms proposed regarding the development of neuromuscular fatigue associated with RSE. As this is highly speculative, the upcoming discussion is to be taken with care and needs further investigation to be confirmed. At the beginning of the precedent section (2.2.3.1), it was proposed that the decrease in power observed during RSE wasn't linear possibly due to differences in fatigue etiology. (Collins et al. 2018) proposed a model (figure 25.A and B) based on the literature postulating that the total neuromuscular fatigue will first increase because of the peripheral compartment and then would rely on both peripheral and central fatigue. While the idea is appealing, the fact that the authors proposed a breaking point with an increase in the rate of fatigue development from the middle of the RSE to the end is surprising and doesn't fit with the observed decrement in performance (i.e. higher rate in performance at the beginning). Rather, it could be hypothesized that the rate of fatigue development is higher during the first phase of the test due to a strong reliance on the anaerobic glycolysis. Through the test, the energy would be more and more furnished by the aerobic system, diminishing the rate of peripheral fatigue development. The central fatigue is even more delicate to interpret as it appears to have different kinetics depending on exercise modalities (i.e. cycling vs running) and that both of its component (i.e. spinal and supraspinal) tend to show different patterns during RSE. However, figure 25.C proposes a modified version of the model of (Collins et al. 2018) that might better fit the development of fatigue during RSE. Again, this proposal still needs to be supported by further experimental data and some limits can already be pointed out as the pattern will probably highly dependent on the "profile" of the participant performing the RSE (i.e. trained, untrained, endurance runner, sprinter...). Furthermore, the only study

(Willis et al. 2018) regarding the neuromuscular function at exhaustion is surprising as it doesn't report any impairment in VA. Therefore, further studies are required in that direction.

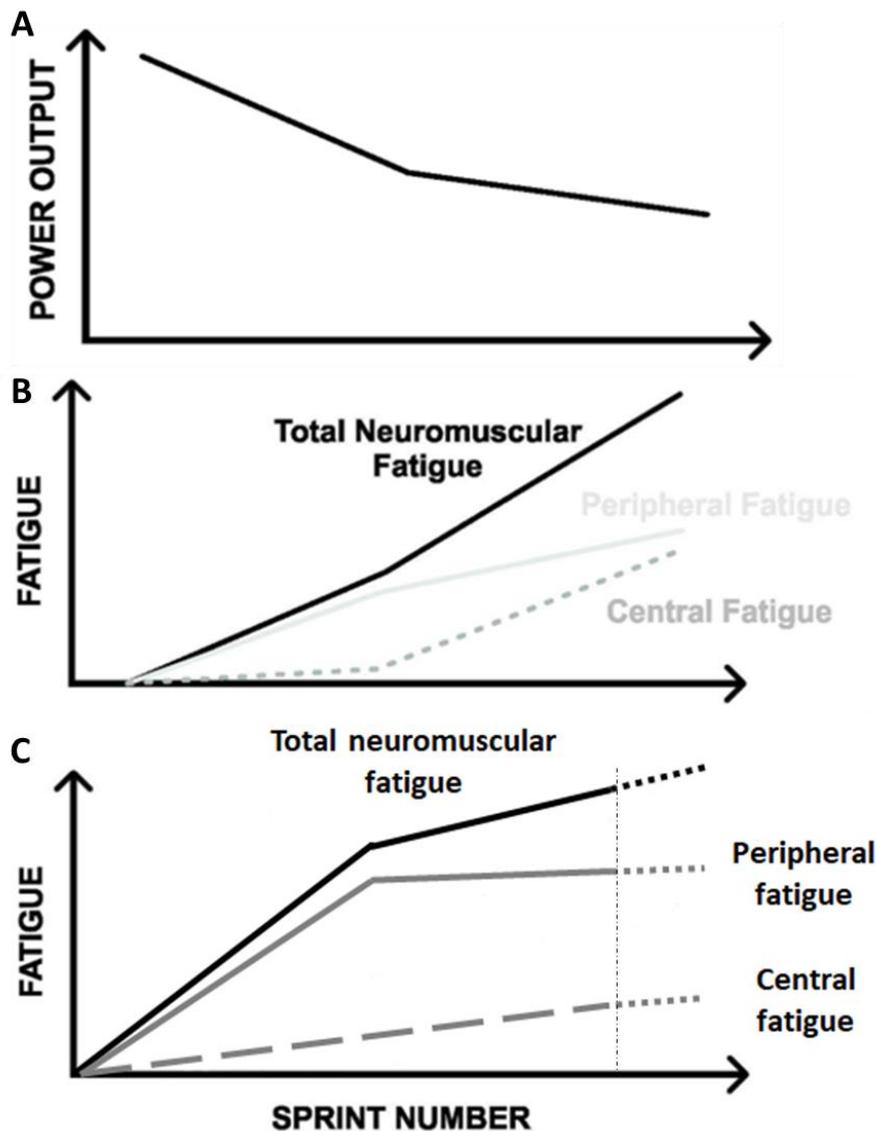


Figure 25. The development of neuromuscular fatigue during a repeated-sprint exercise (RSE). Panel A shows the typical pattern of diminution in sprint performance (here, power output) when ten sprints are repeated with incomplete recovery (< 180s) and panel B is a model proposed by (Collins et al. 2018) to explain the associated origin of neuromuscular fatigue. Surprisingly, the rate of neuromuscular fatigue didn't match the rate of diminution in performance and the amount of central fatigue was paramount to the peripheral fatigue at the end of the RSE. Panel C shows another proposition to explain the same phenomenon, however, the rate of fatigue development would be higher at the beginning of the sprint and the total amount of fatigue would more rely on the peripheral compartment. The dashed line represents the usual number of sprints performed during RSE (~10) and questioned the validity of this model when performed to exhaustion. Interpretation of this model should be made with care as it is highly speculative. Adapted from (Collins et al. 2018).

The next section will try to provide an overview of the actual limitations regarding the evaluation of neuromuscular fatigue induced by RSE.

### 2.3.3 Methodological considerations

Different tools allow the investigation of the neuromuscular function and have been used to highlight the presence of both peripheral and central fatigue associated with RSE in a variety of modalities. As briefly mentioned along the past two sections, those techniques are associated with some limitations and it appears necessary to provide an overview of the situation regarding the potential problems accompanying the evaluation of neuromuscular fatigue in response to RSE.

This section will address the issues of task dependency, joint positioning and muscle state, repeated sprint modalities and time delay.

#### *Task dependency*

As stated earlier, when introducing the concept of fatigue, it is important to remember that fatigue is associated with the task responsible for its origin. And yet most of the current literature used isometric contraction to evaluate the etiology of fatigue induced by a repetition of dynamic contraction such as RSE. Furthermore, RSE is often associated with whole-body exercise (i.e. cycling, running) where participants use a combination of different major muscle groups (Dorel et al. 2012) to produce power. But in order to evaluate fatigue, researchers focus on the MVC developed by a single muscle group (often the knee-extensors) and further concentrate their interpretation from the EMG response of one mono-articular muscle (i.e. *vastus lateralis*). In addition, other parameters have been shown to alter neuromuscular fatigue such as contraction mode (e.g. dynamic, concentric, eccentric...), muscle mass, intensity and duration (Rossman et al. 2012, 2014). Central compartment of fatigue has also been proven to be task-dependent (Carroll et al. 2006a; Taylor and Gandevia 2007). For instance, corticospinal excitability of the biceps brachii is increased during rhythmic compare to tonic motor output (Forman et al. 2016). The findings presented earlier from Hureau et al. (2016) and Monks et al. (2017) are also a good example of the limits associated with the

current means to evaluate NMF. Indeed, the fact that they were not able to detect any changes in fatigue etiology despite an increased recovery time associated with increased power output is questionable. Maybe the overall fatigue that is produced by rhythmic dynamic contractions is not entirely transferable to the isometric mode of contraction for the NMF evaluation. Or maybe the absence of differences regarding fatigue comes from the fact that the total amount of work performed was higher in the condition with longer period of recovery as it was associated with higher power output (i.e. the longer recovery period led to higher power output that could have possibly led to similar amount of fatigue compare to a condition with shorter recovery time but lower power output). This brings the question if the work-to-rest ratio is enough to characterize RSE as it doesn't consider the total work performed. This paragraph provides certainly more questions than it gives answers.

Nevertheless, measuring fatigue using task-specific parameters may be more effective for understanding the development of neuromuscular fatigue in response to repeated sprint exercise.

### *Joint position and muscle state*

This point has been addressed in a recent review by (Collins et al. 2018). The first paragraph will transpose their current opinion on the question whereas the second one will briefly expose some limits of their point of view.

So corticospinal excitability of the upper limb has been shown to be altered depending on the joint position when performing an isometric contraction (Mogk et al. 2014), probably mainly due to spinal changes (Nuzzo et al. 2016). In a series of experiments, Collins et al, (Collins et al. 2017; Collins et al. 2017; Collins and Button 2018) investigated the impact of shoulder position (0 vs. 90° of shoulder flexion) and muscle state (i.e. contracted at 10% MVC or relaxed) on spinal and supraspinal excitability (with the use of M-wave, MEP and CMEP) and reported that both joint and muscle state altered corticospinal excitability of a given muscle (figure 26 provide an overview of their findings). Authors concluded that because the joint position and muscle state affect corticospinal excitability, therefore, if evaluating NMF with MVC, it should be taken care of using a position like the one used during the fatiguing modality.

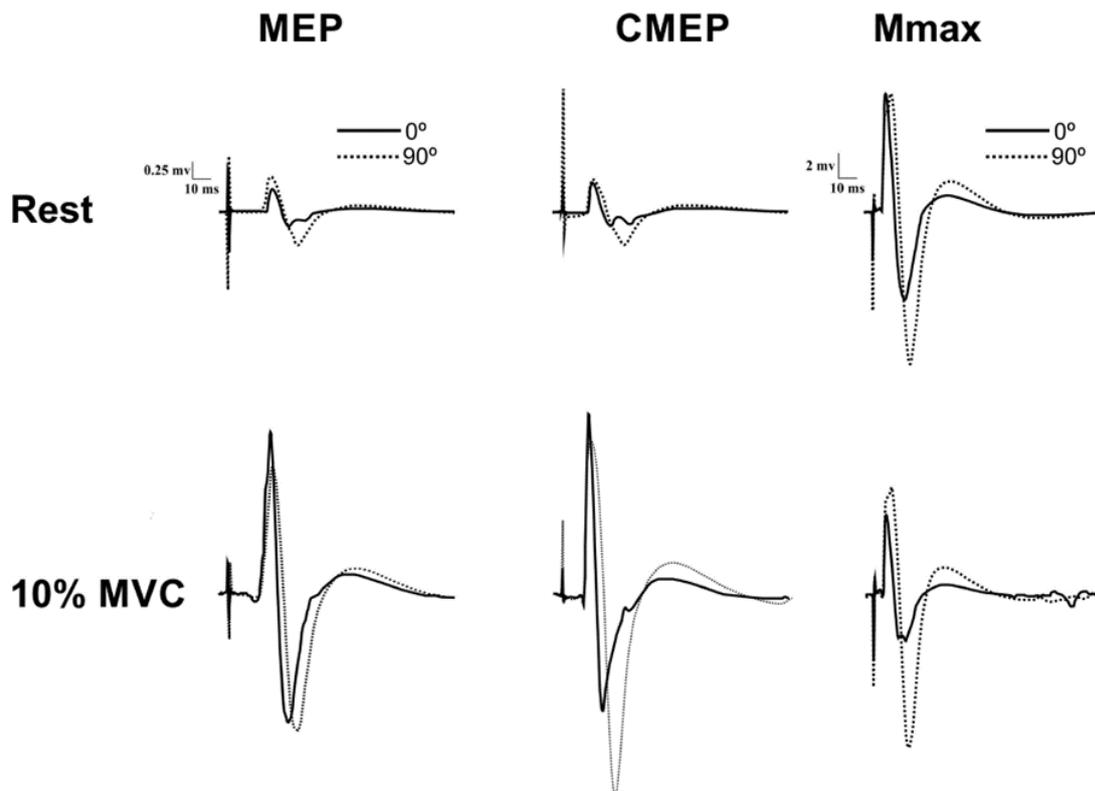


Figure 26. Corticospinal excitability of the biceps brachii assessed either at rest or during a contraction at 10% of maximal voluntary contraction (MVC) force with two positions. Black lines and hashed lines represent respectively  $0^\circ$  or  $90^\circ$  of shoulder flexion. Taken from (Collins et al. 2017)

First, by having a look at their result, it should be noted that mostly the second part of the EMG response seems to be impacted by a change in position and this brings back the point mentioned in the section 2.2.1.1 based on the work of Rodriguez-Falces and Place (2018). Indeed, they explained that the 2<sup>nd</sup> part of the M-wave reflect the termination of the action potential propagation against the tendon and therefore isn't an indicator of muscle membrane excitability (figure 4.A. Section 2.2.1.1). So, when changing the position or the state of a limb, the length of the muscle is likely to change as well, impacting the distance between the motor point and the fiber-tendon junction. This architectural change is likely to be the main reason for the observed change in the so-called "corticospinal excitability" in Collins et al studies. It can be postulated that at a  $90^\circ$  flexion, the biceps brachii is shortened and so is the distance between the motor point and the tendon-fiber junction leading each single-fiber action potentials to reach it in a more synchronous way.

Therefore, additional studies are required investigating only the first phase of the M-wave/CMEP/MEP to see whether the excitability is impaired by the limb position or not. The

main point to assess however is to have the exact same position at each time point of the measurement.

### *Repeated sprint modalities*

Very few studies compared directly the neuromuscular fatigue induced by different modalities of exercise. (Tomazin et al. 2017) reported that similar sessions of running and cycling sprints induced a similar level of peripheral fatigue whereas central fatigue was only detectable after the running modality. They suggested that the current findings in the literature regarding cycling-RSE could not be transposed to running-RSE due to differences in fatigue etiology. No studies reported directly the differences between lower- and upper-limbs and their repercussion on neuromuscular fatigue associated with RSE. However, quite a few reported physiological differences and distinct adaptations to exercises. Upper-body muscles usually involve lower muscle mass and have a larger proportion of type-II muscle fibers (Miller et al. 1993; Koppo et al. 2002), likely relying on different metabolic and cardiovascular responses compare to the legs. For instance, upper-body exercise is known to induce less vascular reactivity (Richardson et al. 2006), shorter blood transit-time but larger diffusion distance and smaller diffusion area (Calbet et al. 2005; Stöggl and Karlöf 2013). Calbet et al. (2009), demonstrated that muscle metabolism elicits systemic perturbation influencing oxygen transport, extraction, and brain function. Therefore, the limb specificities of exercise are likely to produce distinct systemic perturbation impacting neuromuscular fatigue differently. Using 90 submaximal isotonic contractions, it has been shown that knee extensors and elbow flexors have different kinetics regarding fatigue development (Senefeld et al. 2013). In another study, Neyroud et al. (2013b), reported similar force loss after a sustained 50% MVC, the diminution in VA was also similar but peripheral fatigue was higher for the elbow flexors compare to the knee extensors. And Vernillo et al. (2018) showed that the relative strength loss and the associated VA, during a 2 min sustained MVC was higher in the knee extensors compare to elbow flexors but they showed the opposite for peripheral fatigue (i.e. greater diminution of Pt in the elbow flexors compare to the knee extensors). Gruet et al. (2013) also highlighted that upper-limbs are more sensitive to spinal adaptations compare to lower-limbs. Once again, taken together, these results suggest that fatigue etiology is highly dependent on the exercise modality used. Recently, Halperin et al. (2018) compared the responses to upper- and lower-limb RSE in 4 different conditions consisting in ten 10 s-sprints with either

30 or 180 s of recovery but they didn't have any measurement of fatigue development other than the rate of perceived exhaustion (RPE), MVC (post-sprint 5 and 10), and power decrement. Interestingly, considering raw data (i.e. not normalized) authors didn't report any differences in heart rate and RPE between both sprints modalities despite the significant less amount of work performed in the arm conditions. Furthermore, when considering the normalized data (i.e. with the first sprint as reference) no differences were found except for a greater reduction in knee extensors MVC compare to elbow flexors. This suggest that RSE probably leads to differences in fatigue etiology depending on the considered limb as previously suggested with others exercise modalities (Senefeld et al. 2013; Vernillo et al. 2018). However, it should be noted that in the Halperin et al. (2018) study, the applied resistance was different between limbs (i.e. 5% vs. 10% body weight respectively for the arms and legs). Obviously, muscle capabilities are different across limbs and so should be the applied resistance, but this highlight the possible impact that can have the resistance settings on the outcome of RSE. Despite several evidences suggesting that the resistance settings should account for interindividual differences and ergometer specificities (Duche et al. 2002; Jaafar et al. 2015), the majority of studies still conduct cycling sprints using a standard resistance (often 7.5% of body weight), independent of the population, limb or even ergometer tested (Glaister et al. 2005; Hureau et al. 2016). Further studies should be performed to propose an objective way of adapting the resistance during RSE.

Many other factors can influence neuromuscular fatigue development associated with RSE and should be considered. For instance, training status of the participants has been shown to induce changes in the output of the nervous system during muscle contractions (Carroll et al. 2001, 2011; Philpott et al. 2015). Thus, the results obtained during a research protocol highly depend on the recruitment of a specific population (often young and physically active participants...).

### *Time delay*

The classical setup to measure neuromuscular fatigue involve the delivery of a superimposed stimulus during a voluntary contraction thus, requiring the use of a specific ergometer and the necessity to move the participant to it after the completion of the fatiguing task. This has some clear limits as it induces some delay between the end of the task and the measurement of fatigue but also because it prevents researchers to obtain a real kinetics of fatigue etiology

(i.e. often, the study design allows only to have a measure of fatigue pre- and post-exercise, without the possibility to have multiple time points of measurements). Tomazin et al. (2017) reported that usually, this delay is comprised between 45 s and 2.5 min and can be essentially considered as recovery time. This is problematic because neuromuscular parameters of fatigue recover quickly (Bishop and Claudius 2005; Froyd et al. 2013) but even more because depending on its etiology neuromuscular fatigue recover at different rates. Herbert and Gandevia (1999), showed that corticospinal excitability is altered for less than 5 s after maximal motor output compared to later time points. Thus, time delay as short as 5 s may lead to misinterpretation regarding the relative amount of fatigue originating from central vs peripheral compartments. Specific setups have been proposed by different teams to limit this delay and one promising solution appears to combine both sprint and neuromuscular ergometer to avoid the “switch-time” associated to go from one ergometer to another (Doyle-Baker et al. 2017). This method will be presented in the second chapter as a similar model has been developed during the completion of this thesis.

This section presented a brief overview of the concept of neuromuscular fatigue, its origin (i.e. central and/or peripheral) and the potential mechanisms associated with its etiology. It focuses thereafter on the impact of repeated sprint exercise on neuromuscular function and tried to provide information about the current scientific literature and the associated knowledge on the link between repeated sprint exercise and fatigue.

To sum up, repeated sprint exercise induces a combination of both central and peripheral fatigue, however depending on a variety of factors (i.e. upper- or lower- body exercise, work-to-rest ratio, cycling vs running...) fatigue etiology is not constant. Furthermore, some methodological barriers prevent the possibility of investigating fatigue during task completion, often limiting the data collection to pre- and post-exercise.

Moreover, the above-mentioned literature only looked at exercise performed under ecological conditions and fatigue is known to occur more rapidly under deleterious environmental factor, such as, at high altitude (Fan and Kayser 2016), or when blood flow is restricted (Scott et al. 2015).

Thus, investigating the development of neuromuscular fatigue when repeated-sprint exercise is combined with extreme environmental conditions can provide a new experimental model to try to understand the associated physiological mechanisms. In this regard, the next section will bring rapidly some context about the use of hypoxia and blood flow restriction as investigating tools.

### 3 Environmental factors: The Impact of Systemic hypoxia

Hypoxia Can be defined as any state in which oxygen in the lung, blood, and/or tissues is abnormally low compared to sea level. During hypoxic exposure, all the different stages of the oxygen cascade are going to be impacted, from the ambient air to the mitochondria where oxygen is finally used (West et al. 2007). This section will start describing the different processes involved in the oxygen cascade and the acute effect of hypoxia at rest before looking more into details how hypoxia can impact repeated sprint exercise.

#### 3.1 Acute effects of hypoxia at rest

Here, the acute effects of hypoxia on ventilation, pulmonary gas exchange, cardiovascular system, hemoglobin dissociation curve, capillary and mitochondria exchange, and central nervous system will be considered.

##### 3.1.1 From the air to the mitochondria

All cells in the human body depend on oxygen delivery to prosper and, this oxygen comes from the inspired ambient air mainly through the effect of gradient pressure. Noteworthy, never the composition of the air is going to change with altitude, from sea level to the top of Everest ambient air is gas mixture comprised (volume wised) of 20.95% of dioxygen ( $O_2$ ), 78.08% of nitrogen (N) and less than 1% of other gases. However, at sea level, the barometric pressure corresponds approximately to 760 mmHg and as altitude increases, barometric pressure decreases. This pressure diminution will impact the partial pressure of the gases composing the ambient air, including  $O_2$ . Therefore, the partial pressure of a gas composing a mixture can be obtained by multiplying its volume (in % of the total volume of the mixture) by the total pressure of the mixture (in mmHg). For instance, at sea level, the partial pressure of  $O_2$  ( $PO_2$ ) will be 159mmHg ( $0.21 \times 760 = 159$ ). But because the inspired air will be saturated with water vapor passing the trachea and considering that the water vapor partial

pressure at 37°C is 47mmHg, the resulting O<sub>2</sub> pressure of inspired dry air will be 713 mmHg (760-47). Therefore, at sea level, the partial pressure of O<sub>2</sub> when inspired (P<sub>i</sub>O<sub>2</sub>) is 150mmHg ( $[760-47] \times 0.21 = 150$ ). Once O<sub>2</sub> reaches the alveoli, the partial pressure (P<sub>A</sub>O<sub>2</sub>) also depends on the arterial PCO<sub>2</sub> because at this level there is a balance between CO<sub>2</sub> removal from the blood and the alveolar ventilation (i.e. CO<sub>2</sub> extraction from the capillaries and O<sub>2</sub> arrival). P<sub>A</sub>O<sub>2</sub> can be calculated as  $P_{A}O_2 = P_{i}O_2 - 1.25 \times PCO_2$  and with a normal PCO<sub>2</sub> of 40mmHg, PCO<sub>2</sub> is then reduced to 100 mmHg. Then, by diffusion, the O<sub>2</sub> will cross the alveolar-capillary membrane resulting in a drop of ~1 mmHg in a normal lung.

From there, O<sub>2</sub> will be transported in two different ways, either directly dissolved in the plasma or combined with hemoglobin (Hb) but because O<sub>2</sub> doesn't have much affinity for plasma, the majority of it will be transferred in association with Hb and one Hb molecule can transport up to four O<sub>2</sub> molecules bound to its 4 iron atoms. Since a gram of Hb can combine with 1.34mL O<sub>2</sub>, and that a dL of blood contains about 15g of it, Hb transports about 20 mL O<sub>2</sub> per dL of blood. This is the basics of the partial saturation in O<sub>2</sub> (SpO<sub>2</sub>) measurement, if the Hb carry as much oxygen as it can, it means that Hb is fully saturated in O<sub>2</sub> and this is what happens at sea level for a non-pathological person. When the hemoglobin is loaded with oxygen, it is referred to as oxyhemoglobin.

Once the blood reaches the tissue, O<sub>2</sub> will be released through the capillary membrane and at rest, when the blood leaves the peripheral tissues, it carries about 15ml O<sub>2</sub> per dL of blood (venous partial pressure in O<sub>2</sub> is about 40mmHg and oxygen saturation of ~75%) which means that tissues extracted only 5mL of O<sub>2</sub> (figure 27.A). This is enough to meet the energy requirement at rest.

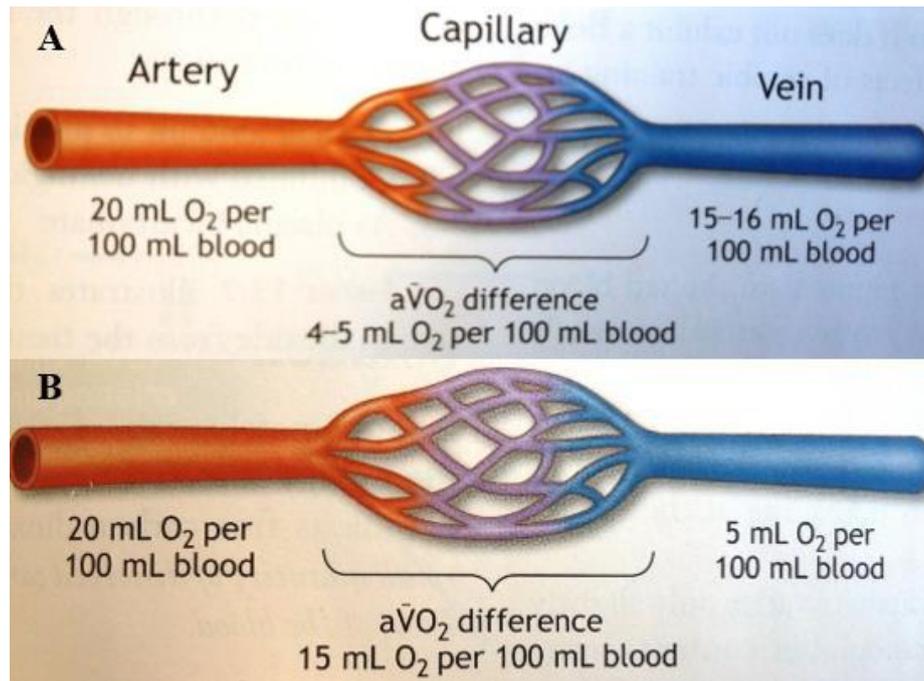


Figure 27. schematic representation of the Oxygen extraction process. Panel A: extraction at rest and panel B: during an aerobic exercise. a $\bar{V}O_2$ , arteriovenous difference. Taken from McArdle et al, (2014).

However, when energy expenditure increases (during exercise for example) a cascade of adjustments will take place in order to assure appropriate oxygen delivery to the tissue, among them, a concomitant increase of pulmonary ventilation and cardiac output, blood flow redistribution, and increased O<sub>2</sub> extraction (figure 27.B).

An important factor during hypoxic exposure is the consideration of the oxyhemoglobin dissociation curve (figure 28) which show the relation between oxygen saturation of the Hb and the PO<sub>2</sub> and because this relation is sigmoidal, increasing the altitude linearly will not have linear repercussion. For instance, at sea level, the P<sub>A</sub>O<sub>2</sub> is about 100 mmHg and the corresponding saturation of the hemoglobin is ~98%, and a 2000m P<sub>A</sub>O<sub>2</sub> drops to 74 mmHg which correspond to a saturation of 90% (West et al. 2007). Because of the shape of the curve, modest altitudes have a low impact on saturation at rest.

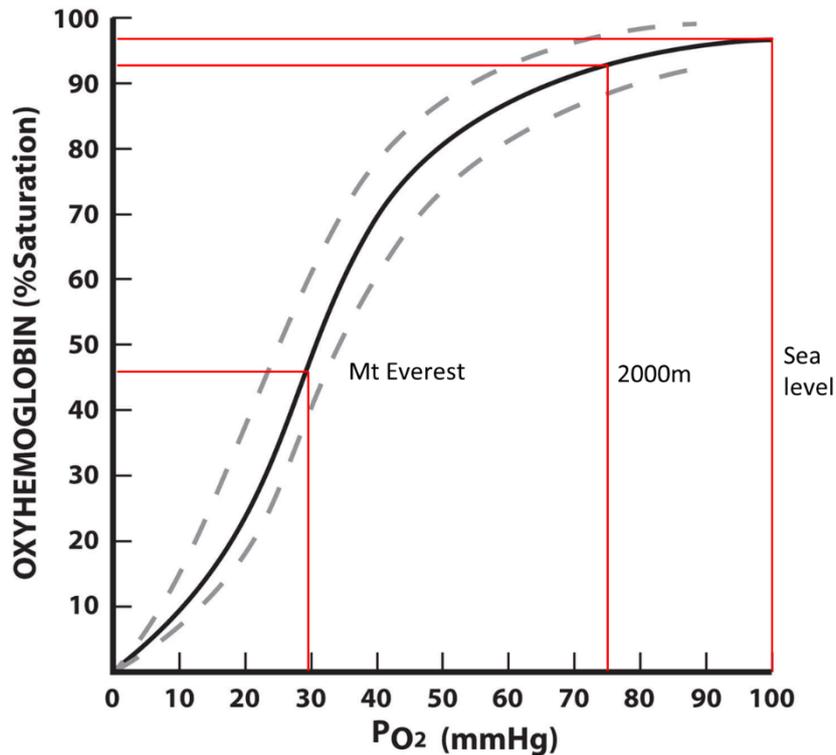


Figure 28. oxyhemoglobin dissociation curve. The dashed line represents a potential shift to the left/right.  $PO_2$ , partial pressure of  $O_2$ .

However, when considering severe altitude leading  $P_{AO_2}$  to be on the steep part of the oxyhemoglobin dissociation curve, few meters of elevation can induce drastic changes in the oxyhemoglobin saturation. Hopefully, some adjustments take place to limit the decrease in oxyhemoglobin saturation. Those adaptations to acute or chronic exposure occur to a certain point and the acute one will be discussed from there.

### *Ventilatory responses*

The first adaptation to acute hypoxic exposure is an increase in ventilation called Hypoxic ventilation response (HVR), it occurs almost instantaneously to reduce the harmful effects of low  $PO_2$  by reducing  $PCO_2$  levels and therefore increasing  $P_{AO_2}$  (i.e.  $P_{AO_2} = P_{iO_2} - 1.25 \times PCO_2$ ). Two types of sensory receptors are involved in the respiratory process. The first ones are peripheral and located in the carotid and aorta, they detect changes in  $PO_2$ . The second types named central are in the medulla and mostly respond to changes in  $PCO_2$  and pH.  $CO_2$  content is also part of the mechanisms controlling the ventilatory responses and because the

HVR act on both the ventilation and the  $PCO_2$ , it is difficult to know the respective part from one another. Indeed, HVR will induce a drop in  $PCO_2$  and therefore, reduced  $CO_2$  drive to breathing limiting the ventilatory response and to see the complete HVR,  $PCO_2$  should be kept constant. HVR also depends on the altitude and is clearer when  $PiO_2$  drops below 100 mmHg (~3000m; Rahn and Otis 1949), consequently, passed this “threshold” any further reduction in  $PiO_2$  will induce a more pronounced HVR.

### *Pulmonary gas exchange*

The  $O_2$  transfer between the alveoli and the capillaries occurs by diffusion through the pulmonary membrane and may be limited at high altitudes. The diffusing capacity depends on the diffusion process itself (i.e. the permeability of the membrane) but also from the necessary time for the  $O_2$  to bind with Hb (figure 29).

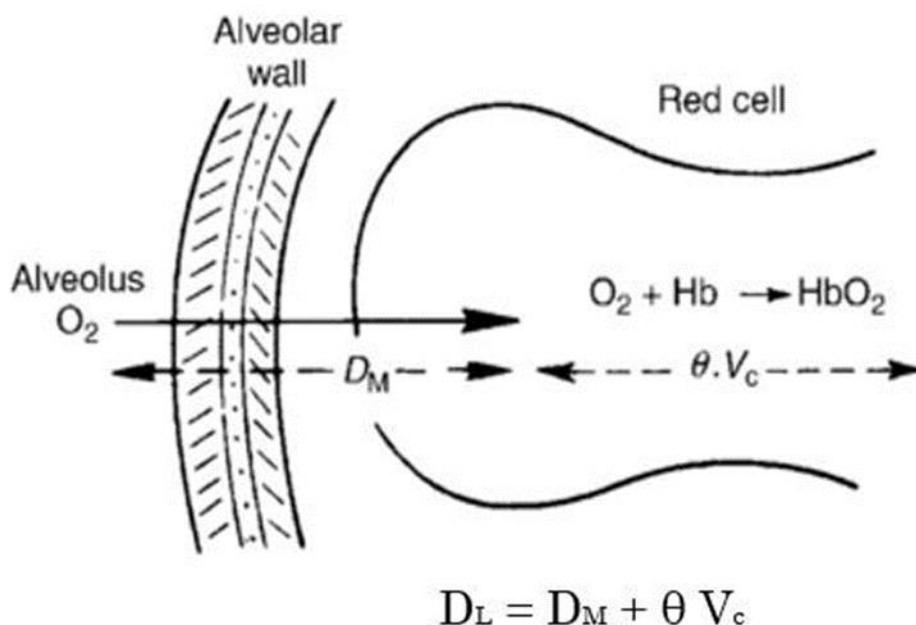


Figure 29. Diffusing capacity of the lung ( $D_L$ ) depends on the diffusion process itself ( $D_M$ ) and the time required for the  $O_2$  to combine with hemoglobin ( $\theta V_c$ ). Adapted from (West et al. 2007).

One way to illustrate the diffusion limitation is to compare the rate of  $O_2$  uptake along the pulmonary capillary between sea level and on the summit of Mount Everest. Figure 30 shows the calculated time course evolution of  $PO_2$  at rest. At sea level (figure 30.A), venous  $PO_2$  is 40 mmHg and  $P_{A}O_2$  is ~100 mmHg, in this condition, it takes about 0.2 s for capillary  $PO_2$  to raise

almost to the value of alveolar  $PO_2$  and there is virtually no difference between alveolar and end-capillary  $PO_2$ . However, when considering a climber on the summit of Mount Everest (figure 30. B), the venous blood arrives in the lungs with a  $PO_2$  of  $\sim 21$  mmHg and the  $P_{A}O_2$  is  $\sim 35$  mmHg. Considering that the time during which the blood will be in contact with the pulmonary capillaries is  $\sim 0.75$  s, the end capillary  $PO_2$  will only be  $\sim 28$  mmHg. This difference of 7 mmHg between alveolar gas and capillary blood indicates a marked diffusion limitation at high altitude.

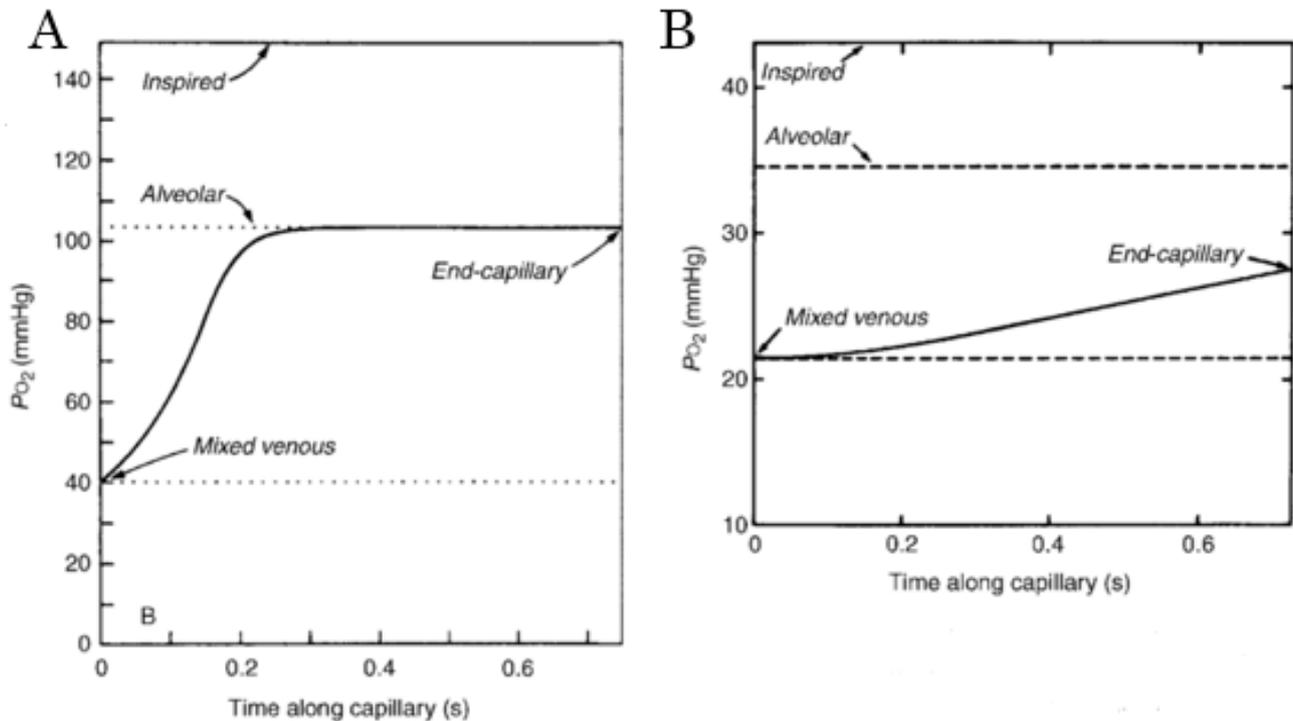


Figure 30. Time course of oxygen pressure ( $PO_2$ ) in a resting human pulmonary capillary. Panel A: at sea level and panel B: at the altitude of Mt Everest. Adapted from (West et al. 2007)

### Cardiovascular system

The cardiovascular system also adapts during acute exposure to hypoxia to counterbalance the lack of oxygen to the tissue. The principal response is an increase in cardiac output mainly resulting from an elevated heart rate (HR) and the higher is the altitude, the higher is the response. Authors reported a concomitant 40-50% increase in HR and cardiac output at rest for simulated altitude comprised between 4000-4600m (Vogel and Harris 1967; Kontos et al. 1967), thus it seems that no changes are observed in stroke volume. Acute hypoxic exposure also caused vasoconstriction at the pulmonary level, increasing vascular resistance and

resulting in pulmonary hypertension. This seems to be caused by contraction of smooth muscle in small pulmonary.

