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On the role of group III/IV muscle afferent feedback on endurance performance, neuromuscular fatigue, and autonomic adjustments to exercise

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Sommario

I meccanismi di feedback afferente ed i riflessi nervosi rappresentano importanti sistemi di regolazione per diversi meccanismi fisiologici, come il controllo motorio, la propriocezione, e gli aggiustamenti autonomici agli stress fisiologici. Nell'ultimo secolo, un numero elevato di studi ha dimostrato in maniera convincente che il feedback afferente mediato dalle fibre dei gruppi III/IV, svolge un ruolo centrale nel mantenimento di un appropriata distribuzione di ossigeno al muscolo. Questo avviene tramite aggiustamenti cardiovascolari e ventilatori volti a ritardare l'insorgenza della fatica. Allo stesso tempo però, questo feedback afferente inibisce la trasmissione degli impulsi dai centri cerebrali verso il muscolo attivo, con un potenziale impatto negativo sulla prestazione fisica. A causa di questo duplice ruolo, il feedback afferente può essere considerato "un'arma a doppio taglio", il cui effetto netto sulla performance e sulla fatica neuromuscolare rimane ancora oggetto di discussione. Nel corso degli anni, lo sviluppo e la scoperta di nuovi metodi e strumenti atti a separare l'influenza dei vari sistemi di regolazione neurocircolatoria, ha permesso di aprire nuovi scenari per comprendere meglio il ruolo del feedback afferente dato dalle fibre dei gruppi III/IV. Ad ogni modo però, la "ridondanza" che esiste nella regolazione di sistemi fisiologici, rende difficile questo compito. In questa tesi, è stata prestata particolare attenzione nel provare a separare gli effetti del feedback afferente dalla potenziale influenza del comando motorio discendente. Quindi, il proposito di questa tesi è quello di delucidare il ruolo del feedback delle fibre dei gruppi III/IV sulla prestazione, la fatica neuromuscolare, e gli aggiustamenti all'esercizio fisico in soggetti giovani e sani. Nel Capitolo 1 è fornita una breve introduzione riguardante la storia delle scoperte nel campo del feedback afferente. Nel Capitolo 2, è stata effettuata una dettagliata revisione della letteratura per fornire le informazioni necessarie a capire il ruolo del feedback afferente negli aggiustamenti autonomici, sulla fatica neuromuscolare, e sulla prestazione. In seguito, nel Capitolo 3 sono presentati gli obiettivi ed ipotesi perseguiti in questa tesi. Nei Capitoli 4, 5, e 6, si possono trovare i risultati degli studi originali sull'argomento in oggetto. In fine, il Capitolo 7 fornisce un sommario ed una visione d'insieme dei nostri risultati, sottolineando punti di forza e limitazioni del nostro lavoro, e fornendo idee per investigazioni future.

Abstract

Afferent feedback mechanisms and reflex arcs represent fundamental regulatory systems for several processes such as motor control, proprioception, and autonomic adjustments to physiological stressors. Nowadays, compelling evidence exists that feedback mediated by group III/IV thin afferent fibers plays a quintessential role in contributing to maintain an appropriate locomotor muscle O_2 delivery by facilitating cardiovascular and ventilatory responses to exercise and eventually delaying the onset of fatigue. Concurrently however, this feedback also inhibits neural drive to the muscle and facilitate central fatigue, which results in a negative effect on performance. Because of the ambivalent and "contradictory" nature of this feedback, the net effect on exercise performance and neuromuscular fatigue is still somewhat unclear. Throughout the years, many advancements have been done in this field through the discovery and optimization of the methods and tools apt to separate the influence of the different regulatory systems that contribute to the neurocirculatory adjustments to exercise. However, the redundancy existing in the regulation of physiological systems, make this endeavor challenging. In this dissertation, particular effort was put into separating the effects of group III/IV afferent feedback, from the potential effects played by the voluntary descending drive. Therefore, the purpose of this dissertation was to elucidate the role of group III/IV afferent feedback on exercise performance, neuromuscular fatigue, and autonomic adjustments to exercise in young healthy individuals. In Chapter 1, a brief introduction about the history of group III/IV afferent feedback is provided. In Chapter 2, a detailed literature review was performed in order to give a comprehensive background on the role of group III/IV afferent feedback on the autonomic adjustments to exercise, the neuromuscular fatigue processes, and exercise performance. In Chapter 3 the aims of the study are highlighted. Successively, Chapters 4, 5, and 6 include the results of the original investigations on this topic. Finally, Chapter 7 provides a summary and an ensemble view of our findings, highlighting strengths and weaknesses of our research, and providing future directions for further studies.

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The results of this dissertation produced the following papers and communications.

Papers

I. **Laginestra FG**, Amann M, Kirmizi E, Giuriato G, Ruzzante F, Pedrinolla A, Martignon C, Barbi C, Tarperi C, Schena F, Venturelli M. Electrically-induced quadriceps fatigue in the contralateral leg impairs ipsilateral knee extensors performance. *Am J Physiol Regul Integr Comp Physiol* 320**,** R747-R756.

II. **Laginestra FG**, Cavicchia A, Vanegas-Lopez JE, Barbi C, Martignon C, Giuriato G, Pedrinolla A, Amann M, Hureau TJ, Venturelli M. Prior involvement of central motor drive does not impact performance and neuromuscular fatigue in a subsequent endurance task. *Med Sci Sport Exerc.*

III. **Laginestra FG**, Favaretto T, Giuriato G, Martignon C, Barbi C, Pedrinolla A, Cavicchia A, Venturelli M. Mechanoreflex-induced chronotropic and ventilatory responses to passive leg movement are potentiated by metabolites accumulation. *Submitted / Under review.*

Posters and oral communications

I. **Laginestra FG**, Amann M, Kirmizi E, Giuriato G, Ruzzante F, Tarperi C, Venturelli M. Electrical stimulation-induced fatigue in the contralateral leg impairs endurance exercise performance. *11th Congresso Nazionale SISMES. Bologna,* 2019.

II. **Laginestra FG**, Favaretto T, Cavicchia A, Giuriato G, Pedrinolla A, Martignon C, Venturelli M. Mechanoreflex-induced chronotropic and ventilatory responses are sensitized by metabolites accumulation. *APS Integrative Physiology of Exercise. Virtual Platform,* 2020.

III. **Laginestra FG**, Cavicchia A, Vanegas-Lopez JE, Barbi C, Pedrinolla A, Amann M, Hureau TJ, Venturelli M. The effect of prior exercise involving central motor drive on subsequent high-intensity knee-extensors endurance performance. *12th Congresso Nazionale SISMES. Padova,* 2021.

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List of abbreviations

^{31}P-MRS	Phosphorus magnetic resonance spectroscopy
BF	Biceps femoris
Ca^{2+}	Calcium ions
CMEP	Cervicomedullary evoked potential
CO	Cardiac output
EMG	Electromyography
EMG_{RMS}	Root mean square of the electromyographical signal
EVO	Electrically-evoked
FBF	Femoral blood flow
FiO_2	Fraction of inspired oxygen
f_R	Breathing frequency
HR	Heart rate
iEMG	Integrated electromyography
KE	Knee-extension
LVC	Leg vascular conductance
MAP	Mean arterial pressure
MEP	Motor-evoked potential
M_{max}	Maximal compound muscle action potential
MRFD	Maximal rate of force development
MRR	Maximal relaxation rate
M_{sup}	Superimposed compound muscle action potential
No-PreF	Non-prefatigued
P_i	Inorganic phosphate
PPO	Peak power output
PreF	Pre-fatigued
RF	Rectus femoris
RMSSD	Root mean square of successive differences
RPE	Rating of perceived exertion
$Q_{tw,pot}$	Potentiated quadriceps twitch force
SV	Stroke volume
TMS	Transcranial magnetic stimulation
VA	Voluntary activation
$\dot{V}_E$	Minute ventilation
$\dot{V}O_2$	Oxygen consumption
$\dot{V}CO_2$	Carbon dioxide production
$\dot{V}_E/\dot{V}CO_2$	Ventilatory equivalent for CO_2
$\dot{V}_E/\dot{V}O_2$	Ventilatory equivalent for O_2
VL	Vastus lateralis
VOL	Voluntary
V_{mean}	Femoral artery mean blood velocity

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CHAPTER 1

Introduction

1.1 Introduction and historical perspective

In 1664, in his "Treatise on Man", French philosopher René Descartes imagined how threads would run from the skin to the brain to transmit signals about perceptions (Descartes, 1664). However, it is not until the first half of the 20th century, that groundbreaking discoveries about the existence of specialized sensory neurons reacting to different stimuli, laid the basis for the study of afferent feedback in the regulation of various bodily functions (Perl, 1994). Even before the discovery of these sensory neurons, the existence of a reflex mediating cardiovascular responses and originating in the skeletal muscle was postulated already by Krogh and Lindhard when they observed that electrically-induced contractions were able to evoke a response in heart rate and blood pressure (Krogh & Lindhard, 1917). However, the first formal investigation on this mechanism stems from the studies by Alam and Smirk in 1937, whom in their seminal work, found that blood pressure response to ischemic exercise was substantially higher compared to the situation in which exercise was performed under freely perfused conditions (Alam & Smirk, 1937). Also, they observed that blood pressure was not returning to baseline values when ischemia was maintained during the post-exercise recovery phase. In a follow-up case study, they showed the heart rate and blood pressure responses in a subject who had an intact motor control in both legs, but one of the legs had a compromised sensory signaling due to a spinal lesion (Alam & Smirk, 1938). This experiment showed that while voluntary exercise evoked a normal, rising, response in both legs, post-exercise ischemia led to a maintenance of the response only in the leg with intact feedback. This represents clear evidence of the existence of a peripheral neural reflex controlling circulatory adjustments. Successively, landmark independent studies from Coote and Kaufman, contributed to establish the nature of this phenomenon and to characterize the properties of the involved afferent fibers (Coote *et al.*, 1971; Kaufman *et al.*, 1983). Specifically, the study by Coote and co-workers found that when the dorsal roots of the spine were sectioned, ventral roots stimulation did not evoke a pressor response in anesthetized cats (Coote *et al.*, 1971). This led to the conclusion that this phenomenon had a reflex nature, and that the "trigger" must have been the metabolic changes in the working muscle. Furthermore, several studies spearheaded by Kaufman and colleagues, characterized the firing properties of these afferent fibers (detailed in later sections of this dissertation) From this point onwards, the studies about the role on group III/IV afferent fibers increased, assuming a pivotal role in our understanding of the hemodynamic and ventilatory adjustment to physical work. Throughout the years, several receptors have been discovered to be involved in metabosensation and mechanosensation, with some of these important discoveries contributing to the assignment of the latest Nobel Prize in physiology to date[1].

However, while the role of group III/IV afferents on hemodynamic adjustments has been known for roughly 100 years, the role they play in fatigue has been only investigated in the last 40 years. Indeed, it was not until the '80s that the importance of these afferents in neuromuscular fatigue started to be recognized with the pioneering work by Bigland-Ritchie and colleagues. In one of their studies, the authors investigated the behavior of motoneuron firing rates through intramuscular electromyography when fatiguing exercise of the *biceps brachii* was followed by either ischemic or freely perfused recovery. Their series of experiments demonstrated that motor neuron firing rates diminished following a prolonged maximal voluntary contraction (Bellemare *et al.*, 1983; Bigland-Ritchie *et al.*, 1986b) but almost displayed a full recovery when the muscle was freely perfused (Bigland-Ritchie *et al.*, 1986b). Contrarily, when recovery was taking place under ischemic conditions, almost no recovery was found in motor neuron firing rates (Bigland-Ritchie *et al.*, 1986b). These results suggested that a reflex mechanism was in play, and that the "sensors" for this mechanism to work, could have been metabosensitive fibers implicated in the cardiovascular response to exercise.

The importance of these afferent fibers is underlined by the discovery that abnormalities in the expression of their functions, is found in several pathologies such as heart failure (Amann *et al.*, 2014; Smith *et al.*, 2020a), hypertension (Barbosa *et al.*, 2016; Sidhu *et al.*, 2019), type 2 diabetes (Roberto *et al.*, 2019), Parkinson's disease (Sabino-Carvalho *et al.*, 2021) and several others (Vianna & Fisher, 2019). This is believed to play an important role in determining the exercise intolerance that accompanies these diseases and may be a potential target for improving clinical outcomes and quality of life of these patients. However, although evidence about the importance of afferent feedback is accumulating, further studies are necessary to understand the role of acute and chronic exercise training in health and disease.

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[1] https://www.nobelprize.org/prizes/medicine/2021/summary/

CHAPTER 2

Background

2.1 Autonomic adjustments to exercise

The physiological response to physical exercise requires the cooperation of different systems in order to maintain an adequate perfusion pressure and meet the metabolic demands of the working muscle. The cardiovascular and pulmonary adjustments to exercise are regulated by the autonomic nervous system, which modulates parasympathetic and sympathetic activity mainly via feedforward and feedback mechanisms. The most important of them are central command, the arterial baroreflex and the exercise pressor reflex, which derives from afferent feedback mechanisms originating in the skeletal muscle (Fisher *et al.*, 2015). An overview of these systems is presented in Figure 2.1. The study of how these mechanisms work in concert and in isolation is not an easy task because of the redundancy within these systems. Redundant physiological mechanisms "operate to govern the behavior of complex systems" in such a way that backup or complementary measures are in place to ensure the correct functioning of the system (Joyner, 2013). The end-result is a complex and integrated interaction of all the neural mechanisms controlling the autonomic adjustments and making sure that the system meets the demands of physical exercise.

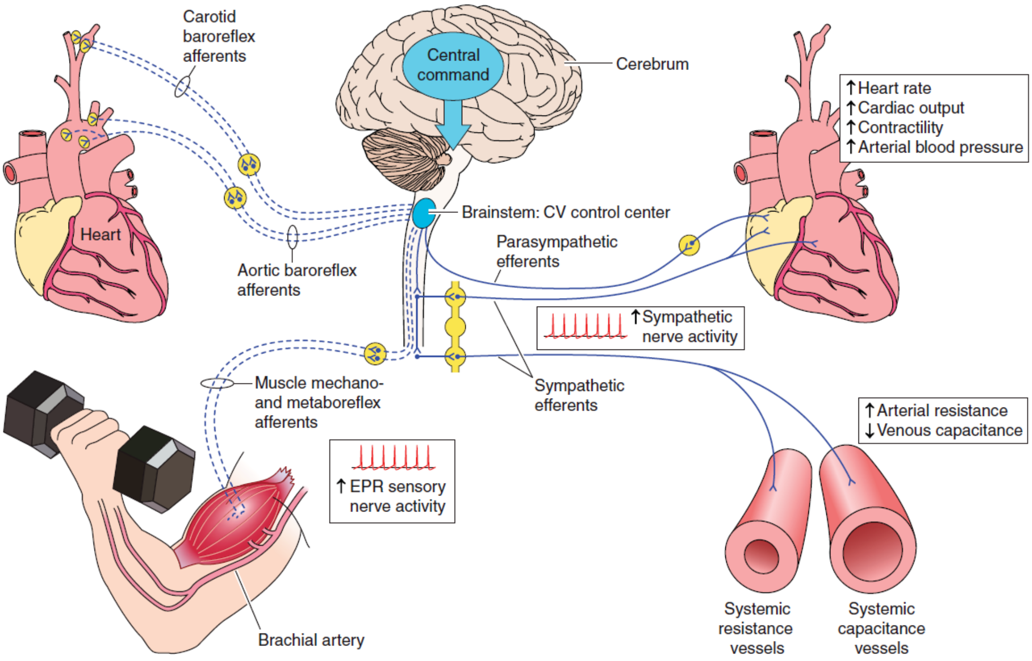

Figure 2.1. Autonomic cardiovascular regulation during exercise. At the onset of exercise descending signals from the brain activate motor and autonomic pathways, decreasing parasympathetic tone and increasing sympathetic drive. Concurrently, also the exercise pressor reflex-mediated responses are sent to the cardiovascular control centers and integrated to participate in these autonomic adjustments. This regulation is further modulated by the arterial baroreflex. Taken from Spranger *et al.*, 2015.

2.1.1 Central command

Central command refers to the feedforward efferent input originating in the higher brain areas, that parallelly activates motor pathways (central motor command) or autonomic branches of the central nervous system, resulting in skeletal muscle contraction, hemodynamic and ventilatory adjustments to exercise. This mechanism, originally called "cortical irradiation", was put forward by Krogh and Lindhard in 1913 (Krogh & Lindhard, 1913), with the observation that some hemodynamic and ventilatory responses increased within 1 s from the onset of exercise. This was later corroborated by studies showing that heart rate increase in anticipation (Ishii *et al.*, 2016) and within the first beat after the start of exercise (Hollander & Bouman, 1975). The rapidity of these adjustments could only be explained by a feedforward regulatory input.

The role of central command has been studied with the use of motor imagery and partial neuromuscular blockade. In the first instance, Williamson and colleagues showed that subjects categorized as "highly hypnotizable" have a higher heart rate, blood pressure response and perceived exertion compared to the low-hypnotizable group during imagined handgrip exercise (Williamson *et al.*, 2002). Neuromuscular blockade using local anesthetics such as tubocurarine, causes a "weakening" of the target muscle, requiring a higher level of central command (and effort) to perform the same work. For example, in a study in which maximal force was reduced by roughly 50%, the response in blood pressure and heart rate at a given absolute load was greater than control condition, while it was the same when a relative intensity was performed (Leonard *et al.*, 1985), highlighting how the hemodynamic adjustments are associated to the levels of central command.

Given the difficulty to obtain direct measures of central command, traditionally, rate of perceived exertion has been used to assess its magnitude (Mitchell, 1990). In fact, central command has been associated with cognitive processes that are effort related (Williamson, 2010). However, the relation between central command and perceived exertion is not straightforward, since the latter may be the result of a complex integration of several other contributors such as feedback mechanisms (Williamson, 2010).

Although many studies have been performed, to elucidate the brain areas associated with central command generation, its neuroanatomy is still elusive. A study utilizing positron emission tomography and bupivacaine injection (*i.e.*, a regional anesthetic) found an increase in the activation of the sensory and supplementary motor areas of the cortex, and the cerebellum during attempted handgrip exercise (Nowak *et al.*, 1999). Other studies utilizing different direct brain stimulation techniques also found subthalamic regions (*e.g.*, hypothalamus) involved in the generation of motor and cardiovascular responses (Eldridge *et al.*, 1985) and the neural circuitry involving the periaqueductal grey (Green & Paterson, 2008). However, given the redundancy in the function of neuroregulatory systems, it is still very challenging to define the exact networks involved in the cardiovascular and respiratory control and a consensus on the *locus* where central command originates does not exist to date.

2.1.2 Arterial baroreflex

The arterial baroreflex is a negative-feedback mechanism which regulates blood pressure on a beat-to-beat basis (Fadel & Raven, 2012; Fisher *et al.*, 2015). It originates from the carotid bodies and aortic arch, where changes in blood pressure stimulate this set of mechanoreceptors, altering sympathetic outflow and ultimately modulating heart rate, stroke volume, and peripheral resistances (Fadel & Raven, 2012). The baroreflex is known to act around a set-point: for example, increases in blood pressure would stretch these mechanoreceptors, which would in turn increase firing of these neuronal structures and cause an increase in vagal tone towards the heart and decreased sympathetic outflow to the periphery (Fadel & Raven, 2012). In healthy conditions, these adjustments will effectively bring blood pressure to the original set point.

In the past, the role of the arterial baroreflex during exercise was debated (Raven *et al.*, 2006). This because a negative feedback loop should not allow for the two regulated variables (heart rate and blood pressure) to increase parallelly as it happens during physical exercise. For this reason, it was initially believed that the baroreflex was not indispensable for the regulation of circulation (Pickering *et al.*, 1972). However, it is now well established that baroreflex is still active during exercise, but its operating set point is shifted towards higher blood pressures without changes in reflex gain (Melcher & Donald, 1981). Successive studies confirmed that the baroreflex stimulus-response curve is shifted upward and rightward during exercise, with this reflex still operating around the prevailing blood pressure (Fadel & Raven, 2012).

Previous studies also showed that both central command and group III/IV afferent feedback play an independent and interactive role on the resetting of baroreflex during exercise (Gallagher *et al.*, 2006). However, the role of central command and group III/IV afferent feedback on baroreflex function are difficult to isolate. Indeed, the vast majority of the studies investigating the role of group III/IV muscle afferents on the baroreflex function, utilized partial or complete limb occlusion to raise the firing of these fibers (Iellamo *et al.*, 1997; Gallagher *et al.*, 2001; Gallagher *et al.*, 2006). However, this approach may not be suited to thoroughly investigate this problem because the accumulation of peripheral fatigue, compromises the muscle response to a given input, requiring higher levels of central command to compensate. To solve this issue, an elegant study recently published used a comprehensive approach involving the combination of voluntary exercise (*i.e.*, requiring central command), electrically-evoked exercise (*i.e.*, without central command), and afferent blockade using fentanyl injection, which does not affect the force-generating capacity and therefore central command (detailed later in the *"Pharmacological afferent blockade"* section) to try to parse out the role of central command and afferent feedback in the baroreflex effects on circulation (Hureau *et al.*, 2018b). The application of neck suction and neck pressure through a collar, simulated a hypertensive and a hypotensive stimulus respectively. Their data showed that the typical upward and rightward shift of stimulus-response curves obtained during electrically-evoked and voluntary exercise were similarly attenuated when afferent feedback was partially blocked. These findings mean that group III/IV afferent feedback plays an important and independent role in baroreflex resetting during exercise, and that this is independent from the involvement of central command.

2.1.3 Exercise pressor reflex

The exercise pressor reflex is a feedback mechanism of autonomic regulation originating in the working skeletal muscle. It is comprised of a metabosensitive branch, sensitive to chemical changes in the intramuscular milieu (*i.e.*, metaboreflex) and a mechanosensitive branch (*i.e.*, mechanoreflex) sensitive to mechanical distortion of the receptors field (Kaufman & Forster, 1996; Fisher *et al.*, 2015). Activation of these afferent neurons evoke an increase in the sympathethic nerve activity and parasympathetic withdrawal, facilitating increases in heart rate, stroke volume, peripheral resistances, all resulting in an increase of blood pressure, and therefore, muscle perfusion pressure (Kaufman & Forster, 1996). All aspects related to this reflex mechanism will be elucidated in the following sections.

Skeletal muscle afferent fibers

The skeletal muscle is innervated by five types of afferent fibers, conveying different kinds of signals to the central nervous system. Group Ia, Ib, and II fibers are large and thickly myelinated fibers, with a conduction velocity between 31 and 120 m/s (Kruger & Light, 2009). These fibers innervate muscle spindles (group Ia, II) and Golgi tendon organs (group Ib) and transmit information to the cerebellum and to the cortex about changes in the length of the muscle and its developed tension. Group III afferents are mainly stimulated by mechanical distortion of the receptive field. These are thinly-myelinated fibers with a conduction velocity of 2.5/30 m/s (Kaufman & Forster, 1996). Finally, group IV afferents are non-myelinated and stimulated by chemical changes happening in the muscle fiber interstitium and their conduction velocity is < 2.5 m/s (Kaufman & Forster, 1996). However, there is no clear-cut distinction between group III and IV fibers, and evidence shows that most of these fibers are polymodal, responding to both mechanical and metabolic stimuli (Kumazawa & Mizumura, 1977). Therefore, it is better to think of these fibers as on a continuum rather than completely distinct.

Classical experiments in this field, demonstrated that group I and II afferent fibers do not partake in the autonomic regulation during exercise (McCloskey & Mitchell, 1972; Tibes, 1977). In fact, when a direct current was passed through the dorsal roots of the spine in anesthetized and decerebrate cats, and selectively blocked these large myelinated afferent fibers, the autonomic adjustments were not impacted (McCloskey & Mitchell, 1972). On the other hand, the application of local anesthetic, which preferentially blocks small afferents, abolished these adjustments. Furthermore, besides cardiovascular and ventilatory adjustment, only group III/IV fibers are also responsible for transmitting pain sensations. This was demonstrated by injection of bradykinin into cats *triceps surae* muscle, which caused a decrease in the firing rate of large (type I and II) afferent fibers, while simultaneously causing an increase in group III/IV afferent fibers firing rates (Mense, 1977). Later, these findings were also confirmed in humans by anesthetizing the skin and selectively blocking group I and II afferent fibers. In this setting, the investigators found that the participants could still feel a painful pressure stimulus, which is likely to be conveyed through group III and IV fibers (Graven-Nielsen *et al.*, 2004).

Anatomy of group III/IV afferent pathway

Group III/IV afferents present a free-nerve ending in the interstitium. These fibers make their first synapse in the dorsal horn of the gray matter of the spine (Kaufman & Forster, 1996). Their axons projects to several spinal and supraspinal sites of the central nervous system. Evidence indicates these afferent fibers mainly synapse onto laminae I and V neurons in the dorsal horn of the spinal cord and their projections are found on the ventromedial nucleus of the thalamus (Craig *et al.*, 1994), on the rostral ventrolateral medulla (Craig, 1995) and on the nucleus tractus solitarii (Craig, 1995; Potts *et al.*, 2003). The nucleus tractus solitarii of the medulla oblongata represent the primary cardiorespiratory control center for the integration of these signals with others coming from the arterial baroreceptors (Aicher & Randich, 1990).

Stimuli and receptors

Group III fibers quickly and strongly respond to the onset of contraction with their discharge rates increasing together with the development of force, and decreasing with fatigue during a static contraction (Kaufman *et al.*, 1983). Mechanoreceptors have been shown to be activated by muscular contractions (Kaufman *et al.*, 1983), tendon/muscle stretch (Hayes *et al.*, 2005), passive dynamic movement (Cui *et al.*, 2006), and compression (Bell & White, 2005). Very little evidence exists about the receptors and channels involved in mechanotransduction. This is mainly due to the lack of commercially available specific antagonists for these channels and receptors (Grotle *et al.*, 2020). Inhibition of mechanogated potassium and cation channels, L-type and T-type calcium channels have been shown to abolish the response to mechanical stimulation and contraction (Hayes & Kaufman, 2001; Hayes *et al.*, 2009). The recent discovery of Piezo1 and Piezo2 channels (Coste *et al.*, 2010) suggest that these channels may be important for the mechanically-activated branch of the exercise pressor reflex (Copp *et al.*, 2016b, a; Sanderson *et al.*, 2019).

Group IV fibers are present in animal and humans in two subsets: metaboreceptors, sensitive to low levels of metabolites, as the ones seen during exercise, and a subset of metabo-nociceptors that are activated during noxious stimulation (Light *et al.*, 2008). Metabolites known to stimulate the metaboreflex are bradykinin (Pan *et al.*, 1993), arachidonic acid (Rotto *et al.*, 1989a), inorganic phosphate (P_i) and lactic acid (Ducrocq & Kaufman, 2020), H^+ (Rotto *et al.*, 1989b), prostaglandins (Mense, 1981), potassium (Rybicki *et al.*, 1985), and adenosine 5'-triphosphate (ATP) (Hanna *et al.*, 2002). Also capsaicin and heat may be involved in the exercise pressor response (Smith *et al.*, 2010). Accordingly, receptors (and pathways) known to play a role in metabosensation are Transient Receptor Potential Vanilloid 1 (TRPV1) receptors, Acid Sensing Ion Channels (ASIC) 1 and 3, Purinergic 2X (P2X) receptors, prostaglandin (PG) receptors, and bradykinin B2 receptors (Kruger & Light, 2009; Grotle *et al.*, 2020).

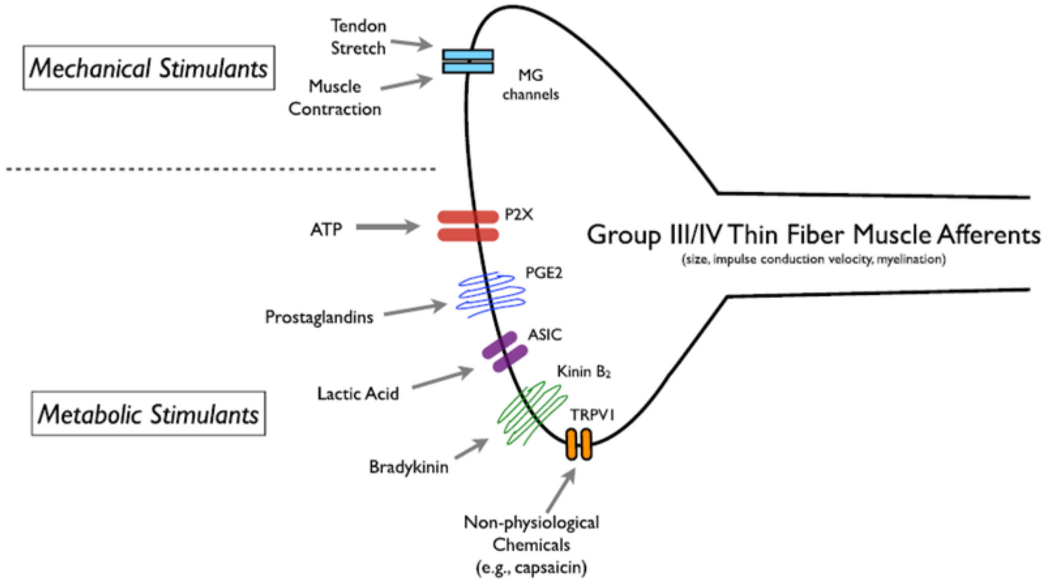

Figure 2.2. Schematic representation of the known stimulants and receptors involved in the exercise pressor reflex. In addition, Piezo1 and Piezo2 mechanoreceptors have been recently discovered to participate in this mechanism. Taken from Greaney *et al.,* 2015.

2.2 The role of group III/IV afferent feedback on autonomic adjustments to exercise

While most of the evidence about the properties and effects of afferent feedback comes from anesthetized and/or decerebrate animal models as described in the previous chapters, different methods have been utilized to study the role of group III/IV afferents in humans or animals *in vivo*. Traditionally, there have been two main ways of studying the role of group III/IV afferent feedback: 1) by increasing the firing of these fibers by different means or 2) by attenuating it through the use of pharmacological agents. In this chapter the main findings on how the afferent-mediated regulation works will be summarized, and emphasis will be put on the methods utilized.

Circulatory occlusion

Traditionally, circulatory occlusion has been utilized to augment firing of metaboreceptors. This can happen post-exercise, isolating its effect from the ones of central command and mechanoreflex, or during exercise, where the synergistic effect of these mechanisms is observed. In humans, a blood pressure cuff is inflated proximally to the exercised muscle to completely or partially arrest circulation to and from the muscle. In animal models, an arterial occluder is used in combination with a flow probe to suddenly reduce and measure blood flow to the hindlimb muscles of the exercising animal (O'Leary *et al.*, 1999; Augustyniak *et al.*, 2001). The "entrapment" of metabolites (or the increased rate of accumulation) in the muscle will then stimulate the firing of metabosensitive afferent fibers. The activation of the metaboreflex evokes an increase in sympathetic nerve activity during exercise, which remains elevated with post-exercise muscle ischemia (Mark *et al.*, 1985; Wallin *et al.*, 1989). This increase in sympathetic outflow is dependent on exercise modality and intensity (Boushel, 2010). Its timing is compatible with the progressive, relatively slow, accumulation of metabolites (Boushel *et al.*, 1998).

It is widely accepted that the activation of the metaboreflex increases blood pressure through increases in sympathetic nerve activity to the peripheral vessels (Victor *et al.*, 1987; Victor & Seals, 1989; Pawelczyk *et al.*, 1997) while its role on central hemodynamics (heart rate, stroke volume, and therefore cardiac output) is more debated. In fact, many studies found that the maintenance of afferent fibers firing through circulatory occlusion, is accompanied by a return to baseline in heart rate. This discrepancy may be related to the "size" of the muscle mass utilized, with larger muscle masses retaining a higher response in heart rate (Pecanha *et al.*, 2017). It is important to note that exercise cessation is accompanied by an immediate increase in parasympathetic drive associated with the cessation of central command (Nishiyasu *et al.*, 1994). Moreover, the rise in pressure that happens when the metaboreflex is activated, will also trigger the engagement of the arterial baroreflex, which would try to buffer the increases in blood pressure by further decreasing heart rate (Nishiyasu *et al.*, 1994; Iellamo *et al.*, 1999). In this scenario, the presence of sympathetic drive caused by the metabolites accumulation in the muscle may be masked by the increase in vagal tone.

