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Conde Alonso Sonia

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FACULTÉ DES SCIENCES SOCIALES ET POLITIQUES

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pour l'obtention du grade de

Docteure ès sciences du mouvement et du sport

par

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Directeur de thèse
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Jury

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IMPRIMATUR

Le Décanat de la Faculté des sciences sociales et politiques de l'Université de Lausanne, au nom du Conseil et sur proposition d'un jury formé des professeurs

- Fabio BORRANI, directeur de thèse, Maître d'enseignement et de recherche à l'Université de Lausanne
- Robin CANDAU, Professeur à l'Université de Montpellier
- Grégoire MILLET, Professeur à l'Université de Lausanne
- Anthony SANCHEZ, Maître de conférence à l'Université de Perpignan

autorise, sans se prononcer sur les opinions de la candidate, l'impression de la thèse de Madame Sonia CONDE ALONSO, intitulée :

« Study of the mechanisms involved in the regulation of O_2 consumption kinetics during exercise »

Marie SANTIAGO DELEFOSSE
Doyenne

Lausanne, le 4 mai 2021

Résumé

Lors d'un exercice à une intensité au-dessous du seuil ventilatoire 1 (V_1), la réponse fondamentale de la cinétique de la consommation de dioxygène ($\dot{V}O_2$) s'élève de manière mono-exponentielle, atteignant un état stable après quelques minutes. Cependant, lors de l'exercice à une charge de travail constante au-dessus de V_1, la cinétique de $\dot{V}O_2$ est caractérisée par un début de l'état stable retardé et une deuxième augmentation de $\dot{V}O_2$ superposée à la réponse initiale de $\dot{V}O_2$.Cette augmentation lente de $\dot{V}O_2$ est appelée la composante lente ($\dot{V}O_{2sc}$). Il a été proposé que cet excès de $\dot{V}O_2$, reflet de l'inefficacité musculaire, provienne principalement des muscles exercitant; cependant, à ce jour, les mécanismes putatifs à cette augmentation sont toujours mal compris. Plusieurs théories ont été proposées, parmi lesquels : a) la combinaison de processus liés à la fatigue nécessitant un recrutement supplémentaire de fibres pour compenser les fibres déjà fatiguées, et b) l'influence potentielle des différents profils métaboliques de différentes populations de types de fibres.

Le but de cette thèse est de clarifier et de nourrir le débat sur les causes de $\dot{V}O_{2sc}$, en particulier pour ces deux derniers paradigmes. Trois expérimentations ont été réalisées pour mesurer la concordance et les interférences de différentes cinétiques de fibres musculaires et la fatigue musculaire avec la $\dot{V}O_{2sc}$.

Les résultats de cette thèse sont les suivants :

1) Lors d'un exercice difficile, l'altération des propriétés neuromusculaires des extenseurs du genou (reflétant les processus de fatigue) n'a été significativement réduite qu'après 20-30 min d'exercice, alors que la $\dot{V}O_{2sc}$ avait fini de croitre. Ce résultat suggère qu'une relation temporelle entre la fatigue et la $\dot{V}O_{2sc}$ ne semble pas exister et, par conséquent, le développement de la fatigue n'est pas une condition essentielle pour le développement de la $\dot{V}O_{2sc}$.

2) La fonction neuromusculaire évaluée à l'aide d'une stimulation double (Ddb, 100 Hz) pendant l'exercice d'extension du genou n'a pas été altérée dans le domaine difficile. En revanche, dans le domaine intense, la diminution significative de la force maximale et du taux maximal de développement de la force lors de la Ddb, reflétaient des processus de fatigue et étaient partiellement corrélées au développement de $\dot{V}O_{2sc}$ relatif. Par conséquent, les résultats suggéraient que la $\dot{V}O_{2sc}$ dans les domaines difficiles et intenses n'est pas le produit d'un mécanisme identique.

3) Afin de construire une nouvelle courbe combinant les principes de Henneman et de superposition, les trois courbes de transitions (repos-modérée, modérée-difficile et difficile-intense) ont été alignées dans le temps et sommées. Les résultats ont montré que globalement les paramètres de la cinétique de la courbe reconstruite n'étaient pas significativement différents d'une transition depuis le repos à un exercice d'intensité intense. Cela suggère que le recrutement supplémentaire de fibres n'était pas présent et que l'apparition de $\dot{V}O_{2sc}$ est au moins liée, sinon le résultat, des différentes propriétés métaboliques des fibres musculaires.

Ces résultats évidence, lors de l'exercice chez l'homme, que les processus de fatigue représentés par des altérations des propriétés neuromusculaires ne sont pas une condition *sine qua non* pour le développement de la $\dot{V}O_{2sc}$ dans le domaine *difficile*, et que l'apparition du $\dot{V}O_{2sc}$ pourrait être le résultat des différents propriétés métaboliques des fibres musculaires.

Abstract

Below the gas exchange threshold (GET), the fundamental response of O_2 consumption ($\dot{V}O_2$) kinetics rises monoexponentially, reaching a steady state after a few minutes. However, at a constant work rate exceeding the GET, the response is characterized by a delayed onset and a second rise in $\dot{V}O_2$ superimposed on the initial $\dot{V}O_2$ response. This slowly developing rise in $\dot{V}O_2$ is termed the slow component ($\dot{V}O_{2SC}$). This excess of $\dot{V}O_2$, a reflection of muscle inefficiency, has been proposed to arise primarily from the exercising muscles; however, to date, the putative mechanisms are poorly understood. Several theories have been proposed, including the combination of fatigue-related processes requiring additional fiber recruitment to compensate for the already fatigued fibers and the potential influence of the different metabolic profiles of different fiber-type populations.

The aim of this thesis is to clarify and nourish the debate on the causes of the $\dot{V}O_{2SC}$, especially for these last two paradigms. Three different experiments were performed to measure the concordance and interferences of different kinetics of muscle fibers and muscle fatigue with the $\dot{V}O_{2SC}$.

The findings of this thesis are as follows:

1) During exercise at heavy intensity, the alteration in the neuromuscular properties of the knee extensors (reflecting fatigue processes) was significantly reduced after only 20-30 min of exercise, while the $\dot{V}O_{2SC}$ was stable. The results suggest that a temporal relationship between fatigue and the $\dot{V}O_{2SC}$ does not appear to exist; therefore, the development of fatigue is not an essential requirement to elicit the $\dot{V}O_{2SC}$.

2) Neuromuscular function assessed through doublet stimulation (Ddb, 100Hz) during knee extension exercise was not altered in the heavy domain. In contrast, in the severe domain, the significant diminution in maximal force and maximal rate of force development during the Ddb, reflected fatigue processes and were partially correlated with the development of the relative $\dot{V}O_2$sc. Therefore, the results suggest that the $\dot{V}O_2$sc in the heavy and severe domains is not the product of an identical mechanism.

3) After constructing a new kinetics curve combining the Henneman and superposition principles, the three different intensity curves (moderate, heavy and severe) were time aligned and summed. The results showed that overall kinetics parameters from the reconstructed curve were not significantly different from one transition to severe-intensity exercise. This suggests that additional fiber recruitment was not present and that the appearance of the $\dot{V}O_2$sc is at least related to, if not the result of, the different metabolic properties of muscle fibers.

These results provide evidence in exercising humans that fatigue processes portrayed by alterations in neuromuscular properties are *not a sine qua non* for the development of the slow component in the heavy domain, and that, the appearance of the $\dot{V}O_2$sc could be the result of the different metabolic properties of muscle fibers.

Dedication

To my father,
an extraordinary person
who left us too soon.

Acknowledgments

Circumstances don't make the man, they only reveal him to himself.

Epictetus

Someone told me that one of the key factors to success in a PhD was to choose the right director. I did not listen to the advice as carefully as I should have. It took me 4 years under the supervision of a dishonest director to realize the importance of assertiveness.

First and foremost, I would like to express my gratitude to the vicissitudes and difficulties I have encountered throughout my life. Because of them, I had the courage to restart a thesis project.

Associate with people who are likely to improve you. Welcome those who you are capable of improving. The process is a mutual one: men learn as they teach.

Seneca

The director

The slow component of the oxygen consumption kinetics changed my life, and it was thanks to Fabio Borrani, the coolest director. I would like to express my deep gratitude and appreciation for accepting me on your team when I was going through one of the hardest moments; for providing guidance and feedback throughout this project; for believing in me and making this PhD possible; for calmly explaining every concept and writing every schema in our bible;) and for your humanity, comprehension, and humility.
Thank you very much for respecting my long training sessions and, more importantly, for showing interest in the results of my races.

The Lausanne University teachers/professors

When I arrived in Switzerland in August 2013, my French had quite deteriorated. I remember going to the university very stressed, with a recording gadget, to transcribe the lessons later at home. Even though I truly enjoyed all the learning during these two years, it was a challenge.
I would like to acknowledge Nicola Place, Davide Malatesta, Gregoire Millet and Jean Marc Vesin for their teaching and character.
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A gem cannot be polished without friction, nor a man perfected without trials.

Seneca

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Last, for the women of my life, my beloved family, it is hard to put into words the gratitude I feel for you. None of this would have been possible without you, your unconditional support and blind faith in me. We are few, but together we are invincible! I cannot avoid thanking the loveliest, cutest and funniest puppy, Theo, who accompanied me through this long pandemic lockdown and lonely period. Thanks to my dear sister in law, Denisse Ariza Pallares, for all the patience, listening, and honesty. Thanks to my mother for showing me, through her example, the wisdom of being organized and facing challenges systematically and for encouraging me in all my projects no matter what. Thank you for, together with my father, instilling values in me such as justice, honesty, humility and hard work. Sista! what can say? You are, have been and will be my great pillar, my best friend and my soul mate. Thank you for our enriching, endless conversations from the kinetics of oxygen consumption, Freud, and Lacan to muscle fibers. Now it is your turn, and you have my full support. Forever.

List of others publications during time of thesis.

1.- Greggio, C., Jha, P., Kulkarni, S. S., Lagarrigue, S., Broskey, N. T., Boutant, M., Wang,X.,**Conde Alonso,S**.,Ofori,E., Auwerx, J. (2017). Enhanced respiratory chain supercomplex formation in response to exercise in human skeletal muscle. Cell metabolism, 25(2), 301-311.

2.- Arribat, Y., Broskey, N. T., Greggio, C., Boutant, M., **Conde Alonso, S**., Kulkarni, S. S., . . . Cantó, C. (2019). Distinct patterns of skeletal muscle mitochondria fusion, fission and mitophagy upon duration of exercise training. Acta Physiologica, 225(2), e13179.

3.- Moreillon, M., **Conde Alonso, S**., Broskey, N. T., Greggio, C., Besson, C., Rousson, V., & Amati, F. (2019). Hybrid fiber alterations in exercising seniors suggest contribution to fast-to-slow muscle fiber shift. Journal of cachexia, sarcopenia and muscle, 10(3), 687-695.

4.- Ofori, E. K., **Conde Alonso, S**., Correas-Gomez, L., Carnero, E. A., Zwygart, K., Hugues, H., . . . Amati, F. (2019). Thigh and abdominal adipose tissue depot associations with testosterone levels in postmenopausal females. Clinical endocrinology, 90(3), 433-439.

List of Publications

1.- Gajanand, T*****., **Conde Alonso, S*.,** Ramos, J. S., Antonietti, J.-P., & Borrani, F. (2020). Alterations to neuromuscular properties of skeletal muscle are temporally dissociated from the oxygen uptake slow component. Scientific reports, 10(1), 7728. doi:10.1038/s41598-020-64395-5

2.- **Conde Alonso, S.,** Gajanand, T., Ramos, J. S., Antonietti, J.-P., & Borrani, F. (2020). The metabolic profiles of different fiber type populations under the emergence of the slow component of oxygen uptake. The Journal of Physiological Sciences, 70, 1-8.

3.- Ramos, J. S*****., **Conde Alonso, S*.,** Gajanand, T., Antonietti, J.-P., & Borrani, F. (2021). The Relationship between the Slow Component of Oxygen Uptake Gain and Changes to the Contractile Properties of the Knee extensors. Sent to the Journal of Physiology.

***** Shared first authorship

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2 List of tables

3 Abbreviations, symbols, and equations.

A	Exponential response amplitude
ACh	Acetylcholine
ADP	Adenosine triphosphate
AP	Action potential
A_s’	Real value of the amplitude of the slow component
ATP	Adenosine triphosphate
Ca^{2+}	Calcium ion
CaO_2	Arterial O_2 content
CF	Correction factor
CO_2	Carbon dioxygen
COPD	Chronic obstructive pulmonary disease
CP	Critical Power
Cp	Creatine phosphate
CT	Contraction time
CvO_2	Venous O_2 content
DHPRs	Dihydropyridine receptors
EMG	Electromyogram
EMS	Electrostimulation
F	Force
F_{opt}	Force at peak power
F_0	Estimated maximal force
G	Gain
GainEnd	Gain at the end
GET	Gas exchange threshold
H	Heavy domain
H^+	Hydrogen ion
H_2CO_3	Carbonic acid
H_2O	Water
HbO_2	Haemoglobin
Hb_{tot}	Total haemoglobin
HCO_3	Bicarbonate

HFF	High frequency fatigue
HHb	De-oxyhemoglobin
HR	Heart rate
Hz	Hertz
iEMG	Integrated electromyogram
LT	Lactate threshold
K^+	Potassium ion
KE	Knee extension
LBF	Leg blood flow
LFF	Low frequency fatigue
M	Moderate domain
MAP	Muscle action potential
MHC	Myosin heavy chain
MLSS	Maximal lactate steady state
MPF	Mean power frequency
MRFD	Maximal rate of force development
RFD	Rate of force development
MRFR	Maximal rate of force relaxation
MRI	Magnetic resonance images
MRT	Mean response time
MU	Motor Unit
MUAP	Motor Unit Action Potentials
MVC	Maximal voluntary contraction
MWVL	M-wave amplitude of vastus lateralis
MWVM	M-wave amplitude of vastus medialis
Na^+	Sodium ion
NIRS	Near–infrared spectroscopy
NMJ	The neuromuscular junction
O_2	Dioxygen
P/O ratio	Reduction of ATP production per mole of oxygen
Pcr	Phosphocreatine
Pi	Phosphate inorganic
P_{max}	Peak power
PNS	Peripheral nerve stimulation

PT	Peak torque single twitch
PT_{MVC}	Amplitude of peak torque
Q	Cardiac output
RFR	Rate of force relaxation
RMS	Root mean square
Rpm	Revolution per minute
S	Severe domain
SFV	Slope of force-velocity relationship
SR	Sarcoplasmic reticulum
SS	Steady state
t	Time
$t_{1/2}$	Half response time of the response
T_2	Transverse relaxation time
TD_p	Time delay for the primary phase
TD_s	Time delay of the slow component
TIT	Twitch interpolation technique
UCP3	Mitochondrial uncoupling protein3
V	Velocity
$\dot{V}O_2(t)$	Oxygen consumption at any point in time
V_0	Estimated velocity of unloaded shortening
VA	Voluntary activation
$\dot{V}CO_2$	Carbon dioxide production
$\dot{V}_E$	Minute ventilation
V_{max}	Maximal velocity
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_{2baseline}$	Oxygen consumption before exercise start
$\dot{V}O_{2max}$	Maximal oxygen consumption
$\dot{V}O_{2peak}$	Peak oxygen consumption
$\dot{V}O_{2rest}$	$\dot{V}O_2$ at rest
$\dot{V}O_{2SC}$	$\dot{V}O_2$ slow component
$\dot{V}O_2SS$	Oxygen consumption steady state
$\dot{V}O_2(t)$	Oxygen consumption at a given time *t*

V_{opt}	Velocity at peak power
W	Watts
WR	Work rate
τ	Time constant
[*x*]	Concentration of *x* (e.g., [Pcr], [Pi])
5s_IMVC	Five-second isometric maximal voluntary contraction
Δx	Difference or temporal change in *x* (e.g., ΔPt)
$\% \Delta$	% difference between GET and VO_{2max}
Subscript c	Cardiodynamic phase (e.g., τ_c, A_c, TD_c)
Subscript p	Phase II or primary response parameter (e.g., τ_p, A_p, TD_p)
Subscript s	Slow component (e.g., τ_s, A_s, TD_s)

4 Theoretical background

4.1 Introduction

Increasing dioxygen (O_2) uptake from the ambient environment and clearing carbon dioxide (CO_2), a product of buffering reactions, the citric acid cycle and oxidative phosphorylation in mitochondria, humans and animals, are able to sustain muscular exercise through adenosine triphosphate (ATP) production. This process, called "gas exchange", and the speed at which it responds to meet the different demands when exercise is started, is defined as oxygen consumption kinetics (Rossiter 2011). The term kinetics has been defined as "the science of the action of force in producing or changing motion" (Chambers English Dictionary). Oxygen consumption ($\dot{V}O_2$) kinetics in human physiology can be defined as "the science of the study of the dynamic VO_2 response to exercise and its subsequent recovery" (Jones and Poole 2013).

In physiological terms, $\dot{V}O_2$ is defined with the Fick equation or principle as the product between the cardiac output and the artery-venous difference in O_2, and it reflects the amount of O_2 that the tissues and cells are able to take up. Therefore, the different speeds of VO_2 kinetics are dependent on the pulmonary, circulatory and muscle systems. The measurement of $\dot{V}O_2$ kinetics in human muscle is an invasive procedure not suitable for routine laboratory use; nevertheless, $\dot{V}O_2$ measurements at the level of the lungs have been shown to accurately reflect the kinetics of O_2 in the working muscles (Barstow and Mole 1987, Whipp and Ward 1990, Jones and Poole 2005).

Fick equation:

$$VO_2 = Q \times (CaO_2 - CvO_2) \qquad \text{Eq 1}$$

where Q is the cardiac output, the product between the stroke volume and the heart rate (HR), and CaO_2 and CvO_2 are the arterial and venous O_2 contents, respectively. Therefore, VO_2 always depends on central oxygen delivery and peripheral O_2 extraction (Jones and Poole 2013). Maximal oxygen consumption ($\dot{V}O_{2max}$) is defined as the maximal rate at which ATP can be synthetized aerobically, and

it symbolizes and yields important information regarding the coordination and capacity of the neuromuscular, cardiovascular and pulmonary systems (Jones and Poole 2013).

4.2 Exercise intensity domains

In exercise physiology, exercise intensity is a fundamental cornerstone of exercise advice, analysis and testing. The most logical procedure to assign relative exercise intensity is to normalize it to a given percentage of $\dot{V}O_{2max.}$ The problem is that this percentage can have dramatic physiological and metabolic variations between subjects. As an example, figure 1 shows the pulmonary $\dot{V}O_2$ of two subjects who were instructed to exercise for 30 min or to the limit of tolerance, both at a work rate (WR) of 85% of $\dot{V}O_2$max. Subject A reached the limit of tolerance after 10 min of effort, while subject B was able to maintain the effort for 30 min; therefore, he was able to maintain a lower relative exercise intensity than subject A.

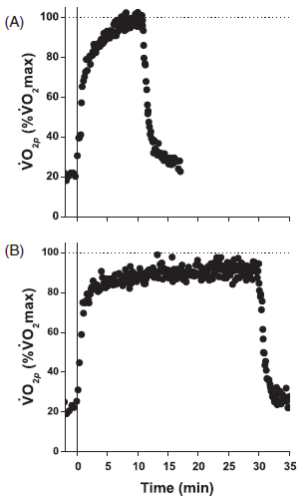

Figure 1 $\dot{V}O_2$uptake of two subjects (A & B) during a constant work rate of 275 W. *Note that subject A reaches the limit of tolerance at 10 min while subject B rode for 30 min. Reproduced from Rossiter 2011.*

This example shows why additional features of physiological stress (more adapted to the fitness level of the subject) should be taken into account. The factors limiting exercise tolerance are well known to be closely related to O_2 exchange, as observed in altitude or pulmonary disorders (Hill, Long et al. 1924, Edwards 1936), and this is one of the reasons why it has been proposed to describe the notion of exercise intensity within the oxygen uptake domain. Whipp, in 1982, (Whipp, Ward et al. 1982) showed that the parameters that describe $\dot{V}O_2$ kinetics, such as gains, time constants and delays (explained later in this rapport), change and adapt their responses at different exercise intensities. Therefore, he argued that parameters that describe $\dot{V}O_2$ kinetics could be used to provide a tool for the assessment of the exercise intensity and the parameters that divide them. Exercise intensity is customarily partitioned into 4 domains based on evoked metabolic responses and $\dot{V}O_2$ kinetics during constant work exercise (Hill, Poole et al. 2002, David C. Poole 2011). Here, the key features that form the central delimitation from which the intensities

are classified are the gas exchange threshold (GET) (Whipp 1987), the critical power (CP) (Poole, Ward et al. 1988), and the $\dot{V}O_{2max}$.

In the *moderate domain*, blood concentrations of lactate and H^+ are stable and confounded with resting concentrations. Nevertheless, at a specific metabolic rate, there is a disproportionate increase in $\dot{V}CO_2$ with respect to $\dot{V}O_2$, defined as the GET. The metabolic event that explains this excess of CO_2 is the rise in blood lactate concentrations above baseline levels, which is why this specific point is also known as the *lactate threshold* (LT) (Connett, Honig et al. 1990). At these WRs, the intensity of exercise cannot be maintained with only oxidative phosphorylation; therefore, the anaerobic lactic system also has to play a role. The rise in lactate, and therefore in H^+, creates a drop in the pH, and; consequently, the pancreas in an attempt to tampon it, secretes bicarbonate. This increase in bicarbonate then pushes the following reaction to the left, generating nonmetabolic CO_2, which in turn activates the ventilatory response, increasing its rate:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3 \qquad \text{Eq 2}$$

where CO_2 is carbon dioxide, H_2O is water, H_2CO_3 is carbonic acid, H^+ is hydrogen ion and HCO_3 is bicarbonate.

This rise in CO_2 affects both pulmonary gas exchange and ventilation and can be conveniently identified as the intersection point of the regression analysis of the slopes of the breath-by-breath values of $\dot{V}CO_2$ plotted against $\dot{V}O_2$ values during an incremental test (figure 2) (Beaver, Wasserman et al. 1986). This rise in CO_2 is used as an index of the LT, i.e., is the increase in blood lactate above resting levels (Wasserman and McIlroy 1964), and delimits the *moderate* and *heavy* domains.

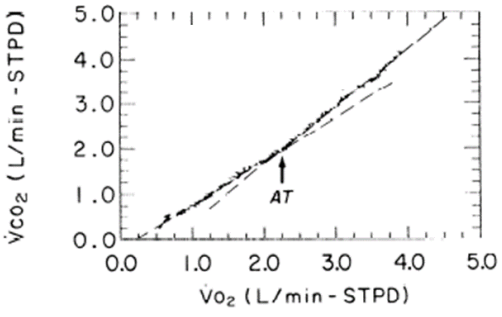

Figure 2 CO_2 production (VCO_2) vs. O_2 uptake (VO_2) showing regression lines for detecting the inflection point (AT point). *STPD, mean standard temperature and pressure in dry conditions. Adapted from Beaver 1986.*

In the *moderate domain*, a steady state (SS) in gas exchange is achieved and can be sustained mainly by aerobic metabolism. After a small increase in blood lactate and a small drop in pH, the values become stable and close to those found at the start of exercise. In fact, even if there is constant

lactate production, this SS is achieved if and only if the removal is equal to the production, regardless of the absolute level (Antonutto and Di 1995). At this intensity, the SS of $\dot{V}O_2$ values in healthy subjects can be attained in only approximately 2-3 min.