### *Hemoglobin and oxygen affinity*

Depending on four different factors, a shift may occur in the oxyhemoglobin curve presented earlier (figure 31), this is known as the Bohr effect and it will influence the hemoglobin affinity for oxygen. Indeed, an increase in  $PCO_2$ , hydrogen ion concentration ( $[H^+]$ ), temperature and the concentration of 2,3-BPG in the red cells will shift the curve to the right. Those factors act through distinct mechanisms,  $CO_2$  and  $[H^+]$  will induce an alteration of the molecular structure of the hemoglobin making its binding site less accessible and therefore decreasing its  $O_2$ -binding affinity (i.e. shift the curve to the right). Because the combination of  $O_2$  and hemoglobin is exothermic, the dissociation of the oxyhemoglobin will be facilitated through an increase of the temperature. Lastly, 2,3-BPG is a glycolytic intermediate of 1,3-BPG which is generated by the glycolytic pathway. 2,3-BPG has a stronger affinity with deoxyhemoglobin than oxyhemoglobin because of its special structure and therefore will shift the oxyhemoglobin curve to the right. Because they don't have mitochondria, red blood cells get their energy through the anaerobic glycolysis and produce 1,3-BPG that can be catalyzed to 2,3-BPG. The balance between generating ATP to support cell metabolism and the need to keep appropriate oxygenation/de-oxygenation status of hemoglobin is thin and is also pH dependent. Thus, an accumulation of  $[H^+]$  will increase 2,3-BPG.

An easy way to characterize the oxygen affinity of hemoglobin is to look at the necessary pressure to obtain a hemoglobin saturation of 50%, this parameter is named  $P_{50}$  (see figure 31). With a right shift of the curve,  $P_{50}$  increases and therefore to have a 50% saturation, a higher  $PO_2$  is required. The concept of the oxyhemoglobin curve is tricky because a right shift (i.e. a decreased oxygen affinity) will facilitate the extraction of oxygen by the tissue and is, therefore, a positive adaptation during exercise in normoxia because the  $P_{AO_2}$  is ~100 mmHg whereas the  $PO_2$  at the tissue level is ~40 mmHg. Thus, the oxygen loading isn't going to be impacted too much and the oxygen extraction will be improved. However, during severe hypoxic exposure because of the already low  $P_{AO_2}$ , a left shift of the curve (i.e. increased oxygen affinity) will be more beneficial (Turek et al. 1973; Bencowitz et al. 1982) improving the oxygen loading in the lung. To illustrate, let's go back to the alpinist climbing Mt Everest, although [2,3-DPG] increases, the HVR will induce a low  $PCO_2$  (~8 mmHg) and respiratory alkalosis (pH: ~7.7) all of which result in a  $P_{50}$  of about 19 mmHg allowing him to survive.

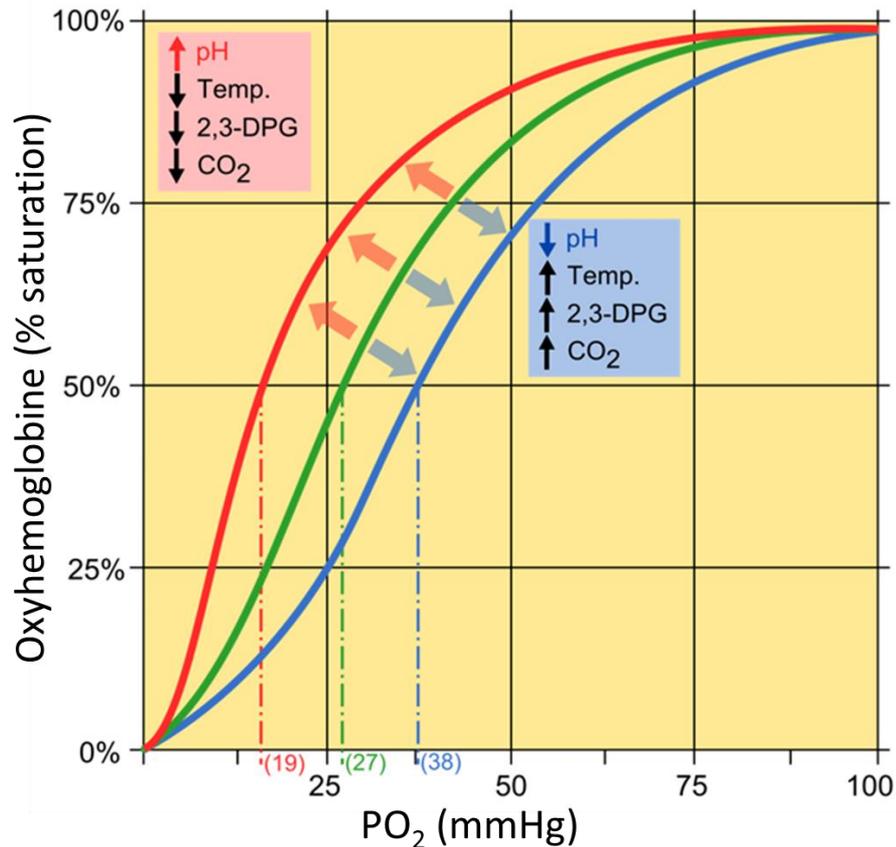


Figure 31. Oxyhemoglobin dissociation curve under normal condition (green), hypoxic exposure (red) and exercise (blue). The associated dashed lines represent the pressure for which the oxygen saturation is 50% ( $P_{50}$ ). Temp, Temperature,  $PO_2$ , Oxygen pressure.

### *From capillaries to the mitochondria*

The mitochondria represent the final step of the oxygen cascade as it is here that the  $O_2$  will be finally transferred to energy.  $O_2$  is then going to move from the capillaries to the tissue whereas  $CO_2$  will move from the tissue to the blood. Because  $CO_2$  has a strong solubility and diffuses 20 times faster than  $O_2$ , it moves easily into the bloodstream and its removal isn't a problem. It is a bit more complicated for  $O_2$ , especially to the muscle, because the distance between capillaries is much greater compared to the lung and so is the diffusion distance. Therefore, the extraction process is thought to be facilitated by the presence of myoglobin (Mb). Mb is an iron- oxygen-binding protein found in muscle tissue, very alike to Hb except that it has only one binding site to link with  $O_2$ , a much higher affinity with it and its dissociation curve is hyperbolic and not affected by  $[H^+]$ ,  $CO_2$ , [2,3-DPG] or temperature (figure 32). This difference arises from different function, whereas Hb transport  $O_2$ , Mb stores it in the muscle

and therefore its  $P_{50}$  is very low (about 5 mmHg). However, it is not a problem due to the extremely low  $PO_2$  of the mitochondria (1 mmHg) that ensure adequate pressure gradient for diffusion.

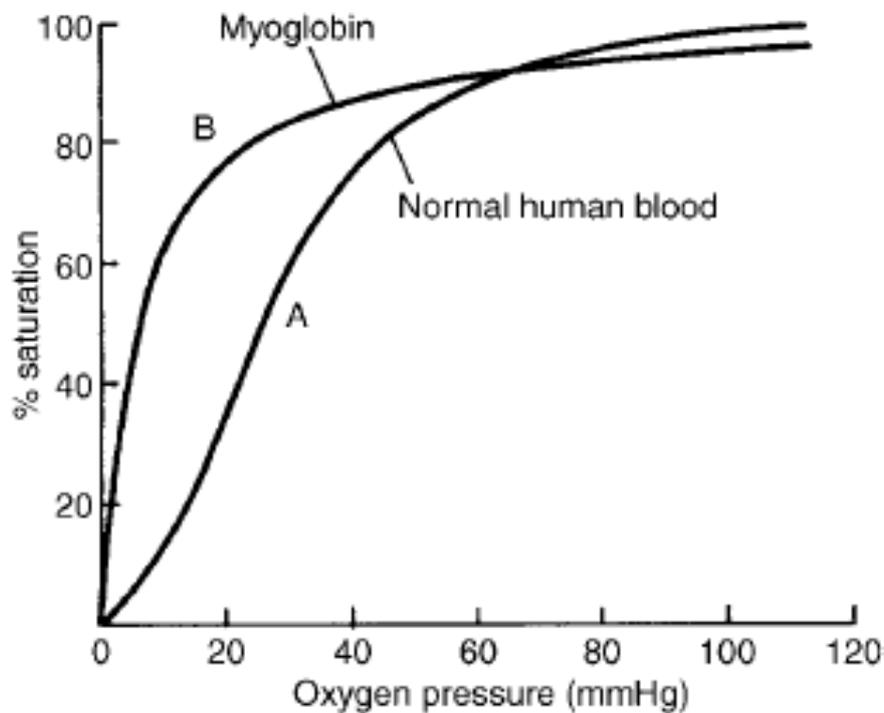


Figure 32. Oxygen dissociation curve for normal blood (A) and myoglobin (B). Taken from West et al. (2007).

### 3.1.2 Acute hypoxia and neuromuscular function

Neurons need energy to maintain the ion gradients across the membrane and they obtain it through the aerobic metabolism, therefore the diminished  $O_2$  level associated with hypoxia can become a limit to the brain function. To answer this, hypoxia triggers a general decreased neuron metabolic demand (Neubauer and Sunderram 2004) but certain areas on the other hand, increase their activity. It is the case of the caudal hypothalamus and the rostral ventrolateral medulla, which have been shown to increase sympathetic and respiratory activity, heart rate and blood pressure (Dillon and Waldrop 1992, 1993). Different methods can be used to investigate how cerebral function is impacted by hypoxia and the upcoming section will try to give an overview of some of them.

One question of interest is to know how the cerebral blood flow (CBF) react to acute hypoxic exposure and this can be assessed using the transcranial Doppler methods to measure blood velocity in proximal intracranial or neck arteries (Verges et al. 2012). Authors found little to no effect at all on acute exposure at rest on the net CBF and this can be explained by the two opposite effect induced by hypoxia. Indeed, hypoxia has been shown to be a vasodilator and therefore one could expect an increased CBF, but as previously explained, hypoxia will also induce hyperventilation associated to a diminution of  $PCO_2$  (hypocapnia) which has a vasoconstrictor effect and result in a diminution of CBF. In fine, acute exposure to hypoxic environments has little to no impact on CBF.

Another noninvasive tool useful to get insight of brain oxygenation is the near-infrared spectroscopy (NIRS), since skin, bones, and tissue are relatively transparent to near infrared spectrum (700 – 900 nm) whereas oxy- and deoxyhemoglobin are strong absorbers, it is possible to determine the relative changes in oxy- and deoxyhemoglobin content by sending near-infrared light through a considered tissue (e.g. muscle). Using this technique, researchers found little to no impact of acute exposure to hypoxia on muscle oxygenation at rest whereas cerebral oxygenation was reduced at  $O_2$  saturation comprised between 77-91% (Peltonen et al. 2007; Ainslie et al. 2007; Rupp and Perrey 2009)

Because of this impaired cerebral oxygenation and the general decrease in neuron metabolic demand when neurons lack oxygen shown by Neubauer and Sunderram (2004), the neuromuscular processes are likely to be impacted. However, no effects have been found regarding MVC, peak twitch or voluntary activation (Szubski et al. 2006, 2007; Perrey and Rupp 2009) suggesting that the maximal force-generating capacity of the muscle is well adapted to hypoxia. Rupp et al. (2012), investigated more precisely the central compartment using TMS and found no changes in MEPs or SPs after 1 hour of exposure to hypoxic air ( $FiO_2 = 0.12$ ), but after 3 hours, MEPs and SPs were increased. This suggests hyperexcitability of both the excitatory and inhibitory corticospinal pathways, however, those adaptations appear to be time dependent. The underlying mechanisms are unclear and require further investigation but hypothesizes can be made regarding a potential impact of HVR and acid-base balance over the first hours and days in hypoxia (Dempsey et al. 1975; Forster et al. 1975), indeed, neuron excitability is sensible to other parameters than hypoxia (e.g.  $PCO_2$ ). Animal studies looking at brain oxygenation responses to hypoxemia also emphasize the specificity of the considered tissue (Almendros et al. 2010) and consequently,

a specific pattern of cerebral tissue deoxygenation could contribute to a time-dependent response.

Electroencephalography (EEG) is a technique measuring directly the electrical activity resulting from the sum of the neuron pool at the scalp level. It has been used to investigate brain activity (Gevins et al. 1995) during various conditions, including hypoxia (Kraaier et al. 1988; Ozaki et al. 1995) and it seems that EEG activity decreases with hypoxic exposure. Furthermore, it seems to be threshold around an O<sub>2</sub> saturation of 75% beyond which EEG reduction is exacerbated (Kraaier et al. 1988; Papadelis et al. 2007; Rozhkov et al. 2009). To illustrate, (Rozhkov et al. 2009) used EEG to measure the balance of brain regulatory structures and reorganization during exposure to severe hypoxia at rest (FiO<sub>2</sub> = 0.08) compared to normoxia. They observed a reorganization of brain activity from the cortico-thalamic level to the limbic-diencephalic level suggesting a reorientation toward important autonomic function. Thus, hypoxia seems to induce a reorganization of brain activity toward survival mechanisms to face oxygen diminution (Goodall et al. 2014b).

In conclusion, it appears that acute exposure to hypoxia at rest induces a decrease of the overall brain activity and a reorganization towards the limbic system to activate autonomic mechanisms responsible for protective adaptation to hypoxic environments. Additionally, corticospinal excitability doesn't seem to be impacted, at least when exposures are shorter than 3 hours. The further section will discuss the potential impact of hypoxia when combined with supra-maximal exercise.

### **3.2 Sprint exercise and hypoxia**

Before presenting the impact of hypoxia on sprint performance, it is necessary to differentiate natural (i.e. terrestrial) from simulated hypoxia. As explained at the beginning of this section, altitude impacts FiO<sub>2</sub> due to the change in barometric pressure. Recently, the development of specific devices allows to easily affect the FiO<sub>2</sub> without having the need to change the pressure. Several mechanisms can be used to either dilute O<sub>2</sub> by adding more azote to a

mixture or to extract oxygen via a pump. Therefore, this section will refer to natural hypoxia as hypobaric hypoxia (HH) and simulated hypoxia as normobaric hypoxia (NH).

This section will start by providing some information's regarding the impact of hypoxia on sprint performance, first on a single sprint and then when sprints are to be repeated. Next the associated response (i.e. physiological, perceptual, neuromuscular, metabolic and biomechanical) will be presented.

### 3.2.1 Single sprint performance

#### *Normobaric hypoxia*

By looking at the first sprint of various study investigating the impact of hypoxia on repeated sprint performance, it can be observed that the performance is not affected either when considering running exercise (Bowtell et al. 2014; Brocherie et al. 2016) or cycling (Balsom et al. 1994; Billaut and Buchheit 2013) for simulated altitude up to 3600m. Globally, the performance of all-out exercise (up to 45 s) are unaffected by the reduction of aerobic power associated with hypoxia even when considering altitude as high as 5000 m (McLellan et al. 1990; Calbet et al. 2003). For instance, Ogura et al. (2006) showed that power output produced during a 40 s all-out test similar between sea-level, 1900 and 3700 m ( $F_{iO_2}$  respectively 16.4 and 12.7 %).

These results suggest that despite the diminution in the aerobic energy production due to hypoxia (Calbet et al. 2003; Ogawa et al. 2005) performance during short-lasting effort up to 60 s is not impacted (Weyand et al. 1999). This can be explained by the low proportion of energy provided by the aerobic pathways during such effort but also because the rate of anaerobic energy release [estimated from muscle lactate concentration or  $O_2$  deficit; (McLellan et al. 1990; Weyand et al. 1999; Calbet et al. 2003)] would compensate for the reduced ATP production from the aerobic pathway (Morales-alamo et al. 2012).

#### *Hypobaric hypoxia*

It has been quantified that aerobic power decrease for ~1% every 100m above 1500 m (Wehrlin and Halle 2006) mainly due to the decrease in partial pressure of  $O_2$  because of diminution in barometric pressure associated with HH. As presented earlier, efforts of short duration (i.e. up to 60 s) are relatively unaffected by this reduced aerobic power. However,

when athletes are tested in laboratory condition (i.e. on a stationary bike and/or treadmill) the impact of the lower air volumetric mass associated to HH isn't considered and therefore doesn't provide a full picture of the impact of terrestrial altitude onto performance.

A good example is, of course, the 1968 Olympic games held in Mexico City at an altitude of 2340 m (barometric pressure 580 mmHg) where a world record of many track events were broken for distances up to 800m (100–400 m, 4 x 100-m and 4 x 400- m relays). In fact, the associated diminution of barometric pressure with terrestrial altitude reduces air density [ $\sim 1\%$  for every 100 m; (Levine and Mehta 2008)] which reduces the aerodynamic resistance and thus decreases energy cost of locomotion task at high-speed (Peronnet et al. 1991; Hollings et al. 2012; Noordhof et al. 2016). Peronnet et al. (1991) showed that the effect of altitude on 400 m running performance is positive (and progressive) up to 2500m. Because the impact of aerodynamic forces is proportional to the square root of the speed, the higher speed associated with shorter sprints (i.e. 100 m) makes altitude beneficial for even higher altitude, up to 4000m.

To conclude, NH or HH doesn't impair single sprint performance physiologically and, furthermore, HH improve sprint performance during a locomotive task due to the lower air resistance associated.

### **3.2.2 Repeated-sprint performance**

To characterize the fatigue development and its kinetics during repeated-sprint exercise (RSE) researchers often use three indices presented in section 2.1.2. The best sprint performance, the mean sprint performance and the fatigue score [either calculated as FI or Sdec, refer to equation 1 and 2; (Girard et al. 2011a)]. Balsom et al. (1994) had participants performed ten 6 s- "sprint" (i.e. participants were asked to maintain 140 revolutions per minute on a stationary bike) in either normoxia or HH (526 mmHg in a low-pressure chamber,  $\sim 3000$  m elevation). They reported that the decline in performance through the test was higher for the HH condition and was associated with lower oxygen consumption and higher blood lactate concentration. Their findings have been confirmed in more recent studies using NH. Either in cycling modalities, where the simulated altitude of  $\sim 2000$ – $3000$  m showed an earlier and larger decrement in peak or mean power output compared to normoxia (Smith and Billaut

2010; Billaut and Buchheit 2013; Billaut et al. 2013). Or when running sprints are performed on a treadmill; for instance, Girard et al. (2015b), showed a larger Sdec (8 vs. 3%) and shorter cumulated distance covered (111 vs. 117 m) during RSE consisting of 5 × 5-s sprints interspaced with 25 s recovery at altitude of ~3600 m ( $\text{FiO}_2 = 13.3\%$ ) compared with normoxia.

(Goods et al. 2014), investigated the effect of several NH level of ~2000, ~3000 and ~4000 m (respectively  $\text{FiO}_2 = 16.4, 14.5$  and  $12.7\%$ ) on running-RSE consisting of 3 sets of 9 × 4 s-sprint interspaced with 16 s recovery for the first 3 repetitions, 26 s for the next 3 and 36 s for the last 3 repetitions. They reported that increased altitude was associated with a significant diminution of mean power output through the test (especially during set 2 and 3) whereas peak power output was relatively well preserved (figure 33).

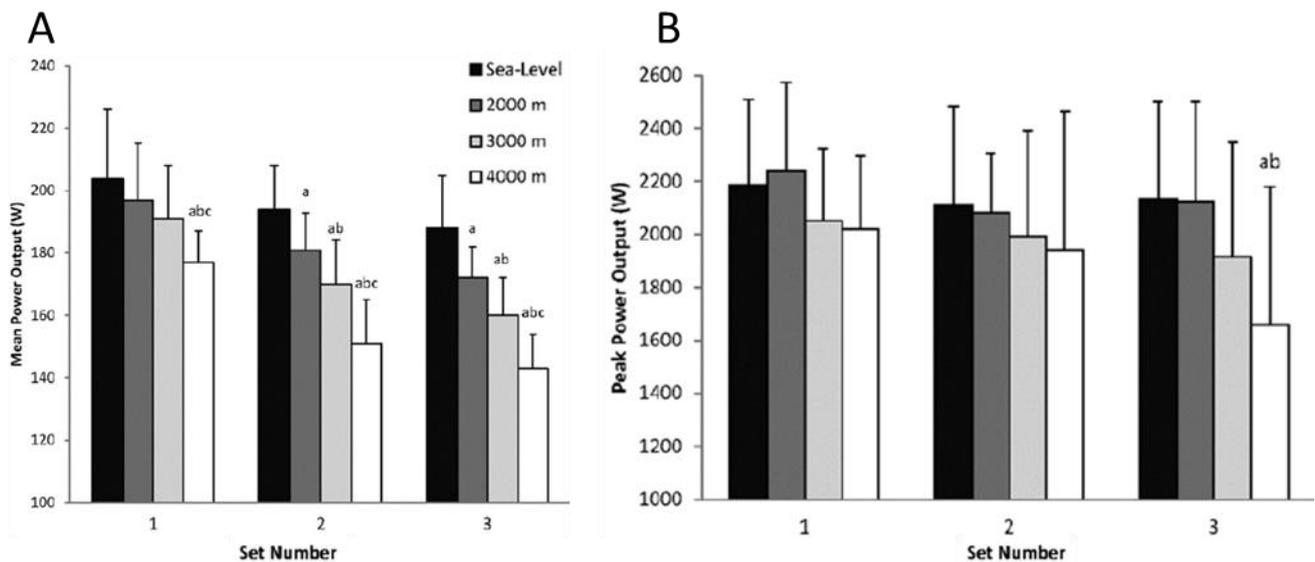


Figure 33. Power output during running repeated sprint exercise performed either at sea level, 2000 m, 3000 m, or 4000 m of simulated altitude. Panel A shows the effect of altitude on Mean power output (values are to be multiplied by ten), whereas panel B shows the effect on peak power output. Data are presented as mean  $\pm$  SD, <sup>a</sup> Significantly different from sea level ( $P < 0.05$ ). <sup>b</sup> Significantly different from 2000 m ( $P < 0.05$ ). <sup>c</sup> Significantly different from 3000 m ( $P < 0.05$ ). Adapted from Goods et al. (2014).

Contrasting results have been observed by Bowtell et al. (2014) where they compared RSA performance (RSE: 10 × 6-s treadmill sprints – 30-s rest) at different level of hypoxia ( $\text{FiO}_2$ : 12%, 13%, 14%, 15%) and reported impaired RSA only during the highest condition (i.e.  $\text{FiO}_2 = 12\%$ , ~4100m).

In addition to the level of hypoxia, and in accordance with the now well-known task dependency paradigm, other parameters are susceptible to influence RSA performance at altitude. Among them, the work-to-rest ratio, the exercise modalities (i.e. running vs. cycling), and the training status. For instance, during RSE consisting of 4 set of 4 × 4 s non-motorized treadmill sprints with 26 s of recovery between sprint and 146 s between sets performed in NH corresponding to ~3200 m ( $FiO_2 = 14.0\%$ ), amateur were unable to achieve their peak speed observed in normoxia during sets 3 and 4 (Morrison et al. 2015a). On the other hand, RSA performance wasn't impaired when professional Australian football players performed an RSE (10 × 4 s non-motorized treadmill sprints with 26 s of recovery) at ~3200 m ( $FiO_2 = 14.0\%$ ) compared to normoxia (Morrison et al. 2015b).

Taken together, these results suggest that unlike single sprint, repeated sprint performance is decreased under both HH and NH condition (i.e. lower averaged mean/peak power output, often associated with a larger decrement score or fatigue index).

### 3.2.3 Responses to sprint in hypoxia

Hypoxia inevitably reduce the oxygen availability to the muscle, limiting the possible energy supply from the oxidative metabolism as shown in literature by lower oxygen consumption during RSE when performed in hypoxic environment therefore placing more demand on anaerobic sources as shown by increased level of blood lactate (Balsom et al. 1994) to provide the required rate of ATP provision (Ogawa et al. 2007). Hypoxia is also associated with lower cerebral oxygenation (Rupp and Perrey 2009). Altogether this will lead to an accumulation of metabolites within the muscle (i.e.  $H^+$ ,  $P_i$ ) and potential failure to fully activate the contracting muscle. Many physiological adjustments (figure 34) are likely to occur as it is often reported that RSE in hypoxia elevates heart rate, minute ventilation, oxygen debt, muscle deoxygenation level and EMG signal (Bowtell et al. 2014). This can affect perceptual, neuromuscular, metabolic and mechanical responses.

#### *Perceptual response*

(Smith and Billaut 2010) had 15 national-level soccer player performed 20 × 5 s cycling sprint with 25 s of recovery. They observed a linear relationship between  $SpO_2$  and RPE ( $r = 0.62$ ,

$p < 0.05$ ). This suggests that arterial hypoxemia could play a role in performance regulation via modulating the sense of effort. Indeed RSE in hypoxia is often associated with slightly higher RPE despite a reduced total work (Goods et al. 2014; Brocherie et al. 2016b). RPE has also been associated to brain cortical oxyhemoglobin during intermittent all-out cycling-exercise and authors concluded that the sense of effort could be integrated at least in part through the dorsolateral prefrontal cortex (Monroe et al. 2016). How exhaustion is perceived during exercise and its link with performance is still unclear in the literature. Further researches are needed to understand if and how it is modulated during exercise in hypoxia.

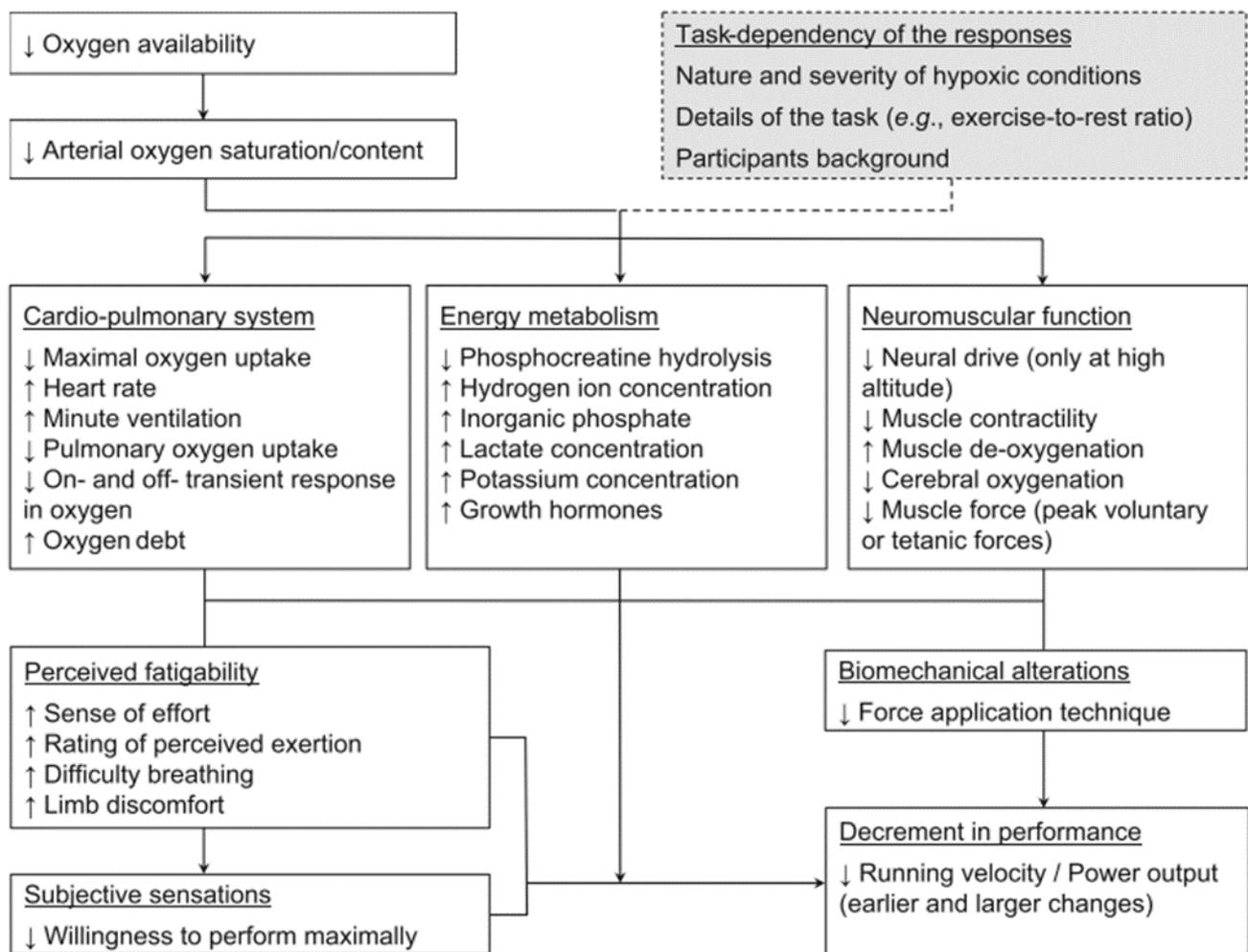


Figure 34. Main acute responses to multiple-sprint exercise in hypoxia. Taken from Olivier Girard, Brocherie, and Millet (2017)

### Metabolic responses

The usage of the near-infrared spectroscopy (NIRS) allows researchers to have a real-time measurement of tissue oxygenation and can, therefore, be used during RSE to see how hypoxia influences muscle and cerebral oxygenation. It appears that the completion of 10 × 10-s cycling sprint with 30 s recovery at an equivalent of 3600 m ( $FiO_2 = 13.3\%$ ) induced similar changes in muscle oxygenation pattern compared to normoxia whereas cerebral oxygenation was more affected through the RSE (Smith and Billaut 2010). Those results were confirmed by Billaut et al. (2013). However Willis et al. (2017) showed that during RSE performed to exhaustion (10-s sprint, 20-s recovery), hypoxia (~3800 m) induced a significant decrease of the oxygenation delta value during the RSE (calculated as maximal value during the sprint minus the minimum value during the same sprint; which is supposed to represent the muscle capacity to extract oxygen). This was associated with a progressive decrease of the raw muscle tissue saturation indicating a limitation of the oxygen transport system. This highlights possible distinct mechanisms when exercise is performed to exhaustion. Billaut and Buchheit (2013) also investigated the impact of hypoxia on the re-oxygenation capacity of the muscle during the recovery period. They showed that under hypoxic condition ( $FiO_2 = 13\%$ ), re-oxygenation was impaired and strongly related to the performance decrement ( $r = 0.78$ ,  $P = 0.002$ ). Because the re-oxygenation rate is often associated to Pcr resynthesis after a single bout of exercise (McCully et al. 1994), it can be postulated that suboptimal re-oxygenation of the active muscle could impair Pcr resynthesis and therefore limit RSE performance. Further study should confirm the result of Billaut and Buchheit (2013).

### *Neuromuscular responses*

It is relatively well established that the impairment in performance during RSE results of changes both within the muscle (peripheral factors) and the alteration in the neural drive to the considered muscle (central factors). One can refer to section [2.3](#) for further details. This section will provide experimental data regarding the impact of hypoxia on fatigue etiology induced by RSE.

Studies usually report a greater attenuation of the EMG activity associated with the exacerbated decline in power output observed during RSE combined with hypoxia (Smith and Billaut 2012; Bowtell et al. 2014). This suggests that central adaptations occur due to the lack of oxygen and could explain the neuromuscular perturbations. However, Girard et al.

(2015c) had EMG activity recorded from 6 muscles (Rectus femoris, Vastus lateralis, Biceps femoris, Gastrocnemius medialis, Gastrocnemius lateralis, and Tibialis anterior) during a running-RSE. They reported that the decline in EMG activity was only exacerbated in the hypoxic condition for the rectus femoris and tibialis anterior, thus, highlighting the muscle dependency. Billaut et al. (2013) had participants performed 3 sets of 5 × 5 s-sprints with 25 s of recovery between sprints and 120 s of rest between sets either in normoxia or in hypoxia (~3100 m,  $FiO_2 = 13.8\%$ ). They reported lower total work and higher decrement score in the hypoxic condition despite similar peripheral fatigue in both normoxia and hypoxia (diminution of quadriceps twitch force without any changes in M-wave). Both hypoxia and normoxia induced a significant diminution of VA (respectively -6.7% and -3.4%) and RMS/Mwave (respectively -15.7% and -7.6%) but the decrease observed in hypoxia was significantly higher. Furthermore, the authors also reported that hypoxia induced a significant systemic ( $SpO_2$ ) and cerebral deoxygenation without differences in muscle oxygenation. These results suggest that under hypoxic condition, central fatigue is exacerbated and could explain the lower total work performed compared to normoxia despite the same peripheral fatigue. From those results, authors concluded that central component acted as a “safety” mechanism to avoid further development of peripheral fatigue.

This exacerbated central fatigue in hypoxia could be explained from two different mechanisms (figure 35). The first one is due to the overall impaired oxygen consumption at the muscle level (Calbet et al. 2015b), that is likely to diminish the amount of energy supplied by the aerobic pathway, therefore increasing the reliance on anaerobic metabolism and leading to an increased accumulation of metabolites, triggering an increased firing rate of the group III and IV muscle afferent impacting the central nervous system both at the spinal and supraspinal level and in fine exacerbating central fatigue (Amann and Calbet 2008). The other potential mode of action would be through a direct effect of cerebral hypoxia onto muscle recruitment, outside the influence of muscle feedback (Goodall et al. 2014b). Hypoxia is associated with hyperventilation that induces arterial hypocapnia (diminution in  $CO_2$ ), which is known to cause cerebral vasoconstriction (Ainslie and Ogoh 2010). Interestingly, 14 days of acclimatization at high altitude attenuates the acute effect of hypoxia on central fatigue but not on peripheral fatigue associated with constant load exercise (Goodall et al. 2014b).

Further study should provide an experimental paradigm to identify the underlying mechanisms behind the development of central fatigue associated with the combination of exercise and hypoxia.

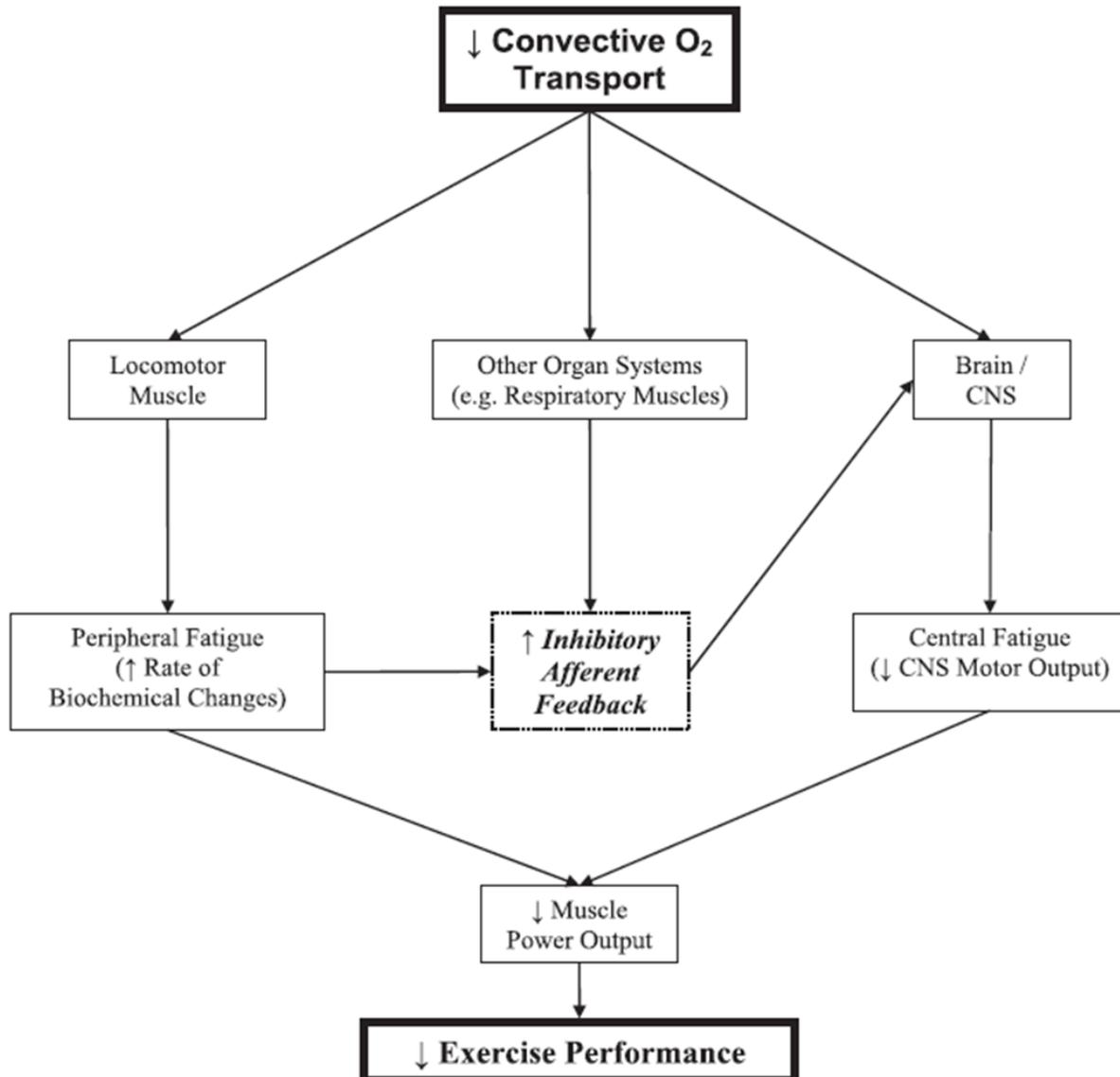


Figure 35. Hypothetical scheme linking oxygen ( $O_2$ ) transport and exercise performance via its effects on fatigue. Taken from Amann and Calbet (2008)

### *The biomechanical manifestation of fatigue*

Recent research suggests that in order to attain higher running speed, it is important to apply more forward-oriented force but not necessarily greater total forces to the ground (Morin et al. 2011b) Using a non-motorized instrumented treadmill Brocherie et al. (2016) compared the effect of RSE (8 × 5 s-sprints with 25 s of recovery) under normoxia and hypoxia (~3600

m) on ground reaction forces. They showed that hypoxia accentuated the inability to apply efficient forces (i.e. forward oriented) and to maintain vertical stiffness and stride frequency.