The role of stroke volume in the hemodynamic adjustments to metaboreflex engagement are less studied. Important studies in animal models demonstrated that the regulation of stroke volume plays a significant role on the responses to metaboreflex activation (O'Leary & Augustyniak, 1998; Sala-Mercado *et al.*, 2006). In these studies, instrumented dogs were allowed to run on a treadmill at different speeds and levels of metaboreflex activation, performed by inflating a balloon in the hindlimb artery, while stroke volume and indices of ventricular contractility were measured. Their results show that an increased ventricular contractility is responsible for the augmented cardiac output response in this model (Sala-Mercado *et al.*, 2006). Studies in humans are not consistent, given the impossibility of using invasive methodologies. In fact, we have studies showing an unchanged stroke volume during post-exercise ischemia (Pawelczyk *et al.*, 1997; Bastos *et al.*, 2000; Lykidis *et al.*, 2008) or elevated (Crisafulli *et al.*, 2003; Crisafulli *et al.*, 2006; Crisafulli *et al.*, 2011). Some of these discrepancies may be explained by the method used to measure stroke volume (photoplethysmography or Doppler ultrasound *vs.* thoracic impedance cardiography).

One of the main questions about the role of the metaboreflex, concerns its role in restoring muscle blood flow. The muscle bed is a very compliant compartment of the vasculature, which has the potential to attract large quantities of blood flow, and cause hypotension. For this reason, the "sympatholysis" (*i.e.*, vasodilation in the working muscle due to local factors) and the sympathetically-mediated vasoconstriction need to be balanced in order to maintain adequate blood flow to support the muscle metabolism, but also maintain perfusion pressure (Venturelli *et al.*, 2022). The importance of this last mechanism is highlighted by Poiseuille's equation:

$$Blood\ flow = \frac{\Pi \cdot r^4 \cdot (P_1 - P_2)}{8 \cdot \mu \cdot L}$$

Where:
Π = pi constant;
r = vessel radius;
P_1-P_2 = pressure differential between two points the vessel (*i.e.*, perfusion pressure);
μ = blood viscosity;
L = length of the vessel;
Since the radius of the vessel "grows" exponentially with the power of 4, it is immediately recognized how a little change in the constriction/dilation balance may cause large changes in blood flow.

As previously mentioned, the engagement of metaboreflex also depends on the type of exercise employed. In fact, during isometric exercise, the metaboreflex engagement predominantly increases blood pressure by the means of peripheral vasoconstriction, due to the necessity of increasing blood pressure to overcome the mechanical obstruction given by the increases of intramuscular pressure on the vessels (Boushel, 2010). On the other hand, during dynamic exercise, which does not represent such a large mechanical impediment to blood flow, the metaboreflex will increase vasoconstriction in the non-active compartments (*e.g.*, renal), therefore increasing perfusion pressure and blood flow to the working muscle (Boushel, 2010). In case blood flow cannot be increased for any reason, the metaboreflex will induce vasoconstriction also in the working muscle. Some authors hypothesize that under physiological conditions, the rise in blood pressure secondary to activation of the metaboreflex should be mediated by increases in central hemodynamics (*i.e.*, cardiac output), while peripheral resistances-driven increases may be a maladaptive mechanism happening in some patient populations (Hammond *et al.*, 2000; Crisafulli *et al.*, 2007; Sala-Mercado *et al.*, 2007)

However, it is important to understand which are the pitfalls of assessing metaboreflex activation using post-exercise circulatory occlusion. Specifically, this method assesses the role of the metaboreflex in a situation of post-exercise recovery and may not reflect the contribution and role of the metaboreflex during exercise. In fact, the complex redundancy existing in the systems contributing to neurocirculatory adjustments may mask the effects on the manipulated variable by using compensatory mechanisms (Secher & Amann, 2012; Bruce *et al.*, 2019). For example, the increase in blood pressure caused by the activation of the metaboreflex is likely to activate the baroreflex response, which will "try" to increase parasympathetic outflow to the heart, to diminish heart rate. Secondly, this method predominantly stimulates subsets of group III/IV afferents which may not be active with physiological concentrations of metabolites reached during exercise (Light *et al.*, 2008; Jankowski *et al.*, 2013; Amann *et al.*, 2015).

Pharmacological afferent blockade

Important evidence on the role of group III/IV afferent feedback in vivo during exercise, comes from studies utilizing pharmacological blockade through different agents. This technique has an important feature compared to other methods because it allows to evaluate the contribution of afferent feedback while other regulatory systems are still in place, representing therefore a more ecological setting compared to other methods such as post-exercise circulatory occlusion. These methods contributed to provide convincing evidence that afferent feedback plays an important role in the hemodynamic and ventilatory regulation to exercise in humans, which is now widely accepted.

Initial studies utilized epidural injection of lidocaine to partially block the communication between the exercising muscles (*i.e.*, lower limbs muscles) and the central nervous system regulatory centers (Mitchell *et al.*, 1989). However, this type of spinal block also affects the ventral roots of the spine and therefore muscle force-generating capacity, requiring a higher level of central motor command and effort to produce the same work. In contrast, the lumbar (between vertebrae L_3 and L_4) intrathecal injection of fentanyl which is a potent μ-receptors agonist, selectively blocks the activity in the ascending sensory pathways (*i.e.*, dorsal roots) without affecting force-generating capacity and central motor drive (Grant *et al.*, 1996; Standl *et al.*, 2001).

This approach has been demonstrated to be able to temporarily block roughly 60% of the response (Hureau *et al.*, 2018b). This "partial" block is due to the fact that group III/IV muscle afferents are comprised of two types of opioid receptors, namely μ and δ (Yaksh & Reddy, 1981) and not all of them possess μ-receptors (Wang *et al.*, 2018). Therefore, the effects seen in these studies may even be somewhat underestimated.

Using this approach, Amann et al. demonstrated that the increases in blood pressure and ventilation during exercise was attenuated with lumbar fentanyl injection in healthy individuals (Amann *et al.*, 2010, 2011). These findings represented the first strong evidence that afferent feedback plays a quintessential role in the regulation of hemodynamic and ventilatory adjustments in humans. Importantly, when afferent feedback was attenuated, leg blood flow was impaired probably due to the lower perfusion pressure. This finding represent strong evidence in support of the idea that the metaboreflex is a flow-raising reflex rather than a pressure-raising one (O'Leary & Joyner, 2006). Furthermore, studies from the same group and other independent groups, applied this method to study these adjustments in different disease populations. Together, these findings showed how abnormal afferent feedback from group III/IV is party accountable for impaired leg blood flow and stroke volume in heart failure patients (Amann *et al.*, 2014; Smith *et al.*, 2020b), and blood pressure response to exercise in hypertensive patients (Barbosa *et al.*, 2016; Sidhu *et al.*, 2019). Importantly, these findings may be crucial to explain the well-established exercise intolerance in these patients.

Exogenous metabolites infusion

In an important *in vitro* study using neurons from mouse dorsal root ganglions, Light et al. determined a combination of metabolites (ATP, protons, lactate) that mimicked the concentrations found in the muscle during rest, exercise, and post-exercise ischemia (Light *et al.*, 2008). This study found two subpopulations of group III/IV afferents: one responding to low levels of metabolites, observed during relatively low-moderate intensity exercise, and another responding to "noxious" levels of metabolites as the ones associated with muscle ischemia. These findings were further confirmed and extended by the experiments of Jankowski et al., who employed an *ex vivo* model consisting of a mouse forepaw muscle, median and ulnar nerve, and dorsal root ganglion (Jankowski *et al.*, 2013). With this method, the authors could infuse this metabolic "soup" directly into the muscle, which represent a more ecological setting to study the responses evoked by afferent signals. However, although these experiments *in vitro* and *ex vivo* provided important information about group III/IV afferent fibers signaling and the receptors involved in the transduction, the missing piece of the puzzle was related to sensations evoked by the stimulation of these afferents. Important insights on this issue were provided in a study by Pollak and colleagues who injected this metabolites mixture in a hand muscle of human participants (Pollak *et al.*, 2014). Consistent with previous findings, low levels of metabolites evoked non-painful sensations as fatigue. However, high levels of metabolites, such as the one found during high-intensity exercise and ischemia, evoked aching and burning sensations. These important results raise an important concern about studies utilizing post-exercise ischemia or hypertonic saline injection to study group III/IV afferent feedback, since the use of these methods may preferentially activate subpopulations of these fibers which may not be active during normal exercise in humans.

Mechanical stimulation

Mechanoreceptors role in cardiorespiratory adjustments to exercise has been investigated through the use of passive stretch (Fisher *et al.*, 2005; Drew *et al.*, 2008a), passive movement (Nobrega & Araujo, 1993; Vianna *et al.*, 2010) or compression (Williamson *et al.*, 1994).

Administration of gadolinium, which is an inhibitor of mechanogated potassium and cation channels, L-type and T-type calcium channels, has been shown to reduce the autonomic adjustments to tendon stretch in cats (Hayes & Kaufman, 2001). Lately, a spider toxin (*i.e.*, GsMTx4) has been found effective in blocking currents deriving from Piezo1 and Piezo2 channels. The use of this toxin in decerebrate rats, has been found to effectively reduce the blood pressure, heart rate, and renal sympathetic response to intermittent contractions and stretch (Copp *et al.*, 2016a; Sanderson *et al.*, 2019).

Passive hindlimb stretch in cats causes an increase in heart rate, which is accompanied by an increase in cardiac sympathetic activity and a pronounced reduction in parasympathetic activity (Matsukawa *et al.*, 1994; Murata & Matsukawa, 2001). These findings are corroborated by the studies by Gladwell and colleagues, who found that passive calf stretch evokes a transient increase in heart rate which is accompanied by a decrease in parasympathetic indices of heart rate variability (Gladwell & Coote, 2002; Gladwell *et al.*, 2005). This change was abolished by cholinergic blockade, strengthening the idea that a decrease in the parasympathetic drive to the heart represent the mechanistic link between this increase in heart rate. Beside this effect on the heart, some studies on animal models showed that passive stretch may have an impact also on peripheral hemodynamic regulation, by increases in muscle and renal sympathetic nerve activity (Victor *et al.*, 1989; Hill *et al.*, 1996; Koba *et al.*, 2006). In humans, Cui et al. found that dynamic wrist stretch elicits transient increases in blood pressure and muscle sympathetic nerve activity (Cui *et al.*, 2006).

As previously mentioned, a subset of group III fibers present polymodal characteristics and it has been shown to be affected by the buildup of metabolites such as bradykinin (Kaufman *et al.*, 1982), byproducts of arachidonic acid metabolism (Rotto & Kaufman, 1988), and lactic acid (Sinoway *et al.*, 1993). Moreover, the responses to mechanoreflex activation may be enhanced when stimulated concomitantly with the metaboreflex. Indeed, previous studies found that heart rate, blood pressure, and muscle sympathetic nerve activity were amplified when passive stretch was superimposed to post-exercise ischemia, suggesting an interaction between the mechanoreflex and the metaboreflex (Cui *et al.*, 2008; Drew *et al.*, 2008a). On the other hand, Fisher et al. showed that heart rate response to static calf stretch was not different after exercise bouts carried out at different intensities, and therefore likely different levels of metabolites accumulation (Fisher *et al.*, 2005). However, given that different subsets of group III/IV muscle afferents respond to different stimuli, there is evidence that mechanically-sensitive fibers may be preferentially activated during movement compared to static stretch (Hayes *et al.*, 2005).

2.3 Neuromuscular fatigue

The study of neuromuscular fatigue has traditionally been one of the central and "hot" topics of research in exercise physiology. This interest is sparked from the fact that the development of fatigue is a common feature in many situations, from the highest-level sporting events, where it can separate a winner from a loser, to the impact it can have on the everyday life of several patients. Therefore, studying the aetiology of fatigue is an important and still somewhat unsolved point in our understanding of this phenomenon.

The seminal work of Angelo Mosso in 1891 entitled *"La fatica"* is often referred to as the first published record attempting to elucidate the mechanisms of fatigue (Mosso, 1891). In his experiments, Mosso demonstrated that the reduction in force-generating capacity was not solely a product of muscular failure, but that it could be also negatively affected by intense mental tasks (*i.e.*, lecturing). Therefore, Mosso enunciated that fatigue entailed both the measurable diminution of muscular force and the non-measurable sensation that accompanies it. While most of the research focused on the first aspects, only recently the role of sensations and perceptions on fatigue received considerable attention (St. Clair Gibson *et al.*, 2003; St Clair Gibson *et al.*, 2018). This "two-domain" concept expressed by Mosso was ahead of its time, and still today, after some re-elaborations possess validity (Enoka & Duchateau, 2016). Indeed, Enoka and Duchateau (2016) suggested that fatigue can be divided into two components: perceived fatigability (*i.e.*, changes in the sensations that regulate the integrity of the performer) and performance fatigability (*i.e.*, the decline in an objective measure of performance over a discrete period). This objective performance decline in a given task is the result of impairments in the neuromuscular system, which is therefore encompassed by the term "neuromuscular fatigue".

Neuromuscular fatigue can be defined as "a reduction in the force-generating capacity of a muscle or muscle group" (Bigland-Ritchie *et al.*, 1986a; Gandevia, 2001). However, this concept is general and there are other traits of fatigue that have been highlighted in successive definitions:

1) The inclusion of the possibility of a decline in velocity, and therefore power, when considering the loss of performance of a muscle or muscle group (NHLBI, 1990).
2) The transient, and therefore reversible, nature of fatigue which distinguishes it from weakness, damage, or the chronic fatigue present in patients populations (NHLBI, 1990).
3) The possibility of having fatigue regardless of whether the task can be successfully sustained or not. This distinguishes "fatigue" from "exhaustion" (Bigland-Ritchie & Woods, 1984; Enoka & Stuart, 1992; Søgaard *et al.*, 2006). Indeed, neuromuscular fatigue starts to develop soon

after the task begins, while exhaustion represent only the endpoint, which is a consequence of fatigue (Barry & Enoka, 2007).

4) The increase in the effort perceived to perform the same work (Enoka & Stuart, 1992).
5) During recovery, force output may be back to optimal levels but some of the components may still be recovering (*e.g.*, prolonged low-frequency force depression) (Edwards *et al.*, 1977).

The expression of voluntary force is the result of a sequence of events happening in the motor pathway (Gandevia, 2001)(Figure 2.3). Firstly, the movement is planned in higher brain centers (upstream from the motor cortex). Subsequently, descending neural drive is transmitted from the motor cortex to the spinal motor neurons and leads to the recruitment of the motor units needed to accomplish the task. The motor unit, which comprise the motor neuron and the fibers it innervates, is the smallest functional (controllable) unit in the motor system. Once the action potentials travel through the motor axons, they reach the neuromuscular junction, where they are transmitted to the muscle fibers. Finally, the propagation along the muscle membrane and the consequent calcium ions (Ca^{2+}) release from the sarcoplasmic reticulum, leads to cross-bridges formation in the actomyosin complex and the production of muscle force. An impairment of neuromuscular function may result from failure at any step of this chain. When failure happens at sites proximal to the neuromuscular junction, this is termed central fatigue, while at or distal to the neuromuscular junction goes under the name of peripheral fatigue (Gandevia, 2001; Allen *et al.*, 2008). Many factors are known to influence the development of neuromuscular fatigue such as exercise modality (*e.g.*, sustained or intermittent) (Bilodeau, 2006), intensity and duration (Behm & St-Pierre, 1997; Place *et al.*, 2009), the fiber type of the tested muscle (Harridge *et al.*, 1996), age and sex of the participants (Hunter, 2009; Sundberg *et al.*, 2019).

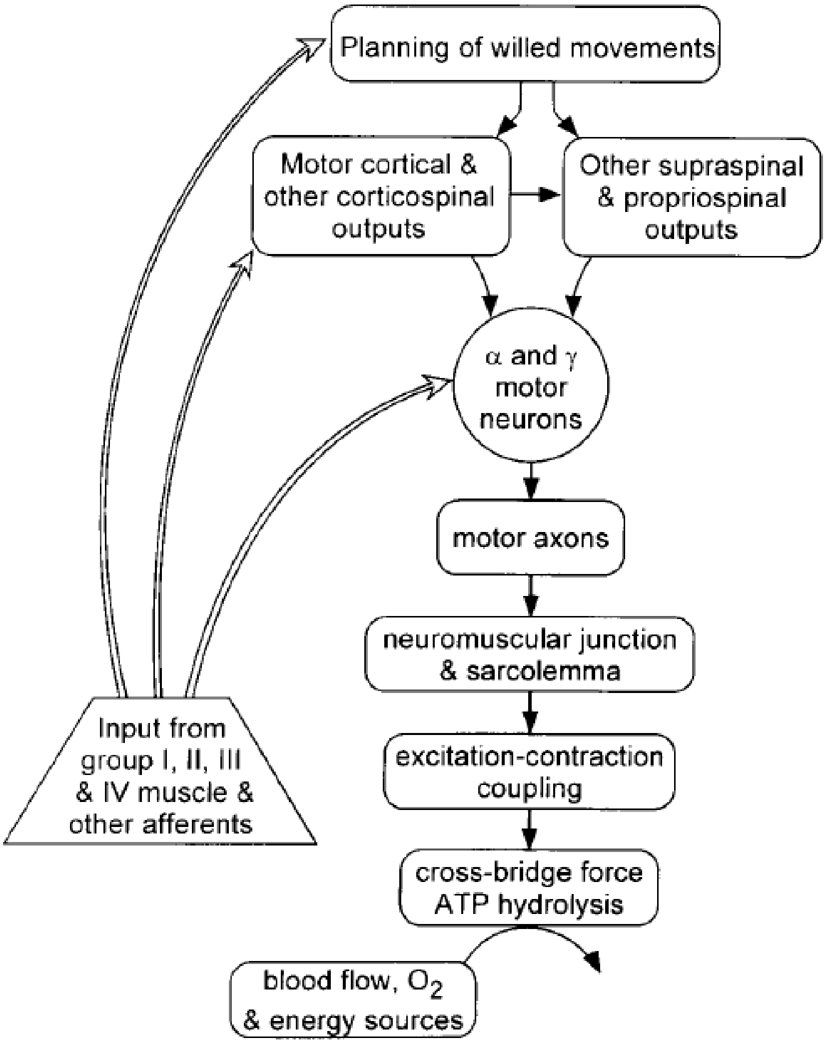

Figure 2.3. Processes involved in the production of voluntary force. The neuromuscular junction represents the point dividing the central from the peripheral component of fatigue. Taken from Gandevia, 2001.

2.3.1 Central fatigue

Central fatigue can be defined as the progressive exercise-induced reduction of voluntary activation of the muscle (Gandevia, 2001; Taylor & Gandevia, 2008). It is usually assessed using the twitch interpolation technique during a maximal voluntary contraction (MVC), developed by Merton in 1954 (Merton, 1954). This technique consists in delivering a supramaximal muscle/nerve stimulation while the subjects is performing an MVC. This supramaximal stimulation, which can be electrical or magnetic in nature, will activate any motor unit which is not recruited or firing fast enough, resulting in an extra twitch in the force output. When this extra twitch (also named superimposed twitch) is apparent, voluntary activation of the muscle is less than

100%, demonstrating a central deficit in the ability of producing force. Voluntary activation of the studied muscle is then calculated by subtracting the ratio between the superimposed twitch and a twitch evoked at rest from the maximal obtainable voluntary activation (*i.e.*, 100%). This technique relies upon the fact that a linear, inverse relationship exists between the superimposed twitch amplitude and the voluntary force at the moment of stimulation (Taylor, 2009). Exercise-induced changes in voluntary activation represent evidence of central fatigue. Even though this technique has some pitfalls, and it has been often subject of scrutiny (de Haan *et al.*, 2009; Dotan *et al.*, 2021), it is nowadays considered as a valid tool to investigate central fatigue.

During a sustained 2-min MVC, Gandevia et al. showed that voluntary force begins to fall almost immediately and superimposed twitch force increases, meaning that central fatigue develops from the first moments of contraction (Gandevia *et al.*, 1996). Furthermore, of the 60% drop in MVC found at the end of contraction, 25% is ascribable to supraspinal fatigue. While measuring voluntary activation when the fatiguing task is maximal is straightforward, assessing central fatigue induced by submaximal contractions is not possible. This leads the investigators to either utilize maximal contractions to induce fatigue, or to place brief MVCs at regular intervals during a submaximal fatiguing protocols (Bigland-Ritchie *et al.*, 1986a). With this latter approach, it has been shown that relatively low intensity contractions (<30% MVC) lead to a substantial loss in voluntary activation and therefore central fatigue. Moreover, Søgaard showed how even the maintenance of a low-intensity elbow flexors contraction (15% MVC) for 40 minutes developed substantial central fatigue and required a disproportionate increase in the rate of perceived exertion (~2/10 to ~8/10) while EMG increased “only” from ~7% to ~20% (Søgaard *et al.*, 2006). A multiple linear regression showed that EMG, the decrease in resting twitch and the decline in voluntary activation contributed significantly to explaining the increase in rate of perceived exertion. Finally, central fatigue occurs sooner and is more pronounced during sustained compared to intermittent contractions (Bilodeau, 2006), and it is also more relevant for low-intensity contractions compared to maximal or high-intensity contractions where the prevalence of the force loss will be peripheral in nature (Bigland-Ritchie *et al.*, 1983; Taylor & Gandevia, 2008).

The central nervous system can modulate force output by increasing/decreasing motor unit recruitment, or by changing the firing rate of the active motor units (Heckman & Enoka, 2012). During repeated (or sustained) maximal isometric contractions, motor units firing rates decline substantially (Taylor & Gandevia, 2008) . This may be the result of a decrease in the excitatory input (*i.e.*, neural drive), modulation of intrinsic properties of the motor neuron, the effects of neuromodulators (*e.g.*, serotonin or noradrenaline) or increase in inhibitory inputs (Taylor & Gandevia, 2008).

Mechanisms of central fatigue

Central fatigue arises when neural drive is suboptimal and not able to fully activate the working muscle (Gandevia *et al.*, 1996). A decrease in motor neurons firing rates appears to be a key factor in the development of central fatigue. Indeed, several studies found a slowing of motor neurons firing rates both using maximal (Bigland-Ritchie *et al.*, 1983) and submaximal fatiguing contractions (Garland *et al.*, 1994). A classical hypothesis (*i.e.*, muscle wisdom hypothesis) suggested that this observed decrease was a mechanism put forward by the muscle to try to preserve its force-generating capacity (Marsden *et al.*, 1983). However, these studies were conducted in a situation in which the muscle was stimulated at rates that are never observed in human muscles. A later study at physiological ranges (30-15 Hz), failed to confirm this hypothesis, and suggested that slowing of motor neuron firing rates does not "protect" against fatigue, but rather it enhances it (Fuglevand & Keen, 2003). As previously mentioned, three mechanisms are known to potentially contribute to this phenomenon: a decrease in the excitatory input, modulation of intrinsic properties of the motor neuron, and an increase in inhibitory inputs (Taylor & Gandevia, 2008).

Descending excitatory drive and corticospinal excitability

Descending drive from the motor cortex may become suboptimal during fatiguing exercise. This means that either the drive from the cortex decreases during fatiguing exercise, or that it becomes less able to appropriately drive the spinal motor neurons, which may have become less sensitive to the synaptic input (Taylor & Gandevia, 2008).

Insights into this issue come from studies investigating the role of the excitability of the corticospinal pathway during fatiguing exercise. Corticospinal excitability can be defined as the efficacy of the corticospinal pathway to relay neural signals from the higher brain areas to the exercising muscle (Weavil & Amann, 2018). Two non-invasive procedures have been traditionally employed in combination to understand the role of cortical and spinal excitability in fatigue. Firstly, when a transcranial magnetic stimulation (TMS) is applied to the contralateral motor cortex, a near synchronous, short-latency EMG response called motor-evoked potential (MEP) is observed in the target muscle. This response is typically normalized by the maximum compound muscle acion potential (M_{max}) to account for changes in peripheral transmission. Increases in MEP amplitude or area represent an augmented overall corticospinal excitability while conversely, smaller MEPs represent a decrease. However, this observed response does not allow to parse out the contribution of cortical *vs.* spinal mechanisms. For this reason, another magnetic or electrical stimulation can be administered to the cervicomedullary junction, near the pyramidal decussation to bypass the motor cortex. Since this stimulation activates a similar portion of large corticospinal axons as TMS, it is largely monosynaptic, and is not subject to presynaptic inhibition as other measures, it is sensitive to changes in motor neuron excitability (McNeil *et al.*, 2013). The observed response is termed cervicomedullary motor-evoked potential (CMEP). Therefore, beside serving as a measurement of spinal excitability, it can be used as a normalization factor for MEPs to “isolate” cortical changes.

As previously said, sustained submaximal isometric contractions lead to an increase in the electromyographical activity for a constant force output (Søgaard *et al.*, 2006). If TMS is administered during the task, an increase in MEP is progressively observed (Gandevia *et al.*, 1996; Taylor *et al.*, 1996). This increase is consistent with an augmented excitatory drive, recruiting fresh motor units, and acting as a compensatory mechanism for the loss in spinal excitability and contractile failure at the muscle level (Taylor & Gandevia, 2008). However, when a single pulse TMS is applied on top of a sustained, fatiguing MVC, a twitch-like response is found indicating that motor cortical output is suboptimal (Taylor *et al.*, 2006). Therefore, supraspinal fatigue develops even though excitability of the motor cortex increases.

In vivo evidence shows that during sustained maximal isometric contractions the motor neurons become less responsive to synaptic input (*i.e.*, motor neuron excitability decreases) (Søgaard *et al.*, 2006). This observation is corroborated by studies on animal preparations showing that even when a constant current is injected into the motor neuron, there is a physiological slowing in its firing rates (Brownstone, 2006). Also, when the subjects are asked to maintain a steady firing rate (obtained through instant feedback from single motor unit recordings), mean EMG signal increases, implying that a higher excitatory input is required to drive the motor neurons that have become less responsive to synaptic input (Johnson *et al.*, 2004). Furthermore, CMEP responses during a sustained maximal (Butler *et al.*, 2003) or submaximal (McNeil *et al.*, 2011a) elbow flexors and knee-extensors (Finn *et al.*, 2018) are drastically reduced. Also, since corticospinal stimulations follow the Henneman's size principle, small CMEPs are more impacted than larger CMEPs (McNeil *et al.*, 2011a). This because the motor neurons pool involved in small CMEPs is recruited earlier in the contraction, being more subject to the negative effects of repetitive activation.

Intracortical inhibitory mechanisms

When a TMS pulse is applied during a contraction, the motor-evoked potential is followed by a period of silence in the EMG signal lasting ~200-300ms, which is termed silent period. While historically the silent period was thought to represent intracortical inhibitory mechanisms, it is now accepted that it represents both spinal (earlier part) and cortical (latter part) mechanisms (Weavil & Amann, 2018). These intracortical inhibitory mechanisms are governed by γ-aminobutyric acid (GABA), which is an inhibitory neurotransmitter present in the central nervous system, which hyperpolarize the post-synaptic neuron making it less likely to fire in response to a given input (Rosenthal *et al.*, 1967).

During a fatiguing contraction the silent period lengthens (Søgaard *et al.*, 2006; Taylor & Gandevia, 2008; Vernillo *et al.*, 2018) or stays the same (Goodall *et al.*, 2012; Sidhu *et al.*, 2017) and its recovery time is related to task duration (Gruet *et al.*, 2013)A previous study using intermittent quadriceps contractions showed that exercising with attenuated feedback from group III/IV afferents shortened the silent period, demonstrating a role of these afferent in increasing intracortical inhibition (Hilty *et al.*, 2011b). This effect was later confirmed during whole body cycling (Sidhu *et al.*, 2018).

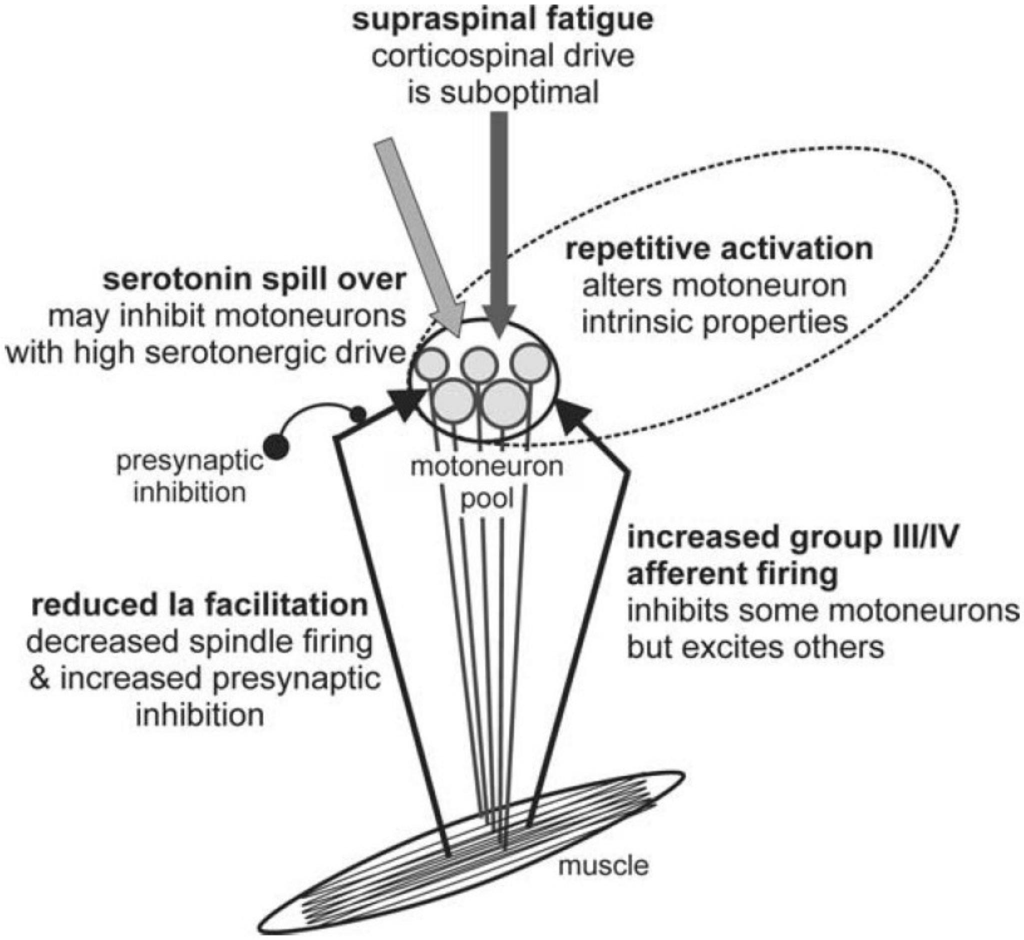

Figure 2.4. Possible mechanisms contributing to central fatigue during exercise. Taken from Taylor *et al.*, 2016.

Afferent-mediated inhibition and disfacilitation

Another cause of central fatigue may be the changes in excitatory (group Ia) and inhibitory (group Ib, groups III/IV, and Renshaw cells) inputs coming from afferent feedback. While group Ib fibers and Renshaw cells activity is reduced during fatiguing exercise and do not appear to play a substantial role, group Ia and group III/IV afferent have been shown to be significantly involved in fatigue. The effects of group III/IV on fatigue will be discussed in a later section (see "The role of group III/IV afferent feedback on central fatigue").

Group Ia afferent feedback is known to have a facilitatory effect on the motor neuron pool (Taylor *et al.*, 2016). Using microneurography, it has been shown that these fibers decrease their discharge rates during a sustained fatiguing contraction of the ankle dorsiflexors (Macefield *et al.*, 1991). This decrease would in turn lead to less excitatory drive on the motor neuron pool (*i.e.*, disfacilitation). The role of group Ia fibers on the motor neuron pool comes predominantly from studies utilizing short and long-latency reflexes pathways by peripheral nerve stimulation with weak currents (Duchateau & Hainaut, 1993). However, this method has several caveats (Gandevia, 2001). Interestingly, a recent study using distal tendon vibration to increase group Ia discharge while concurrently assessing motor neuron excitability found that exercise-induced impairment of CMEPs was not ameliorated by an increase in the firing of these afferents (McNeil *et al.*, 2011b). These results represent evidence that changes in group Ia firing rates may not be of primary importance in determining motor neuron responsiveness.