In the *heavy domain*, lactatemia rises and the pH drops in a more pronounced way; however, after a while (approximately 10 min), both reach an equilibrium, as the rates of appearance of lactate in blood are counterbalanced by the rates of its removal from blood. This delay in the SS is accompanied by 2 features represented by a progressive rise in $\dot{V}O_2$, termed the $\dot{V}O_2$ slow component ($\dot{V}O_{2SC}$), and sustained (but stable) metabolic acidosis.

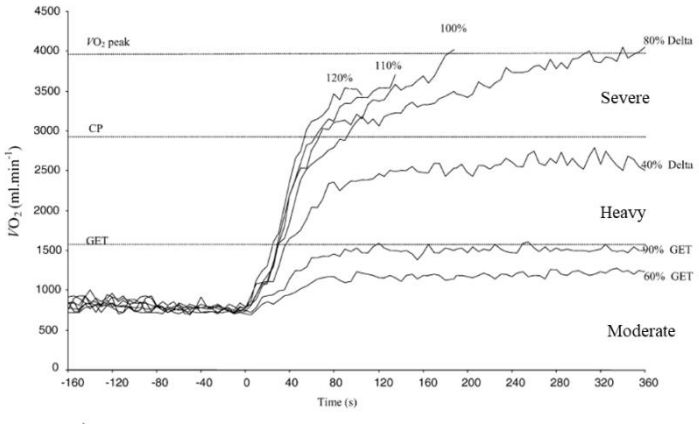

Figure 3 Pulmonary VO_2 response to exercise in the moderate-, heavy- and severe-intensity domains. *The dashed horizontal lines represent the VO_2 measured at the participant's gas exchange threshold (GET), the estimated VO_2 at the critical power (CP) and the VO_2 peak determined in the preliminary ramp test. Adapted from Wilkerson* et al. *2004.*

The CP is defined as the highest WR that can be sustained for a prolonged period of keeping stable the blood acid-base status, or more properly, the highest metabolic rate at which intramuscular creatine phosphate (Cp) and H^+ are still stabilized after an initial adaptation (Jones, Wilkerson et al. 2008). Functionally, or physiologically, the CP is very close to the concept of maximal lactate steady state (MLSS) (Housh, Devries et al. 1991). Even though the methods for determining the CP and MLSS are completely different, both typically occur at approximately 50%**Δ** (Pringle and Jones 2002), although Pringle and Jones (Pringle and Jones 2002) found that MLSS underestimates the CP by approximately 20 W. This metabolic state point is used to differentiate the *heavy* and *severe* domains.

Above the CP, in the *severe domain*, an SS in $\dot{V}O_2$ is never achieved, blood lactate and H^+ accumulate, and muscle phosphocreatine (PCr) is progressively depleted (Jones, Wilkerson et al. 2008). What characterizes this domain is a clear, progressive reduction in work efficiency, with the appearance of a $\dot{V}O_{2SC}$ that decreases with intensity and causes $\dot{V}O_2$ to rise to its maximum until the exercise is terminated (figure 4). The higher the WR above the CP, the faster the projection of the $\dot{V}O_{2SC}$ and the shorter the exercise can be maintained before exhaustion (Gaesser and poole 1996).

$\dot{V}O_{2max}$ is the last demarcator that delimits the *severe* from the *extreme* domain. The term $\dot{V}O_{2max}$ was defined by Hill and Lupton in 1923 (Hill and Lupton 1923) as the highest amount of O_2 uptake during maximal exercise that could not be increased even with increments in the workload.

In the *extreme domain*, there is no time for an SS, even though the $\dot{V}O_{2SC}$ is not discernable due to the rapidity in achieving the maximum rate of O_2 consumption (2-4 min in healthy subjects (Hill, Poole et al. 2002). Two scenarios are plausible: the subject stops the exercise either because of exhaustion or because he has reached $\dot{V}O_{2max}$.

4.3 $\dot{V}O_2$ kinetics and exercise intensity in the different domains

- Moderate domain

At the onset of a constant, moderate-intensity WR exercise, three different phases that correspond to distinct physiological events can be differentiated when looking at the pulmonary oxygen uptake kinetics (figure 3&4).

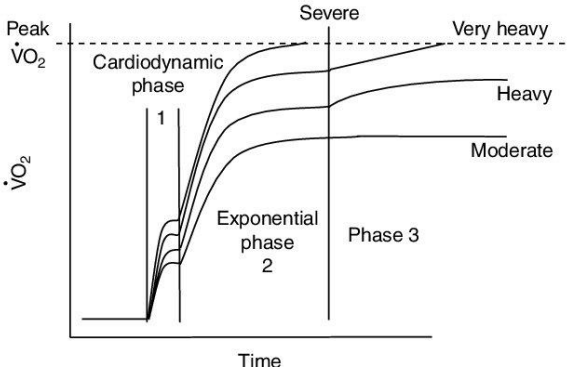

Figure 4 Oxygen uptake kinetic response to exercise in children. *Taken from Samantha G. Fawkner & Neil Armstrong 2003.*

The first phase, called the *cardiodynamic phase (phase I)*, does not represent increased muscle O_2 consumption but, is the result of blood flow into the lungs resulting from the sudden increase in venous return from exercise onset as a result of muscle contraction. The exact inflection point where this phase ends is not always easy to recognize; therefore, some groups have decided to eliminate it from consideration by omitting data collected from the onset of the exercise to a specific time point (Whipp, Ward et al. 1982). Indeed, Whipp and colleagues (Whipp, Ward et al. 1982) demonstrated that, on average, the exponential response began approximately 20 s after exercise onset. Later, Krustrup and colleagues (Krustrup, Jones et al. 2009) demonstrated that the mean transit time from muscle capillaries to the lung was approximately 17 s before exercise and that the removal of this phase did not distort the fidelity of the relationship between muscle $\dot{V}O_2$ kinetics and pulmonary $\dot{V}O_2$ kinetics. Therefore, as a consensus, the

first 20 s of the pulmonary $\dot{V}O_2$ signal, which does not reflect muscle O_2 consumption, are removed from the analysis.

After this cardiodynamic phase, $\dot{V}O_2$ increases in an exponential fashion, describing *phase II, or the primary component*, which quite closely represents the muscle $\dot{V}O_2$ kinetics after exercise onset (Barstow, Scremin et al. 1996, Krustrup, Jones et al. 2009). The amplitude of the primary phase is proportional to the exercise intensity, and $\dot{V}O_2$ increases as a linear function of WR with a slope or gain (G) ($\mathbf{\Delta}\dot{V}O_2/\mathbf{\Delta}W$) of 10 ml/min/W on average for cycle ergometry (Wasserman and Whipp 1975). Relatively rapidly (within 2-3 min) after the beginning of exercise, a new SS called *phase III* is achieved (Whipp, Ward et al. 1982, Jones and Poole 2005).

- Heavy domain

At this intensity, the amplitude increases according to the exercise intensity, and the primary phase length is reflected by a larger time constant (τ). τ represents the time taken to achieve 63% $\mathbf{\Delta}\dot{V}O_2$ (Jones and Poole 2013) (this term will be explained in more detail later in the manuscript). As mentioned before, in this domain, the attainment of an SS is delayed, and the time to achieve an SS increases greatly with the increases in WRs, leading to the appearance of an excess of oxygen consumption, called the $\dot{V}O_2sc$ (Whipp and Wasserman 1972). This secondary $\dot{V}O_2$ elevation becomes apparent after approximately 90 to 120 s and is superimposed on phase III (Poole, Barstow et al. 1994).

- Severe domain

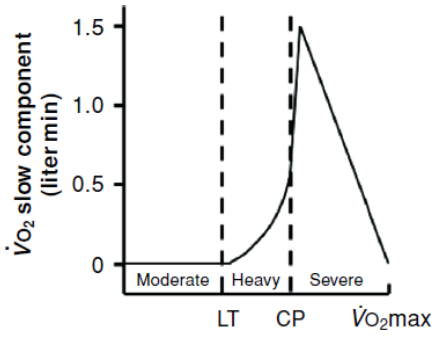

Figure 5 Magnitude of the slow component during incremental exercise. *Taken from Poole, D. C., & Jones, A. M. (2011). Oxygen Uptake Kinetics (Vol. 72, pp. 1810–65).*

In the severe-intensity domain, as in the heavy-intensity domain, there is an increase in the amplitude of the primary component proportional to the exercise intensity and a slowdown of $\dot{V}O_2$ kinetics. A specific feature in this domain is the progressive diminution in the $\dot{V}O_2sc$, as shown in figure 5, and the inevitable progress of the $\dot{V}O_2$ towards the $\dot{V}O_{2max}$ boundary.

Talking strictly about the kinetics of $\dot{V}O_2$, related to different intensities or exercise domains, in the moderate or extreme domain, the kinetics can be

represented by a primary component modeled by an exponential. In contrast, in the heavy and severe domains, the appearance of the slow component adds an exponential relation to the model. However, the reasons are different, because in the moderate domain, there is not enough metabolic challenge for the development of the $\dot{V}O_{2SC}$, and in the extreme domain, there is not enough time to develop it. Hence, the heavy and severe exercise domains are better fit with two exponential terms.

4.4 Modeling $\dot{V}O_2$ kinetics

The exact date when $\dot{V}O_2$ kinetics were signaled as a scientific investigation field is difficult to define, but it has been argued that it could be when O_2 itself was discovered. Between 1772 and 1774, Joseph Priestly and Carl Wilhelm Scheele independently discovered oxygen (Sternbach and Varon 2005). Both communicated this discovery to Antoine Lavoisier in Paris. Three years after Priestly's visit, Lavoisier named the gas "oxygine" because when different substances burned in it, their oxides dissolved in water and formed acids (in Greek, oxygen "acid former" (Lavoisier 1777)). Years later, Lavoisier (figure 6) and the mathematician Pierre La Place, created an innovative device that revealed that the quantity of "that gas" decreased while CO_2 and heat were produced when a guinea pig was sealed within. He made the epic statement "*eminently respirable gas (O_2) that enters the lung, leaves it in the form of chalky aeroform acid (CO_2)…in almost equal volume*" (Lavoisier 1789) Lavoisier concluded that respiratory combustion took place in the lungs and that the heat evolved was passed into the blood for transport throughout the body. Unfortunately, he could not investigate this conclusion further, as he was guillotined during the French Revolution despite being an eminence and his service to science (Sprigge 2002).

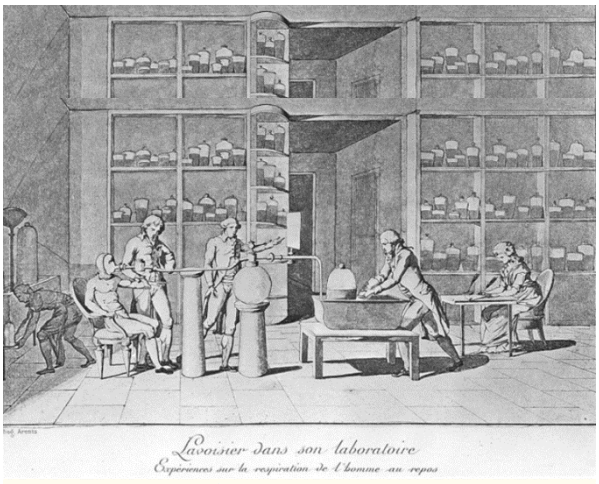

Figure 6 Lavoisier measuring the respiration of a subject at rest, as drawn by his wife, who is depicted herself at the table on the far right. *Adopted from: Wellcome Library London (Grimaux 1888). From Schoffelen, P.F.M; Plasqui,G 2018.*

This event represents the earliest form of indirect calorimetry, i.e., the quantification of O_2 consumption and CO_2 production by measures of the difference in O_2 and CO_2 contents between the air inspired and expired, along with minute ventilation.

However, this type of calorimetry was a closed-circuit version, as the individual was breathing air within a sealed system and was Humphry Davy (Davy 1800), the first person to use open-circuit spirometry (breathing ambient air) (Sprigge 2002). He collected his expired air for 1 min over a series of 20 experiments and compared those data with the data from his ambient inspired air. Davy was able to measure his own resting VO_2 (484 ml/min) and CO_2 (447 ml/min), which are perhaps quite high values for a resting person; however, he was probably not resting while performing his experiments (Sprigge 2002).

In 1911, the British scientist Claude Douglas and his colleagues took part in an expedition to Pikes Peak in Colorado. Douglas is mainly remembered for confirming that the concentration of hemoglobin in blood increases with altitude but also because, in preparation for the expedition, he developed the gold-standard method against which other methods are compared today, the Douglas method (Cunningham 1964). The Douglas method consists of a rubber-lined cloth bag capable of holding 10 to 50 L of gases. This bag was fitted with straps so that it could be fastened around the shoulders, with the possibility of being used during physical activities (figure 8). The bag was connected to a three-way tap, which in turn was connected to a mouthpiece placed between the lips (figure 7). After respiratory equilibrium of the subject was achieved, the three-way valve was turned, and the succeeding expirations were collected inside the bag for a certain period. Then,

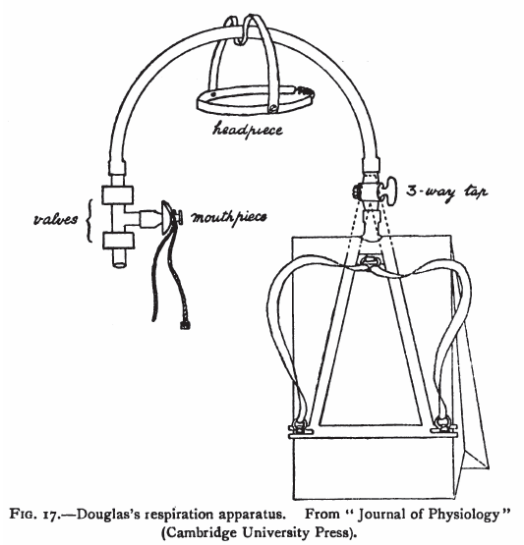

FIG. 17.—Douglas's respiration apparatus. From "Journal of Physiology" (Cambridge University Press).

***Figure 8 From the respiratory exchange of animals and man**. By August Krogh, 1916, page 43*

***Figure 7 The Douglas bag, 1970, skiing. Attributed to an unnamed 1970 article**. By Astrand and Rodahl.*

the air contained inside the bag was mixed, analyzed and measured by connecting the bag to a gas meter to obtain the O_2 and CO_2 concentrations. Oxygen uptake and CO_2 production could be calculated from the difference between the inspired and expired air in the bag, taking into account the temperature and barometric pressures. The Douglas bag is, therefore, called a SS gas exchange analyzer.

Nevertheless, it was not until 1913 that August Krogh and Johannes Lindhard published a revolutionary study describing the changes in ventilation, respiratory exchange and circulation that occurred during a non-SS but during the first minutes of light or heavy cycling exercise (Krogh and Lindhard 1913). They had subjects exercise with sudden and violent exertions or with lower loads and longer periods. The results showed that the O_2 absorption values for the 2-8-, 4-6- and 4-5-s durations were 200, 250 and 750 cubic centimeters (cc), respectively, in contrast with the 300 cc found in control resting conditions. They concluded from these experiments that the O_2 absorption was not abrupt (i.e., not in a square-wave manner, in which target demand is attained instantaneously when the change in requirements occurs); in contrast, O_2 absorption took place gradually (figure 9).

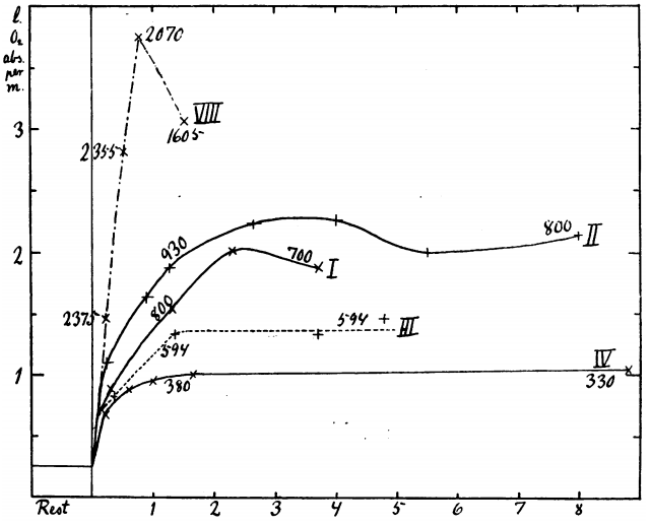

***Figure 9 Curves showing oxygen absorption before and during work**. Figures along curves kg. m. per min. From Lindhard and Krogh 1913*

In 1924, two years after being awarded the Nobel Price (for his discovery of the production of heat in muscle), Archibald Vivian Hill (figure 10) used a portable Douglas bag to measure O_2 and CO_2 while subjects were walking or running around an 85 m grass track (Hill, Long et al. 1924, Bassett 2002). Hill and colleagues collected gases for periods of half a minute, using a system with several bags and taps for instantaneous switching, making it possible to accurately measure the rapid alterations in O_2 uptake and CO_2 output at the beginning and at the end of exercise (Hill, Long et al. 1924).

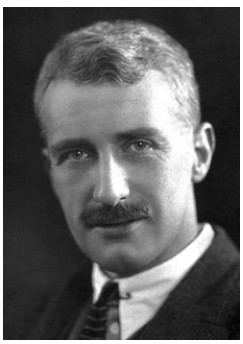

***Figure 10 Nobel laureate Archibald Vivian Hill (1886-1977).** Pictured in 1927.*

Hill's works in the field of sports performance are worth highlighting, since owing to them, the scientific discipline of exercise physiology was established (Hill, Long et al. 1924, Bassett 2002).

Hill and colleagues were the first to demonstrate the clear exponential nature of the $\dot{V}O_2$ response at exercise onset, the concept of VO_{2max} and the concept of anaerobic energy production during exercise (Hill, Long et al. 1924). Indeed, the concept of the O_2-independent metabolic pathway was unknown, and the overall consensus was that energy was provided by an aerobic metabolism in a "pay-as-you-go" manner. Hill and colleagues demonstrated the existence of another method of energy production, the "buy-now-and-pay-later method", called oxygen debt (this term is explained in more detail later in this manuscript).

Although Hill and colleagues demonstrated the exponential nature of $\dot{V}O_2$, they never modeled it. A few years later, in 1951, Franklin M. Henry (Henry 1951) hypothesized that when there was no limitation to O_2 delivery, O_2 uptake could be determined by the amount of oxidizable substrate present, which in turn would be determined by the rate of muscular work.

Henry formulated the idea with the assumption that, during constant work, *a* units of substrate *x* will be produced. Some proportion, *c*, of this substrate *x* will be oxidized, but the rest will be added to the previous *a* units. Therefore, if *c* does not change, the rate of the accumulation of substrates will have an exponential form, and consequently, the rate of oxygen consumption will be

$$\dot{V}O_2(t) = a_0(1 - e^{-kt}) \qquad \text{Eq 3}$$

where $\dot{V}O_2(t)$ is the oxygen consumption at time *t*, a_0 is the SS of $\dot{V}O_2$ and *k* is a velocity constant (later established as time constant τ). With this system, a_0 will increase linearly with the WR.

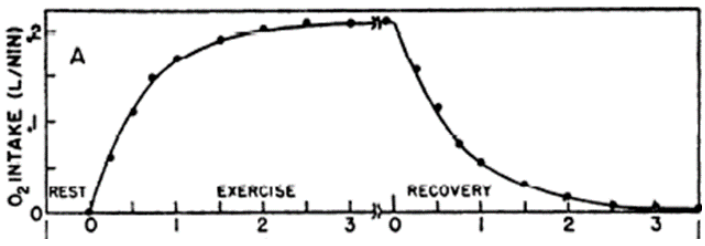

Figure 11 Exercise and recovery curves. *Experimental points represent the average of 12 individuals measured at the lightest bicycle workload. Adapted from F.M. Henry 1951.*

Henry measured O_2 uptake in 12 subjects and found close agreement of the theoretical curve with the experimental results, as shown in figure 11. However, it is important to note that the subjects exercised at relatively light workloads.

Three years later, Henry showed that at higher loads, the time for O_2 to reach the SS was increased and suggested a two-component exponential equation (Henry and DeMoor 1956):

$$\dot{V}O_2(t) = a_i\left(1 - e^{-k_i t}\right) + a_{ii}\left(1 - e^{-k_{ii} t}\right) \qquad \text{Eq 4}$$

where $\dot{V}O_2(t)$ is the oxygen consumption at time *t*, a_i is the SS of $\dot{V}O_2$ and *k* is a velocity constant.

In 1967, Wasserman and colleagues (Wasserman, Kessel et al. 1967) measured the metabolic effects, circulation and respiration responses of three different work intensities and durations in healthy male subjects.

At that time, gas analyzers had improved dramatically with the advancement of technology, and pulmonary gas exchange could be analyzed on a breath-by-breath basis. They determined moderate, heavy and very heavy (another nomenclature for severe) intensities, and the results showed that the time to reach an SS in $\dot{V}O_2$ was related to intensity. Indeed, a true SS was reached after 4 min in the moderate domain but was delayed for 10 min in the heavy domain and was never reached in the very heavy domain.

Five years later, J. Whipp and Karlman Wasserman (Whipp and Wasserman 1972), in a study performed to determine the effect of intensity in $\dot{V}O_2$ kinetics, confirmed that at high intensities, the non-SS phase was made with two exponentials, one being rapid and the other much slower. In their study, subjects performed a six-exercise test consisting of 10 min of rest followed by cycling at 50, 75, 100, 125, 150 and 175 W at 60 rpm for a minimum of 6 min each (figure 12). The results showed that the difference in $\dot{V}O_2$ between 3 and 6 min ($\mathbf{\Delta}\dot{V}O_{2\,(3\text{-}6)}$) was close to zero, and an SS in $\dot{V}O_2$ was attained by the third minute of exercise. In contrast, at high WRs, ($\mathbf{\Delta}\dot{V}O_{2\,(3\text{-}6)}$) increased as the WR increased, showing a delayed process, which affected the kinetics and WRs. Moreover, the increase in ($\mathbf{\Delta}\dot{V}O_{2\,(3\text{-}6)}$) coincided with the rise in lactate, reaffirming the delay in SS

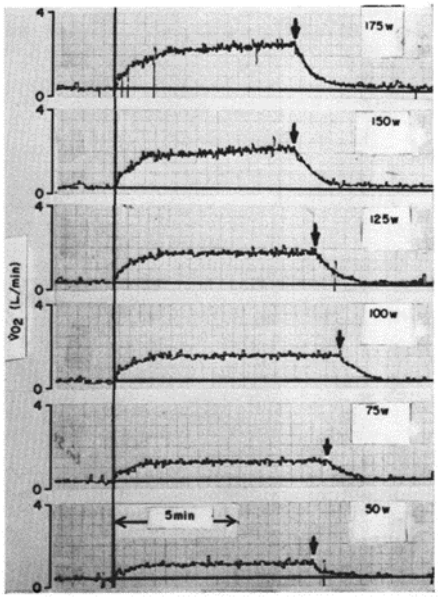

Figure 12 VO_2 of a subject during six constant-load exercises*. Solid vertical line indicates the start of exercise. Arrows indicate the time exercise stopped. From Whipp and Wasserman 1972.*

beyond 3 min in work above the "anaerobic threshold". Whipp and Wasserman also clarified why the studies of Magaria (Margaria, Manglli et al. 1965) and Di Prampero (Prampero, Davies et al. 1970) have contradictory results. Both investigated exercises lasted for only 2 min, not giving enough time for a true SS to be installed.