To conclude, the performance of a single sprint performed in normobaric hypoxia is not impacted because of an increased anaerobic rate of energy released to compensate for the decline in aerobic ATP furniture (relatively limited in such exercise). Furthermore, when a single sprint is performed at terrestrial altitude, performance is improved due to the decline in aerodynamic drag. With the repetition of multiple sprints, performance is negatively impacted in high-altitude (>3000m) both in normobaric or hypobaric hypoxia compared to normoxia. This impairment might be explained by a combination of factors such as a decrease of oxygen transport, increased metabolic (i.e. slower muscle re-oxygenation) and cardiorespiratory stress (heart rate, ventilation), plus impairment of the neuromuscular function at both peripheral and central level.

## 4 Environmental factors: The Impact of blood flow restriction

Blood flow restriction (BFR), also named KAATSU or vascular occlusion, is a method based on the application of external pressure on proximal part of the limb to induce a reduction of arterial blood flow whilst occluding venous return. It induces an impairment of muscular oxygen delivery and metabolite removal, therefore adding supplemental stress to the exercising muscle that may trigger physiological adaptation beneficial for performance when repeated chronically (i.e. BFR training). In addition to the effect of this metabolic stress, changes in the intra- and extracellular environment induced by BFR exercise have been associated with a diminution of MVC (Cook et al. 2007; Loenneke et al. 2013) which is an indicator of muscle fatigue.

Most of the literature regarding BFR and exercise focuses on resistance modalities (i.e. weight training) whether it is to understand mechanisms to acute exposure or before and after a training intervention. It is well established that this exercise modality leads to muscular adaptations inducing an increase in one repetition maximum (1 RM) and cross-sectional area despite lower intensities (between 20-40% of 1RM) compared to traditional resistance training where loads upwards of 70% of 1RM are recommended (American College of Sports Medicine 2009). For a review of the mechanisms involved in muscle hypertrophy, one can refer to several reviews that have been published (Loenneke et al. 2014b; Pearson and Hussain 2015; Scott et al. 2015).

It has been shown that BFR during low-intensity resistance exercise resulted in severe hypoxia of the muscle (Manini and Clark 2009). It is then possible that many of the positive gains found with BFR training are linked to the reduced oxygen availability more than the reduced blood flow per se. To answer this question, (Manimmanakorn et al. 2013) compared the effect of low load resistance training combined with either localized hypoxia (induced using BFR) or systemic hypoxia (breathing hypoxic air to generate an arterial blood oxygen saturation of 80 %) vs. a control group without added stimulus. They showed that both hypoxic stimuli resulted in improved muscular strength (MVC) and endurance (30s MVC and the number of repetitions to exhaustion at 20% 1 RM), compared to training with normal oxygen delivery to the muscles. These gains were associated with an increase in CSA of the leg extensor muscles and changes in voluntary neural activation (measured by raw RMS) in both groups, relative to control. However, the increase in RMS was substantially larger in the

BFR group suggesting that muscular hypoxia per se might not be the only phenomenon responsible for NMF adaptation and therefore highlighting different adaptation mechanisms between localized vs. systemic hypoxia. Nevertheless, further studies are required as the authors underlined that they didn't measure O<sub>2</sub> delivery to the muscle during training sessions. Indeed, it may be possible that O<sub>2</sub> delivery was maintained in the systemic hypoxic condition despite a lower arterial O<sub>2</sub> saturation by an elevation in the limb muscle blood flow, thereby limiting the hypoxic environment in the muscle.

Subsequently, to the success of BFR combined with resistance training methods, researchers transferred the BFR technique to aerobic exercise thinking that the hypoxia-induced environment plus the increased metabolic stress could also create physiological responses that would improve aerobic and anaerobic performance. Authors showed improvements in maximal oxygen uptake as well as time to exhaustion following submaximal aerobic exercise with BFR (Abe et al. 2010). Following these encouraging results and probably the development of repeated sprint training in hypoxia (RSH, an upcoming section will focus on reviewing the potential mechanisms of this training methodology) authors combined BFR during an acute session of repeated sprint exercise to exhaustion (Willis et al. 2018) or during submaximal sprint training (i.e., 60-70% of maximal sprint speed, Behringer et al. 2017). In the latter study, authors found an amelioration of the 100m sprint time after training combined with BFR compared to without, but they were unable to detect significant changes between physiological parameters. (Willis et al. 2018) demonstrated a diminution of the total number of sprints performed to exhaustion when combined with BFR, thus suggesting a direct effect of BFR on the development of muscle fatigue.

While the literature about muscular changes following BFR training or acute BFR exercise is consequent (for review, (Pope et al. 2013; Wilson et al. 2013; Pearson and Hussain 2015), there is still a lack of data regarding how the NMF might be impacted. Therefore, the upcoming section will present a review of the literature regarding the impact of BFR on fatigue development and potential associated mechanisms as well as some practical recommendation for the use of BFR during exercise.

## 4.1 Fatigue etiology

Russ et al. (2003) measured the acute impact of ischemia (230mmHg, 16 subjects; 8men, 8 women) on NMF of the dorsiflexor (*tibialis anterior*) and reported an effect of BFR alone, increasing MVC force (+6.3% in men and 4.2% in women). However, this augmentation wasn't associated with any other acute NMF indices (potentiated or un-potentiated Pt, central activation ratio, M-wave amplitude, Db10, Db50 or Db10/Db50 ratio...) making the interpretation of this result difficult. They also compared the combination of isometric exercise using a protocol of 4 min of 5-s MVCs, with a 5-s rest between contractions (i.e., 24 contractions) either associated with BFR (i.e., 230mmHg) or not. They reported that BFR induced greater central and peripheral fatigue in both men and women (greater reduction in VA, M-wave amplitude and area, stimulated tetanic force at 10 and 50Hz as well as the low-frequency ratio). Hogan et al. (1994) conducted on surgically isolated dog muscle (i.e., cut from the sciatic nerve) where authors reported similar fatigue during intermittent stimulation in ischemic (no blood flow) or hypoxemic (no O<sub>2</sub>, but normal blood flow) conditions. It is important to note that because of the muscle was isolated, those results are likely to miss the potential central impact of BFR. Based on this study, Russ et al. (2003) concluded that "the effect of ischemia is most likely due to the absence of oxygen, and not the restriction of blood flow per se".

When considering dynamic exercise associated with BFR, many reports in the literature using surface EMG measurements showed an increase of the motor unit recruitment (i.e., more activation of the fast twitch fibers) during low intensity exercise with BFR relative to the same exercise without (Takarada et al. 2000, 2002; Yasuda et al. 2009). Even if this increase may not always be observed (Wernbom et al. 2009; Kacin and Strazar 2011) it can be hypothesized that this change in motor unit recruitment could induce significant changes in the development and etiology of neuromuscular fatigue.

Whereas quite a few studies have recorded EMG (iEMG or RMS) during BFR exercise, only 8 (see table 1) incorporated NMF evaluation such as the ITT technique or assessment of corticospinal parameters using TMS allowing a deeper understanding of the peripheral vs. central origin of fatigue.

**Table 1.** Published studies on the impact of blood flow restriction (BFR) exercise on neuromuscular function (n = 8)

	Author (Year)	Subjects / muscle group ♂ = male ♀ = female.	Training/Acute design (sessions, type, content)	BFR modalities	Meaningful result
1	Moore et al, (2004)	Untrained ♂ Elbow flexors	Training study 24 sessions, 8 wks.  50% 1RM: 2x(2x10 + 1xfailure) r=1min passive recovery. R= 5min passive recovery Rq: Contralateral limb served as control	BFR, N = 8 100mmHg during blocks 7cm cuff width No BFR between blocks  CON, N = 8	+10% maximal voluntary contraction (MVC), +22% 1 RM -21% peak twitch (PT) -52% PT/MVC  +23% 1 RM
2	Karabulut et al, (2010)	Untrained ♂ Knee extensors	Acute exercise Constant resistance/velocity at 20% 1RM  5x20 rep r=30s Rq: MVCpre without BFR, MVCpost with BFR	BFR, N = 14 1.44xSystolic blood pressure (sBP) 5cm cuff  CON, N = 14	-13% voluntary activation (VA), -44% PT, -12% EMGrms  -19% PT, +3 %EMG amp.
3	Cook et al., (2013)	Active ♂ Knee extensors	Acute exercise Low-load (LL)/High-load (HL) (20% vs. 70% torque max) 3 sets to <b>failure</b> r=30s	LLBFR, N = 8 5cm cuff inflated continuously at 180mmHg  LL, N = 8  HL, N = 8	Similar decrease in MVC, Db and no changes in VA for all conditions  Higher EMGrms compare to both LL session, no changes between LL and LLBFR
4	Brandner et al., (2015)	Untrained ♂ Elbow flexors	Acute exercise HL (80% 1RM): 4x6 to 8 reps r= 150s  LL (20% 1RM): 1x30 + 3x15 reps r=30s	LL + BFRconti, N = 10 10.5cm cuff 0.8xsBP continuously  LL + BFRinter, N = 10 10.5cm cuffs, At 1.3 x sBP during exercise Deflated during recovery  LL, N = 10  HL, N = 10	MEP increased at post 5, 20, 40 and 60min compare to HL, LL, and LL+BFRinter  MEP increased at post 5, 20 and 40min compare to HL  MEP increased at post 5 and 20min compare to HL No changes
5	Broxterman et al., (2015)	Active ♂ Handgrip muscles	Acute exercise 1.5 contraction/relaxation cycle at 85% of peak power to exhaustion	OCC + occ, N = 6 pressure > 275mmHg during exercise + recovery  OCC, N = 6 pressure > 275mmHg	Shorter time to exhaustion, higher Deoxy-(Hb+Mb), lower oxy (HB+MB) and lower %sat (Hb+Mb) in OCC conditions compare to CON

			Rq: pressure used induced total occlusion (OCC)	during exercise CON + occ, N = 6, pressure > 275mmHg during recovery  CON, N = 6	reductions in Db100, MVC and VA greater in OCC vs. CON  Increase in tot-(Hb+Mb)  Increase in tot-(Hb+Mb)
6	Cook et al., (2017)	untrained 3 ♂, 3 ♀ Knee extensors	Training study 18 sessions, 6 wks Leg press + Leg extension 20% 1RM:2 (2x25+1xfailure) r=30s R=150s  Leg press + Leg extension 70% 1RM:2 (2x25+1xfailure) r=30s R=150s  No training	BFR, N = 6 5.5cm cuff 180/200mmHg deflated between exercise  HL, N = 6  CON, N = 6	Increased 1RM and CSA no changes neuromuscular function  Increased 1RM and CSA no changes neuromuscular function  No changes
7	Husman et al., (2018)	Resistance-trained ♂ Knee extensors	Acute exercise (30% 1RM): 1x30 + 3x15 reps r=30s	BFR, N = 17 10cm cuff at 60% of total arterial occlusion pressure  CON, N = 17	BFR exacerbated: - MVC decreased more from the 1 <sup>st</sup> set to the 1 <sup>st</sup> min of recovery - Peripheral fatigue decreased more from the 1 <sup>st</sup> set to at least 4 min of recovery. - VA appeared and was exacerbated after the 4 <sup>th</sup> set. - the increase in VL and VM RMS
8	Willis et al., (2018)	Physically active 6 ♂, 5 ♀ Knee extensors	Acute session Repeated sprint cycling test to exhaustion. 10 s-sprint with 20 s-recovery at 20W. Resistance for the sprint set at 8% body weight	BFR <sub>60</sub> , N = 11 10cm cuff at 60% of total arterial occlusion pressure  BFR <sub>45</sub> , N = 11 10cm cuff at 45% of total arterial occlusion pressure  CON, N = 11	Overall effect of BFR decreasing MVC, RMS/Mwave and VA post  BFR <sub>60</sub> exacerbated the loss in MVC and VA compared to BFR <sub>45</sub>

The first study evaluating NMF using the ITT was performed by Moore et al. (2004) and investigated the effect of an 8 weeks low-intensity resistance training period performed with or without BFR on elbow flexors properties. Subjects served as their own controls as their arms were randomly assigned to either the occluded or control condition. Authors reported

an increase of MVC and a decrease of resting PT only for the arm trained with occlusion whereas no changes in VA or potentiated PT were identified before and after training for both limbs. They concluded that low-intensity resistance training in combination with BFR increased muscle strength and causes changes in indices of NMF.

Sadly, the authors didn't measure acute responses to compare the fatigue induced by both exercise's condition. Furthermore, the specific design of their protocol (i.e. having one arm trained in the occluded vs. the other one in the non-occluded condition) might have blunted some central adaptations as it has been shown that exercising one limb will improve the homologous muscles. This phenomenon is known as the contralateral strength training effect and can be responsible for up to half the increase in strength of the trained side mainly due to central adaptations (Carroll et al. 2006b).

Cook et al. (2017), also investigated the effects of BFR training by comparing 6 weeks of low load resistance training with BFR (20% 1RM, intermittent BFR at ~190mmHg) vs. medium load training (70% 1RM) on knee extensors muscle. However, authors were unable to replicate the finding from Moore et al (2004) on the arm flexors and didn't detect any significant changes in NMF for both training groups. The limb specificities, as well as the low number of participants and the inclusion of both men and women (i.e., n=6, 3 men and women in both groups), might explain those results

Karabulut et al. (2010) compared NMF after 5 sets of 20 repetitions at 20% of 1 RM performed with or without BFR. They reported a decrease of MVC after both conditions however the post-exercise MVC was 18% lower in the BFR condition. Regarding NMF parameters, PT was more depressed in the occluded condition (-44% vs. -19% in CON) and VA was only impacted after BFR (-13%). They concluded that BFR exacerbated fatigue following low-intensity exercise and that both central (diminution of VA) and peripheral (diminution of twitch torque) factors were responsible. However, the fact that the authors found the baseline (i.e. without any fatigue) value for VA of approximately 80% might indicate some methodological bias. For example, they didn't use the corrected formula (as presented previously in the section 2.2.1 of this manuscript) to calculate VA. This result could also suggest that the subjects weren't properly familiarized with the ITT technique. Cook et al. (2013) looked at the impact of BFR combined with different exercise intensities performed to exhaustion instead of work matched between conditions. They compared NMF impairment (MVC, VA, potentiated doublets at 166Hz and RMS during the first vs. last contraction during the fatiguing task) after high-load (70% dynamic peak torque) or low-load with or without BFR (20% dynamic peak torque, occlusion was obtained via a 5cm cuff inflated at 180mmHg).

Interestingly, authors reported no significant effect of conditions, meaning that the impairment in NMF was similar between exercise modalities (e.g., MVC: -37%; DB166: -40%). Furthermore, they didn't detect any changes in VA after exhaustion and EMGrms was lower during low-load conditions compare to high-load (either pre- or post-exercise) and increased over time in all 3 conditions. Authors concluded that significant peripheral fatigue occurred whatever the considered condition and that high-load exercise requires higher muscular activation compared to low-load exercise with BFR. A limit from this study might be the low number of subjects (n=8) determined as stated by the author "to detect significant decreases in isometric force immediately after high-load and low-load combined with BFR fatigue protocols". While the important effect size of force decrement certainly allows the authors to detect such changes, it is possible that differences in the amount and etiology of fatigue required a higher number of participants to be detected. This makes interpretation of non-significant results often difficult in sport science as most of the study include a relatively modest number of subjects.

A few years later, Brandner et al. (2015) used TMS to investigate whether an acute bout of resistance exercise with (BFR) induced changes in corticospinal excitability (i.e. MEP) and intracortical inhibition (i.e. SICI). Their main findings were a greater increase in MEP amplitude from 5 to 60min after low load (20% 1RM) exercise combined with continuous BFR (80% of systolic blood pressure) compare to all their other conditions (high-load at 80% 1RM, low-load alone or low-load combined with intermittent BFR at 130% of systolic blood pressure). Furthermore, they didn't detect any change in SICI whatever the considered condition. From these results, they achieved that continuous BFR added to low load resistance exercise causes hyperexcitability of excitatory corticospinal circuits and that the pattern of cuff application is an important factor contributing to corticospinal excitability and recommend the use of low-pressure continuous BFR during exercise training to promote central adaptations. A limit from this study comes from the fact that the authors didn't measure the fatigue triggered by the different bouts of exercise. Furthermore, the changes in neural function have been recorded from 5 to 60min post exercise, therefore they are probably not representative of what occurred during exercise.

In a more integrated approach, Broxterman et al. (2015) completed four constant power handgrip tests to exhaustion (at 85% of peak power) under control, occlusion, control plus post-exercise occlusion and occlusion + post-exercise occlusion conditions. All occlusion conditions were at supra-systolic pressure (>275mmHg) and it should be noted that this level of pressure could no longer refer to BFR but to total occlusion. They reported a shorter time

to exhaustion for the occluded conditions associated with higher deoxy-hemoglobin/myoglobin and lower saturation, oxy- and total-hemoglobin/myoglobin measured by NIRS. The reductions in MVC, Db100, and VA were significantly greater for occlusion than for control and for occlusion + occlusion than for control + occlusion. They concluded that blood flow occlusion during exercise reduced O<sub>2</sub> delivery and exacerbated the development of both peripheral and central fatigue. Furthermore, maintaining the blood flow occlusion stimulus worsened the magnitude of peripheral and central fatigue.

Adding more insight to the time course and fatigue etiology, Husmann et al. (2018) performed NMF evaluation before, during and after a resistance training session consisting in four sets of dynamic knee extensions at 30% of 1RM. The loss of MVC during BFR ( $-45 \pm 14\%$ ) condition were more pronounced compared to without BFR ( $-24 \pm 12\%$ ). The main results reported by the authors are an exacerbation of the exercise-induced fatigue with BFR, mainly due to peripheral factors with major impairments during the early stage of the exercise protocol whereas central factors appeared only at the end of the fourth stage.

While still focusing on the impact of BFR on neuromuscular fatigue and tissue oxygenation, Willis et al. (2018) used a novel approach based on the growing literature about repeated sprint performed in hypoxia and try to apprehend BFR exercise from a new perspective. Indeed, they compared a repeated sprint test (RST) performed on a cyclo-ergometer under three conditions (control, with BFR at 45% or 60% of total occlusion pressure). They reported a diminution of sprint performance (number of repetitions and total work) concomitant with a lower peak oxygen consumption and a lower muscular TSI under BFR conditions. NMF (MVC, VA, and RMS/M-wave) was more altered after RST performed with BFR and significantly different between 45 and 60% BFR. No changes were detected on the low-frequency ratio (Db10/Db100) and authors didn't report Db100, Db10, PT and M-wave. These results suggest that BFR would exacerbate central indices of fatigue. However, some parameters of peripheral fatigue are not reported (i.e., M-wave, Db100, Db10 or PT) and the 3 minutes delay observed between exercise cessation and the NMF evaluation makes the detection of fatigue hazardous. Furthermore, the number of sprints performed under the control condition (approximately  $30 \pm 14$ ) seems relatively high compared to previous studies using the same exercise methodology with trained cyclists (i.e.,  $9 \pm 4$  for Faiss et al. 2013b). This associated with the relatively low peak oxygen consumption ( $2.72 \pm 0.59$  L) whereas participants reached approximately VO<sub>2</sub> max after the 5<sup>th</sup> sprint of RSE involving 5 x 6-s sprints interspaced with 24s of recovery (McGawley and Bishop 2015) would suggest

that participants might have adopted a pacing strategy making difficult to interpret those results.

Taken together, the literature confirms an acute effect of BFR combined with dynamic exercise on neuromuscular fatigue. Indeed, most of the results are in accordance and show an exacerbated decrease in peripheral (i.e., Pt, Db100, Db10/Db100 ratio) and central indices (i.e., VA, MVC) associated with BFR when exercise is not performed to exhaustion. In the only study reporting M-wave (Husmann et al. 2018), no changes were associated with BFR indicating that sarcolemma excitability was well preserved. Contrarily, low-frequency fatigue and force responses to electrical stimulation were more altered during BFR exercise suggesting an impairment of the contractile properties due to alterations distal to the sarcolemma, such as inhibition of the cross-bridge cycle, reduced myofibrillar sensitivity to calcium ( $\text{Ca}^{2+}$ ), and/or impaired  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum (SR) (Allen, 2008).

The decrease in VA reported by the authors (Karabulut et al. 2010; Broxterman et al. 2015; Husmann et al. 2018; Willis et al. 2018) indicates that BFR promotes the development of central fatigue. While this diminution may arise from several mechanisms (Gandevia 2001), the inhibitory feedback from the group III/IV muscle afferents likely plays a role in BFR conditions by decreasing motor unit firing rate (Butler et al. 2003). This interpretation is corroborated by the decrease in RMS during MVC reported (Karabulut et al. 2010; Willis et al. 2018). It is important to note that from the authors reporting RMS, two different methodologies are used. It is either measured during MVC pre- and post-exercise (Karabulut et al. 2010; Willis et al. 2018) or it is reported during the fatiguing task (Husmann et al. 2018). As reported during MVC, RMS is an indicator of the “maximal” central drive that a participant can produce pre- and post- a fatiguing task, and therefore, can be interpreted as a central index of fatigue. However, when reported during the fatiguing task (Husmann et al. 2018) RMS indicates how much central drive is necessary for this specific task, therefore, during low load exercise with BFR, RMS increased more compared to the same exercise without BFR, indicating that the exercise modality is more challenging. Observing an increase RMS during exercise and a decrease during MVC after this same exercise seems then logical.

From the only study providing insight about the timeline of fatigue development (Husmann et al. 2018), it seems that peripheral compartment is the first involved with drastic changes in

Pt torque (approx. -50 %) after the first set whereas VA is still at its baseline value. The decrease in VA appears significant only after the last set of exercise and remain so up to 8 min after exercise cessation. Lastly, from the two studies that compared different mode of BFR application, both reported different adaptation from the central compartment. (Willis et al. 2018) showed that MVC and VA were more impaired at 60% BFR compared to 45% and Brandner et al. (2015), reported exacerbated hyperexcitability of the motor cortex after cessation of exercise with continuous BFR at 80% of systolic BP compare to intermittent at 130% systolic BP. It can be hypothesized that by increasing the relative intensity of BFR, oxygen delivery to the muscle would be more limited whereas the impaired venous return would already be maximal therefore highlighting two linked mechanisms responsible for the NMF impairment. However, more studies investigating NMF associated with BFR are required to fully understand the potentials mechanisms behind it. Another question arises from different results obtained through different exercise modalities, indeed Cook et al. (2013), performed low-load resistance exercise to exhaustion and failed to show any subsequent adaptation of NMF by adding BFR to it. On the other hand, (Willis et al. 2018) observed exacerbated MVC and VA decrement with BFR during a repeated sprint session to exhaustion without detecting changes on peripheral parameters. This contradiction reinforces the need for further studies investigating the impact of BFR on NMF during exercise performed to exhaustion.

Overall, the current literature demonstrates that blood flow occlusion exacerbated the development of both peripheral and central fatigue. Therefore, the upcoming section will focus on the potential BFR induced mechanisms responsible for acute impairments of the neuromuscular system.

## **4.2 Acute mechanisms of Blood Flow Restriction impacting fatigue**

The understanding of the precise induced mechanisms responsible for physiological adaptations when BFR is combined with exercise is still in progress but some major factors impacting fatigue can be identified (see figure 36 for an overview). BFR suppress venous return limiting clearance of metabolic byproducts and, reduces arterial blood flow causing hypoxia to the tissue but also the deprivation of nutrients and (Kawada 2005) which is contributing to exaggerated levels of metabolic stress and impacts fatigue development either

directly, or by acting on other mechanisms such as i) the increased recruitment of fast-twitch muscle fibers, ii) increased group III/IV muscle afferents iii) increased production of reactive oxygen species, and iv) potentially inducing muscle damages (refer to the previous section regarding the physiological aspect of fatigue).

It has been confirmed using NIRS measurements that muscle oxygen levels during exercise with BFR is diminished (Kawada 2005; Manini and Clark 2009; Larkin et al. 2012). The lack of oxygen is then associated with an increase of the reliance on the anaerobic metabolism for ATP rephosphorylation, increasing phosphocreatine depletion, inorganic phosphate production and lactate acid response to BFR exercise as well as decreasing muscle pH (Takarada et al. 2000; Takano et al. 2005; Suga et al. 2009). Although reduced pH doesn't seem to impact force and velocity capacity of single muscle fibers by itself (Allen et al. 2008), results from (Karatzaferi et al. 2008) suggests that it might have additive effects when combined with Pi at close to physiological temperatures. Concomitantly to this increase of metabolites production from the muscle, venous return is suppressed by BFR (or at least limited depending on occlusion and exercise modalities), therefore exacerbating metabolic perturbations in the working muscle and directly impacting fatigue etiology via the peripheral and/or central mechanisms. Indeed, as previously discussed in this manuscript, the rate of phosphocreatine hydrolysis and concomitant inorganic phosphate accumulation is one of the main contributors to exercise-induced impairments in Ca<sup>2+</sup> handling (Allen, 2008) and the most likely explanation regarding the development of peripheral fatigue during low-intensity BFR exercise (Husmann et al. 2018). However, this metabolic response isn't limited to peripheral compartment and is also going to induced other adaptations.

Previous reports in the literature showed higher motor unit recruitment and activation of type II muscle fibers using EMG recordings during low-load BFR resistance exercise compared to a work matched protocol without BFR (Karabulut et al. 2007). This is probably because of premature fatigue of oxidative fibers and can be mechanistically explained through the stimulation of group III and IV muscle afferents as it has been documented that the intramuscular accumulation of metabolites increases their firing frequency (Adreani et al. 1997). Authors proposed that this increased feedback could induce an alpha motoneuron inhibition at the spinal level resulting in a compensatory response to maintain the development of muscular force via an increased muscle fiber recruitment to protect against conduction failure (Yasuda et al. 2009; Loenneke et al. 2011). However, Wernbom et al.

(2009) and Kacin and Strazar (2011) failed to detect such increased in EMG pattern between BFR and control trials. It is important to note that both studies used knee extensors exercise to exhaustion (30% of 1RM and 15% MVC respectively) instead of work matched conditions in both BFR and control conditions. These results suggest that BFR doesn't increase muscle recruitment more at exercise failure and could be explained by the fact that whatever the condition, when reaching exercise failure, group III and IV muscle afferent firing rate are already at their maximal potential.

Sensory afferent feedback has also been implicated as determinants of muscle fatigue during exercise (Amann et al. 2011; Kennedy et al. 2015) by firing to the cortical and/or subcortical area during exercise under hypoxic/ischemic conditions. This has been previously linked to the impairment of muscle activation and corticomotor excitability (Gandevia et al. 1996). Results presented earlier (Brandner et al. 2015; Broxterman et al. 2015; Husmann et al. 2018) and showing an impairment of central parameters with BFR reinforce the potential role of group III and IV muscle afferents in modulating corticomotor excitability in occluded conditions of exercise.

Previous research investigating the impact of hypoxia and the following reperfusion associated with arterial occlusion showed an increase in ROS production in skeletal muscle (Korthuis et al. 1985; Clanton 2007). Furthermore, the interstitial concentration of metabolites produced by active muscle cells plays an important role in vasodilatation of arteriolar vascular smooth muscle (Korthuis 2011). It could, therefore, be expected that the combination of BFR and exercise could exacerbate the production of ROS. As they've been associated with fatigue development (Allen et al. 2008), it can be hypothesized that BFR exercise-induced fatigue could partly result from the presence of ROS within the considered muscle. However, Takarada et al. (2000) and Goldfarb et al. (2008) both failed to report any markers of ROS after a low load resistance exercise with BFR. Pope et al. (2013) mentioned that their results could be explained by the significant difference regarding the time spent under vascular occlusion. Indeed, BFR exercise usually lasts less than 20 minutes and may not be enough to elevate ROS levels whereas Korthuis et al. (1985) used a much longer occlusive period (4 hours). But interestingly, in their study, Goldfarb et al. (2008) compared the ROS responses to resistance exercise between moderate-load (70% of 1RM), low-load with BFR (30% of 1RM) and BFR only. They reported that moderate-load exercise to exhaustion and BFR only for the same duration increased blood oxidative stress marker to a similar extent whereas, low-load exercise plus BFR to exhaustion attenuated the oxidative stress response. These

results confirm that both high-intensity resistance exercise (McBride et al. 1998; Bloomer et al. 2007) and BFR alone (even for a shorter period of time) demonstrates oxidative stress markers within the circulatory system but questioned the potential mechanisms impacting ROS production when BFR is combined with low-load exercise. A potential explanation for this surprising result would be the modalities used to induced BFR, indeed they inflated the cuff to 20 mmHg below the acute systolic pressure at rest (no reports of cuff width or mean pressure used). During dynamic exercise, intramuscular blood pressure and total blood flow increase (Saltin et al. 1998) and, this could have led to overcome the BFR induced resistance to venous outflow and arterial inflow allowing the removal of muscle metabolites and adequate oxygen delivery to the muscle, reducing ROS production despite the presence of the occlusion cuff. Nevertheless, further researches are necessary to understand whether ROS production is part of the responses induced by BFR exercise or not.

It has also been proposed that exercising with BFR may result in ischemia-reperfusion muscle damage dependent on the duration and severity of ischemia with irreversible damage being seen between 4 and 6 h of occlusion (Blaisdell 2002). It is important to note that Ischemia-reperfusion injury has been often examined in a surgical setup involving complete blood flow occlusion to a limb during surgeries to decrease bleeding. During ischemia, the depletion of nutrients and the decreased muscle oxygenation will result in a buildup of metabolites and if the ischemic condition is maintained, it can lead to cell necrosis (Wang et al. 2011). Reperfusion of blood flow to the considered area would induce damages due to an increase of ROS in the mitochondria (Wang et al. 2011). However, this kind of response is limited when considering BFR exercise as they typically last between 5 and 20 minutes. Umbel et al. (2009) and Wernbom et al. (2012) still reported increased muscle damage following acute exercise combined with BFR compared to without. In a recent review, Loenneke et al. (2014a) concluded that the available evidence doesn't support the fact that BFR exercise induces muscle damage. Indeed, both studies concluding that BFR exercise induced muscle damages presented some methodological flaws. First, both used contralateral limb as control condition and matched the number of contractions performed to exhaustion under BFR condition to the free flow limb, therefore, comparing muscle damage induced by exercise to volitional exhaustion vs. submaximal exercise. Furthermore, the differences reported by Umbel et al. (2009) regarding the magnitude of delayed onset muscle soreness (DOMS) appeared to be within their error of the measurement (2.3%, Loenneke et al. 2014a). Wernbom et al. (2012) reported a significantly increased percentage of muscle

fibers with elevated tetranectin staining (considered by the author as a marker of muscle damage) in the BFR limb exercising to exhaustion compared to the control limb completing submaximal exercise after 24h. As mentioned by Loenneke et al. (2014a), it has been suggested that tetranectin may play a role in the fibrinolytic response (i.e. a process that prevents blood clots) and is not necessarily reflective of muscle damages. This added to the fact that BFR exercise has been associated with an increase in fibrinolytic potential (Madarama et al. 2010; Clark et al. 2011), it seems possible that elevated tetranectin staining reflects more an increased of fibrinolysis and not necessarily damages to the muscle. Taken together, these results suggest that BFR is unlikely to produce muscle damage that would impact fatigue development.

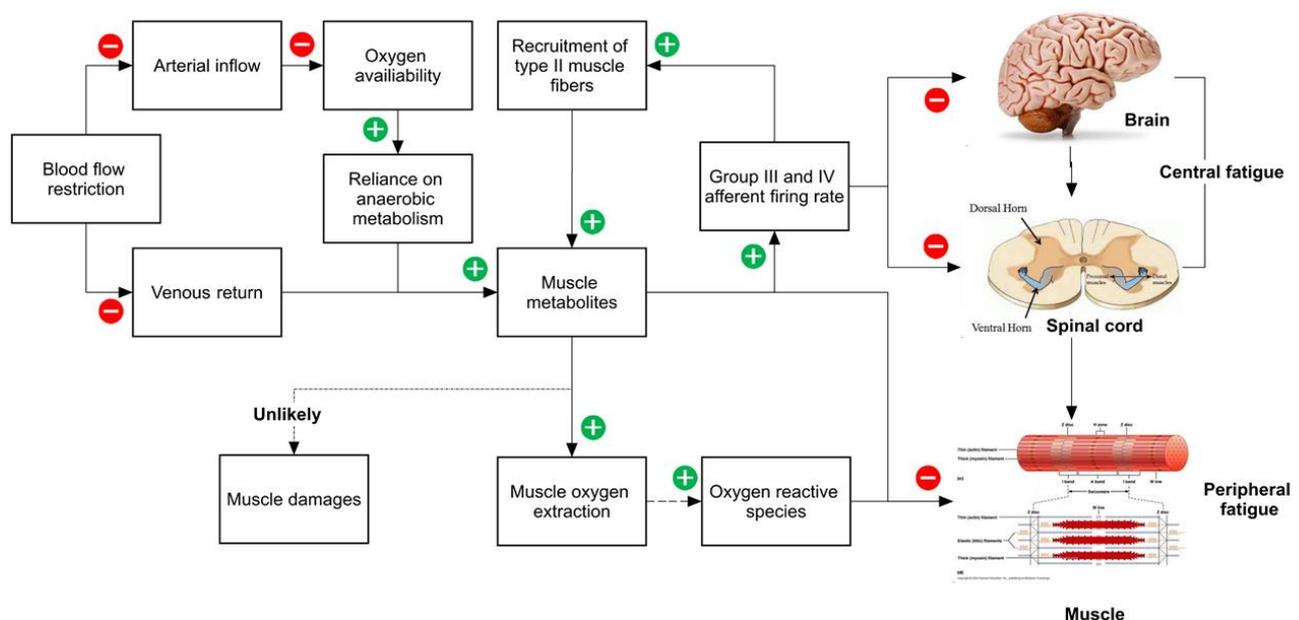


Figure 36. Proposition of potential mechanisms induced by blood flow restriction and impacting the development of neuromuscular fatigue. Positive green signs denote an increase whereas negative red signs denote an impairment.

### Conclusion

Due to mechanical constraint, BFR impeded limb venous outflow, trapping metabolites produced by the exercising muscle, and concomitantly reduced arterial inflow, limiting oxygen delivery to the tissue, increasing the reliance on anaerobic metabolism contributing to the exacerbation of metabolite accumulation. This augmented metabolic stress will directly impair NMF via peripheral factors (e.g.  $\text{Ca}^{2+}$  handling affected by Pi) but also impact muscle

recruitment through increased firing rate of group III and IV muscle afferent which in fine leads to corticomotor adaptations. Production of reactive oxygen species might also be impacted by metabolites accumulation induced by BFR exercise, therefore playing a role in fatigue etiology. However, further studies are needed in order to fully understand the potential mechanisms of ROS. Lastly, it seems unlikely that BFR would exaggerate exercise-induced muscle damages.

### 4.3 Cuff application

Currently, there is no standardized recommendation for the application of BFR during exercise. It can involve the application of a tourniquet (Shinohara et al. 1998), an inflatable cuff (Takano et al. 2005) or elastic wraps (Loenneke and Pujol 2009) at the top of a limb to reduce blood flow to the muscle. (Scott et al. 2015) suggest that differences between cuffs are more related to cuff width rather than cuffs materials, it is, therefore, the first parameter to consider when applying BFR. A wide range of cuffs size has been previously used by researchers (5 to 18.5 cm for the legs and 3 to 12 cm for the arms; Fahs et al. 2012) and it appears that for similar restrictive pressure, wider cuffs cause greater rating of perceived exhaustion and pain associated with lower total worked performed when exercising under BFR conditions (Rossow et al. 2012). Although, (Jessee et al. (2016) reported that wider cuffs (12 cm) restrict arterial blood flow at lower pressures compared to narrow cuffs (5 cm) showing that it is easier to reach the desired level of occlusion. However, depending on the considered limb and exercise modality, wide cuff could inhibit the normal range of motion of some participants. It has also been showed that muscular stimulus may be attenuated directly below the cuff (Kacin and Strazar 2011) even if such a result needs confirmation. Another finding from (Loenneke et al. 2012), is that occlusive pressure to reach the same percentage of arterial occlusion strongly relies on limb's circumference which is, therefore, an effective parameter to predict the required pressure to restrict arterial blood flow. In the same study, the authors reported that some participants did not reach complete arterial occlusion with narrow cuffs on the legs even at pressures of 300mmHg. Based on those results, we could recommend the use of wide cuff when exercise is performed with lower limbs whereas narrow cuffs seem more appropriate to upper limb exercise.

Even if some studies used restrictive pressures above 200 mmHg (Takarada et al. 2000; Broxterman et al. 2015), it is now accepted that to trigger physiological adaptation during

training, BFR should be high enough to occlude venous return from the muscle but low enough to allow arterial inflow to the muscle (Loenneke et al. 2014b). This highlights the need to individualize the occlusive pressure, rather than using an absolute value, considering the size of the limb as well as its size. While some studies have attempted to normalize BFR relative to brachial systolic blood pressure, probably due to the convenience of the measurement (Cook et al. 2007; Manini et al. 2011), there is no evidence to suggest that this is a valid method for the estimation of BFR on the lower limbs. The best way to account for inter-individual differences is probably to consider BFR as a percentage of the pressure required to reach total arterial occlusion. This involves the use of a Doppler probe to determine which pressure is required to no longer detect arterial pulse (Willis et al. 2018). Laurentino et al. (2012) using 80% of total arterial occlusion pressure during low-load resistance training (20% 1RM) reported similar strength and hypertrophic gain compared to traditional high-load training (80% 1RM). Further Results from Loenneke et al. (2015b) reported that individualized pressure of 50% estimated arterial occlusion pressure maximized EMG amplitude and promote acute loss of torque during and following low-load knee extension exercise. Noteworthy, 60% of estimated arterial occlusion pressure didn't cause further changes in those parameters. Authors, therefore, concluded that pressure corresponding of 50% estimated arterial occlusion permitted optimal physiological adaptation.

Because measurement of total occlusion pressure requires specific equipment and procedures not always compatible with a practical recommendation for training, Loenneke et al. (2013) provided a table based on the equivalence between individual's thigh circumference (lower limb only) and required pressure to reach approximately 60% of total arterial occlusion. While methods using elastic wraps procure easy access to BFR training, application of the restrictive stimulus is often based on a subjective pressure scale (0 indicating no pressure and 10 indicating intense pressure with pain; Wilson et al. (2013) making difficult to standardized occlusion pressure. Therefore, the use of this method should be restricted to practical training and not used for the research-based protocol.