Exercise-induced changes in neurotransmitters

Neurotransmitters are molecules used by the central nervous system to transmit messages between neurons and other target cells. Exercise-induced changes in the concentrations of various neurotransmitters such as serotonin, dopamine, and noradrenaline have been associated with central fatigue. Since the original formulation of the hypothesis that changes in brain serotonin concentration affect perception of effort and fatigue (Newsholme *et al.*, 1987), contrasting findings have been reported in the literature. Studies on rats, demonstrated that administration of a serotonin agonist or antagonist respectively impaired and improved performance (Bailey *et al.*, 1993). Accordingly, a study in humans found that when a serotonin reuptake inhibitor (*i.e.*, paroxetine) was administered before the completion of a cycling performance task, subjects presented a lower performance than under placebo conditions (Wilson & Maughan, 1992). On the other hand, successive studies failed to demonstrate a role for serotonin on performance (Meeusen *et al.*, 2001; Piacentini *et al.*, 2002; Roelands *et al.*, 2009). The contrasting findings are attributable to the complexity of the serotonin system and suggest that it is unlikely that serotonin plays a central role in fatigue by its own. However, it is plausible that serotonin may exert an influence on the fatiguing processes by working in concert with other neurotransmitters such as dopamine and noradrenaline and that a role for high environmental temperature may have a role in this interaction (Roelands & Meeusen, 2010).

More recently, the interest for a role of serotonin on fatigue has surged again. Indeed, in vitro experiments on a turtle spinal cord model, suggested that the increase in the extracellular concentrations of serotonin (*i.e.*, serotonin spillover) may reduce motor neuron excitability (Perrier & Cotel, 2008; Cotel *et al.*, 2013). This finding has found some confirmations in humans, where administration of a serotonin reuptake inhibitor improved induced a greater degree of exercise-induced drop in voluntary activation during fatiguing exercise, that was accompanied by a decrease in the motor neuron excitability measured by peripheral nerve stimulations (*i.e.*, F-waves). Altogether, these findings mean that serotonin spillover may not act on supraspinal sites as initially thought, but rather on spinal excitability, by modulating intrinsic motor neuron properties.

2.3.2 Peripheral fatigue

Peripheral fatigue, also called "contractile fatigue", is the result of biochemical changes happening distally to the neuromuscular junction leading to excitation-contraction coupling failure (Allen *et al.*, 2008). Contrarily to central fatigue, larger contributions of peripheral fatigue to the exercise-induced decline in force production happen at higher exercise intensities (Taylor & Gandevia, 2008). This observation may be related to muscle perfusion and restriction of blood flow happening during muscle contractions due to the pressure exerted by the muscle on the vasculature (Barcroft & Millen, 1939). Importantly, higher contraction forces cause a higher hindrance to blood flow (Barcroft & Dornhorst, 1949) which is of paramount importance in maintaining O_2 delivery to the muscle, but also for the washout of metabolic by-products away from the muscle. Indeed, several studies demonstrated how the rate of development of fatigue is tightly related to O_2 delivery (Amann & Calbet, 2008). A suboptimal O_2 delivery, due to lower oxygen content (Amann *et al.*, 2006b) or due to less blood flow (Broxterman *et al.*, 2014), results in an exacerbation of the fatigue processes. Moreover, recovery of neuromuscular function does not take place when the muscle is held ischemic after exercise (Broxterman *et al.*, 2015a) or when the muscle is reperfused by deoxygenated blood (Hogan *et al.*, 1999), underlying the importance of O_2 delivery in peripheral fatigue.

While most mechanistic evidence for the development of peripheral fatigue comes from studies on isolated fibers (Allen *et al.*, 2008), peripheral fatigue is usually measured *in vivo* by observing the exercise-induced changes in the force produced by an electrical single, doublet, or tetanic supramaximal stimulation (Behm *et al.*, 1996). A single nerve/muscle stimulation results in a mechanical force response (*i.e.*, muscle twitch) and an electrical event (*i.e.*, muscle compound action potential, M-wave). The latter is given by the spatial summation of individual action potential arising from the depolarization of the motor axons and it is thought to reflect the propagation of action potentials across the sarcolemma (Rodriguez-Falces & Place, 2018). The assessment of peripheral fatigue is usually performed with a supramaximal intensity compared to the one needed to evoke a maximal response in twitch force and M-wave. This because with the development of neuromuscular fatigue, the threshold for axonal excitation may increase (Kernell & Monster, 1982). Therefore, the maximal stimulation intensity obtained pre-exercise may not be enough to recruit the same motor units post-exercise, resulting in a lower twitch force and an overestimation of exercise-induced peripheral fatigue. For this reason, a supramaximal intensity (usually between 120% and 200% of the intensity evoking a plateau) is used. Moreover, when assessing the force response to electrical stimulation, it is important to consider that this response is not only influenced by fatigue but also potentiation and that these behaviors may coexist (Rassier & Macintosh, 2000). Potentiation is a phenomenon deriving from previous activation of the muscle, which phosphorylation or the myosin light chain (particularly in type II fibers) and increases Ca^{2+} sensitivity of the acto-myosin complex (Blazevich & Babault, 2019). For practical applications, it is therefore recommended to perform 2/3 MVCs in order to achieve full potentiation (Kufel *et al.*, 2002).

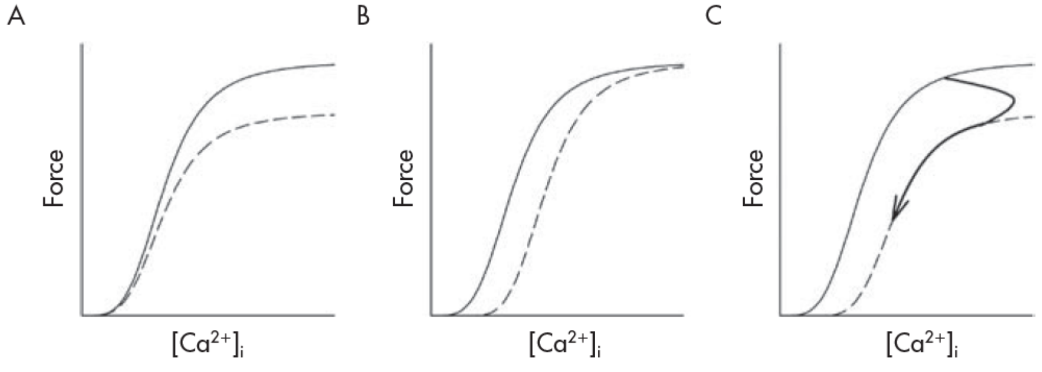

Figure 2.5. Representation of the changes in the relation between force and intracellular calcium ions concentration $[Ca^{2+}]_i$. Solid line represents unfatigued condition while dashed line represent the fatigued condition. A) Decreased cross-bridge force production (dashed line). B) Right-shift of the relation due to reduced myofibrillar Ca^{2+} sensitivity. C) Combined effect of decreased cross-bridge force and reduced myofibrillar Ca^{2+} sensitivity. The arrow illustrates the typical pattern change during fatigue induced by repeated tetanic contractions in a fast-twitch rodent fiber. Taken from Williams & Ratel, 2009.

Excitation-contraction coupling

The chain of events going from the depolarization of the muscle membrane (*i.e.*, sarcolemma) to the initiation of cross-bridges formation is defined as excitation-contraction coupling (Dulhunty, 2006). When acetylcholine is released by the alpha motor neuron in the synaptic cleft at the level of the neuromuscular junction, it binds to receptors on the sarcolemma (Allen *et al.*, 2008). This is the signal for the opening of ion channels allowing the passing of Na$^+$ and K$^+$ from one side of the muscle membrane to the other, which results in its depolarization and the generation of an action potential that travels through the transverse tubular system. Here dihydropyridine receptors, activated by the propagating action potential, interact with the sarcoplasmic reticulum Ca^{2+} release channels (*i.e.*, ryanodine receptors) and cause the release of Ca^{2+}into the cytosol (Dulhunty, 2006).. This is the signal for the opening of ion channels allowing the passing of Na$^+$ and K$^+$ from one side of the muscle membrane to the other, which results in its depolarization and the generation of an action potential that travels through the transverse tubular system (Westerblad *et al.*, 1990). Here dihydropyridine receptors, activated by the propagating action potential, interact with the sarcoplasmic reticulum Ca^{2+} release channels (*i.e.*, ryanodine receptors) and cause the release of Ca^{2+}into the cytosol (Dulhunty, 2006). Once Ca^{2+} is released, it binds to troponin, leading to a change in conformation in the tropomyosin, which exposes the myosin binding sites on the actin filament (Williams, 2009). Finally, the myosin attaches to the actin filament creating a cross-bridge and hence, producing force (Williams, 2009). Finally, when the motor neuron discharge ceases, the sarcolemma is repolarized and all these processed are reversed. Relaxation finally happens by the reuptake of Ca^{2+} into the sarcoplasmic reticulum which brings Ca^{2+} levels in the myoplasm to baseline levels (Williams, 2009). Disruptions in any of these processes lead to excitation-contraction coupling failure and peripheral fatigue development.

Mechanisms of peripheral fatigue

A reduction in the intrinsic muscle force production with fatigue may be the results of two phenomena: a failure in the neuromuscular transmission of the action potentials at the level of the sarcolemma or the impairment of the contractile processes downstream from it (Place *et al.*, 2010). As previously mentioned, changes in sarcolemmal excitability are usually monitored *in vivo* by observing the M-wave response to supramaximal electrical stimulation. Typically, a decrease in the M-wave amplitude/area has been interpreted as evidence of a decrease in sarcolemmal excitability, even though this notion has recently been challenged (Rodriguez-Falces & Place, 2018). However, a change in sarcolemmal excitability is not a mandatory prerequisite for fatigue. Indeed, most studies show an unchanged M-wave response after fatiguing exercise (Place *et al.*, 2010), although M-wave behavior is also related to the exercise modality and intensity of the task (Rodriguez-Falces & Place, 2018).

On the other hand, the mechanisms by which force production may be impaired downstream from the sarcolemma are essentially three: a decrease in the myofibrillar force-generating capacity, an impaired myofibrillar Ca^{2+} sensitivity, and an impaired Ca^{2+} release from the sarcoplasmic reticulum (Allen *et al.*, 2008; Olsson *et al.*, 2020). Intracellular Ca^{2+} is the signaling molecule that dictates the start and the end of a muscle contraction, and it determines the number of cross bridges that can be formed. The progressive accumulation of several metabolites and glycogen depletion, may cause excitation-contraction coupling failure and inhibition of the contractile machinery by any of the three abovementioned mechanisms (Allen *et al.*, 2008).

Acidosis

Traditionally, muscle acidosis has been considered as one of the main causes of fatigue. During high-intensity exercise, the rate of pyruvate formation by glycogen breakdown exceed the rate of oxidation, leading to formation and accumulation of lactic acid, which is then dissociated in lactate and hydrogen ions (H^+)(McArdle, 2010). This increase in H^+ causes an increase in the acidity of the metabolic milieu, as witnessed by a decrease in pH. The notion that acidosis plays an important role in the impairment of contractile mechanisms stems from the observation that lactic acid increases in parallel with fatigue development, and that at exhaustion high values of lactic acid are found in the blood and in the muscle (Sahlin *et al.*, 1976). This decrease in pH may reach values around ~6.2-6.5 in the muscle (Sahlin *et al.*, 1976; Wilson *et al.*, 1988; Broxterman *et al.*, 2017a). Moreover, *in vitro* evidence shows that peak power of muscles fibers is reduced by 18% when pH is low. The mechanism by which a low pH may disturb contractile apparatus, is thought to be a competition between H^+ and Ca^{2+} for the binding sites on troponin C, lowering the sensitivity for Ca^{2+} and therefore reducing force output (Fabiato & Fabiato, 1978). Moreover, acidosis may also inhibit the activity of some enzymes (ATPases), leading to a higher ATP cost of contraction (Fitts, 2016). However, the impact of acidosis on fatigue is controversial and it has also recently been debated in an exchange of views (Fitts, 2016; Westerblad, 2016). Most of the experiments demonstrating a role of acidosis on muscle fatigue derives from experiments carried out at non-physiological temperatures (<15°C) due to previous methodological constraints of muscle fibers handling (Pate *et al.*, 1995). Later studies at physiological temperatures, demonstrated that this effect is rather small, and it may not be relevant in the development of fatigue (Westerblad *et al.*, 2002). Moreover, the decrease in pH is not always present when a decline in force is found, and the recovery of the muscle function is dissociated by the recovery of pH, with the former recovering more promptly than the latter.

Inorganic phosphate

During high-intensity exercise bouts, the maintenance of intramuscular ATP concentration is maintained by the breakdown of phosphocreatine in creatine and inorganic phosphate (P_i). The accumulation of P_i has as important inhibitory role on the cross-bridge force production and myofibrillar Ca^{2+} sensitivity (Allen *et al.*, 2008). In fact, since P_i is released in the transition from low-force to high-force cross bridges, an increase in its concentration will lead to fewer high-force cross-bridges and a lower myofibrillar force production (Takagi *et al.*, 2004). Moreover, experiments on skinned fibers demonstrated that accumulation of P_i shifts the typical sigmoid relation existing between force production and Ca^{2+} concentration towards the right, meaning that myofibrillar Ca^{2+} sensitivity is decreased and a higher concentration of Ca^{2+} is required to produce the same force (Allen *et al.*, 2008). As with H^+, also P_i accumulation present an effect that is temperature-dependent. While the direct inhibitory role of P_i on myofibrillar force capacity decreases with increasing temperatures (Allen *et al.*, 2008), its role on Ca^{2+} sensitivity acquires more importance when approaching physiological temperatures (Debold *et al.*, 2006).

A third negative effect of P_i on fatigue is represented by the so called "calcium-phosphate precipitation" phenomenon. When Pi is accumulated in the cytosol of the fiber, it may enter the sarcoplasmic reticulum and bind to Ca^{2+}. When this happens, less Ca^{2+} will be free and available for release. This has been shown by Westerblad &

Allen by intracellular injections of P_i in unfatigued fibers and observing a concomitant decrease in Ca^{2+} concentration and force production (Westerblad & Allen, 1996).

Glycogen content

Another potential mechanism of peripheral fatigue is the depletion of energy substrates, such as glycogen stores. Classical studies demonstrated a close association between exercise capacity and glycogen depletion, which is known to influence the rate at which ATP can be regenerated. However, the hypothesis of a causal role of low energy stores in inducing fatigue is unlikely, because the relation between low glycogen and decreased muscle function is still present after recovery, when ATP levels are expected to be normal (Chin & Allen, 1997; Ørtenblad *et al.*, 2013). On the other hand, a role for low glycogen on Ca^{2+} handling mechanisms has been hypothesized. Accordingly, in a previous study, Ørtenblad and colleagues provided either a carbohydrates-rich diet or water to skiers right after the end of an intense, glycogen-depleting time-trial performance on ~20km (Ørtenblad *et al.*, 2011). Muscle biopsies were performed before and right after exercise, as well as after a 4h-recovery. Glycogen content and sarcoplasmic reticulum vesicles Ca^{2+} release were measured at all time points. Their results show that right after exercise, there was no difference between groups in the sarcoplasmic reticulum vesicles Ca^{2+} release. However, after 4 hours, glycogen had recovered only in the group that ingested the carbohydrate-rich diet, in which also the sarcoplasmic reticulum vesicles Ca^{2+} release returned to the pre-exercise levels. These findings are corroborated by experiments in skinned muscle fibers, where ATP levels can be kept high and constant (Ørtenblad *et al.*, 2013). Indeed, a study by Nielsen and coworkers showed that in this model, the number of contractions needed to reach a 50% decline in force production (*i.e.*, endurance performance) is correlated only with the glycogen content present within the myofibrils (Nielsen *et al.*, 2009). Since low energy availability is not an issue in this model, these findings indicate that glycogen depletion may contribute to fatigue by causing a decrease in the sarcoplasmic reticulum Ca^{2+} release.

2.3.3 The role of group III/IV afferent feedback on peripheral fatigue and performance

In previous chapters, the role of group III/IV afferents-mediated feedback was highlighted in its importance on raising ventilation, central and peripheral hemodynamics. These adjustments assure the matching between O_2 delivery and demand (Dempsey *et al.*, 1996). Importantly, a diminished blood flow and O_2 delivery to the muscle impair exercise performance and accelerate the rate of development of central and peripheral fatigue. The consequences of this impairment are especially clear when the performance task is longer than a few minutes at high exercise intensities (Amann & Calbet, 2008). Contrarily, a performance task that is too long in duration (and therefore at relatively low intensity) may "mask" the effects of low O_2 delivery, because the lower concentration of O_2 in the blood may be compensated by increasing cardiac output and leg blood flow (Amann & Calbet, 2008)

When exercise is performed with afferent blockade, the result is an accelerated rate of fatigue development, because the "missing" feedback leads to a significant hypoventilation (and consequent arterial hypoxemia) and hypoperfusion of the working muscle. Also, evidence shows that group III/IV afferent feedback is important for the maintenance of ATP cost of contraction (Broxterman *et al.*, 2017b). Therefore, the lack of the typical inhibition in central motor drive observed with attenuated feedback (detailed in the following sections) is counteracted by a lower O_2 delivery and muscle efficiency, with a net result of an unchanged performance. Given these results, is afferent feedback an important factor determining exercise performance? To test this hypothesis, Hureau and colleagues performed a study in which concomitant to afferent blockade, O_2 delivery was aided by the breathing of a mixture containing 100% fraction of inspired O_2 (Hureau *et al.*, 2019). Breathing of a hyperoxic mixture, maintained O_2 delivery to the muscle as shown by the leg O_2 delivery data (calculated as femoral blood flow multiplied by arterial O_2 content). Their findings showed that under this experimental condition, performance in a 5-km cycling time-trial was effectively improved compared both to normoxia or the hyperoxic condition with intact afferent feedback. These findings represent convincing evidence that group III/IV afferent-mediated feedback represents an important determinant of endurance exercise performance.

2.3.4 The role of group III/IV afferent feedback on central fatigue

Firing of group III/IV afferent fibers can cause the slowing of motor neurons firing rates and impact central fatigue. Evidence for this proposition initially came from single-limb studies utilizing post-exercise circulatory occlusion after fatiguing exercise (Bigland-Ritchie *et al.*, 1986b; Gandevia *et al.*, 1996). Specifically, in a previous study, Gandevia and colleagues asked their participants to perform a 2-min MVC of the elbow flexors while periodically measuring voluntary activation and TMS-induced motor evoked potentials and silent periods (Gandevia *et al.*, 1996). At the end of the contraction, a blood pressure cuff was inflated proximally to the elbow flexors, preventing any blood flow to and from the muscle, and impeding its recovery. Interestingly, both motor evoked potentials and silent periods recovered to baseline values as soon as the contraction stopped. However, voluntary activation and force remained depressed for as long as the muscle was kept ischemic. This result implies that even though the neural pathway appears to have recovered, the output from the motor cortex is still impaired and not able to drive the muscle fully. Also, albeit CMEPs were not measured in this study, there is evidence that this variable also recovers as soon as the contraction is stopped (Butler *et al.*, 2003). Indeed, while CMEPs were severely reduced after a 2-min elbow flexors MVC, a suprasystolic circulatory occlusion at the end of the exercise was not able to maintain this reduction, and CMEPs were recovered within 15 s (Butler *et al.*, 2003). Therefore, it is plausible that group III/IV afferent feedback-mediated impairments of voluntary descending drive may happen upstream of the motor cortex. However, when thoracic motor evoked potentials were measured during a 2-min MVC of the knee extensors, which are arguably a more representative muscle group for daily life and locomotion, motoneuronal excitability was not affected by the fatiguing task, nor from the successive circulatory occlusion (Kennedy *et al.*, 2016). However, in this study motoneuronal excitability was measured during an EMG-matched contraction and therefore possibly underestimated. Indeed, in a successive study that measured this variable with a paired pulse paradigm, and therefore abolishing the role of descending motor drive, motoneuronal excitability was severely depressed with fatigue (Finn *et al.*, 2018).

Further evidence that group III/IV muscle afferents exert an inhibitory role on motoneuronal output during whole-body exercise comes from a series of studies in which the participants performed high-intensity cycling to exhaustion under control conditions or pharmacological blockade of afferent feedback (Amann *et al.*, 2008; Amann *et al.*, 2009; Amann *et al.*, 2011). Overall, a common finding from these studies was that the EMG signal during exercise (estimate of central motor drive), was less restricted when feedback from the legs was attenuated. Interestingly, a follow-up study demonstrated that this inhibitory influence may also affect a remote muscle group not involved in the exercise task (Sidhu *et al.*, 2014). However, it is important to note that using surface EMG to estimate neural drive to the muscle present some limitations. In fact, even though the EMG signal is monotonically related to neural drive to the muscle, its amplitude is influenced by many factors such as electrode location, subcutaneous fat thickness, distribution of motor units' conduction velocities and the detection system, resulting in significant amplitude cancellation (Farina *et al.*, 2004). Low-threshold motor units have been shown to be the most affected by this phenomenon, rendering EMG amplitude estimates relatively insensitive to changes in these motor units (Farina *et al.*, 2014). Moreover, even though the two terms share many features and are often used as synonyms, it is necessary to make a distinction between neural drive and motor neuronal output (*i.e.*, EMG). For example, if the overall motor pathway excitability is diminished by any intervention, more neural drive (input) is necessary into the motor neurons for a given level of EMG (output), to compensate for the diminished excitability (Weavil & Amann, 2018).

As previously briefly mentioned, inhibitory influences of group III/IV afferent feedback may act at a higher level than the motor cortex (*i.e.*, higher brain areas involved in movement planning). However, given the impossibility of currents methods to investigate this portion of the pathway, effort has been put into studying the effects on motor cortical and spinal excitability. To accomplish this aim, Sidhu et al. investigated the changes in the abovementioned variables after high-intensity cycling exercise performed with intact and attenuated feedback using intrathecal fentanyl injection (Sidhu *et al.*, 2017). While in the control trial with intact feedback corticospinal excitability remained unchanged, during cycling with attenuated feedback it increased. Since CMEPs were unaltered (*i.e.*, motoneuronal excitability was preserved), an increase in motor cortical excitability took place. Therefore, since this increase was prevented in the control condition, the authors concluded that afferent feedback compromises motor cortical excitability. Follow-up studies from the same authors extended these findings discovering that this depression in motor cortical excitability may be related to intracortical inhibitory mechanisms and more specifically to $GABA_B$-mediated inhibitory networks. (Sidhu *et al.*, 2018).

Although these studies imply a role for central fatigue in this phenomenon, voluntary activation may not be a sensitive tool to support it. Indeed, most studies failed to find differences in voluntary activation between conditions. This could be due to many reasons, such as the delayed assessment of fatigue which typically recover in short time frames (Carroll *et al.*, 2017) or motor control issues of the subjects post-fentanyl which impair their ability to perform adequate MVCs (Blain *et al.*, 2016).

2.4 Models of neuromuscular fatigue on performance

The previous sections presented and discussed the mechanisms behind neuromuscular fatigue. However, although these mechanisms are widely accepted, there is no consensus on how they are integrated to explain the reasons why humans stop exercising. Classical models of endurance performance only considered physiological aspects related to O_2 delivery to explain fatigue and exhaustion. Many studies nowadays, have demonstrated that energy availability is not a limiting factor in performance. In fact, when subjects reach their volitional exhaustion point in a performance test, they still possess large energy reserves (Morales-Alamo *et al.*, 2015) and their capacity to express power is still largely above the requirements of the exercise (Marcora & Staiano, 2010).

2.4.1 Psychobiological model of performance

The psychobiological model of performance proposed by Marcora, based on Brehm's motivational intensity theory (Brehm & Self, 1989) is founded on the concept that endurance performance is regulated mainly by the two psychological constructs: potential motivation and perception of effort (de Morree & Marcora, 2015)Potential motivation refers to the maximum effort a subject is willing to invest to succeed in a task, while perception of effort is "the conscious sensation of how hard, heavy, and strenuous the task is" (de Morree & Marcora, 2015). During a time-trial, where the subject is free to choose the pacing strategy, this choice will depend on: 1) perception of effort, II) potential motivation, III) knowledge of the distance/time to cover, IV) knowledge of the distance/time elapsed, V) previous experience (Marcora, 2010). During time-to-exhaustion tests the subject will disengage from task when the level of effort reaches the maximum tolerated level. In this model, the shorter time-to-exhaustion after physiological (*i.e.*, pre-fatigue, hypoxia) or psychological (*i.e.*, mental fatigue) manipulation is explained by a higher perceived effort for the same workload, leading to maximal effort in less time. Physiologically, a higher level of perception of effort is explained by a higher level of central command (Williamson, 2010). Central command is defined as "the parallel activation of the locomotor and autonomic circuits in the central nervous system that simultaneously increase motor activity as well as arterial pressure, cardiac rate, and ventilation" (Waldrop *et al.*, 1996). According to the psychobiological model, perception of effort is solely generated by feedforward mechanisms. Indeed, concomitantly to the motor and autonomic signals, an "efferent copy" of this message (*i.e.*, corollary discharge) is sent to other sensory areas of the brain where it generates perception of effort. Interestingly, various corollary discharge neuronal circuits have been found in animals (Poulet & Hedwig, 2007).

Even though this model is attractive especially because of its simplicity, it relies on the assumption that afferent feedback does not play a role in the generation of effort perception. However, there is no established consensus about this issue and a clear relation between central command and perceived exertion has never been established so far. Contrarily, different authors provided evidence that perception of effort is also associated with feedback from the muscles, heart and lungs (Amann *et al.*, 2008; Romer & Polkey, 2008), general discomfort, pain, and thermal stress (Cabanac, 2006). Moreover, studies using hypnosis to manipulate perception of effort only partially support this concept. In fact, a previous study found that when highly-hypnotizable subjects cycled while perceiving to perform uphill cycling, the cardiovascular variables were elevated compared to the control situation (Williamson *et al.*, 2001b). This would appear to reinforce the idea that perceived effort can operate independently from afferent feedback. However, when perception of effort was decreased, by having the subjects believe that were cycling downhill, the cardiovascular response was not decreased below the requirements of the metabolic needs, suggesting that a regulatory system is still in place (*i.e.*, afferent feedback). Current evidence suggests that perception of effort the result of a complex interaction of feedforward and feedback mechanisms (Galbo *et al.*, 1987; Amann *et al.*, 2008; Williamson, 2010; Abbiss *et al.*, 2015).

2.4.2 Critical threshold of fatigue

Many studies from different groups found that the magnitude of exercise-induced peripheral fatigue after single-limb or whole-body exercise does not exceed a certain task-specific value (Amann *et al.*, 2006a; Amann & Dempsey, 2008; Vanhatalo *et al.*, 2010; Hureau *et al.*, 2014; Hureau *et al.*, 2016). For example, in a study from 2006, Amann and colleagues modified arterial content of O_2 by having their subjects breathe different gas mixtures from hypoxic (15% FiO_2) to hyperoxic (100% FiO_2). Exercise performance was measured with a 5-km cycling time-trial and fatigue was assessed pre-post by magnetic femoral nerve stimulation. Not surprisingly, performance was enhanced during the hyperoxic condition, and gradually decreased with decreasing levels of arterial O_2 content. The same trend was presented by mean power output and EMG, which, with the due limitations (Farina et al., 2004), may be reflective of neural drive to the muscle. However, interestingly, the levels of peripheral fatigue found at the end of all trials was strikingly similar (~34% decline in $Q_{tw,pot}$). Further insights came from a successive study from the same laboratory when the same 5-km time-trial was preceded by pre-induction of different levels of fatigue (Amann & Dempsey, 2008). Pre-fatigue was induced by having the subjects cycle for a certain time and intensity, known to induce a predefined level of peripheral fatigue based on a previous trial. Similarly to the previous study, even though performance was impacted proportionally to the levels of starting fatigue (*i.e.*, higher pre-fatigue, slower completion time), once again, the levels of fatigue reached at the end of the time-trial were similar across conditions. Importantly, the level of peripheral fatigue is tightly associated to the accumulation of metabolites in the muscle (Blain *et al.*, 2016; Broxterman *et al.*, 2017a). A consistent level of metabolites accumulation at exhaustion is found also in other studies using other methodologies such as ^{31}P-MRS (Jones *et al.*, 2008b; Chidnok *et al.*, 2013).Together, these observations led the authors to hypothesize that peripheral fatigue was a variable regulated by the central nervous system, which would in turn lead to a conscious/subconscious modulation of central motor drive in order to preserve the muscle homeostasis and prevent excessive accumulation of potentially harmful levels of metabolites. This concept is usually referred to as "critical threshold of fatigue", which represent a task-dependent threshold that the central nervous system does not allow to surpass under physiological conditions. The role of muscle "sensors" for this process to work in this scenario, is attributed to group III/IV afferent fibers. Further support for this idea comes from studies employing pharmacological blockade of group III/IV afferent feedback. Indeed, when afferent feedback is attenuated, subjects are able to surpass the critical threshold, finishing the trial with higher intramuscular metabolic perturbations (Blain *et al.*, 2016). Interestingly, these authors often reported that during the trials with attenuated feedback, subjects often present ambulatory and motor control issues (Amann *et al.*, 2009). Although anecdotal, this may represent evidence that afferent feedback is in place to prevent excessive, and potentially dangerous, levels of metabolites accumulation.

2.4.3 Sensory Tolerance Limit

The concept of a "sensory tolerance limit" was initially proposed by Gandevia, who proposed the idea that there is an hypothetical threshold, whereby continuing to exercise would become sufficiently unattractive (Gandevia, 2001). Several studies to date have demonstrated that exercise performance can be limited by feedback from previously fatigue remote muscles, respiratory muscle fatigue, pain in exercising and non-exercising muscles, and corollary discharges associated with central command.

For example, previous studies demonstrated that fatiguing exercise performed in a remote muscle has the potential to impact a subsequent endurance performance task. On this note, Amann et al. conducted a study in which dynamic single-leg knee-extension to exhaustion at 85% of the peak power output previously determined, was preceded by the same performance on the contralateral leg (Amann *et al.*, 2013). Prior fatiguing exercise on the contralateral leg caused the subsequent endurance performance to be drastically impaired. Also, exhaustion was accompanied by lower levels of peripheral fatigue. The interpretation of the authors suggests that when the contralateral leg was prefatigued, afferent feedback arose from both the exercising, and the recovering leg. Moreover, also the higher involvement of respiratory muscles and other organs may have contributed to the increase in the ensemble afferent feedback. Therefore, in this condition, the sensory tolerance limit was reached before than when exercise was carried out in rested (non-prefatigued) conditions. On the same note, a study from Johnson and colleagues found similar results when cycling exercise was performed after fatiguing arm cranking (Johnson *et al.*, 2015). Importantly, all these studies utilized a voluntary fatiguing exercise to induce pre-fatigue. Therefore, fatigue associated with descending neural drive to the muscle cannot be excluded as a potential cause of impairment for performance.

Another factor that may contribute to reaching the sensory tolerance limit is the involvement of metabonociceptors. On this note, studies utilizing hypertonic saline infusion in the exercising limb demonstrated a shorter time to exhaustion (Smith *et al.*, 2020c; Norbury *et al.*, 2021). Interestingly, also an impaired exercise performance is found also when the contralateral leg is injected with hypertonic saline (Deschamps *et* *al.*, 2014) or is made ischemic (Aboodarda *et al.*, 2020), highlighting how noxious stimulus also exacerbates fatigue and accelerate the rate at which the subjects reach the sensory tolerance limit.