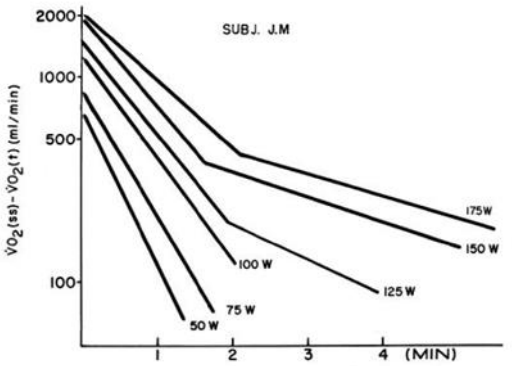

***Figure 13 Semilogarithmic model**. Rate of change in VO_2 as a function of time for the subject described in figure 7. From Whipp and Wasserman (1972).*

Whipp and Wasserman also proposed the classic $\dot{V}O_2$ model. With this model, they represented an excess of $\dot{V}O_2$ compared with the VO_2 predicted from the $\dot{V}O_2$-WR relationship during submaximal exercise, i.e., the differences between the predicted and the measured $\dot{V}O_2$ value. The break point of the slope of the line represents the start of the second component (figure 13).

In 1982, Whipp and colleagues (Whipp, Ward et al. 1982) performed an interesting study in which the cardiorespiratory phase was defined. They had six subjects perform square-wave exercises at intensities below the anaerobic threshold, starting from complete rest or from unloaded pedaling (0-W). Each exercise was performed 8 times by each subject, and breath-by-breath data for the eight tests were interpolated for the first time, time aligned and averaged ensemble. The averaging of several tests permitted the visualization of the different patterns more clearly, as fluctuations from breath-by–breath data were reduced. To characterize the kinetic behavior of $\dot{V}O_2$, they proposed three different models: in model 1, the exponential response started at the onset of the 100 W exercise, time delay (TD) = 0; in model 2, they incorporated a TD; and in model 3, the exponential response started only at the inflection point of the response (figure 14). The results showed that from rest

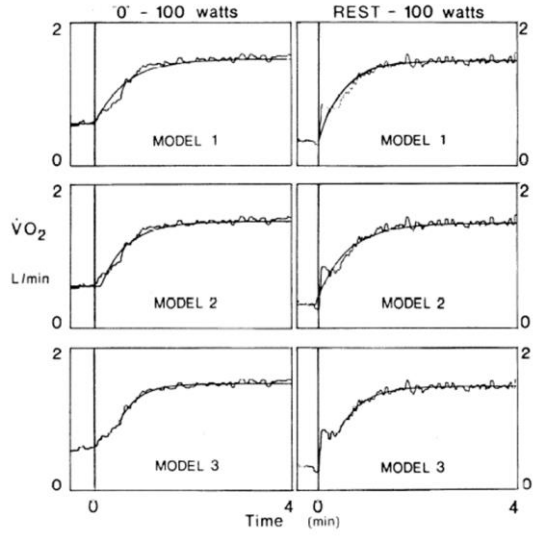

***Figure 14 Best fits of single exponential models 1 (top), 2 (middle) and 3 (bottom) to averaged breath-by-breath responses of VO_2 to 100-W exercise from 0-W baseline (left) or from rest (right).** From B.J. Whipp* et al.*, 1982.*

(not from unloaded pedaling), an abrupt initial response was apparent after 20 s; thereafter, the response developed exponentially to a new SS. As mentioned in chapter 5.3, this increase in pulmonary $\dot{V}O_2$ does not represent O_2 uptake by the tissues but a muscle-to-lung transit delay. Whipp and colleagues termed this first response "cardiodynamic" and concluded that model 3 provided the most accurate description of the exponential behavior responses of $\dot{V}O_2$, CO_2 and $\dot{V}E$ during phase II starting from the inflection point from phase I to phase II.

To wrap up, this study was key in VO_2 kinetics, as Whipp et al. (Whipp, Ward et al. 1982) identified and defined the three different phases of $\dot{V}O_2$ kinetics: phase I, rapid increase in blood flow perfusing the lungs; phase II, increasing in O_2 uptake from the exercising muscles; and phase III, an SS in $\dot{V}O_2$.

A few years later, in 1991, Barstow and colleagues (Barstow and Molé 1991) performed a study with the purpose of deciphering whether the fast component (phase II) behaved as a linear first-order system, i.e., whether $\dot{V}O_2$ increased as a unitary function with the WR or the $\dot{V}O_2$ profile ascribed to a single exponential process. If so, it would suggest that the extra O_2 cost would arise solely from the slow process ($\dot{V}O_{2SC}$) having no impact on the speed or magnitude of phase II. Barstow and colleagues proposed two different models. In model 1 (parallel model, Eq 5), there was no TD between the fast and slow components; in contrast, in model 2 (model serial, Eq 6), the second exponential component started after a second independent TD (figure 15). With these two models, what Barstow and colleagues tried to demonstrate was that, if the two processes began together, the second TD would ultimately converge to that for the first one, and Eq 2 would be reduced to model 1.

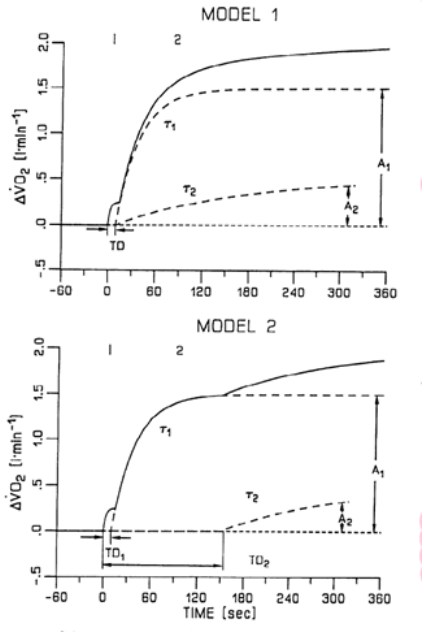

Figure 15 Schematic of the 2 double exponential models. *A, Amplitudes or gains; τ, time constant; TD, time delay from the onset of exercise to the beginning of the respective exponential processes; 1 and 2, phases of the responses. From Barstow and Molé 1991.*

$$\dot{V}O_2(t) = A_1\,(1\text{-}\exp^{-(t-TD)/\tau_1}) + A_2\,(1\text{-}\exp^{-(t-TD)/\tau_2}) \qquad \text{Eq 5}$$

$$\dot{V}O_2(t) = A_1\,(1\text{-}\exp^{-(t-TD1)/\tau_1}) + A_2\,(1\text{-}\exp^{-(t-TD2)/\tau_2}) \qquad \text{Eq 6}$$

(equations explained in detail on the next page)

Barstow and colleagues had subjects exercise at different intensities below and above the GET. The results showed that responses for exercise below the GET were well fit with model 1, confirming a monoexponential response.

On the other hand, when exercise was done above the GET, model 2 (Eq 6) was a better fit, confirming that the second component did not begin coincident with the first but rather later into exercise. Instead, it seems that the authors did not take into account the degrees of freedom of the two models in the comparison. In fact, the greater the number of parameters in the equation, the better the fit but also the greater the degrees of freedom, something that should be taken into account when making the comparison.

As a recapitulation, for most of the 20th century, research papers about $\dot{V}O_2$ kinetics assumed that VO_2 followed a nonlinear first-order system. However, further studies demonstrated that this first-order nonlinearity of $\dot{V}O_2$ was valid only for exercise within the moderate-intensity domain, represented with an exponential function.

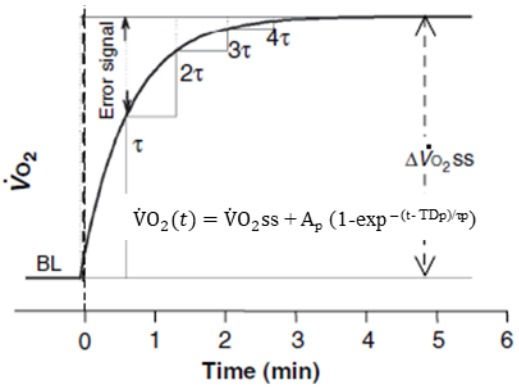

Figure 16 Kinetics of VO_2 in the moderate domain. *BL is baseline; $\dot{V}O_2(t)$ is the O_2 consumption at any time point in time; $\dot{V}O_2ss$ is the $\dot{V}O_2$ at steady state; A_p, is the amplitude of the primary phase; and (1-exp $^{-(t-TDp)/\tau p)}$) is the exponential function describing the rate at which $\dot{V}O_2$ rises towards the SS amplitude. Adapted from D.C. Poole and A.M. Jones (2011).*

An exponential response of a system is in accordance with a difference between the instantaneous and required values (in this case of $\dot{V}O_2$) and the feedback control of the response until the error signal is eliminated (figure 16) (Jones and Poole 2013).

Today, most publications describe the $\dot{V}O_2$ response under the GET with the following equation:

$$\dot{V}O_2(t) = \dot{V}O_{2\,baseline} + A_p\ (1\text{-}exp^{-(t-TDp)/\tau p}) \qquad \text{Eq 7}$$

where $\dot{V}O_2(t)$ is the oxygen consumption at any point in time and $\dot{V}O_{2\,baseline}$ is the oxygen consumption before exercise starts. A_p is the SS amplitude of the $\dot{V}O_2$ response of the primary phase, and the term (1-exp $^{-(t-TDp)/\tau p}$) is the exponential function describing the rate at which $\dot{V}O_2$ rises towards the SS amplitude. t is time, and TDp is the TD before the start of the exponential term, i.e., the time it takes oxygen to travel from the muscle capillaries to the pulmonary capillary bed (Rossiter 2011). Indeed, as

mentioned before, after an abrupt increase in exercise intensity, the result of oxygen extraction from the muscles will not be reflected in pulmonary gas exchange for the period of this TD (Whipp, Ward et al. 1982). This TDp is not constant and normally takes from 10 (during exercise) to 20 s in resting conditions (Krustrup, Jones et al. 2009). Of course, this TDp is inversely proportional to the cardiac output; the larger Q is, the smaller the TD. Finally, τp is the time constant of the primary phase, i.e., the time that $\dot{V}O_2$ takes to reach 63% of the amplitude of the action potential (AP), and can take from 10 to >100 s.

The smaller the τ value, the faster the $\dot{V}O_2$ kinetics, which is very important in regards to the O_2 deficit, since the metabolic perturbations (e.g., **Δ**[H^+] or **Δ**[Lactate] or **Δ**[PCr]) will be minimized (Poole and Jones 2012). For instance, elite cyclists (Barstow and Molé 1991) and marathon runners (Jones and Poole 2009) could achieve $\dot{V}O_2$SS within 30 or 40 s. On the other hand, patients suffering from pulmonary or cardiac diseases could require several minutes to reach SS and will therefore incur a larger O_2 deficit associated with premature fatigue (Poole and Jones 2012).

The oxygen deficit is represented as the shaded area in figure 17. The absolute size of this area (deficit) is the product of $\mathbf{\Delta}\dot{V}O_2$ and the speed of the $\dot{V}O_2$ response, represented by τ. Therefore, the faster the $\dot{V}O_2$ response is, the smaller τ and the O_2 deficit are. In contrast, unhealthy individuals with a slow response (larger τ) will incur a greater degree of metabolic perturbation, such as an increase in lactic acid production and PCr degradation,

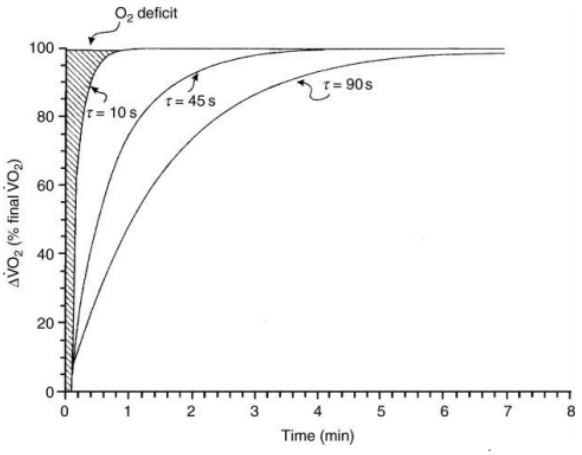

Figure 17 Schematic representation of the O_2 deficit. *The range of τ values given represent measures of a racehorse (10 s), a sedentary human (45 s) and a cardiac patient (90 s). The shaded area represents the O_2 deficit for a τ of 10 s. Note that the area l becomes larger as the VO_2 kinetics become slower. From Poole, Kindig* et al. *2005.*

because in the transition from rest to any WR (below the GET), the vertical distance between the baseline $\dot{V}O_2$ and the $\dot{V}O_2$ required at SS must be met from the energy stores within the muscle, principally PCr hydrolysis and anaerobic glycolysis (Poole, Kindig et al. 2005).

Contrary to moderate, heavy and severe domains are better fit with two exponential functions; consequently, the independent variables A, TD and τ are elected to partition $\dot{V}O_2$ kinetics into discrete components.

Therefore, as seen in figure 18, the $\dot{V}O_{2SC}$ has its own independent TDs, A_s and τ_s. These differences are made because the $\dot{V}O_{2SC}$ does not commence when exercise begins, but instead appears sometime after following the onset of exercise. In addition, this is a requisite to clarify why, the kinetics are slower in the heavy compared with the moderate domain (Koga, Shiojiri et al. 1999, Borrani, Candau et al. 2001, Pringle, 2003 #25).

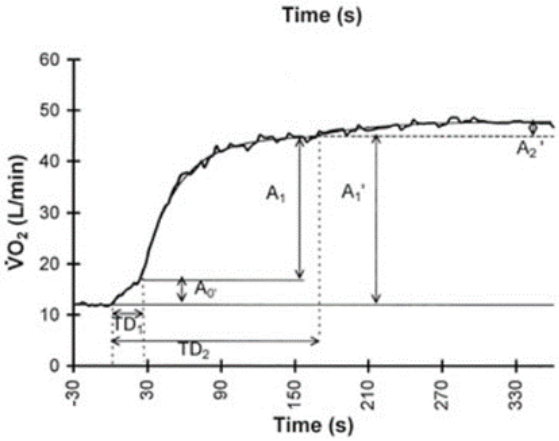

Figure 18 Example of the VO_2 response (thick line) and model (thin line) for a horse during heavy-intensity exercise*. TD_1 and TD_2, independent time delays; A'_0, value of* A_0 *(amplitude of* phase I *response) at* TD_1*. $A'_1 = A'_0 + A_1$ (amplitude of* phase II *response) and represents the physiologically relevant amplitude. A'_2, magnitude of the VO_{2SC} at the end of exercise; $EEVO_2$, net increase in VO_2 at the end of exercise. Modified from* I. Langsetmo et al. *1997.*

The three exponential functions are described with the following equations:

$$\dot{V}O_2(t) = \dot{V}O_2\text{baseline} + A_c\,(1\text{-}e^{-(t/\tau c)}) \quad \text{Phase I cardiodynamic phase}$$

$$+ A_p\,(1\text{-}e^{-(t\,-\,TDp)/\tau p}) \quad \text{Phase II primary component}$$

$$+ A_s\,(1\text{-}\,e^{-\,(t\text{-}TDs)/\tau s}) \quad \text{Slow component}$$

where $\dot{V}O_2(t)$ is the oxygen consumption at any point in time and $\dot{V}O_2$baseline is the oxygen consumption before exercise starts. Ac, Ap and As are the amplitudes for the cardiodynamic, primary and slow components, respectively. τ_c, τ_p and τ_s are the time constants, and TD_p and TD_s are the TDs. Because the asymptotic value of the exponential equation describing phase III may represent a higher value than the one actually reached, the actual amplitude at the end of exercise for the $\dot{V}O_{2SC}$ is described as A_s' and is calculated as follows:

$$A_s' = A_s\,(1\text{-}e^{-(T.end\text{-}TDs)/\,\tau s}) \quad \text{Eq 8}$$

As mentioned before, during the XX century, the $\dot{V}O_2$ kinetics were modeled with first-order multiexponential (FOME) model.

However, at the beginning of the XXI century, two new models were proposed by Luis A.P. de Lima (de Lima, Raison et al. 2018, de Lima, Achiche et al. 2020).

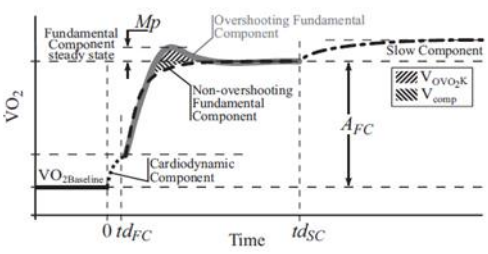

Figure 19 VO_2 responses to the work rate step on-transient, with the overshoot phenomenon either present (gray line) or absent (dashed line) in the fundamental component. From de Lima 2018.

Over the last few years, some studies have reported a remarkable VO_2 overshoot phenomenon ($O\dot{V}O_2K$) in phase II, or the fundamental phase, of the $\dot{V}O_2$ kinetics of step-on transient responses (figure 19) (Hoogeveen and Keizer 2003, Koppo, Whipp et al. 2004). This phenomenon is observed during constant load exercises in well-trained endurance athletes during the first 2 min of exercise. De Lima and colleagues proposed in their study (de Lima, Raison et al. 2018) a mixed multiexponential (MiME) model, combining a first-order model for the cardiodynamic and slow components with a second-order model for the fundamental phase, as a better overall fitting of $\dot{V}O_2$ kinetics. As hypothesized, the results showed that the MiME model presented is more adequate than the FOME model in explaining the $\dot{V}O_2$kinetics, regardless of the presence of $O\dot{V}O_2K$.

In 2020, de Lima and colleagues (de Lima, Achiche et al. 2020) proposed another new model composed of two second-order simultaneous components (SOSCs) for a better overall fit for both the $O\dot{V}O_2K$ phenomenon and the delayed response of the slow augmentation of $\dot{V}O_2$ of the $\dot{V}O_{2SC}$. The results confirmed SOSCs as a better alternative to a FOME model.

Nevertheless, to date, it has not been confirmed that any of the proposed models are better than the actual multiexponential model, as they have still not been utilized.

5 Origin of the $\dot{V}O_2sc$

5.1 Historical precedence

The first authors reporting the existence of the $\dot{V}O_{2SC}$ were probably Hill and Lupton (Hill and Lupton 1923) when they found an increase in the VO_2 of 320 ml/min in a subject running at a constant speed of 14.4 km/h. The authors attributed this to a painful blister causing inefficient movement. The fact that the running speed represented 86% of $\dot{V}O_{2max}$ suggests that these data probably represent the first observation of the $\dot{V}O_{2SC}$ in humans.

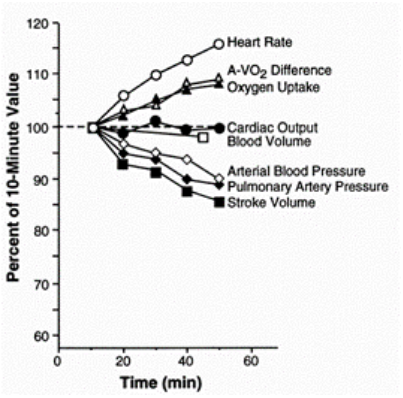

Figure 20 Cardiovascular drift. *Adapted from Coyle and Gonzalez- Alonso 2001.*

Importantly, the $\dot{V}O_{2SC}$ should not be confused with the modest $\dot{V}O_2$ drift (~200 ml) that may occur during moderate-intensity exercise for a duration of more than 60 min (figure 20). After the first 10-15 min of a constant-rate, moderate-intensity exercise at 50-75% of $\dot{V}O_{2max}$, a slow and progressive change over time in some of the cardiovascular measures occurs. The arteriovenous difference in O_2 and HR increases progressively while the stroke volume and mean arterial and pulmonary pressures decrease, resulting in a cardiac output that is relatively constant but an increase in O_2 uptake. This rise in $\dot{V}O_2$ is not associated with an increase in blood lactate; it is observed mostly in warm environments (Montain and Coyle 1992), and the mechanism after this cardiovascular drift remains controversial. One hypothesis is that this drift is caused by peripheral displacement of the blood volume to the cutaneous blood bed, causing a drop in arterial and central venous pressure and stroke volume (Ekelund and Holmgren 1964). Another hypothesis is that hyperthermia causes an increase in sympathetic nervous system activity, which decreases the ventricular filling time, the end-diastolic volume and, as a consequence, the stroke volume (Coyle and Gonzalez-Alonso 2001)

The $\dot{V}O_{2SC}$ should not be confused with the gradual rise in $\dot{V}O_2$ normally seen during submaximal negative work, such as downhill running. Dick & Cavanaught (Dick and Cavanagh 1987) proposed that this drift in $\dot{V}O_2$ while performing low-intensity downhill running is related to the damage that occurs in the muscle with this form of exercise. They suggested that with this type of exercise, some

muscle fibers are damaged and are no longer able to maintain or generate force; therefore, other motor units (MUs) must be recruited to maintain the WR. However, the damaged fibers continue to utilize oxygen, resulting in an increase in $\dot{V}O_2$ up to 10% and associated with an increase in muscle electromyography.

The first publication in the literature as evidence of the $\dot{V}O_{2SC}$ was in 1961 by Astrand and Salting (Åstrand and Saltin 1961). In their study, the profile bouts of exercise at different intensities showed how the $\dot{V}O_{2SC}$ drives $\dot{V}O_2$ towards $\dot{V}O_{2max}$ and exhaustion (figure 21). The figure shows the data of one of the subjects in whom the slow component can be observed in the two last less-intense WRs.

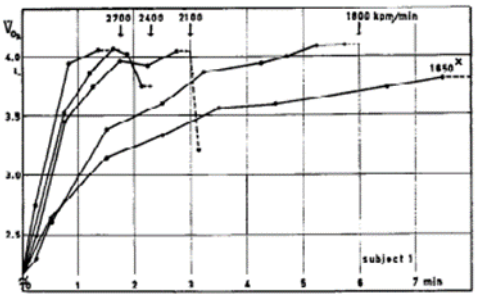

Figure 21 VO_2 responses for one subject during five different exercise intensities. *Arrows indicate the point of maximum fatigue. Note the existence of a slow component when the VO_2 after the 3rd minute of exercise continues to rise at 275 and 300 W. Adapted from Astrand and Saltin 1961.*

After the work of Astrand and Saltin (Åstrand and Saltin 1961), a multitude of other authors established evidence about the existence of the slow component, including Whipp et al. (Whipp 1994).

As mentioned before, the appearance of the $\dot{V}O_{2SC}$ causes an augmentation in $\dot{V}O_2$, which confers a diminution in the muscular efficiency and is characterized by a rise in the G ($\Delta\dot{V}O_2$/ Δwork) above the GET. This increase in the G is inversely proportional to the efficiency. This reduced muscle efficiency should not be ignored since it could account for as much as a 1.0-1.5 L/min increase in $\dot{V}O_2$ in the severe domain (Poole, Schaffartzik et al. 1991).

The slow component phenomenon causes a delay in SS achievement, limiting the individual's capacity to tolerate exercise at high intensity for a prolonged period of time. Thus, preventing the emergence or limiting the magnitude of this component is crucial for the success of athletes competing at high intensity or for individuals in which a high WR is necessary to reduce the risk of the onset of diseases, such as hypolipoproteinemia or hypertriglyceridemia (Slentz, Houmard et al. 2007).