Although further researches are needed in order to make a precise recommendation for the use of optimal BFR stimuli, principal factors to consider are cuffs width and the circumference of the limb and if possible BFR should be individualized based on total arterial occlusion pressure. Preliminary results suggest that pressure equivalent to 50% to 80% of total arterial occlusion pressure could be optimal for muscular adaptation (Laurentino et al. 2012; Loenneke et al. 2014b; Fatela et al. 2016).

## 5 Repeated sprint training in hypoxia

This section will try to provide a brief overview of a new training method based on the use of hypoxia to develop repeated sprint performance brilliantly named repeated sprint training in hypoxia (RSH).

For a review of more classical training methods to improve repeated sprint performance, one can refer to the existing literature (Bishop et al. 2011; Petrakos et al. 2016; Rumpf et al. 2016).

Historically, altitude/hypoxic training has been used to improve endurance performance after return to sea level by seeking mainly hematological adaptations. With the subsequent development of technological means (i.e. normobaric hypoxia) a lot of different methods involving the use of hypoxia to increase performance emerged (figure 37), focusing on another adaptive pathway (i.e. within the muscle).

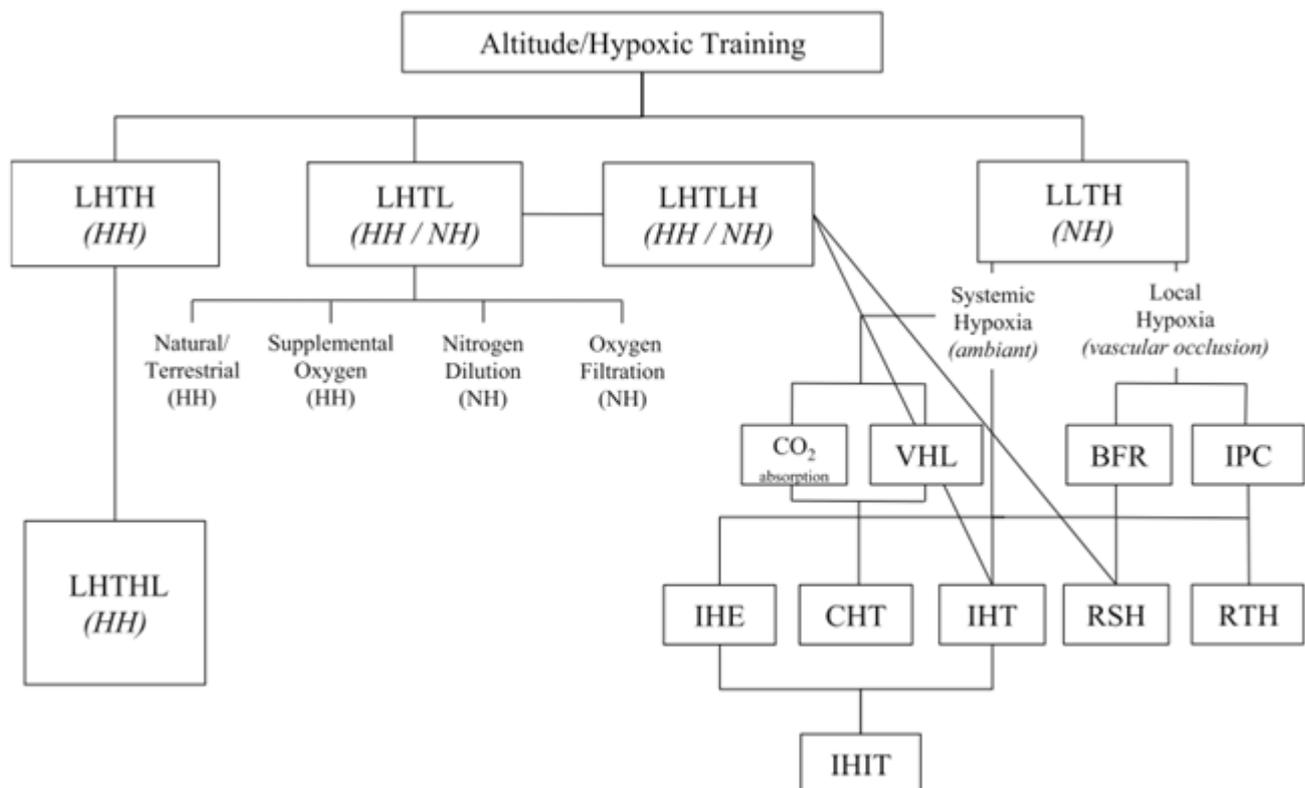


Figure 37. Updated panorama of the different hypoxic/altitude training methods. Taken from (Girard et al. 2017). BFR blood flow restriction, CHT continuous hypoxic training, CO<sub>2</sub> absorption rebreathing with a mask, HH hypobaric hypoxia, IHE intermittent hypoxic exposure, IHIT IHE during interval-training, IHT interval hypoxic

training, IPC ischemic pre-conditioning, LHTH live high-train high, LHTL live high-train low, LLTH live low-train high, LHTHL live high-train high and low, LHTLH live high-train low and high, NH normobaric hypoxia, RSH repeated sprint training in hypoxia, RTH resistance training in hypoxia, VHL voluntary hypoventilation at low lung volume.

RSH is based on the repetition of short (<30 s) maximal sprints associated with incomplete recovery (<60 s) in hypoxia (Faiss et al. 2013a). The difference with interval hypoxic training is the maximal intensity associated with the sprint exercise allowing to maintain very high recruitment of type II muscle fibers through the training session. By adding hypoxia to this type of exercise, oxygen delivery will be impaired and subsequently the capacity of the oxidative pathway to provide ATP. It has been shown earlier in this manuscript (section 2.1.2) that aerobic metabolism provides up to 40% of the energy during the latter stage on RSE in normoxia (Girard et al. 2011a), consequently hypoxia is likely to increase the stress on anaerobic glycolysis to compensate for ATP production which might trigger an up-regulation of this energy pathway with training. Faiss et al. (2013b) were the first to report that compared to repeated sprint training in normoxia, RSH induced beneficial molecular adaptation at the muscle level [e.g. +55% of mRNA concentration of HIF-1 $\alpha$ , +35% of carbonic anhydrase III (CA3) and +20 % of monocarboxylate transporter-4 (MCT-4)].

## **5.1 Potential mechanisms**

Because RSH is a relatively new training method (Faiss et al. 2013b), its underlying mechanisms are still to be fully understood. Acute hypoxia is known to trigger sympathetic discharge in the skeletal muscle enhancing vasoconstrictor activity during exercise (Hanada et al. 2003). But despite this mechanism, the same authors didn't detect any changes in blood flow in the resting muscle. Furthermore, when hypoxia is combined with submaximal exercise, it induces compensatory vasodilatation increasing blood flow to limit the alteration in oxygen delivery to the exercising muscle (Casey and Joyner 2012). Whereas this phenomenon is quite well established during submaximal exercise, it is more controversial at supramaximal intensity. Calbet et al. (2015b), had participants performed a 30 s all-out cycling exercise in normoxia or hypoxia ( $PiO_2 = 73$  mmHg) and analyzed separately the first 10 s of the all-out exercise (referred as "sprint") and the whole 30 s. During the first 10 s they

reported a 22% decrease of leg oxygen delivery associated to 27% increase in oxygen extraction in the hypoxic condition, which led to the same oxygen consumption for both hypoxic and normoxic condition. However, authors reported a decrease in power output during the first 10 s in hypoxia ( $499 \pm 86$  W) compared to normoxia ( $567 \pm 90$  W) which isn't in accordance with previous literature showing that supramaximal exercise up to 60 s is not impacted by hypoxia (Weyand et al. 1999). Furthermore, the average power of 500W produced over 10 s appear quite low to be assimilated to a maximal sprint. When considering the whole 30 s all-out exercise, authors reported again a 7% difference in power output between hypoxia ( $461 \pm 52$  W) and normoxia ( $494 \pm 47$  W). Peak leg blood flow was reduced by 5% in hypoxia ( $7.04 \pm 0.92$  L·min<sup>-1</sup>) compared to normoxia ( $6.66 \pm 0.95$  L·min<sup>-1</sup>), and this was associated with a 28% diminution of peak leg oxygen delivery and a 15% increase of oxygen extraction resulting in a peak oxygen consumption by the leg muscle decreased by 23% in hypoxia. This study suggests that when combined to all-out exercise, hypoxia would result in an increased oxygen extraction capacity of the muscle to limit the overall reduced oxygen delivery to the active muscle. Recently, two studies recorded the combination of RSE and hypoxia on blood flow measured by ultrasound Doppler technique. The first one was conducted on lower limb muscles during cycling-RSE and failed to detect any changes in blood flow with hypoxia (Willis et al. 2017). It can be noted that the range of the value obtained was huge due to a methodological problem. The second one, performed by the same team, was conducted on upper limb muscle and detected a trend for higher blood flow level post exercise in the hypoxic condition (3800 m, FiO<sub>2</sub> = 13.12%) compared to normoxia (respectively  $779 \pm 318$  and  $605 \pm 549$  mL·min<sup>-1</sup>), again, the huge range of values obtained are denoted by the high  $\pm$  SD. Taken together, these results highlight the relative blur associated with the potential mechanisms of RSH. Indeed, one of the main hypotheses is that the stress added by hypoxia would trigger compensatory vasodilatation associated with higher blood flow that would benefit more to the type-II muscle fibers than the type-I muscle fibers (Faiss et al. 2013a). RSH would, therefore, lead to further muscular adaptation through the oxygen-sensing pathway (HIF-1 $\alpha$ ) to improve oxygen extraction (i.e., capillary-fiber ratio, myoglobin content and oxidative enzyme activity such as citrate synthase) compare to normoxia (Hoppeler and Vogt 2001). This would improve in fine the Pcr resynthesize, known to be closely related to repeated sprint performance. Those adaptations are likely to occur through specific muscle transcriptional responses as previously mentioned and shown by Faiss et al. (2013b). They reported an increase of CA3, MCT-4, lactate dehydrogenase (LDH) and mRNA concentration of HIF-1 $\alpha$ , with a concomitant decrease of mRNA expression of

PGC-1 $\alpha$  and MCT-1. This suggests a shift from aerobic (PGC-1 $\alpha$  and MCT-1) to the anaerobic glycolytic pathway (CA3, MCT-4, LDH) and would explain why an exercise involving high recruitment of type II muscle fibers is more efficient compared to IHT.

This remains to be demonstrated as it is still not acted that repeated sprint in hypoxia leads to compensatory vasodilation to the exercising muscle. However, the promising results in the growing literature regarding RSH and performance improvement suggest that beneficial physiological adaptations are more likely to occur.

## **5.2 Effects on performance**

A good indicator of the popularity of this method is the growing body of literature (i.e. 25 papers) available since the first study published in 2013 and its use by many professional sports (e.g. Welsh Rugby Union, Roland Garros training center, Aspire Football Academy, Australian footballers). Table 2 presents the review of all studies that investigated the impact of RSE on sprint performance and included a control group plus a meta-analysis. Overall, only two studies (Goods et al. 2015; Montero and Lundby 2015) reported no beneficial effect of RSH compared to RSN (i.e. similar improvement in RSA performance). All the other found further improvement using hypoxia. For instance, using an open-loop design, authors reported a 38 % increase in the number of cycling-sprints performed to exhaustion in well trained cyclist (Faiss et al. 2013b) and a 58% increase in the number of double poling sprint performed by cross-country skiers (Faiss et al. 2015) after six to eight RSH sessions. In more classical close-loop design (i.e. a determined number of sprints) the meta-analysis from Brocherie et al. (2017) revealed a small to moderate beneficial effect of RSH on mean sprint time and/or mean power output compared to RSN. Individual results from all studies are summarized in table 2.

To conclude, RSH appears to be a promising method to improve repeated sprint ability in a variety of population. It can be used relatively easily compared to more classical hypoxic training methods where hematological adaptation is the main outcome. The mechanisms responsible for the improved performance are still unclear but likely originate from within the muscle. Further researches are however required to elucidate that question and to confirm the range of population susceptible to benefit from such training intervention.

**Table 2.** Published studies on training intervention with RSH. From (Millet et al. 2019)

	<b>Author (Year)</b>	<b>Subjects</b> ♂ = male, ♀ = female.	<b>Training protocol (sessions, type, altitude, content)</b>	<b>Groups</b>	<b>Meaning differences (P &lt; 0.05)</b>
1	Faiss et al. (2013b)	Moderately trained cyclists ♂	8 sessions, 4 wk., cycling, 3000m (NH). 3x5x10s - 20s passive recovery.	RSH, N = 20 RSN, N = 20 CON, N = 10	+6% Pmean sprints + <b>38% more sprints during RSE.</b> +7% Pmean sprints, no more sprints during RSE. NS changes. No RSE improvement.
2	Galvin et al. (2013)	Rugby players ♂	12 sessions in 4 wk., treadmill, 3500m (NH). 10x6s - 30s passive recovery.	RSH, N= 15 RSN, N = 15	<b>+33% Yo-Yo</b> +14% Yo-Yo
3	Gatterer et al. (2014)	Football players ♂	7-8 sessions in 5 wk., running, 3000m (NH). 3x10sx4.5 m round-trip shuttle - 20s passive recovery.	RSH, N = 5 RSN, = 8	+20% Yo-Yo IR, <b>-38% slope fatigue curve during RSA.</b> +21% Yo-Yo IR, +9% slope fatigue curve during RSE.
4	Gatterer et al. (2015)	Football players ♂	8 sessions in 12 days, shuttle-run, 3300m, (NH). RSE = 6x40-m (6x20-m back & forth, 20s passive recovery.	RSH, N = 7 RSN, N = 7	<b>Improvement RSE 0.91% mean time (s)</b> Improvement RSE 0.39% mean time (s)
5	Brocherie et al. (2015)	Well trained youth football players ♂	10 sessions in 5 wk., racing, 2900m (NH). 5x4x5s - 45s passive recovery.	RSH, N = 8 RSN, N = 8	<b>RSH vs. RSN; -4% Time of 1<sup>st</sup> sprint, -4% time of the cumulative sprint, -2% Time of 1<sup>st</sup> sprint, -2% Cumulative sprint time.</b>
	Faiss et al. (2015)	Highly trained XC-skiers	6 sessions in 2 wk., double-pole ergometer cross-country skiing, 3000m (NH).	RSH, N = 9	+25% Pmax Sprints <b>+57% more sprints during RSE.</b>

6		(11 = ♂, 6 = ♀)	4x5x10s - 20s passive recovery.	RSN, N = 8	+21% Pmax sprints, no more sprints during RSE.
7	Kasai et al. (2015)	Lacrosse players ♀	8 sessions in 4 wk., ergo-cycle, 3000m, (NH). 2x10x7s - 30s passive recovery.	RSN, N = 16 RSN, N = 16	<b>+5% Pmax sprints on ergometer after RSH</b> vs. 1.5% in RSN. +9.7% Pmean Sprints vs. 6% = $\dot{V}O_{2Max}$ in both groups.
8	Goods et al. (2015)	Australian football players ♂ « semi-elite »	15 sessions in 5 wk., ergo-cycle, (3000m, (NH)). 3x9x5s -active recovery (self-selected pace).	RSH, N = 9 RSN, N = 10	+ 4.7% Pmax and + 10.3% Pmean  + 8.6% Pmax and + 13.6% Pmean sprints  -1.1% Pmax and 1.4% Pmean, for the controlled group.  + 2.3% (RSH), + 1.8% (RSN), + 1.1% (controlled) on the average time of sprints (running) between before and after Training.
9	Brocherie et al. (2015b)	Elite field hockey players	LHTL, 6 sessions in 2 wk., running, 3000m, (NH). 4x5x5s - 25s passive recovery.	RSH, N = 11 RSN, N = 12	<b>Cumulated sprint time decreased in LHTLH and LHTL but not in LLTL and remained significantly reduced at Post-2 in LHTLH only.</b>  Same increase in Hbmass and YR2
	Montero and Lundby (2016)	Moderately trained endurance athletes ♂	12 sessions in 4 wk., ergo-cycle, 3000m (NH). 4x5x10s - 20s active recovery.	Crossover RSN - RSH, N = 15	RSH Post 1 (3-5 days); +6.7% PPO, +14% MPPO, +12.1% MPO. Post 2 (12 days); +6.8% PPO, +18.7% MPPO, +18.1% MPO.

10					RSN Post 1 (3-5 days); +7.2% PPO, +16.3% MPPO, +9.9% MPO. Post 2 (12 days); +6.9% PPO, +16.3% MPPO, +14.2% MPO.  No differences between RSH & RSN  RSH-induced increase in $\Delta$ [tHb] during RS in hypoxia compared with normoxia.
11	Hamlin et al. (2017)	Well-trained rugby players ♂	6 sessions in 3 wk., ergo-cycle, 3000m (NH).  Followed by 3 post-tests in 2 wk., then 2 sessions in 1 wk. Followed by 2 Post-tests in 2 weeks.  4x5x5s - 25s active recovery.	RSH, N = 8 RSN, N = 10	<b>- 2.0%, - 2.2%, - 1.6% RSE post 3 wk., post 4 wk., post 5 wk., respectively, in favor of RSH</b>  No difference between the groups on the performance of the YYIR1.
12	Brocherie et al. (2016a)	Elite field hockey players	LHTL, 6 sessions in 2 wk., running, 3000m, (NH).  4x5x5s - 25s passive recovery.	RSH, N = 11  RSN, N = 12	RSH vs. RSN; <b>-3.6% difference in RPE (average), -7.8% difference in overall peripheral discomfort and -23.2% difference in lower-limb discomfort</b>
13	Trincat et al. (2017)	Competitive swimmers (9 = ♂, 7 = ♀)	6 sessions in 2 wk that included 2x16x15m with 30s send off.  RSH-VHL low-level active recovery.	RSH-VHL, = 8  RSN, = 8	RSH-VHL <b>+35% number of sprints increased and [La] max</b> compared to RSN
14	Kasai et al. (2017)	University sprinters ♂	5 consecutive days, 3000m (NH) vs normoxia, 2 sessions/day  Morning: RS (5x6s - 24s recovery) 3x + (4x20s 5-15 min)	RSH, N = 9  RSN, N = 9	<b>Running time 0-10m improved (before, 1.39 ± 0.01s; after, 1.34 ± 0.02s, P&lt;0.05)</b> <b>Increase in PCr content (31.5 ± 1.3 to 38.2 ± 2.8 mM, P&lt;0.05).</b>

			Afternoon: 3 x 5x6s – 36s recovery + 4x20s – 40s passive recovery		
15	Brocherie et al. (2017)	Meta-analysis		N = 202	<b>Pmean during RSE further enhanced (P = 0.05) with RSH vs RSN.</b>  Best performance improved to the same extent between RSH and RSN.
16	Oriishi et al. (2018)	College 400-800m runners ♀	4 sessions in 6 days., running, RSH = LH (200m), TH (3000m)  RSN = LLTL	RSH = 7  RSN = 8	<b>Pmax in the MART +2.5% Lactate Concentration decreased (p&lt;0.05) at submax velocities.</b>
17	van der Zwaard et al. (2018)	Elite field hockey players ♂	LHTLH, 6 sessions in 2 wks. 3000m, (NH).  4x5x5s - 25s passive recovery	LHTLH = 6  LHTL = 6  LLTL = 6	<b>LHTLH +35% Succinate dehydrogenase, LHTLH and LHTL improved combination of fiber size and oxidative capacity.</b>
18	Fornasier-Santos et al. (2018)	Highly trained rugby union players ♂	RSH-VHL 8 sessions in 4 wks., running  2-3x8x40m on 30s- Semi-active recovery (walking)	RSH-VHL, = 11  RSN, = 10	<b>Number of sprints increased +64%</b>
19	Brechbuhl et al. (2018)	Well trained tennis players (16 = ♂, 4 = ♀)	5 sessions in 12 days, 3000m, (NH).  4 x 5 “sport specific” sprints – 50 s passive recovery	RSH, N = 9  RSN, N = 9	<b>Time to exhaustion and OBLA improved. +13.8% in ball accuracy testing.</b>
20	Wang et al. (2018)	Recreational-active ♂	8 sessions in 4 wk., ergo-cycle, 3000m, (NH).  β-Alanine supplementation (NB) or normoxia placebo (NP) in both RSH or RSN groups.	RSH-NB = 10 RSH-NP = 9  RSN-NB = 11 RSN-NP = 8	<b>+4% PO in RSE for RSH vs RSN groups.</b>

			3x5x10s - 20s active recovery.		
21	Brocherie et al. (2017b)	Elite field hockey players ♂	6 sessions, running, 3000m (NH). 4x5x5s - 25s passive recovery.	LHTLH, = 8 LHTL, = 11 LLTL, = 9	<b>Larger LHTLH-induced adaptations in molecular responses in O2 signaling (HIF-1α) and transport (VEGF, Mb) and mitochondrial biogenesis and metabolism (PGC-1α, TFAM, CS) compared with LHTL and LLTL.</b>
22	Gatterer et al. (2019)	Amateur team sports players ♂	9 sessions, x3 wk. 2200m, (NH). RSH: 3x5x10s -20s 5-min recovery SIH: 4x30s - 5-min recovery	RSH, N = 6 SIH, N = 5	<b>RSE running time improved by -0.14 s and -0.11 s after RSH and SIH (p = 0.012). RSH improved reoxygenation during RSE</b>
23	Kasai et al. (2019)	University sprinters ♂	6 consecutive days, 3000m (NH) vs normoxia, 2 sessions/ day Morning: RS (5x6s – 24s recovery) 3x + (4x20s 5-15 min) Afternoon: 3 x 5x6s – 36s recovery + 4x20s – 40s passive recovery	RSH, N = 10 RSN, N = 9	<b>+ 3% max pedaling frequency and Pmax. Increases in muscle glycogen (+79.9%) and Pcr (+3.9%).</b>
24	Beard et al. (2018)	Elite Rugby union players ♂	4 sessions, cycling, 3000m (NH). 3x8x10s - 20s passive recovery	RSH, N = 10 RSN, N = 9	<b>RSA Pmax +6.3% Pmean+ 6.5% improved</b>
25	(Beard et al. 2019)	Elite Rugby union players ♂	4 sessions, double poling, 3000m (NH). 3x8x10s - 20s passive recovery	RSH, N = 18 RSN, N = 18	<b>RSA Pmax +13% Pmean+ 11% improved</b>
	Woorons et al. (2019)	Highly trained Jiu-Jitsu fighters	RSH-VHL shuttle sprint running. 2x6s - 15s passive recovery.	RSH-RSN, N = 10	<b>SPO2 lower (89.8 vs 97.7%; p &lt;0.01) and cerebral oxygenation (-6.1±5.4 vs -</b>

26		(7 = ♂, 3 = ♀)		<p><b>1.5±6.6 au) were lower than in RSN.</b></p> <p><b>VO<sub>2</sub> higher during recovery periods.</b></p> <p>RSE performance not impaired with RSH-VHL.</p>
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The significantly ( $P < 0.05$ ) larger benefits of RSH are presented in **bold**.

NH, Normobaric Hypoxia; Con, Control group without RS training; RSA, Repeated Sprints Ability; RSE, Repeated Sprint Exercise; RSH, Repeated Sprint Training in Hypoxia; RSN, Repeated Sprint Training in Normoxia; SIH, Sprint Interval Training in hypoxia; PO, Power Output; Pmax, maximal PO; Pmean, Mean PO; LT4, Lactic Threshold, i.e. lactate concentration of 4 mmol. L<sup>-1</sup>, Yo-yo IR, Yo-Yo IR test; Pcr, Phosphocreatine; [tHb], total hemoglobin/myoglobin; VHL, voluntary hypoventilation; OBLA, the onset of blood lactate accumulation; La, Blood lactate concentration.

## **Synthesis and objectives**

The ability to repeat short maximal exercises (i.e. sprints < 10 s) interspaced with incomplete recovery (< 60 s) is considered as a good performance index in a variety of intermittent sports (e.g. team or racket sports). This modality of exercise is extremely strenuous for the neuromuscular system as it relies mostly on the anaerobic metabolism during the first sprints. Towards the exercise, the power output developed during each sprint decrease concomitantly to the related energy expenditure. This is associated with a progressive switch from the anaerobic to the aerobic pathway as the number of sprints increase.

As with any exercise, fatigue will gradually develop from the beginning of the test until completion of the task. For the purpose of this work, fatigue has been defined as “a response that is less than the expected or anticipated contractile response, for a given stimulation”. The current methodology to evaluate neuromuscular fatigue involves the use of electrical or magnetic stimulations superimposed during an isometric voluntary contraction or delivered in the relaxed muscle to assess muscle properties (i.e. peripheral fatigue) and determine the ability of the central nervous system to fully activate the exercising muscle (i.e. central fatigue). While it is obviously a limit to bound fatigue measurement to an isolated muscle group especially when the fatiguing task is induced by whole body exercise and involves the use of multiple synergist muscle groups, it is still the most reliable way of investigating fatigue etiology. This procedure is however relatively complicated to pursue when considering locomotive exercise such as repeated sprint. Indeed, the execution of an isometric contraction requires the use of a specific ergometer and thus, implies to move the subject from the fatiguing task (e.g. non-motorized treadmill, cycloergometer) before measuring the induced fatigue. This procedure usually takes between 30 s and 3 min, which is rather problematic as depending on its origin, fatigue can recover quickly and at different rates. This is likely to result in an underestimation of fatigue and probably to a misinterpretation of its etiology as central fatigue usually recover more rapidly than its peripheral component. Another issue resulting from the inherent delay of fatigue measurement is the difficulty to obtain complete kinetics of fatigue development. Therefore, researchers often limit the evaluation to two time-points before and after task completion or propose complex experimental setups where participants need to performed multiple session of exercise to extrapolate fatigue kinetics from it. Despite those limitations, a few studies characterized fatigue etiology during repeated sprint exercise, and while some discrepancies persist, it seems that the fatigue induced by repeated-sprint exercise is mainly peripheral during the early stage of fatigue whereas central fatigue only appears after a few repetitions. It is relatively hard to determine when central fatigue occurs precisely because of the limited time

points of measurements through the test. Physiologically, those results could be explained by the reliance on the anaerobic glycolysis during the first part of the repeated sprint exercise, indeed, this source of energy produces a lot of metabolites which are closely related to the development of peripheral fatigue notably due to an accumulation of inorganic phosphate affecting the excitation-contraction coupling. Metabolites accumulation and mechanical changes within the muscle are also associated to muscle afferents (i.e. often referred as group III and IV muscle afferents) that can have inhibitory or excitatory activity at the spinal and supraspinal level. It is therefore hypothesized that muscle afferents are responsible for the later development of central fatigue observed during exercise.

Recently, a new training method has been developed to improve repeated sprint ability based on the completion of training session associated with hypoxic stress (i.e. either using terrestrial or simulated altitude). This method has been used for the past five years and presents interesting results especially with intermittent-sport athletes. However, the associated publications are often limited to performance measurements and only a few studies investigate the underlying physiological mechanisms potentially responsible for the observed benefits on sprint performance. While the main hypothesis relies on hypoxia-induced muscular adaptation through the oxygen-sensing pathway to improve oxygen extraction, and in fine the phosphocreatine resynthesizes, it is known that hypoxia also impacts the development of neuromuscular fatigue and especially the central compartment. Therefore, a potential mechanism associated with better performance post-training could arise from central adaptation to hypoxic stimulus. Nevertheless, the actual impact of hypoxia on neuromuscular function is not fully understood as two potential mechanisms have been proposed to explain the exacerbated central fatigue. Indeed, hypoxia impairs oxygen consumption of the muscle and decreases the amount of energy furnish by the aerobic pathway. This is likely to increase the reliance on anaerobic mechanisms and the production of the associated metabolites, leading to an exacerbation of the group III and IV muscle firing rate known to be partly responsible for the development of central fatigue by impacting the central nervous system both at the spinal and supraspinal levels. The second mode of action of hypoxia would be through a direct effect on brain oxygenation. The diminution in CO<sub>2</sub> level associated with hypoxia-induced hyperventilation is responsible for cerebral vasoconstriction which reduces the brain oxygen delivery resulting in a diminution of brain activity and central motor drive during exercise. However, by using systemic hypoxia (i.e. terrestrial or simulated altitude), it isn't possible to isolate those mechanisms from one another as both the muscles and the brain will be impacted by the overall lack of oxygen.

Two main objectives can be drawn from this synthesis of the current literature and will articulate the next chapter.

The purpose of the first study was to use two different modalities of hypoxia to isolate both potential mechanisms responsible for the exacerbation of central fatigue when performing exercise under hypoxic exposure. In this regard, neuromuscular fatigue induced by repeated sprint exercise combined with systemic hypoxia (i.e. induced via breathing a hypoxic gas mixture) and/or localized hypoxia (i.e. obtained via the use of blood flow restriction), were compared.

The second study aimed to assess some methodological problems associated with the evaluation of neuromuscular function as traditionally done in the literature. Therefore, a new ergometer was developed and build in the lab to allow participants to perform both the fatiguing task and the neuromuscular evaluation on the same ergometer. Furthermore, it also allows the characterization of muscle fatigue via the evolution of dynamic parameters of muscle capabilities calculated from the linear relationship of the torque-velocity profile. A reliability study of the ergometer is proposed.

## **Chapter 2 – Experimental data**

# **1 Study 1: Neuromuscular evaluation of arm-cycling repeated sprints under hypoxia and/or blood flow restriction.**

Neuromuscular function during arm cycling sprint combined with Hypoxia and/or blood flow restriction.

## **1.1 Presentation**

This study aimed to characterize fatigue etiology induced by a repeated sprint exercise performed to exhaustion with the upper limbs under different hypoxic stress. It has been shown that when combined to hypoxia, central fatigue is exacerbated potentially due to two mechanisms. Either a direct effect of lower oxygen availability at the brain level inducing an overall diminution of cortical activity, or an indirect effect from lower oxygen availability to the muscle during exercise, increasing the rate of metabolites production and the firing rate of group III and IV muscle afferent feedback at the spinal and supraspinal level impairing corticospinal excitability. Because previous studies used only systemic hypoxia (i.e. breathing hypoxic gas mixture), it is not possible to dissociate the two potential mechanisms as both the considered muscle and the brain are under hypoxic stress.

Therefore, the idea was to dissociate systemic hypoxia and localized hypoxia of the limb (i.e. using BFR to reduce the oxygen delivery to the working muscle) to see if both conditions would lead to an exacerbated central fatigue compare to normoxia or if one would prevail. Furthermore, the literature regarding sprints and the combined use of BFR is rather scarce so a secondary objective was to characterized fatigue induced by such exercise and if the combination of both systemic plus localized hypoxia would exacerbate fatigue even more.

## 1.2 The Study

### **Neuromuscular evaluation of arm-cycling repeated sprints under hypoxia and/or blood flow restriction.**

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## Abstract

**Purpose:** This study aimed to determine the effects of hypoxia and/or blood flow restriction (BFR) on an arm-cycling repeated sprint ability test (aRSA) and its impact on elbow flexor neuromuscular function.

**Methods:** Fourteen volunteers performed an aRSA (10s sprint / 20s recovery) to exhaustion in four randomized conditions: normoxia (NOR), normoxia plus BFR ( $N_{BFR}$ ), hypoxia ( $FiO_2=0.13$ , HYP) and hypoxia plus BFR ( $H_{BFR}$ ). Maximal voluntary contraction (MVC), resting twitch force (Db10), and electromyographic responses from the elbow flexors (*biceps brachii* [BB]) to electrical and transcranial magnetic stimulation were obtained to assess neuromuscular function. Main effects of hypoxia, BFR, and interaction were analyzed on delta values from pre- to post-exercise.

**Results:** BFR and hypoxia decreased the number of sprints during aRSA with no significant cumulative effect (NOR:  $16\pm 8$ ;  $N_{BFR}$ :  $12\pm 4$ ; HYP:  $10\pm 3$  and  $H_{BFR}$ :  $8\pm 3$ ;  $P<0.01$ ). MVC decrease from pre- to post-exercise was comparable whatever the condition. M-wave amplitude ( $-9.4\pm 1.9\%$  vs.  $+0.8\pm 2.0\%$ ,  $P<0.01$ ) and Db10 force ( $-41.8\pm 4.7\%$  vs.  $-27.9\pm 4.5\%$ ,  $P<0.01$ ) were more altered after aRSA with BFR compared to without BFR. The exercise-induced increase in corticospinal excitability was significantly lower in hypoxic vs. normoxic conditions (e.g. BB motor evoked potential at 75% of MVC:  $-2.4\pm 4.2\%$  vs.  $+16.0\pm 5.9\%$ , respectively,  $P=0.03$ ).

**Conclusion:** BFR and hypoxia led to comparable aRSA performance impairments but with distinct fatigue etiology. BFR impaired the muscle excitation-contraction coupling whereas hypoxia predominantly affected corticospinal excitability indicating incapacity of the corticospinal pathway to adapt to fatigue as in normoxia.

**Keywords:** corticospinal excitability, transcranial magnetic stimulation, occlusion, neuromuscular fatigue, BFR.

**Abbreviation:**

<b>1RM</b>	One-repetition maximum
<b>AMT</b>	Active motor threshold
<b>ANOVA</b>	Analysis of variance
<b>aRSA</b>	Repeated arm-cycling sprint ability test
<b>BB</b>	Biceps brachii
<b>BFR</b>	Blood flow restriction
<b>CMEP</b>	Cervicomedullary motor evoked potentials
<b>CSP</b>	Cortical silent period
<b>EMG</b>	electromyography
<b>EMS</b>	Electrical muscle stimulation
<b>ENS</b>	Electrical nerve stimulation
<b>ERT</b>	Estimated resting twitch
<b>ESM</b>	Electronic supplementary material
<b>FiO<sub>2</sub></b>	Fraction of inspired oxygen
<b>GABA</b>	Gamma-aminobutyric acid
<b>H<sub>BFR</sub></b>	Hypoxia with BFR
<b>HYP</b>	Hypoxia
<b>MEP</b>	Motor evoked potential
<b>Mmax</b>	Amplitude of the muscle compound action potential
<b>mRNA</b>	Messenger ribonucleic acid
<b>M<sub>SUP</sub></b>	amplitude of the muscle compound action potential during maximal voluntary contraction
<b>MVC</b>	Maximal voluntary contraction
<b>M-wave</b>	Muscle compound action potential
<b>N<sub>BFR</sub></b>	Normoxia with blood flow restriction
<b>NIRS</b>	Near infra-red spectroscopy
<b>NME</b>	Neuromuscular function evaluation
<b>NOR</b>	Normoxia
<b>PFC</b>	Pre-frontal cortex
<b>Pmax</b>	Maximal power
<b>POST</b>	After Repeated arm-cycling sprint ability test

<b>PRE</b>	Before Repeated arm-cycling sprint ability test
<b>RMS</b>	Root mean square
<b>RSA</b>	Repeated sprints ability
<b>SD</b>	Standard deviation
<b>SICI</b>	Short-interval intracortical inhibition
<b>SIT</b>	Superimposed twitch
<b>SpO<sub>2</sub></b>	Peripheral arterial oxygen saturation
<b>TB</b>	Triceps brachii
<b>TMS</b>	Transcranial magnetic stimulation
<b>TSI</b>	Tissue saturation index
<b>TTE</b>	Time to exhaustion
<b>VA</b>	Voluntary activation
<b>VA<sub>TMS</sub></b>	Voluntary activation assessed with TMS
<b><math>\eta_p^2</math></b>	Partial eta-squared

## Introduction

Repeated sprints ability (RSA) is characterized by the athlete's capacity to repeatedly produce maximal efforts for a short period of time (<10 s) interspersed with incomplete (<60 s) recovery and has been defined as an important factor of team-sport performance and intermittent activities efficiency (Girard et al. 2011a).

Sprint repetition induces fatigue due to a complex interaction between muscular (peripheral) and neural (central) responses leading to an impairment of the neuromuscular function (Girard et al. 2011a). Peripheral fatigue is usually described as a decrease in peak twitch force induced by electrical stimulation whereas central fatigue is characterized by a diminution of voluntary activation (VA) using the interpolated twitch technique (Shield and Zhou 2004). More recently, transcranial magnetic stimulation (TMS) has been used to calculate VA and this method provides additional information (e.g. motor evoked potential [MEP] or cortical silent period [CSP]) about the fatigue that occurs upstream of the motor cortex (Todd et al. 2016).

Collins et al. (2018) proposed that knee extensor neuromuscular fatigue throughout a RSA exercise mainly refers to peripheral impairments during the first five sprints (e.g. peak twitch diminution) but then relies on both peripheral and central perturbations. While the literature about RSA-related neuromuscular function predominantly focuses on the legs, researchers (Pearcey et al. 2016) have also investigated the impact of a RSA test on neuromuscular parameters of the elbow flexors since RSA with the upper limbs is of particular interest in multiple sport activities (e.g. kayak, judo, nordic skiing) and in some rehabilitation modalities. Using a determined number of sprints (i.e. 10), they concluded that arm-cycling sprints impacted neuromuscular function by increasing spinal excitability (e.g. increase in cervicomedullary motor evoked potentials [CMEP] from sprint 1 to sprint 10) and decreasing supraspinal excitability (e.g. decrease in MEP/CMEP ratio from sprint 1 to sprint 5). Gruet et al. (2013) also demonstrated that upper limbs were more sensitive to spinal adaptations compared to the lower limb. Furthermore, muscle metabolism also elicits systemic perturbation that could influence oxygen transport, extraction, and brain function (Calbet et al. 2009). Considering the smaller muscle mass and the differences in muscle typology (Koppo et al. 2002) involved with arm exercise compare to the legs, it may result in different systemic perturbation, highlighting limb specificities regarding the development of

neuromuscular fatigue. There is a lack of information regarding what are the underlying mechanisms limiting RSA performed to exhaustion in the upper limbs.