Finally, also respiratory muscle fatigue and the sensory aspect of breathlessness that is associated with it, may contribute to reaching the sensory tolerance limit. During intense exercise, the metabolic needs of respiratory muscles drastically increase, and by triggering the respiratory metaboreflex, has the potential to compromise muscle blood flow and therefore accelerated the rate of fatigue development (Harms *et al.*, 1997). The importance respiratory afferent feedback is particularly evidenced by studies that manipulated work of breathing by using a mechanical ventilator. When work of breathing was reduced, the rate of development of fatigue was reduced, and performance improved (Harms *et al.*, 2000). Vice versa, performance was impaired by a faster rate of fatigue development when the work of breathing was increased (Harms *et al.*, 2000). Secondly, the sensation of breathlessness, is believed to be the result of integration of respiratory afferent feedback and corollary discharges to the medullary respiratory centers (Laviolette *et al.*, 2014).

Collectively, this model expands on the theory of the critical threshold of peripheral fatigue and integrates in it the role of feedforward mechanisms and other sources of afferent feedback. The result is an interaction of several inputs into the central nervous system, which ultimately contribute to regulate exercise performance.

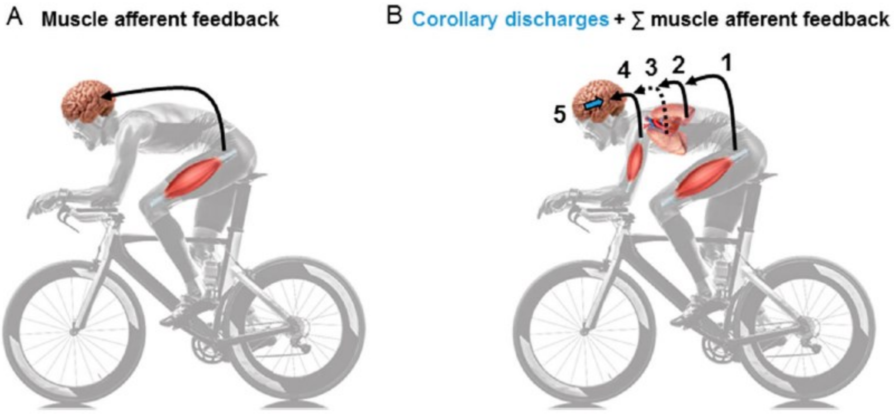

Figure 2.6. Schematic representation of the differences between A) the "critical fatigue threshold" and B) the "sensory tolerance limit" theory. The critical threshold of fatigue model proposes that afferent feedback from the working muscles largely determine exercise performance. The sensory tolerance limit suggests that feedback from the 1) working muscles, 2) respiratory muscles, 3) other organs, and 4) remote muscles not directly involved in the exercise are integrated in the brain together with 5) the corollary discharges associated with central command and ultimately determine central motor drive. Taken from Hureau *et al.,* 2018.

2.4.4 Central governor model

The central governor model theory originated from Ulmer (1996). This idea stated that a control system in the brain ensure a "teleoanticipatory" optimization of performance in order to avoid physiological disturbances by integrating feedback with motor learning and desired objectives. This model, successively refined and enunciated by Noakes and colleagues (Noakes *et al.*, 2004), states that exercise performance is regulated in a way that the strain generated from it does not threaten homeostasis and cause damage to the heart. As a consequence, exercise is never voluntarily performed to the limits of capacity. This regulation takes place by integrating afferent information from muscles and other organs in the brain, which in turn regulates muscle recruitment and power output. The integration of all the different signals then results in a perception of "fatigue". In a race, this integration provides information to the athlete on whether the power output can be decreased or increased. In this way, a continuous subconscious adjustment of the power output deriving from earlier experience, knowledge of exercise duration, and afferent feedback conveying information about the metabolic state of the body. Therefore, the pacing strategy employed by athletes in a race, is the result of a continuous adjustments through both conscious and subconscious mechanisms.

Even though this theory is appealing and led physiologists to consider the role of the brain in determining the limits of endurance performance, it has been criticized numerous times (Ekblom, 2009; Shephard, 2009a, b; Inzlicht & Marcora, 2016). One of the fundamental points of this model is that muscle recruitment is constrained in order to avoid organs damage. However, many studies have shown that strenuous exercise can cause various degrees of damage to different organs (Zavorsky, 2007; Shephard, 2009b). Moreover, also other fundamental points that were also part of the initial formulation of the model have been heavily questioned and criticized (Shephard, 2009b).

2.4.5 "Flush" model

The flush model was enunciated to help explain fatigue and pacing strategy in all endurance competitions, with a special reference to ultra-endurance events (Millet, 2011). This model resembles Noakes' Central Governor Model, presenting the determinants of endurance performance following an analogy with the toilet flush. In this model, four components are present:

- The buoy
- The filling rate
- The emptying rate
- The security reserve

The buoy represents the rating of perceived exertion. This will increase or decrease during endurance activities, due to filling and emptying rates. The filling rate is influenced by both feedforward and feedback mechanisms. Emptying rates in this analogy are represented by all the types of strategies that can be employed by the athletes to delay the onset of fatigue. Finally, the security reserve sets the upper limit of exertion, or in the flush analogy, the water overflow, with the aim of avoiding catastrophic failure.

In this model, the subject (or the athlete) sets the pace based on previous experience, as proposed by Ulmer (1996), which will provide an initial "filling rate". Successively, mechanical, chemical, and nociceptive feedback information as well as the levels of central command will contribute to modify the filling rate. On the other hand, several strategies such as using dissociative thoughts (Raglin, 2007), nutritional approaches (Chambers *et al.*, 2009), or hypnotic manipulations (Williamson *et al.*, 2001a) could be employed by the subject in order to decrease the "water level".

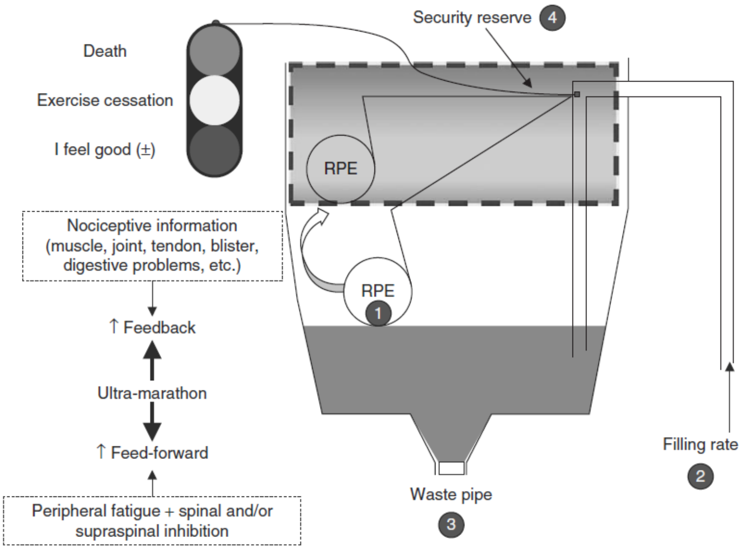

Figure 2.7. Schematic diagram of the flush model. Taken from Millet, 2011.

CHAPTER 3

Aims & hypotheses

3.1 Aims

The specific aims of this dissertation were to:

1) Determine whether electrically-induced peripheral fatigue in the contralateral limb impact the subsequent exercise performance of the ipsilateral muscle.

2) Determine whether the involvement of central motor drive during prior fatiguing exercise plays a role on the subsequent endurance exercise performance and fatigue development.

3) Determine whether mechanoreflex-induced responses are influenced by the concurrent activation of the metaboreflex.

3.2 Hypotheses

To accomplish our aims, we tested the following hypotheses:

1) Electrically-induced contralateral fatigue would compromise the subsequent exercise performance of the ipsilateral muscle and that exercise-induced decline in peripheral fatigue at the end of the performance task in the latter, would be smaller than in the control condition

2) A voluntary fatiguing protocol (*i.e.*, with central motor drive) would impair endurance performance more than the electrically-evoked one (*i.e.*, without central motor drive). Consequently, exercise-induced decline in peripheral fatigue would be lower due to the less work performed.

3) Our hypothesis was that hemodynamic and ventilatory responses to passive leg movement would be higher when metabolites were trapped in the muscle.

CHAPTER 4

Study I

Electrically-induced quadriceps fatigue in the contralateral leg impairs ipsilateral knee extensors performance

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4.1 Abstract

Muscle fatigue induced by voluntary exercise, which requires central motor drive, causes central fatigue that impairs endurance performance of a different, non-fatigued muscle. This study investigated the impact of quadriceps fatigue induced by electrically-induced (no central motor drive) contractions on single-leg knee-extension (KE) performance of the subsequently exercising ipsilateral quadriceps. On two separate occasions, eight males completed constant-load (85% of maximal power-output) KE exercise to exhaustion. In a counterbalanced manner, subjects performed the KE exercise with no pre-existing quadriceps fatigue in the contralateral leg on one day (No-PreF), while on the other day, the same KE exercise was repeated following electrically-induced quadriceps fatigue in the contralateral leg (PreF). Quadriceps fatigue was assessed by evaluating pre- to post-exercise changes in potentiated twitch force ($\Delta Q_{tw,pot}$; peripheral-fatigue), and voluntary muscle activation (ΔVA; central-fatigue). As reflected by the 57±11% reduction in electrically-evoked pulse force, the electrically-induced fatigue protocol caused significant knee-extensors fatigue. KE endurance time to exhaustion was shorter during PreF compared to No-PreF (4.6±1.2 vs 7.7±2.4 min; $p<0.01$). While $\Delta Q_{tw,pot}$ was significantly larger in No-PreF compared to PreF (-60% vs -52%, $p<0.05$), ΔVA was greater in PreF (-14% vs -10%, $p<0.05$). Taken together, electrically-induced quadriceps fatigue in the contralateral leg limits KE endurance performance and the development of peripheral fatigue in the ipsilateral leg. These findings support the hypothesis that the crossover-effect of central fatigue is mainly mediated by group III/IV muscle afferent feedback and suggest that impairments associated with central motor drive may only play a minor role in this phenomenon.

4.2 Introduction

The progressive reduction in the ability to exert muscle force or power during exercise is defined as muscle fatigue (Bigland-Ritchie & Woods, 1984) and has a central and a peripheral component. Central fatigue describes the impairment of the neural drive to a muscle or muscle group, while peripheral fatigue entails biochemical changes occurring at or distally to the neuromuscular junction (Gandevia, 2001). Previous studies demonstrated that peripheral fatigue in one limb may induce changes in the central nervous system (CNS), potentially affecting exercise performance a remote muscle group (Rattey *et al.*, 2006; Amann *et al.*, 2013; Kennedy *et al.*, 2013, 2014; Sidhu *et al.*, 2014; Johnson *et al.*, 2015). This "crossover" effect of fatigue is usually secondary to a compromised activation of the remote muscle group by the CNS. Although several factors have been identified as possible contributors (Halperin *et al.*, 2015), the mechanistic basis for this phenomenon is still unclear.

In previous investigations, pre-induced fatigue in the one limb has been shown to directly affect neuromuscular function and exercise performance during the successive exercise bout carried out with a previously rested muscle (Amann *et al.*, 2013; Aboodarda *et al.*, 2020; Finn *et al.*, 2020). For example, voluntary knee-extension (KE) exercise induced fatigue in the contralateral leg impaired spinal motoneuronal output and endurance performance of the ipsilateral quadriceps (Amann *et al.*, 2013). A reduction in endurance performance and a faster decline in voluntary muscle activation (VA) were also found when arm-cranking exercise was performed prior to leg cycling exercise (Johnson *et al.*, 2015). Since exercising with prior fatigue entails increased afferent feedback signaling arising from both the active and recovering muscles, strong evidence suggests that group III/IV afferent-mediated inhibition of central motor drive may play a pivotal role in the crossover effect of central fatigue and the impairment of performance (Amann *et al.*, 2013; Sidhu *et al.*, 2014). Importantly, when leg cycling exercise is carried out with pharmacologically blocked group III/IV afferent feedback from the lower limbs, the decrease in VA in a non-exercising, remote muscle (*i.e.*, elbow flexors) is prevented, emphasizing the importance of these sensory neurons in mediating the crossover effect of fatigue (Sidhu *et al.*, 2014). However, controversy exists on the role of group III/IV on the crossover of central fatigue. For example, Kennedy and colleagues (Kennedy *et al.*,

2015) did not find any decrease in VA or maximal voluntary force when ischemia was maintained on the contralateral limb after a 2-min isometric fatiguing task. Also, it is important to note that all the above-mentioned studies utilized voluntary exercise to induce prior fatigue.

When voluntary exercise is used to pre-fatigue a limb, the effects associated with central motor drive may, by itself, impair performance during subsequent tasks. In fact, parallel to the activation of spinal motor neurons directly involved in the exercise, a "copy" of the neural signal (*i.e.*, corollary discharge) is sent from the premotor areas to the sensory areas of the brain, ultimately facilitating effort perception and potentially impairing exercise performance (Poulet & Hedwig, 2007; Hureau *et al.*, 2018a). Moreover, several studies using different neurophysiological approaches have shown that the activity in different brain areas, such as the prefrontal cortex (Berchicci *et al.*, 2013), the thalamus and the insula (Hilty *et al.*, 2011a) progressively increases during fatiguing voluntary exercise, and possibly contributes to the development of fatigue and, ultimately, exercise cessation. Furthermore, repetitive central motor pathway activation associated with voluntary fatiguing exercise may, by itself, reduce motoneuron excitability (Weavil *et al.*, 2016; Finn *et al.*, 2018; Weavil & Amann, 2018; D'Amico *et al.*, 2020). On the other hand, the pre-induction of fatigue through electrical stimulation of the limb that is successively exercised compromises exercise performance by causing peripheral fatigue that diminishes the ability of the muscle to respond to a given motor input. Therefore, this approach may confound the potentially performance limiting effects of increased group III/IV afferents discharge (Gagnon *et al.*, 2009).

To circumvent these issues, we used electrical muscle stimulation to induce quadriceps fatigue in the contralateral leg immediately prior to the start of voluntary KE exercise with the ipsilateral leg. This strategy elevated, compared to the control trial performed without pre-existing fatigue in the contralateral leg, ensemble afferent feedback to the CNS during KE exercise. We hypothesized that 1) electrically-induced peripheral fatigue in the contralateral leg would compromise exercise performance of the ipsilateral leg, 2) end-exercise peripheral fatigue would be lower when exercise is executed with pre-existing fatigue in the contralateral leg.

4.3 Methods

Subjects and ethical approval

Eight active healthy young males (age: 26 ± 1 years; height: 179 ± 2 cm; body mass: 76 ± 2 kg) were recruited for the purpose of this study. All subjects were right-leg dominant. The study was approved by the local ethical committee of the University of Verona (#30444) and was carried out following the Declaration of Helsinki. All subjects read and signed an informed consent before enrolling in the study. All the experiments were performed following the safety procedure for exercise testing in the scenario of COVID-19 (Venturelli *et al.*, 2020).

Study design

The subjects reported to the laboratory on four occasions, interspersed by at least 48 hours between sessions. Also, the two finals visits were separated by a minimum of 72 hours. Following preliminary familiarization trials conducted to practice exhaustive KE exercise, on the first experimental day, anthropometric data were collected, and the subjects were familiarized with the study instrumentation and procedures including exhaustive voluntary KE exercise and electrically-evoked muscle contractions to the limit of tolerance. On a separate occasion (Day 2) a single-leg KE incremental test to exhaustion ($15W + 5W \cdot min^{-1}$) was administered on the dominant leg to determine the individual peak power output (PPO). On the two following visits, the subjects carried out a constant-load exercise to exhaustion on the single-leg KE ergometer at 60 RPM and 85% PPO in a non-prefatigued condition (No-PreF) or with pre-induced fatigue in the contralateral leg (PreF). The induction of fatigue (detailed in a following section: see *"Pre-fatiguing protocol"*) was achieved by electrical stimulation on the non-dominant, contralateral limb. The order of the two experimental sessions was counterbalanced. In both sessions, participants were evaluated for neuromuscular function before and right after the end of the single-leg KE exercise. The constant-load test was terminated when the subjects were unable to maintain 50 RPM for more than 10 s. At the beginning of each constant-load exercise test, the ergometer was manually accelerated by a member of the research team, to avoid initial peak force outputs.

Exercise responses

Pulmonary gas exchange ($\dot{V}O_2$ and $\dot{V}CO_2$,) and ventilatory ($\dot{V}_E$) responses were measured breath-by-breath with a metabolic cart (Quark b^2, Cosmed, Italy). Before each session, after an appropriate warm-up, the gas analyzer and the turbine flowmeter were calibrated according to the instructions of the manufacturer. The data were averaged over the last 30s of each minute. Rate of perceived exertion (RPE) was obtained at rest and after every minute of exercise using the Borg's modified CR10 scale (Borg, 1998).

During the voluntary KE exercise, a Doppler ultrasound (Logic 7 Doppler system; General Electric Medical Systems, Milwaukee, USA) equipped with a 12-14 MHz linear array transducer, was utilized to assess femoral blood flow and vascular conductance, which may have an influence on the development of neuromuscular fatigue (Dempsey *et al.*, 2008). Femoral artery blood velocity (V_{mean}) and arterial diameter were measured distally from the inguinal ligament and proximal to the bifurcation of the superficial and deep femoral artery. V_{mean} was measured using the same probe utilizing a frequency of 5 MHz with the probe positioned and maintained at an insonation angle of 60° or less and the sample volume was centered and maximized according to vessel size. Then, femoral blood flow (FBF) was calculated as $V_{mean} \cdot \pi \cdot (\text{arterial diameter} \cdot 2^{-1})^2 \cdot 60$.

A non-invasive thoracic impedance cardiograph (Physio Flow®, Manatec, Strasbourg, France) was utilized to measure heart rate (HR) and estimate stroke volume. Cardiac output (CO) was calculated as stroke volume · HR. The validity and reliability of this method has previously been established (Richard *et al.*, 2001). The data of the last 30s of each minute were averaged. Systolic and diastolic blood pressures were measured manually, by means of a sphygmomanometer and an adult size cuff at each minute of exercise. Mean arterial pressure (MAP) was calculated as: diastolic blood pressure + 1/3 · (systolic blood pressure – diastolic blood pressure). Leg vascular conductance (LVC) was calculated as FBF · MAP^{-1}.

Neuromuscular function assessment of the ipsilateral limb

Surface electromyography. Vastus lateralis electromyography (EMG) was continuously recorded with a wireless system (ZeroWire, Aurion, Italy). Two surface Ag/AgCl electrodes (Blue sensor, Ambu®, Ballerup, Denmark) were attached to the skin with a 2-cm inter-electrode distance. The electrodes were placed longitudinally, in line with the underlying muscle fibers arrangement, at two-thirds of the distance between the anterior iliac spine and the lateral part of the patella (Hermens *et al.*, 2000) Before electrodes application, the skin was shaved and cleaned with alcohol in order to minimize impedance. The placement of the electrodes was marked to allow a consistent re-positioning of the electrodes between the two experimental sessions. The EMG transmitter connected to the electrodes was secured with adhesive tape to avoid movement-induced artefacts. The raw EMG signal was amplified, band-pass filtered (10 - 1000 Hz) and digitized online at a 5 kHz sampling frequency. Acquisition of the EMG data was done using a computer-based data acquisition and analysis system (hardware: PowerLab 16/30; ML880, ADInstruments, Colorado Springs, CO and software: LabChart 6, ADInstruments, Colorado Springs, CO). Burst onset was established when the EMG signal rose >2.5 SD above baseline noise. On the contrary, burst offset was determined by the signal declining below the same threshold. Integrated electromyography (iEMG) for each muscle contraction was calculated and averaged over the last 30s of each minute and normalized to the EMG signal obtained during an MVC performed before the exercise.

Femoral nerve stimulation. The motor nerve was stimulated with the anode placed between the greater trochanter and the iliac crest and the cathode placed over the femoral nerve in the femoral triangle via a constant current electrical stimulator (Digitimer DS7AH, Welwyn Garden City, United Kingdom). Electrical stimuli were delivered through rectangular (50x90 mm) self-adhesive electrodes (Myotrode Plus, Globus G0465). The evoked twitch force was measured by a force transducer (model UU2; DaCell, Korea) previously calibrated, connected to a custom-made chair through a noncompliant strap placed around the subject ankle. The subjects were seated with a 90° knee flexion. The output from the force transducer was amplified (INT2-L, London Electronics Limited, Sandy Bedfordshire, United Kingdom), and recorded at a sampling rate of 5 kHz. Once the electrodes were in place, stimulation intensity was increased by 25-mA increments until the size of the evoked twitch and compound muscle action potential (M-wave) demonstrated no further increase (*i.e.*, amplitude of maximal M-wave; M_{max}). Stimulation intensity was set at 125% of this value (range utilized: 343 – 469 mA) and was kept constant throughout the experimental session.

For the evaluation of quadriceps function, we measured potentiated twitch force ($Q_{tw,pot}$) 2-s after a 5-s MVC of the knee extensors and repeated this procedure 6 times (Venturelli *et al.*, 2019). These MVCs were interspersed by at least 60 s of rest. VA was assessed using the interpolated twitch technique by comparing the force produced during a superimposed single twitch on the MVC with the potentiated single twitch delivered 2 s afterwards (Venturelli *et al.*, 2019). VA was then calculated as [1-(superimposed twitch force/$Q_{tw,pot}$) · 100]. Peak force, maximal rate of force development (MRFD) and maximal rate of relaxation (MRR) were analyzed for all $Q_{tw,pot}$. Peak force was calculated as the highest value reached for every $Q_{tw,pot}$. MRFD and MRR represent the maximal force increase and decrease, respectively, occurring between 10 and 90% of the peak force of $Q_{tw,pot}$.

Electrically-induced fatiguing protocol

In PreF, neuromuscular electrical stimulation was used to fatigue the contralateral quadriceps prior to the voluntary KE exercise to exhaustion of the ipsilateral leg. After completion of the neuromuscular assessment of the right leg, subjects were accommodated on the single-leg KE ergometer. After adequate preparation of the skin, two 50 x 90 mm self-adhesive electrodes (Myotrode Plus, Globus G0465) were positioned on the left quadriceps. The left ankle was then connected to the force transducer via a non-compliant velcro strap to measure the force evoked by the train pulses. The anode was placed on the proximal part of the rectus femoris while the cathode was placed about 3 cm above the patella. To elicit the train pulses and to administer the stimulation, we used a custom-made system previously used in our lab (Giuriato *et al.*, 2020). Briefly, the same constant current electrical stimulator previously mentioned was interfaced with an Arduino microcontroller (Arduino Uno, Arduino, Italy) to generate the train pulses. The stimulation intensity was progressively increased to find the maximal tolerable intensity. The subjects were given instructions to indicate their discomfort levels on a visual analogue scale (VAS) from 0 to 10, where 0 was no discomfort and 10 maximal discomfort that they considered tolerable for at least 5 minutes. When the maximal tolerable intensity was reached, the ramp protocol was interrupted, and the electrically-induced fatiguing protocol began. Stimulation intensity was kept constant throughout the trial. Every train of stimulation consisted of twenty 1 ms square wave pulses, duty cycle 20% (0.2s on / 0.8s off) delivered at 100Hz to evoke a tetanic contraction. The decrease in the force evoked by the train pulses was expressed as the difference between the average of the peak force reached in the first 5 and last 5 contractions (Figure 4.1, panel A). Once the subjects reached their subjective point of intolerable discomfort, the stimulation was terminated, and the performance test of the ipsilateral leg started within 10 s.

Statistical analysis

For the neuromuscular assessments, where a pre-post design was applied, a two-way ANOVA with repeated measures (conditions: No-PreF and PreF, time: pre and post exercise) was performed to evaluate differences between means. For the in-exercise data, also a two-way repeated measures ANOVA was carried out in which the last time point was the subjective time to exhaustion. If the sphericity assumption was violated, the Greenhouse-Geisser correction coefficient was reported. When a significant condition x time interaction was found, pairwise differences were identified using Bonferroni post-hoc test correction for multiple comparisons. A two-tailed paired samples t-test was used to assess the differences in time to exhaustion between conditions, to compare baseline data for the neuromuscular assessment, and to assess the difference between the force of the first five vs last five evoked contractions. Significance level was set at $\alpha \leq 0.05$. Data are expressed as mean ± SD unless otherwise stated. Statistical analysis was performed using IBM SPSS Statistics 24 (IBM Corp©, 2016). Graphs and figures were made with GraphPad Prism 6.0 (GraphPad Software, Inc., 2012).

4.4 Results

Electrically-induced fatiguing protocol

Electrically-evoked quadriceps contractions significantly decreased the evoked force response to the train pulses by 57 ± 11% (from 318 ± 124 N to 136 ± 54 N, $t_7 = 6.16$, $p < 0.01$, Figure 4.1, panel B). Furthermore, the point of intolerable discomfort was reached after 6.2 ± 1.5 min.

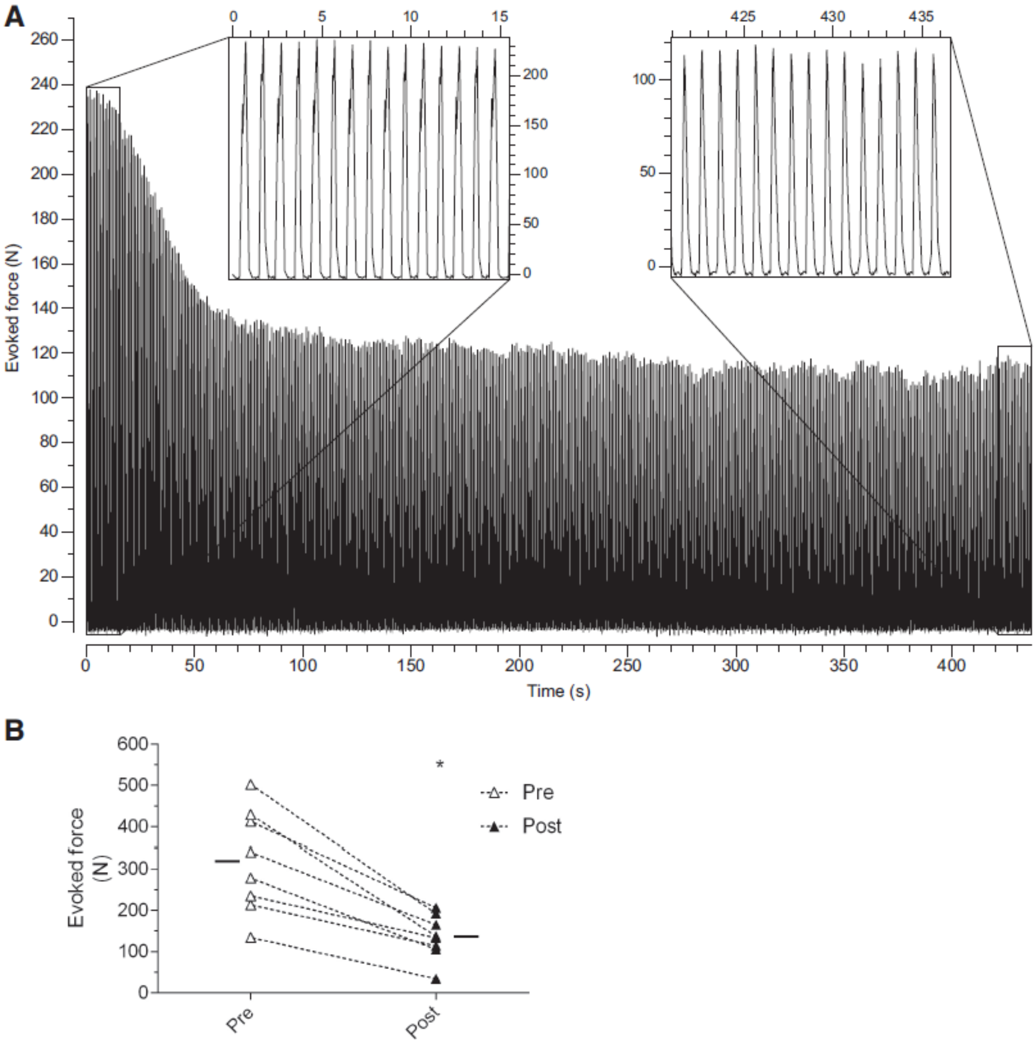

Figure 4.1. A) Representative quadriceps force output obtained from a subject during the pre-fatigue protocol using electrically-evoked knee-extensors contractions. B) Individual (triangles) and mean (-) changes in the evoked force during the electrical stimulation protocol. Δ = average of the evoked peak force reached in the first 5 contractions, ▲ = average of the evoked peak force achieved during the last 5 contractions. * = significantly different than Pre ($p < 0.05$). n=8.

Exercise Performance and Neuromuscular Function

Time to exhaustion was 38 ± 13% shorter in PreF compared to No-PreF (4.6 ± 1.2 vs 7.7 ± 2.4 min, $t_7 = 5.01$, $p < 0.01$). The changes in neuromuscular function for the two trials are presented in Figure 4.2. At baseline, quadriceps MVC was similar between conditions (625 ± 111 N vs 618 ± 101 N, $t_7 = 0.46$, $p = 0.66$). Immediately after the two protocols, quadriceps MVC was reduced both in No-PreF (from 625 ± 111 N to 391 ± 76 N) and in PreF (from 618 ± 101 N to 430 ± 51 N) with a significant difference between the two conditions ($F_{1,7} = 9.3$, $\eta_p^2 = 0.57$, $p = 0.02$). Pre-exercise $Q_{tw,pot}$ was not different between experimental days (191 ± 47 N vs 189 ± 43 N, $t_7 =$

1.22, p = 0.26). Also, $Q_{tw,pot}$ was reduced after both trials with a higher exercise-induced decline ($F_{1,7}$ = 24.7, η_p^2 = 0.78, p < 0.01) after No-PreF (191 ± 47 N to 75 ± 20 N) compared to PreF (189 ± 43 N to 87 ± 21 N). Furthermore, while not being different at baseline (No-PreF: 92 ± 3% vs PreF: 91 ± 4%, t_7 = 1.29, p = 0.24), the decrease in VA was more accentuated ($F_{1,7}$ = 8.3, η_p^2 = 0.54, p = 0.02) in PreF (from 91 ± 4% to 78 ± 7%) compared to No-PreF (from 92 ± 3% to 83 ± 5%). Finally, within-twitch variables showed a significant greater reduction in MRFD ($F_{1,7}$ = 5.4, η_p^2 = 0.44, p = 0.05) after No-PreF (5.24 ± 1.44 N/ms to 1.57 ± 0.56 N/ms) compared to PreF (5.10 ± 1.44 N/ms to 2.10 ± 0.81 N/ms) and also in MRR ($F_{1,7}$ = 17.6, η_p^2 = 0.72, p < 0.01) between No-PreF (1.05 ± 0.38 N/ms to 0.32 ± 0.13 N/ms) and PreF 1.00 ± 0.28 N/ms to 0.40 ± 0.18 N/ms).