5.2 Putative causes of the $\dot{V}O_{2SC}$

To explain the phenomenon of the $\dot{V}O_{2SC,}$ several putative mechanisms have been proposed. One of the first and most obvious was the accumulation of lactate concentration in the blood, since the $\dot{V}O_{2SC}$ has been reported to emerge at exercise intensities above the LT. The physiological basis of this increase in $\dot{V}O_2$ accompanying the accumulation of blood lactate was assumed to be the result of the stimulation of glycogenesis or gluconeogenesis (McArdle, Katch et al. 2001, Shulman and Petersen 2009). However, some studies have shown that the small $\dot{V}O_2$ cost of the Cori cycle process is not enough to support the notion that the emergence of the $\dot{V}O_{2sc}$ results from an elevated level of blood lactate (Whipp 1994, Gaesser and poole 1996). Additionally, it has been argued that the decrease in pH, rather than the mere accumulation of lactate in blood, contributes to the increased magnitude of the $\dot{V}O_2$sc (Wasserman, Whipp et al. 1974, Stringer, Wasserman et al. 1994, Gaesser and poole 1996, Xu and Rhodes 1999). This decrease in pH (lactic acidosis) allows hemoglobin to readily release and deliver oxygen to the active muscles, which in turn allows the increase in $\dot{V}O_2$ to meet energy demands. This phenomenon is known as the "Bohr effect", which promotes the right shift of the oxyhemoglobin dissociation curve or the decrease in oxygen affinity of hemoglobin (Riggs 1988). However, this hypothesis has been argued by researchers to be an insufficient explanation of the excess oxygen evident during heavy exercise from the predicted $\dot{V}O_2$-WR relationship above the LT (Whipp 1994, Gaesser and poole 1996).

Despite the correlation reported between the $\dot{V}O_{2SC}$ time course and the magnitude of the blood lactate concentration (Casaburi, Storer et al. 1987, Poole, Ward et al. 1988), later studies employing an infusion of epinephrine and blood lactate (Gaesser 1994) eliminated lactate as a major contributor to the development of the appearance of the $\dot{V}O_{2SC}$. Furthermore, other possible causes, such as the increase in catecholamines (Gaesser and poole 1996); exercising muscle temperature (Hagberg, Mullin et al. 1978, Whipp and Wasserman 1986); metabolic acidosis (Zoładź, Duda et al. 1998); and respiratory (Poole, Ward et al. 1990), cardiac (Poole, Wagner et al. 1995) and auxiliary muscle work (Cross, Morris et al. 2010), were considered putative mediators of the $\dot{V}O_{2SC}$. Nevertheless, the landmark study of Poole et al. (Poole, Schaffartzik et al. 1991) disproved many of the mechanisms mentioned above, with the

discovery that approximately 86% of the $\dot{V}O_{2SC}$ originates from the exercising limbs. To show this, they had subjects exercise in the moderate and severe domains and simultaneously measured the pulmonary and leg $\dot{V}O_2$ by thermodilution. During severe-intensity exercise, both pulmonary and leg $\dot{V}O_2$ increased, and these increases were highly correlated.

The notion that the $\dot{V}O_{2SC}$ predominantly originates in the "exercising limb" was further supported by others (Krustrup, Jones et al. 2009). For instance, during repeated two-legged isometric contractions of the quadriceps at 30% of maximum voluntary contraction (MVC) for 6 s with 4 s of rest in between series until exhaustion, a twofold increase in $\dot{V}O_2$ occurred. Blood samples were taken from the femoral vein and artery, and blood velocity was recorded by ultrasound Doppler from the same artery. The results showed that the increase in energy demand resulted from an increase in blood flow and oxygen extraction by 54% and 34%, respectively (Vollestad, Wesche et al. 1990).

These investigations, which point to a significant increase in blood flow and oxygen uptake by the working muscles at the intensities where the $\dot{V}O_{2sc}$ is expected to happen, indeed suggest that the $\dot{V}O_{2sc}$ predominantly arises from the exercising limbs due to an increase in energy demands to maintain force production.

Since it was shown that the $\dot{V}O_{2sc}$ originates mainly within the exercising limb rather than from central factors such as cardiac, ventilatory, auxiliary muscle work or metabolic stimulation at sites outside the exercising limb (Poole, Schaffartzik et al. 1991), some of the proposed mechanisms have been either weakened or eliminated.

Even though the results from Poole et al (Poole, Schaffartzik et al. 1991) suggested that the increase in leg $\dot{V}O_2$ could account for 86% of the pulmonary $\dot{V}O_2$sc and muscle appears to be the location of slow component development, the exact mechanisms causing this $\dot{V}O_{2SC}$ are still under debate (Jones and Poole 2005, Borrani, Malatesta et al. 2009).

Different experiments, such as glycogen depletion in type I muscle fibers (Krustrup, Söderlund et al. 2004), slow twitch fiber blockade (Krustrup, Secher et al. 2008), and others performed with electromyography (ShlNohara and Moritanl 1992), supported the idea of the involvement of fast twitch muscle fiber activation patterns in the development of the $\dot{V}O_{2SC}$.

The reason why all these authors agree with the idea of type II muscle fibers being involved, or even the culprit, in the development and appearance of the $\dot{V}O_{2SC}$ is founded in the Henneman principle. Muscle fiber activation is not aleatory and follows the "size principle" of MU recruitment (Henneman E 1981). The Henneman principle states that MUs and their fibers are recruited in an orderly manner. MUs containing slow-twitch, fatigue-resistant muscle fibers have the lowest threshold for activation and will be recruited first. This type of MU contains type I slow twitch fibers, which have greater mitochondrial density, greater capacity to resynthesize ATP via oxidative phosphorylation (Jackman and Willis 1996), more myoglobin content and a higher fiber/capillarity ratio (Gollnick, Armstrong et al. 1972). At the other end of the spectrum, MUs innervating type IIx fibers have fewer mitochondrial content and lower oxidative enzyme activity (Meyer, Brown et al. 1985), are less efficient (Wendt and Gibbs 1973) and have greater ATP cost of force production than their type I counterparts (Stienen, Kiers et al. 1996, Han, Proctor et al. 2001). Youn Soo Han and colleagues (Han, Proctor et al. 2001) reported that the reserve capacity for ATP consumption for fibers expressing myosin heavy chain 2X (MHC_{2x}) was lower than that for fibers expressing slow MHC. MHC_{2x} fibers, on the other hand, have a higher tension cost, which is one of the reasons why they are less energy efficient.

As expected, during moderate-intensity exercise below the LT, the majority, or even all, of the muscle work would be produced by the MUs that innervate the slow-twitch fibers. On the other hand, when the intensity is above the LT, the power production will come from both the MUs innervating type I fibers and the MUs innervating the fast-twitch, type II fibers (Saunders, Evans et al. 2000).

Glycogen content (VØLLESTAD and BLOM 1985, Krustrup, Söderlund et al. 2004) and electromyographic studies (Mateika, Duffin et al. 1994) have demonstrated that type II fibers are activated in the intensity domain associated with the $\dot{V}O_{2SC}$. However, there is still a debate in the current literature about the mechanistic link between the recruitment of less efficient type II fibers and the excess cost of the $\dot{V}O_{2SC}$ (Poole and Jones 2012).

Since then, three main research paradigms have been explored:

- The idea of the central O_2 delivery limitation being the principal cause of the alteration in $\dot{V}O_2$ kinetics.

- The idea of the influence of the different kinetics of fiber type and MU recruitment on the development of the $\dot{V}O_{2SC}$.
- The idea that the development of fatigue in fibers during exercise triggers the recruitment of other fibers (of higher hierarchy and lower economic efficiency) to maintain exercise intensity and consequently allows the appearance of the $\dot{V}O_{2SC}$.

In the following chapter, these three paradigms will be developed in more detail.

6 Three different paradigms

6.1 - Central O_2 delivery limitation

Certain authors have hypothesized that the $\dot{V}O_{2SC}$ could be the result of inadequate O_2 delivery to the working muscles. Some have hypothesized that with a prior "warm up" bout of exercise above the GET, perfusion could be improved due to the vasodilating effects of acidosis.

In 1989, Gauche and colleagues (Gausche, Harmon et al. 1989) were the first to provide evidence that a prior bout of heavy exercise altered the $\dot{V}O_2$ kinetics of supra-GET-intensity exercise. Although these investigators did not measure acid-base variables, this event raised the possibility that the accelerated kinetics after a heavy exercise bout were a consequence of the vasodilating effects of acidosis. In 1996, Gerbino et al (Gerbino, Ward et al. 1996), focusing on alterations in acid-base status, established that a prior bout of heavy (but not moderate)-intensity exercise could accelerate the overall $\dot{V}O_2$ kinetics (reducing τp) in a second bout of heavy exercise performed 6 min later. They proposed two factors that could explain this phenomenon: vasodilatation at the start of the second bout and an acidemia-induced Borg shift of the hemoglobin dissociation curve. This speculation was based upon the fact that the elevation of blood lactate and consequent acidosis caused by high-intensity exercise would still be present in the second bout of exercise. This residual acidosis is associated with vasodilation and with the right shift in the oxyhemoglobin dissociation curve (rightwards facilitates the release of O_2 from hemoglobin to the tissues).

The problem with the study of Gerbino (Gerbino, Ward et al. 1996) is that they used a simple exponential function to model the kinetics from the first 25 s to the end of the exercise. With this approach, the

"resultant" effective τ_p may be reduced as a consequence of an acceleration of the primary kinetics or due to a reduction in $\dot{V}O_{2SC}$ amplitude.

A few years later, it was revealed that with a hyperoxia protocol and prior heavy exercise, $\dot{V}O_2$ kinetics were faster and the $\dot{V}O_2$sc was decreased compared with normoxia. Both VO_2 kinetics and the $\dot{V}O_2$sc were faster, but even faster was the adaptation of $\dot{V}O_2$ when the arterial O_2 content was increased, leading to the conclusion that O_2 transport acted as the rate-limiting step (Macdonald, Pedersen et al. 1997).

On the other hand, other groups, such as Burnley et al. and Koppo et al. (Burnley, Jones et al. 2000, Koppo and Bouckaert 2001), used more complex modeling procedures to provide insights regarding the physiological mechanisms after the overall acceleration of $\dot{V}O_2$ kinetics. Both confirmed that the acceleration of $\dot{V}O_2$ kinetics found by the Gerbino study (Gerbino, Ward et al. 1996) with prior heavy exercise was a consequence of the increase in the amplitude of the primary phase and the reduction in the $\dot{V}O_{2SC}$ rather than a faster primary phase.

Another important factor to consider for the calculation of the amplitude of the primary phase is that it could be influenced by the elevated baseline due to the priming exercise. To verify the influence of the baseline value, Burnley and colleagues (Burnley, Doust et al. 2001) extended the duration of the recovery between bouts from 6 to 12 min, concluding that the absolute $\dot{V}O_2$ amplitude at the end of the primary phase was a consequence of an increase in the net amplitude of the primary phase response itself, independent of the baseline $\dot{V}O_2$.

Ninety percent of the studies support the idea that prior exercise creates residual blood acidosis that accelerates the overall kinetics, increasing the primary component amplitude and reducing the $\dot{V}O_{2SC}$ amplitude without changes in τ_p. Notwithstanding, if the initial conditions of exercise regarding O_2 availability are modified, the results can be very different. The hydrostatic gradient is well known to play a considerable role in the supply of additional perfusion pressure above heart level (Convertino, Goldwater et al. 1984). Indeed, when subjects are in the supine position, the blood flow and, by implication, O_2 delivery to the exercising muscles is reduced because the gravitational assistance is attenuated. For instance, it has been shown that VO_{2peak} and ventilatory threshold are reduced in the

supine position compared with the upright position (Hughson, Xing et al. 1991, Hughson, Cochrane et al. 1993). These results are in line with the study of Rossiter and colleagues (Rossiter, Ward et al. 2001), who had subjects exercise in the prone position and noticed that a prior bout of heavy exercise reduced τ_p (46.6 ± 6.0 s vs 40.7 ± 8.4 s) and the amplitude of the $\dot{V}O_{2SC}$. Additionally, Scheuermann (Scheuermann, Bell et al. 2002) found in a study with young (26 years) and older (65 years) individuals that prior heavy exercise accelerated the overall $\dot{V}O_2$ kinetics, represented by a decrease in the mean response time ((MRT), i.e., the sum of TD and τ) from 52.0 ± 4.3 to 40.6 ± 2.3 in the older population, with no significant changes in the younger population (29.9 ± 3.1 s vs 28.3 ± 1.7 s). The authors concluded that muscle O_2 consumption (in older and young adults) is limited by intracellular processes within the exercising muscle when muscle blood flow and O_2 delivery are adequate. The same conclusions have been found comparing the effect of a prior heavy exercise bout on a moderate-intensity second bout in older and young populations, with reductions in τp from 38 to 30 s and from 26 to 25 s, respectively (DeLorey, Kowalchuk et al. 2004). In the same manner, others have found significant effects of prior exercise during supine cycling, with τp being reduced from 38 to 24 s without changes in $\dot{V}O_{2SC}$ amplitude (Jones, Berger et al. 2006). Additionally, when performing arm cranking, when the arm was positioned above the level of the heart, $\dot{V}O_2$ availability was compromised (Koppo and Bouckaert 2005).

In addition, in certain pathologies in which muscle O_2 delivery is impaired, $\dot{V}O_2$ kinetics can be accelerated when the capacity of O_2 delivery is increased by arterial oxygen content or by increased cardiac output. For example, in hypoxemic chronic obstructive pulmonary disease (COPD) patients, the administration of supplemental O_2 during moderate constant-load exercise results in enhanced oxidative metabolism and faster $\dot{V}O_2$ kinetics (Palange, Galassetti et al. 1995). Another example is found in work with chronic heart failure patients (Grassi, Marconi et al. 1997), where it was found that "prior exercise", even if it did not affect the speed of $\dot{V}O_2$ kinetics, was effective in speeding up the convective O_2 flow to muscles during the second transition bout. In contrast, Paterson and colleagues (Paterson, Cunningham et al. 1994) found some speed effects of "prior exercise" in heart transplant subjects with a decrease in τp from 77 ± 26 to 46±17 s.

Multiple mechanisms have been proposed to explain the changes in $\dot{V}O_2$ kinetics following prior exercise. These mechanisms include the following:

-Muscle temperature. During prior heavy exercise interventions, muscle temperature can be elevated during the second bout, possibly affecting the dynamics of the $\dot{V}O_2$. Nevertheless, Koga et al (Koga, Shiojiri et al. 1997) reported no significant reduction in the $\dot{V}O_{2SC}$ after a 3°C increase in leg muscle temperature with hot-water-perfused pants, concluding that elevated muscle temperature does not contribute to the $\dot{V}O_{2SC}$ in heavy exercise. Neither Koppo et al (Koppo, Jones et al. 2002) found any significant difference in the $\dot{V}O_{2SC}$ when muscle temperature was manipulated. They measured intramuscular temperature and $\dot{V}O_2$ simultaneously during heavy exercise bouts, and on a separate day, they raised muscle temperature to the same level of prior exercise (37.3°C) before the participants performed a unique bout of heavy exercise. The reduction in the $\dot{V}O_{2SC}$ was observed only with prior exercise, concluding that elevating muscle temperature per se had no effects on $\dot{V}O_2$kinetics.

- Increased enzyme activity. Pyruvate dehydrogenase is a key component of metabolic inertia, especially between glycolysis and the Krebs cycle. Pyruvate dehydrogenase is the first enzyme of the pyruvate dehydrogenase complex that intervenes in the step from pyruvate to acetyl-CoA, and its administration results in less PCr degradation and lactate accumulation during transitions from rest to submaximal exercise. Campbell-O'Sullivan et al. (Campbell-O'Sullivan, Constantin-Teodosiu et al. 2002) showed that after a prior exercise bout at 55% of $\dot{V}O_{2max}$, there was a stockpiling of acetyl groups with a concomitant increase in speed on the $\dot{V}O_2$ response during the second bout of exercise. The authors therefore proposed that prior exercise enhances substrate availability (acetyl-CoA) and subsequently accelerates the $\dot{V}O_2$ kinetics in the second bout of exercise with a reduction in anaerobic ATP synthesis. Nevertheless, others have found opposite results (Bangsbo, Gibala et al. 2002, Grassi, Hogan et al. 2002). They did not find any impact on the τ of the primary component, as expected, with the administration of dichloroacetate (known to increase the active form of the pyruvate dehydrogenase complex) or any anaerobic ATP provision.

- MU recruitment. Barstow and Mole (Barstow, Jones et al. 1996) showed that subjects with a higher percentage of type I fibers had a larger amplitude of the first component and a smaller $\dot{V}O_{2SC}$

during heavy cycling exercise, hence faster primary $\dot{V}O_2$ kinetics. Therefore, changes in the primary phase and $\dot{V}O_{2SC}$ have been proposed to be related to changes in the fiber recruitment pattern. For example, Burnley et al. (Burnley, Doust et al. 2002) reported an increase in the integrated electromyogram (iEMG) in three different muscle groups (gluteus maximus, vastus lateralis, and vastus medialis) in the second bout of heavy exercise, while the mean power frequency (MPF) was unchanged. This increase in the iEMG was accompanied by an increase in the amplitude of the primary component. These results suggested that the increased primary phase amplitude is related to a greater recruitment of MUs at the onset of exercise. Nevertheless, other studies did not find any relation between the second bout of heavy exercise and the iEMG response (Scheuermann, Hoelting et al. 2001).

DiMenna (DiMenna, Wilkerson et al. 2008) examined the interaction between pedal rate and prior exercise. They found that τ_p was speeded only when severe-intensity priming exercise was done at high pedal rates (115 rpm). The authors concluded that the effect of priming at high pedal rates was presumably through specific priming effects on type II muscle fibers.

The above studies show that there is no consensus on MU recruitment theory.

As originally proposed by the Gerbino and Macdonald groups (Gerbino, Ward et al. 1996, Macdonald, Pedersen et al. 1997), prior exercise accelerates the $\dot{V}O_2$ response as a consequence of vasodilatation in the active muscle caused by the accumulation of metabolites. As mentioned before, metabolic acidosis causes a rightward shift in the oxyhemoglobin dissociation curve, increasing O_2 availability. However, several more studies indicate that acidosis is probably not the cause of the altered $\dot{V}O_2$ response after prior exercise. Indeed, Burnley and colleagues (Burnley, Doust et al. 2002) showed that regardless of whether the prior exercise in the form of constant-work, heavy-intensity exercise (6 min Δ50% with 3.4 mmol/L lactate concentration) or a 30-s all-out cycle sprint (6.4 mmol/L), the magnitude of the increase in the primary component or the reduced amplitude of the $\dot{V}O_{2SC}$ were similar. Koppo and Bouckeart (Koppo and Bouckaert 2000, Koppo and Bouckaert 2002) verified that even moderate-intensity prior exercise causes a reduction in the $\dot{V}O_{2SC}$ or increases the amplitude of the primary phase, showing that metabolic acidosis is not a necessary condition to elicit a reduction in the

$\dot{V}O_2$sc. More recently, a study showed that baseline blood lactate concentration was still elevated 60 min after prior exercise, while the $\dot{V}O_2$ kinetics effect persisted for no more than 45 min (Burnley, Doust et al. 2006). The results of this study suggested that blood lactate and the $\dot{V}O_{2SC}$ are not temporally linked. These studies indicate that a high blood lactate concentration or a low muscle pH are not sine qua non conditions to see the effects of prior exercise on $\dot{V}O_2$ kinetics.

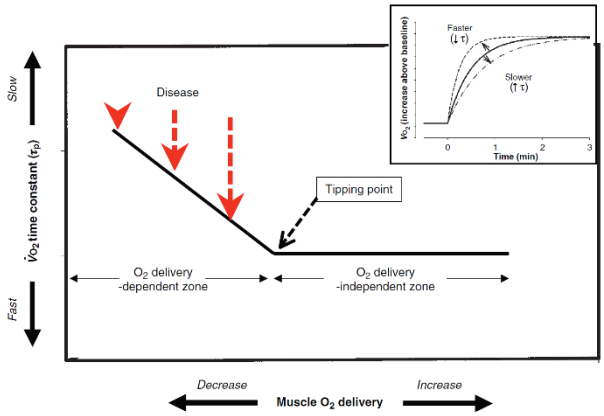

Figure 22 O_2-delivery-dependent and -independent regions. *From Poole & Jones 2011*

Therefore, as shown in figure 22, it can be concluded that in young and healthy subjects, as they lie to the right of the tipping point, "prior" exercise does not have any effect on τp or the final value of $\dot{V}O_2$. Even so, the amplitude of the primary phase is increased by the vasodilatation effect, and therefore, the $\dot{V}O_{2SC}$ is decreased by this effect.

On the other hand, under certain conditions where O_2 availability is compromised, as in COPD, type II diabetes or aging, the "prior exercise effect" can accelerate the primary phase, not only with the reduction in the $\dot{V}O_{2SC}$ and the increase in the primary phase but also with the reduction in τ. However, there is no consensus on the exact mechanism in which this works.

6.2 - Studies supporting different kinetics of muscle fiber types being involved in the development of the $\dot{V}O_2$sc

Mammalian skeletal muscle is composed of different cell populations with different metabolic and mechanical characteristics, mitochondrial contents and contractile proteins (Pette and Staron 1997). Kushmeric and colleagues (Kushmerick, Meyer et al. 1992) compared the $\dot{V}O_2$ of the two different muscle fiber types, demonstrating that the mechanism of the control of cellular respiration is quantitatively and qualitatively different in fast and slow muscle fibers. Additionally, Stienen and colleagues showed in their study (Stienen, Kiers et al. 1996) using single human muscle fibers during isometric contraction that ATP consumption depends on the myosin isoform composition. Specifically, the ATP consumption in fast IIx fibers was fourfold higher than that in slow type I fibers. In addition,

Willis and Jackman (Willis and Jackman 1994) showed in rabbit fibers that mitochondrial respiration was 27% higher in type IIb fibers than in type I fibers. These differences between the slow- and fast-twitch fibers may have an impact on the economy of mitochondrial oxidative phosphorylation kinetics during heavy-intensity aerobic exercise and thus contribute to the appearance of the $\dot{V}O_{2SC}$.

In 1996, Barstow and colleagues (Barstow, Jones et al. 1996) were the first authors to investigate the relationship between muscle fiber type used during exercise and $\dot{V}O_2$ kinetics. They had nine subjects with different fitness levels exercise at **Δ**50% and took muscle biopsies of the vastus lateralis for the determination of fiber type. As seen in figure 23, the subjects with a higher percentage of type I muscle fibers had different response kinetics than subjects with a lower percentage of type I muscle fibers and were characterized by a larger primary phase. Their results showed that the percentage of type I muscle fibers was significantly correlated with the $\dot{V}O_{2SC}$ (r=-0.83). This finding is in line with other studies that also reported that the percentage of type I muscle fibers is associated with an improved efficiency or reduced $\dot{V}O_2$ for the same WR in cycling (Coyle, Sidossis et al. 1992) or in running (Bosco, Montanari et al. 1987).

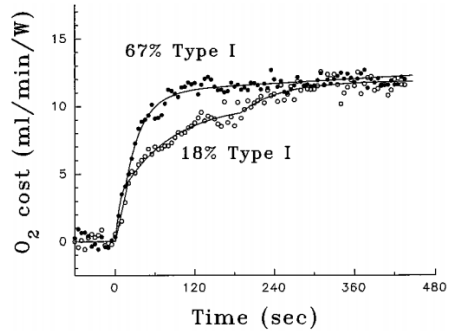

Figure 23 Relationship between muscle fiber type and the kinetics of VO_2 (normalized as O_2 cost) during heavy exercise. *From Barstow* et al. *1996.*

Russell et al. (Russell, Wadley et al. 2002) compared trained vs nontrained individuals and found similar results as Barstow (Barstow, Jones et al. 1996), reporting that both the percentage type I muscle fibers and markers of aerobic fitness were significantly correlated with the relative magnitude of the $\dot{V}O_{2SC}$.

Additional proof indicating that type II fibers are less efficient and have a greater $\dot{V}O_2$ cost was provided by Horowitz and colleagues using the gross efficiency parameter (Horowitz, Sidossis et al. 1994). Gross efficiency is known to reflect whole-body oxygen consumption and energy expenditure from all bodily processes. In their study, at identical oxygen consumption, trained cyclists with a higher percentage of type I fibers (73 ± 3%) were able to generate 9% more power during a 1-h performance bout, showing higher gross efficiency than equally well-trained cyclists with a smaller percentage of type I fibers (48 ± 2%).