In the last few years, repeated sprint training in hypoxia has received growing interest in light of a probable, although debated (Goods et al. 2015) optimization of the gains in terms of performance compared to repeated sprint training performed in normoxia (Brocherie et al. 2017a). It is then likely that physiological adaptations induced by a single session in hypoxia are more prone to promote gains when repeated chronically. As a specificity, it has been shown that short intermittent bouts of sprints (15 x 5-s leg cycling / 25-s passive recovery) combined with acute hypoxia ( $SpO_2 \sim 84\%$ ) induce greater decline in cerebral oxygenation, quadriceps muscle activation and cycling performance than in normoxia, but with no difference in the magnitude of quadriceps fatigue (Billaut et al. 2013). The authors suggested that the central nervous system regulated the development of peripheral muscle fatigue, acknowledging however that it was not possible to rule out a direct effect of  $O_2$  desaturation on reducing motor output. Hence, from the only study exploring neuromuscular function pre-post a RSA test performed in hypoxia vs normoxia (Billaut et al. 2013), it is not possible to determine if the origin of fatigue differs and how far performance limitation is predominantly due to systemic, brain and/or muscle hypoxia. One way to evaluate the respective weight of localized vs systemic hypoxia in the genesis and the amount of exercise-induced fatigue would be to dissociate and combine these distinct environmental stressors during a session of RSA.

Localized hypoxia can be achieved by blood flow restriction (BFR) after the inflation of occlusion cuffs in the proximal portion of the limbs (Willis et al. 2018). This technique has been used for training purposes in combination with low-load resistance exercise, to promote hypertrophy and increase muscle strength due to significant hypoxic stimuli and greater accumulation of metabolites in the exercising muscles (Scott et al. 2015). A study from Fatela et al. (2016) using electromyographic (EMG) indices showed that the use of BFR combined to resistance exercise could enhance muscle activity (e.g. increased EMG root mean square [RMS]) and may increase neuromuscular fatigue. The exact hypoxic response to BFR exercise is not well understood but preliminary evidence suggests that BFR may acutely i) lead to enhanced hypoxia-inducible factor-1 $\alpha$  mediated cell signaling through an increase of its mRNA expression (Taylor et al. 2016a) as classically seen in response to hypoxia (Maxwell 2005) and/or ii) increase corticospinal excitability, possibly due to altered sensory feedback via group III and IV afferents (Brandner et al. 2015). Hemodynamic responses are

known to differ between the upper and lower limb. For example, arm exercise is known to elicit larger increases in arterial blood pressure than leg exercise (Calbet et al. 2015a) potentially inducing differences in response to BFR. Whether BFR in upper limbs may specifically modify arm-cycling performance, the amount of fatigue and/or its etiology after high-intensity arm-cycling sprints needs to be clarified. Also, it remains unknown if the combination of BFR plus hypoxia would have cumulative deleterious effects on RSA performance and neuromuscular function.

The purpose of this study was to determine the effects of repeated maximal intensity arm-cycling sprints on elbow flexor neuromuscular function when exercise is performed to exhaustion in normoxia vs. hypoxia and/or BFR. It was first hypothesized that arm-cycling performance would be more impacted under both hypoxia and/or BFR compared to normoxia. Secondly, it was hypothesized that during exhaustive exercise, BFR would predominantly affect peripheral parameters of fatigue due to the reduction in blood flow restraining the removal of muscle metabolites, while hypoxia would rather exacerbate central fatigue through a direct brain-hypoxic effect.

## Material and Methods

### Participants

Fourteen healthy volunteers (10 men and 4 women: age  $26 \pm 4$  yrs; height  $181 \pm 6$  cm; mass  $76 \pm 9$  kg) provided written informed consent after being informed of the procedures and risks involved in the current study. All participants were physically active (at least 4 h of training per week), unacclimated to high altitude (no sojourn above 2,000 m of altitude over the past 3 months) and completed the magnetic stimulation safety checklist for TMS. Two participants were excluded for TMS (one had a metal implant in the head and the other previously had a serious head injury) making 12 participants for TMS parameters only. Participants were asked to refrain from intense physical activity on the day before testing and to refrain from having a meal or drinking caffeinated beverages in the 2 h preceding the tests due to potential effects on exercise responses. This study was conducted according to the seventh revision of the Declaration of Helsinki (2013) and approved by the local ethics committee [CER-VD; protocol number: PB\_2016-01170 (138/15)].

### Study design

A preliminary session was first conducted to familiarize participants with sprint exercise on the arm cycloergometer, and with neuromuscular function evaluation (NME) procedures. Individual pressure inducing total brachial artery occlusion was also assessed during this session following 15 min of seated rest (see description below).

At least 3 days after the preliminary session, four experimental sessions (3 to 4 days apart) were performed for each subject in a randomized order (to limit potential training effect) at a similar time of the day and under standard environmental conditions (temperature  $24 \pm 2^\circ\text{C}$ , relative humidity  $41 \pm 5\%$ , and barometric pressure  $731 \pm 4$  mmHg) in a normobaric hypoxic chamber (ATS Altitude, Sydney, Australia) located in the laboratory at an altitude of 380 m (Lausanne, Switzerland). At the beginning of each session, electrical muscle stimulation (EMS), electrical nerve stimulation (ENS), as well as TMS sites and intensities, were assessed to allow the elbow flexors of the right arm to be investigated (see Fig. 1A for an overview). A standardized isometric warm-up was completed with repetitions of 5-s isometric

contractions / 5-s rest with intensities self-adjusted by the participant to progressively reach maximal voluntary contraction (MVC) during the last three trials. After a subsequent 6 min recovery period, participants performed the NME (see Fig. 1B). After another 6 min recovery period, participants engaged in a repeated arm-cycling sprint ability test (aRSA) to exhaustion. The aRSA test was conducted under either normoxia (NOR), normoxia with BFR (NBFR), hypoxia (HYP, with an inspired fraction of oxygen  $FiO_2 = 0.13$ ) or hypoxia with BFR (HBFR,  $FiO_2 = 0.13$ ). The  $FiO_2$  of 0.13 was chosen based on previous literature showing no impact on single sprint performance but changes in cerebral oxygenation (Smith and Billaut 2010). Post NME were measured 2 min after aRSA. This 2-min period was standardized between the end of the aRSA and the beginning of the post-test to adjust the setup. TMS, ENS, and EMS to assess corticospinal excitability and peripheral fatigue were achieved during NME. In addition, arterial ( $SpO_2$ ), the pre-frontal cortex (PFC) and *biceps brachii* (BB) oxygenation (using near-infrared spectroscopy) were monitored and EMG signals of the BB and *triceps brachii* (TB) muscles were measured continuously throughout sessions. Each session lasted ~120 min and was executed in a single semi-blinded fashion, as participants were naïve regarding altitude level but were not blinded for the blood flow restriction conditions.

## Experimental session

### *EMG, stimulation sites and intensities assessment*

Electromyography recordings. EMG signals of the BB and TB muscles were recorded during the whole session with three pairs of bipolar silver chloride surface electrodes (10-mm diameter, Type 0601000402, Contrôle Graphique, France), the reference electrode being placed on the elbow. The recording electrodes were secured lengthwise to the skin over the muscle belly following SENIAM recommendations, with an inter-electrode distance of 20 mm. A low resistance (<5 k $\Omega$ ) between the two electrodes was verified after shaving, gently abrading the skin and cleaning it with isopropyl alcohol. Electrode positions were marked using a permanent marker during the first session to ensure a reproducible position through subsequent visits. EMG and force signals were amplified, analog-to-digitally converted, band-pass filtered (10-500 Hz, input impedance = 200 M $\Omega$ , common mode rejection ratio = 85 dB, gain = 1000) and recorded at a sampling rate of 2 kHz using BioAmp (octal bio-amplifier

ML138, ADInstruments, Bella Vista, Australia) and PowerLab system (16/35, ADInstruments,). Data were stored and analyzed offline with LabChart 8 software (ADInstruments).

*Peripheral muscle and nerve stimulation.* For both EMS and ENS, square-wave pulses (respectively 100  $\mu$ s and 200  $\mu$ s duration) were delivered via high-voltage constant-current stimulators (DS7A; Digitimer, Hertfordshire, UK). Single ENS was delivered percutaneously to the brachial plexus via a 20-mm diameter cathode electrode (Ag-AgCl, Controle Graphique Medical, Brie-Comte-Robert, France) pressed into the supraclavicular fossa and a 50×90-mm anode gel pad electrode (Durastick Plus, DJO Global, Vista, USA) placed on the acromion. ENS intensity started at 10 mA and was incremented by step of 10 mA every 5 seconds up to the maximal amplitude of BB M-wave was reached. Stimulation intensity corresponding at 130% of this latter was used thereafter.

Since ENS to the brachial plexus also recruits the antagonist's muscle, paired-pulse EMS (10 Hz) was used instead to obtain the force response of the BB. It was delivered percutaneously via a 45×45-mm cathode pad gel electrode (Durastick Plus, DJO Global, Vista, USA) placed over the BB motor point found using a stimulator pen (Compex SA, Ecublens, Switzerland) and a 50×90-mm anode gel pad electrode (Durastick Plus, DJO Global) positioned over the bicipital tendon (2-3 cm proximal to the elbow). Initially, EMS intensity was set at 20 mA and was augmented by step of 10 mA every 5 seconds to reach the maximal amplitude of force response. Again, 130% of the defined optimal intensity was used for the whole experiment.

*Transcranial magnetic stimulation.* A magnetic stimulator (Magstim BiStim<sup>2</sup>, The Magstim Company, Dyfed, UK) was used to stimulate the motor cortex during voluntary isometric elbow flexion. Single and paired (3-ms interspaced) TMS pulses were delivered with a circular coil (135-mm outside diameter) held tangentially to the skull. Participants wore a swim cap, secured to the skin, on which lines were drawn between the preauricular points and from *nasion* to *inion* to identify the vertex. Every 1.5 cm from 3 cm anterior to 3 cm posterior to the vertex were demarcated along the *nasal-inion* line and also to 2 cm left from this line. At each point, a stimulus was delivered at 50% of maximal stimulator power output during 5-s isometric elbow flexion at 10% MVC. The optimal stimulus site was defined as the one eliciting the largest MEP in the BB and a MEP as small as possible in the TB. The position was drawn directly on the swim cap to ensure reproducibility of the stimulus throughout the session. The optimal TMS site was defined for each session.

The active motor threshold (AMT) was defined from multiple sets of contractions (6 x 5s on / 10 s off) at 10% MVC during which participants received a TMS starting at 25% of maximal stimulator output and increasing by 5% after each set, up to the specific intensity eliciting at least 4 MEP visually recognizable and  $\geq 50 \mu\text{V}$  out of the 6 contractions in the BB (Forman et al. 2015). The intensity for eliciting MEP during PRE and POST neuromuscular evaluations was set at 140% of AMT to avoid underestimating evoked responses (Temesi et al. 2014). Short interval intracortical inhibition (SICI) indices were obtained by administering two TMS pulses interspaced by 3 ms during contractions at 10% MVC. The first (conditioning) stimulation was set at 70% of AMT and the second (test) stimulation was 140% of AMT.

#### *Arm cycling position and dynamic warm-up*

Participants sat upright, with hips and knees flexed at  $90^\circ$  in front of a cycloergometer (Excalibur, Lode, Groningen, The Netherlands) mounted on a table and equipped with hand grips. The crankset center was on the same level as the chest and arms were nearly straight and shoulders at  $90^\circ$  when the grips reached the further point. Participants were authorized to use their feet to stabilize body position. Participants performed a 6-min bout on the cycloergometer at 1 W/Kg. After a 3 min resting period, they subsequently performed 2 maximal sprints for 10 s, interspaced again with a 3 min recovery. During each sprint, the cycloergometer was set in 'Wingate' mode and the torque factor (i.e. resistance) was set at  $0.4 \text{ Nm}\cdot\text{kg}^{-1}$  (adjusted from pilot testing). Strong verbal encouragements were given during the sprints to promote maximal efforts.

#### *Arm repeated sprint ability test*

The aRSA test consisted of a repetition of 10-s sprints interspersed with 20 s of active recovery (20 W) up to task failure (i.e. volitional stop or inability to maintain a cadence  $>70$  rpm). Participants were instructed to go "as hard as you can" from the start of every sprint. The mean power of the first sprint of the aRSA test had to reach at least 95% of the best sprint performed during the warm-up in order to limit any pacing strategies.

#### *Neuromuscular function evaluation*

Participants were in a similar position as described above but the right arm was positioned in an isometric ergometer equipped with a strain gauge (Captels, St. Mathieu deTreviers, France) with the shoulder and elbow flexed at 90° allowing measurement of the force evoked or produced voluntarily by the elbow flexors.

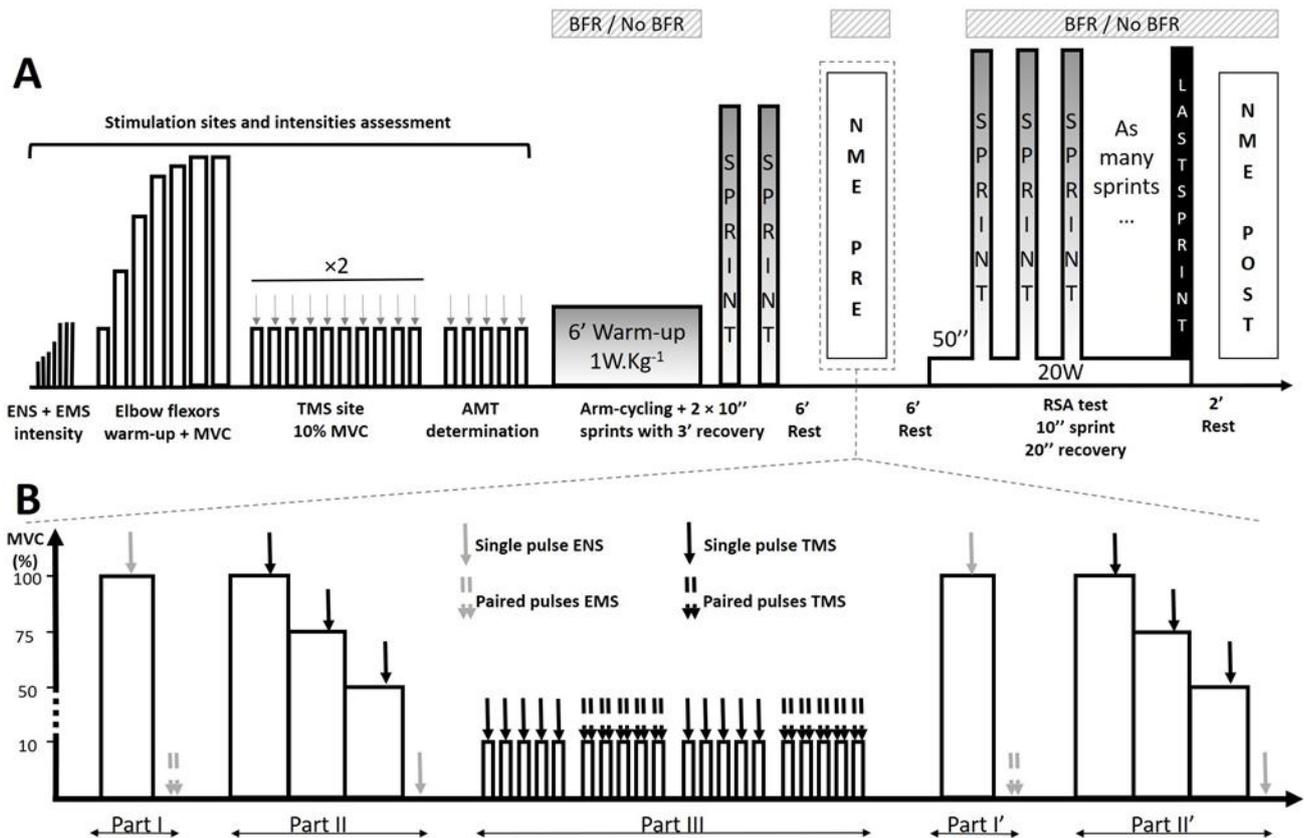


Figure 1. A: Protocol overview. B: Details on neuromuscular function evaluation (NME) performed PRE and POST exercise. AMT, active motor threshold; aRSA, arm-cycling repeated sprint ability; BFR, blood flow restriction; EMS, electrical muscle stimulation; ENS, electrical nerve stimulation; MVC, maximal voluntary contraction; TMS, transcranial magnetic stimulation

As described in Fig. 1B, NME was separated into three distinct parts. It consisted in (I) a supramaximal single-pulse ENS delivered during a brief MVC of the elbow flexors and a paired-pulse EMS delivered 3 s after MVC to assess elbow flexors contractile properties; (II) a voluntary contraction of the elbow flexors with TMS at different torque levels without rest between them (i.e. MVC, followed directly by 75% and 50% MVC all in a continuous way, refer to Mira et al. (2017) for methodology of the technique) to assess corticospinal excitability, CSP, and cortical VA. A single ENS was then delivered 3 s after the last

contraction to assess sarcolemma excitability; (III) 20 contractions at 10% of MVC force (3-s on / 3-s off) with TMS, where contractions 1-5 and 11-15 were superimposed by a single pulse TMS to measure MEP and contractions 6-10 and 16-20 superimposed by paired-pulse TMS to assess SICl (Rothwell et al. 2009). Parts I and II were then performed again (I' and II', respectively) to complete the testing procedure. This testing procedure lasted ~4 min.

#### *Near-infrared spectroscopy measurements*

Tissue saturation index was estimated in the BB ( $TSI_{BB}$ ) and the PFC ( $TSI_{PFC}$ ) throughout testing sessions using 3x2 wavelengths (760 and 850 nm) multichannel continuous wave NIRS systems based on spatially resolved spectroscopy (respectively Portamon and Portalite, Artinis Medical Systems, the Netherlands) with 30, 35, 40 mm optode distance (between receiver and transmitters). The probes were secured on the skin using double-sided tape and covered to prevent ambient light from disturbing the optode. Elbow flexor muscle oxygenation was assessed from the left BB, and the left PFC oxygenation was assessed between Fp1 and F3 locations according to the international 10–20 EEG system. Data were recorded continuously at the maximum frequency for each device (10 Hz for Portamon and 50 Hz for Portalite) and filtered at 10Hz with a 4th-order low-pass zero-phase Butterworth filter (cutoff frequency 0.2 Hz).

#### *Cardiorespiratory parameters.*

Arterial oxygen saturation ( $SpO_2$ ) was recorded continuously during the session by pulse oximetry at the ear lobe (Radical-7, Masimo Corporation, Irvine, CA, USA).

#### Blood flow restriction

Narrow (3 cm wide) nylon specialized cuffs were applied to the most proximal portion of the arms. The cuff pressure was regulated by a rapid cuff inflator system (E20 Hokanson, Bellevue, WA, USA). At the beginning of the familiarization session, the cuffs were inflated to 50 mmHg and the pressure was slowly raised until the arterial flow was no longer detected during inflation. The pulse (arterial blood flow) was detected using a hand-held bidirectional Doppler probe (Linear probe L12-5L60N; Software EchoWave II 3.4.4, Telemed Medical Systems, Milano, Italy) placed on the brachial artery. This operation was performed two to three times and resting arterial occlusion pressure was set as the mean cuff pressure at which

a pulse was not present. It was then used 45% ( $95.4 \pm 11.9$  mmHg) of this value during the BFR conditions as recommended by Loenneke et al. (2014). The cuffs were inflated during the dynamic warm-up, 5-sec prior to the beginning of neuromuscular testing and 5-sec before the first sprint of the aRSA test with a 6-min wash-out period between them.

### Data analysis

All the data were analyzed blinded. SpO<sub>2</sub> and TSI were averaged respectively over a period of 5 and 10 s at the end of the 20 W period before the first sprint and during the last sprint of the aRSA test. The maximal force was measured during part II of the NME as the maximal force before any TMS stimulation (Fig. 1B). The amplitude of the M-wave was measured from the superimposed single pulse ENS during MVC ( $M_{SUP}$ ) and in relaxed muscles, 3 s after the contraction ( $M_{max}$ ). EMG root mean square ( $EMG_{RMS}$ ) was calculated during MVC over a 500-ms period before TMS delivery and normalized using  $M_{SUP}$ .

MEPs of the BB were recorded using TMS superimposed on maximal ( $MEP_{100}$ ) or submaximal (75%, 50%, e.g.  $MEP_{50}$ ) contractions and were normalized by  $M_{SUP}$ . SICI were normalized by MEP obtained on the same level of contraction (i.e. 10%). CSP were manually analyzed as the time between the TMS delivery and the return of pre-stimulation EMG activity.

Force amplitude for paired EMS at 10 Hz (Db10) was determined as the maximal force measured after paired EMS in relaxed muscle 3 s after the end of MVC.  $VA_{TMS}$  was quantified by measuring force responses to TMS. Superimposed twitch (SIT) amplitude evoked during contractions at 100, 75, and 50% MVC, and the y-intercept of the linear regression between SITs and voluntary force allowed to quantify the estimated resting twitch (ERT). When linear regression was not linear ( $R^2 < 0.9$ ), ERT was excluded and  $VA_{TMS}$  was not calculated for the considered set of contractions. Hence, one subject was excluded from  $VA_{TMS}$  measurements, otherwise,  $R^2$  were acceptable permitting  $VA_{TMS}$  to be determined in 11 subjects using the equation:

$$VA_{TMS} = [1 - (SIT \times ERT^{-1})] \times 100$$

The number of sprints was defined as the total sprints performed by the subject before exhaustion, no cut off (e.g. %Pmax) was applied and the best sprint (mean power over 10 s) during the aRSA test was calculated.

Statistical analysis was performed on the raw values for sprint performance indices (number of sprints and mean power output of the best sprint) whereas delta ( $\Delta$ ) values were used between PRE and POST, expressed in percentage of PRE to evaluate the effect of *altitude* and/or *occlusion* on MVC force, EMG<sub>RMS</sub>, VA<sub>TMS</sub>, Db10, Mmax, MEP, SICI, SpO<sub>2</sub>, and TSI.

### Statistical analysis

All statistical procedures were completed on Statistica v10 (Statsoft, Tulsa, OK). Grubbs test was used to determine potential outliers and when such value was detected, it was deleted. If more than one data was determined as outliers or missing for the same period of analysis (i.e. PRE or POST), participants were excluded of the variable analysis (cf. parameters of the F-value).

Normality of the distributions and homogeneity of the variances were confirmed using Shapiro-Wilk normality tests and the Levene's tests, respectively. Two-way ANOVAs (*altitude\*occlusion*) with repeated measures were performed to evaluate the effect of an aRSA performed in different conditions on performance, MVC force, EMG<sub>RMS</sub>, VA<sub>TMS</sub>, Db10, Mmax, MEP, SICI, SpO<sub>2</sub>, and TSI. Post-hoc Tukey's tests were applied to determine a difference if the ANOVA revealed a significant main or interaction effect. Statistical significance was set at  $P < 0.05$ . Effect sizes are reported as partial eta-squared ( $\eta_p^2$ ) for ANOVAs [with interpretation thresholds fixed at 0.02, 0.13, 0.26 for respectively, small, medium and large, (Cohen 1988)]. All data are presented as mean  $\pm$  SD.

Two additional statistical analyses were performed. Since their results are not the focus of the study but may be of interest for some readers, they are depicted in the electronic supplementary material (ESM) to not overload the article. 1) Two-way ANOVAs (*altitude\*occlusion*) with repeated measures were performed on PRE-values only to evaluate the acute effect of *altitude* and *occlusion* before the start of the fatiguing test (see results in ESM I). And 2) T-tests for dependent samples were performed on data PRE/POST aRSA test in NOR condition only to describe variables evolution in terms of absolute values rather than delta in this "control" condition (see results in ESM II).

## Results

### Performance and saturation indices

As shown in Fig. 2A, *occlusion* had a significant effect on aRSA test performance decreasing the number of sprints ( $10 \pm 4$  in BFR vs.  $13 \pm 7$  without BFR,  $F_{(1,13)}=9.73$ ,  $P<0.01$ ,  $\eta_p^2=0.43$ ). *Altitude* also affected performance, ( $9 \pm 3$  sprints in hypoxia vs.  $14 \pm 6$  in normoxia,  $F_{(1,13)}=14.30$ ,  $P=0.002$ ,  $\eta_p^2=0.52$ ). *Altitude\*occlusion* interaction did not reach significance but tended to show an attenuation of BFR effect when combined with hypoxia ( $F_{(1,13)}=3.41$ ,  $P=0.09$ ,  $\eta_p^2=0.21$ ). The same result occurred when considering the total work (Time  $\times$  Mean power, not presented here).

Mean power output of the best sprint (Fig. 2B) was impacted by *occlusion* ( $F_{(1,13)}=7.65$ ,  $P=0.02$ ,  $\eta_p^2=0.37$ ), but was not impacted by *altitude* ( $F_{(1,13)}=2.70$ ,  $P=0.12$ ) or *altitude\*occlusion* ( $F_{(1,13)}=0.07$ ,  $P=0.79$ ). The decrease in MVC force after exercise was not impacted by *occlusion* ( $F_{(1,11)}=3.39$ ,  $P=0.09$ ), *altitude* ( $F_{(1,11)}=2.37$ ,  $P=0.15$ ), or *altitude\*occlusion* ( $F_{(1,11)}=3.03$ ,  $P=0.11$ ). Data are presented in Table 1.

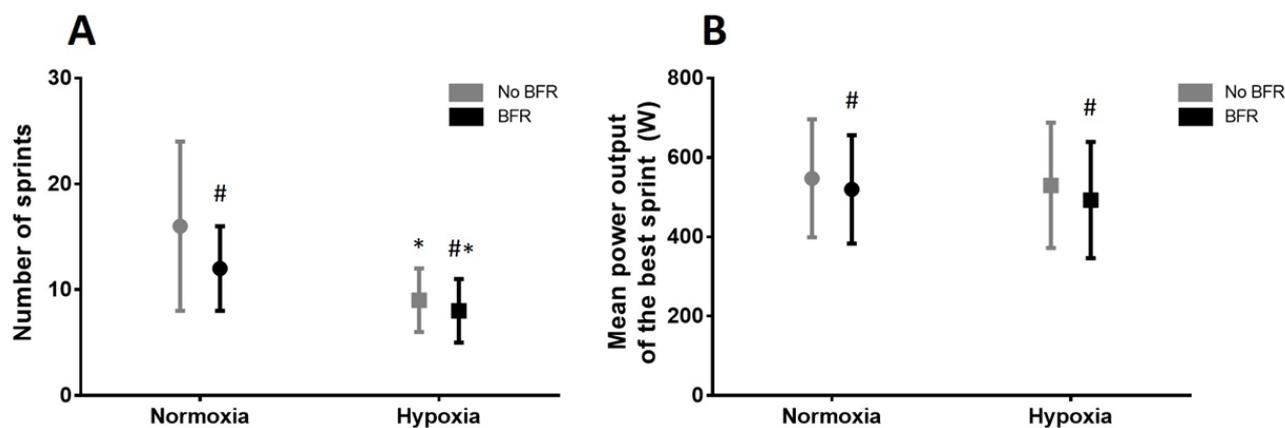


Figure 2 Performance on the arm-cycling repeated sprint ability test depending on the condition: normoxia without blood flow restriction (NOR), normoxia with BFR (NBFR), hypoxia without BFR (HYP) and hypoxia with BFR (HBFR). A: Number of sprints completed until exhaustion. B: Best single sprint performance as mean power output over the 10-s period. # indicates a significant main effect of occlusion and \* indicates a significant main effect of altitude. Statistical significance set at  $P < 0.05$ .

Fig. 3 shows that  $\Delta\text{SpO}_2$  and  $\Delta\text{TSl}_{\text{PFC}}$  from the end of the warm-up to the end of aRSA were not impacted by *occlusion* ( $F_{(1,13)}=0.45$ ,  $P=0.51$  and  $F_{(1,13)}=2.12$ ,  $P=0.17$ , respectively),

*altitude* ( $F_{(1,13)}=2.92$ ,  $P=0.11$  and  $F_{(1,13)}=0.17$ ,  $P=0.69$ , respectively) or *altitude\*occlusion* ( $F_{(1,13)}=2.21$ ,  $P=0.51$  and  $F_{(1,13)}=0.17$ ,  $P=0.68$ , respectively).

$\Delta\text{TSI}_{\text{BB}}$  was not impacted by *occlusion* compared to without BFR ( $F_{(1,13)}=1.43$ ,  $P=0.25$ ) but decreased with *altitude* ( $-7.6 \pm 6.8\%$  vs.  $+1.3 \pm 7.1\%$  in normoxia,  $F_{(1,13)}=35.8$ ,  $P<0.01$ ,  $\eta_p^2=0.73$ ). The interaction *altitude\*occlusion* was significant ( $F_{(1,13)}=5.61$ ,  $P=0.03$ ,  $\eta_p^2=0.30$ ) and the post-hoc analysis showed different evolution in NOR ( $+1.9 \pm 8.6\%$ ) vs. HYP ( $-10.3 \pm 7.4\%$ ,  $P<0.01$ ,  $d=1.52$ ) and vs. HBFR ( $-4.8 \pm 5\%$ ,  $P=0.02$   $d=0.96$ ).

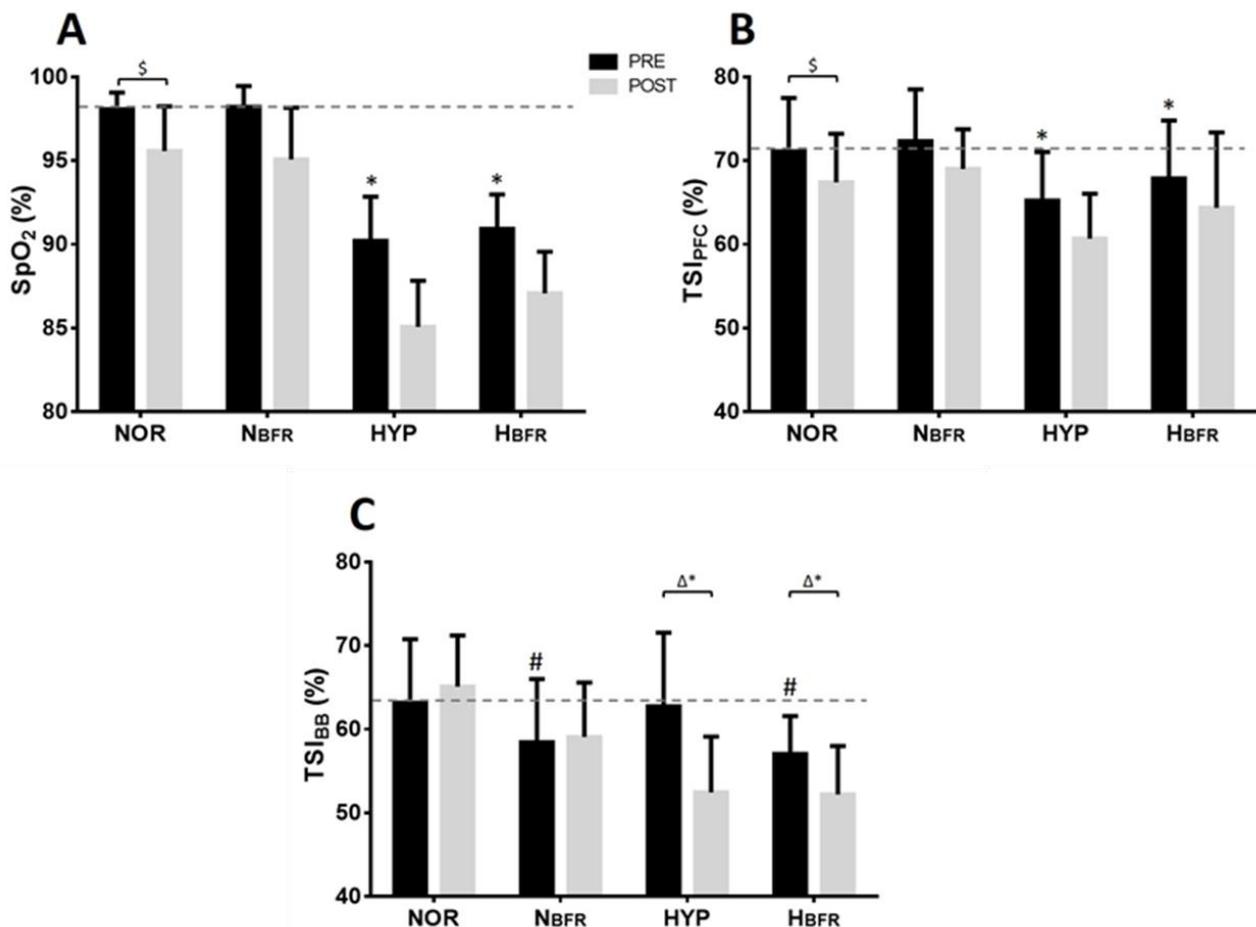


Figure 3 Arterial and tissue oxygenation PRE and POST arm-cycling repeated sprint ability test. A: Peripheral arterial oxygen saturation (SpO<sub>2</sub>). B: Tissue saturation index of the biceps brachii (TSI<sub>BB</sub>). C: Tissue saturation index of the pre-frontal cortex (TSI<sub>PFC</sub>). Dotted lines help to refer to values observed in control (NOR) condition PRE exercise. \$ indicates a significant main effect of time, # indicates a significant main effect of occlusion, \* indicates a significant main effect of altitude and  $\Delta^*$  indicates a significant main effect of altitude on the delta from PRE to POST aRSA test. Statistical significance set at  $P < 0.05$ .

## Peripheral indices

An example of raw data from typical subjects are presented in Fig.4.  $\Delta M_{max}$  from PRE to POST exercise was more impacted with BFR ( $-9.4 \pm 1.9\%$ ) compared to without BFR ( $+0.8 \pm 2.0\%$ ,  $F_{(1,13)}=14.03$ ,  $P<0.01$ ,  $\eta_p^2=0.52$ ) but was not affected by *altitude* ( $F_{(1,13)}=0.98$ ,  $P=0.34$ ) or *altitude\*occlusion* ( $F_{(1,13)}=0.23$ ,  $P=0.64$ ).

$\Delta Db_{10}$  was significantly greater from PRE to POST exercise in *occlusion* conditions ( $-40.8 \pm 4.7\%$  vs.  $-27.9 \pm 4.5\%$  without BFR,  $F_{(1,13)}=10.73$ ,  $P<0.01$ ,  $\eta_p^2=0.45$ ) but was not impacted by *altitude* ( $F_{(1,13)}=3.43$ ,  $P=0.09$ ) or *altitude\*occlusion* ( $F_{(1,13)}=1.01$ ,  $P=0.37$ ).

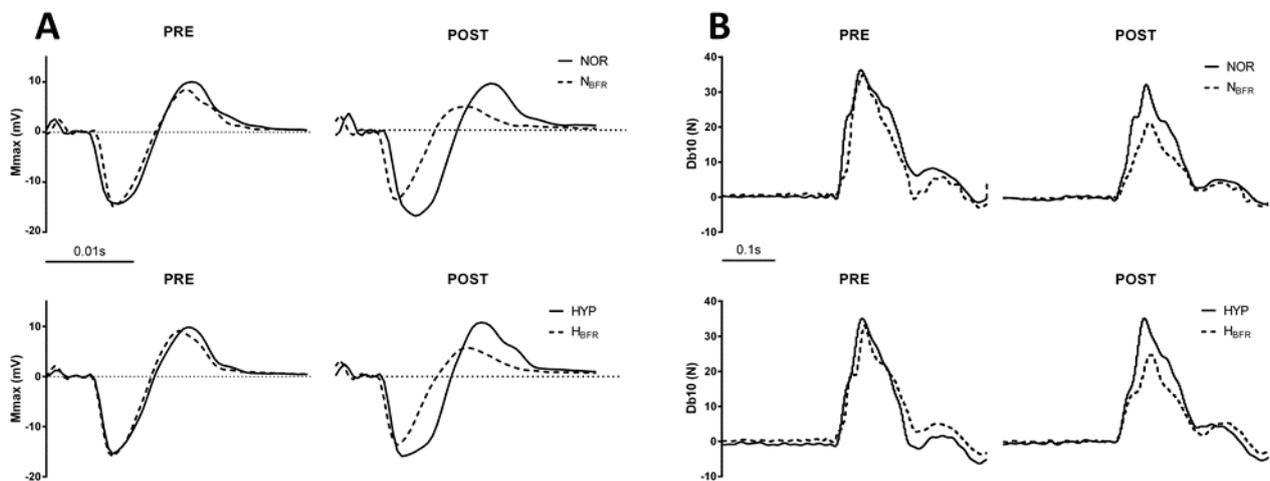


Figure 4 Peripheral fatigue indices PRE and POST arm-cycling repeated sprint ability test performed in 4 conditions: normoxia without blood flow restriction (NOR), normoxia with BFR (NBFR), hypoxia without BFR (HYP), and hypoxia with BFR (HBFR). A: amplitude of the M-wave ( $M_{max}$ ) measured from the relaxed muscles 3 s after maximal voluntary contraction. B: force amplitude for paired electrical muscle stimulation at 10 Hz ( $Db_{10}$ ) in relaxed muscle 3 s after maximal voluntary contraction. Presented as raw data from a typical subject.

## Central indices

$\Delta VA_{TMS}$  from PRE to POST exercise was not impacted by any factors (*occlusion*:  $F_{(1,10)}=0.25$ ,  $P=0.62$ ; *altitude*:  $F_{(1,10)}=0.95$ ,  $P=0.35$ ; *altitude\*occlusion*:  $F_{(1,10)}=0.92$ ,  $P=0.36$ ).

$\Delta EMG_{RMS}$  was not impacted by *occlusion* ( $F_{(1,10)}=0.41$ ,  $P=0.54$ ) or *altitude\*occlusion* ( $F_{(1,10)}=0.31$ ,  $P=0.59$ ) but was significantly less increased with *altitude* ( $+3.5 \pm 4.2\%$  in hypoxia vs.  $+18.3 \pm 4.1\%$  in normoxia,  $F_{(1,10)}=5.09$ ,  $P=0.05$ ,  $\eta_p^2=0.34$ ).