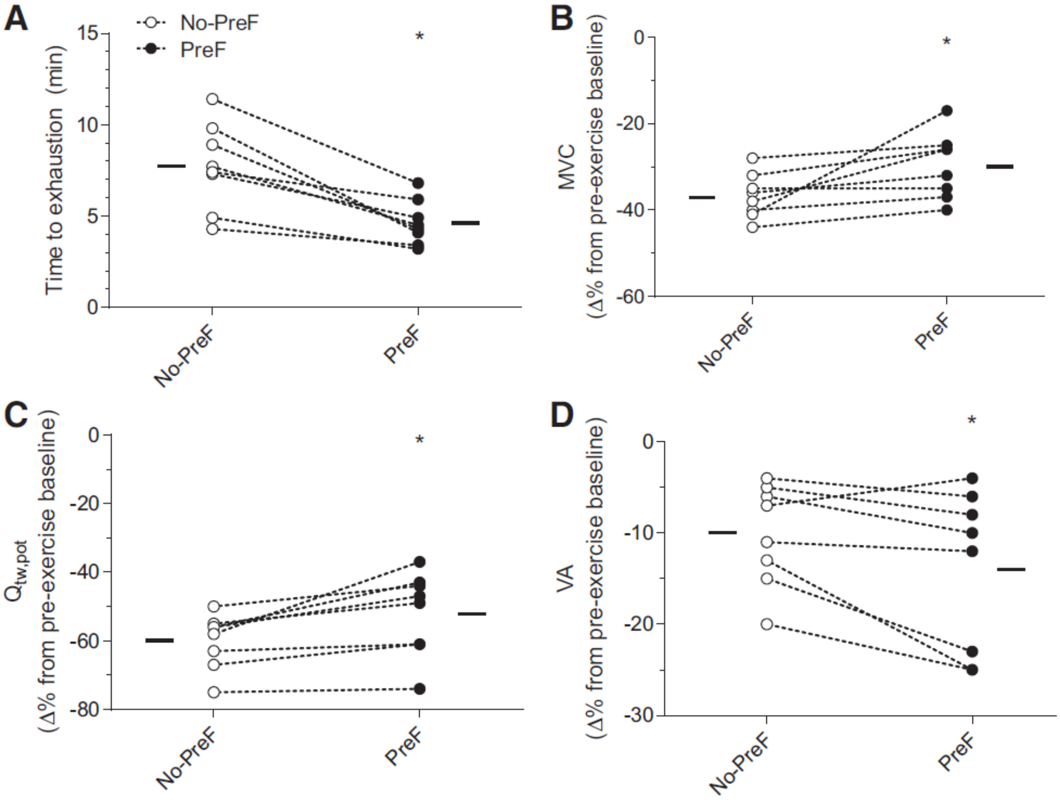

Figure 4.2. Individual (circles) and group mean (-) data showing the changes in A) time to exhaustion, B) maximal voluntary contraction (MVC), C) potentiated twitch force ($Q_{tw,pot}$), and D) voluntary muscle activation (VA) under control condition (No-PreF, ○) and under experimental condition (PreF, ●, *i.e.*, exercise performed with prior fatigue induced by electrical stimulation in the contralateral leg). * = significantly different from No-PreF (p < 0.05); n=8.

Ventilatory and hemodynamic variables

The ventilatory and hemodynamic responses collected during both performance trials are reported in Figure 4.3. A condition x time interaction was found for HR ($F_{4,28}$ = 7.3, η_p^2 = 0.51, $p < 0.01$), MAP ($F_{4,28}$ = 9.0, η_p^2 = 0.56, $p < 0.01$), VO_2 ($F_{1.7,11.8}$ = 9.6, η_p^2 = 0.58, $p < 0.01$), VCO_2 ($F_{4,28}$ = 5.3, η_p^2 = 0.43, $p < 0.01$), and V_E ($F_{4,28}$ = 16.1, η_p^2 = 0.70, $p < 0.01$) during the successive knee-extensors performance test with all variables being heightened at *min 0*. Also, the two-way ANOVA showed a main effect of time ($F_{4,28}$ = 196.9, η_p^2 = 0.97, $p < 0.01$) and condition ($F_{1,7}$ =8.2, η_p^2 = 0.54, $p < 0.02$) for CO. FBF also presented a significant main effect of time ($F_{4,28}$ = 155.4, η_p^2 = 0.96, $p < 0.01$) and condition ($F_{1,7}$ =19.1, η_p^2 = 0.73, $p < 0.01$). Finally, a significant effect of time ($F_{4,28}$=143.8, η_p^2 = 0.96, $p < 0.01$) and condition ($F_{1,7}$ =22.4, η_p^2 = 0.76, $p < 0.01$) was also found for LVC.

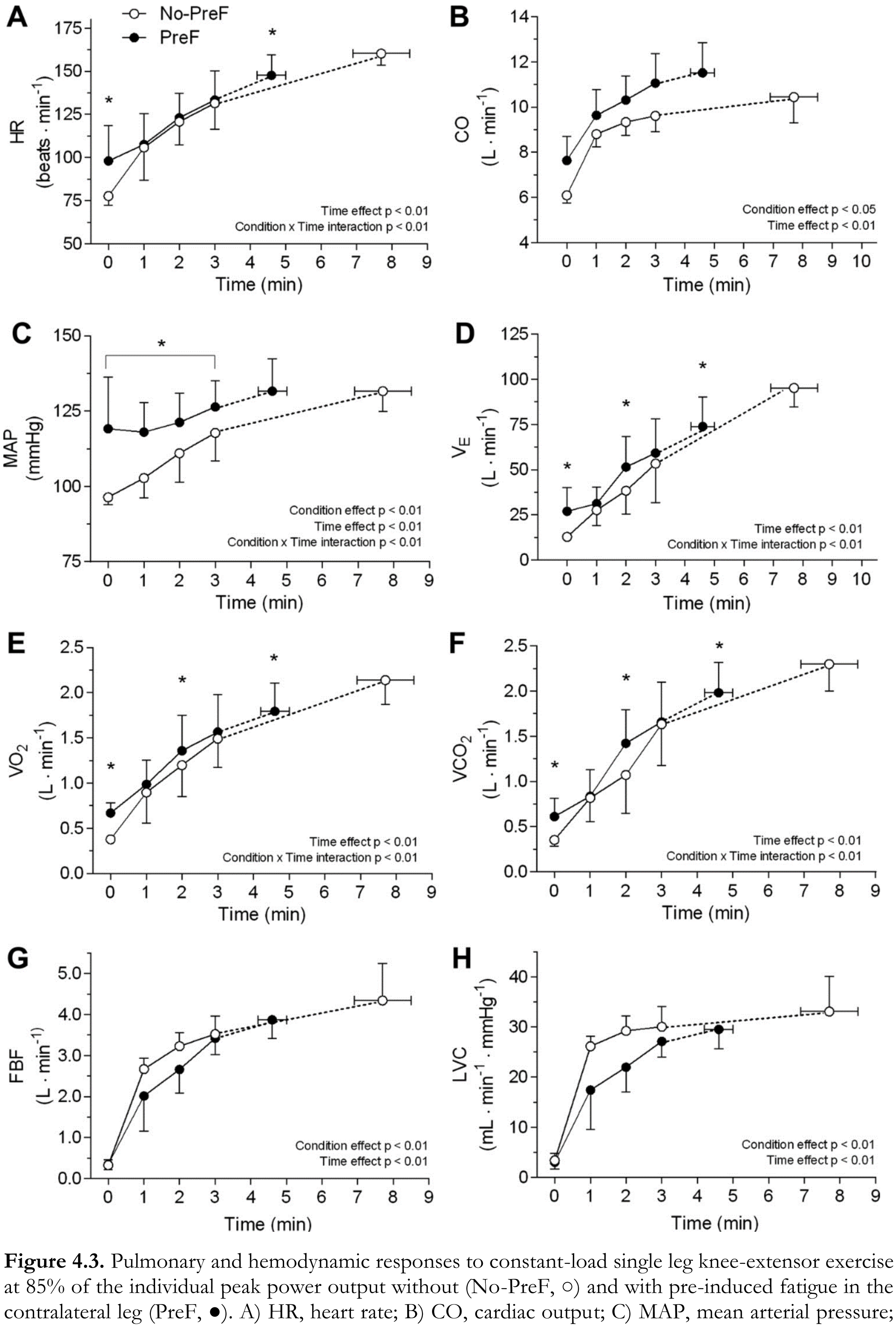

Figure 4.3. Pulmonary and hemodynamic responses to constant-load single leg knee-extensor exercise at 85% of the individual peak power output without (No-PreF, ○) and with pre-induced fatigue in the contralateral leg (PreF, ●). A) HR, heart rate; B) CO, cardiac output; C) MAP, mean arterial pressure; D) VE, minute ventilation; E) VO2, oxygen consumption; F) VCO2, carbon dioxide production; G) FBF, femoral blood flow; H) LVC, leg vascular conductance. * = significantly higher than No-PreF ($p < 0.05$). The exhaustion point is presented as mean ± SEM (horizontal bars); n=8.

M-waves characteristics, Integrated Electromyography and Rate of Perceived Exertion

Peak-to-peak M-wave amplitude was similar between experimental days (No-PreF: 10.02 ± 1.37 mV vs PreF: 9.78 ± 0.35 mV, t_7 = 0.4, p = 0.69). Sarcolemmal excitability was maintained from pre- to post-exercise in all trials as indicated by unchanged M-wave characteristics (peak-to-peak amplitude: No-PreF: from 10.02 ± 1.37 mV to 9.97 ± 1.41 mV; PreF: from 9.78 ± 0.35 mV to 9.74 ± 0.47 mV, $F_{1,7}$=0.46, η_p^2 = 0.01, p = 0.84)

The iEMG data (Figure 4.4, panel A) showed a main effect of time ($F_{3,21}$ = 35.7, η_p^2 = 0.84, p < 0.01) and condition ($F_{1,7}$ = 8.0 η_p^2 = 0.54, p = 0.03). Finally, apart from the exhaustion time point, RPE was different throughout exercise ($F_{3,21}$ = 12.9, η_p^2 = 0.65, p < 0.01, Figure 4.4, panel B). RPE was higher in PreF at *min 1* and continued to increase at the same rate as in No-PreF until reaching the maximal value at the point of exhaustion.

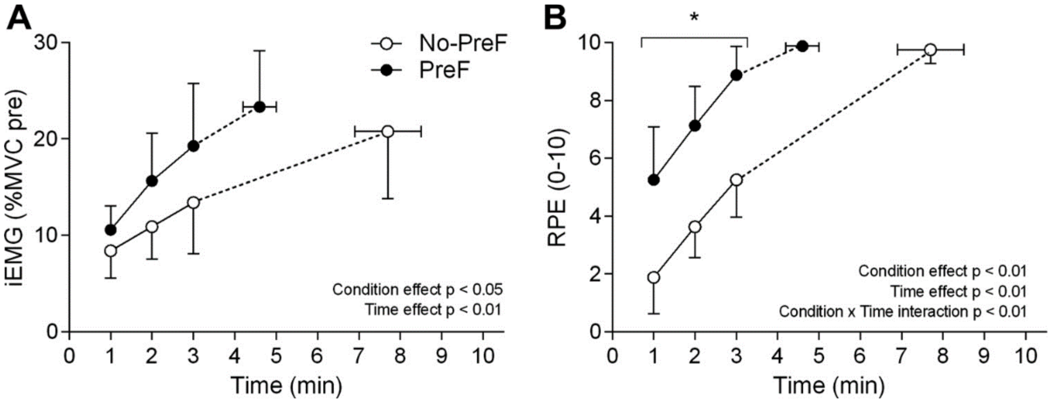

Figure 4.4. A) Integrated electromyography (iEMG), and B) rate of perceived exertion (RPE) during constant-load single leg knee-extensor exercise at 85% of the individual peak power output without (No-PreF, ○) and with pre-induced fatigue in the contralateral leg (PreF, ●). Mean values for iEMG during each concentric knee extension were averaged during the last 30s of every minute and normalized to the EMG activity during an MVC performed before exercise. The exhaustion point is presented as mean ± SEM (horizontal bars); n=8.

4.5 Discussion

The purpose of this study was to investigate whether electrically-induced quadriceps fatigue in the contralateral leg would affect endurance performance and fatigue development of the subsequently exercising ipsilateral leg. In agreement with our hypothesis, we found that endurance time to exhaustion was 38% lower, and end-exercise quadriceps fatigue ($\Delta Q_{tw,pot}$) 8% less when the exercise was performed with pre-existing fatigue. Since the magnitude of the effects associated with contralateral fatigue induced by voluntary exercise (Amann *et al.*, 2013) is only slightly larger compared to the one observed in the current study, together, these results suggest that central motor drive and associated CNS effects might only play a minor role in the crossover effect of central fatigue and further support the hypothesis that group III/IV-mediated afferent feedback is the main determinant of this phenomenon.

Evidence that crossover of central fatigue is mediated by group III/IV muscle afferent feedback

Previous studies have shown that group III/IV muscle afferent feedback-mediated inhibition affects exercise performance and VA in a remote, non-exercising muscle (Amann *et al.*, 2013; Sidhu *et al.*, 2014). However, the involvement of central command in the induction of fatigue may have been a confounding factor in these studies. We circumvented this caveat in the current study by employing electrically-evoked KE exercise to induce fatigue in the contralateral quadriceps. This paradigm allowed us to study the group III/IV-mediated crossover of central fatigue in a condition in which confounding influences associated with central motor drive and volitional effort were eliminated during the process of pre-fatiguing a remote muscle.

Since group III/IV muscle afferents play an important role in regulating the cardiovascular and ventilatory response to exercise independently from central command (Kaufman & Hayes, 2002; Boushel, 2010), the augmented cardiorespiratory response found in PreF (Figure 4.3) represents indirect evidence that electrically-induced fatigue in the contralateral leg successfully raised group III/IV afferents firing during the successive performance test. It is, however, important to recognize that pain associated with the electrical stimulation likely also contributed to the increase in the cardiorespiratory response at the beginning of the fatiguing task in PreF (Aboodarda *et al.*, 2020; Smith *et al.*, 2020c).

An experimental setup and conceptual framework similar to the current study was previously used to raise ensemble afferent feedback and evaluate its effect on exercise performance and neuromuscular function in a remote muscle (Amann *et al.*, 2013). In that study, voluntary KE exercise to exhaustion in the contralateral leg was employed to increase group III/IV afferents firing immediately prior to the start of constant-load KE exercise to exhaustion in the ipsilateral leg. Although the magnitude of end-exercise quadriceps fatigue was similar in the current (Figure 4.2, panel C) and the previous (Amann *et al.*, 2013) study, the impact on endurance performance was slightly less pronounced when pre-fatigue was induced via electrically-evoked (*i.e.*, current study) compared to voluntary (Amann *et al.*, 2013) KE exercise [-38% (range: 20-58%) vs -49% (range: 33-75%)]. Although speculative, the larger impact on performance in the previous study might, at least in part, be due to the involvement of central command in the pre-fatigue process. Specifically, the impact on performance might not only have resulted from the group III/IV-mediated inhibition (as was the case in the current study), but additionally from CNS-related implications associated with central motor drive.

Studies utilizing post-exercise circulatory occlusion (PECO) following fatiguing voluntary isometric muscle contractions document that metabo-nociceptive muscle afferent feedback only impairs neuromuscular function in the rested antagonist (Kennedy *et al.*, 2015) or proximal (Finn *et al.*, 2020) ipsilateral muscle, but not in the contralateral homologous (Kennedy *et al.*, 2015). Based on these observations, the authors concluded that nociceptive group III/IV muscle afferents, a subset of group III/IV muscle afferents not active during freely perfused conventional exercise (Light *et al.*, 2008), are not responsible for the crossover effect of fatigue to the contralateral limb but may reduce central motor drive to an ipsilateral muscle (Kennedy *et al.*, 2015). The current study employed a different exercise paradigm during the pre-fatigue task (*i.e.,* rhythmic contractions following electrical muscle stimulation) and might therefore have engaged a different subset of group III/IV muscle afferents. This could, at least in part, account for the discrepancy between the current study and these earlier observations. Importantly, however, the recruitment pattern during electric muscle stimulation (*i.e.*, earlier recruitment of high threshold, fatigue-prone motor units) does not follow the Henneman size principle (Rodriguez-Falces & Place, 2013) and the exercise-induced intramuscular changes might therefore not only have differed from that during voluntary exercise (Vanderthommen *et al.*, 2003), but also from that during PECO. Taken together, these observations suggest that the role of group III/IV-mediated muscle afferent feedback in the crossover of fatigue is highly dependent on the exercise modality utilized for the pre-fatigue task (and consequently the subtype of muscle afferents activated), and the outcome measures (*e.g.*, VA/MVC *vs* time to task failure). Also, our findings and those of others (Halperin *et al.*, 2015) suggest that the impact of the crossover of central fatigue may be more relevant in the context of submaximal, rhythmic contractions compared to changes in the neuromuscular function obtained during brief, maximal, isometric contractions.

The mechanism by which group III/IV muscle afferent feedback modulates the crossover in central fatigue is not completely clear. Interestingly, however, electrically-induced muscle fatigue has been shown to affect supraspinal areas of the CNS (Papaiordanidou *et al.*, 2010; Alexandre *et al.*, 2015). A study based on near-infrared spectroscopy showed that when electrical stimulation was used to induce plantar flexor fatigue, the decline in VA, measured in the stimulated limb, was mirrored by a decline in both somatosensory and motor cortex activity (Alexandre *et al.*, 2015). Since indices of spinal excitability, quantified by H-reflex, were unchanged, and cortical activity (changes in oxy- and deoxy-hemoglobin) decreased, these findings suggest that afferent feedback may also act at a higher level than the motor cortex, compromising the synaptic input into it. However, to the best of our knowledge, no study has measured whether central impairments are also detected in a rested muscle when electrically-induced contractions are performed on the contralateral side. Therefore, even though Alexandre et al. (Alexandre *et al.*, 2015) evaluated neuromuscular function on the same limb on which fatigue was electrically induced, we speculate that this effect may carry over to the contralateral muscles. In fact, various crossover effects between the two sides have been identified and are possibly mediated by shared neural networks between the two brain hemispheres (*e.g.* transcallosal connections) (Baumer *et al.*, 2002) and between upper and lower limbs (Huang & Ferris, 2004).

It is recognized that electrical stimulation involving high frequency and wide pulses may depolarize sensory neurons and therefore recruit descending spinal motor neurons by an evoked afferent volley (Collins, 2007; Barss *et al.*, 2018). Even though we cannot exclude that central fatigue in the current study was also influenced by spinal factors, previous studies suggest that electrically-induced muscle contractions do not cause spinal fatigue (Boerio *et al.*, 2005; Alexandre *et al.*, 2015; D'Amico *et al.*, 2020). Specifically, fatigue induced by electrical stimulation of the plantar flexors (Boerio *et al.*, 2005) or the adductor pollicis (D'Amico *et al.*, 2020) does not change spinal/motoneuronal excitability. Therefore, supraspinal mechanisms are likely to be the main determinants of central fatigue evoked by electrically-induced muscle contractions.

Evidence that central fatigue restricts the development of peripheral fatigue and impairs exercise performance of a remote muscle

Central fatigue develops during constant-load, dynamic endurance exercise until a subject reaches his/her sensory tolerance limit, a point where the CNS fails to sufficiently activate the exercising muscle and task failure occurs (Gandevia, 2001; Hureau *et al.*, 2018a). Although numerous factors contribute to the development of central fatigue and the attainment of the sensory tolerance limit during exercise, group III/IV muscle afferent feedback, triggered by the intramuscular metabolic perturbations associated with peripheral fatigue (Blain *et al.*, 2016), has been identified as a key determinant (Gandevia, 2001; Matkowski *et al.*, 2011; Amann *et al.*, 2013; Hureau *et al.*, 2018a).

In the current study, task failure occurred sooner, and the sensory tolerance limit was reached with less peripheral fatigue in the ipsilateral leg, during PreF compared to No-PreF. We hypothesize that, compared to No-PreF, the additional source of afferent feedback during PreF facilitated the development of central fatigue and accelerated the attainment of the sensory tolerance limit which reduced exercise time to task failure and, as a consequence, end-exercise peripheral fatigue. Specifically, since the discharge frequency of group IV muscle afferents remains elevated for up to 15 min following electric muscle stimulation (Darques *et al.*, 1998), our results suggest that during PreF, inhibitory afferent feedback was arising from both the electrically fatigued and the voluntarily exercising quadriceps.

Given the differences in V_E between the two trials, it is reasonable to assume that the work carried out by the respiratory muscles was higher during PreF. Thus, a potentially stronger respiratory metaboreflex during PreF cannot be excluded as a factor contributing to the exacerbated development of fatigue and the compromised performance during this trial. However, although respiratory muscle work can negatively affect endurance performance and fatigue development during heavy whole-body exercise (Romer *et al.*, 2006; Amann *et al.*, 2007), single-leg exercise is characterized by a significant cardiac reserve allowing to satisfy the metabolic demand of all working muscles, even at maximal intensities. We therefore consider it unlikely that the relatively small difference in V_E between PreF and No-PreF had a significant impact on performance or fatigue.

Considerations on the use of voluntary activation as an index of central fatigue crossover

The crossover effect of central fatigue is often illustrated by the decrease in VA in the non-exercised muscle (Todd *et al.*, 2003; Rattey *et al.*, 2006; Martin & Rattey, 2007). The design utilized in the present study prevents the use of changes in VA as an indication of fatigue crossover. Specifically, in order to use differences in the exercise-induced fall in VA between conditions as an indicator of fatigue crossover, the assessment should be carried out in the fresh, rested muscle immediately after the pre-fatiguing bout is performed. If the assessment is done at the end of the fatiguing task, as in the present study, VA data may be confounding, since they would result from the sum of the pre-fatiguing and the voluntary exercise tasks. Instead, relevant information could be extrapolated by the rate of development of central fatigue (ΔVA/exercise time). For instance, in two recent studies (Johnson *et al.*, 2015; Aboodarda *et al.*, 2020) the exercise-induced fall in VA was the same across conditions, but the pre-fatigued groups reached it in a significantly shorter time. This finding, in line with the outcome of the current study, agrees with the concept of a crossover of central fatigue, suggesting that more central motor drive was needed to overcome inhibitory feedback, ultimately leading to a premature termination of exercise.

4.6 Conclusions

The current study provides further evidence that group III/IV muscle afferent feedback plays a crucial role in the development of central fatigue and therefore in limiting endurance exercise performance of a remote muscle. Since the augmented firing of these afferent fibers was obtained bypassing central command, it is suggested that impairments associated with repetitive activation of the central motor pathway may only play a minor role in this phenomenon.

Acknowledgements and disclosures

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CHAPTER 5

Study II

Prior involvement of central motor drive does not impact performance and neuromuscular fatigue in a subsequent endurance task

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5.1 Abstract

Purpose: This study evaluated whether central motor drive during fatiguing exercise plays a role in determining performance and the development of neuromuscular fatigue during a subsequent endurance task.

Methods: On separate days, 10 males completed 3 constant-load (80% peak-power output), single-leg knee-extension trials to task failure in a randomized fashion. One trial was performed without pre-existing quadriceps fatigue (CON), and 2 trials were performed with pre-existing, quadriceps fatigue induced either by voluntary (VOL; involving central motor drive) or electrically-evoked (EVO; without central motor drive) quadriceps contractions (~20% maximal voluntary contraction (MVC)). Neuromuscular fatigue was assessed via pre-post changes in MVC, voluntary activation (VA), and quadriceps potentiated twitch force ($Q_{tw,pot}$). Cardiorespiratory responses and rating of perceived exertion were also collected throughout the sessions. The two pre-fatiguing protocols were matched for peripheral fatigue and stopped when $Q_{tw,pot}$ declined by ~35%.

Results: Time-to-exhaustion was shorter in EVO (4.3±1.3min) and VOL (4.7±1.5min) compared to CON (10.8±3.6min, *p*<0.01) with no difference between EVO and VOL. ΔMVC (EVO:-47±8%, VOL:-45±8%, CON:-53±8%), $\Delta Q_{tw,pot}$ (EVO:-65±7%, VOL:-59±14%, CON:-64±9%), ΔVA (EVO:-9±7%, VOL:-8±5%, CON:-7±5%) at the end of the dynamic task were not different between conditions (all *p*>0.09). Compared to EVO (10.6±1.7) and CON (6.8±0.8), rating of perceived exertion was higher (*p*=0.05) at the beginning of VOL (12.2±1.0).

Conclusion: These results suggest that central motor drive involvement during prior exercise plays a negligible role on the subsequent endurance performance. Therefore, our findings indicate that peripheral fatigue-mediated impairments are the primary determinants of high-intensity endurance performance.

5.2 Introduction

During high-intensity exercise, several neural mechanisms cooperate to sustain muscle activity and delay the development of neuromuscular fatigue. Throughout the years, several concepts have been proposed in order to understand the metabolic and neural mechanisms determining exercise tolerance (Burnley & Jones, 2018; Hureau *et al.*, 2018a; St Clair Gibson *et al.*, 2018; Staiano *et al.*, 2018). Among these, the sensory tolerance limit theory postulates that central motor drive and associated corollary discharges, and feedback (*i.e.*, afferent activity) mechanisms, both contribute to achieving a threshold at which exercise becomes unsustainable (Gandevia, 2001; Hureau *et al.*, 2018a). However, the relative contribution of feedforward and feedback mechanisms on the impairment of exercise performance (and development of neuromuscular fatigue) has been traditionally proven elusive to demonstrate.

Central motor drive, defined as the voluntary neural drive to the exercising muscles, may potentially impact exercise performance and fatigue development in two ways: 1) by diminishing the excitability of motor pathway through repetitive activation (Taylor & Gandevia, 2008), and 2) by transmitting a "copy" of the motor command (*i.e.,* corollary discharges) to sensory areas of the brain, ultimately influencing the perception of effort and facilitating central fatigue (Poulet & Hedwig, 2007; Marcora & Staiano, 2010). On the other hand, feedback from mechano- and metabosensitive group III/IV muscle afferents has been proven to exert a strong inhibitory role through projections at different levels of the central nervous system, ultimately facilitating central fatigue and reducing motoneuronal output (Amann *et al.*, 2020).

While the effects of prior exercise on a successive bout have been studied from a metabolic perspective (Jones *et al.*, 2008a; Layec *et al.*, 2009), relatively little is known about the contribution of neural factors in determining the subsequent endurance performance. By inducing various degrees of locomotor muscle fatigue prior to a 5-km cycling time trial, Amann and Dempsey (2008) showed a dose-dependent effect of pre-existing peripheral fatigue on the subsequent exercise performance. Notably, despite significant differences in exercise performance and pre-existing levels of quadriceps fatigue, exercise-induced locomotor muscle fatigue at the termination of the time trials was similar, indicating that afferent feedback from the exercising muscles may have contributed to a reduction in central motor drive and muscle activation in an attempt to preserve the active muscle homeostasis (Amann & Dempsey, 2008). Furthermore, using a different paradigm (*i.e.,* pre-fatigue induced in a contralateral limb), previous studies suggested, together, that afferent feedback may be a more relevant contributor to fatigue development and exercise tolerance than central motor drive during dynamic exercise (Amann *et al.*, 2013; Laginestra *et al.*, 2021). However, no study clearly isolated the role played by central motor drive due to the methodological challenges associated with the confounding influence of group III/IV muscle afferent feedback.

One approach to discern the role of central motor drive from group III/IV muscle afferents is to use electrically-evoked contractions. Nevertheless, given the different motor unit recruitment strategies between voluntary and electrically-evoked contractions, fatigue mechanisms and metabolite accumulation kinetics may differ (Vanderthommen *et al.*, 2003; Barss *et al.*, 2018). In turn, different levels of peripheral fatigue between conditions, would also require different levels of neural drive in order to compensate for the fatigued motor units. Also, since group III/IV muscle afferent activity is related to the intramuscular metabolic perturbation, and given its strong association with peripheral fatigue (Blain *et al.*, 2016; Broxterman *et al.*, 2017a), it is imperative to match peripheral fatigue levels between voluntary and electrically-evoked contractions in order to isolate the effect of central motor drive on the development of fatigue during exercise.

Therefore, the aim of this study was to determine whether the involvement of central motor drive during fatiguing exercise plays a role on the subsequent endurance exercise performance and fatigue development. We hypothesized that a voluntary fatiguing protocol (*i.e.*, with central motor drive) would impair endurance performance more than the electrically-evoked one (*i.e.*, without central motor drive). Moreover, as a consequence of the less work performed in the voluntary exercise, we hypothesized that participants would develop less peripheral fatigue at exhaustion compared to the electrically-evoked condition.

5.3 Methods

Subjects

Ten healthy young males (age: 22.7 ± 1.3 years; height: 177 ± 5 cm; weight: 72.4 ± 7.0 kg) volunteered to participate in this study. The study conformed to the principles of the declaration of Helsinki and was approved by the local ethical committee of the University of Verona.

In the first session, after providing a written informed consent, participants completed a screening questionnaire to evaluate risk for transcranial magnetic stimulation (TMS) (Rossi et al., 2009). The contraindications to TMS are metallic or electronic implants, substance abuse, skin irritations, epilepsy/seizures. Also, all subjects were asked to complete the Waterloo Footedness Questionnaire Revised (WFQ-R) for determination of leg dominance (Van Melick et al., 2017). Two subjects reported to be left leg dominant. Before each visit, subjects were required to abstain from caffeine (≥12h), vigorous exercise (≥24h), and food (≥2h). The subjects were tested in the same temperature-controlled room (22-24 °C) and at the same time of the day to avoid changes related to circadian rhythms (Douglas *et al.*, 2021).

Study design

Subjects visited the laboratory on 5 occasions. All tests were performed with at least 72 hours of rest between sessions. During the first session, all participants were thoroughly familiarized with the experimental procedures, including the single leg knee-extensor exercise (KE) and pre-fatigue protocols. On a separate day, the subjects performed an incremental (10 W + 10 W/min, 60 rpm) single leg knee-extension test to volitional exhaustion to determine peak power output (W_{peak}) of the dominant leg. Task failure was determined when the subject could not maintain 50 RPM despite strong verbal encouragement. During the subsequent 3 visits, subjects performed a constant-load performance task on the single-leg knee extension ergometer at 60 RPM and 80% W_{peak}, preceded by voluntary (VOL) or evoked (EVO) pre-fatigue of the quadriceps muscle (Figure 5.1). Pre-fatigue induction (detailed in a later section: see *"Pre-fatigue protocol"*) was achieved by submaximal isometric contractions. The order of the 3 experimental sessions was randomized. In all sessions, the neuromuscular function of the dominant leg was assessed before and immediately after the end of the single-leg knee extension exercise. Before the beginning of the neuromuscular function assessment, the same standardized warm-up was performed. The performance task was terminated when the cadence dropped <50 RPM despite strong verbal encouragement. The ergometer was manually accelerated by a member of the research team until reaching 60 RPM to avoid initial force spikes.

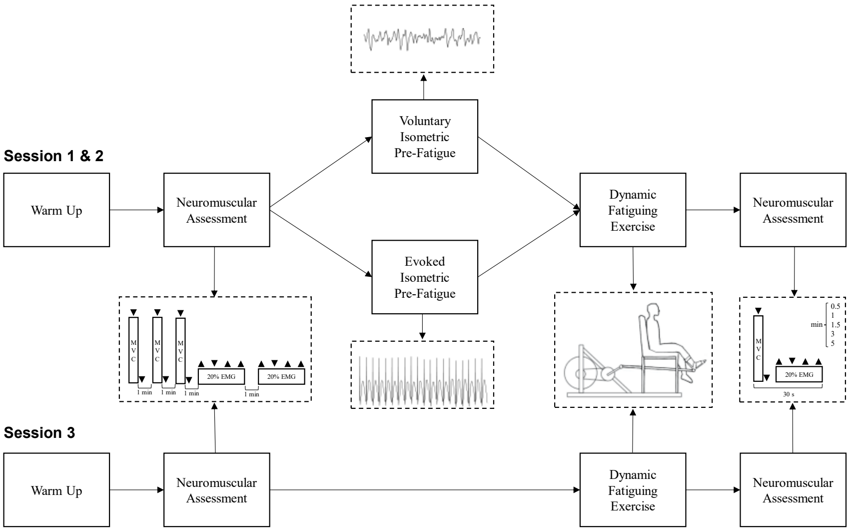

Figure 5.1. Schematic representation of the study protocol. Session 1, 2, and 3 were randomized. Femoral nerve stimulations and transcranial magnetic stimulations during the 20% EMG contractions were delivered in a random order. The neuromuscular assessment post-exercise was repeated at *min* 0.5, 1, 1.5, 3, and 5. MVC: maximal voluntary contraction, EMG: electromyographical activity. ▼= femoral nerve stimulation; ▲ = transcranial magnetic stimulation.