A few years later, Pringle and colleagues (Pringle, Doust et al. 2003) tested the hypothesis that muscle fiber type influences τp and $\dot{V}O_{2SC}$ amplitude. They took muscle biopsies from fourteen subjects for histochemical determination and performed square-wave cycling tests at moderate, heavy and severe intensities. Their hypotheses were verified when they found that type I fibers were significantly negatively correlated with $\dot{V}O_{2SC}$ amplitude for heavy (r= -0.74) and severe (r=-0.64) exercise and with the τ of the primary component (r=-0.68). Deley and colleagues (Deley, Millet et al. 2006) observed that after prefatiguing by electrostimulation of type II fibers, $\dot{V}O_{2SC}$ amplitude was significantly reduced. They concluded that the recruitment of type II fibers may be, at least in part, involved in the $\dot{V}O_{2SC}$ phenomenon. The authors made this assumption based on the idea that fatigued type II fibers (recruited predominantly during the electromyostimulation (EMS) protocol (Vanderthommen, Depresseux et al. 1997)) were unable to replace exhausted type I fibers, and as a consequence, there was a diminution of the additional O_2 uptake and an exercise interruption. These results are in line with the study of Carter (Carter, Pringle et al. 2004), who reported an altered response in $\dot{V}O_2$ kinetics during heavy-intensity exercise with an increase in the amplitude of the primary component and a decrease in the amplitude of the $\dot{V}O_{2SC}$ after a protocol aimed at glycogen depletion in type II fibers. Four years later, Krustrup (Krustrup, Secher et al. 2008) tested the hypothesis that energy turnover and therefore the ATP cost were higher for type II fibers than for type I fibers with a partial neuromuscular blockade of the latter. They confirmed that muscle O_2 uptake was 20% higher and the MRT was also significantly longer in type II fibers, supporting the idea that type II fibers had slower kinetics and greater ATP cost than type I fibers during dynamic exercise.

Further, as reported by Young-Soo Han et al. (Han, Proctor et al. 2001), type II muscle fibers have a lower reserve capacity for ATP consumption than type I muscle fibers. The reserve capacity is defined as [1- ratio of ATP_{iso} to ATP_{max}], where ATP_{iso} is the rate of ATP consumption in single fibers during isometric contraction and ATP_{vmax} is the upper limit of ATP consumption. With such an energetic imbalance under conditions of high workloads (where energy utilization increases in proportion to the WR), the higher ATP cost and the lower ATP reserve capacity may explain, in part, the greater fatigue

susceptibility of fibers expressing MHC_{2x}. This fact can have important implications in the development of the $\dot{V}O_{2SC.}$

All these outcomes suggest that the $\dot{V}O_2$ primary component is related to type I fibers and that the $\dot{V}O_{2SC}$ is associated with type II fibers, although no conclusions about these relationships are stated in the literature.

The pulmonary $\dot{V}O_2$ signal homogenizes any oxidative response diversity within the activated pool of MUs and therefore fibers during any exercise that requires the activation of a heterogeneous fiber population. However, if exercise is performed with transitions of two or more steps of sufficiently different intensity to activate different fiber populations, it is possible, with the pulmonary $\dot{V}O_2$ signal, to unveil which pool of MUs are activated at that specific moment and/or intensity (Brittain, Rossiter et al. 2001).

The work-to-work transition protocol could be an ingenious tool to disentangle the factors that potentially influence the control of $\dot{V}O_2$ kinetics. If exercise is started from a higher metabolic baseline, then it could be possible to see alterations in the cardiovascular function affecting O_2 delivery (Hughson and Morrissey 1982, MacPhee, Shoemaker et al. 2005) but also to see alterations in MU recruitment patterns (Brittain, Rossiter et al. 2001), two of the most popular theories. For instance, a transition from unloaded to moderate-intensity cycling would be expected to be commanded by the recruitment of type I muscle fibers that are positioned low in the recruitment hierarchy (Henneman 1957). On the other hand, if the transition is made from the moderate to the severe domain, recruitment would be done mainly by the muscle fibers positioned at the higher edge of the recruitment hierarchy, type II (Brittain, Rossiter et al. 2001). In 1970, di Prampero et al. (Prampero, Davies et al. 1970) studied the rate of O_2 uptake of an exercise of high intensity when it was started from rest or from moderate intensity (i.e., work-to-work transitions). They found faster $\dot{V}O_2$ half-time kinetics (time needed for $\dot{V}O_2$ to increase from rest to half of its peak value) in the work-to-work transitions ($t_{1/2}$=17 s) than in the rest-to-work transitions ($t_{1/2}$=30 s). A few years later, the same group (Davies, Prampero et al. 1972) showed similar results. The $\dot{V}O_2$ half-life was reduced in the transition from mild to heavy work compared with the transition from rest to mild or heavy work levels.

In 1977, the group of Diamond (Diamond, Casaburi et al. 1977) studied $\dot{V}O_2$ kinetics with WRs that fluctuated, in a sinusoidal manner, from 25 W to higher values but always within the moderate domain. At these intensities, similar to those of work-to-work transitions, they argued that the mechanisms controlling the rate of $\dot{V}O_2$ should be the same as those involved in mild to heavier transitions. In contrast, instead of having faster $\dot{V}O_2$response kinetics, the response they found was slower than that found in the transitions from rest to work, with a half time of 34 s. These results appeared to conflict with those obtained by di Prampero (Prampero, Davies et al. 1970) and Davies et al. (Davies, Prampero et al. 1972).

Hughson and Morrisey (Hughson and Morrissey 1982) published a work in an attempt to clarify the controversy among the previous papers of the di Prampero, Davies and Diamond groups (Prampero, Davies et al. 1970, Davies, Prampero et al. 1972, Diamond, Casaburi et al. 1977). They used a different protocol in which the subjects exercised in transitions from rest to 80% GET and from rest to 40% GET, followed by a stage at 80% GET and finally from rest to 40% GET followed by an intensity at 120% GET. The authors observed that $\dot{V}O_2$ kinetics significantly slowed when the transition was from the upper region compared with the lower region of the moderate domain. A few years later, Yamamoto, Hughson and other researchers (Yamamoto, Hughson et al. 1991) proposed an explanation for this slow response in work-to-work transitions. They studied the autonomic control of the HR during cycle ergometer exercises at different submaximal intensities below the GET. Their findings were that from rest to 60% GET, the HR was controlled by the parasympathetic nervous system, while the sympathetic nervous system took control when the intensity exceeded the GET. After this finding, Yamamoto and colleagues argued that the cause of the slow response of $\dot{V}O_2$ between 60 and 100% GET was due to the slow sympathetic activation after the removal of vagal tone (Rowell and O'Leary 1990), creating this biphasic response between these 2 intensities.

In 1987, Morton and colleagues (Morton 1987) revisited the previous studies mentioned above and suggested several methodological considerations that could explain these bizarre and contradictory results. For example, he mentioned the experimental design modeling procedures and the limited intensity ranges. After the work of Morton (Morton 1987), new studies were developed in an attempt to

elucidate what the issue was. Several authors again performed experiments taking into account his considerations. For instance, di Prampero et al. (Prampero, Mahler et al. 1989) eschewed mixing chamber collection and used breath-by-breath technology. In addition, they used a cycle ergometer instead of running (kinetics are faster in running than in cycling (Martinez, Modrego et al. 1993, Hill 2000)) to equal the $\dot{V}O_2$ kinetics rate to normalize the test.

On the other hand, they did not remove the cardiodynamic phase to avoid contamination. Their findings supported those of the Hughson team (Hughson and Morrissey 1982), who showed slow kinetics when transitions were made from an elevated $\dot{V}O_2$ baseline.

In 2001, Britain et al (Brittain, Rossiter et al. 2001) published a paper regarding the effects of an elevated baseline in $\dot{V}O_2$ kinetics in the moderate domain. They found the presence of slower kinetics in the upper ($\dot{V}O_2$ $\tau_p \approx 40$ s) than in the lower moderate region ($\dot{V}O_2$ $\tau_p \approx 25$ s), confirming that $\dot{V}O_2$ is not dynamically linear even below the LT. In addition, the novel finding was that the G was the lowest in the lower region, the highest in the upper region and intermediate for the full transition. They explain this phenomenon appealing to the different properties of the fiber recruitment pool, as shown by Henneman et al (Henneman, Somjen et al. 1965).

In 2005, MacPhee and colleagues (MacPhee, Shoemaker et al. 2005) used a new technology (near–infrared spectroscopy (NIRS)), providing information about the change in concentrations in muscle microvascular oxy-(HbO_2), deoxy-(HHb), and total (Hb_{tot}) hemoglobin (hemoglobin/myoglobin) during dynamic exercise to determine why work-to-work transitions were slower in the moderate domain. Specifically, they examined the adaptation of $\dot{V}O_2$ to a change in WR initiated from different regions of the moderate domain while at the same time measuring the adaptation of leg muscle blood flow (LBF), HR, and local muscle HHb during two-legged knee extension exercise. The major new findings of their study were that during a knee extension (KE) exercise in the upper region of the moderate domain, the kinetics of pulmonary $\dot{V}O_2$ and HR, G, femoral artery blood flow and leg vascular conductance were slowed, while the amplitude of HHb was higher. They concluded that the increase in HHb suggests an increase in O_2 extraction to compensate for the slower femoral artery blood flow response and therefore prevent further slowing of $\dot{V}O_2$ uptake. They attributed this

slower $\dot{V}O_2$ uptake in the upper domain than in the lower moderate domain to a slowness in the activation of intracellular oxidative metabolism, a slower activation of muscle enzymes or a delayed activation of the pyruvate dehydrogenase complex in the work-to-work transitions.

Wilkerson and Jones (Wilkerson and Jones 2006) investigated the work-to-work effect into intensities ranging from the unloaded to moderate (80% GET), moderate to heavy (40%Δ) and heavy to severe (100%$\dot{V}O_2$ peak) domains, including the assessment of HR kinetics and surface iEMG. Interestingly, they found a complete dissociation between $\dot{V}O_2$ and HR kinetics as the $\dot{V}O_2$ τ_p progressively lengthened when the WR was increased. The authors showed faster HR kinetics during both moderate to severe and heavy to severe intensities than during unloading to moderate transition. This provides evidence that the slower work-to-work kinetics were not due to slow HR response and/or bulk O_2 delivery limitation but were associated with iEMG evidence of differences in muscle activation and the recruitment of different muscle fibers. However, there was an increase in the iEMG during the first 30 s of severe exercise and then a levelling off in all cases. This supports the idea of an increase in rate coding of already recruited muscle fibers in the severe domain but also shows a higher recruitment of type II muscle fibers that was masked by higher muscle temperatures achieved at the end of heavy exercise. The authors explained this by arguing that, at the end of the baseline period of heavy-intensity exercise and during the severe-intensity exercise, all the available muscle mass was possibly recruited; consequently, the increased iEMG is the result of an increased rate coding in the already recruited MUs (Wilkerson and Jones 2006). Britain et al (Brittain, Rossiter et al. 2001) also observed an increase in the G when the WR was started from an elevated baseline, which is consistent with the theory of an increase in the proportional contribution of fibers with lower oxidative efficiency.

In line with this, Keir and colleagues (Keir, Benson et al. 2016) found, with a ramp incremental exercise protocol, a curvilinear, rather than linear, relationship between τ and the mean response with the WR. They explain these different kinetics as a consequence of the progressive recruitment of higher-order muscle fibers being kinetically slower and metabolically less efficient. The progressive recruitment of type II fibers could contribute to a time-dependent increase in $\dot{V}O_2$ for a given WR. They also found counterintuitive the fact that, in athletes and young people, the G was correlated with the

aerobic fitness status (Barstow, Jones et al. 2000), with values of 10.5-12.0 ml/min^{-1}/W^{-1} for trained cyclists (Boone, Koppo et al. 2010), 9-10 ml/min^{-1}/W^{-}1 for healthy young adults (Hansen, Casaburi et al. 1988) and 6.5-8.510 ml/min^{-1}/W^{-}1 for older adults and patients with chronic diseases (Toyofuku, Takaki et al. 2003, Gravelle, Murias et al. 2012). On the other hand, in step exercise, the SS G has been shown to be independent of age or fitness status (Grey 2014) but to be increased with a greater baseline WR. Increases in baseline WR also increase τp values, reflecting slow $\dot{V}O_2$ kinetics, although these increases are attenuated in trained adults. The authors explain these changes by two facts: first, individuals with greater mitochondrial content have faster $\dot{V}O_2$ kinetics and could, therefore, see the G increase as exercise proceeds; second, MU recruitment demands increase along with the WR, and more type II fibers, which are less efficient and have slower kinetics, are recruited. Therefore, in healthy trained subjects, the larger G may reflect fast kinetic properties in active muscle fibers rather than a paradoxical work inefficiency. In subjects with chronic diseases, the low G could reflect a compounding inability to activate oxidative metabolism at a sufficient rate to match changes in the WR, showing lower $\dot{V}O_2$.

Another explanation regarding why the highly trained subjects have greater G than their nontrained counterparts was highlighted by Boone and colleagues (Boone, Koppo et al. 2010). They found that during ramp exercises, but not during step incremental exercises, cyclists showed a higher G in $\dot{V}O_2$ parallel with a higher iEMG/WR. In contrast, there were no differences between exercises in either of these variables in physically active students. Boone and colleagues argued that the difference could be explained by an overshoot in $\dot{V}O_2$ triggered by a temporary reduction in muscle mechanical efficiency due to an "overrecruitment" of muscle fibers in the transition to moderate exercise during ramp exercise. This overshoot, in the non-SS during a constant WR, has been suggested to reduce the muscular efficiency due to the production of activation heat (in non–cross-bridge activities). This heat arises from the movement of calcium in and out of the sarcoplasmic reticulum (SR) (Barclay 1996). At the same time, this overrecruitment could also, in return, reduce the metabolic impairments in each individual fiber because of the wider spread of force development. Consequently, the fatigue in each

fiber is delayed so that it can participate for longer periods to increase the external WR. In that manner, this overrecruitment could be seen as an advantage despite a small loss in muscle efficiency.
In 2007, the same group, (Wilkerson and Jones 2007) implemented a new protocol with full transitions to moderate, full to heavy and work-to-work transitions from moderate to heavy. The novel finding was that the amplitude of the $\dot{V}O_{2SC}$ during heavy exercise was reduced in the work-to-work transitions. The authors explained it as an increase in the proportional contribution of type II fibers to force production in the transition from moderate to heavy exercise.

As shown in these different studies, there is no consensus about why the $\dot{V}O_2$ kinetics are slower during work-to-work transition protocols, nor is there a consensus about what triggers the reduction in muscular efficiency or the rise in the G above the LT that characterizes the $\dot{V}O_{2SC}$.

6.3 - Studies supporting fatigue as a trigger in the development of the $\dot{V}O_2$sc

One of the most prevalent hypotheses explaining the appearance of the $\dot{V}O_{2SC}$ is the progressive increase in MU recruitment during supra-LT exercise (Whipp 1994). Some authors support the idea that the recruitment of type II, fast-twitch, and less efficient fibers is involved in the development of the $\dot{V}O_2$sc to compensate for the deficiency of type I slow-twitch fibers during heavy-intensity exercise (Gaesser and poole 1996). This increase in recruitment is necessary to maintain force production to counter muscle fatigue and results in an increased reliance on poorly efficient type II fibers (Crow and Kushmerick 1982, Hunter, Newcomer et al. 2001, Han, Geiger et al. 2003) However, there is no agreement in the literature concerning the appearance of muscle fatigue as the main reason for the $\dot{V}O_{2SC}$.

One of the first studies showing fatigue as a candidate for the appearance of the $\dot{V}O_{2SC}$ came from the study of Shinohara and Moritani (Moritani, Sherman et al. 1992). The study showed that the decrease in efficiency, reflected as an increase in $\dot{V}O_2$ during constant power output, was correlated with an increase in the iEMG. Shinohara and Moritani argued that the increases in $\dot{V}O_2$ could be caused by a progressive recruitment of fast MUs to compensate for the reduced power output of the fatigued muscle fibers. However, a few years later, Scheuermann and colleagues (Scheuermann, Hoelting et al. 2001) found different results. Subjects performed step increases in exercise bouts from moderate to heavy

intensity for 8 min. The $\dot{V}O_{2SC}$ was evident 120 s after the onset of exercise, with no corresponding changes in the iEMG or MPF. The authors concluded that the lack of association between the $\dot{V}O_{2SC}$ and both parameters was inconsistent with the hypothesis that the $\dot{V}O_{2SC}$ was a consequence of the recruitment of additional MUs.

In 2001, Borrani and colleagues (Borrani, Candau et al. 2001) performed spectral analysis of the electromyogram (EMG) signal to test the hypothesis of the $\dot{V}O_{2SC}$ being partially induced by the recruitment of type II muscle fibers. By having subjects exercise at 95% of $\dot{V}O_2$max and measuring the EMG activity in the vastus lateralis, gastrocnemius lateralis, and soleus muscles, they concluded that there was a concomitance between the beginning of the $\dot{V}O_{2SC}$ and the increase in the MPF. In addition, the time of the MPF increase onset in the soleus muscle came later than that in the other two muscles. Interestingly, the soleus is known to have a high percentage of type I fatigue-resistant muscle fibers. Borrani and colleague also observed that the beginning of the $\dot{V}O_{2SC}$ corresponded with the beginning of the rise in the MPF in the vastus lateralis and gastrocnemius lateralis but was postponed for the soleus. They therefore concluded that low-efficiency type II fiber recruitment partially clarifies the existence of the $\dot{V}O_{2SC}$.

Later, in 2010, Hirai and colleagues (Hirai, Roseguini et al. 2010) showed that high pedal frequencies, expected to enhance fast-twitch muscle fibers, were associated with a greater $\dot{V}O_{2SC}$ amplitude and greater surface EMG portrayed by the root mean square (RMS). As the authors mentioned before, there are other studies supporting the fact that the increase in the iEMG signal is correlated with the consistent progressive increase in the recruitment of type II muscle fibers during the development of the $\dot{V}O_{2SC}$ (Perrey, Betik et al. 2001, Sabapathy, Schneider et al. 2005).

One of the limits of EMG data is that they provide information from only a specific zone of superficial muscle, and movement artifacts or signals from other muscles can interfere with the signal. In recent years, another technique has been used to determine muscle use during exercise. For example, Saunders and colleagues (Saunders, Evans et al. 2000) used magnetic resonance imaging (MRI). They evaluated EMGs and MRI transverse relaxation times (T2) during two 15-min bouts of cycling at low and high intensities to determine muscle activity. Proton T2 has been shown to increase during exercise and is

highly correlated with increases in the iEMG (r=0.99, P < 0.05) (Adams, Duvoisin et al. 1992). Saunders and colleagues found that during constant-rate exercise below the GET, there was no significant increase in muscle activity and no $\dot{V}O_{2SC}$. In contrast, during high-intensity cycling, $\dot{V}O_2$, T2, EMG activity and MPF rose significantly from 3 to 15 min. Another pivotal finding was that $\dot{V}O_2$ and muscle T2 during high-intensity cycling were related (r = 0.63).

Perhaps one of the most clear examples showing the link between the $\dot{V}O_{2SC}$ and the recruitment of fatigued fibers is the study of Vanhatalo and colleagues (Vanhatalo, Poole et al. 2011). Subjects performed 2 different protocols, i.e., 3 min of all-out cycling, where all the fibers should be recruited, and a constant-WR high-intensity exercise. Even if there is no time for the VO_{2SC} to develop during supramaximal exercise, as mentioned before, they argued that the dramatic increase in the O_2 cost of power production during all-out exercise could be considered a slow-component-like phenomenon. The results showed a rise in $\dot{V}O_{2SC}$ amplitude with a progressive decline in muscle activation measured with an iEMG. On the basis of these results, Vanhatalo and colleagues suggested that the $\dot{V}O_{2SC}$ could be generated by the high O_2 cost of the fatigued muscle fibers, which do not contribute any further to the power output but continue to consume O_2.

In line with this hypothesis, Zolaz and colleagues (Zoladz, Gladden et al. 2008) observed an $\dot{V}O_{2SC}$-like response in canine isolated gastrocnemius muscle. In the preparation of the isolated muscle, muscle fibers were maximally activated by electrical stimulation, corresponding to ~60–70% of the muscle peak $\dot{V}O_2$ so that no progressive recruitment of fibers was possible. Curiously, at the same time, they observed a constant $\dot{V}O_2$ value in the presence of a diminution of force output reflected in the increase in the $\dot{V}O_2$/force, calling that phenomenon a "mirror image" of the $\dot{V}O_{2SC}$. They explain this fact with different arguments: the muscle was becoming less efficient with fatigue or the fatigued fibers had greater metabolic cost for recovery processes while they contributed little, if any, to power output. However, after this publication, Borrani and colleagues (Borrani, Malatesta et al. 2009) wrote a letter to the editor expressing their disagreement with the interpretation of the results by Zolaz and colleagues. Borrani and colleagues argued that if the "real" $\dot{V}O_{2SC}$ is an increase in O_2 consumption during a constant power output that reflects a decrease in the efficiency of muscle contraction, the unique solution to

maintain the same power output is the recruitment of additional MUs. The conclusion of Zolaz and colleagues about the reduced efficiency of muscle contraction not being related to the progressive recruitment of muscle fibers seemed questionable to Borrani and colleagues.

In 2011, Canon and colleagues (Bowen, Cannon et al. 2012) published a study with the aim of discerning whether muscle fatigue precedes the $\dot{V}O_2$sc. More specifically, they aimed to determine whether the magnitude and time course of muscle fatigue are related to the kinetics of the $\dot{V}O_2$sc. They used a protocol consisting of a constant-WR exercise for 8 min at 80% LT, Δ20% and Δ60% followed by a maximal isokinetic effort maintained for 5 s in isokinetic mode to measure velocity-specific peak torque (Pt) and power at 60, 90 and 120 rpm. Their results showed that force production dropped significantly within 3 min of exercise onset, but there was no further reduction between 3 and 8 min, although this was when the $\dot{V}O_2$sc was most evident. This finding suggested that muscle fatigue precedes the development of the $\dot{V}O_2$sc but that the two variables are not temporally related. Canon and colleagues concluded that fatigue was probably the mechanism needed to initiate VO_2sc development, but fatigue did not participate in $\dot{V}O_2$sc progression. In addition, the recruitment of additional muscle fibers was not necessary for the development of the $\dot{V}O_2$sc, but a reduction in the mechanical efficiency of fatigued fibers was involved.

Later, in 2016, Keir and colleagues (Keir, Copithorne et al. 2016) wanted to analyze the relationship between peripheral muscle fatigue and the $\dot{V}O_2$sc; more specifically, they analyzed whether the magnitude of peripheral muscle fatigue was associated with $\dot{V}O_2$sc amplitude. To assess that hypothesis, they implemented a protocol consisting of a pre-exercise neuromuscular assessment followed by the exercise intervention and finished by a postexercise muscular assessment. The exercise intervention consisted of a constant-WR exercise at 80% LT (moderate intensity) for 18 min and five constant-WR tests at Δ60% (severe intensity) completed for 3, 8, 13 and 18 min, with a second 18-min cycling trial to collect EMG data.