As shown in Table 1, exercise-induced adaptations in MEP were not affected by *occlusion* (e.g.  $\Delta\text{MEP}_{75}$ :  $F_{(1,9)}=2.78$ ,  $P=0.13$ ) or *altitude\*occlusion* (e.g.  $\Delta\text{MEP}_{75}$ :  $F_{(1,9)}=0.33$ :  $P=0.58$ ) but exercise-induced increase in corticospinal excitability was significantly lower with *altitude* (e.g.  $\Delta\text{MEP}_{75}$ :  $-2.4 \pm 4.2\%$  in hypoxia vs.  $+16 \pm 5.9\%$  in normoxia,  $F_{(1,9)}=6.78$ ,  $P=0.03$ ,  $\eta_p^2=0.43$ ).

$\Delta\text{CSP}$  from PRE to POST exercise was not impacted by any factors during contractions at 100, 75 or 50% MVC. The same was observed for SICI.

**Table 1** Effects of an arm-cycling repeated sprint ability test to task failure on elbow flexors, saturation index, and neuromuscular function under different combination of hypoxia and blood flow restriction

	Conditions				Factors		
	NOR	N <sub>BFR</sub>	HYP	H <sub>BFR</sub>	Altitude	Occlusion	Altitude × occlusion
					<i>P</i> value	<i>P</i> value	<i>P</i> value
<b>Performance</b>							
TTE (s)	460 ± 220	340 ± 100	250 ± 70	220 ± 70	<b>0.002</b>	<b>0.008</b>	0.088
Sbest (W)	547 ± 149	520 ± 137	530 ± 158	493 ± 147	0.124	<b>0.0160</b>	0.786
$\Delta\text{MVC}$ (%)	-16.5 ± 9	-10.5 ± 12.1	-11.1 ± 10.4	-9.8 ± 11.1	0.152	0.093	0.110
<b>Peripheral indices</b>							
$\Delta M_{\text{max}}$ (%)	0.5 ± 14.6	-11.6 ± 10.9	1.1 ± 6	-7.2 ± 12.3	0.339	<b>0.002</b>	0.643
$\Delta\text{Db10}$ (%)	-29.3 ± 23.2	-46.5 ± 19.8	-26.6 ± 18	-35 ± 19.8	0.087	<b>0.006</b>	0.332
<b>Central indices</b>							
$\Delta\text{VA}_{\text{TMS}}$ (%)	-3.4 ± 2.8	-1.3 ± 3.3	-3.5 ± 6.7	-4.0 ± 5.6	0.352	0.627	0.360
$\Delta\text{EMG}_{\text{RMS}}$ (%)	18.2 ± 26.5	18.5 ± 18.9	7.8 ± 20.9	-0.8 ± 20.2	<b>0.048</b>	0.538	0.588
$\Delta\text{MEP}_{100}$ (%)	20 ± 36.4	7.2 ± 11.4	2.6 ± 17.7	-5.9 ± 13.9	<b>0.051</b>	0.182	0.742
$\Delta\text{MEP}_{75}$ (%)	23.2 ± 33.8	8.8 ± 15.5	-0.3 ± 21.8	-4.6 ± 17.1	<b>0.028</b>	0.130	0.580
$\Delta\text{MEP}_{50}$ (%)	15.7 ± 27.2	12.9 ± 12.3	4.7 ± 25.3	-8.7 ± 10.1	<b>0.030</b>	0.072	0.52
$\Delta\text{CSP}_{100}$ (%)	-1.7 ± 8.3	5.3 ± 14.9	3.8 ± 6.2	8.1 ± 11.1	0.097	0.064	0.702
$\Delta\text{CSP}_{75}$ (%)	-2.5 ± 9.4	3.9 ± 13.5	4.0 ± 9.0	3.7 ± 8.7	0.200	0.327	0.240
$\Delta\text{CSP}_{50}$ (%)	-5.4 ± 10.4	0.6 ± 11.4	-1.1 ± 8.0	1.9 ± 6.2	0.310	0.130	0.487
$\Delta\text{SICI}$ (%)	4.3 ± 16.6	7.5 ± 20.3	6.6 ± 21.3	-3.1 ± 18.6	0.407	0.579	0.211

Data are presented as a delta from PRE to POST exercise (except for time to exhaustion and the best sprint performance). Values are presented as mean (SD). Statistical significance was set at  $P < 0.05$  and is highlighted by bold type. NOR normoxia without blood flow restriction, N<sub>BFR</sub> normoxia with blood flow restriction, HYP hypoxia without blood flow restriction, H<sub>BFR</sub> hypoxia with blood flow restriction, TTE time to exhaustion, Sbest best sprint performed (in mean power), MVC maximal voluntary contraction force, M<sub>max</sub> maximal M-wave, Db10 force evoked by a 10 Hz doublets, VA<sub>TMS</sub> voluntary activation assess by TMS, EMG<sub>RMS</sub> EMG root mean square, MEP<sub>100</sub>, MEP<sub>75</sub>, MEP<sub>50</sub> motor evoked potential, respectively, during MVC, submaximal contraction at 75 and 50% of MVC, CSP<sub>100</sub>, CSP<sub>75</sub>, CSP<sub>50</sub> cortical silent period, respectively, during MVC, submaximal contraction at 75, and 50% of MVC, SICI short-interval intracortical inhibition during contraction at 10% MVC

## Discussion

The purpose of this study was to determine the effects of systemic hypoxia and/or localized hypoxia induced by BFR on performance and neuromuscular function adaptations induced by an arm-cycling RSA test. The main finding is that the number of sprints performed was similarly decreased when exercise is performed under hypoxia ( $FiO_2 = 0.13$ ) and/or moderate BFR (45% of resting full ischemia). Regarding the neuromuscular function, BFR impacted the peripheral compartment, specifically leading to an impairment of the muscle excitation-contraction coupling. Contrarily, hypoxia led to lower corticospinal excitability compared to normoxia, potentially indicating an incapacity of the corticospinal pathway to adapt to fatigue as in normoxia.

### aRSA test Performance

The number of sprints performed to exhaustion decreased both with *altitude* and *occlusion* without any clear cumulative effect, indicating that both hypoxic stressors negatively impacted the ability to repeat arm-cycling sprints. It has been shown that partial occlusion impacts muscular endurance during low-load resistance training (up to 40% of 1RM), decreasing the number of repetitions completed at a given intensity (Wernbom et al. 2006). To our knowledge, this is the first study demonstrating such a diminution when repeated sprints are performed with BFR in the arms. Regarding the impact of hypoxia on RSA performance, the topic is more widely documented and the decreased number of sprints performed was expected (Girard et al. 2017). It is yet to determine if the mechanisms responsible for the decrease in the number of sprints are similar for the two modalities.

The mean power of the best sprint performed during each session was lowered by *occlusion* but not by *altitude*, leading to speculation that BFR immediately negatively impacts the ability of the neuromuscular system to produce maximal force (cf. MVC, Db10). This could be, at least partly, related to PRE-exercise lower levels of muscle oxygenation with BFR (refer to ESM I), which is not visible with *altitude* only. These observations reinforce the assumption that hypoxia *per se* does not impact short isolated sprint performance (Girard et al. 2017).

### Oxygen saturation indices

At the end of the aRSA test, both  $SpO_2$  and  $TSI_{PFC}$  decreased in similar ways from warm-up level, no matter the condition. However, since both variables were impacted negatively at rest by *altitude* (see ESM I), it is likely that the level of deoxygenation at the end of exercise was exacerbated with hypoxia (Fig 3A). In addition,  $TSI_{BB}$  decreased with *altitude* whereas no changes were noticed in normoxia. It means that despite not being affected by *altitude* at rest, muscular oxygenation imbalance was exacerbated with *altitude* compared to normoxia. This implies that the vascular system was not able to match the muscular demand in oxygen during exercise in hypoxic conditions. This supports previous studies that have demonstrated no peripheral vasodilation during leg cycling repeated sprint in hypoxia (Willis et al. 2017). However, further researches are needed regarding the question of peripheral vasodilatation during intermittent supra-maximal exercise in hypoxia in the upper limb as the present results don't allow us to investigate this hypothesis. Interestingly, while impacting muscle oxygenation at rest, BFR had no major additional effect during exercise (Figure 3C). This could be explained by the fact that muscle activity during aRSA test could induce an increase of intramuscular pressure that may reach levels high enough to impede blood flow by itself, therefore bypassing the effect BFR may have had. This is well known with isometric contractions (Sadamoto et al. 1983) and also occurs during dynamic isolated exercise at 50% of 1RM (Wernbom et al. 2006). It should also be noted that the current study used 45% of the pressure corresponding to total occlusion at rest as recommended by Loenneke et al. (2014) to promote muscle adaptation during resistance training and previously used on lower limb muscle during high-intensity repeated-sprint exercise (Willis et al. 2018). It has been shown that during a dynamic exercise, arterial pressure and muscular vasodilation are correlated with the exercise intensity (Saltin et al. 1998). Therefore, in this study, it is likely that supramaximal exercise intensity induced vasodilatation and an increase in arterial pressure sufficient to counteract the action of BFR on tissue oxygenation parameters. Taken together, these results show different oxygenation perturbations regarding the tissue and the use of BFR or hypoxia.

### Neuromuscular parameters

No matter the condition, the aRSA test induced comparable changes in MVC from PRE to POST (mean  $\Delta MVC$  of  $-12 \pm 10.7\%$  over conditions, see ESM II for absolute responses on NOR condition only). To note that the tendency towards the lower impact of BFR on  $\Delta MVC$

could be partially related to the fact that BFR already has an effect at rest (i.e. before the fatiguing exercise, see ESM I), therefore limiting the possible range of force decrease. These results are in accordance with previous literature showing similar force decrement in the elbow flexors (-9%) after a set of 10 repeated sprints in normoxia without BFR (Pearcey et al. 2016). In the present study, despite a lower number of sprints performed in hypoxia and/or BFR condition, the decrease in MVC seems to be consistent, indicating a similar global level of fatigue. Thus, only the rate of fatigue seems to be accelerated rather than its amounts as shown by the shorter TTE (table 1). After a set of fifteen 5-s leg cycling sprints performed either in normoxia or hypoxia, Billaut and Buchheit (2013) observed similar peripheral fatigue (-54.3% in peak twitch) whereas central indices were more impacted in hypoxia (VA: -3.4% in normoxia and -6.7% in hypoxia; EMG<sub>RMS</sub> :-7.6% in normoxia and -15.7% in hypoxia). The authors concluded that muscle recruitment and performance were regulated by the central nervous system to restrict the peripheral fatigue generated. However, only systemic hypoxia was used in that previous study. Accordingly, it is unknown if the fatigue etiology would be different depending on local and/or systemic hypoxic stresses.

Peripheral and central fatigue are known to both be partly accountable for the decrease of MVC force after leg cycling sprints (Girard et al. 2011a) and arm cycling sprints (Pearcey et al. 2016). The present study showed a similar decrease in Db10 from PRE to POST (-29.8% in NOR) in line with previous results of Pearcey et al. (2016) in normoxia in the same muscle group (-27.6% for peak twitch with a 5s delay between the end of the sprint and the measurements). However, this decrease in Db10 was exacerbated in the present BFR conditions. Also, sarcolemma excitability was well preserved in NOR and HYP conditions while Mmax decreased when exercise was performed with BFR. Together, these observations underline a BFR-induced specific fatigue etiology through challenging peripheral perturbations. A decrease in Db10 suggests that peripheral fatigue occurred at and/or beyond the sarcolemma and could partly relate to impairments in the muscle excitation-contraction coupling potentially due to an intracellular accumulation of H<sup>+</sup> (Bishop et al. 2004) although debated (Allen et al. 2008), a reduction in blood lactate removal (Thomas 2004) and a Phosphocreatine breakdown (Glaister 2005). The known effect of BFR developing an accumulation of muscle metabolites (Loenneke et al. 2011), which is strongly related to the onset of peripheral fatigue (Kirkendall 1990) could explain the higher decrease of  $\Delta$ Db10 under BFR conditions. In the literature, Mmax impairment is explained by the change in ion concentrations across the muscle membrane. During ecological exercise, protecting mechanisms prevent a loss of excitability (refer to Allen et al. [2008] for a review

of the mechanisms involved). However, the use of BFR is known to induce muscular damages (Umbel et al. 2009) leading to sarcolemmal permeability (Wernbom et al. 2012) and therefore could imbalance the ion distribution across the sarcolemma explaining the diminution of  $M_{max}$ . The link between sarcolemmal excitability and sarcolemmal permeability has been previously reported during exercise (Piitulainen et al. 2008). It is likely that BFR impact peripheral factors at rest due to tissue deoxygenation but also because of an impairment of the metabolite's removal *per se* during exercise.

As a global index of central fatigue, the present study showed a decrease of  $VA_{TMS}$  after exhaustive dynamic exercise of the elbow flexors in normoxia without BFR (refer to ESM II for details). Comparable  $VA_{TMS}$  decrements were observed whatever the condition. Exercising with hypoxia can lead to cerebral perturbation and exacerbated central fatigue (Goodall et al. 2010). It looks like the present experimental conditions were not constraining enough to aggravate the diminution of VA measurements in comparison to normoxia. The reason might be the relatively moderate systemic hypoxic stimulus used ( $FiO_2=0.13$ ) compared to other studies (e.g. Goodall et al. [2010],  $FiO_2=0.10$ ) and the moderate muscle mass involved in the task (i.e. arms vs. legs) preventing  $SpO_2$  to decrease towards critical values for the central nervous system (e.g. 85-90% vs ~75-80% for Goodall et al. [2010] to fully activate motoneurons). Hence, with higher systemic constraints, these authors reported an exacerbated central fatigue with less peripheral fatigue for a similar decrease in MVC (i.e. modified fatigue etiology compared to normoxia) what could, however, explain the tendency of *altitude* to limit the decrease in  $\Delta Db_{10}$  force in the current study.

MEP amplitude in the BB tended to increase from PRE to POST aRSA test in normoxic conditions at 100, 75 or 50% of the MVC. Pearcey et al. (2016) showed that during the first 5 sprints, MEP amplitude decreased before returning to resting values after the 10<sup>th</sup> sprint. However, participants stopped the exercise before exhaustion maybe preventing them to increase MEP value even higher than the resting values. In another study (Fernandez-del-Olmo et al. 2013) MEP amplitude at 75 and 50% of MVC increased after a set of two 30-s Wingate test. It has been hypothesized that the increase in MEP could be an adaptation from the corticospinal pathway to compensate for a decrease in force production due to peripheral fatigue (Gruet et al. 2013). In hypoxia, MEP did not increase as in normoxia. This indicates lower exercise-induced excitability of the motor cortex in hypoxia compared to normoxia and potentially incapacity of the corticospinal pathway to adapt as in normoxia. Similar results were observed for  $\Delta EMG_{RMS}$  during MVC in the BB with lower increases in hypoxia compared to normoxia potentially indicating an incapacity of the central nervous system to adapt fully

during acute hypoxic exposure. Those results can be related to the effect of hypoxia on TSI<sub>PFC</sub> leading to lower cerebral oxygenation levels and altered corticospinal excitability (although these perturbations are not marked enough to impact more global central indices such as VA). In the present study, BFR did not induce any measurable central adaptations in comparison to without BFR as observed by Brandner et al. (2015), who mentioned the potential impact of BFR on III and IV afferents that would modulate corticospinal excitability. This might be explained by the relatively low pressure used to elicit BFR and the specificity of the exercise (as discussed above in the “Oxygen saturation indices” section) therefore limiting the impact of BFR on sensory feedback. No differences were found regarding the impact of an aRSA test performed with or without *altitude* and *occlusion* on SICI results, suggesting that neither hypoxia and/or BFR would impact directly intracortical inhibition through GABA<sub>A</sub> receptors activity. In accordance with previous studies measuring neuromuscular parameters after locomotive tasks in normoxia (Jubeau et al. 2014), CSP was not lengthened after aRSA test either by *occlusion* or *altitude* suggesting that intracortical inhibition through GABA<sub>B</sub> receptors activity was not impacted at exhaustion. Despite a moderate specific impact of *altitude* on fatigue etiology, evidence converges to consider that exercising within hypoxia rather than normoxia or BFR challenges predominantly central parameters and it can be hypothesized that this effect would be exacerbated with lower FiO<sub>2</sub> level than in the present study.

### Methodological considerations

As a limitation, the neuromuscular function was not able to be assessed immediately at the end of the aRSA tests. We must acknowledge that this could have induced some underestimation regarding both the peripheral (Froyd et al. 2013) and central parameters of fatigue (Mira et al. 2017). Further research should definitively explore the time-course of recovery of the neuromuscular function after high-intensity exercise coupled with different hypoxic stressors. However, the time between exhaustion and post-exercise neuromuscular tests was strictly standardized to 2 min as previously used in the literature (Jubeau et al. 2017) to ensure similar testing conditions no matter the session.

## Conclusion

The number of sprints performed during an aRSA test and the decline in the ability to produce force after exercise decreases to a similar extent when exercise was performed under hypoxia and/or moderate BFR. However, fatigue etiology differs depending on hypoxic modalities. BFR specifically led to an impairment of the muscle excitation-contraction coupling probably due to immediate muscle deoxygenation and impairment in metabolites removal ability during exercise, whereas hypoxia predominantly impacted corticospinal excitability in line with lower levels of cerebral oxygenation, potentially indicating incapacity of the corticospinal pathway to adapt to fatigue as in normoxia. Further investigations are needed to understand the recovery time course of central and peripheral components and the potential exacerbated impact of higher systemic/localized hypoxic doses on fatigue etiology.

### List of electronic supplementary material

Electronic supplementary material is available in the Annex part.

ESM I. Effects of hypoxia and/or blood flow restriction (BFR) on elbow flexors, saturation index, and neuromuscular function at rest.pdf

ESM II. Effects of an arm-cycling repeated sprint ability test in normoxia without blood flow restriction on elbow flexors neuromuscular function and saturation indices.pdf

### Conflict of Interest

The authors declare that they have no conflict of interest.

### Author Contributions

GP.M., F.B., N.P., and T.R. designed the study methodology. A.P. and S.W. collected the data and analyzed the results. A.P., S.W., and T.R. drafted the article. All authors reviewed and revised the work. All authors reviewed the final article and approved it for submission.

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### 1.3 Synthesis

The purpose of this study was to investigate neuromuscular fatigue induced by an arm cycling repeated sprint exercise completed under different hypoxic condition (i.e. systemic and/or localized).

The number of sprints performed through the test and the overall amount of fatigue measured were similarly impaired by systemic and localized hypoxia, however distinct mechanisms of fatigue seemed to occur depending on the condition. The use of BFR exacerbated the peripheral origin of fatigue through an impairment of the EC coupling probably due to the accumulation of metabolite. Whereas systemic hypoxia impaired corticospinal excitability probably because of a direct hypoxic effect on the brain as shown by a decrease of cortex oxygen saturation both at rest and during exercise.

Some limits are inherent to this study as the level of hypoxia induced by BFR could not be normalized to the one obtained with systemic hypoxia. Therefore, one can wonder if the differences observed could arise from different levels of hypoxic stress induced in both conditions. Furthermore, the specific modalities used to induce localized hypoxia impaired both oxygen delivery to the muscle and venous return, leading to two potential mechanisms inducing an accumulation of metabolites. Because the literature regarding repeated sprint combined with BFR was limited, the percentage of BFR was based on previous recommendations for resistance strength training and therefore might not be optimal for repeated sprint exercise.

A second study published from the same experiment showed different vascular and oxygenation responses across hypoxic conditions. BFR increased vascular resistance and restricts vasodilation whereas hypoxia likely induces compensatory vasodilation in the working muscles (Willis et al. 2019).

Taken together, and despite the associated limits, those results support a direct effect of hypoxia on the brain that would impair the central component of fatigue during repeated sprint exercise. Nevertheless, further studies should confirm those results, notably by reducing the delay between the end of exercise and the fatigue measurements.

## **2 Study 2: A new ergometer to investigate neuromuscular fatigue**

### **2.1 Presentation**

One of the main objectives of this thesis was to develop an innovative ergometer based on the one validated by Doyle-Baker et al. (2017) to evaluate neuromuscular fatigue kinetics during a repeated sprint exercise without any delay.

As a “novel” way to assess the evolution of muscle capabilities during fatigue, recent studies have interpreted Torque-Velocity (T-V) relationship PRE and POST fatigue (Krüger et al. 2019).

Therefore, the objectives were to build and to evaluate the reliability of this ergometer. Two separate analysis were performed, one on the neuromuscular function parameters and another one on the T-V profile indices. The idea would be to separates both analysis into two distinct papers, because the introduction and methodological parts would be quite similar, in this thesis both have been combined into one entity. To not overload the result section, not all the results are presented.

## 2.2 The Study

### Introduction

Fatigue is commonly experienced especially when it comes to physical activities and is usually quantified as a diminution of maximal voluntary contraction (MVC). This study will focus on performance fatiguability, which will be defined as exercise-induced changes resulting in a force output that is less than anticipated for a given voluntary contraction or stimulation (Macintosh and Rassier 2002). Its origin may come from the contractile capabilities and/or the ability of the nervous system to activate the muscle involved in the task (Enoka and Duchateau 2016). Fatigue is therefore a multifactorial phenomenon as its origin depends on multiple causes. Furthermore, the specificity of the task is known to be determinant in the relative contribution of the different mechanisms to fatigue (Enoka and Stuart 1992). Intensity, duration of exercise, mode and speed of contraction are among the variables that can influence fatigue etiology.

A big part of the literature investigating neuromuscular function (NMF) after fatigue used single-joint contraction (either isometric or dynamic) as exhaustive task allowing quick assessment of the NMF after exercise termination. While being practical, this method is quite far from daily life physical activities where dynamic whole-body exercises are usually involved. In this exercise modalities, a greater muscle mass is recruited, and cardiorespiratory demands are greater compared to single-joint exercise (Sidhu et al. 2013). These differences are likely to exacerbate the importance of respiratory muscle work, core and brain temperature, cerebral and muscle oxygenation and therefore impacting fatigue etiology (Nybo and Nielsen 2001; Romer and Polkey 2008; Secher et al. 2008; Rasmussen et al. 2010). More specifically, Sidhu et al. (2012) showed that corticospinal responses after sustained cycling exercise are different from single-joint exercise emphasizing the importance of exercise modalities. Understanding the underlying mechanisms of fatigue during whole-body exercise would give precious information to the actual debate regarding what is responsible for exercise cessation.

Previous studies have performed NMF evaluation before and after physical activities like running (Millet et al. 2011), cycling (Amann et al. 2007) and even soccer (Brownstein et al. 2017) by transferring the subject to an instrumented chair. However, the necessary delay to transfer the subject from the bike/treadmill to the chair is often between one or two minutes

and unlikely faster than 30 s (Tomazin et al. 2017). This is rather problematic because fatigue is known to recover rapidly, especially after short duration exercise (i.e. within 15s Taylor et al. 2000). Therefore, there is a risk of underestimating the amount of fatigue and potentially misinterpreting its etiology. Recently, an innovative ergometer that allows the subject to cycle and to perform isometric contraction alternatively on the same device has been developed and validated for steady-state exercise (Doyle-Baker et al. 2017). This device enables proper NMF evaluation (including MVC and interpolated twitch technic, Shield and Zhou 2004) while limiting the delay from the end of exercise (< 5 s) and seems very much appropriate to assess NMF during repeated supramaximal exercise [i.g. repeated sprints exercise (RSE)].

RSE is characterized by the succession of short maximal exercise (i.e. short enough to allow the subject to maintain maximal power output through the whole sprint; often establish as <10 s) interspaced with incomplete (<60 s) recovery (Girard et al. 2011a). This type of exercise has been recently investigated by researchers but a limitation present through all the studies is the inherent delay to switch from the sprint ergometer to the NMF one (Tomazin et al. 2017). Another recent approach is to use the sprint itself to quantify fatigue in a more adapted way that what's usually done (i.e. mean or peak power).

Lower limbs dynamic force production capabilities can be well described using linear and polynomial relationships that are obtained between torque and velocity [torque-velocity (T-V) relationship] and between power output and velocity [power-velocity (P-V) relationship], respectively (Samozino et al. 2007). This relation can be obtained using different exercise modality including on a stationary cycle ergometer (Arsac et al. 1996). From the two extremums of the T-V relationship, the force production capacities at high and low velocities are represented by the maximal theoretical torque produced at null velocity ( $T_0$ ) and the maximal theoretical angular velocity produced under zero load ( $V_0$ ) (Samozino et al. 2007). Those characteristics (i.e.  $T_0$ ,  $V_0$ ) can be used to evaluate the potential distinct impact of fatigue on force and/or velocity capabilities of the muscle.

Recently, (Krüger et al. 2019), used this methodology to characterized fatigue pre- and post-different exercise-modalities (a 30 s all-out exercise, a 10 min cycling exercise at severe intensity and a 90 min cycling exercise at moderate intensity) and compared it to the classical isometric method (i.e. MVC decrement). They reported distinct evolution patterns as for example  $P_{max}$  decreased more than MVC after the all-out exercise whereas it decreased less than MVC when considering the 10- and 90-min exercise. This is to be related to the study from (Monks et al. 2017), presented in the literature review and showing that by

modulating the recovery time, the repeated sprint performance (i.e. power output decrement) wasn't associated to any change in neuromuscular fatigue assessed via MVC. This suggests that the results obtained from isometric contraction can't characterize the dynamic function of the muscle.

Therefore, the purpose of this study was to validate intra- and inter-day reliability of neuromuscular function and force-velocity profile obtained from RSE performed on a similar cyclo-ergometer as the one presented by (Doyle-Baker et al. 2017).

## Methods

### Participants

Thirteen subjects (age:  $27 \pm 5$  years old, weight:  $66 \pm 8$  kg, height:  $175 \pm 9$  cm) gave written informed consent after being informed of the procedures and risks involved in the current study. Subjects were physically active (at least 2 training sessions per week) and did not present any pathology. Participants were asked to refrain from intense physical activity 24 hours before testing and to refrain from having a meal or drinking caffeinated beverages in the 2 h preceding the tests due to potential effects on exercise responses. This study was conducted according to the seventh revision of the Declaration of Helsinki (2013) and approved by the local ethics committee.

### Study design

A preliminary session was first conducted to familiarize participants with sprint exercise and NMF evaluation procedures on the specific cyclo-ergometer.

At least 48 hours after the familiarization, two experimental sessions (3 to 4 days apart) were performed for each subject at a similar time of the day and under standard environmental conditions in the laboratory. At the beginning of each session (an overview is provided in figure 1), electrical nerve stimulation (ENS) intensity was determined to allow the knee extensors of the right leg to be investigated using the interpolated twitch technic. During the first part, participants then performed a standardized warm-up consisting in the repetitions of 4 blocks of isometric contractions (intensity increasing from 50N to 70% of estimated MVC) interspaced with cycling (power ranking from 75 to 125W). They finished the warm-up with 2

MVC. After a 5 min recovery period, participants performed the NMF evaluation twice. And after another 5 min recovery period, participants performed the torque-velocity profile, which consisted of the realization of 2 maximal sprints at different torque factors (i.e.  $0.4 \text{ N}\cdot\text{kg}^{-1}$  and  $0.8 \text{ N}\cdot\text{kg}^{-1}$ ). After a subsequent 50 min recovery period, participants performed the second part consisting of the same procedure as previously described (i.e. Warm-up, MVC, NMF evaluation and T-V profile) to measure intra-day reliability. After a 10 min recovery period following the T-V profile, participants performed the RSE consisting of five 10 s sprint with 20 s of passive recovery. NMF evaluation was performed before (PRE), immediately after sprint 2 (INT) and sprint 5 (POST), and 2 minutes after the end of the last sprint (POST2). Participants took part in a second session corresponding to the second part of the first session (i.e. after the 50 min break) to obtain inter-day reliability of the torque-velocity profile, the NMF evaluation at rest and during the RSE. EMG signals of the vastus lateralis (VL) and the rectus femoris (RF) muscles were measured continuously throughout the sessions.

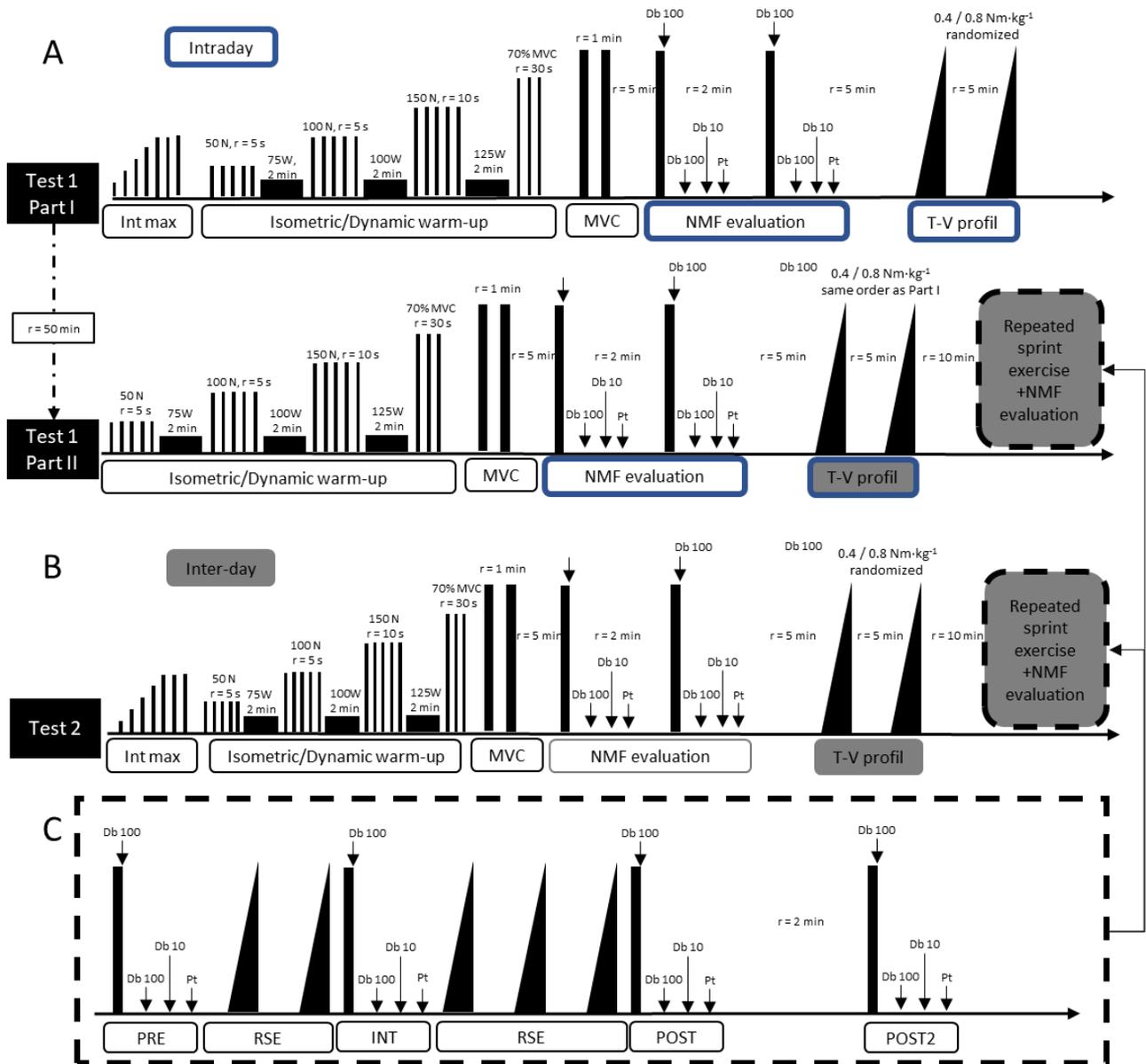


Figure 1. Overview of the study design. Session one and two are respectively shown in panel A and B. Panel C represent the neuromuscular function (NMF) evaluation. Int max: maximal intensity for electrical nerve stimulation, MVC: Maximal voluntary contraction, Db100: paired-pulse electrical nerve stimulation at 100 Hz, Db10: paired-pulse electrical nerve stimulation at 10 Hz, Pt: single electrical nerve stimulation, T-V: torque-velocity relationship, RSE: repeated sprint exercise.

## Experimental session

### *EMG and intensities assessment*

EMG signals of the VL and RF muscles were recorded during the whole session with two pairs of bipolar silver chloride surface electrodes (10-mm diameter, Type 0601000402, Contrôle Graphique, France), the reference electrode being placed on the patella. The recording electrodes were secured lengthwise to the skin over the muscle belly following SENIAM recommendations, with an inter-electrode distance of 20 mm. A low resistance (<5 k $\Omega$ ) between the two electrodes was verified after shaving, gently abrading the skin and cleaning it with isopropyl alcohol. Electrode positions were marked using a permanent marker during the first session to ensure a reproducible position through subsequent visits. EMG signals were amplified, analog-to-digital converted, band-pass filtered (10-500 Hz, input impedance = 200 M $\Omega$ , common mode rejection ratio = 85 dB, gain = 1000) and recorded at a sampling rate of 2 kHz using BioAmp (octal bio-amplifier ML138, ADInstruments, Bella Vista, Australia) and PowerLab system (16/35, ADInstruments). Data were stored and analyzed offline with LabChart 8 software (ADInstruments).

Square-wave pulses of 100  $\mu$ s duration were delivered via high-voltage constant-current stimulators (DS7A; Digitimer, Hertfordshire, UK) percutaneously to the femoral nerve via a 20-mm diameter cathode electrode (Ag-AgCl, Contrôle Graphique Medical, Brie-Comte-Robert, France) pressed into the inguinal crease and a 50 $\times$ 90-mm anode gel pad electrode (Durastick Plus, DJO Global, Vista, USA) placed on the gluteal fold. ENS intensity started at 10 mA and was incremented by step of 10 mA every 5 seconds up to the concomitant reach of a plateau for force and M-wave amplitude (both VL and RF). Stimulation intensity corresponding at 120% of the one at the beginning of the plateau was used thereafter.

Single and paired-pulse ENS at 10 and 100 Hz were used to obtain different neuromuscular parameters details below (respectively Pt, Db10 and Db100).

### *The innovative ergometer*

The ergometer (figure 2) was built from a recumbent bike linked to an electromagnetically braked Velotron system (Racermate Inc, Seattle, WA) and powered by Wingate Velotron Software (Racermate Inc). The crank and the right pedal were lockable via automated

motorized units piloted by a customized software created using LabVIEW (LabVIEW, National Instrument, Austin, Texas, USA). Both seat height and length were adjustable permitting an optimal cycling position and an approximately 90 degrees angle for the ankle, knee, and hips in the locked position (in this position, crankset were parallel to the ground). To limit trunk movements during the experiments, participants were secured to the bike via a racing seat belt. Instrumented pedals (I-crankset, Sensix, Poitiers, France) recorded the angle position of the pedals as well as raw forces and moments in the three dimensions (400Hz). Force in the x-axis of the right pedal was sampled at 200 Hz and sent to PowerLab system (16/35, ADInstruments) for synchronization with EMG signals via a National Instruments card (National Instruments, Austin, Texas, USA). Power was instantaneously calculated from the pedals and display in the customized software.

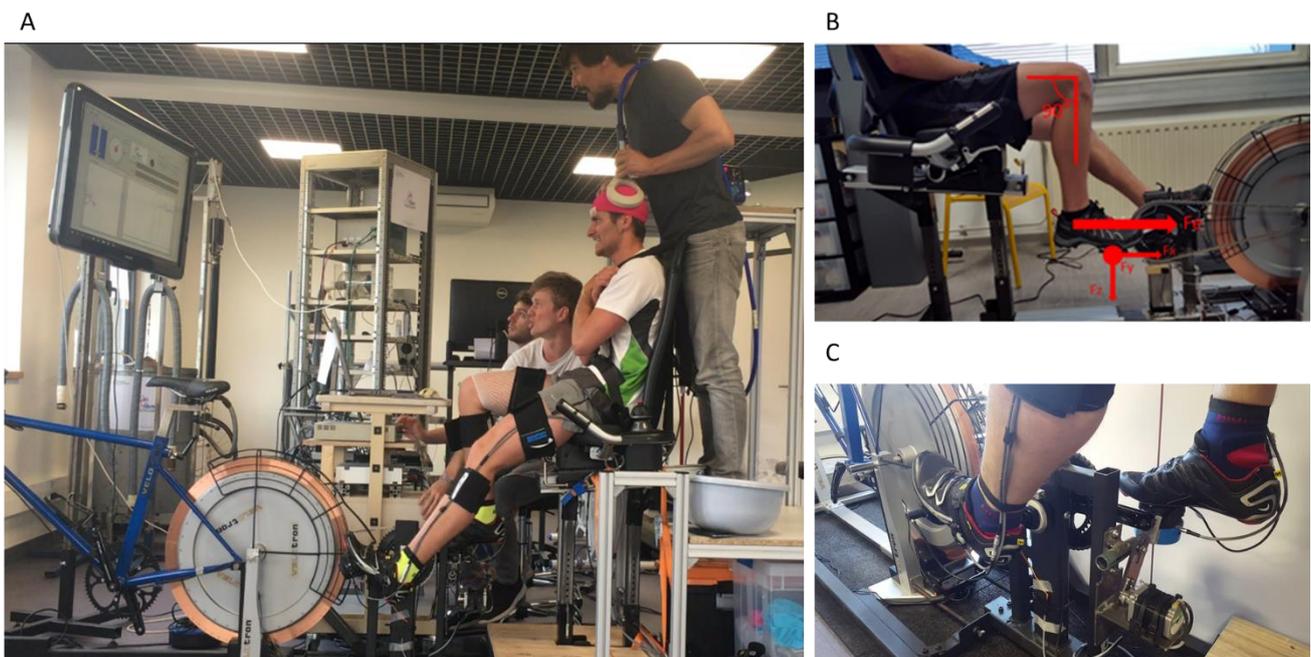


Figure 2. Pictures of the specific ergometer build from a recumbent bike and a velotron flywheel (Racermate Inc). Panel A represents an overview of the experimental set up in the laboratory environment. A screen allows the display of visual feedback to the subject. Panel B shows the participant position when the system is locked, the big red arrow represents the effective force measured during MVC whereas the mark provides the different force axis measured by the pedal ( $F_x$ ,  $F_y$ , and  $F_z$ ) and used to calculate the power. Panel C shows a closer view of the locking system. A short video is provided as supplementary material.