Neuromuscular function assessment

Surface electromyography. Vastus lateralis (VL) and rectus femoris (RF) electromyography (EMG) were continuously recorded with a dual bioamplifier (ML135, ADInstruments, Australia). On the VL, two surface Ag/AgCl electrodes (PG10C; Fiab, Vicchio, Italy) were attached to the skin with a 20-mm inter-electrode distance. The electrodes were placed longitudinally, in line with the underlying muscle fibers arrangement, at two-thirds of the distance between the anterior iliac spine and the lateral part of the patella (Hermens *et al.*, 2000). On the RF, the same electrodes were positioned with a monopolar belly-tendon configuration with the active electrode positioned at 50% of the distance between the anterior iliac spine and the superior part of the patella. The reference electrode was placed on the patellar tendon. Also, two electrodes were placed on the biceps femoris (BF) at 50% of the distance between the ischial tuberosity and the lateral epicondyle of the tibia (Hermens *et al.*, 2000). Before the application of the electrodes, the skin was shaved, abraded with sandpaper, and finally cleaned with an alcohol swab in order to minimize impedance. The electrodes location was marked on the skin with indelible ink to maintain it constant across visits. During the stimulus-response curve protocol (detailed under "*Transcranial magnetic stimulation*"), EMG signal was acquired from RF and BF due to the limited capacity of EMG channels available on our amplifier (2 channels). Once this procedure was completed, wires were switched back from BF to VL. The raw EMG signal was acquired at 2 kHz sampling frequency and stored for offline analysis. Acquisition of the EMG data was done using a computer-based data acquisition and analysis system (hardware: PowerLab 16/30; ML880, ADInstruments, Bellavista, NSW, Australia and software: LabChart8, ADInstruments, Bellavista, NSW, CO Australia). EMG data were analyzed with an in-house built MATLAB routine (MATLAB 2020b, Mathworks, USA). Briefly, the raw EMG signal was bandpass filtered (10-450 Hz) with a 4th order finite impulse response filter and full-wave rectified. Afterwards, a 250ms baseline was detected between each knee-extension and contraction onset was defined as the point when the signal deviated by 3 SD from baseline. The same algorithm was applied to find contraction offset. For each muscle contraction, average root mean square of the VL was calculated and normalized by the maximum EMG_{RMS} obtained during the last three MVC performed during the neuromuscular assessment. Maximum EMG_{RMS} was calculated from a 500ms window preceding the superimposed electrical stimulation. Successively, data contained in the last 20s of each minute were averaged together.

Femoral nerve stimulation. The femoral nerve was stimulated with the anode placed between the greater trochanter and the iliac crest and the cathode placed over the femoral nerve in the femoral triangle via a constant current electrical stimulator (Digitimer DS7AH, Welwyn Garden City, United Kingdom). Electrical stimuli (duration: 1 ms, voltage: 400V) were delivered through squared shape (50x50 mm) self-adhesive electrodes (Myotrode Plus, Globus G0466). The electrode sitting on the femoral nerve was cut in a 20x20mm square to provide a more focal stimulation point. The evoked twitch force was measured by a force transducer previously calibrated. The output from the force transducer was amplified (INT2-L, London Electronics Limited, Sandy Bedfordshire, United Kingdom), and recorded at a sampling rate of 2 kHz. Once the electrodes were in place, stimulation intensity was increased by 25-mA increments until the size of the evoked twitch and compound muscle action potential (M-wave) demonstrated no further increase (*i.e.*, amplitude of maximal M-wave; M_{max}). Stimulation intensity was set at 125% of this value and was kept constant throughout the experimental session.

To evaluate quadriceps neuromuscular function, a potentiated twitch was delivered 2s after a 4-s MVC of the knee-extensors. This procedure was repeated 6 times and each MVC was interspersed by 60s of complete rest. Since full potentiation of Q_{tw} may not be achieved in the first 2/3 MVC (Kufel *et al.*, 2002), only the last 3 $Q_{tw,pot}$ were considered. Voluntary activation (VA) was then assessed using the interpolated twitch technique by comparing the force produced during a superimposed twitch on the MVC with the potentiated single twitch delivered 2-s afterwards. %VA was calculated as: (1 - superimposed twitch force / $Q_{tw,pot}$) · 100. Maximal rate of force development (MRFD) and maximal rate of relaxation (MRR) were calculated for each $Q_{tw,pot}$ as the highest positive and negative derivatives of the force during the rising/declining phases respectively.

Transcranial magnetic stimulation. Single-pulsed transcranial magnetic stimulation (TMS) was applied on the contralateral motor cortex using a double cone concave coil and a magnetic stimulator (Magstim 200^b, Magstim Co. Ltd, Whitland, UK) to elicit motor evoked potentials (MEPs) and contractions in the knee extensors. The coil was manually controlled by an experienced researcher throughout the protocol. Subjects wore a lycra cap on which lines were drawn between the preauricular points and from nasion to inion to identify the vertex. For hotspot determination, ~3s-contractions were performed at 20% MVC in which a stimulus at 60% of the maximum stimulator output was delivered at various points situated laterally and posteriorly relatively to the vertex. Finally, the coil was placed at the site evoking the largest RF MEP amplitudes with minimum BF MEP amplitude. The hotspot position was stored and maintained throughout the session using a neuronavigation system (SofTaxic Navigator system, Electro Medical Systems, Bologna, Italy).

Stimulation intensity was determined by the stimulus-response curve method (Vernillo *et al.*, 2018). Subjects performed brief contractions (~3 s) of the knee extensors while TMS was delivered at each of the following randomly ordered stimulus intensities: 20, 30, 40, 50, 60, 70, and 80% of the maximal stimulator output. In case the participant did not reach a clear plateau in the stimulus-response curve, also 90%

of maximal stimulator output was used. Each intensity was repeated four times and stimuli were delivered at 12-s intervals. Optimal stimulus intensity was defined as the lowest intensity eliciting maximal RF MEP amplitude with minimal antagonist response. Mean stimulus intensity was similar across sessions (EVO: 72 ± 11%, VOL: 71 ± 14%, CON: 72 ± 10%).

To quantify exercise-induced changes in corticospinal excitability, participants were asked to contract the knee-extensors at an intensity corresponding to 20% of the EMG activity obtained during an MVC. Visual live feedback was provided by using a custom-made program written in MATLAB, which calculated the root mean square of the EMG signal in real time over a 200ms window. During this contraction, three transcranial magnetic stimulations and one femoral nerve stimulation were delivered in a randomized order at 3-s intervals from each other.

Exercise responses

Pulmonary gas exchanges (VO_2 and VCO_2) and minute ventilation (V_E) were measured breath-by-breath with a metabolic cart (Quark b^2, Cosmed, Italy). Before each session, after an appropriate warm-up, the gas analyzer and the turbine flowmeter were calibrated according to the instructions of the manufacturer. The data were averaged over the last 20s of each minute. During the voluntary knee-extension exercise, a Doppler ultrasound (Logic 7 Doppler system; General Electric Medical Systems, Milwaukee, WI) equipped with a 12–14MHz linear array transducer was utilized to assess femoral blood flow and vascular conductance of the exercising limb. Femoral artery blood velocity (V_{mean}) and arterial diameter were measured distally from the inguinal ligament and proximal to the bifurcation of the superficial and deep femoral artery. V_{mean} was measured using the same probe utilizing a frequency of 5MHz with the probe positioned and maintained at an insonation angle of 60 or less, and the sample volume was centered and maximized according to vessel size. Then, femoral blood flow (FBF) was calculated as: $V_{mean} \cdot \pi \cdot (\text{arterial diameter} / 2)^2 \cdot 60$. A noninvasive thoracic impedance cardiograph (PhysioFlow, Manatec, Strasbourg, France) was utilized to measure heart rate (HR).

A continuous-wave single-distance near-infrared spectroscopy system (NIMO, Nirox, Brescia, Italy) was used to monitor tissue oxygenation index in the exercising leg. The probe was placed on the belly of the VL muscle, immediately proximally to the EMG electrodes. It was then covered and secured in place by an elastic strap. The area of probe placement was marked with indelible ink to keep the position constant across visits.

Perceived exertion was assessed using the Borg 6-20 scale (Borg, 1998). Subjects were previously properly familiarized with the use of the Borg scale. During the performance test, RPE assessment was performed at each minute.

Pre-fatiguing protocols

The pre-fatiguing protocols consisted in continuously performing 1-min contraction cycles until a ~35% reduction in $Q_{tw,pot}$ from pre-exercise values was achieved. Each 1-min cycle entailed a 50 s isometric contraction at 20% MVC, and 10 s in which a MVC was performed in order to assess global, central, and peripheral fatigue (figure 5.2) through the interpolated twitch technique. The same contraction timing was applied for both VOL and EVO. The choice of including a MVC to assess peripheral fatigue stems from the observation that voluntary and evoked exercise at the same force output, present different degrees of twitch potentiation (Jubeau *et al.*, 2010). Since exercise-induced neuromuscular fatigue has been shown to be best captured by potentiated twitch force (Kufel *et al.*, 2002) a MVC is necessary to achieve the same level of potentiation. In the EVO session, the same pair of electrodes used for peripheral nerve stimulation were utilized for the administration of the electrically-evoked fatiguing task. In this protocol, two stimulators were used to perform the fatiguing electrical stimulation (50 s) and subsequent fatigue assessment (10 s). One stimulator (Digitimer DS7A, Welwyn Garden City, UK), interfaced with PowerLab (PowerLab 16/30; ML880; ADInstruments, Colorado Springs, CO), was used to elicit pulse trains (square-shaped, frequency: 40 Hz, pulse width: 500 μs, 400V, range 12-34 mA) apt to evoke a tetanic contraction. One member of the research team manually held the electrode in place in the femoral triangle. The second stimulator (Digitimer DS7AH, Welwyn Garden City, UK) was set with supramaximal settings as described previously (see *"Femoral nerve stimulation").* After the 50-s tetanic contraction, an operator manually moved the cables from one stimulator to the other for the fatigue assessment.

Visual feedback was provided to maintain the target force. Once the fatigue level was reached, the limb performing the exercise was occluded via a custom-made rapid inflation system and a blood pressure cuff (~300 mmHg), which was released at the onset of single-leg knee extension exercise. The duration of the transition between the isometric and the dynamic exercise was standardized to 30 s.

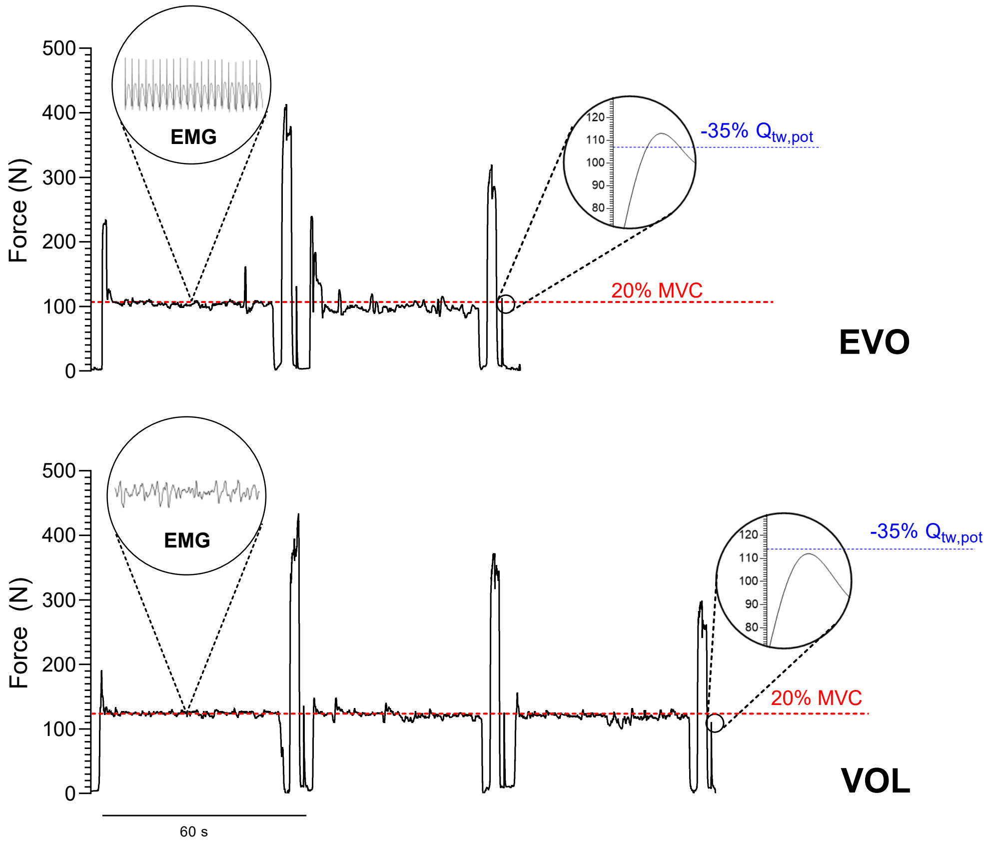

Figure 5.2. Force profile of a representative subject during A) electrically-evoked (EVO) and B) voluntary (VOL) pre-fatigue, The force target (red dotted line) was set at 20% of maximal voluntary contraction (MVC) while the exercise was stopped when potentiated quadriceps twitch force ($Q_{tw,pot}$) decreased by ~35% (blue dotted line). Each pre-fatiguing cycle consisted of 50s of isometric contraction and 10s for the fatigue assessment.

Statistical analysis

For the exercise-induced changes in neuromuscular function and the data collected during the pre-fatigue trials, a two-way ANOVA for repeated measures was performed to find differences between means. The same test was also applied for the in-exercise data, in which inputs were the time points were data was present for all participants and the last point was the individual exhaustion point. A one-way ANOVA was used to test differences in exercise performance between conditions, and to compare baseline data for the neuromuscular function. When a significant condition x time interaction was found, pairwise differences were identified using Bonferroni post-hoc test correction for multiple comparisons. Sphericity was not assumed and, the Greenhouse-Geisser correction coefficient was applied to adjust the resulting *p*-

values when appropriate. Significance level was set at $\alpha \leq 0.05$. Data are expressed as mean ± SD unless otherwise stated. Statistical analysis, graphs and figures were made with GraphPad Prism 8.0 (GraphPad Software, Inc., 2018).

5.4 Results

Pre-fatiguing protocol

Both EVO and VOL were successfully stopped at similar levels of peripheral fatigue (EVO: -38% ± 4%, VOL -38% ± 3%, $t_9 = 0.08$, $p = 0.94$). As expected, the number of pre-fatiguing cycles needed to achieve this target were significantly lower in EVO compared to VOL (2.1± 0.6 vs 3.1 ± 0.7, $t_9 = 4.74$, $p = 0.01$). The reduction in MVC was also similar between conditions (EVO: -34 ± 7% vs VOL: -35 ± 10%, $t_9 = 0.49$, $p = 0.64$) while VA declined significantly less in EVO compared to VOL (-4 ± 4% vs -8 ± 6%, $t_9 = 2.68$, $p = 0.03$). Other variables collected during the pre-fatiguing protocol are presented in Table 1.

		EVO	VOL			
Neuromuscular parameters					*p*	
$Q_{tw,pot}$ (% change from baseline)		-38 ± 3	-38 ± 4		0.94	
MVC (% change from baseline)		-34 ± 7	-35 ± 10		0.64	
VA (% change from baseline)		-4 ± 4	-8 ± 6 *		**0.03**	
Cardiorespiratory variables and local tissue saturation				*p* (Time)	*p* (Condition)	*p* (Interaction)
HR (bpm)	Baseline	79 ± 12	78 ± 11	**<0.01**	**<0.01**	**<0.01**
	End PreF	97 ± 20	120 ± 26 *			
$\dot{V}_E$ (L/min)	Baseline	12 ± 2	11 ± 1	**<0.01**	**0.02**	**0.01**
	End PreF	22 ± 5	33 ± 15 *			
$\dot{V}O_2$ (L/min)	Baseline	0.37 ± 0.06	0.38 ± 0.05	**<0.01**	0.18	0.23
	End PreF	0.67 ± 0.15	0.74 ± 0.17			
$\dot{V}CO_2$ (L/min)	Baseline	0.32 ± 0.59	0.30 ± 0.04	**<0.01**	**0.03**	**0.02**
	End PreF	0.61 ± 0.19	0.82 ± 0.31 *			
$\dot{V}_E/\dot{V}O_2$	Baseline	30 ± 5	28 ± 4	**0.03**	0.08	**0.01**
	End PreF	30 ± 3	43 ± 18 *			
$\dot{V}_E/\dot{V}CO_2$	Baseline	35 ± 5	35 ± 4	0.57	0.21	0.08
	End PreF	34 ± 4	38 ± 5			
TOI (%)	Baseline	66 ± 6	65 ± 4	**<0.01**	0.30	0.54
	End PreF	53 ± 12	51 ± 8			

Table 5.1. Changes in neuromuscular, cardiorespiratory, and hemodynamic responses from the beginning to the end of each pre-fatiguing protocol. "End PreF" represents the individual time point when the pre-fatiguing task was interrupted. EVO: evoked; VOL: voluntary; $Q_{tw,pot}$: potentiated quadriceps twitch force; MVC: maximal voluntary contraction; VA: voluntary activation; HR: heart rate; $\dot{V}_E$: minute ventilation; $\dot{V}O_2$: oxygen consumption; $\dot{V}CO_2$: carbon dioxide production; $\dot{V}_E/\dot{V}O_2$: ventilatory equivalent for O_2; $\dot{V}_E/\dot{V}CO_2$: ventilatory equivalent for CO_2; TOI: tissue oxygenation index. Statistical significance was set at $p \leq 0.05$. * = different than the other condition. Number of participants (n) = 10.

Exercise performance and neuromuscular function

Time to exhaustion was significantly reduced by 60% in EVO (4.3 ± 1.3 min) and 56% in VOL (VOL: 4.7 ± 1.5 min) compared to CON (10.8 ± 3.6 min, $F_{(1.2,10.4)} = 22.9$, $p < 0.01$) with no difference between the two types of pre-fatiguing protocol (EVO and VOL, $p > 0.99$).

Exercise-induced changes in neuromuscular variables are presented in Figure 3. At baseline, MVC were similar between conditions (EVO: 626 ± 93 N, VOL: 654 ± 111 N, CON: 648 ± 124 N, $F_{(1.9,17)} = 2.6$, $p = 0.11$). Same results were found for pre-exercise $Q_{tw,pot}$ (EVO: 189 ± 20 N, VOL: 194 ± 22 N, CON: 189 ± 21 N $F_{(1.8,16.6)} = 2.9$, $p = 0.09$) and VA (EVO: 96 ± 2, VOL: 96 ± 2%, CON: 96 ± 2% $F_{(1.8,16.1)} = 0.3$, $p = 0.74$). Finally, MRFD (EVO: 5897 ± 865 N/s, VOL: 6113 ± 747 N/s, CON: 1618 ± 803 N/s) and MRR (EVO: 2156 ± 549 N/s, VOL:2338 ±494 N/s CON: 2127 ± 314 N/s) were also similar at baseline in all conditions (MRFD: $F_{(2.0,\ 17.7)} = 2.3$, $p = 0.13$, MRR: $F_{(1.7,15.5)} = 3.2$, $p = 0.08$) and declined to similar values post-exercise (Table 2).

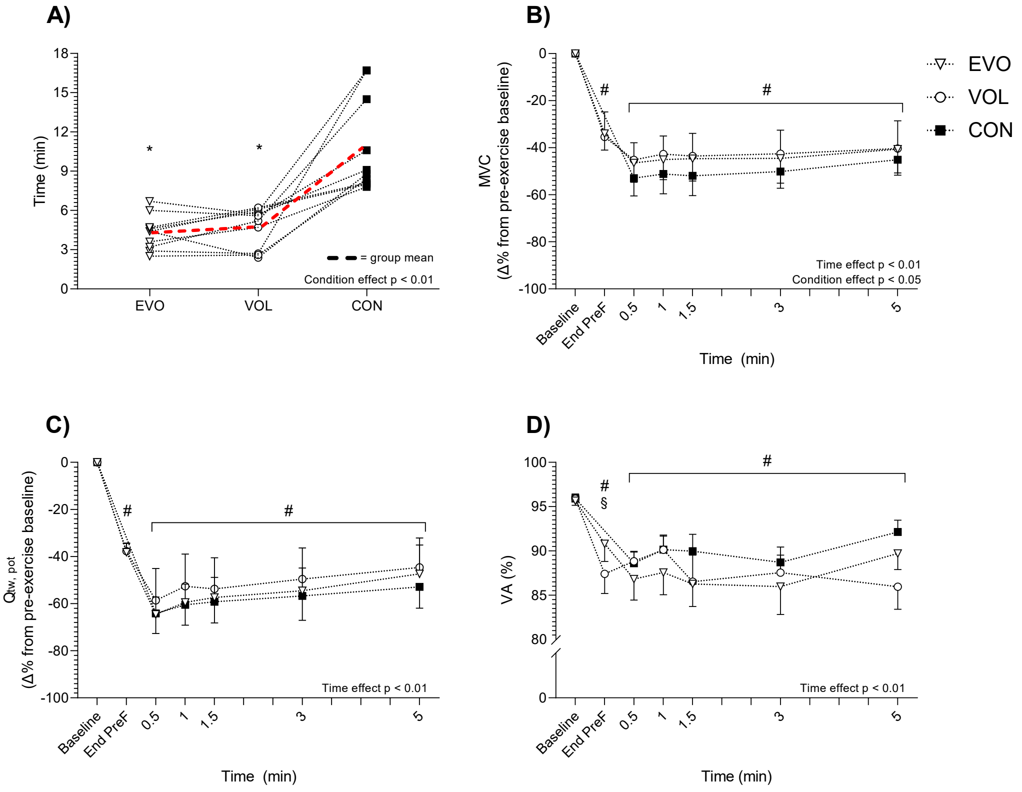

Figure 5.3. Exercise-induced changes in A) time to exhaustion, B) maximal voluntary contraction (MVC), C) potentiated twitch force ($Q_{tw,pot}$), and D) voluntary activation (VA) after electrically-induced (EVO, ∇) and voluntary (VOL, ○) fatigue, or control condition (CON, ■). The red dotted line in panel A) represents the mean value for each condition. * = significantly different than CON; # = significantly different than baseline values; § = VOL significantly higher than EVO. Simple effects are presented following Bonferroni post hoc correction for multiple comparisons. Statistical significance was set at $p \leq 0.05$. Data are presented mean ± SD except for panel D, where for clarity purposes, data are presented as mean ± SEM. number of participants (n) = 10.

		Baseline	*min 0.5*	*min 1*	*min 1.5*	*min 3*	*min 5*	*p* (Condition)	*p* (Time)	*p* (Interaction)
In-twitch parameters										
MRFD (Δ% baseline)	EVO	-	-71 ± 6	-63 ± 7	-60 ± 8	-56 ± 10	-50 ± 12	**<0.01**	0.19	0.14
	VOL	-	-68 ± 12	-62 ± 12	-59 ± 15	-56 ± 14	-50 ± 11			
	CON	-	-72 ± 10	-67 ± 10	-64 ± 10	-62 ± 12	-58 ± 12			
MRR (Δ% baseline)	EVO	-	-70 ± 10	-63 ± 8	-60 ± 9	-50 ± 14	-40 ± 18	**<0.01**	0.70	0.12
	VOL	-	-65 ± 16	-60 ± 12	-55 ± 15	-55 ± 15	-50 ± 19			
	CON	-	-66 ± 16	-63 ± 17	-60 ± 19	-57 ± 17	-51 ± 19			
Electromyography										
RF M_{max} (mV)	EVO	23.8 ± 2.4	26.8 ± 2.4	27.2 ± 2.1	27.2 ± 2.3	27.3 ± 2.8	27.2 ± 2.4	**<0.01**	0.57	0.35
	VOL	23.6 ± 2.1	26.8 ± 2.7	27.0 ± 2.8	26.7 ± 3.1	26.7 ± 3.2	26.4 ± 3.0			
	CON	22.6 ± 2.3	26.5 ± 2.4	27.0 ± 2.1	26.9 ± 2.4	26.7 ± 2.7	26.6 ± 2.7			
RF MEP / M_{sup} (% baseline)	EVO	100 ± 0	108 ± 17	107 ± 18	106 ± 21	104 ± 17	106 ± 14	0.77	0.13	0.21
	VOL	100 ± 0	98 ± 14	102 ± 13	96 ± 11	96 ± 11	97 ± 11			
	CON	100 ± 0	94 ± 18	92 ± 17	92 ± 15	95 ± 14	90 ± 21			
VL M_{max} (mV)	EVO	7.4 ± 2.5	8.9 ± 3.1	8.9 ± 3.0	8.8 ± 2.9	8.8 ± 3.0	8.4 ± 2.8	**0.01**	0.48	0.18
	VOL	8.8 ± 3.3	9.7 ± 2.8	9.6 ± 2.7	9.5 ± 2.8	9.5 ± 2.8	9.2 ± 2.9			
	CON	8.1 ± 3.2	9.2 ± 3.2	8.7 ± 3.2	8.6 ± 3.1	8.7 ± 3.0	8.4 ± 3.1			
VL MEP / M_{sup} (% baseline)	EVO	100 ± 0	100 ± 38	102 ± 31	105 ± 44	98 ± 37	102 ± 29	0.72	0.38	0.47
	VOL	100 ± 0	101 ± 27	104 ± 28	95 ± 22	94 ± 22	96 ± 25			
	CON	100 ± 0	87 ± 31	83 ± 28	84 ± 19	90 ± 18	89 ± 32			

Table 5.2. Exercise-induced changes in the in-twitch parameters, M-wave characteristics, and corticospinal excitability indices. EVO: evoked; VOL: voluntary; CON: control; MRFD: maximal rate of force development; MRR: maximal rate of relaxation; RF: rectus femoris; M_{max}: maximal M-wave; MEP: motor evoked potential; M_{sup}: superimposed M-wave; VL: vastus lateralis. Number of participants (n) =10.

Cardiorespiratory and hemodynamic variables

Metabolic, ventilatory, central, and peripheral hemodynamics data obtained during the dynamic knee-extension exercise are presented in Figure 4. Starting the bout with pre-induced fatigue resulted in significant condition x time interaction in HR ($F_{(2.8,25.5)} = 12.4, p < 0.01$), V_E ($F_{(2.9,26.4)} = 5.1, p < 0.01$), $\dot{V}O_2$ ($F_{(3.0,26.9)} = 14.0, p < 0.01$), $\dot{V}CO_2$ ($F_{(3.1,27.8)} = 9.4, p < 0.01$), , $V_E/\dot{V}O_2$ ($F_{(3.3,29.8)} = 3.9, p = 0.02$), $V_E/\dot{V}CO_2$ ($F_{(2.8,24.8)} = 3.2, p = 0.05$). All variables but $V_E/\dot{V}CO_2$ presented significantly higher responses in both EVO and VOL at the beginning of the exercise task (*min 0*, all $p < 0.05$) while similar values were found at exhaustion (all $p > 0.19$). Finally, only a time effect was found for FBF ($F_{(1.6, 14.5)} = 19.0, p < 0.01$).

Tissue oxygenation index

A significant interaction effect (condition x time) was found for TOI values ($F_{(2.27, 20.4)} = 40.9$, $p < 0.01$), which were significantly lower in both pre-fatigue conditions at the start of the dynamic exercise. However, TOI reached similar values in all conditions at exhaustion (all $p > 0.99$).

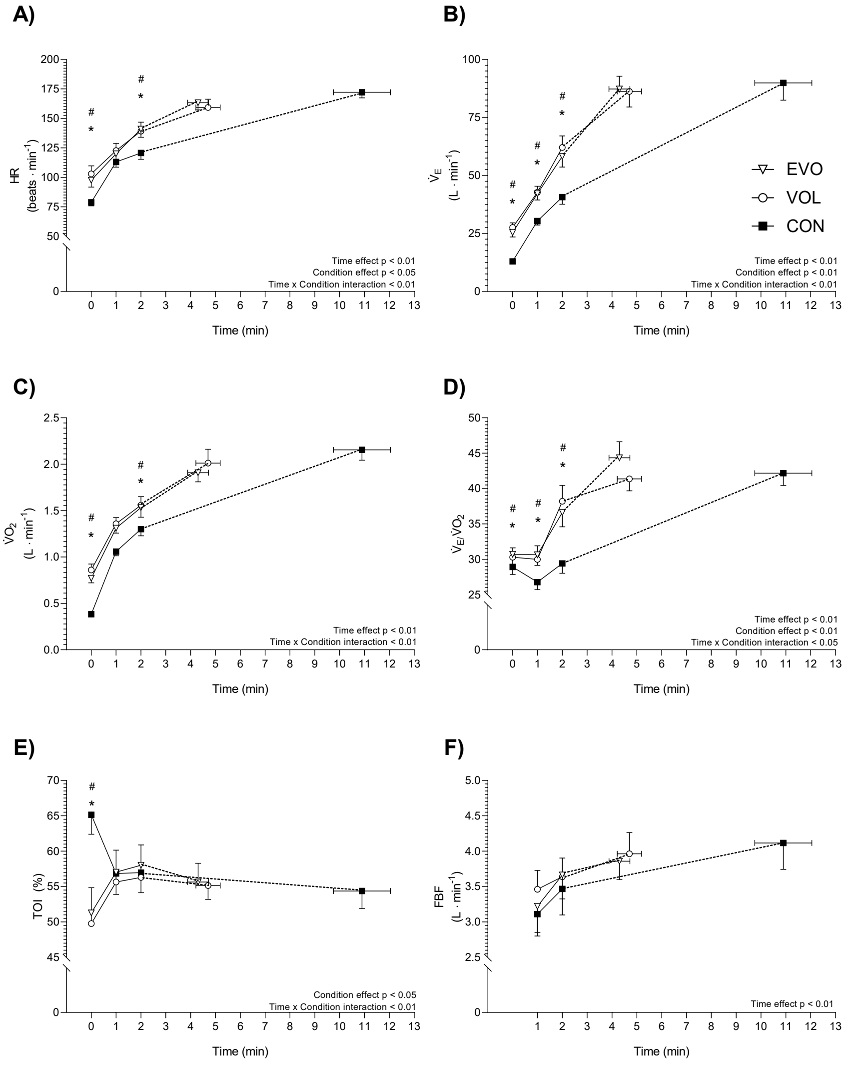

Figure 5.4. Pulmonary and hemodynamic responses to constant-load single leg knee-extensor exercise at 80% of the individual peak power output performed after electrically-evoked (EVO, ∇) and voluntary (VOL, ○) fatigue, or control condition (CON, ■). A) HR: heart rate; B) V_E: minute ventilation; C) $\dot{V}O_2$: oxygen consumption; D) $V_E/\dot{V}O_2$: ventilatory equivalent for O_2; E) TOI: tissue oxygenation index, F) FBF: femoral blood flow. * = VOL significantly higher than CON; # = EVO significantly higher than CON. Simple effects are presented following Bonferroni post hoc correction for multiple comparisons. Statistical significance was set at $p \leq 0.05$. Data are presented mean ± SEM (vertical and horizontal bars). Number of participants (n) = 10.

M-wave amplitude, corticospinal excitability, and electromyography

Exercise-induced changes in M-wave peak-to-peak amplitude for RF and VL are presented in Table 2. No change was found between conditions nor between pre and post exercise for corticospinal excitability indices (Table 2).

During exercise, EMG_{RMS} increased significantly throughout the trial from the first minute until exhaustion (Figure 5). Also, a significant time x condition interaction effect was found ($F_{(1,9,17,5)} = 5.5$, p = 0.01) with the pairwise comparison showing both pre-fatigue conditions having higher values of EMG activity compared to CON at *min 1* of the dynamic exercise task. No difference was found between conditions at the time of exhaustion (all p > 0.99).

Rating of perceived exertion

A condition x time interaction effect was found in the RPE response ($F_{(1.93,17.4)}$ = 23.9, p < 0.01). Pairwise comparison showed that other than a difference between CON (6.8±0.8) and both pre-fatiguing protocols (simple effects: EVO: 10.6 ± 1.7, p < 0.01, VOL: 12.2 ± 1.0, p < 0.01) at the end of *min 1* a significant difference was found between EVO and VOL (p = 0.05), with no difference between all three conditions at exhaustion.