In contrast to the isokinetic model of Cannon (Cannon, White et al. 2011), Keir and colleagues (Keir, Benson et al. 2016) utilized a protocol with low (10 Hz) and high frequencies (50 Hz) of electrical stimulation aiming to assess central and/or peripheral fatigue. Their results showed that peripheral

muscle fatigue, measured by electrically stimulated muscle torque loss, was coincident with the development of the $\dot{V}O_2$sc. The greater depression found in force during low frequencies (10 Hz) indicated high-intensity muscle fatigue, which is a hallmark mechanism that occurs beyond the neuromuscular junction (NMJ) (Keir, Benson et al. 2016). In addition, the reduction in the 10/50 Hz ratio with time confirmed that the decrease in muscle torque was mediated by a peripheral mechanism. Additionally, Place et al. (Lepers, Maffiuletti et al. 2002, Place, Lepers et al. 2004) examined the time course of neuromuscular fatigue by measuring voluntary and evoked muscle contractions. They found that changes in peripheral mechanisms, such as a reduction in twitch force or contraction time (CT), were observed at the beginning of exercise (Lepers, Maffiuletti et al. 2002). In contrast, central activation and M-wave properties, estimated by the twitch interpolation technique (TIT) (Merton 1954), were significantly reduced only at the end of the exercise. Although Place and colleagues did not measure the $\dot{V}O_{2SC}$, their results interestingly show that peripheral fatigue was observed at the beginning of the exercise, when the $\dot{V}O_2$sc developed. Moreover, Decorte et al. (Decorte, Lafaix et al. 2012) found after an intermittent constant-load intense cycling test that most of the alterations in mechanical and EMG responses to femoral nerve stimulations occurred during the first half of the exercise, but voluntary activation (VA) (central drive) was present only at the end of the exercise. These data indicate that central and peripheral fatigue have different kinetics and that, if fatigue is associated with the $\dot{V}O_2$sc, it is peripheral fatigue.

In the study of Keir et al (Keir, Benson et al. 2016), the highest amplitudes of the $\dot{V}O_2$sc were associated with the largest reductions in muscle torque. The authors concluded that because the time course and muscle fatigue were related to $\dot{V}O_2$sc development, muscle fatigue appears not only as an initiator of the $\dot{V}O_2$sc but also as a mechanism by which it progresses with time. Surprisingly, the surface EMG data showed no changes during the period in which the $\dot{V}O_2$sc and muscle fatigue developed. The authors explained this fact by indicating that when exercised muscle fatigues, it concurrently needs more O_2 to sustain the same power output. The strong association between peripheral fatigue and VO_{2SC} amplitude in the absence of any changes in muscle activation suggests that the fatigued muscle fiber pool remains capable of generating the required power to continue exercise but with a greater O_2 cost. In conclusion,

this study provided evidence in humans of the association between muscle fatigue and the development of the $\dot{V}O_{2SC}$. The absence of any changes in muscle activation indicated that muscle fatigue may come from metabolic causes (Bongbele 1990) rather than from excitation-contraction issues.

Nevertheless, despite the weight of the evidence supporting the hypothesis of progressive recruitment of more muscle fibers due to fatigue, there is still no certainty in the literature, since other groups found no change in the iEMG.

For example, Scheuermann (Scheuermann, Hoelting et al. 2001) argued that the information given by the iEMG indicates the overall recruitment of MUs but does not provide confirmation about the specificity of the recruitment pattern of type I or II fibers. However, this information could be inferred from the frequency content (power density spectrum) of the EMG signal (Tesch, Komi et al. 1983, Kupa, Roy et al. 1995). Using both treatments, they found a lack of association between the $\dot{V}O_{2SC}$ and the changes found in the iEMG or MPF during heavy constant-WR exercise, concluding that there was no additional recruitment of type II fibers. Alternatively, they suggested that the $\dot{V}O_{2SC}$ reflects either a progressive uncoupling of the mitochondrial P/O ratio or a progressive increase in ATP requirements. In contrast, Borrani and colleagues, after performing a treadmill test at 95% of the velocity associated with VO_{2max}, found concomitance between the beginning of the VO_{2sc} and the beginning of the MPF (Borrani, Candau et al. 2001).

Alejandro Lucia and colleagues (Lucía, Hoyos et al. 2000) tested the neuromuscular factors implicated in the $\dot{V}O_{2SC}$ in professional cyclists. They found no significant changes in the iEMG or MPF after cycling at 80% of $\dot{V}O_2$max for 20 min even if a significant but small $\dot{V}O_{2SC}$ was shown, arguing that, probably after years of highly demanding training, the slow MUs of professional cyclists have greater resistance to fatigue. Similarly, Tordi et al (Tordi, Perrey et al. 2003) found no significant differences in the recruitment pattern measured by the iEMG in well-trained cyclists who performed 2 6-min bouts of cycling at 85% $\dot{V}O_2$max separated by "prior" exercise of 3 30-s all-out bouts of the Wingate test.

Krustrup (Krustrup, Söderlund et al. 2004) established an alternative approach to the common EMG to evaluate the recruitment pattern—the measurement of Cp and glycogen content in successive muscle biopsies during exercise. Their results showed that additional type I and II fibers were recruited from 3

to 6 min in intense supramaximal exercise in association with the appearance of the $\dot{V}O_{2SC}$. The glycogen depletion pattern confirmed that both type I and II fibers were active during high-intensity exercise, while only type I fibers were recruited during moderate-intensity exercise, with no $\dot{V}O_{2SC}$ presence. They concluded that during high-intensity exercise, the recruitment of additional fibers contributes to the development of the VO_{2SC}.

Thistlethwaite and colleagues (Thistlethwaite, Thompson et al. 2008) showed with an ingenuous experimental design that prefatigue was not the cause of the appearance of the $\dot{V}O_{2SC}$. They used KE exercises as an alternative way to manipulate the recruitment pattern of quadriceps muscle fibers. Indeed, MRI studies have indicated that KE exercises are limited to the quadriceps muscles (Richardson, Frank et al. 1998). Koga and colleagues (Koga, Poole et al. 2005) demonstrated that the gain in the primary rise in the $\dot{V}O_2$ response is higher in KE exercises than in cycling at the same relative intensity. Therefore, KE exercises allow for greater muscle mass activation of both type I and II fibers compared with the prior bout of cycling exercise. As discussed above, prefatiguing the muscle will probably result in an increase in the recruitment of type II, less-efficient fibers, altering the primary phase or the amplitude of the $\dot{V}O_2$ response in the second bout of heavy cycling exercise. The authors designed two different protocols. In the first protocol, subjects performed 6 min of heavy constant-load exercise at Δ50%. In the second protocol, subjects performed 6 min of exhaustive bilateral KE exercises at 30 contractions/min with a resistance to elicit twice the active muscle mass recruitment compared to that recruited during the heavy cycling exercise, followed by 6 min of heavy constant-load exercise at Δ50%. Contrary to the expected results, τ_p, the gain in the primary response and the amplitude of the $\dot{V}O_{2SC}$ were quite similar between the protocols, showing that the "prefatigue" protocol with the KE exercises was not an absolute condition for the appearance of the $\dot{V}O_{2SC}$.

Hopker and colleagues (Hopker, Caporaso et al. 2016) also showed that the $\dot{V}O_{2SC}$ is not supported by fatigue involvement. They used an experimental design to isolate the effects of the reduced maximal voluntary power from the metabolic stress effects. They induced locomotor muscle fatigue using a protocol consisting of 100 intermittent drop jumps (Skurvydas, Dudoniene et al. 2002). This protocol is known to cause prolonged locomotor muscle fatigue by disrupting the excitation-contraction coupling,

especially in type II muscle fibers, but at the same time with a pause of 20 s that allows the recovery of the ATP and PCr spent during each jump. In that way, it was possible to differentiate locomotor muscle fatigue from the metabolic stress associated with high-intensity aerobic exercise. The results of the study showed that locomotor muscle fatigue (tested by the reduction in power in the maximal voluntary cycling power test) was not associated with the development of the $\dot{V}O_2$sc. In fact, the magnitude of the $\dot{V}O_{2SC}$ was not significantly different between the experiment with prefatigue and the control condition. Very recently, do Nascimento and colleagues (do Nascimento Salvador, Souza et al. 2018) published a study regarding the problem of the cause–effect relationship between the $\dot{V}O_2$sc and fatigue. Because prior heavy- or severe-intensity exercise has been shown to result in a reduction in the $\dot{V}O_2$sc during the next bout of severe-intensity exercise (Koppo and Bouckaert 2002, Jones, Koppo et al. 2004), they designed a study to verify whether prior severe-intensity exercise attenuates the muscle fatigue accompanying the reductions in the $\dot{V}O_{2SC}$ in the subsequent bout of severe-intensity exercise. To test this hypothesis, they used a protocol consisting of instantaneous switching between constant-WR and isokinetic cycling to measure reductions in Pt at 3 and 8 min during severe-intensity exercise with and without priming exercise. Their results showed that with prior severe-intensity exercise, the $\dot{V}O_{2SC}$ was reduced and that the decrease in force was larger at 8 min than at 3 min regardless of whether the protocol was performed with or without prior exercise. The authors concluded that because there was no difference in the decline in force with or without priming exercise, the results refute a cause-effect relationship between muscle fatigue and the $\dot{V}O_2$sc.

7 Neuromuscular fatigue

7.1 Definition

Skeletal muscles continuously contract in daily life; nevertheless, they cannot contract continuously without the development of fatigue. Muscle fatigue is a fundamental functional characteristic of skeletal muscle, defined as a decrease in force or power-generating capacity during prolonged muscle activity (McLester 1997, Gandevia 2001).

7.2 Pathway of the stimulus from the neuromuscular system to the muscle cell

Fatigue is a complex and multifactorial phenomenon. The exact mechanisms of the decrease in muscle performance are complicated to discern in humans, and changes could occur at multiple sites at the same time, as shown in figure 24, from the motor cortex to the contractile apparatus (Place, Yamada et al. 2010).

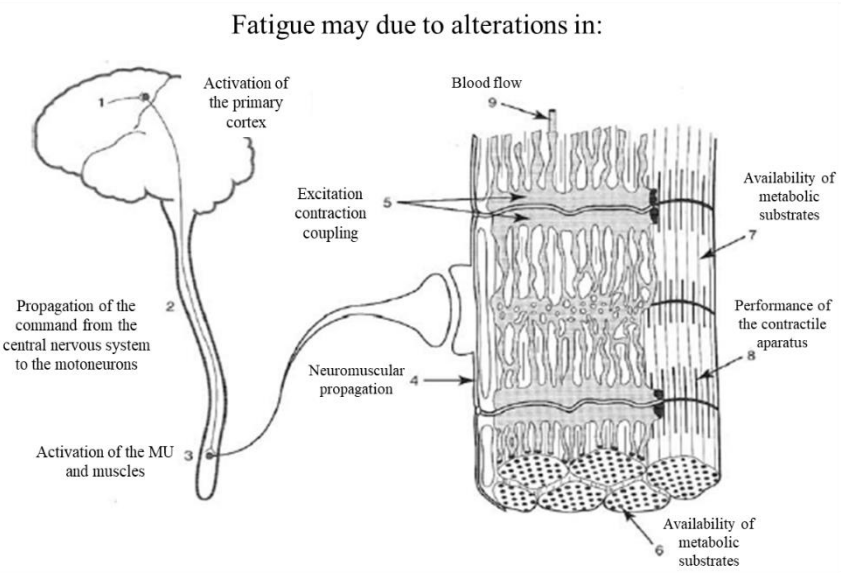

Figure 24. Sites where neuromuscular alterations could occur to cause fatigue. *Modified from Bigland-Ritchie 1981.*

Therefore, it seems necessary first to describe the pathway that the central stimulus follows, from the neuromuscular system to the muscle cell, and second to describe what happens to each site under normal conditions, i.e., in the absence of fatigue.

During exercise (or movement), skeletal muscle cells are activated in the central motor areas through a motor nerve impulse. This impulse travels from the presynaptic neuron through the alfa-motoneurons until it reaches the axon terminals (figure 25). Then, it stimulates voltage-gated calcium channels, leading to an influx of Ca^{2+} into the terminal button. This process triggers the exocytosis of acetylcholine (ACh) from synaptic vesicles to the synaptic cleft. ACh then diffuses through the sarcolemma and binds to its receptors located in the motor endplate of the muscle fiber. When enough ACh binds to its receptors, Na permeability increases, and depolarization results. The AP at that time propagates across the sarcolemma and T-tubules to the interior of the muscle cell, triggering the release of stored Ca^{2+} from the SR to the cytoplasm.

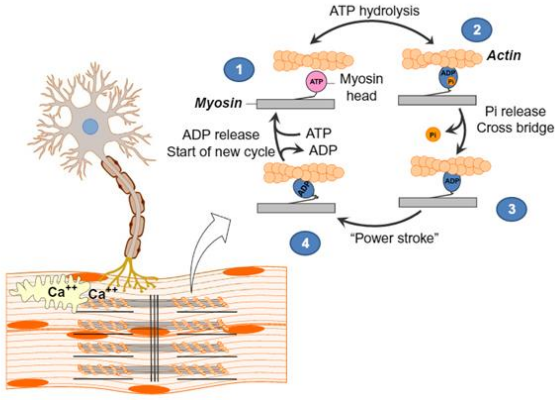

Figure 25 Molecular mechanism of muscle contraction. *An action potential travels through the transverse tubule system and the sarcoplasmic reticulum, resulting in the release of Ca ions. Ca ions bind to troponin C, resulting in conformational changes that allow myosin to bind to actin, producing muscle contraction. From Zsolt Radák, in The Physiology of Physical Training, 2018.*

Ca^{2+} subsequently binds to the complex protein troponin C. This protein is attached along actin filaments and twisted around the tube-shaped protein tropomyosin. The binding of Ca^{2+} to troponin C triggers a conformational change in the tropomyosin protein, pulling it out and releasing the active actin sites that are normally hidden. Currently, the globular heads of myosin are able to attach to their previously unseen binding sites. However, myosin heads have to be activated before the cross-bridge cycle can begin. This activation occurs when ATP bound to the myosin head is hydrolyzed into ADP and inorganic phosphate (Pi). The energy liberated from the hydrolysis of ATP activates the myosin head, forcing it into the cocked position. This latter position of myosin enables spontaneous binding to actin available sites (cross-bridge formation) and provokes the tilt of the head of myosin, bringing both filaments close to each other. This event is known as a power stroke and is developed in two separate phases (Taylor and Trenlham 1979):

a) With Pi release, the bond between both filaments goes from low-force states to high-force states. The large decline in free energy (Taylor and Trenlham 1979, Pate and Cooke 1989, Kawai and Halvorson 1991) (approximately half of the energy available from ATP hydrolysis) and the decline in

force in solutions where Pi is added to the bath (Cooke, Franks et al. 1988) confirm this hypothesis. The tilt of the myosin head, i.e., the power stroke, is produced by the release of the ADP, and then the myosin head pivots, sliding the thin filament towards the center of the sarcomere. Experiments in which ADP was added to the muscle fiber bath solution produced increases in isometric tension, which was suggested to be due to a higher number of attached cross-bridges and decreases in isotonic shortening velocity (Cooke, Franks et al. 1988), probably from the larger number of myosin heads that were not dissociated from ATP (Cooke, Franks et al. 1988).

b) Once the power stroke has been performed, cross-bridge detachment occurs when another ATP binds to the myosin head, causing a weakness in the actin-myosin link. Then, ATP is hydrolyzed by the enzyme ATPase into ADP and Pi molecules. The energy released during ATP hydrolysis is used to change the angle of the myosin head into a cocked position, and myosin is again ready to bind to actin sites if they are available. Actin and myosin have a high affinity at this stage (McLester 1997).

The cycle of coupling and uncoupling continues as long as the Ca^{2+} concentration is sufficient. The Ca^{2+} concentration will drop when there is no nerve stimulus and therefore no AP. Then, Ca^{2+} will be pumped back to the SR, troponin C will be deactivated, and tropomyosin will cover the anchoring sites of actin. The myofilaments will then be in a relaxed position (McLester 1997).

Considering the events mentioned above, fatigue could potentially arise at several sites in this pathway. For simplicity and to be able to more deeply describe these sites, the processes occurring inside the spinal cord, i.e., located before the NMJ, will be defined as neural or central, while processes occurring after the NMJ will be defined as intramuscular or peripheral.

7.3 Possible sites of neuromuscular fatigue

- Central fatigue

To discern whether the brain can drive human muscles during MVCs, it is necessary to determine first whether the motor neuron pool has been excited enough by human volition. VA has been defined as the level of voluntary drive during an effort (Gandevia 2001). The level of VA (%VA), i.e., central fatigue, is normally identified with the TIT (Merton 1954). The TIT is considered the gold-standard method to evaluate, noninvasively, the ability to maximally activate MUs. In 1954, with the

TIT, Merton (Merton 1954) showed evidence that voluntary contraction activates the contractile apparatus to the fullest.

The TIT consists of the comparison of the increment in muscle force produced during MVC while an electrical (or magnetic) stimulus is applied to a nerve trunk, with the force increment being the same as that when the muscle is potentiated in the resting state. The principle of the method resides in that if all the muscle fibers are fully recruited, an extramotor volley will not superimpose any twitch in the record. Indeed, during MVC, electrical stimulations do not yield a twitch but a normal AP (Merton 1954, Gandevia 2001). When there is an increase in the superimposed twitch, the descending drive to the motoneuron is not maximal, and therefore, central fatigue exists. The more MUs recruited and the faster they are firing, the smallest the superimposed twitch will be.

To measure VA through the TIT, the following formula is normally used:

$VA = 100 \times [1 - (\text{superimposed twitch/potentiated resting twitch})]$ (Taylor 2009)

Once its know that the fatigue is central rather than peripheral, spinal and supraspinal adaptations can be evaluated using transcranial magnetic stimulations or corticospinal magnetic or electric stimulation, respectively.

- Transcranial magnetic stimulation

The first method of transcranial electric stimulation was devised almost 30 years ago by Merton and Morton (Merton and Morton 1980). They showed that a high voltage current above the scalp was able to activate the motor cortex and evoke contraction in the contralateral muscles, but it was very painful. Five years later, Barker et al (Barker, Jalinous et al. 1985) designed an electromagnetic stimulator (transcranial magnetic stimulation) that solved this problem. In brief, the stimulator consists of a condenser of electric current that is discharged through a coil and produces a magnetic field that, through the scalp, induces an electric field in turn. This electric stimulation leads to depolarization membrane potentials in the nearby cortical tissue under the coil, affecting neural activity patterns (Reithler, Peters et al. 2011). With a circular coil and electrical current flowing clockwise, the left hemisphere is preferably excited. Turning the coil so that the current is counterclockwise, the right

hemisphere is preferably activated. With an eight-shaped coil, the area of intersection of both rings has to be on the motor area to be stimulated. Brief muscle contractions in the contralateral muscle targeted are observable via motor evoked potentials and recorded with electrodes located near the relevant muscle (Reithler, Peters et al. 2011).

- Corticospinal magnetic or electric stimulation

Spinal tracks can also be stimulated by passing a high-voltage stimulus between the mastoids or by magnetic stimulation over the back of the head. This stimulus activates the corticospinal track at the cervicomedulary junction and evokes motor responses in the arm muscles. With stimulation over the thoracic spine or the cervicomedullary junction, responses in the leg can be elicited (Taylor and Gandevia 2004). This method, which activates the corticospinal output at a subcortical level, is valuable for the investigation of the behavior of the motor pathway and allows a better interpretation of the responses evoked in the cortex (Taylor and Gandevia 2004).

- Peripheral fatigue

In elite athletic individuals who are well motivated, the most common form of muscle fatigue is peripheral fatigue (Allen, Lamb et al. 2008). Most of the research in this area implies that peripheral fatigue is the major limiting factor (McLester 1997). Nevertheless, it is important to note that there are two different types of peripheral fatigue: high-frequency fatigue (HFF) and low-frequency fatigue (LFF).

In 1977, Richard Edwards and colleagues started to study muscle fatigue in normal subjects. To measure it objectively, methods using electrical stimulation were developed (Edwards, Young et al. 1977). To obtain the maximum force from the muscle, it was necessary to stimulate the muscle at approximately 50 Hz. In contrast, if the goal was to obtain the maximum duration of the contraction, it was important to reduce the frequency to approximately 20 Hz. This event raised the idea that the level of fatigue was dependent on the frequency of the stimulation, and HFF and LFF were characterized (Jones 1979).

LFF was characterized by a higher relative loss of force at low frequencies of stimulation than at high frequencies and very slow recovery, taking hours or even days in severe cases. Indeed, the effects could persist even if the disturbance of the muscle disappeared (Jones 1996).

This type of fatigue is reported during high-intensity exercises as well as during submaximal repetitive contractions (Edwards, Hill et al. 1977), in exercises where the active muscle is stretched (Newham, Mills et al. 1983) or when the muscle is exercised isometrically at a long length (Jones, Newham et al. 1989).

This type of fatigue was usually associated with a reduction in Ca^{2+} release, although it has also been suggested that decreases in Ca^{2+} myofibrillar sensitivity could play an important role (Millet, Martin et al. 2011). Another explanation is that the series of elements damaged during stretching exercises may be the middle sarcomeres of the fiber, which are elongated by the stronger sarcomeres at the ends of the fiber (Jones, Newham et al. 1989).

HFF was characterized by a higher loss of force at high stimulation frequencies (80-100 Hz) than at low frequencies and a rapid recovery once the frequency was reduced. This loss of force is attributed to an accumulation of extracellular K^+ or a decrease in extracellular Na^+ (Millet, Martin et al. 2011). Another characteristic of this type of fatigue is the decrease in amplitude and the slowing down of the waveform of the muscle action potential (MAP). Certainly, accumulations of K^+ in the extracellular spaces would prevent the propagation of the AP through the sarcolemma (Jones 1996).

To differentiate between LFF and HFF, the ratio of LFF to HFF is commonly used. In this way, a lower ratio means LFF, and a higher ratio means HFF.

Several steps have been proposed to be implicated in the development of peripheral fatigue, including impairments in the NMJ, sarcolemma, transverse tubules, SR, Ca^{2+} and H^+ ion concentrations and byproducts of ATP hydrolysis.

In the next sections, each step will be described in detail:

- Neuromuscular transmission

The NMJ, or the motor endplate, is the site where the motor nerve AP is transformed into an MAP to initiate muscle contraction, i.e., neuromuscular transmission. The NMJ is the synaptic cleft formed between the interface of the motor nerve axon and the muscle fiber, also known as the presynaptic and postsynaptic terminal, respectively (Moczydlowski 2016). The arrival of central commands in the form of APs at the presynaptic (axon) terminal signals vesicles containing the neurotransmitter ACh to move towards the presynaptic membrane. These vesicles then fuse with the presynaptic membrane and release ACh into the synaptic cleft, which then binds to its receptors, triggering the opening of both sodium (Na^+) and potassium (K^+) channels located at the postsynaptic terminal of the sarcolemma of the muscle fiber being innervated. This allows Na^+ and K^+ ions to rush into and out of the muscle cell, respectively, which leads to the generation of postsynaptic APs or MAPs (Gandevia 2001).

Studies have shown that during exercise-induced fatigue, this neuromuscular transmission may be impaired due to factors such as a reduction in the ACh released (Wu and Betz 1998) from the axon terminal and the desensitization of ACh receptors at the postsynaptic membrane (Magleby and Pallotta 1981).

To quantify the amount of electrical activity produced by activated MUs during a voluntary muscle contraction, iEMGs are commonly used. iEMG recordings from surface electrodes represent a very complex summation of varying numbers of motor APs. This method permits indirect measurements of neuromuscular fatigue (Moritani and DeVries 1978). An increase in iEMGs has been shown to reflect the recruitment of additional MUs and an increase in the MU coding rate as the strength of a muscle contraction increases (Lippold 1952).