### *Force measurement*

This design allowed the participants to performed voluntary isometric knee extension within 5 seconds after the end of a cycling task. Crank and pedals were locked such as the participant's right leg was in a position where ankle, knee, and hips formed approximately a 90° angle (figure 2B). From this position, it was asked the participant to produce maximal force parallel to the ground for 3 to 5 s. The assignment given to the subject was “to push their foot forward” to maximize the x-axis force (parallel to the ground) and preferentially recruit the knee extensors muscles. A screen was displayed in front of the participant with real-time visual feedback including force production and orientation and the possibility to adjust guidelines for submaximal contractions. A video of the bike utilization will be associated with this manuscript. The isometric force was analyzed offline using LabChart 8 software (ADInstruments).

### *Power measurement*

Friction force was applied and maintained constant by the electromagnetic brake of the velotron system controlled by the Wingate software (Racermate Inc). Torque in the crankset referential was calculated from raw forces and angular position obtained from the pedals. Instantaneous power was therefore determined as the efficient torque multiply by the angular velocity. Mean power was automatically calculated at the end of each sprint to have visual feedback on real-time performance decrement.

From the instantaneous power, data were cut and averaged downstroke per downstroke automatically allowing to export, instantaneous power (at 400Hz) and averaged power, speed, and torque for each downstroke.

## Measurements

### *Warm-up and MVC*

The warm-up consisted of a succession of isometric contraction interspaced with pedaling. It had both the objectives to get the participant ready to produce maximal exercise but also to

check that he was comfortable with the locking/unlocking process. The entire warm-up is presented in figure 1.

Two MVC were performed after the warm-up without any superimposed stimulation and served as a reference for the NMF evaluation to ensure that participants didn't refrain from producing maximal force due to the expectation of the stimulation.

#### *Neuromuscular function evaluation*

After 5 min of recovery, participants were asked to perform MVC, after they plateaued at their maximal force, a superimposed paired-pulse ENS at 100Hz was delivered. Then, in relaxed muscles, Db100 was delivered 2 s after the end of the MVC followed by Db10 and Pt (with a 2 s delay between each) to assess knee extensors contractile properties and sarcolemma excitability (figure 1C). Strong verbal encouragements were given to the subject to ensure maximal force production during MVC. This testing procedure lasted ~15s.

#### *Torque-velocity profile.*

Friction force was applied through the sprint by the electromagnetic brake of the velotron system controlled by the Wingate software (Racermate Inc). Two sprints were performed at different resistances (i.e. low:  $0.4 \text{ N}\cdot\text{kg}^{-1}$ , and high:  $0.8 \text{ N}\cdot\text{kg}^{-1}$ ) to widen the range of point and improve the relationship. Average torque and velocity per downstroke during the acceleration phase were plotted into T-V and P-V relationship using least-square linear and second-order polynomial regression, respectively (Samozino et al. 2007).

#### *Repeated sprint exercise*

The RSE test started after a 10 min resting period following the NMF evaluation. It consisted of 5 repetitions of 10-s sprints interspaced with 20 s of passive recovery. Participants were instructed to go "as hard as you can" from the start of every sprint. The mean power of the first sprint of the RSE test had to reach at least 95% of the isolated sprint performed during the Torque-velocity profile (at  $0.8 \text{ N}\cdot\text{kg}^{-1}$ ) to limit any pacing strategies. During each sprint,

the cycloergometer was set in 'Wingate' mode and the torque factor (i.e. resistance) was set at  $0.8 \text{ N}\cdot\text{kg}^{-1}$ . Strong verbal encouragements were given during the sprints to promote maximal efforts.

Almost instantaneously (0.5 s) after the 2nd and last sprints, the crank and the right pedal were locked automatically to allow participants to perform their MVC. To prevent them to arrive too rapidly in the locking system, a countdown was given 3 s before the end of each sprint. The neuromuscular evaluation took 15 s to be completed, as a safety margin and to compensate for the 5-s MVC, recovery time was increased to 25 s between sprint 2 and 3.

### Data analysis

MVC force was measured during each NMF evaluation as the maximal force before any ENS stimulation. Force amplitude for Db100, Db10, and Pt was determined as the maximal force measured after ENS in relaxed muscle after the end of MVC. The ratio between Db10 and Db100 (LF/HF) was calculated as an index of low-frequency fatigue (Verges et al. 2008). The amplitude of the M-wave was measured peak to peak from the superimposed single pulse ENS delivered in the relaxed muscles ~6 s after the contraction ( $M_{\text{max}}$ ). The duration of the M-wave was measured as the time from peak to peak ( $M_{\text{dur}}$ ). EMG root mean square was calculated for both vastus lateralis ( $\text{RMS}_{\text{VL}}$ ) and rectus femoris ( $\text{RMS}_{\text{RF}}$ ) during MVC over a 500-ms period before ENS delivery and normalized using  $M_{\text{max}}$  of the respective muscle ( $M_{\text{maxVL}}$  and  $M_{\text{maxRF}}$ ). Voluntary activation (VA) was calculated using the formula from Strojnik and Komi (1998):

$$\text{VA (\%)} = [1 - \text{SIT (F}_{\text{base}}/\text{MVC})/\text{Db100}] \times 100$$

Where SIT is the superimposed force induced by the paired-pulse stimulation during the MVC and  $F_{\text{base}}$  is the force right before the stimulation.

$T_0$  was determined as the intercept of the T-V relationship in the force axis and  $V_0$  as the intercept of the T-V relationship in the velocity axis (Samozino et al. 2007). Both values were determined either by combining the sprints at low and high resistances ( $T_{0c}$  and  $V_{0c}$ ) or from a single sprint at high resistance only ( $T_{0.8}$  and  $V_{0.8}$ ).  $P_{\text{mean}}$  was determined as the mean

power obtained during the sprint at high resistance. Pmax was determined as the most powerful downstroke during the sprint at high resistance.

During RSE, Pmean of each sprint were averaged (Smean) and the best overall sprint power (Sbest) was determined. Decremental score (Sdec) was calculated using the equation provided by (Girard et al. 2011a):

$$Sdec (\%) = (1 - Smean/Sbest) \times 100$$

Total work (Wtot) was calculated as the sum of the work obtained from each sprint during the RSE.

For the neuromuscular function evaluation indices, intraday reliability was determined by comparing the average values obtained during test 1 before and after the 50 min break. Inter-days and inter-sprints reliability were determined by comparing the values from the RSE.

For the biomechanical indices, intraday reliability was determined by comparing the values obtained during test 1 before and after the 50 min break and inter-days reliability was calculated from test 1 and test 2 isolated T-V relationship. Inter-sprints reliability was determined by comparing the RSE performed on test 1 and test 2. An overview is provided in figure 1.

### Statistical analysis

All data are reported as mean  $\pm$  SD. Normal distribution was assessed with the Shapiro-Wilk's test. A two-way repeated measures ANOVA (*exercise*  $\times$  *session*) was used to compare the values obtained from the two RSE at different time points (i.e. PRE, INT, POST, POST2 for neuromuscular parameters and during each sprint for the biomechanical indices). Effect sizes are reported as partial eta-squared ( $\eta_p^2$ ) for ANOVAs. Post-hoc Tukey's tests were applied to determine a difference if the ANOVA revealed a significant main or interaction effect. As recommended by Hopkins (2000), relative reliability was assessed with a mixed-model intra-class correlation coefficient (ICC) at the 95% confidence level. An ICC  $>$  0.75 represents excellent reliability and from 0.60 to 0.74 represents good reliability. ICC was not calculated for voluntary activation due to a ceiling effect, which occurs when there is little variability in the scores of all subjects as they approach the maximum possible value (Todd et al. 2004). Absolute reliability was determined through typical error expressed as

coefficients of variation (CV)  $\pm$  95% confidence intervals (CI; Hopkins 2000). Both ICCs and CVs were calculated using log-transformed data in the online spreadsheet created by Hopkins (Hopkins 2015). Statistical significance was set at  $P < 0.05$  for all tests, and all ANOVAs were completed on Statistica v10 (Statsoft, Tulsa, OK).

Because a participant broke the left pedal during his 2<sup>nd</sup> session, only ten people (including him) completed both sessions. It was possible to get the value from the right pedal to obtain the force responses to electrical stimulation, but it wasn't possible to compute the power. Therefore, intraday reliability of the neuromuscular function evaluation is determined from 13 participants, whereas, inter-day reliability is calculated from 10 participants. All the power indices have been calculated on 9 participants.

## Results

### Neuromuscular parameters

#### *Intraday reliability*

Table 1 provides the results of intraday reliability for all neuromuscular parameters including the change in mean between test 1 part I and test 1 part II. All the indices present excellent reliability with ICCs all above 0.92 (i.e. LF/HF) and CVs under 6.9% (i.e. RMS<sub>VL</sub>).

Table 1. Intraday reliability of the neuromuscular indices measured on the new cycloergometer.

	Test 1 part I Mean ± SD	Test 1 part II Mean ± SD	% change in mean (95% CI)	CV (95% CI)	ICC (95% CI)
<b>MVC (N)</b>	275 ± 70	264 ± 52	-2.3 (-7.4; 2.9)	6.4 (4.5; 10.7)	0.96 (0.87; 0.99)
<b>Db100 (N)</b>	148 ± 29	144 ± 31	-2.5 (-5.8; 0.8)	4.0 (2.9; 6.7)	0.97 (0.91; 0.99)
<b>Db10 (N)</b>	121 ± 25	112 ± 22	-7.0 (-10.2; -3.7)	4.2 (3.0; 7.0)	0.97 (0.89; 0.99)
<b>Pt (N)</b>	91 ± 19	87 ± 17	-4.8 (-9.1; -0.3)	5.6 (4.0; 9.3)	0.95 (0.86; 0.99)
<b>VA (%)</b>	98.6 ± 0.9	97.4 ± 2.9	-1.3 (-3.2; 0.6)	2.3 (1.6; 3.8)	
<b>LF/HF (%)</b>	82 ± 11	79 ± 10	-4.6 (-8.0; -1.0)	4.4 (3.1; 7.3)	0.92 (0.75; 0.97)
<b>RMS<sub>VL</sub> (%Mmax)</b>	78 ± 32	76 ± 29	-1.6 (-7.0; 4.2)	6.9 (4.9; 11.7)	0.97 (0.91; 0.99)
<b>RMS<sub>RF</sub> (%Mmax)</b>	58 ± 0.13	60 ± 12	2.7 (-2.2; 7.8)	5.9 (4.2; 9.9)	0.94 (0.82; 0.98)
<b>MmaxVL (mV)</b>	13.2 ± 2.5	12.7 ± 1.9	-3.3 (-6.6; 0.3)	4.3 (3.0; 7.1)	0.95 (0.84; 0.98)
<b>MmaxRF (mV)</b>	7.3 ± 1.3	6.9 ± 1.4	-5.0 (-8.6; -1.2)	4.7 (3.3; 7.8)	0.95 (0.86; 0.99)
<b>MdurVL (ms)</b>	7.98 ± 1.72	8.19 ± 1.63	3.0 (-1.1; 7.4)	4.9 (3.5; 8.3)	0.95 (0.85; 0.99)
<b>MdurRF (ms)</b>	9.75 ± 2.56	9.94 ± 2.55	4.8 (1.3; 8.5)	3.9 (2.8; 6.8)	0.98 (0.93; 0.99)

Values are presented as mean ± SD; CI: confidence interval; MVC: maximal voluntary contraction force; Db100: force evoked by a 100Hz doublets; Db10: force evoked by a 10Hz doublets; Pt: force evoked by a single stimulation; VA: voluntary activation assess by the interpolated twitch technique; LH/HF: ratio between Db10/Db100; VL: Vastus lateralis; RF: rectus femoris; RMS: root mean square; Mmax: potentiated M-wave; Mdur: duration of the Mmax.

*Inter-days reliability*

The time between the end of a sprint and the beginning of the neuromuscular fatigue evaluation (i.e. MVC) was  $4.6 \pm 1.5$  s.

The Anova didn't detect a *session* main effect for any of the variables (e.g. VA:  $F_{(1,9)}=0.07$ ,  $P=0.53$ ; Pt:  $F_{(1,9)}=0.42$ ,  $P=0.53$ ).

All variables but Mmax ( $F_{(3,24)}=0.72$ ,  $P=0.55$ ) and VA ( $F_{(3,27)}=1.70$ ,  $P=0.18$ ) presented a main effect of *exercise* and decreased over time. MVC:  $F_{(3,27)}=19.25$ ,  $P<0.01$ ,  $\eta_p^2=0.68$ ; Db100:  $F_{(3,27)}=25.25$ ,  $P<0.01$ ,  $\eta_p^2=0.74$ ; Db10:  $F_{(3,27)}=14.89$ ,  $P<0.01$ ,  $\eta_p^2=0.62$ ; Pt:  $F_{(3,27)}=16.80$ ,  $P<0.01$ ,  $\eta_p^2=0.65$ ; LF/HF:  $F_{(3,27)}=4.13$ ,  $P=0.016$ ,  $\eta_p^2=0.31$ . Results are presented in figure 3. No interactions *exercise*  $\times$  *session* were significant.

Table 2 provides the results of inter-days reliability for PRE and POST values only (not to overload the paper), including the change in mean between test 1 and test 2. overall the indices present excellent reliability with ICCs above 0.75 except for LF/HF [ICC PRE: 0.74 (0.26; 0.93) and POST: 0.83 (0.42; 0.96)]. CVs tend to be lower for PRE compared to POST measurement [e.g. CV for Db100: PRE, 7.1% (4.9; 13.4); POST, 13.5% (8.9; 27.5)].

Table 2. Inter-days reliability of the neuromuscular indices measured during a repeated sprint exercise on the new cycloergometer.

		Test 1 Mean $\pm$ SD	Test 2 Mean $\pm$ SD	% change in mean (95% CI)	CV (95% CI)	ICC (95% CI)
<b>MVC</b>	PRE	278 $\pm$ 38	287 $\pm$ 57	2.0 (-3.7; 8.0)	5.8 (4.0; 10.9)	0.92 (0.72; 0.98)
	(N) POST	224 $\pm$ 29	237 $\pm$ 46	4.9 (-3.6; 14.1)	8.7 (5.9; 16.4)	0.81 (0.40; 0.95)
<b>Db100</b>	PRE	130 $\pm$ 31	135 $\pm$ 32	3.6 (-3.4; 11.1)	7.1 (4.9; 13.4)	0.94 (0.79; 0.99)
	(N) POST	102 $\pm$ 26	113 $\pm$ 27	11.4 (-3.0; 27.8)	13.5 (8.9; 27.5)	0.82 (0.40; 0.96)
<b>Db10</b>	PRE	94 $\pm$ 18	97 $\pm$ 22	2.1 (-3.9; 8.4)	6.2 (4.2; 11.5)	0.95 (0.80; 0.99)
	(N) POST	71 $\pm$ 14	75 $\pm$ 18	5.1 (-6.8; 18.5)	11.7 (7.7; 23.6)	0.81 (0.36; 0.95)
<b>Pt</b>	PRE	69 $\pm$ 16	72 $\pm$ 20	2.2 (-9.0; 14.8)	12.1 (8.2; 23.3)	0.87 (0.56; 0.97)
	(N) POST	52 $\pm$ 10	55 $\pm$ 14	5.6 (-7.1; 20.0)	12.5 (8.3; 25.3)	0.80 (0.33; 0.95)
<b>LF/HF</b>	PRE	73.4 $\pm$ 9.5	72.5 $\pm$ 10.5	-1.5 (-8.4; 6.0)	7.5 (5.1; 14.1)	0.74 (0.26; 0.93)
	(%) POST	69.1 $\pm$ 9.9	65.6 $\pm$ 11.5	-5.6 (-13.1; 2.6)	8 (5.3; 15.8)	0.83 (0.42; 0.96)
<b>VA</b>	PRE	98.4 $\pm$ 1.4	98.5 $\pm$ 1.6	0.1 (-1.7; 2.0)	1.9 (1.3; 3.4)	
	(%) POST	99.0 $\pm$ 2.1	97.9 $\pm$ 2.4	-1.1 (-3.7; 1.7)	2.5 (1.7; 4.9)	
	PRE	13.3 $\pm$ 2.1	14.8 $\pm$ 3.4	10.3 (-0.2; 22.0)	10.4 (7.1; 19.8)	0.78 (0.33; 0.94)

<b>Mmax</b>						
<b>VL</b>	POST	13.5 ± 2.2	14.4 ± 3.1	6.2 (-0.4; 13.2)	6.6 (4.5; 12.3)	0.91 (0.69; 0.98)
<b>(mV)</b>						
<b>Mmax</b>	PRE	7.0 ± 1.5	7.5 ± 1.8	7.0 (-2.4; 17.3)	9.5 (6.5; 18.0)	0.89 (0.61; 0.97)
<b>RF</b>						
<b>(mV)</b>	POST	7.3 ± 1.5	7.7 ± 1.7	5.1 (-6.3; 17.9)	12.0 (8.1; 23.1)	0.81 (0.40; 0.95)

Values are presented as mean ± SD; CI: confidence interval; MVC: maximal voluntary contraction force; PRE: before the first sprint; POST: immediately after the 5<sup>th</sup> sprint; Db100: force evoked by a 100Hz doublets; Db10: force evoked by a 10Hz doublets; Pt: force evoked by a single stimulation; VA: voluntary activation assess by the interpolated twitch technique; LH/HF: ratio between Db10/Db100; VL: Vastus lateralis; RF: rectus femoris; RMS: root mean square; Mmax: potentiated M-wave.

## Biomechanical parameters

### *Intraday reliability*

Table 3 provides the results of intraday reliability of the biomechanical parameters including the change in mean between test 1 part I and test 1 part II. Only V0.8 has bad reliability as shown by an ICC of 0.42 (-0.29; 0.83).

Table 3. Intra- and inter-days reliability of the biomechanical indices measured during isolated sprints on the new cycloergometer.

	<b>Test 1 part I</b>	<b>Test 1 part II</b>	<b>% change in</b>	<b>CV (95% CI)</b>	<b>ICC (95% CI)</b>
	<b>Mean ± SD</b>	<b>Mean ± SD</b>	<b>mean (95% CI)</b>		
<b>Intraday</b>					
<b>Pmean (W)</b>	549 ± 81	542 ± 74	-1.2 (-6.6; 4.5)	5.3 (3.5; 10.4)	0.91 (0.65; 0.98)
<b>Pmax (W)</b>	733 ± 126	714 ± 129	-2.8 (-7.3; 1.9)	4.5 (3.0; 8.7)	0.96 (0.84; 0.99)
<b>T0<sub>c</sub> (Nm)</b>	92.8 ± 17.8	89.0 ± 18.1	-4.3 (-8.9; 0.6)	4.7 (3.1; 9.2)	0.96 (0.85; 0.99)
<b>V0<sub>c</sub> (rad.s<sup>-1</sup>)</b>	23.8 ± 1.7	24.0 ± 1.5	0.8 (-2.6; 4.4)	3.3 (2.2; 6.4)	0.83 (0.42; 0.96)
<b>T0.8 (Nm)</b>	94.3 ± 21	91.2 ± 18.6	-3.0 (-11.0; 5.7)	8.2 (5.5; 16.3)	0.90 (0.63; 0.98)
<b>V0.8 (rad.s<sup>-1</sup>)</b>	23.6 ± 2.2	23.0 ± 1.6	-2.4 (-9.2; 4.9)	6.9 (4.6; 13.5)	0.42 (-0.29; 0.83)
<b>Inter-days</b>					
	Test 1	Test 2			
<b>Pmean (W)</b>	549 ± 81	555 ± 72	1.3 (-4.5; 7.5)	5.6 (3.8; 11.0)	0.89 (0.59; 0.97)
<b>Pmax (W)</b>	714 ± 129	748 ± 123	5.1 (0.4; 10.1)	4.3 (2.9; 8.4)	0.96 (0.85; 0.99)
<b>T0<sub>c</sub> (Nm)</b>	92.8 ± 17.8	90.6 ± 20.5	-3.0 (-7.8; 2.0)	4.8 (3.2; 9.3)	0.97 (0.87; 0.99)

<b>V0c (rad.s<sup>-1</sup>)</b>	23.8 ± 1.7	24.2 ± 1.9	1.5 (-4.1; 7.4)	5.3 (3.6; 10.4)	0.58 (-0.08; 0.89)
<b>T0.8 (Nm)</b>	94.3 ± 21.0	91.7 ± 25.2	-3.8 (-11.9; 5)	8.4 (5.6; 16.7)	0.93 (0.71; 0.98)
<b>V0.8 (rad.s<sup>-1</sup>)</b>	23.6 ± 2.2	24.2 ± 2.9	2.5 (-5.3; 10.9)	7.5 (5.0; 14.9)	0.57 (-0.1; 0.88)

Values are presented as mean ± SD; CI: confidence interval; Pmean: mean power during the sprint; Pmax: most powerful downstroke; T0c: torque determined as the intercept of the torque-velocity relationship in the force axis (T0) for 2 sprints combined; V0c: velocity determined as the intercept of the torque-velocity relationship in the velocity axis (V0) for 2 sprints combined; T0.8: T0 obtained from a single sprint at a resistance of 0.8 N·kg<sup>-1</sup>; V0.8: V0 obtained from a single sprint at a resistance of 0.8 N·kg<sup>-1</sup>.

### *Inter-days reliability*

Inter-days reliability values of the biomechanical indices obtained on isolated sprints are provided in table 3. V0c and V0.8 present both poor relative reliability with ICC of respectively 0.58 (-0.08; 0.89) and 0.57 (-0.1; 0.88).

The Anova didn't detect a *session* main effect for any of the variables (e.g. Pmean:  $F_{(1,8)}=0.07$ ,  $P=0.79$ ; V0:  $F_{(1,8)}=0.02$ ,  $P=0.91$ ) or any interaction *exercise* × *session* (e.g. Pmax:  $F_{(1,8)}=0.07$ ,  $P=0.79$ ; V0.8:  $F_{(1,8)}=0.02$ ,  $P=0.91$ ).

All variables presented a main effect of *exercise* and decreased over time. Pmean:  $F_{(4,32)}=57.50$ ,  $P<0.01$ ,  $\eta_p^2=0.88$ ; Pmax:  $F_{(4,32)}=38.24$ ,  $P<0.01$ ,  $\eta_p^2=0.83$ ; V0.8:  $F_{(4,32)}=6.86$ ,  $P<0.01$ ,  $\eta_p^2=0.46$ ; T0.8:  $F_{(4,32)}=6.45$ ,  $P<0.01$ ,  $\eta_p^2=0.45$ . Figure 4 presents the evolution of the variables.

Inter-days reliability values of the biomechanical indices obtained during repeated sprints are provided in table 4. V0.8 and T0.8 present good relative reliability with ICC for the first sprint of respectively 0.73 (0.19; 0.93) and 0.80 (0.35; 0.95).

Table 4. Inter-days reliability of the biomechanical indices measured during a repeated sprint exercise on the new cycloergometer.

		<b>Test 1</b> <b>Mean ± SD</b>	<b>Test 2</b> <b>Mean ± SD</b>	<b>% change in</b> <b>mean (95% CI)</b>	<b>CV (95% CI)</b>	<b>ICC (95% CI)</b>
<b>Pmean</b> <b>(W)</b>	Sprint 1	540 ± 76	530 ± 69	-1.6 (-4.2; 1)	2.5 (1.7; 4.8)	0.98 (0.91; 1.0)
	Sprint 5	370 ± 38	371 ± 38	0.2 (-3.8; 4.4)	3.8 (2.5; 7.4)	0.90 (0.61; 0.98)
<b>Pmax</b> <b>(W)</b>	Sprint 1	700 ± 119	678 ± 110	-3 (-7.1; 1.3)	4.1 (2.7; 8.0)	0.96 (0.85; 0.99)
	Sprint 5	520 ± 102	492 ± 69	-4.8 (-13.6; 5.0)	8.6 (5.6; 18.3)	0.81 (0.31; 0.96)
<b>T0.8</b> <b>(Nm)</b>	Sprint 1	85.9 ± 18.1	83.4 ± 20.0	-3.5 (-15.5; 10.2)	13.0 (8.6; 26.3)	0.80 (0.35; 0.95)
	Sprint 5	73.2 ± 18.6	75.0 ± 13.8	3.8 (-8.1; 17.4)	11.9 (7.9; 24.1)	0.80 (0.33; 0.95)
<b>V0.8</b> <b>(rad.s<sup>-1</sup>)</b>	Sprint 1	24.5 ± 5.1	24.4 ± 3.6	0.4 (-9.5; 11.4)	10.0 (6.7; 20.1)	0.73 (0.19; 0.93)
	Sprint 5	20.4 ± 4	20.6 ± 3.5	1.6 (-8.6; 12.9)	10.2 (6.8; 20.5)	0.76 (0.26; 0.94)
<b>Wtot</b> <b>(kJ)</b>	-	22.0 ± 2.3	22.1 ± 2.3	0.5 (-2.9; 4.0)	3.2 (2.2; 6.3)	0.93 (0.73; 0.98)
<b>Sdec</b> <b>(%)</b>	-	17.9 ± 4.4	16.2 ± 4.2	-9.4 (-21.2; 4.2)	13.7 (9.1; 28.0)	0.81 (0.36; 0.95)

Values are presented as mean ± SD; CI: confidence interval; Pmean: mean power during the sprint; Pmax: most powerful downstroke; T0.8: torque determined as the intercept of the torque-velocity relationship in the force axis; V0.8: velocity determined as the intercept of the torque-velocity relationship in the velocity axis; Wtot: total work performed during the 5 × 10-s sprints; Sdec: decrement score calculated from Pmean over the sprint repetition.

## Discussion

The purpose of this study was to validate the reliability of a new ergometer builds to investigate neuromuscular fatigue during a repeated sprint exercise. Two different approaches were combined to characterize fatigue, a classical neuromuscular function evaluation using isometric MVC and the interpolated twitch technique to determine fatigue etiology and a biomechanical description of fatigue using muscle capabilities determined directly from dynamic exercise (i.e. the sprint itself). Overall, the reliability of the fatigue indices is excellent intraday (i.e. all ICC > 0.90 and CV < 7%) and very good inter-days (i.e. ICC ranging from 0.74 to 0.95 and CV from 1.9 to 13.5%). Only V0 provides poor reliability and will be discussed later.

During repeated sprint exercise, mean power output decreased as soon as the second sprint and neuromuscular fatigue was detected from the first measure (i.e. MVC decreased after the second sprint) proving that this ergometer is a reliable and valid tool to investigate fatigue.

### The ergometer

It is important to keep in mind the task dependency of fatigue, depending on the exercise modality, the fatigue induced can have different etiology and characteristics. Yet, most of the studies investigating fatigue induced by whole-body exercise (e.g. running, Goodall et al. 2014a) use isometric contraction because it is possible to combine it with the interpolated twitch technique to evaluate fatigue etiology. However, the relationship between isometric and dynamic force production isn't that clear, indeed, Krüger et al. (2019) compared the fatigue induced by different dynamic exercise and their impact on both isometric and dynamic force production. They reported distinct fatigue responses notably when considering high-intensity exercise (i.e. 30-s all-out exercise) where the decrease in power output was more pronounced than the decrease in MVC, suggesting that fatigue could be underestimated if only based on isometric measurement. Furthermore, whole body exercise often required the use of different large muscle group (Dorel et al. 2012) whereas isometric force is usually obtained by isolating one, which could partly explain the differences observed in power diminution compared to MVC. However, it is not yet possible to estimate fatigue etiology from dynamic contractions. Therefore, dynamic and isometric measurements seem to be

complementary and should both be used to describe fatigue mechanisms induced by the dynamic task.

But this raises the question of time delay and experimental setup as it has been shown that fatigue recovers quickly and at a different rate depending on its etiology and/or amount (Herbert and Gandevia 1999; Froyd et al. 2013). Indeed, as the fatigue and evaluation task are not performed on the same device, it induces an inherent time delay for switching to one another which result in a “recovery” period making difficult to obtain kinetics of fatigue development. To avoid this, researchers elaborate complex experimental setup. For instance, Hureau et al. (2016) had participants performed RSE on 5 different days (i.e. 1, 4, 6, 8, or 10 sprints) with NMF evaluation before and after each session to be able to build a kinetics from it. Krüger et al. (2019) had to use two sessions to compare one modality of exercise (i.e. one session to evaluate the impact of fatigue on dynamic contractions and another one to evaluate the same exercise on isometric contraction). While it is doable (but not optimal) to obtain kinetics under one specific condition, it becomes much harder if one wants to compare fatigue etiology in different environmental conditions for example.

With this ergometer, it is possible to assess fatigue using both dynamic parameters ( $T_0$  and  $V_0$  obtained from the T-V profile determined during a sprint) and indices obtained from isometric MVC and the use of superimposed electrical stimulation (i.e. Pt, Db100...). It is therefore possible to obtain complete kinetics of fatigue in a single session.

### Measurement reliability

Doyle-Baker et al. (2017) previously validated a similar ergometer allowing reliable inter-days measurement of NMF during incremental exercise. The results presented in this study are similar with very good to excellent reliability for all the parameters in conditions without fatigue (i.e. PRE exercise). In this study, results of the fatigued condition (i.e. POST) are presented as raw value whereas Doyle-Baker et al. (2017) decided to present them as a percentage of PRE values (likely reducing the variability). Despite this difference, the results of the present study provide overall very good relative reliability with ICC all above 0.74, however, the CVs are often higher when considering POST values and therefore, it could be better to use relative changes instead of absolute one if the purpose of the study is to compare fatigue induced by different conditions performed on different days. The overall excellent intraday

reliability also suggest that it would allow better interpretation of the results to consider delta value from PRE to POST exercise rather than raw value.

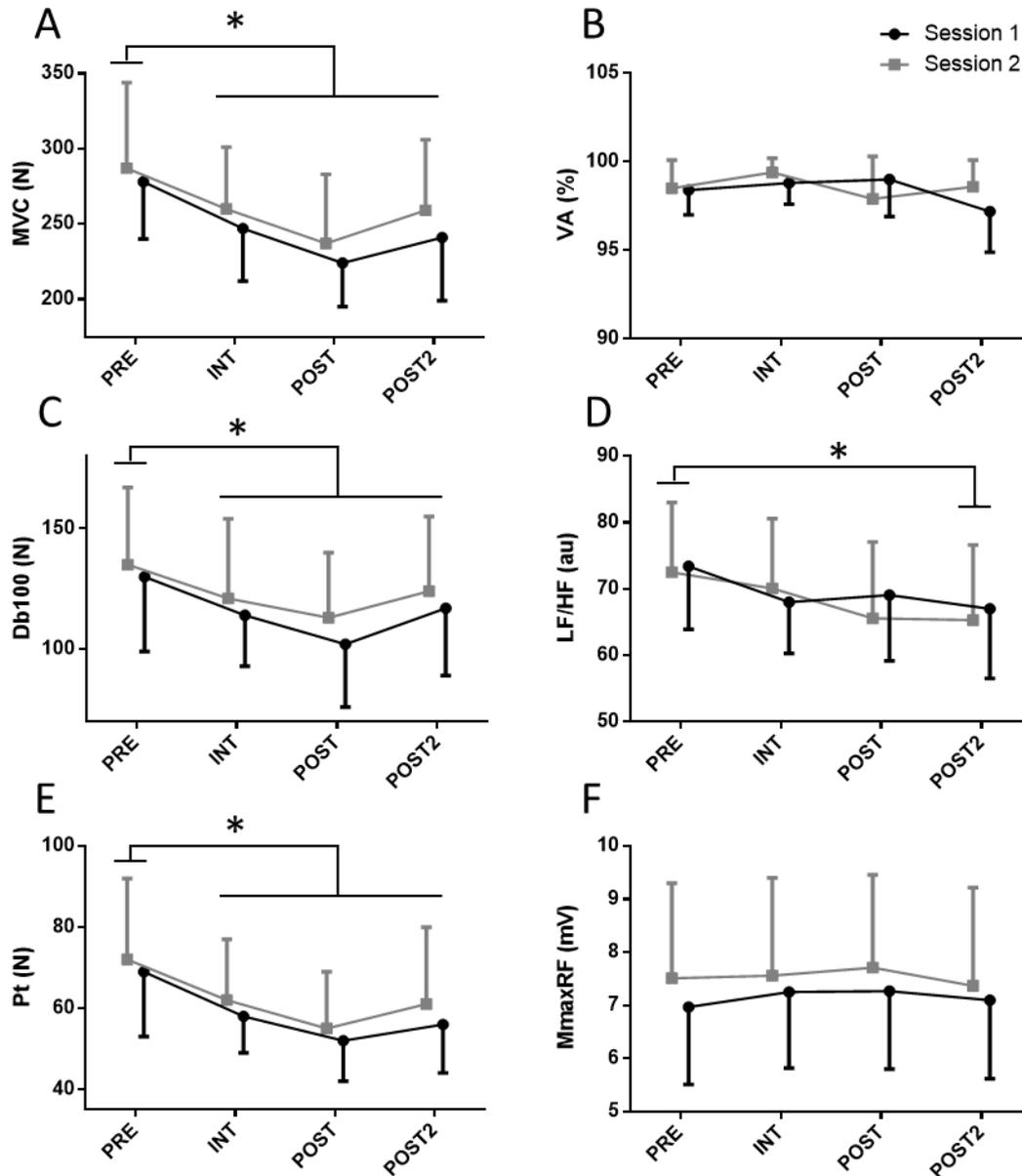


Figure 3. Neuromuscular function measured during two sessions of a repeated sprint exercise consisting of 5 times 10-s sprints interspaced with 20 s of passive recovery. Maximal voluntary contraction force (MVC, panel A), voluntary activation (VA, panel B), evoked force by 100Hz-doublet (Db100, panel C), low frequency fatigue ratio (LF/HF, panel D), evoked force by single electrical nerve stimulation (Pt, panel E) and amplitude of the electromyographic response of the rectus femoris to a Pt (MmaxRF, panel F). All measures were performed before (PRE), during (INT) immediately after (POST) and 2 min after (POST2) the exercise. \* denotes a significant difference from PRE,  $P < 0.05$ .

The results of the biomechanical parameters (i.e.  $P_{mean}$ ,  $T_0$ , and  $V_0$ ) provides some differences.  $P_{mean}$  and  $P_{max}$  have both excellent intra- and inter-days reliability whereas  $T_0$  and  $V_0$  have very good reliability only when combining two sprints at low and high resistances. The problem comes from the fact that the resistance chosen in the present protocol is based on what is traditionally applied on a normal bike. Therefore, the resistance used (i.e.  $0.8 \text{ N}\cdot\text{kg}^{-1}$ ) is likely too important for this setup and prevent the participant to reach high speed during his sprints. This will shift the range of point of the T-V relationship towards the force side, impairing the quality of the linear regression and making the extrapolation of  $T_0$  and  $V_0$  difficult (especially  $V_0$ , Jaafar et al. 2015). This is even more problematic when exercise induces fatigue as it will also reduce the neuromuscular capabilities, making difficult to overcome the flywheel inertia and to produce maximal speed. This is likely to exacerbate the left shift of the range of point on the T-V relationship (i.e. more points with high forces and low speed) making  $V_0$  and  $T_0$  less reliable.

One way to overcome this issue would be to determine individually the optimal resistance for each participant depending on his muscular capabilities and the specificity of the ergometer. This has been the topic of another study under the reviewing process.

### Repeated sprint exercise and fatigue

The purpose of the ergometer is to be able to detect fatigue development during RSE. The results obtained in the present study show that sprint performance is impacted as soon as the second sprint (i.e.  $P_{mean}$  and  $P_{max}$  diminution, see figure 4). Neuromuscular function evaluation showed a diminution of MVC and peripheral indices of fatigue (i.e.  $Db_{100}$ ,  $P_t$ , figure 3) as soon as the first measure performed after the second sprint whereas voluntary activation wasn't impacted through the test. Because no changes were detected in  $M_{max}$ , those results can be interpreted as an impairment of the EC coupling probably because of an accumulation of  $P_i$ . Indeed, RSE is known to be dependent on the anaerobic glycolysis (Gaitanos et al. 1993) which produces a lot of metabolites impacting the different steps involved in the EC coupling (REF).

These results suggest that fatigue appears as soon as the first sprint and is mainly peripheral from the early stage of fatigue. This is in accordance with previous literature showing that peripheral fatigue seems to develop from the early stage of exercise whereas central fatigue

appears later (Collins et al. 2018). The small number of sprints performed in the present study is likely to explain the absence of central fatigue.

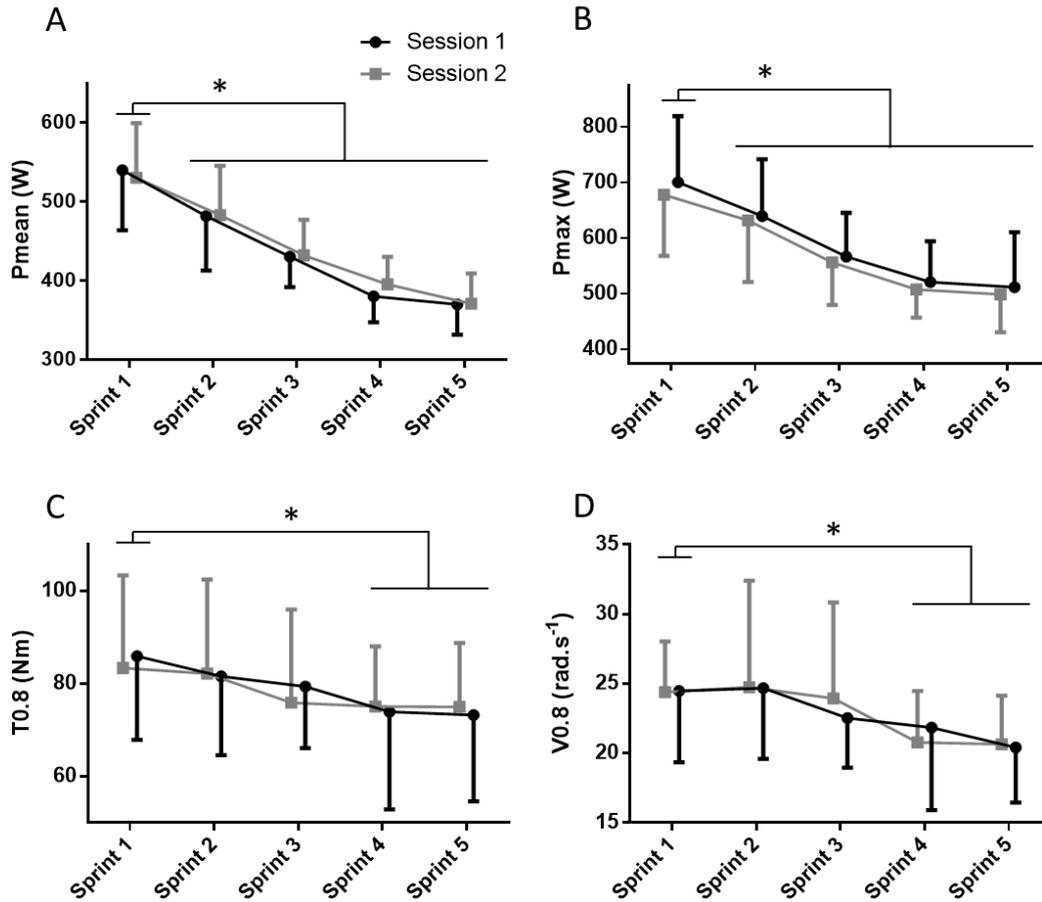


Figure 4. Biomechanical indices measured during two sessions of a repeated sprint exercise consisting of 5 times 10-s sprints interspaced with 20 s of passive recovery. Average power output from the sprints (Pmean, panel A), most powerful downstroke during the sprint (Pmax, panel B), torque determined as the intercept of the torque-velocity relationship in the force axis (T0.8, panel C) and velocity determined as the intercept of the torque-velocity relationship in the velocity axis (V0.8, panel D). \* denotes a significant difference from sprint 1,  $P < 0.05$ .