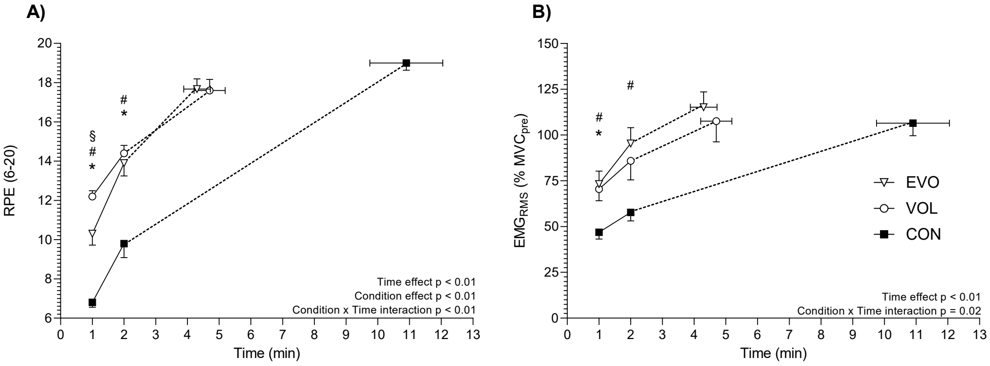

Figure 5.5. A) Rating of perceived exertion (RPE) and B) electromyography during constant-load single leg knee-extensor exercise at 80% of the individual peak power output performed after electrically-evoked (EVO, ∇) and voluntary (VOL, ○) fatigue, or control condition (CON, ■). * = VOL significantly higher than CON; # = EVO significantly higher than CON; § = VOL significantly higher than EVO. Simple effects are presented following Bonferroni post hoc correction for multiple comparisons. Statistical significance was set at $p \leq 0.05$. Data are presented mean ± SEM (vertical and horizontal bars). Number of participants (n) = 10.

5.5 Discussion

This study investigated whether the involvement of central motor drive during prior exercise plays a role in determining endurance performance and the development of fatigue during a subsequent exercise bout. To accomplish our aim, we matched the levels of peripheral fatigue between two pre-fatiguing protocols encompassing voluntary or electrically-evoked contractions before performing a high-intensity endurance task. Contrary to our initial hypothesis, we found that there was no difference in exercise performance between the two conditions. Consequently, all three conditions reached similar levels of fatigue at the end of the performance task. These findings indicate that fatigue mechanisms related to central motor drive involvement have a negligible impact on exercise performance and suggest that peripheral fatigue-mediated effects are the principal determinants of high-intensity endurance performance.

The potential role of central motor drive on endurance performance

The involvement of central motor drive during exercise may have an impact on fatigue development through factors associated with repetitive activation of the motor pathway (Taylor & Gandevia, 2008) or by increasing corollary discharges towards sensory areas of the brain that in turn increase perception of effort which can ultimately limit performance (Marcora & Staiano, 2010). In an effort to minimize the role of repetitive activation (either orthodromic or antidromic) of the motor neurons pool (Heroux *et al.*, 2016), the frequency of electrical stimulation in our protocol mimicked the mean discharge rates of human motor neurons (Pucci *et al.*, 2006).

In this study, indirect insights about whether our protocol was able to manipulate the overall central motor drive activation, may be gained by the RPE results. Since central motor drive and associated corollary discharges are elusive physiological factors to measure, RPE may, in some cases, represent an indirect surrogate for central command (Williamson, 2010).

After the first minute of the performance task (Figure 5), RPE was significantly lower in EVO compared to VOL. Since our exercise model (*i.e.*, time to exhaustion) excludes any possibility of pacing, and afferent feedback was presumably similar by design, this observation may indicate that our pre-fatigue intervention resulted in higher levels of central command for VOL compared to EVO at the beginning of the dynamic task. Furthermore, since the dynamic exercise began 30-s after the termination of the pre-fatiguing task and the recovery of the central nervous system from any central command-induced fatigue is not known, it is likely that this effect may even be underestimated. The mechanisms behind a lower RPE in EVO compared to VOL are not certain. However, a few mechanisms may be involved in this phenomenon. In fact, afferent feedback and corollary discharges are integrated within the somatosensory areas of the brain (Christensen *et al.*, 2007). Therefore, the lower RPE in EVO may be explained by either a "lack" of corollary discharges compared to VOL, or by a direct modulating effect of the electrical stimulation protocol on these somatosensory areas of the brain. Support for this idea comes from a recent study in which electrical stimulation of the elbow flexors altered the relation between RPE and produced force during a successive voluntary contraction, resulting in a reduced RPE for the same task performance and an improved performance for the same RPE (Monjo *et al.*, 2020). Besides, another potential explanation for our data may lie in the fact that any potential decrease in excitability of the central motor pathway caused by its voluntary, repetitive activation, would require an increase in the level of neural drive necessary to maintain a given workload (Taylor & Gandevia, 2008). Regardless, the observation that no difference was found in exercise performance between EVO and VOL even though the two conditions likely required an overall different level of central command activation, represents indirect evidence that central command-related factors may be less important than afferent feedback in determining exercise tolerance during high-intensity efforts. Importantly, we do not exclude that these factors may acquire a more prominent role in exercise of longer durations or when central command involvement is protracted for longer periods of time.

Finally, at the end of the pre-fatiguing task, both conditions presented a decrease in VA, which was, however, ~4% more pronounced in VOL than EVO (Table 1). In contrast with our results, a recent study found that only when the subjects performed the exercise voluntarily, VA of a hand muscle was impaired, suggesting that voluntary descending drive is necessary to lower VA in this muscle (D'Amico *et al.*, 2020). However, similar to our data, other previous studies also found a decrease in VA after electrically-evoked contractions (Boerio *et al.*, 2005; Zory *et al.*, 2005; Papaiordanidou *et al.*, 2010). This discrepancy may be explained by the size of the tested muscle (smaller *vs* larger) and also its location (upper *vs* lower limbs). It is in fact known that several neurophysiological properties are affected by these factors (Vernillo *et al.*, 2018; Rozand *et al.*, 2019).

Evidence that exercise performance is limited by peripheral fatigue-mediated mechanisms

Studies utilizing prior exercise revealed how starting the exercise task with pre-existing fatigue in the exercising (Amann & Dempsey, 2008; Gagnon *et al.*, 2009; Hureau *et al.*, 2014) or contralateral (Amann *et al.*, 2013; Morgan *et al.*, 2019; Laginestra *et al.*, 2021) limb impacts performance regardless of the modality used to induce fatigue. However, to the best of our knowledge, a direct comparison between the consequences of voluntary and electrically-evoked pre-fatigue on a subsequent exercise performance has never been performed. Our pre-fatigue protocol allowed us to isolate the effect of the voluntary central motor drive, from the peripheral fatigue-related impairment happening at the muscular level (which by itself would require a higher central motor drive) and the group III/IV muscle afferents-mediated effects. The finding that time to exhaustion was not significantly different between VOL and EVO, suggests that peripheral factors play a pivotal role in determining exercise tolerance. Moreover, since it is widely accepted that at volitional exhaustion the muscle still possesses an important functional reserve (Morales-Alamo *et al.*, 2015; Martin-Rincon *et al.*, 2021), it is reasonable to expect that our subjects did not terminate the exercise because of the muscles' inability to perform the required work. Rather, it is more plausible that inhibitory feedback coming from group III/IV muscle afferents played an important role in exercise termination. The observation from this and previous studies (Amann *et al.*, 2006a; Amann & Dempsey, 2008; Hureau *et al.*, 2019; Azevedo *et al.*, 2021) that exercise cessation coincides with similar and consistent levels of decline in $Q_{tw,pot}$ (Figure 3C) supports the concept of the existence of a "critical threshold of peripheral fatigue". This idea proposes that metabolic perturbation (and therefore peripheral fatigue) is a tightly monitored and regulated variable, which, through group III/IV afferents-mediated feedback, lead the central nervous system to restrict motoneuronal output and promote central fatigue, in order to limit the disturbance to the muscle homeostasis. This feedback is thought to act at supraspinal level, both on the mechanisms "driving" motor cortical activation situated upstream from the motor cortex (Taylor & Gandevia, 2008; Amann *et al.*, 2020), and on its excitability (Sidhu *et al.*, 2018).

In our study, the two pre-fatiguing protocols led the muscle to carry out different amounts of work. However, it is important to note that our goal was actually to match the level of peripheral fatigue induced by the VOL and EVO protocols, given the tight relationship between $Q_{tw,pot}$ and intramuscular metabolic perturbation measured in muscle biopsies or with phosphorus magnetic resonance spectroscopy from pre to post fatiguing exercise (Blain *et al.*, 2016; Broxterman *et al.*, 2017a). In this scenario, group III/IV muscle afferent feedback was likely similar between conditions, allowing us to isolate the effect of having no, versus substantial, central motor drive activation between EVO and VOL, as expected by design.

Pre-induced fatigue does not alter corticospinal excitability

In this study, no change was found in corticospinal excitability between conditions nor time. This finding is in agreement with previous studies utilizing dynamic tasks (Sidhu *et al.*, 2012; Aboodarda *et al.*, 2020), suggesting that contrarily to what happens in the upper limbs (McNeil *et al.*, 2011b), the net excitability of the motor pathway is not affected by fatiguing tasks in the lower limbs. However, even though our stimulation protocol does not allow us to draw conclusions about any eventual differential changes happening at cortical or motoneuronal levels, previous studies showed that the unchanged excitability may be the result of contrasting facilitatory and inhibitory phenomena happening at these sites (Weavil *et al.*, 2016). Furthermore, we acknowledge that the TMS intensities utilized in our study would be more likely to recruit pools of higher-threshold motor units (McNeil *et al.*, 2011a), possibly neglecting any potential effect on low-threshold motor units, which may be more relevant for endurance tasks.

5.6 Conclusions

Our results suggest that the involvement of central motor drive during prior exercise plays a negligible role on the subsequent high-intensity endurance performance. This indicate that any potential impact of central motor drive on the central nervous system is not sufficient to impact exercise performance substantially. Rather, this type of exercise is primarily limited by peripheral fatigue-mediated impairments.

Acknowledgments and disclosures

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No conflicts of interest, financial or otherwise, are declared by the author(s). The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation, and do not constitute endorsement by ACSM.

CHAPTER 6

Study III

Mechanoreflex-induced chronotropic and ventilatory responses to passive leg movement are potentiated by metabolites accumulation

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6.1 Abstract

BACKGROUND: Previous studies in animal models showed that exercise-induced metabolites accumulation may sensitize the mechanoreflex-induced response. The aim of this study was to assess whether the magnitude of the central hemodynamic and ventilatory adjustments evoked by isolated stimulation of the mechanoreceptors in humans are influenced by the prior accumulation of metabolic byproducts in the muscle.

METHODS: Twenty healthy young subjects performed two exercise bouts consisting of 5-min of intermittent isometric knee-extensions performed 10% above the previously determined critical force. Post-exercise, the subjects recovered for 5 minutes either with a suprasystolic circulatory occlusion applied to the exercised quadriceps (PECO trial) or under freely-perfused conditions (CON trial). Afterwards, 1-min of continuous passive leg movement was performed. Central hemodynamics, pulmonary data, and electromyography from exercising/passively-moved leg were recorded throughout the trial. Root mean square of successive differences (RMSSD, index of vagal tone) was also calculated.

RESULTS: Δpeak responses of heart rate (ΔHR) and ventilation (ΔV_E) to passive leg movement were higher in PECO compared to CON (ΔHR: 6±5 *vs.* 2±4bpm, $p=0.01$; 3.9±3.4 *vs.* 1.9±1.7L·min^{-1},$p=0.02$). Δpeak of mean arterial pressure (ΔMAP) was significantly different between conditions (5±3 *vs.* -3±3mmHg, $p<0.01$). Changes in RMSSD with passive leg movement were different between PECO and CON ($p<0.01$), with a decrease only in the former (39±18 to 32±15 ms,$p=0.04$). No difference was found in all the other measured variables between conditions ($p>0.05$).

CONCLUSION: These findings suggest that mechanoreflex-mediated central hemodynamic and ventilatory adjustments are sensitized by metabolites accumulation and that this effect may be mediated by a transient decrease in vagal tone.

6.2 Introduction

Cardiovascular and ventilatory adjustments to physical exercise are achieved through the cooperation of different mechanisms. The most important are a feedforward efferent mechanism (*i.e.*, central command), the arterial baroreflex, and the exercise pressor reflex, which is a negative-loop feedback mechanism originating from the working muscle, entailing a mechanosensitive and a metabosensitive branch (Fisher *et al.*, 2015). This feedback from the working muscles is sent to the central nervous system through group III/IV afferent fibers, which convey information about the mechanical distortion of their receptive fields (group III), and metabolic changes happening in the intramuscular milieu (group IV) (Fisher *et al.*, 2015). While the involvement of group IV fibers on hemodynamic regulation is well established (Boushel, 2010), the role of mechanosensitive afferent fibers is more difficult to demonstrate. In the last decade, strong evidence for an important role of these fibers in the hemodynamic regulation came using intrathecal fentanyl injection (a potent opioid receptors agonist), which partially blocks afferent feedback to the central nervous system. Indeed, blocking afferent feedback resulted in a substantial decrease in the chronotropic response that typically accompanies passive leg movement (Trinity *et al.*, 2010). Furthermore, using the same pharmacological approach in healthy volunteers (Amann *et al.*, 2010) and a spinal cord injury model, in which afferent feedback is intrinsically interrupted (Venturelli *et al.*, 2012), it has been shown that muscle afferent fibers play a pivotal role also in the ventilatory adjustments to exercise (Amann *et al.*, 2010) and passive movement (Venturelli *et al.*, 2012), respectively.

Classical studies carried out in animal models indicate that metabolic changes in the exercising muscle may sensitize the response to mechanical stress (Rotto & Kaufman, 1988; Rotto *et al.*, 1990). In humans, this issue is more controversial. A previous study utilizing passive stretch of the wrist during post-exercise ischemia, found an increase in the blood pressure response and sympathetic activation (Cui *et al.*, 2008). Similarly, other investigators found a vagally-mediated transient increase in heart rate (HR) when static stretch of the calf muscles was superimposed to circulatory occlusion of the limb (Drew *et al.*, 2008a). However, Fisher et al. showed that HR response to static calf stretch was not different after exercise bouts carried out at different intensities, and therefore likely different levels of metabolites accumulation

(Fisher *et al.*, 2005). Moreover, since different subsets of group III/IV muscle afferents respond to different stimuli, there is evidence that mechanically-sensitive fibers may be preferentially activated during movement compared to static stretch (Hayes *et al.*, 2005). Therefore, static stretch and dynamic movements may yield different outcomes in terms of autonomic control.

Recently, a study found that when passive cycling was coupled with circulatory occlusion of the lower limbs, chronotropic and ventilatory responses were augmented (Lis *et al.*, 2020). However, as highlighted by (Fernandes & Vianna, 2020), the accumulation of metabolic byproducts and the presence of electromyographic recordings are of paramount importance to parse out alternative hypotheses when studying the interaction between metabo- and mechanoreflex. On this note, an adequate exercise-induced metabolic perturbation is necessary to activate the group III/IV afferent pathway. Given that metabolites accumulation is highly dependent on the intensity domain in which exercise is performed (Jones *et al.*, 2008b), prescription based on percentages of maximal strength may not represent an ideal method, since this does not relate to metabolic benchmarks of energy production (Kellawan & Tschakovsky, 2014).

The aim of this study was to evaluate mechanoreflex-induced central hemodynamic and ventilatory responses when a passive, mechanical stimulation was superimposed over circulatory occlusion compared to when recovery is allowed to take place under freely perfused conditions. Our hypothesis was that mechanoreflex-induced responses would be higher when exercise-induced metabolites were trapped in the muscle by circulatory occlusion.

6.3 Methods

Subjects and ethical approval

Twenty young healthy subjects (10 males / 10 females) were recruited for this study (age: 24 ± 4 years, height: 169 ± 9 cm, weight: 66 ± 13 kg). All subjects were non-smokers and none of them was taking medications as determined by a health questionnaire. They were instructed to report to the lab after having refrained from alcohol and caffeine (≥12h), food (≥2h), and physical exercise (≥24h). The subjects were tested in the same temperature-controlled room (22-24 °C). Moreover, the last experimental session for females was performed during the early follicular phase (days 1-5 from menstruation onset) to avoid potential effects of estrogen hormones on hemodynamic responses (Wenner & Stachenfeld, 2020). Leg dominance was established based on the self-reported foot used to kick a ball. All subjects but two reported being right-leg dominant. Written informed consent was obtained from each participant after a detailed verbal and written explanation of the experimental procedures. The study complied with the Declaration of Helsinki and was approved by the local ethical committee of the University of Verona (IRB #30444).

Experimental design and procedures

The subjects were asked to report to the lab on three different occasions. On the first experimental day, the subjects familiarized with the study procedures and with the performance of isometric knee-extensors maximal voluntary contractions (MVC). The instructions for the performance of MVCs were to push "as hard and as fast as possible", to reach a force plateau in the shortest time possible. A schematic representation of the study protocol is reported in Figure 1.

On the 2nd experimental day, the subjects performed an all-out isometric knee-extensor test, in order to estimate critical force employing a 60% duty cycle (contraction/relaxation: 3s/2s) (Burnley, 2009). The five minutes all-out test consisted of 60 knee-extension MVCs using the same duty cycle abovementioned. During the test, participants were verbally encouraged by the members of the research team to ensure maximal effort.

The subjects were seated with a 90° knee flexion on a custom-built chair, with their ankle linked to the force transducer and a steel bar through a noncompliant strap, which was placed 2-cm above the lateral malleolus. The length from the middle of the strap to the center of the patella was measured as the moment arm. Extraneous movement of the upper body was avoided by two-crossed belts over the chest while the hips were stabilized by an additional belt. An audio recording signaled the start and stop of each contraction and the subjects were able to see the force feedback on a wall projected ~3m in front of them. No information was given to the subjects concerning the time elapsed or remaining.

On the 3rd and last experimental session, the subjects were asked to perform two identical exercise bouts in which the recovery was done either without (CON) or with post-exercise circulatory occlusion (PECO). Circulatory occlusion was obtained by inflating a tourniquet cuff (DTC-506. Daesung Maref, South Korea) around the proximal part of the exercising thigh at a suprasystolic pressure (300mmHg) with a custom-made rapid cuff occlusion system (<0.5s to full occlusion). These two bouts were interspersed by a 30 min rest and were carried out in a counterbalanced manner to minimize any eventual carry-over effect. Each bout started with a 5-min baseline period. Then, a MVC was performed to normalize EMG signals. Once the hemodynamic values returned to baseline, 5 minutes of rhythmic isometric knee-extension (3s on / 2 s off) were performed at an intensity that was 10% above critical force, which was determined in the previous experimental visit. At the end of the exercise, the subjects started the 5-min recovery period either with or without PECO. Finally, the lower leg of the subjects was passively moved for 60 s at a frequency of 1 Hz. All passive movement procedures were performed by the same member of the research team, moving the subjects' lower leg through the range of motion defined by 90 and 180° knee joint angles (where the fully extended knee joint is defined as 180°).

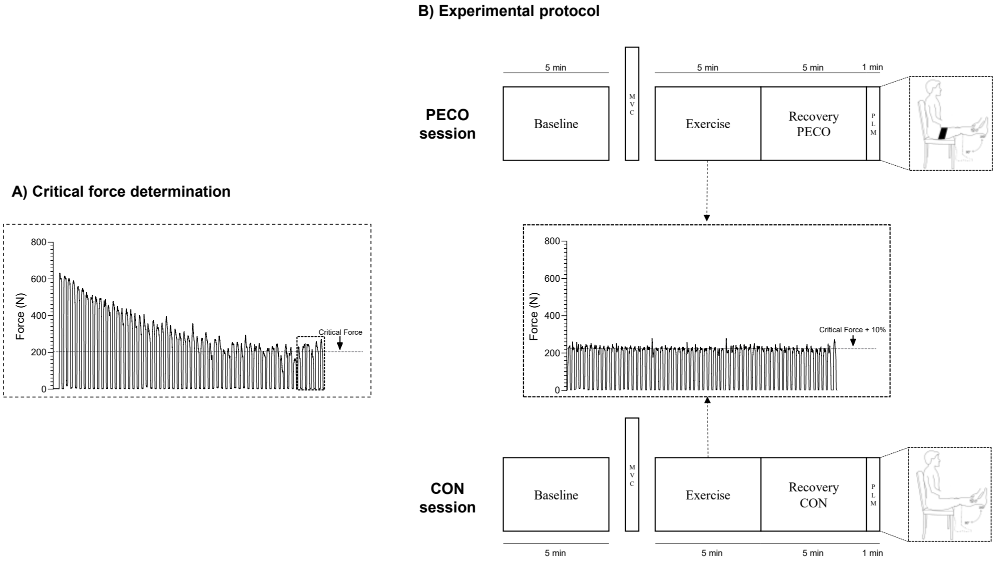

Figure 6.1. Schematic representation of the study protocol. After the familiarization session (not shown) two sessions were carried out in separate days (A and B). Critical force (dashed line) in panel A was calculated as the mean force expressed in the last 6 contractions (dashed square). In the second session, post-exercise circulatory occlusion (PECO) or freely perfused (CON) conditions were performed in a counterbalanced manner with a ~30-min rest in between. Exercise intensity was set at 10% above critical force (dashed line). MVC: maximal voluntary contraction; PLM: passive leg movement.

Central hemodynamics and pulmonary data

Blood pressure, cardiac output (CO), and stroke volume (SV) were measured on a beat-by-beat basis using a finger photoplethysmography device (Finapres model 2300; Ohmeda, Englewood, CO, USA). The left hand was held on a custom-made support, at the level of the heart and a photoplethysmographic cuff was placed on the fourth finger. A software extension (Non-Invasive Cardiac Output, ADInstruments, Australia) was added to the LabChart 8 software to apply the Modelflow algorithm to the raw beat-by-beat data from the non-invasive blood pressure measurement device. Mean arterial pressure (MAP) was calculated as diastolic blood pressure + 1/3 (systolic – diastolic blood pressure). HR was calculated beat-by-beat from the electrocardiographic signal collected with a dual bioamplifier (ML135, ADInstruments, Australia) at 2 kHz.

Pulmonary gas exchange ($\dot{V}O_2$ and $\dot{V}CO_2$,) minute ventilation ($\dot{V}_E$), breathing frequency (f_R) and tidal volume (V_T) were measured breath-by-breath with a metabolic cart (Quark b^2, Cosmed, Italy). Before each session, after an appropriate warm-up, the gas analyzer and the turbine flowmeter were calibrated according to the instructions of the manufacturer.

Isometric force, surface electromyography, and rating of perceived exertion

Isometric force was measured by a force transducer (model UU2; DaCell, Korea) previously calibrated, connected to a custom-made chair through a noncompliant strap placed around the subject ankle. The subjects were seated with a 90° knee flexion. The output from the force transducer was amplified and filtered with a 20 Hz low-cut filter and recorded at a sampling rate of 2 kHz.

Vastus lateralis electromyography (EMG) was continuously recorded with a dual bioamplifier (ML135, ADInstruments, Australia). Two surface Ag/AgCl electrodes (PG10C; Fiab, Vicchio, Florence, Italy) were attached to the skin with a 20-mm inter-electrode distance. The electrodes were placed longitudinally, in line with the underlying muscle fibers arrangement, at two-thirds of the distance between the anterior iliac spine and the lateral part of the patella (Hermens *et al.*, 2000). Before the application of the electrodes, the skin was shaved, abraded with sandpaper, and finally cleaned with an alcohol swab in order to minimize skin impedance. The raw EMG signal was amplified and digitized online at a 2 kHz sampling frequency. Acquisition of the EMG data was done using a computer-based data acquisition and analysis system (hardware: PowerLab 16/30; ML880, ADInstruments, Bellavista, Australia and software: LabChart 8, ADInstruments, Bellavista, Australia).

Rating of perceived exertion (RPE) was obtained at every minute using the 6-20 Borg scale (Borg, 1975). RPE has previously been associated with central command (Williamson, 2010).

Data analysis

All central hemodynamics and breath-by-breath data were linearly interpolated to 1-s intervals and time-aligned to the onset of passive leg movement. Successively, data from the 30 s before passive leg movement was averaged and represented baseline values. Pulmonary data was checked visually for eventual aberrant breaths. When an aberrant breath was found, it was eliminated from the analysis and data from the two adjacent breaths were linearly interpolated. All hemodynamics and pulmonary data were smoothed using a 3-s rolling average. Statistical analysis for the passive leg movement-induced responses was performed on the Δpeak values from baseline.

Root mean square of successive differences (RMSSD) was calculated from the R-R intervals of the electrocardiogram during the 60-s period preceding the onset of the passive movement and the first 15 s afterwards. This time point was chosen because changes in vagal tone may be expected here based on previous literature (Drew *et al.*, 2008a). RMSSD is a recommended time-domain measure of short-term HR variability and it is sensitive to changes in vagal tone (Task-Force, 1996) and relatively free of respiratory influences (Hill & Siebenbrock, 2009).

EMG data were analyzed with an in-house built MATLAB routine (MATLAB 2020b, Mathworks, USA). The raw EMG signal was bandpass filtered (10-500 Hz) with a 4th order, zero-phase, Butterworth filter and full-wave rectified. For the exercise EMG, a 500ms baseline was detected between contractions, and onsets were set when the signal rose by >3SD from baseline values. The same algorithm was applied to find contraction offset. For each muscle contraction, the root mean square (RMS) was calculated and normalized by the highest 500ms EMG_{RMS} obtained during a MVC performed before exercise. Moreover, the EMG_{RMS} of the 30s period preceding passive leg movement, and the 60s of passive leg movement were calculated to ensure that no active contractions were performed.

Critical force was calculated as the average of the mean force exerted during the last 6 contractions (Burnley, 2009).

Statistical analysis

Two-tailed paired sample t-tests were employed to find differences in the Δpeak responses to passive leg movement between PECO and CON and between rest and passive leg movement within each condition for EMG measurements only. The same test was used also to assess baseline hemodynamic, ventilatory, and metabolic data between conditions. Successively, a two-way (condition x time) ANOVA for repeated measures was performed to find eventual differences between conditions during the three phases of the protocol for all the cardiorespiratory variables (rest – exercise – recovery) and for RMSSD (baseline – movement). If a significant treatment x time interaction was found, pairwise differences were identified using Bonferroni post-hoc test correction for multiple comparisons. RMSSD data from one subject was removed from the analysis because it was >5 SD compared to the average data. Statistical analysis and figures were made with GraphPad Prism 8.0 (GraphPad Software, Inc., 2012). Significance level was set at $\alpha < 0.05$. Data are expressed as mean ± SD unless otherwise stated.

6.4 Results

Maximal voluntary contraction, critical force, and exercise intensity

In our participants, MVC was 595 ± 158 N. Critical force was 195 ± 57 N, which was equivalent to 33 ± 8% MVC. Exercise intensity for PECO and CON was 214 ± 62 N (equivalent to 37 ± 9% MVC).

Central hemodynamics and root mean square of successive differences

Rest, exercise, and recovery data for the two conditions are presented in Table 1. All variables were similar between conditions at rest and during exercise (all $p>0.05$). During recovery after exercise, HR, SV, and CO returned to baseline in both conditions, while MAP remained elevated during PECO.

Δpeak values for central hemodynamics variables during passive leg movement are presented in Figure 2. A significant difference in HR behavior was found ($p = 0.01$) whereby HR increased by ~8% in PECO (77 ± 14 to 83 ± 17 bpm) and ~3% in CON (75 ± 11 to 78 ± 12 bpm). Furthermore, the change in SV was similar between conditions (PECO: 67 ± 15 to 74 ± 16 mL, CON: 69 ± 18 to 77 ± 19 mL, $p = 0.47$). However, together these adjustments did not translate into a different increase in CO (PECO: 5.05 ± 1.00 to 5.60 ± 1.07 $L{\cdot}min^{-1}$, CON: 5.08 ± 0.98 to 5.54 ± 1.07 $L{\cdot}min^{-1}$, $p = 0.39$). A divergent response in MAP ($p < 0.01$) was found during passive leg movement with PECO (103 ± 8 to 108 ± 9 mmHg) and in CON (92 ± 7 to 89 ± 9 mmHg).

A significant condition x time interaction was found in RMSSD ($p<0.01$, Figure 4). Follow- up pairwise comparison showed that RMSSD during the first 15 s of passive leg movement was different than baseline in PECO only (39 ± 18 to 32 ± 15 ms, $p=0.04$).

		Baseline	Exercise	Recovery
HR	PECO	77 ± 11	97 ± 18 *	77 ± 14 *
(bpm)	CON	78 ± 13	96 ± 19 *	75 ± 11 *
SV	PECO	72 ± 15	76 ± 16	67 ± 16
(mL)	CON	71 ± 18	79 ± 17	69 ± 18
CO	PECO	5.4 ± 1.2	7.2 ± 1.4 *	5.1 ± 1.0 *
($L \cdot min^{-1}$)	CON	5.5 ± 1.2	7.5 ± 1.4 *	5.1 ± 1.0 *
MAP	PECO	91 ± 7	112 ± 9 *	103 ± 8 * §
(mmHg)	CON	91 ± 6	112 ± 9 *	92 ± 7 * §
V_E	PECO	13.5 ± 3.5	20.6 ± 5.3 *	11.4 ± 4.8 *
($L \cdot min^{-1}$)	CON	14.8 ± 4.5	21.6 ± 6.6 *	10.4 ± 2.5 *
$\dot{V}O_2$	PECO	6.3 ± 1.4	10.1 ± 2.3 *	4.5 ± 0.9 *
($mL \cdot kg^{-1} \cdot min^{-1}$)	CON	6.6 ± 1.6	10.0 ± 2.0 *	4.6 ± 0.8 *
$\dot{V}CO_2$	PECO	5.3 ± 1.2	9.0 ± 2.4 *	4.0 ± 1.2 *
($mL \cdot kg^{-1} \cdot min^{-1}$)	CON	5.7 ± 1.7	9.1 ± 2.3 *	3.8 ± 0.7 *
$V_E/\dot{V}CO_2$	PECO	36.6 ± 4.5	33.1 ± 3.2 *	38.5 ± 6.5 *
	CON	37.2 ± 4.6	34.1 ± 3.5 *	37.2 ± 4.8 *

Table 6.1. Hemodynamic and ventilatory data at baseline, during exercise, and recovery movement in post-exercise circulatory occlusion (PECO) or freely perfused recovery (CON). Passive leg movement started at the end of the recovery. HR: heart rate; SV: stroke volume; CO: cardiac output; MAP: mean arterial pressure; V_E: minute ventilation; $\dot{V}O_2$: oxygen consumption; $\dot{V}CO_2$: carbon dioxide production $V_E/\dot{V}CO_2$: ventilatory equivalent for CO_2. * = significantly different from previous time point ($p<0.05$); § = significantly different from the other condition ($p<0.05$). n=20.

Pulmonary variables

Rest, exercise, and recovery data for the variables of interest are presented in Table 1.

Furthermore, Δpeak values for ventilatory variables in response to passive leg movement are presented in Figure 3. During passive leg movement, Δpeak for V_E increased by ~40% in PECO (11.4 ± 4.8 to 15.3 ± 5.9 $L \cdot min^{-1}$) and only ~20% in CON (10.4 ± 2.5 to 12.3 ± 2.9 $L \cdot min^{-1}$) with this difference being statistically significant ($p=0.02$). Concurrently, the increase in V_T was not significantly different ($p = 0.15$) between PECO (0.87 ± 0.40 to 1.06 ± 0.52 L) and CON (0.72 ± 0.28 to 0.77 ± 0.29 L). Finally, also Δpeak response in f_R was not different ($p = 0.22$) between

PECO (14.8 ± 5.2 to 21.2 ± 5.3 breaths·min^{-1}) and CON (16.4 ± 5.2 to 21.2 ± 5.2 breaths·min^{-1}).