The changes in the strength of a muscle contraction are shown in two ways: first, the greater the contraction is, the greater the number of MUs recruited, and second, the faster these MUs repetitively contract, the larger the rise in frequency. These two factors would increase the iEMG output, showing a linear relationship (Lippold 1952). Nevertheless, the interpretation of iEMGs must be done with caution, as they could be influenced by factors such as temperature, showing an overestimation of the frequency, or changes in intracellular K^+, showing an underestimation of the frequency values (Petrofsky and

Laymon 2005). The increase in iEMG signal during maintained contractions is assumed to reflect a failure in muscle contractility. Indeed, in this scenario, active fibers exert progressively less force; consequently, to compensate for this effect, new MUs are recruited, and/or the MUs will fire with an increased frequency (Lippold, Redfearn et al. 1960). On the other hand, during maximal contractions, all MUs are assumed to be active, and the natural effect of continued contraction in this situation is a reduction in both muscle tension and iEMG activity (Komi and Rusko 1974).

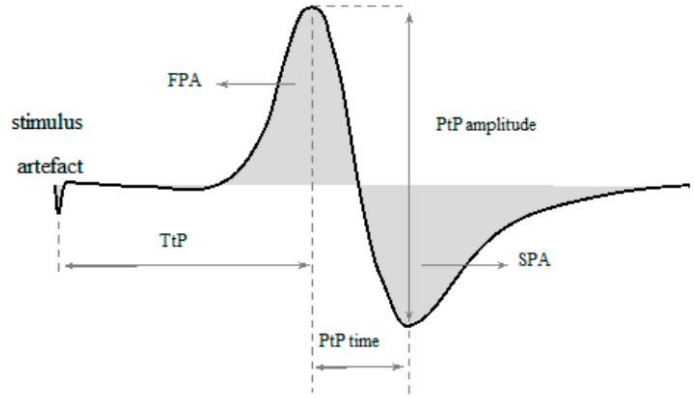

Figure 26. General representation of an M-wave and some of the commonly extracted parameters. *Peak-to-peak amplitude (PtP), time between peaks (PtP time), time to peak (TtP), first peak area (FPA), second peak area (SPA). From Ibitoye, Estigoni* et al.*2014.*

The M-wave amplitude (figure 26) is essentially a reflection of the magnitude of the sum of individual motor unit action potentials (MUAPs).

The M-wave has been analyzed to quantitatively investigate the relationship between electrical evoked contractions and muscle fatigue (Ibitoye, Estigoni et al. 2014).

- Excitation-contraction coupling

i) Sarcolemma

When an MAP is generated from the influx and efflux of Na^+ and K^+ ions, respectively, it is actively conducted through the sarcolemma. During physical exercise, the repeated firing of APs leads to changes in intra- and extracellular Na^+ and K^+ concentrations, which could impair sarcolemma excitability (Cheng, Place et al. 2018). The cause of this deficient excitability has been proposed to be linked with changes in the K^+ gradient across cells. Several studies have found an increase in K^+ concentration during MVC (Vyskočil, Hnik et al. 1983, Sjogaard, Adams et al. 1985). Others have shown that the extracellular K^+ concentration could rise from 4 mM in resting conditions to above 10 mM under high-intensity conditions (Juel, Pilegaard et al. 2000).

In rested muscle fibers, such a change in K^+ concentration would result in impaired AP propagation by increasing the excitation threshold of the T-tubule (Cheng, Place et al. 2018). Consequently, if the

sarcolemma AP is sufficiently reduced, the T-tubule charge will not be induced, which in turn will inhibit Ca^{2+} release from the SR (Rios and Pizarro 1988, Ríos, Ma et al. 1991).

ii) Transverse tubules

The primary role of the T-tubule system is well known to allow the sarcolemma AP to propagate into the core of the muscle cell. Ion flux takes only seconds to occur (Allen, Westerblad et al. 1992), the density of Na^+- K^+ pumps is lower than that of sarcolemma and the lumen of T-tubules is of a smaller volume relative to its surface (Venosa and Horowicz 1981). For all these, failure conduction in T-tubules has been proposed to be due to the accumulation or deficiency of Na^+ and K^+ (Allen, Westerblad et al. 1992).

The T-tubule membrane expresses high levels of voltage sensors and dihydropyridine receptors (DHPRs). If no impairment occurs, AP triggers a change in DHPRs, which in turn leads to the release of Ca^{2+} from the SR to the mycoplasma (Allen, Lamb et al. 2008).

iii) SR

SR Ca^{2+} release fails with fatigue, and it is not known whether this occurs because of changes in the degree of voltage sensor activation, through the influence of muscle metabolites or due to a depletion of Ca^{2+} inside the SR (Allen, Lamb et al. 2008). The magnitude of Ca^{2+} transients depends on SR Ca^{2+} release but also on all Ca^{2+} buffers in the cell, such as troponin C, parvalbumin, the SR Ca^{2+} pump, or calmodulin.

The muscle relaxes when the elevated Ca^{2+} is pumped back into the SR by the SR Ca^{2+} pumps. These pumps are known to be sensitive to the metabolic and ionic changes that appear during fatigue processes; nevertheless, the contribution of the changing pump properties to the slowing of relaxation in fatigue is still uncertain (Allen, Lamb et al. 2008).

Regardless of the role of the T-tubule or the SR in fatigue, a disruption in Ca^{2+} concentration does occur and will be explained in the next section.

- Metabolic changes

i) Ca^{2+}

As mentioned before, a failure in excitation-contraction coupling is one of the possible sites of the development of peripheral fatigue. Nevertheless, fatigue could be caused by a failure in AP

propagation, in the coupling mechanism between the AP and the release of Ca^{2+} or in Ca^{2+} regulation at the level of contractile elements.

Several authors (Blinks, Rüdel et al. 1978, Allen, Lee et al. 1989, Györke 1993) have demonstrated that the amplitude of Ca^{2+} transients decreases as fatigue develops.

Two main cellular mechanisms have been proposed to explain the impaired function of muscles during fatigue: (a) reduced Ca^{2+} release from the SR (Allen, Lannergren et al. 1995) and (b) reduced Ca^{2+} sensitivity of the myofilaments (Godt and Nosek 1989).

Studies on reduced Ca^{2+} release started with the work of Eberstein and Sandow (Eberstein 1963). They fatigued intact muscle with repeated tetani until the force was strongly reduced and then increased the level of activation with the perfusion of caffeine (known to directly stimulate the SR to release Ca^{2+}). The conclusion was that the decline in tension could be, at least partially, overcome with the facilitation of Ca^{2+} release. Indeed, the rise in tetanic Ca^{2+} concentration activates contractile proteins. Caffeine increases the opening of SR Ca^{2+}-release channels, increasing the myoplasmic Ca^{2+} concentration and therefore overcoming much fatigue. Westerblad and Allen (Westerblad and Allen 1991) performed similar experiments with caffeine. They concluded that reduced Ca^{2+} sensitivity and its release could be involved in the later stages of fatigue development but that the tension decline during early stages strongly indicates a reduction in the maximum tension-generation capacity. Indeed, metabolic changes induced by fatigue, such as an increase in H^+ by anaerobic glycolysis and increased Pi by the breakdown of PCr, could have deleterious effects. These effects will be discussed in greater detail later in this section.

In 1989, Godt and Nosek (Godt and Nosek 1989) demonstrated that the changes produced in the intracellular milieu by hypoxia and fatigue have direct deleterious effects on Ca^{2+} sensitivity and the F_{max} of the contractile apparatus. The decreases in Ca^{2+} sensitivity were due to competition between H^+ and Ca^{2+} at the actin filament, as H^+ acts directly at troponin C (Robertson, Glenn et al. 1979). Nevertheless, they concluded that the cellular acidification accompanying fatigue was not sufficient to fully explain the corresponding decline in force.

ii) ADP

ADP is known to inhibit the dissociation of ATP with the actomyosin complex during the cross-bridge cycle (Siemankowski, Wiseman et al. 1985). Indeed, White et al (White 1977) found that ADP at 0.05 mmol/L in solution inhibited actomyosin dissociation. In addition, ADP has been shown to cause an increase in isometric tension in fiber preparation as a result of the inhibition of myosin detachment (White 1977). Similarly, others (Cooke and Pate 1985) found that with ADP concentrations similar to those observed in fatigue muscles, the isometric tension increased by 2%, and the maximum isotonic shortening velocity decreased by 5%. The authors suggested that the results are a consequence of a higher number of attached cross-bridges and a larger number of myosin heads not dissociated from myosin by ATP.

iii) Pi concentration

Pi is the product of the breakdown of ATP (ADP+Pi) and PCr (CR+Pi); therefore, during intense skeletal muscle activity, the concentration of Pi will rise quantitatively. Compelling evidence in the literature has reported an increase in the intracellular concentration of Pi under fatigue and its correlation with limiting performance.

During high-intensity physical activity, PCr breaks down to Cr and Pi. PCr has a small effect on contractile properties (Murphy, Stephenson et al. 2004); in contrast, Pi has been shown to cause deleterious effects in myofibrillar force production, Ca^{2+} sensitivity, and SR Ca^{2+} release (Allen, Lamb et al. 2008). Most models of cross-bridge action propose that within the cross-bridge, the myosin head is weakly bound to actin filaments in the first step, and in the second phase, when Pi is released, the myosin head binds strongly (Takagi, Shuman et al. 2004). This proposal implies that increases in Pi will inhibit the transition to high-force cross-bridge states, and consequently, fewer cross-bridges would be in high-force states during fatigue development (Hibberd, Dantzig et al. 1985).

Evidence regarding the low-force and high-force states was provided by Martyn and Gordon (Martyn and Gordon 1992). They found that with Pi elevation, the maximum force declined but that stiffness was reduced only at levels of Pi higher than 10 mol/L. They explained this phenomenon as Pi elevation causing a shift of the cross-bridges to a low-force-producing, strongly attached Pi-bound state, which takes place before Pi release. The major cause of the reduced tetanic force appears to be the precipitation

of Pi with Ca^{2+} inside the SR. Posterino and colleagues (Posterino and Fryer 1998) tested the effects of increased Pi concentration on the SR Ca^{2+} content using a skinned fiber preparation and stimulating it with caffeine. The results confirmed that Pi enters the SR, binds to Ca^{2+} and precipitates. In this situation, the capacity of SR Ca^{2+} reuptake is greatly increased. Similarly, Westerblad and Allen (Westerblad and Allen 1996) directly injected Pi inside the muscle, expecting to see a decrease in force and Ca^{2+} sensitivity. Surprisingly, on the contrary, they found a drastic reduction in SR Ca^{2+} release with the corresponding decline in force. They concluded that most of the injected Pi probably entered the SR and precipitated as CaPi, reducing Ca^{2+} release.

iv) Glycogen

Glycogen depletion is associated with reductions in force and lower Ca^{2+} release during fatigue (Chin and Allen 1997). This association between low muscle glycogen and impaired muscle function has been attributed to the tempering role of glycogen in the release of Ca^{2+} from the SR (Duhamel, Perco et al. 2006). Interestingly, the role of glycogen depletion during fatigue has been shown to be present mainly at moderate and heavy intensities (Black, Jones et al. 2017).

7.4 Mechanical responses of skeletal muscle

Traditionally, MVC has been used to measure the peak force. Nevertheless, as mentioned above, performing MVC depends on the subject's volition, becoming a vulnerable measure under the possible influence of several factors. In contrast, the use of electrical stimulation techniques to evoke the isometric involuntary twitch is completely independent of the subject's skills or motivation, and as a result, these techniques have been used as the preferred method.

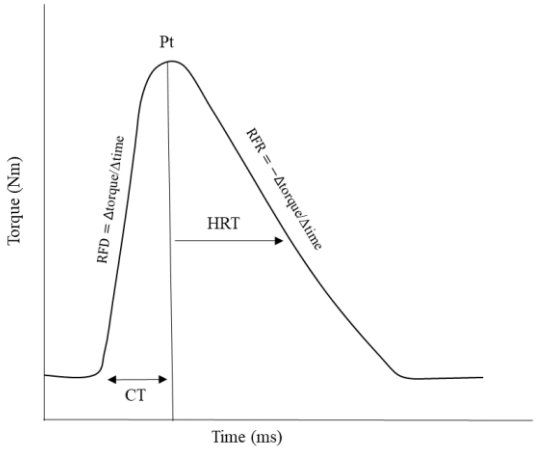

***Figure 27** Measurement of the peak twitch torque (Pt), rate of force development (RFD), contraction time (CT), half-relaxation time (HRT) and rate of force relaxation of a twitch.*

During electrical nerve stimulation, two neuromuscular properties can be evaluated: electrical signal transmission through the M-wave and the mechanical response of the twitch. The mechanical response can be isometric (through the twitch response) or dynamic (through a force–velocity test), depending on the test performed.

- Twitch mechanical responses

Muscular strength is defined as the production of maximal contractile force against a resistance in a single contraction (Coulson 2017), and Pt is the universal standard parameter used to measure muscular strength (figure 27). Pt represents the maximum torque produced during the twitch response. Attention was also paid to the temporal parameters during the twitch response. For instance, the CT is the time from the start of the contraction to the Pt, or the half relaxation time (HRT), which is defined as the time from Pt to a 50% decline in Pt. Reductions in Pt, as well as prolongation in the CT and the augmentation of the HRT, have been shown to be due to an increase in the duration in the intracellular Ca^{2+} transient rather than an impairment at the cross-bridge level (Allen, Lannergren et al. 1995).

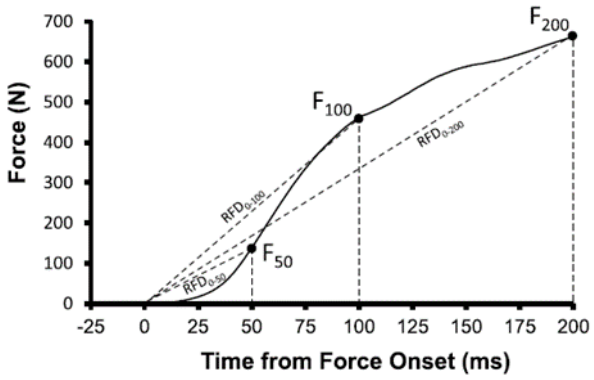

Figure 28 Common measurements of the rising force-time curve*. Force at specific time points (F50, F100, etc.) and overlapping RFD measurement all starting from force onset (RFD (0-50), RFD (0-100), etc.). Adapted from N.A. Maffiuletti* et al. *2016*

One other interesting parameter is the rate of force development (RFD), which expresses the explosiveness of the muscle during the twitch and can be defined as the rate of the rise in the contractile force, or torque, at the onset of contraction (figure 28) (Aagaard, Simonsen et al. 2002). The in vivo RFD has been defined as the slope of the torque–time curve (Δtorque/Δtime) obtained during twitch conditions (Andersen and Aagaard 2006). Once the contraction onset has been defined, the RFD can be measured notably during the initial phase over periods from 0 to 50 and 0 to 100 and 200 milliseconds (ms), as shown in figure 28. The RFD is mostly measured in single-joint tasks, such as elbow flexion/extension or knee extension/flexion, using a commercial isokinetic dynamometer with a rotational torque transducer (Maffiuletti, Aagaard et al. 2016).

The highest rate (or the steepest part of the curve) of the produced force is named the maximum rate of force development (MRFD) and is measured with the derivative of the development of force (dF/dt; derivative of force as a function of time). This parameter seems to be well related to sport-specific performances (Tillin, Pain et al. 2013) and could detect acute changes in neuromuscular function (Andersen and Aagaard 2006).

The RFD has been associated with the fiber type composition of the muscle (Andersen and Aagaard 2006) and with neuromuscular activation mechanisms such as high initial firing rates (Klass, Baudry et al. 2008). This neuromuscular property is thought to be limited by the amount of Ca^{2+} released from the SR and by the rate of transition from the weakly bound state to the high-force state when Pi is released from the actomyosin complex (Maffiuletti, Aagaard et al. 2016).

Finally, the rate of force relaxation (RFR), which is the ability to quickly relax the contracted muscle during the twitch, has often been investigated. The MRFD has also been evaluated by using the maximum rate of force relaxation (MRFR), i.e., the maximum value for –dF/dt (derivative of force as a function of time). The RFR, MRFR and HRT are highly dependent on the intrinsic properties of the muscles. Indeed, relaxation occurs when Ca^{2+} is pumped back into the SR, and Ca^{2+} is pumped back in faster in type II fibers than in type I fibers (Rossi and Dirksen 2006). Under fatigue conditions, the RFR declines more than the force and more in type II fibers than in type I fibers. This observation alludes to a slower dissociation of actin from myosin with fatigue, which could reflect a reduced rate of Ca^{2+} reuptake from the SR pumps or a deficit in the cross-bridge detachment rate (Westerblad and Allen 1993, Westerblad and Allen 1994). However, even if the rate of the dissociation of Ca^{2+} from myosin could be a limiting step, it has been shown to be too fast to be a limiting factor (Allen, Lannergren et al. 1995).

- Dynamic mechanical responses

During locomotion, the skeletal muscles of the different limbs are required to perform concentric and eccentric contractions against different loads; consequently, from a functional point of view, the analysis of the force (F)–velocity (V) relationship is important in the study of muscle fatigue.

Several studies performed during the first half of the previous century showed that the force of individual muscles and muscle groups decreases with velocity in a nonlinear manner (hyperbolic) (Hill 1938) (figure 30). However, more recent studies performed on maximum-performance multijoint tasks have suggested a linear relationship between F and V in the entire body (Bosco, Belli et al. 1995) or in the limbs (Vandewalle, Peres et al. 1987) (figure 29). The method more utilized to obtain the F-V

relationship is based on manipulating an external load to provide different values of F and V to establish a linear regression model:

$$F(V) = F_0 - aV_0 \qquad \text{Eq 9}$$

where F_0 is the F-intercept corresponding to the maximum isometric F (i.e., F at zero V), while *a* is the slope that corresponds to the ratio F_0/V_0, where V_0 is the V-intercept (V at zero F).

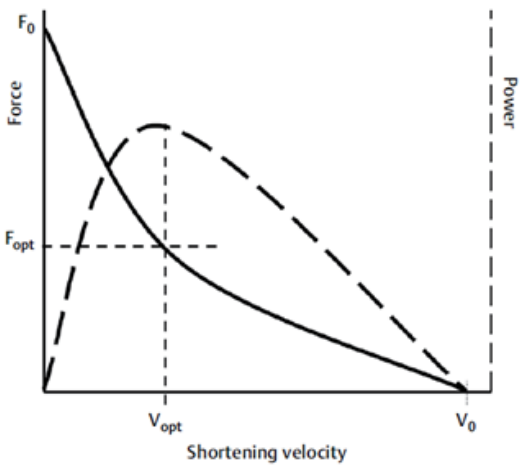

Figure 30 F-V relationship for a muscle or monoarticular movements. *The solid line represents the typical F-V relationship; the dashed line represents the corresponding P-V relationship obtained from a hypothetical muscle or muscle group. F0, force at null velocity; V0, velocity at null force; Pmax, peak power; Fopt and Vopt, optimal force and velocity for Pmax, respectively. From Jaric, S 2015.*

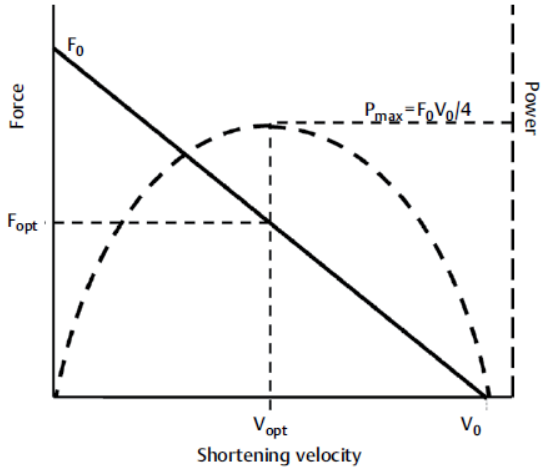

Figure 29 F-V relationship for a multijoint task. *The linear solid line represents the linear F-V relationship; the dashed line represents the corresponding parabolic P-V. F0, force at null velocity; V0, velocity at null force; Pmax, peak power; Fopt and Vopt, optimal force and velocity for Pmax, respectively. From Jaric, S 2015.*

Velocity at null force or maximal unloaded velocity (V_0) is attained during maximal unloaded contractions where the requirement for the strongly bound high-force state of the cross-bridge is low and the overall cycle rate is maximal. V_0 corresponds to the intercept with the velocity axis in the force-velocity relationship (figure 30) and can be determined from the extrapolation of the force–velocity relationship to zero load (Hill 1938). V_0 appears to be highly correlated with the actomyosin ATP hydrolysis rate (Bárány 1967) and limited by the ADP dissociation step (Fitts 2008). In rat skeletal muscle, fiber V_0 has been shown to be higher in fast glycolytic fibers than in slow oxidative fibers. This difference has been attributed to higher ATPase (the enzyme that catalyzes the decomposition of ATP into ADP) activity in the fast glycolytic myosin isozymes (Fitts, McDonald et al. 1991).

Maximal force at theoretical null velocity (F_0) is attained during maximal isometric contractions, where the velocity is null. Contrary to V_0, F_0 is measurable, and it corresponds to the intercept with the force axis in the force-velocity relationship (figure 30). Reductions in force have been related to decreases in Ca^{2+} sensitivity and release and with the precipitation of Ca^{2+} with Pi inside the SR (Martyn and Gordon 1992, Westerblad and Allen 1996).

As body movement is dependent on the capacity to generate power, muscle fatigue may be better related to changes in this neuromuscular property. The greatest values for fibers, isolated muscles and monoarticular movements are obtained at contraction velocities and loads of 20 to 40% of V_0 and F_0, respectively (Duchateau and Hainaut 1984) (figure 29). In contrast, when there is a linear relationship (figure 30), the greatest values for power (P_{max}) are found at 50% V_0 and F_0 (figure 30).

$$P_{max} = (F_0 V_0)/4 \qquad \text{Eq 10}$$

Since power is the product of force and velocity, higher values will be found in type II muscle fibers than in type I muscle fibers (Fitts 2008). In addition, reductions in P_{max} are mostly explained by decreases in F_0; V_0 is a late event in the development of fatigue and plays a relatively small role in the loss of power (Jones, De Ruiter et al. 2006).

8 Purpose of the thesis

In view of the previous discussion, it appears that for young and healthy subjects, the O_2 supply deficit is not a preponderant factor in the development of the $\dot{V}O_2sc$, which is why "prior" exercise does not have any effect on τ or the final value of $\dot{V}O_2$ in this population.

On the other hand, regarding the paradigms of muscular fatigue or the differences in metabolic kinetics between slow and fast fibers as the trigger(s) of the slow component, the results remain contradictory.

Therefore, the aim of this thesis was to clarify and nourish the debate of the causes of the $\dot{V}O_{2SC}$, especially for these last two paradigms. Our interest was in deciphering whether fatigue or the different metabolic response profiles of different fiber-type populations are the real culprit, or the trigger, for the appearance and development of the $\dot{V}O_2sc$.

Different experiments were conducted to shed light on that question. Since the $\dot{V}O_2sc$ occurs during high-intensity exercise and is linked with changes in metabolic concentrations that may produce alterations in neuromuscular properties, the latter might be considered the mediator of the former. Neuromuscular function evaluation, such as peripheral nerve stimulation, has been extensively used to explore the complex relationship between exercise and fatigue.

Therefore, the aim of the first experiment (chapter 9) was to quantify the alteration of neuromuscular properties of knee extensors during heavy exercise and to see whether these impairments covary, as a function of time, with $\dot{V}O_{2SC}$ amplitude.

The hypothesis was that $\dot{V}O_2sc$ amplitude would correlate with the change in neuromuscular properties of knee extensor muscles, depicted by a decrease in evoked Pt.