Further work should focus on establishing complete kinetics of fatigue etiology in order to determine when exactly central fatigue starts to occur. In their recent review, Collins et al. (2018) proposed a model in which performance decrement follows distinctive pattern with a rapid loss of power in the early sprints and a slower rate at the end of the RSE. It would be interesting to perform such exercise to exhaustion on the developed ergometer with several NMF evaluation throughout the test to confirm this nonlinear decrease in power output and see if there is any relationship between fatigue etiology and this rate of performance decrement.

## Conclusion

The present study shows the very good relative and absolute reliability of the developed ergometer and confirmed that it is an adapted tool to evaluate neuromuscular fatigue using isometric contraction associated with superimposed electrical stimulation to determine fatigue etiology. It also allows to perform dynamic exercise providing information regarding the impact of fatigue on muscle force and velocity production capabilities. Results also highlight the needs to individualize sprint resistance to the participant and to consider the specificity of the ergometer.

By removing the delay between the fatiguing task and the fatigue measurement, this original setup brings new possibilities to assess more precisely neuromuscular function during cycling and to obtain a continuum in fatigue origin through whole body exercise.

## 2.3 Synthesis

The current literature investigating neuromuscular fatigue associated with repeated sprint exercise usually have 2 or 3 time-points of measurement through the session mostly because of methodological limits. Indeed, the common methodology to investigate neuromuscular function often implies the delivery of superimposed electrical stimulation during a voluntary contraction and therefore, the use of a specific ergometer. Because repeated sprints in laboratory environments are mostly performed on non-motorized treadmill or cyclo-ergometer, it is necessary to move the participant after the completion of the fatiguing task, to the designed ergometer to investigate fatigue. This is rather problematic due to the delay (often comprised between 30 s and up to 3 min) induced between the task completion and fatigue measurement as it has been shown that fatigue recovers quickly and at different rates depending on its etiology (e.g. central or peripheral).

Another limitation from the current methodology to assess neuromuscular fatigue is to use only isometric contraction to characterize fatigue development induced by whole-body dynamic exercise. It has been shown that power output decrement is not necessarily correlated to a diminution of isometric MVC, questioning the relevance of such measurement. It is however possible to characterize muscle capabilities to produce force and velocity through the linear relationship linking those two indices and to look at the impact of fatigue on those characteristics. To do so it is necessary to obtain force and velocity measurement during maximal exercise.

From those limitations, the objective was to develop a device allowing to perform the fatiguing task and the neuromuscular evaluation on the same ergometer to avoid the associated delay to switch from one another. The idea was to make possible the completion of multiple measurements during a single session of repeated sprint to obtain full kinetics of the development of fatigue to investigate its etiology.

The above section presents the concept and the reliability of the designed ergometer. Its development represented a huge part of the job achieved during this thesis however its validation is just a step forward to many other projects. Conjointly to its development, a method to determine the individualized optimal sprint resistance depending on individual muscle capabilities and ergometer specificities has been established and is currently under

reviewing process. From there a new protocol was conducted to evaluate the neuromuscular fatigue associated with a repeated sprint exercise and to determine precisely fatigue etiology by having measurement interspaced between each sprint repetition.

## **Chapter 3 – Analysis and outlooks**

## 1 General discussion

This thesis was constructed around three main objectives that aimed to answer a lack of information in the scientific literature or some methodological issues regarding the neuromuscular evaluation to assess fatigue induced by a repeated-sprint exercise (RSE) and the potential adaptation when combined to hypoxic stress.

The first study meant to isolate the two potential mechanisms responsible for the exacerbation of central fatigue previously reported when performing RSE under hypoxic stress (Billaut et al. 2013) and determined if both really played a role. Indeed, hypoxia is believed to affect either directly the central motor drive by a reduction of brain oxygen delivery (due to the combination of the reduced amount of oxygen and the hypocapnia induced by the hyperventilation response to hypoxia) that would reduce the overall brain activity. Or through increased reliance on anaerobic metabolism induced by the diminution of oxygen delivery to the working muscle limiting the aerobic pathway. This would lead to an accumulation of metabolites known to increase the firing rate of group III and IV muscle afferents and impacting the central nervous system at the spinal and supraspinal level to finally exacerbate central fatigue (Amann and Calbet 2008).

To achieve this objective, two different modalities of hypoxia were applied. The commonly used systemic hypoxia (classically obtained by breathing a hypoxic gas mixture,  $FiO_2 = 0.13$  in the present case) and the localized hypoxia obtained via the use of blood flow restriction (BFR). The idea was to create a hypoxic paradigm where in one condition, both the brain and the muscle would be held in hypoxia whereas, on the blood flow restriction condition, only the muscle would be. If central fatigue is exacerbated only in systemic hypoxia, it is likely that the brain hypoxic effect is mainly responsible for the exacerbation of central fatigue.

The results, while promising, were not as clear as expected. The number of sprints performed during the repeated sprint test and the MVC were similarly decreased under both hypoxic modalities, but fatigue etiology differed. BFR led to a more pronounced impairment of the muscle excitation-contraction coupling (i.e. peripheral fatigue) probably due to immediate muscle deoxygenation and impairment in metabolites removal ability during RSE. Whereas systemic hypoxia predominantly impacted corticospinal excitability (i.e. central fatigue) in line with lower levels of cerebral oxygenation, potentially indicating incapacity of the corticospinal

pathway to adapt to fatigue as in normoxia. Therefore, those results suggest that hypoxia would be more likely to impact central fatigue via a direct brain hypoxic effect.

However, some limits remain and prevent to conclude clearly regarding the main objective. First, this study failed to report any exacerbation of VA decrement with both hypoxic modalities compared to normoxia as reported by Billaut et al. (2013). A potential explanation for this discrepancy could be that in the present study, exercise was performed to exhaustion, likely diluting any potential difference between conditions. Indeed, the decrease in MVC was similar in all condition despite the smaller number of sprints performed when exercise was combined with hypoxic modalities. It would be interesting to compare neuromuscular fatigue for the same number of sprints performed in all condition and see if this would change the results. Another limitation is the lack of data regarding RSE performed with blood flow restriction and the fact that localized hypoxia couldn't be normalized regarding the systemic condition (i.e. muscle oxygenation at rest is not impacted by hypoxia, making difficult to use NIRS to quantify the localized hypoxic level compared to hypoxia). Thus, the level of BFR was based on a recommendation for resistance training (Loenneke et al. 2014b) and might not be optimal for sprint exercise. Indeed, RSE is likely to induce more cardiovascular strain than resistance training especially when considering the arms (Calbet et al. 2015a) and might counteract the BFR effect. Furthermore, hemodynamic responses are likely different between localized and systemic hypoxic modalities (Willis et al. 2019), therefore the increase in peripheral fatigue associated with blood flow restriction might originate from an impairment of metabolites removal per se rather than a hypoxic effect. Lastly, the 2 minutes delay between the end of the repeated sprints test and the neuromuscular evaluation probably led to an underestimation and a misinterpretation of fatigue etiology and could also explain the smaller decrement in VA observed in the normoxic condition (i.e. -4.2%) compared to the -5.6% reported by Pearcey et al. (2016). This delay was standardized to 2 minutes to be sure that arterial blood flow was detected and recorded via Doppler ultra-sound, results are presented by Willis et al. (2019).

Overall and despite the limitations, those results are promising, and further studies are required to understand fatigue etiology when repeated sprint exercise is performed combined to hypoxic stimulus.

From this first study and the literature review, a critical point when assessing fatigue is the associated delay between the end of the fatiguing task and the neuromuscular evaluation that can be assimilated as a recovery period. Because fatigue is known to recover quickly

and at different rates depending on its origin, this will likely impact the amount of fatigue measured as well as its interpretation. While some methodological improvements have been made since the first use of electrical superimposed stimulation to assess VA by Merton (1954), the concept is still the same and requires the participant to perform a single-joint isometric MVC in a specific setup to record the twitch force both during the MVC and in the relaxed muscle. This has led most of the study to focus on fatigue induced by isometric contractions and although it provides an easy and controllable fatiguing task that can be performed on the same experimental setup used for the fatigue evaluation, this mode of contraction is quite far from daily life activities or sports performance. Because the type of activity carried out will impact the physiological stress and the induced fatigue (i.e. the task dependency principle) the results obtained from single-joint isometric fatiguing task can't be transferred to dynamic whole-body exercise. However, when considering this type of exercises, a major limitation arises from the necessary time to switch participants from the fatiguing task (i.e. often performed on a treadmill or a cycloergometer) to the isometric ergometer for fatigue assessment. This limitation is inherent to any study that investigates neuromuscular function induced by RSE with a delay between the end of the task and the evaluation generally comprised between 30 s and 3 min (Tomazin et al. 2017). Keeping in mind the task dependency, another limitation appears to be the evaluation mode of fatigue itself. It appears questionable to focus the quantification of fatigue development induced by whole body exercise only to a decrease of isometric MVC. In a recent study, Krüger et al. (2019) compared the impact of a 30-s all-out cycling test on both isometric and dynamic contractions. To characterize muscle capabilities during dynamic contraction, they had participants perform a 7-s sprint before and after (i.e. 10 s) the fatiguing task and calculated biomechanical indices (Samozino et al. 2007) reflecting the muscle capabilities to produce torque ( $T_0$ ), velocity ( $V_0$ ) and power ( $P_{max}$ ). They compared the decrease induced by the fatiguing exercise on  $P_{max}$  and  $T_0$  to the isometric MVC and  $V_0$  to the rate of force development (i.e. an indicator of muscle explosiveness obtained from isometric contractions). They reported different diminutions for MVC and  $P_{max}$  and  $V_0$  and RMFD (MVC: -20.3%,  $P_{max}$ : -40%,  $P < 0.05$ ).  $T_0$  presented similar decrease than MVC ( $T_0$ : -19.3%) however when considering individual value, no correlation between the two variables were detected. Their results suggest that isometric and dynamic measurements are not impacted to the same extent by all-out exercise and probably don't share the same underlying physiological mechanisms, however, only isometric contraction allows assessment of VA. This highlights the complementarity of the two measurements and the scientific interest to develop a single

ergometer allowing to measure isometric MVC and dynamic muscle properties during short sprints.

From this analysis, the purpose of the second part of this thesis was to build and validate such ergometer. The results obtained and presented previously showed that this new device provides excellent intraday reliability of neuromuscular function measurement ( $ICC > 0.92$  for all parameters) and very good inter-days reliability during RSE ( $ICC > 0.74$  for all parameters). However, the results obtained on the biomechanics measures present some limits. Whereas the reliability of the measured power is excellent (e.g. inter-days reliability of  $P_{mean}$ :  $ICC > 0.9$ ), the determination of  $T_0$  and  $V_0$  required the combination of two sprints at different resistances to provide reliable data. This is explained by the fact that the resistance used during the RSE (i.e.  $0.8 \text{ N}\cdot\text{kg}^{-1}$ ) was based on what's used in the current literature for exercise performed on classical cycloergometer and was likely too high for a majority of participants, preventing them to reach their maximal velocity. This would lead to too many points with high torques and slow velocity, impairing the extrapolation of  $T_0$  and  $V_0$ , especially in a fatigued state. This issue was addressed during a parallel project conducted to determine the optimal sprint resistance load depending on individual muscle capabilities and the specificity of the ergometer. Preliminary results from a second protocol on the recumbent bike performed with 11 participants report an optimal load of  $0.64 \pm 0.07 \text{ N}\cdot\text{kg}^{-1}$ , confirming that  $0.8 \text{ N}\cdot\text{kg}^{-1}$  was too high. Further experimental work should therefore assess the reliability of this new ergometer using an appropriate resistance load.

Nevertheless, the development of this ergometer will open a wide range of possibilities to investigate the impact of RSE on neuromuscular function. Not only the delay to switch from one ergometer to another induced an underestimation of fatigue but it also prevents to performed multiple measurements during the test to obtain a precise kinetic of fatigue development. Some authors interspaced a measure in the middle of the RSE or used a complex experimental design having the participants to perform multiple RSE on different days with a variable number of sprints to build a kinetic. For instance, Hureau et al. (2016) used 5 RSE consisting in 1, 4, 6, 8 and 10 sprints with pre- and post- measurement to establish the evolution of fatigue. While this methodology is doable, it is certainly not optimal, especially if the purpose is to compare the impact of different experimental conditions such as environmental factors or training intervention. The use of this ergometer is obviously not limited to sprint exercise and can also be used to investigate the impact of continuous or incremental exercise on the neuromuscular function.

Most of the current knowledge regarding fatigue induced by whole-body exercise and its impact on neuromuscular function has been established using isometric MVC with a significant delay between the end of the task and the evaluation. The validation of the present ergometer represents a step forward for the neuromuscular evaluation and the associated understanding of fatigue etiology.

The experimentations conducted during this thesis brings more outlooks than they provide clear answers and some of the projects started during those last years are still under progress and awaits

The next part focus on providing a few perspectives for the short to mid-term following the completion of this work.

## 2 Perspectives

Surely, the validation of the new ergometer provides a lot of potential projects and some of them have already started.

The first one aimed to characterize fatigue etiology during RSE and investigate the relationship between power decrement and the development of peripheral and central fatigue. In their review, Collins et al. (2018) highlighted that the decrease in power output during RSE is nonlinear with a higher rate of fatigue development (mainly peripheral) during the early stage of exercise. There is also a discrepancy in the literature regarding the onset of central fatigue, where some authors reported its development only after 2, 6 or 10 sprints whereas others didn't report any change after 3 sets of 5 sprints (Tomazin et al. 2017). Another question arises from the fact that most studies use a predetermined number of sprints. This likely induces participants to reach different levels of fatigue at the end of the test depending on their physiological profile and training status and prevent the investigation of fatigue etiology at task failure. Therefore, the purpose of the protocol was to investigate fatigue etiology induced by the repetitions of 10-s sprints interspaced with 30-s recovery until the average sprint power output decrease below 70% of the first sprint. During each recovery period, neuromuscular function was assessed using the interpolated twitch technique. The sprints were performed at an individualized optimal resistance load to ensure reliability of biomechanical determinants of muscle capabilities (i.e.  $T_0$  and  $V_0$ ). The associated hypothesis is that peripheral fatigue follows the nonlinear profile of power decrement (i.e. rapid decrease toward the early stage of RSE then plateaued) whereas central fatigue starts its development when peripheral fatigue has plateaued.

During this thesis, a partnership has been developed with the military department to assess the effectiveness of a repeated sprint training protocol in hypoxia to improve the physical performance of mountain infantrymen. The evaluation consists of several basic fitness tests applied to the specific military population (e.g. assault course) performed pre- and post- the training intervention to evaluate the effectiveness of RSH protocol with this population. The actual number of participants was too low to include this work in the thesis ( $n= 5$  in both experimental and control group). This project served as a basis for an upcoming PhD starting next year and should therefore be completed shortly.

Finally, the last perspective aims to characterize the evolution of neuromuscular fatigue after RSH. This is part of a much bigger project involving the investigation of several aspects of potential physiological adaptation to RSH including neuromuscular function, metabolic changes within the muscle measured via nuclear magnetic resonance spectroscopy (e.g. Pcr depletion and resynthesize, pH) and biopsies as well as lactate kinetics during exercise recovery. The objective of this project is to provide experimental data to identify the underlying mechanisms responsible for performance improvement following sprint training in hypoxia. Hopefully, ethics approval should arrive soon.

### 3 Conclusion

Fatigue may originate from any site involved in the motor command chain from the brain to the muscle. The interpolated twitch technique first described by Merton in 1954 is still the most widely used tool to assess neuromuscular fatigue induced by exercise as it provides information regarding both the central and peripheral component of fatigue.

Repeated sprint exercise is known to induce mainly peripheral fatigue during the early sprints whereas central fatigue only appears towards the end of the task. However, fatigue etiology is impacted by environmental conditions such as systemic or localized hypoxia. Results from the first study show that hypoxia and blood flow restriction impaired similarly sprint performance and the force production ability. However, hypoxia exacerbates the development of central fatigue due to a direct brain hypoxic effect diminishing corticospinal excitability whereas blood flow restriction worsened peripheral fatigue via impairment of the muscle excitation-contraction coupling because of an impairment of metabolites removal.

The use of the interpolated twitch technique requires to perform an isometric contraction to obtain insight into fatigue etiology and therefore involves the use of a specific ergometer. When investigating neuromuscular function during whole-body exercise, it is therefore necessary to switch between the ergometer where the fatiguing task is being performed (i.e. treadmill or cycloergometer) to the one to evaluate fatigue. This inevitably leads to a delay between the end of the fatiguing task and the neuromuscular fatigue evaluation that is assimilated to a recovery period and thus to an underestimation of fatigue and a misinterpretation of its etiology as the kinetics of recovery differs depending on its origin. Furthermore, the use of single-joints isometric contraction to assess fatigue induced by dynamic whole-body exercise has been questioned.

To answer those issues the second part of this work has been dedicated to the validation of an innovative ergometer allowing to perform both the fatiguing task and the neuromuscular function evaluation on the same device. Therefore, the delay is reduced to less than five seconds to switch from dynamic to isometric mode and fatigue can be characterized by both the interpolated twitch technique associated with isometric contraction and the determination of muscle capabilities to produce force and velocity during dynamic contraction.

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## **Annexes**

**Arm-cycling repeated sprints under hypoxia and/or blood flow restriction.**

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## Electronic supplementary material part I: Effects of hypoxia and/or blood flow restriction

(BFR) on elbow flexors, saturation index, and neuromuscular function at rest.

Two-way ANOVAs (*altitude\*occlusion*) with repeated measures were performed on PRE-values only to evaluate the acute effect of *altitude* and *occlusion* before the start of the fatiguing test. As for the analysis presented in the article, Post-hoc Tukey's tests were applied to determine a difference if the ANOVA revealed a significant main or interaction effect. Statistical significance was set at  $P < 0.05$ . Effect sizes are reported as partial eta-squared ( $\eta_p^2$ ) for ANOVAs (with interpretation thresholds fixed at 0.02, 0.13, and 0.26 for respectively, small, medium, and large). All data are presented as mean  $\pm$  SD).

### Results

As presented in the table below, MVC was lower with *occlusion* ( $F_{(1,11)}=6.60$ ,  $P=0.03$ ,  $\eta_p^2=0.37$ ), but was not impacted by *altitude* ( $F_{(1,11)}=0.25$ ,  $P=0.63$ ) or *altitude\*occlusion* ( $F_{(1,11)}=1.66$ ,  $P=0.22$ ) before exercise.

Pre values of SpO<sub>2</sub> (Fig. 3A) and TSI<sub>PFC</sub> (Fig.3C) were not impacted by *occlusion* ( $F_{(1,13)}=2.24$ ,  $P=0.16$  and  $F_{(1,13)}=1.44$ ,  $P=0.25$ , respectively) or *altitude\*occlusion* ( $F_{(1,13)}=0.39$ ,  $P=0.54$  and  $F_{(1,13)}=0.32$ ,  $P=0.58$ , respectively) but were decreased with *altitude* (SpO<sub>2</sub> in normoxia:  $98 \pm 1\%$ , hypoxia:  $91 \pm 2\%$ ,  $F_{(1,13)}=197.1$ ,  $P<0.01$ ,  $\eta_p^2=0.94$  and TSI<sub>PFC</sub> in normoxia:  $72 \pm 6\%$ , hypoxia:  $67 \pm 6\%$ ,  $F_{(1,13)}=10.1$ ,  $P=0.01$ ,  $\eta_p^2=0.44$ , respectively). TSI<sub>BB</sub> (Fig. 3B) decreased at baseline with *occlusion* ( $58 \pm 6\%$  and  $63 \pm 8\%$ , respectively, with and without BFR,  $F_{(1,13)}=6.80$ ,  $P=0.02$ ,  $\eta_p^2=0.34$ ) but was not impacted by *altitude* ( $F_{(1,13)}=0.80$ ,  $P=0.39$ ) or *altitude\*occlusion* ( $F_{(1,13)}=0.18$ ,  $P=0.68$ ).

Baseline Mmax was not impacted by *occlusion* ( $F_{(1,13)}=2.20$ ,  $P=0.16$ ), *altitude* ( $F_{(1,13)}=1.27$ ,  $P=0.28$ ) or *altitude\*occlusion* ( $F_{(1,13)}=0.06$ ,  $P=0.80$ ).

Db10 decreased at PRE with *occlusion* ( $F_{(1,13)}=14.1$ ,  $P<0.01$ ,  $\eta_p^2=0.52$ ) and increased with *altitude* ( $F_{(1,13)}=9.0$ ,  $P=0.01$ ,  $\eta_p^2=0.41$ ). The interaction *altitude\*occlusion* was not significant ( $F_{(1,13)}=0.33$ ,  $P=0.58$ ).

Baseline  $VA_{TMS}$  was not impacted by *occlusion* ( $F_{(1,10)}=1.62$ ,  $P=0.23$ ), *altitude* ( $F_{(1,10)}=1.15$ ,  $P=0.31$ ) or *altitude\*occlusion* ( $F_{(1,10)}=0.47$ ,  $P=0.51$ ).

MEP, SICI, and  $EMG_{RMS}$  were not impacted, no matter the intensity of contraction. CSP was not impacted at PRE by *occlusion* (e.g.  $CSP_{75}$ :  $F_{(1,10)}=0.79$ ,  $P=0.39$ ) or *altitude\*occlusion* (e.g.  $CSP_{75}$ :  $F_{(1,10)}=0.92$ ,  $P=0.36$ ) but decreased with *altitude* (e.g.  $CSP_{75}$ : normoxia:  $127 \pm 23$  ms, hypoxia:  $117 \pm 22$  ms,  $F_{(1,10)}=6.69$ ,  $P=0.03$ ,  $\eta_p^2=0.40$ ).

**Supplementary Table I.** Effects of hypoxia and/or blood flow restriction (BFR) on elbow flexors, saturation index and neuromuscular function at rest.

	Conditions				Factors		
	NOR	N <sub>BFR</sub>	HYP	H <sub>BFR</sub>	<i>altitude</i> P-value	<i>occlusion</i> P-value	<i>Altitude*occlusion</i> P-value
<b>Performance</b>							
MVC (N)	229 ± 58	220 ± 60	235 ± 59	218 ± 57	0.625	<b>0.026</b>	0.225
SpO <sub>2</sub> (%)	98 ± 1	98 ± 1	90 ± 3	91 ± 2	<b>&lt;0.001</b>	0.158	0.544
TSI <sub>BB</sub> (%)	63 ± 8	58 ± 8	63 ± 9	57 ± 5	0.390	<b>0.022</b>	0.680
TSI <sub>PFC</sub> (%)	71 ± 6	72 ± 6	65 ± 6	68 ± 7	<b>0.007</b>	0.251	0.580
<b>Peripheral indices</b>							
M <sub>max</sub> BB (mV)	16.8 ± 5.4	14.9 ± 5.5	17.6 ± 5.3	16 ± 6.5	0.280	0.162	0.807
Db10 (N)	31 ± 13	27 ± 9	37 ± 11	31 ± 14	<b>0.010</b>	<b>0.002</b>	0.577
<b>Central indices</b>							
$VA_{TMS}$ (%)	96.2 ± 2.3	94.9 ± 2.8	95.8 ± 2.9	93.4 ± 4.4	0.148	0.087	0.430
$EMG_{RMS}$ (AU)	0.079 ± 0.039	0.070 ± 0.03	0.071 ± 0.021	0.075 ± 0.031	0.819	0.711	0.226
MEP <sub>100</sub> (AU)	0.70 ± 0.33	0.65 ± 0.25	0.66 ± 0.25	0.69 ± 0.25	0.981	0.923	0.325
MEP <sub>75</sub> (AU)	0.93 ± 0.47	0.85 ± 0.3	0.86 ± 0.32	0.86 ± 0.34	0.621	0.441	0.567
MEP <sub>50</sub> (AU)	0.92 ± 0.38	0.91 ± 0.35	0.86 ± 0.36	0.94 ± 0.37	0.871	0.351	0.498
CSP <sub>100</sub> (ms)	112 ± 20	103 ± 18	102 ± 22	101 ± 19	0.063	0.269	0.554
<b>CSP<sub>75</sub> (ms)</b>	131 ± 24	122 ± 22	116 ± 22	117 ± 22	<b>0.027</b>	0.393	0.360
CSP <sub>50</sub> (ms)	143 ± 27	138 ± 27	130 ± 23	133 ± 24	0.086	0.822	0.502
SICI (AU)	0.53 ± 0.16	0.48 ± 0.15	0.53 ± 0.22	0.55 ± 0.18	0.303	0.608	0.107

Values are presented as mean (SD); NOR : normoxia without blood flow restriction; N<sub>BFR</sub>: normoxia with blood flow restriction; HYP: hypoxia without blood flow restriction; H<sub>BFR</sub>: hypoxia with blood flow restriction; MVC: maximal voluntary contraction force; SpO<sub>2</sub>: arterial oxygenation; TSI : tissue saturation index; BB: *biceps brachii*; PFC: pre-frontal cortex; M<sub>max</sub>: maximal M-wave; BR: *brachioradialis*; Db10: force evoked by a 10Hz doublets;  $VA_{TMS}$ : voluntary activation assess by TMS;  $EMG_{RMS}$ : EMG root mean square; MEP<sub>100</sub>, MEP<sub>75</sub>, MEP<sub>50</sub>: motor evoked potential respectively during MVC, submaximal contraction at 100, 75, and 50%, of MVC force; CSP<sub>100</sub>, CSP<sub>75</sub>, CSP<sub>50</sub>,: cortical silent period respectively during MVC, submaximal contraction at 100, 75, and 50% of MVC force; SICI: short interval intracortical inhibition during contraction at 10% MVC force. Statistical significance was set at  $P < 0.05$ .

## Interpretation

As expected, SpO<sub>2</sub> and TSI<sub>PFC</sub> were significantly reduced at rest in conditions with simulated *altitude* (FiO<sub>2</sub>: 0.13). However, despite lower systemic O<sub>2</sub> availability in these conditions, resting TSI<sub>BB</sub> (~30 min) was well preserved. This is in accordance with previous literature showing that acute passive exposure to hypoxia, even at lower FiO<sub>2</sub> than the one used here, only marginally affect resting muscle oxygenation status contrarily to its effect on systemic and brain oxygenation (Rupp and Perrey 2009; Rupp et al. 2013). As BFR immediately impaired muscle TSI in the present study, while preserving systemic and cerebral oxygenation, we are confident our experimental design was effective to induce either dissociated or coupled local *versus* systemic hypoxic stress. MVC decreased with acute exposure to BFR. As an indicator of muscle excitation-contraction coupling efficiency, Db10 force was also depressed so that one may argue that acute local muscle hypoxia likely impaired MVC through peripheral limitations, as previously reported (Racinais et al. 2007). Systemic hypoxia had an acute effect on PRE-values, surprisingly increasing Db10 force and decreasing CSP during contractions, indicating that hypoxia could impact the muscle excitation-contraction coupling and the activity of the GABA<sub>B</sub> receptors, limiting intracortical inhibition. However, those results did not transfer to more global parameters such as MVC or VA<sub>TMS</sub>.

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## **Electronic supplementary material part II: Effects of an arm-cycling repeated sprint ability test in normoxia without blood flow restriction on elbow flexors, saturation index, and neuromuscular function.**

T-tests for dependent samples were performed on data PRE and POST aRSA test in NOR condition only to describe variables evolution in terms of absolute values rather than delta in this “control” condition. Statistical significance was set at  $P < 0.05$ . Effect sizes are reported as Cohen’s  $d$  ( $d$ ) for t-tests (with interpretation thresholds fixed at 0.2, 0.6, 1.2, and 2.0 for, respectively, small, moderate, large, and very large). All data are presented as mean  $\pm$  SD.

### **Results**

Data are shown in the table below.

MVC decreased by 16.7% from PRE to POST aRSA test ( $P < 0.01$ ,  $d = 0.73$ ).  $SpO_2$  and  $TSl_{PFC}$  decreased respectively by 2.6% ( $P < 0.01$ ,  $d = 1.28$ ) and 5.2% ( $P < 0.01$ ,  $d = 0.62$ ) while  $TSl_{BB}$  was not impacted ( $P = 0.42$ ) from PRE to POST aRSA test in NOR (Fig. 3).  $M_{max}$  was not impacted by the aRSA test ( $P = 0.84$ ), but  $Db_{10}$  decreased by 29.8% ( $P < 0.01$ ,  $d = 0.83$ ).  $VA_{TMS}$  decreased by 4.3% after aRSA test in NOR ( $P < 0.01$ ,  $d = 2.46$ ). MEP tended to increase in the BB after exercise ( $MEP_{100}$ : +16%,  $P = 0.15$ ,  $d = 0.48$ ;  $MEP_{75BB}$ : +17%,  $P = 0.08$ ,  $d = 0.47$ ;  $MEP_{50BB}$ : +17.4%,  $P = 0.09$ ,  $d = 0.58$ ). The aRSA test did not impact CSP (e.g.  $CSP_{100}$ :  $P = 0.81$ );  $EMG_{RMS}$  ( $P = 0.21$ ) or  $SICI$  ( $P = 0.90$ ).

**Supplementary Table II.** Effects of an arm-cycling repeated sprint ability test to task failure in normoxia without blood flow restriction on elbow flexors, saturation index, and neuromuscular function.

	PRE	POST	P-value	Cohen's d
<b>MVC (N)</b>	229 ± 58	191 ± 52	<b>&lt; 0.001</b>	0.73
<b>SpO<sub>2</sub> (%)</b>	98 ± 1	96 ± 3	<b>0.003</b>	1.28
TSl <sub>BB</sub> (%)	63 ± 8	65 ± 6	0.416	0.29
<b>TSl<sub>PFC</sub> (%)</b>	71 ± 6	67 ± 6	<b>0.004</b>	0.62
<b><u>Peripheral indices</u></b>				
Mmax (mV)	16.8 ± 5.4	16.6 ± 5.4	0.839	0.03
<b>Db10 (N)</b>	31 ± 12.9	21.7 ± 10.1	<b>0.001</b>	0.83
<b><u>Central indices</u></b>				
<b>VA<sub>TMS</sub> (%)</b>	96.3 ± 1.4	92.1 ± 2.1	<b>&lt; 0.001</b>	2.46
EMG <sub>RMS</sub> (AU)	0.08 ± 0.04	0.09 ± 0.04	0.210	0.27
MEP <sub>100</sub> (AU)	0.76 ± 0.26	0.88 ± 0.28	0.146	0.48
MEP <sub>75</sub> (AU)	1.01 ± 0.39	1.18 ± 0.37	0.082	0.47
MEP <sub>50</sub> (AU)	1.0 ± 0.25	1.18 ± 0.37	0.095	0.58
CSP <sub>100</sub> (ms)	112 ± 20	112 ± 22	0.811	0.03
CSP <sub>75</sub> (ms)	131 ± 24	128 ± 20	0.367	0.17
CSP <sub>50</sub> (ms)	143 ± 27	135 ± 23	0.101	0.36
SICI (AU)	0.53 ± 0.16	0.55 ± 0.14	0.672	0.08

Values are presented as mean ± SD; MVC: maximal voluntary contraction force; SpO<sub>2</sub>: arterial oxygenation; TSI: tissue saturation index; BB: *biceps brachii*; PFC: pre-frontal cortex; Mmax: maximal M-wave; Db10: force evoked by a 10Hz doublets; VA<sub>TMS</sub>: voluntary activation assess by TMS; EMG<sub>RMS</sub>: EMG root mean square; MEP<sub>100</sub>, MEP<sub>75</sub>, MEP<sub>50</sub>: motor evoked potential respectively during MVC, submaximal contraction at 100, 75, and 50% of MVC; CSP<sub>100</sub>, CSP<sub>75</sub>, CSP<sub>50</sub>: cortical silent period respectively during MVC, submaximal contraction at 100, 75, and 50% of MVC force; SICI: short interval intracortical inhibition during contraction at 10% MVC force. Statistical significance was set at P < 0.05.

## **Fatigue neuromusculaire induite par répétition de sprints – Etiologie et impact de l'hypoxie.**

### Résumé :

La capacité à répéter des efforts de courte durée et d'intensité maximale est considérées comme un indicateur de la performance dans de nombreux sports intermittents (sports collectifs, sports d'opposition). Ce travail de thèse s'est focalisé sur l'étude de la fatigue neuromusculaire induite par une répétition de sprints et de l'impact que peut avoir l'hypoxie sur le développement de celle-ci. Il est établi qu'en condition normale l'origine de cette fatigue est d'avantage musculaire (périphérique) dès les premiers sprints alors qu'une fatigue dites centrale, correspondant à une incapacité du système nerveux centrale à recruter le muscle de manière optimale, apparait lors des derniers sprints. La réalisation de ce type d'effort en hypoxie a pour effet d'exacerber l'apparition de la fatigue, notamment centrale, de deux manières potentielles. Soit via une diminution de la quantité d'oxygène fournit au cerveau, ce qui aurait un effet direct diminuant l'activité cérébrale et donc la commande motrice nécessaire à l'exercice. Soit via la réduction de l'arrivée de l'oxygène au niveau musculaire, qui engendrerait une diminution de la part d'énergie produite par le métabolisme aérobie, qui serait redirigé vers le métabolisme anaérobie, connu pour produire davantage de déchets métaboliques. Ces derniers sont liés à des voies afférentes qui inhibe de manière indirect la commande motrice. L'un des objectifs était de pouvoir isoler ces deux mécanismes grâce à la mise en place d'une hypoxie musculaire localisée afin de voir si cela suffirait à induire une augmentation de la fatigue centrale. Les résultats présentés suggèrent que les deux types d'hypoxies diminuent la performance en sprint de manière similaire mais via des mécanismes distincts. L'hypoxie générale impact d'avantage le développement de la fatigue centrale via un effet direct sur le cerveau alors que l'hypoxie localisée augmente surtout la fatigue périphérique via l'accumulation de métabolites. Cependant la méthode utilisée dans cette étude et classiquement dans la littérature induit un délai entre la fin du sprint et la mesure de fatigue, ce qui peut induire une sous-estimation et une mauvaise interprétation de l'étiologie de celle-ci et ne permet pas d'établir une cinétique précise du développement de la fatigue. C'est pourquoi suite à cette première étude, l'objectif était de développer une méthode permettant des mesures régulières et sans délai de la fatigue neuromusculaire. Ce travail présente le développement et la validation d'un nouvel ergomètre permettant des mesures de la fonction neuromusculaire intégrées pendant un exercice de sprint répétés sur vélo.

## **Neuromuscular fatigue and repeated sprint exercise – etiology and impact of hypoxia.**

### **Abstract:**

The ability to repeat short maximal sprints is considered as a good performance index in a variety of intermittent sports (e.g. team or racket sports). This work focused on the neuromuscular fatigue induced by repeated sprint exercise and the impact of hypoxia on its development. In normoxic condition, it is rather established that fatigue mainly originates from the muscle itself (i.e. peripheral) during the early stage of the task whereas central fatigue (i.e. the incapacity of the central nervous system to fully activate the muscles) arise during the latter stage of exercise. When performed under hypoxic condition, central fatigue is exacerbated due to two potential mechanisms. The first one implies a direct brain hypoxic effect that would reduce the brain activity and therefore the motor drive to the exercising muscles. The second one would be through a decrease in oxygen delivery to the muscle, decreasing the energy furnished by the aerobic pathway, increasing the reliance on anaerobic metabolism and leading to the production of more metabolites. Those are known to increase the firing rate of muscle afferents inhibiting the central drive. One of the objectives of this thesis was to isolate those mechanisms thanks to the use of localized muscle hypoxia obtain through blood flow restriction and to determine if exercising in that condition would increase the development of central fatigue. The present results suggest that systemic and localized hypoxia impaired similarly sprint performance but with distinct mechanisms. Systemic hypoxia exacerbates the development of central fatigue through a direct brain hypoxic effect whereas blood flow restriction increased peripheral fatigue due to the accumulation of metabolites. However, the methodology used for this study and classically in the literature involves a delay between the end of the repeated sprint exercise and the fatigue measurement. This is problematic as fatigue is known to recover quickly and at different rates depending on its origin, thus leading to a probable underestimation and misinterpretation of fatigue etiology. Furthermore, it prevents the possibility of having kinetics of fatigue development allowing to determine more precisely the apparition and the evolution of central and peripheral fatigue. Therefore, following the first study, the aim was to propose a new methodological approach to allow several measures of fatigue during the exercise without any delay. This work presents the development and the validation of a new ergometer making possible to assess neuromuscular function during repeated sprint cycling exercise.