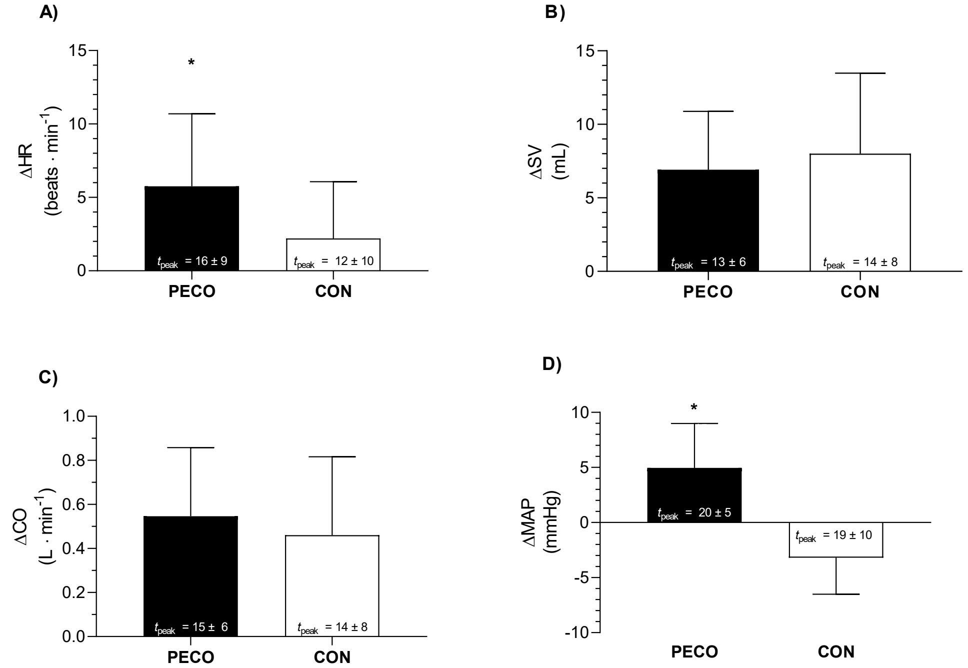

Figure 6.2. Peak changes in central hemodynamics variables during passive leg movement with post-exercise circulatory occlusion (PECO) or freely perfused recovery (CON). * = significantly different than CON. The time when the peak occurred tpeak is expressed in seconds. A) HR: heart rate; B) SV: stroke volume; C) CO: cardiac output; D) MAP: mean arterial pressure. Statistical significance was set at $p \leq 0.05$. Data are presented mean ± SD. Number of participants (n) = 20.

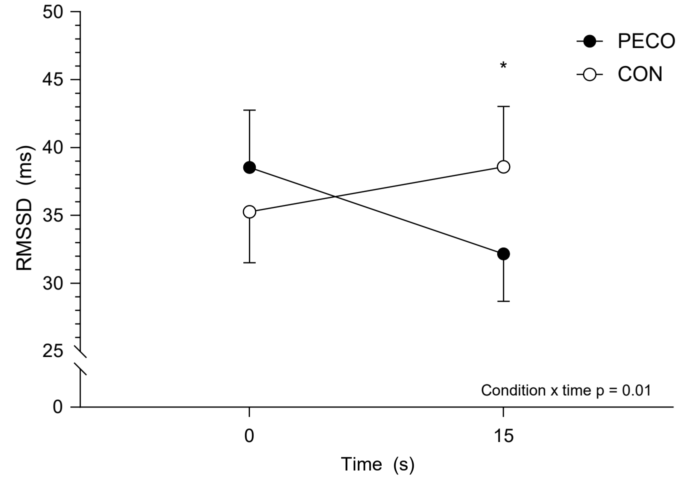

Figure 6.3. Root mean square of successive differences (RMSSD) from baseline to 15s after the onset of the passive leg movement. * = significantly different than the previous time point in PECO. Statistical significance was set at $p \leq 0.05$. Data are presented mean ± SEM. Number of participants (n) = 19.

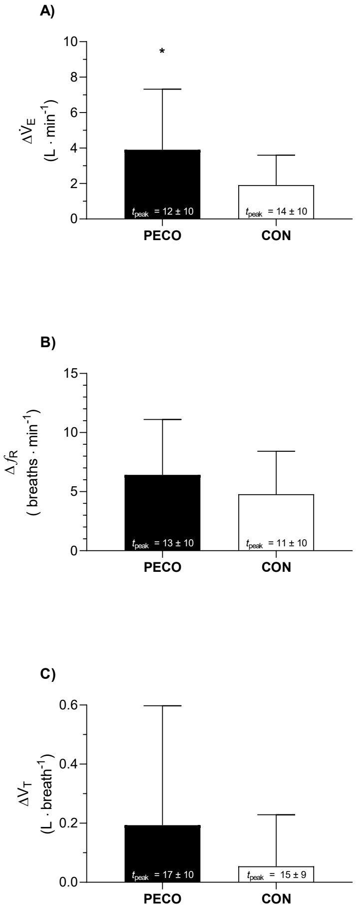

Figure 6.4. Peak changes in ventilatory variables during passive leg movement with post-exercise circulatory occlusion (PECO) or freely perfused recovery (CON). * = significantly different than CON. The time when the peak occurred t_{peak} is expressed in seconds. A) VE: minute ventilation; B) fR: breathing frequency; C) VT: tidal volume. Statistical significance was set at $p \leq 0.05$. Data are presented mean ± SD. Number of participants (n) = 20.

Electromyography and rate of perceived exertion

EMG activity during passive leg movement was not different from the preceding resting period in both conditions PECO (2.35 ± 1.89% to 2.37 ± 1.87%, p=0.30) or CON (2.40 ± 1.90% to 2.40 ± 1.91%, p=0.93). $\mathrm{EMG}_{\mathrm{RMS}}$ and RPE during exercise are presented in Figure 5. $\mathrm{EMG}_{\mathrm{RMS}}$ increased over time in both conditions (pooled values from *min 1* to *min 5*: 39.6 ± 13.0% to 45.0 ± 20.5%, $p < 0.01$), with no difference between PECO and CON (p= 0.97). Also, RPE demonstrated the same behavior by increasing over time (pooled values from *min 1* to *min 5:* 8.9 ± 1.5 to 13.3 ± 2.2, $p < 0.01$) with no difference between conditions (p=0.33).

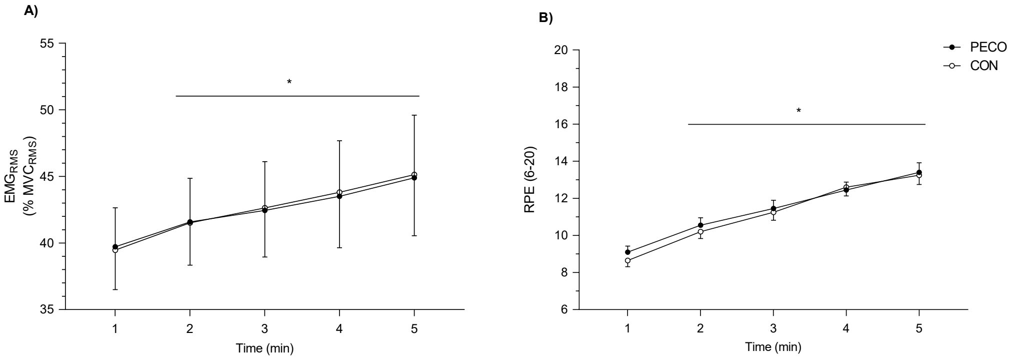

Figure 6.5. A) Electromyography and B) rating of perceived exertion (RPE) during intermittent isometric knee-extensions performed 10% above critical force. Since no differences were found between conditions, data were pooled to display the effect of time. * = significantly different than the previous time point. Statistical significance was set at $p \leq 0.05$. Data are presented as mean ± SD. Number of participants (n) = 20.

6.5 Discussion

The primary objective of this investigation was to study whether the central hemodynamic and ventilatory adjustments to passive leg movement (*i.e.,* mechanoreflex) interact with the effects caused by intramuscular metabolites accumulation (*i.e.,* metaboreflex). The main findings of this study were that chronotropic (ΔHR) and ventilatory (ΔV_E) responses to passive leg movement were higher when the previously exercised muscle was maintained ischemic compared to a situation in which the muscle was freely perfused. These higher responses were accompanied by a larger decrease in the vagal tone at the onset of the passive movement. This finding supports the concept that the cardiorespiratory response to mechanical stimulation, is potentiated when metabolites are accumulated in the muscle and suggest that this phenomenon is mediated by a transient decrease in the parasympathetic drive.

The effects of combined metabo- and mechanoreflex activation on central hemodynamics

Whether an interaction between the mechanical and metabolic branches of muscle afferents exists in humans is still controversial with studies demonstrating an influence (Cui *et al.*, 2008; Lis *et al.*, 2020) and others demonstrating no influence (Fisher *et al.*, 2005; Venturelli *et al.*, 2017). In humans, isolated muscle mechanoreceptors stimulation through passive stretch (Gladwell & Coote, 2002; Gladwell *et al.*, 2005), and dynamic movement (McDaniel *et al.*, 2010; Venturelli *et al.*, 2017) has been shown to be able to evoke a transient HR and blood pressure response (Cui *et al.*, 2006). Given the rapidity of this response, and the fact that infusion of anticholinergic drugs (*i.e.,* glycopyrrolate) abolishes it, this chronotropic adjustment is attributed to an effect on vagal withdrawal (Gladwell *et al.*, 2005).

Our results showed a transient but significantly higher increase in HR response (~8% *vs.* ~3%) to passive leg movement when ischemia was maintained on the moved leg after exercise. However, this difference was too small to result in a higher CO response compared to CON, limiting its functional importance in this model. Importantly, the results from EMG data suggest that there was no involvement of central command during the passive leg movement and indicate that the observed effect is due to a reflex mechanism. Interestingly, we also observed a significant decrease in RMSSD only in PECO in response to passive leg movement (Figure 4). This result agrees with the results of a previous study in which static stretch superimposed on PECO transiently decreased RMSSD (Drew *et al.*, 2008a) and supports the idea that the mechanism between the mechano- and metaboreflex interaction, may be represented by a transient decrease of the vagal tone during the first seconds after movement onset. Therefore, the sensitization of group III fibers, together with the sympathoexcitation due to higher group IV afferents firing, may be a sufficient stimulus to temporarily increase HR and MAP. In fact, previous studies showed how this interaction may lower baroreflex sensitivity and, therefore, its ability to control changes in HR (Drew *et al.*, 2008a; Drew, 2017). The observation that HR was not different between PECO and CON during recovery (Table 1) agrees with previous studies showing that PECO alone does not cause enough sympathoexcitation to override the parasympathetic reactivation happening with the cessation of central command (Nishiyasu *et al.*, 1994; Iellamo *et al.*, 1999). Finally, a recent study by Pecanha and colleagues showed that activation of the mechanoreflex during post-exercise plays a role in attenuating heart rate recovery, highlighting the importance of these mechano-sensitive fibers on the cardiac parasympathetic branches of the autonomic nervous system (Pecanha *et al.*, 2021).

The effects of combined metabo- and mechanoreflex activation on ventilatory drive

The role of muscle afferents stimulation on ventilatory responses is not univocal with previous studies using various approaches, yielding inconsistent results, also dependent on the modality to stimulate group III/IV afferents. For example, passive calf stretch leads to a non-significant increase in V_E (Bruce & White, 2012), while bilateral passive leg movement has been found to provoke a ~6 $L \cdot min^{-1}$ increase in V_E (Bell & Duffin, 2006). However, while the use of large muscle masses may be more appropriate because of the evidence that the magnitude of afferent feedback is related to the size of the involved muscle mass (Iwamoto & Botterman, 1985) it also makes the contribution of central command harder to parse out, given the possible involvement of postural, stabilizing muscles, not directly involved in the passive movement.

In this study, we found that passive movement of a single leg with PECO was accompanied by an increase in peak ΔV_E that was almost twofold the one observed during CON. The fact that neither ΔV_T nor Δf_R were significantly different between PECO and CON may suggest that neither factor alone is responsible for the increased response in V_E in this model, but that may be the result of changes in both variables, which are known to be regulated following distinct inputs (Forster *et al.*, 2012). This larger increase in V_E during the combined activation of metabolically and mechanically sensitive afferents, may signify that the effects of the two pathways are hyperadditive. In fact, while it is well accepted that post-exercise ischemia is unable to sustain ventilatory drive in and of itself (Bruce *et al.*, 2019), evidence from recent studies suggests that inputs from different regulatory mechanisms (*e.g.* central command, central chemoreflex) need to act synergistically in order to increase the ventilatory responses to muscle afferents stimulation (Silva *et al.*, 2018; Lam *et al.*, 2019; Wan *et al.*, 2020a). In an elegantly designed study, Lam and colleagues recently showed that when PECO was superimposed to the previously exercised contralateral leg, the ventilatory response to a successive exercise bout (*i.e.*, involving central command) was accentuated, with the "excess" response attributable to enhanced afferent firing (Lam *et al.*, 2019). Accordingly, a study from Silva et al., demonstrated that when the subjects were breathing a hypoxic mixture, the ventilatory response to passive leg movement was amplified compared to the passive movement alone, demonstrating an interaction when the chemoreflex and the mechanoreflex were stimulated together (Silva *et al.*, 2018).

Experimental considerations

In this study, we decided to use the critical intensity model to establish the exercise intensity for our protocol, instead of basing it on a fixed %MVC. To the best of our knowledge, this approach is novel in the study of autonomic adjustments to metabolic and mechanical stimulation. Our choice stems from the observation that metabolites accumulation depends on the metabolic domain in which exercise is performed (Jones *et al.*, 2008b). In fact, given the high inter-subjects variability in the levels of %MVC at which critical intensity is located (Burnley, 2009; Kellawan & Tschakovsky, 2014), a fixed %MVC would likely create very different metabolic perturbations, which could be extremely high for a subject or extremely low for another (Kent-Braun *et al.*, 1993). Accordingly, a study using ^{31}P magnetic resonance spectroscopy demonstrated how the kinetics of changes in the intramuscular metabolic milieu differ when exercise is performed above, or below critical intensity (Jones *et al.*, 2008b). Also, it is becoming increasingly recognized that absolute muscle strength influences the pressor response to exercise (Notay *et al.*, 2018; Lee *et al.*, 2021), which may also partly be explained by this proposition. In fact, the levels of blood flow occlusion caused by higher absolute forces would, in turn, cause longer time under ischemia and different rates of metabolites accumulation. Therefore, we believe that the approach used in our study is more suitable to compare physiological responses that are highly dependent on metabolites accumulation, and we advocate for future studies to apply this concept to further investigate the role of intensity domains on autonomic adjustments to exercise.

6.6 Conclusions

In conclusion, in this study we have shown that mechanoreflex-induced responses by passive leg movement are sensitized by the metabolic conditions of the muscle in young adults. Our data suggest that these transient responses may result from of a decreased vagal tone.

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CHAPTER 7

General Discussion

7.1 Recap

The purpose of this dissertation was to give new insights on the role that group III/IV afferent feedback plays on exercise performance and autonomic adjustments. It is well known that group III/IV afferent feedback play a quintessential role in determining central and peripheral fatigue, which will have contrasting effects on performance. While the increase of the firing of these fibers will facilitate central fatigue, with a negative impact on performance, it will also try to delay the onset of fatigue by increasing the cardiovascular and ventilatory responses, in an attempt to preserve O_2 delivery to the working muscle and therefore "buffer" peripheral fatigue development. The studies in this dissertation provide new insights on this double role, by studying the effects on exercise performance (Chapter 4 and 5) and on the autonomic adjustments to exercise (Chapter 6).

In the first two studies we aimed to isolate the contribution of group III/IV afferent feedback from possible negative effects that the involvement of voluntary descending drive may have on performance. This was done by using two pre-fatigue models: a contralateral (Chapter 4) and an ipsilateral one (Chapter 5). Prior-induction of fatigue allows to raise afferent feedback compared to control condition. In the last study of this dissertation, the interaction between the metabolically and mechanosensitive branches of group III/IV afferent feedback was investigated. This was accomplished by studying the passive movement-induced hemodynamic and ventilatory adjustments in isolation, or when metabolic stress was superimposed on the passively moved limb.

7.2 Discussion of the main findings

In Chapter 4, it was shown that fatigue induced without the involvement of voluntary effort, is able to impair the endurance performance of a contralateral, non-exercised muscle. Specifically, time to exhaustion was ~40% lower in the pre-fatigued condition. Interestingly, the levels of peripheral fatigue at the end of this trial, were substantially lower compared to the condition in which fatigue was not pre-induced. A similar result, when leg cycling performance was preceded by arm cranking has previously been interpreted to go against the concept that fatigue development is regulated inside the boundary of a critical threshold (Johnson *et al.*, 2015). However, this study does not disprove the notion that fatigue is regulated to a critical threshold, but rather, it demonstrates that exercise performance can be terminated before the attainment of a critical threshold. To disprove the critical threshold of fatigue notion, a study should have a condition in which the experimental condition surpasses the levels of fatigue of the control condition in which the subjects exercise to volitional exhaustion (Broxterman *et al.*, 2015b). Findings such as the ones by Johnson *et al.* (2015) and our findings can exhaustively be explained by the sensory tolerance limit model of fatigue. Indeed, as outlined in Chapter 2.4.2, feedback mechanisms from a previously exercised muscle, together with heightened feedback from other districts, contribute to reaching the sensory tolerance limit in a shorter time in a subsequent trial. This feedback from other working districts, such as the respiratory muscles, is hard to parse out. Indeed, the "respiratory muscles metaboreflex" has been previously documented to play an important role in high-intensity endurance performance, by diverting and "stealing" blood flow from the working muscles and accelerating the rate of development of peripheral fatigue (Harms *et al.*, 1997; Dempsey *et al.*, 2008). Even though comparing responses across different studies present obvious challenges and limitations, some insights may be gained by closely examining the study presented in Chapter 4, and the study by Amann *et al.* (2013). The two studies employed similar endurance performance tasks but differed in the way in which pre-fatigue was induced (*i.e.*, electrically vs voluntarily). The performance decrease in the two studies is relatively similar (Chapter 4: [-38% (range: 20-58%) vs Amann *et al.* (2013): -49% (range: 33-75%)]. Initially, it was speculated that these differences may be potentially due to the involvement of central motor drive (page 58). However, in Chapter 5 we showed that factors associated with central motor drive do not appear to play a crucial role in high-intensity, single-limb endurance performance. Therefore, the small difference in magnitude between the two studies, may actually be due to the involvement of the respiratory metaboreflex. Indeed, ventilation and heart rate at the beginning of the endurance performance task were roughly two-fold and ~30% higher when fatigue was pre-induced by voluntary exercise to exhaustion. Interestingly, in Chapter 5, when the two-pre-fatigue modalities did not result in dramatic changes between conditions at the start of the endurance task (Figure 5.4), also performance was not different between conditions.

In Chapter 5, we found that the involvement of central motor drive does not play a significant role in impairing the successive exercise performance. Indeed, endurance performance was equally impaired by ~55/60% independently from which method was utilized in the induction of fatigue before exercise. The purpose of the study was to manipulate the overall involvement of central motor drive throughout the session (from beginning of pre-fatigue to the end of the fatiguing task), in a way that yields different levels between conditions, while maintaining the afferent component as similar as possible. This was done using a novel condition-matching paradigm, based on the tight relationship between intramuscular metabolic perturbation and exercise-induced decline in muscle potentiated twitch force (Blain *et al.*, 2016; Broxterman *et al.*, 2017a). These above cited studies utilized pre to post exercise assessments of both variables to provide evidence of this relationship. Moreover, perhaps even more convincingly, unpublished findings by the same group (Hureau et al., personal communication by the authors) strengthened these results and found a strong association between peripheral fatigue and intramuscular metabolic perturbation also during fatiguing exercise. In other words, the kinetic of metabolites accumulation and of the progressive exercise-induced development of peripheral fatigue follow similar patterns in time when exercise is performed in the bore of a magnet (*i.e.*, ^{31}P-MRS). Taken together, these findings support our choice of preferring to match peripheral fatigue levels instead of other parameters such as exercise-induced decline in MVC (which is influenced also by volition), or total work (*i.e.*, force-time integral).

In Chapter 6, our findings showed that the chronotropic and ventilatory responses to rhythmic mechanical stimulation are amplified when the passive leg movement is superimposed on the post-exercise circulatory occlusion that keep exercise-induced metabolites in the muscle. Interestingly, these adjustments seem to be mediated by a change in the parasympathetic tone happening in the first phase after the onset of the passive mechanical stimulation. This finding agrees with previous studies showing that exercise-induced metabolites accumulation sensitize the response of the mechanically-sensitive fibers (Rotto *et al.*, 1990; Herr *et al.*, 1999) and support the concept that the mechanoreflex is particularly important for the autonomic adjustments at the onset of exercise. Studies in humans on this topic so far have found inconsistent results with one study concluding that previous metabolites accumulation does not increase the typical mechanoreflex-induced responses (Fisher *et al.*, 2005) and others suggesting the opposite (Cui *et al.*, 2008; Drew *et al.*, 2008a). Interestingly, the studies finding an interaction, proposed two different mechanisms for this interaction. According to the studies by Drew and colleagues, passive calf stretch decreases spontaneous baroreflex sensitivity (Drew *et al.*, 2008a) and the gain of the carotid-cardiac baroreflex function curve (Drew *et al.*, 2008b). This in turn results in lower control on HR, and a decrease in the parasympathetic tone. Also, the administration of aspirin, which inhibits the formation of thromboxane (*i.e.*, a metabolite involved in the exercise pressor reflex and sensitization of group III fibers (Rotto & Kaufman, 1988)) increases the carotid baroreflex sensitivity and results in lower vagal inhibition (Drew *et al.*, 2013). The results from these studies are in line with our findings, in which metabolites accumulation resulted in an increase in HR that was accompanied by a decrease in RMSSD, which is an index of vagal tone. On the other hand, a study by Cui *et al.* (2008) showed an increase in sympathetic nerve activity with a short latency from the movement onset (1 to 3 s). The lack of any direct measurement of sympathetic activity does not allow us to exclude a potential influence of sympathetic drive on the observed increase in chronotropic and ventilatory responses.

7.3 Experimental considerations

Given the well-established differences in neuromuscular fatigue development (Ansdell *et al.*, 2019; Ansdell *et al.*, 2020), in our first two studies we employed only male subjects. Therefore, these results may not be generalizable to the female population. Interestingly, as a result of their series of studies, Ansdell et al. speculated that the limitation in exercise tolerance may be shifted in females from the working muscles to the lungs, given the physiological and anatomical differences (Ansdell *et al.*, 2020). In other words, there may be a sex-difference in the relative contribution of the afferent feedback from the working muscles and other districts, such as the heart and the lungs, in the achievement of the sensory tolerance limit. This represents an interesting scenario for future investigations.

In Chapter 5, the lack of cervicomedullary or thoracic stimulation does not allow us to discern whether there was no change in both cortical and spinal responses, or the observed effect in corticospinal excitability is the result of contrasting data which result in a null net change. On this note, previous studies demonstrated that single-limb isometric (Gandevia *et al.*, 1996; Søgaard *et al.*, 2006) and dynamic exercise (Pageaux *et al.*, 2015). However, strong evidence exists also for a concurrent decrease in spinal excitability during fatiguing exercise (Pageaux *et al.*, 2015; Finn *et al.*, 2018). The lack of an increase in MEP/M_{max} in our study may be related to one limitation that is important to acknowledge. Indeed, the intensity for the TMS was chosen by assessing the complete individual stimulus-response curve at 20% MVC and successively selecting the lowest intensity that concurrently evoked the largest MEP response in RF while minimizing the response in the antagonist muscle (*i.e.*, BF) (Temesi *et al.*, 2014). However, maximation of MEP responses may lead to observe a "ceiling" effect by choosing an intensity that lies on the "plateau" part of the stimulus-response curve. Hence, another method of determination for the stimulator intensity (based on the active motor threshold, or by choosing the intensity corresponding to the maximal slope in the curve) may have allowed to increase the sensitivity to spot differences in corticospinal excitability.

Even though group III/IV afferents are thought to inhibit motoneuronal output and facilitate central fatigue, these results are often not corroborated by findings in voluntary activation of the muscle. The reasons for this observation are still unclear and experimental evidence is lacking. However, there may be various reasons explaining this. Firstly, there may be physical constraints in the experimental setup that only allow to measure voluntary activation with a certain time-lag from when the fatiguing exercise is terminated, and therefore some recovery may have already taken place. Furthermore, voluntary activation can only be assessed during maximal voluntary contractions. Even though the mechanisms are not clear and experimental data is lacking, we speculate that this type of contraction may not be adequate to study the effects of group III/IV afferents. Indeed, empirical evidence suggests that the majority of studies demonstrating an impairment in performance with increased afferent feedback, predominantly involve endurance tasks, where rhythmic submaximal contractions are repeated in time (Halperin *et al.*, 2015). Moreover, group III/IV afferents-mediated inhibitory feedback should not be seen as a "hard boundary" that cannot be overridden. As speculated by Bishop et al. (2010) it is reasonable in fact to believe that conscious regulation may overcome the inhibitory feedback for a brief period. This would explain the "end spurt" at the end of a race, or the lack of an influence during MVCs.

Finally, in Chapter 6 we utilized the critical force model to establish exercise intensity. To the best of our knowledge, this study is the first study utilizing such approach to study the autonomic adjustments to exercise. Similarly to the study in Chapter 5, in this experiment the attention was focused into creating the right assumptions in order to test our hypothesis. Specifically, we recognize the importance of the levels of muscle metabolic perturbation when observing responses in the autonomic adjustments. In other words, the autonomic response is proportional to the level of the intramuscular metabolic perturbation created (Cornett *et al.*, 2000). However, it is important to specify that we do not claim that the method employed in our study guarantees equal or comparable levels of metabolites perturbation. However, given the very distinct behavior of metabolites accumulation across intensity domains, and the intersubject variability regarding the %MVC at which critical intensity lies, we believe that this is still a more appropriate method than using a fixed %MVC.

7.4 Future perspectives

Our model employed in Chapter 5 (*i.e.*, matching participants for peripheral fatigue development) is an interesting tool that has potential to be applied in many other settings, when it is necessary to account for the role of afferent feedback. For example, it may be argued that the impact of central motor drive on performance may be dose-dependent in regards of the time in which central motor drive is involved, and that longer involvements would yield different results compared to our study presented in Chapter 5. A future study set to test this hypothesis should involve central motor drive for different time periods while simultaneously controlling for the development of peripheral fatigue, before performing an endurance performance task. The assessment of cortical and motor neuronal excitability responses during the pre-fatiguing process and the exercise performance may also help understand whether a different modulation happens at cortical and spinal responses.

Another future investigation should exploit this paradigm to investigate the contribution of central command in the autonomic adjustments to exercise. Previous studies tried to tackle this issue using evoked vs. voluntary contractions (Iellamo *et al.*, 1997; Fisher & White, 2003; Fisher *et al.*, 2015). However, the results of these studies are constrained by the fact that due to different motor neurons recruitment strategies involved in these studies, the level of metabolic perturbation is different between conditions, adding two confounding factors: 1) peripheral fatigue renders the muscle less responsive to the neural input, such that more central command is necessary for a given workload, and 2) the two conditions will provide different levels of afferent feedback.

Finally, the interaction between different autonomic regulatory systems, is an important and interesting current topic of investigation. In fact, while traditionally there has been interest in isolating the contribution of each system, in the last years more and more studies are currently investigating the combined effects of two or more systems together (Hureau *et al.*, 2018b; Silva *et al.*, 2018; Lam *et al.*, 2019; Wan *et al.*, 2020a; Wan *et al.*, 2020b). Specifically, Lam *et al.* (2019) used an elegant setup to study the interaction between the metaboreflex and the central command in a more "ecological" setting compared to traditional studies. Indeed, while usually PECO is administered with the subject at rest, these authors applied it on the previously exercised, contralateral leg, while the hemodynamic and ventilatory responses are assessed in the subsequent performance, while there is concurrent activation of the central command. A follow-up study from ours illustrated in Chapter 6, could try to exploit this setup by studying the combined effects of mechanical stimulation when it is superimposed on central command, by comparing the autonomic adjustments to exercise while more muscle mass is moved/stretched than the sole exercising one (*i.e.*, passive movement of the limbs while performing rhythmic handgrip exercise).

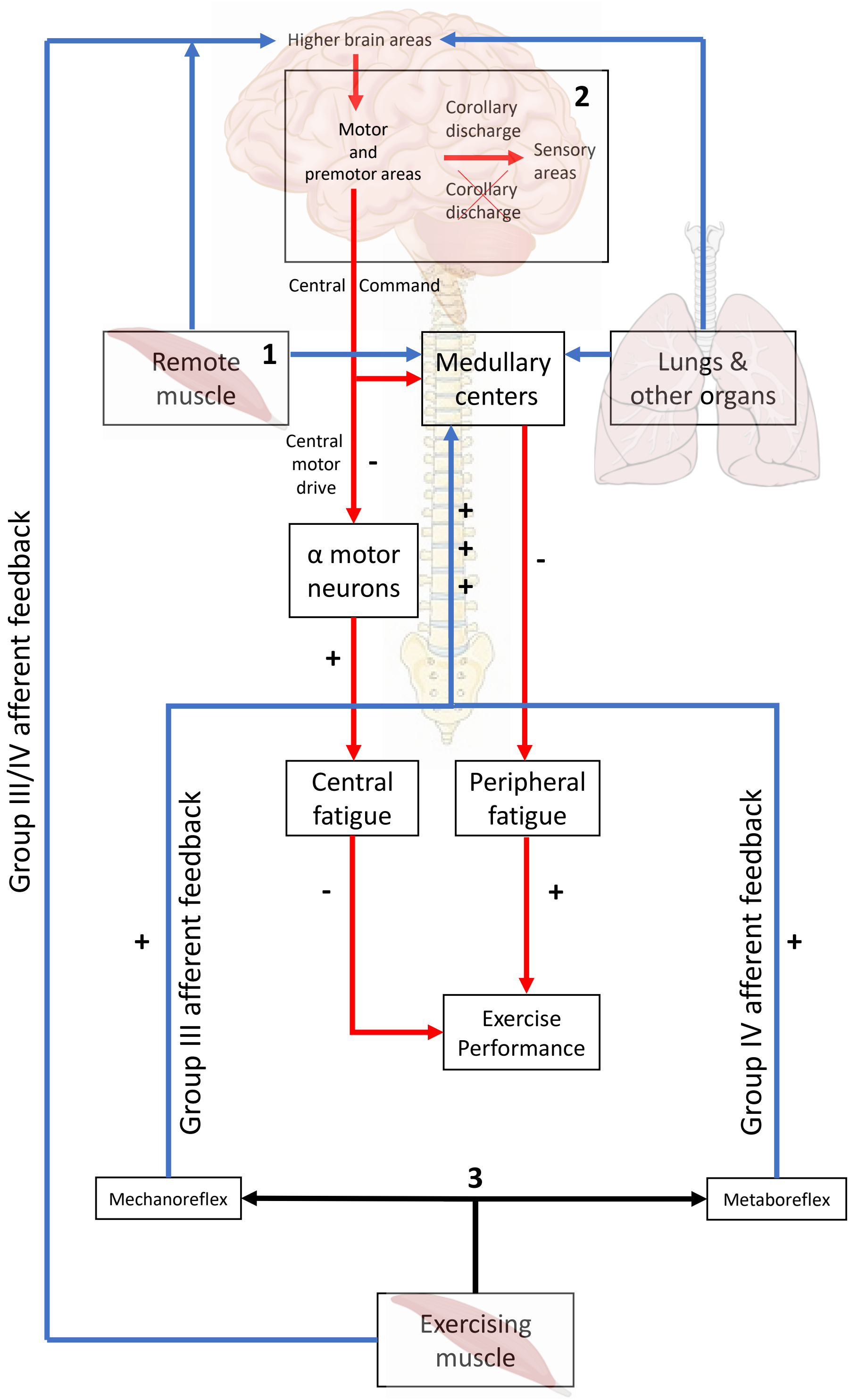

Figure 7.1. Visual schematic representation of the findings of this dissertation and their integration in the existing literature. Blue and red arrows represent the afferent and efferent pathways, respectively. The numbers 1, 2, and 3, represent the study of this dissertation in which that mechanism has been investigated. 1) Afferent feedback from a previously fatigued muscle (bypassing central command) impairs endurance performance. 2) High-intensity endurance performance is equally impaired regardless of the prior involvement of central motor drive and corollary discharges. 3) The combined effect of metaboreflex and mechanoreflex interaction result in a potentiated hemodynamic and ventilatory response.

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