The second experiment (chapter 10) was conducted to explore whether exercise intensity was the trigger of the relationship between fatigue and the $\dot{V}O_2sc$. The apparent discrepancies regarding the involvement of fatigue in the development of the $\dot{V}O_2sc$ may also be related to other causes, such as the measurement according to the stimulation methods (Jones, Bigland-Ritchie et al. 1979), according to the protocol used (isometric vs. dynamic (Krüger, Aboodarda et al. 2019)), or the time of data acquisition (during exercise or with or without delay after exercise (Froyd, Millet et al. 2013)). Thus, this original experiment aimed to measure muscular fatigue before, during and after 10 min of constant KE exercises

at different intensities. In addition, to reveal the time course and nature of fatigue and the relationship with the development of the $\dot{V}O_{2SC,}$ neuromuscular function was evaluated in a static and dynamic way before, after and during the course of these exercises.

With this strategy, the goal was to answer two questions. First, what are the time course and the nature of the changes in fatigue that develop during moderate, heavy and severe bouts of dynamic exercise? Second, what is the concordance between muscle fatigue and the development of the $\dot{V}O_{2SC}$ during these different exercise intensities?

Finally, the last experiment (chapter 11) was carried out to clarify and add more evidence regarding whether $\dot{V}O_2$sc development was related to fatigue processes or was genuinely the product of the differences in metabolic properties of different muscle fibers. The purpose was to be able to manipulate MU recruitment by applying the work-to-work protocol (DiMenna, Wilkerson et al. 2008) to discern which kind of muscle fibers were recruited under each type of exercise intensity. In addition, by combining the Henneman and the superposition principles, a new curve, formed with three different intensities, was constructed to ascertain whether it was similar to a final severe-intensity kinetic curve. The reconstructed $\dot{V}O_2$ kinetics curve from multiple transitions was hypothesized to have identical kinetics to a simple transition at the same final intensity.

9 Article I : Alterations to neuromuscular properties of skeletal muscle are temporally dissociated from the oxygen uptake slow component

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ABSTRACT

To assess if the alteration of neuromuscular properties of knee extensors muscles during heavy exercise co-vary with the $\dot{V}O_2sc$ ($\dot{V}O_2$ slow component), eleven healthy male participants completed an incremental ramp test to exhaustion and five constant heavy intensity cycling bouts of 2, 6, 10, 20 and 30minutes. Neuromuscular testing of the knee extensor muscles were completed before and after exercise. Results showed a significant decline in maximal voluntary contraction (MVC) torque only after 30 minutes of exercise (-17.01%±13.09%; $p<0.05$) while single twitch (PT), 10 Hz (P10), and 100 Hz (P100) doublet peak torque amplitudes were reduced after 20 and 30 minutes ($p<0.05$). Voluntary activation (VA) and M-wave were not affected by exercise, but significant correlation was found between the $\dot{V}O_2sc$ and PT, MVC, VA, P10, P100, and P10/P100 ratio, respectively ($p<0.015$). Therefore, because the development of the $\dot{V}O_2sc$ occurred mainly between 2-10 minutes, during which neuromuscular properties were relatively stable, and because PT, P10 and P100 were significantly reduced only after 20-30 minutes of exercise while $\dot{V}O_2sc$ is stable, a temporal relationship between them does not appear to exist. These results suggest that the development of fatigue due to alterations of neuromuscular properties is not an essential requirement to elicit the $\dot{V}O_2sc$.

9.1 Introduction

At the onset of constant power exercise, the muscles requirements for ATP re-synthesis increase immediately following exercise onset. The same cannot be said about the oxygen uptake ($\dot{V}O_2$) response that instead, displays a sluggishness to fully activate metabolism(Grassi, Pogliaghi et al. 2003, Jones and Poole 2005, Hughson 2009). During exercise below the lactate threshold, $\dot{V}O_2$ rises mono-exponentially to a new steady-state (Xu and Rhodes 1999, Jones and Poole 2005) and from unload pedalling, the rise of $\dot{V}O_2$ increases as a linear function of work-rate(Hansen, Sue et al. 1987) . However, during constant-load exercise completed at intensities above the lactate threshold, the $\dot{V}O_2$ response becomes more complex with a second rise in $\dot{V}O_2$, developing slowly, which is superimposed onto the initial $\dot{V}O_2$ response (Whipp and Wasserman 1972). This slowly developing rise in $\dot{V}O_2$, termed the slow component of $\dot{V}O_2$ ($\dot{V}O_2sc$), results in a greater end-exercise $\dot{V}O_2$ than that predicted by the sub-LT$\dot{V}O_2$-power output relationship. It has been proposed that the inefficiency which leads to the $\dot{V}O_2sc$ originates primarily from the active muscles (Poole, Schaffartzik et al. 1991). However, the reason for this observed inefficiency in the muscle is not clear and may potentially result from reduction of ATP production per mole of oxygen (P/O ratio), diminution of the energy yield per unit of hydrolysed ATP, alteration of neuromuscular properties of muscle filament to produce force, and/or deterioration of the motor pattern of the motion (Allen, Lamb et al. 2008). However, the potential link between the alteration of neuromuscular properties of muscle filament and progressive muscle inefficiency, and therefore the $\dot{V}O_2sc$, is not well explored. The capability of muscle to produce force progressively declines during high-intensity exercise when fatigue gradually develops (Keir, Copithorne et al. 2016). It is widely accepted that alterations of the metabolic milieu of locomotor muscles are mainly responsible for the decline in force. Indeed, neuromuscular properties of knee extensor muscles are sensitive to the accumulation of muscle metabolites such as adenosine diphosphate (ADP), inorganic phosphate (P_i), hydrogen ion (H^+), and magnesium ion (Mg^{2+}). Muscular force production is reduced by increases in [P_i], [Mg^{2+}], and [H^+] while augmented by an increase in [ADP] (Ament and Verkerke 2009). Additionally, increased [ADP] reduces cross-bridge cycling rate (Ament and Verkerke 2009).

Since the $\dot{V}O_2sc$ occurs during high-intensity exercise, and because high-intensity exercise is always associated with changes in metabolite concentration that may produce an alteration in neuromuscular properties of muscle filament, the latter may be considered a putative mediator of the $\dot{V}O_2sc$ (in line with current views;(Rossiter 2011, Poole and Jones 2012, Cannon, Bimson et al. 2014, Grassi, Rossiter et al. 2015)). Standardised investigative methods of neuromuscular function, such as peripheral nerve stimulation (PNS), have been extensively used to explore the complex relationship between exercise and fatigue. For instance, using PNS, Decorte and colleagues showed that during exhaustive constant-load cycling at 80% of maximum aerobic power output, neuromuscular properties were significantly reduced as early as 20% of the total duration of cycling, indicating a potential link with the $\dot{V}O_2sc$ (Decorte, Lafaix et al. 2012).

Although, little is known about the possible relationship between the $\dot{V}O_2sc$ and the alteration of neuromuscular properties of knee extensors, Keir and colleagues(Keir, Benson et al. 2016) in 2016 showed a significant association between the decrements in muscle torque and the $\dot{V}O_2sc$, without changes in muscle activation over the course of the exercise. Also in an in vivo study using cycle ergometry, Cannon and colleagues have shown that changes in velocity-specific peak power generated in the initial minutes of exercise were correlated to the $\dot{V}O_2sc$ measured between three and eight minutes of heavy and severe exercise (Cannon, White et al. 2011). Results from the same working group suggest that the $\dot{V}O_2sc$ during heavy exercise arises from both contractile and mitochondrial sources(Bowen, Cannon et al. 2012). Furthermore, using self-paced dynamic concentric extension/flexion of the knee and interleaving voluntary and electrically evoked contractions, Froyd and colleagues have shown, even without measuring directly VO_2 kinetics, that fatigue progresses with similar dynamics to those expected of the $\dot{V}O_2sc$ during an approximately 6-min time trial (Froyd, Millet et al. 2013). However, these findings do not show the mechanism linking the alteration of neuromuscular properties of knee extensors, per se, and the $\dot{V}O_2sc$.

The aim of the present study was to quantify the alteration of neuromuscular properties of knee extensors during heavy exercise and to see if these impairments co-vary, as function of time, with the $\dot{V}O_2sc$

amplitude. The hypothesis was that the $\dot{V}O_2$sc amplitude correlates with the change in neuromuscular properties of knee extensor muscles, depicted by a decrease in evoked peak torque.

9.2 Methods

Participants

Eleven healthy, recreationally active, male participants (mean ± SD, age 27 ± 6.6 years, body mass 76 ± 7.6 kg, and height 179 ± 8.1 cm) were recruited for this study. The participants were provided with a participant information sheet outlining the procedures involved, time commitment, and requirements of the study. Participants were screened using a self-administrated pre-exercise health questionnaire designed to identify those who may be at risk of an adverse event during exercise. Participants were advised of their right to withdraw from the study at any time without disadvantage.

Participants were asked to avoid, in the 24h preceding a testing session, strenuous physical activity, alcohol, tobacco, and caffeine. Furthermore, participants were asked not to consume any food for the 3h preceding a test and to arrive fully hydrated. All tests were completed at a similar time of day (± 1h). The study was approved by the local humans Ethics Committee and conformed to the latest revision (2013) of the Declaration of Helsinki. All participants provided written informed consent prior to participation.

Experimental Design

This study involved each participant attending six separate laboratory sessions, with at least a 48h interval between tests, over a three-week period. All tests were completed in an air-conditioned (21ºC ± 1ºC) exercise physiology laboratory. The first session involved an incremental ramp test on a cycle ergometer (Velotron, RacerMate, Seattle, WA, USA). This test was used to assign a work-rate for the subsequent five experimental sessions during which constant work-rate exercise was completed. Following the incremental ramp test, participants were familiarized with the procedure to be used to evaluate neuromuscular function. The five experimental sessions (Figure 31A) involved participants cycling for different durations of time in a random order at an identical power (heavy domain, see below). Neuromuscular evaluation was performed before exercise, and within 1-minute of completing

constant work-rate exercise. This was completed to determine the central and peripheral fatigue through neural and neuromuscular properties of the knee extensor muscles.

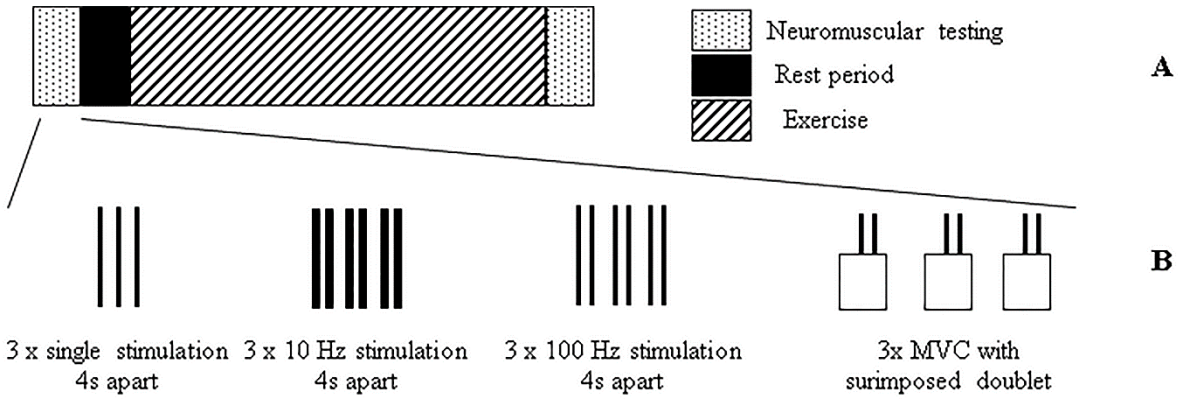

Figure 31 Description of events completed during experimental testing (Figure A) and during neuromuscular testing (Figure B). *Neuromuscular tests (dotted box) were completed prior to the rest period (filled box) and after exercise (box with diagonal lines). Neuromuscular testing involved three single stimulations (single solid lines) followed by three stimulations at 10 Hz (thick-double solid lines) and then three stimulations at 100 Hz (thin-double solid lines). Each stimulation had a four second separation. Finally, three MVCs were completed with superimposed 100 Hz doublets applied (empty box with thin-double solid lines), each separated by a minute rest period.*

Testing procedures

Incremental ramp test

Incremental ramp exercise test was completed in order to determine the gas exchange threshold (GET) and peak oxygen consumption ($\dot{V}O_{2peak}$). After a three-minute rest period seated on the cycle ergometer, participants performed six minutes of baseline cycling at 60 watts, after which, the work rate was increased by the rate of 30 watts each minute until reaching the limit of tolerance. The ergometer allows participants to cycle at a constant power output independent of pedal rate, though participants were asked to maintain a pedal rate of 85 revolutions per minute (rpm). Verbal encouragement was provided throughout the test. The test was terminated when the pedal rate dropped by more than 10 rpm (i.e. 75 rpm). All cycle tests were completed on an electromagnetically braked cycle ergometer where the seat and handlebars were fully adjustable both vertically and horizontally. The horizontal and vertical direction of both the seat and handlebars were adjusted to suit each participant and were recorded following the ramp test and replicated for subsequent tests. Pulmonary gas exchange and ventilation were measured from the beginning of the rest period until cessation of the test.

Step transition tests

Each participant attended a total of five experimental sessions during which cycling at a constant-load were completed. The test began with a 5-minute rest period before participants completed three minutes of unloaded cycling (20 watts). At the end of the three minutes, an immediate transition to the work rate equal to 30%Δ (GET plus 30% of the difference between the work rate at the GET and $\dot{V}O_{2peak}$; heavy exercise) was imposed with the duration altered at each session (2, 6, 10, 20, 30 minutes). Constant power was maintained at 85 rpm and was maintained for the duration specified for each of the tests.

Neuromuscular Evaluation

Neuromuscular evaluation (Figure 31B) consisted of (1) 3 x single supra maximal electrical stimulations, each separated by four seconds, (2) 3 x paired at 10 Hz (two stimulation pulses separated by 100 ms) and 3 x paired at 100 Hz (two stimulation pulses separated by 10 ms) electrical stimulations, each separated by four seconds, and (3) 3 x five-second isometric maximal voluntary contraction (MVC) tests of the knee extensor muscles during which a 100 Hz doublet was superimposed to the MVC. A one-minute rest period separated each MVC. Strong, standardised, verbal encouragement was provided throughout the MVC. In order to increase the contact between the electrode and the skin during all electrical stimulations, a pressure was applied to the cathode electrode using a wooden handle with a rubber end. Note that during post exercise, each sequence was repeated only one time in order to diminish the possible effect of recovery time. Less than one minute was required to position the participant for testing after exercise.

Measurements

Pulmonary gas exchange

During all tests, pulmonary gas exchange was continuously measured using a computerised system (MetaMax 3b, Cortex, Leipzig, Germany). The system used an infrared sensor and an electrochemical cell to measure fractional concentrations of CO_2 and O_2 in expired gas. A digital transducer turbine assessed inspired and expired gas volume. A capillary line was used to continuously sample gas concentration. The transducer and the capillary line were securely attached to the facemask, which was

firmly fitted to the participants face using Velcro straps. Immediately before each exercise test, the gas analysers were calibrated with gases of known concentration (O_2=14.01 %, CO_2 =6.03%), and the turbine volume transducer was calibrated using a three-litre Rudolph syringe (Cortex, Leipzig, Germany).

$\dot{V}O_{2peak}$ was noted as the highest 30-second average value attained during the incremental ramp test. The GET was determined using a number of measurements: (1) visual examination for the first disproportionate increase in CO_2 production ($\dot{V}CO_2$) from $\dot{V}CO_2$ versus $\dot{V}O_2$ graph, (2) an increase in ventilatory equivalent of oxygen ($\dot{V}_E/\ \dot{V}O_2$) without increase in ventilatory equivalent of carbon dioxide ($\dot{V}_E/\ \dot{V}CO_2$), and (3) increase in partial pressure of end-tidal oxygen with no decrease in partial pressure of end-tidal carbon dioxide. Subsequently, the work rate that would require 30%Δ was calculated and assigned for the experimental tests after accounting for the mean response time for $\dot{V}O_2$ during ramp exercise (2/3 of the ramp rate was subtracted from the work rate at the gas exchange threshold and $\dot{V}O_{2peak}$, i.e. 20 watts) (Whipp, Davis et al. 1981, Bailey, Romer et al. 2010).

PNS

Electrical stimulation was delivered using a high-voltage stimulator (model DS7, Digitimer Stimulator, Hertfordshire, UK). Low intensity stimulation (~ 20mA) was used to locate the femoral nerve by means of a cathode ball electrode (0.5 cm diameter) which was manually pressed into the femoral triangle and maneuvered until the femoral nerve was properly located (determined by observing contraction of the leg). A 5cm diameter cathode electrode (American Imex, CA, USA) was then placed on the site after it was cleaned with an alcohol wipe. The anode, a rectangular electrode (18 x 7 cm, American Imex, CA, USA), was placed opposite the cathode in the gluteal fold. Both the cathode and anode electrodes were worn during exercise and therefore both were taped to the skin using micropore tape (3M Micropore, St. Paul, MN, USA) to limit movement. To determine maximal stimulation, single electrical stimulations (rectangular pulse, 1ms duration, 400 V) were delivered to the nerve and progressively increased until a plateau in the twitch torque and M-wave amplitude were achieved. The current that achieved plateau was increased by 20%, which was then used for subsequent tests.

Torque measurement

The evaluation of neuromuscular function was conducted on the right knee extensor muscles with participants seated in a Biodex isokinetic dynamometer (Biodex Medical Systems Inc., Shirley, NY, USA). The hip and knee angles were fixed at 90º (0º = full knee extension) with the ankle strapped to the lever arm of the Biodex. The rotational axis of the dynamometer was aligned with the lateral epicondyle of the femur, found after palpation. Two crossover straps were placed firmly across the shoulders to limit upper body movement and one strap was placed midway across the thigh of the right leg. Participants were asked to cross their arms across their chest during testing. Adjustments made to the seat position and to the lever arm of the Biodex were recorded for each participant during familiarisation and reproduced for subsequent tests.

Electromyography Recordings

Once participants were seated, the right vastus medialis (VM) and vastus lateralis (VL) muscles were palpated and prepared for electromyogram signal (EMG) recording. To reduce impedance, the skin around the belly of the muscles was shaven, lightly abraded (3M Red Dot Trace Prep, Ontario, Canada) and cleaned using 70% isopropyl alcohol wipes (Kendall Company, Mansfield, MA, USA). One pair of silver-chloride electrodes (3M Red Dot, St. Paul, MN, USA) of 10 mm diameter with an interelectrode (center to center) distance of 2 cm were then placed lengthwise over the prepared muscle. The ground electrode was placed over the patella of the right leg. EMG and torque signals were recorded through chart software (v. 5.5.6, ADInstruments, Sydney, Australia). EMG signals were amplified with a bandwidth frequency ranging from 1.5 Hz to 2 kHz (common mode rejection = 90 dB; impedance = 100 MΩ; gain = 1000). The myoelectric and mechanical responses were digitised on-line at a sampling frequency of 2000 Hz and stored for off-line analysis.

Data Analysis

Oxygen uptake kinetic analysis

The breath-by-breath $\dot{V}O_2$ data from each of the 30%Δ tests were initially examined to exclude errant breaths caused by coughing, swallowing, sighing, etc., and those values lying more than three standard

deviations from the model $\dot{V}O_2$ were considered outliers and were removed. The breath-by-breath data from the different exercise durations were subsequently linearly interpolated to provide second-by-second values, and, for each individual, repetitions from different durations were time aligned to the start of exercise and the ensemble averaged.

The primary component (phase 2) kinetics were isolated to identify the mono-exponential region and modelled by the following equation:

$$\dot{V}O_2(t) = \dot{V}O_{2b} + A_p \cdot (1 - exp^{\left(-\frac{t-td_p}{\tau_p}\right)}) \cdot U \qquad \text{Eq (1)}$$

Where $\dot{V}O_2(t)$ represents $\dot{V}O_2$ at a given time t; U= 0 for $t < td_1$ and U = 1 for $t \geq td_1$

$\dot{V}O_{2b}$ is the $\dot{V}O_2$ during unloaded cycling defined as the mean $\dot{V}O_2$ measured over the final 90 seconds of baseline pedaling; A_P is the asymptotic amplitudes for the primary phases; τ_P is the time constant, and td_P represents the time delay. Since the focus of this study was the $\dot{V}O_2sc$, the cardiodynamic phase was removed from analysis (Weissman, Jones et al. 1982, Paterson and Whipp 1991), and therefore, not modelled, in order to ensure that the early initial component did not influence the results (Whipp, Ward et al. 1982). Initially, the fitting window extended from 20 seconds (i.e., at the end of phase I) to 80 seconds (only 60 s into the exercise). The window was lengthened iteratively in order to attain four series of the initial window length. For each window length, the parameters of the model were determined with an iterative procedure by minimising the sum of the mean squares of the differences between the model $\dot{V}O_2$ and actual $\dot{V}O_2$.

Identification of the end of the primary phase was completed using H.B. Rossiter criteria consideration (Rossiter, Ward et al. 2001, Murgatroyd, Ferguson et al. 2011).

As such, the amplitude of the slow component at time 2, 6, 10, 20, and 30 minutes were assigned the value (A_{SX}) and were defined as the difference between the value of $\dot{V}O_2$ at a given time and the sum of the primary phase and the $\dot{V}O_{2b}$ at the same given time.

$\dot{V}O_2sc$ was also described as a percentage of the primary component ($\dot{V}O_2sc$ %) since this ratio would provide information regarding the loss of efficiency.

Neuromuscular Function Analysis

From the EMG trace of single stimulations, peak-to-peak amplitude (M-waves) of the VL (MWVL) and VM (MWVM) were measured. Peak torque (PT) was determined from the torque signal of the single twitch. The highest torque achieved during MVC in their respective conditions were taken as the MVC torque. The PT of doublet stimulations were quantified and termed P10 and P100 for 10 Hz, and 100 Hz, respectively. In addition, the P10-to-P100 ratio (P10/P100) was calculated to assess for the occurrence of low or high frequency fatigue.

The voluntary activation (VA) level was calculated by expressing any increment in torque evoked during maximal isometric contractions (superimposed twitch) as a fraction of the amplitude of the response evoked by the potentiated doublet (Merton 1954).

In agreement with the work by Strojnick and colleagues, the following correction factor (CF, the ratio between the torque just before the superimposed doublet divided by MVC peak torque) was used in order to take into account the possibility that the superimposed twitch was not necessarily applied when the torque level was at the true maximal voluntary force (Strojnik and Komi 1998).

$$VA = 100 - \left[CF \cdot \left(\frac{superimposed\ doublet}{potentiated\ doublet}\right)\right] \cdot 100 \qquad \text{Eq (2)}$$

All data presented are the average of three measurements in pre, and a single measurement on post.

Data and Statistical Analysis

Data were normalised by expressing the measures taken immediately after exercise as a percentage change relative to before exercise. This was completed to avoid day-to-day variations in measures that may occur. Normality test (Kolmogorov-Smirnov) and F-test of equality of variances were completed to test for normal distribution and equality of variance. . One-way repeated measures analysis of variance (ANOVA) was used to test the effect of exercise duration on measurers of neuromuscular function. When a significant main effect was found, significant differences were located using Tukey's post hoc analysis test. Pearson correlation coefficient was used to assess relationships between the change of $\dot{V}O_2$sc % and changes to neuromuscular parameters. Analyses were completed with Box and Tidwell

tests, and the Theil method (Theil nonparametric regression technique). The Box and Tidwell test assesses whether the association between the slow component and fatigue is linear or not, and therefore related to time. In contrast, Theil's regression highlights, in a qualitative way, the points that are distant from the linear relationship. For all tests, significance was set at $p < 0.05$. Data are expressed as mean ± SD.

9.1 Results

Oxygen Uptake Kinetics

Mean $\dot{V}O_{2peak}$ was 3.95 ± 0.18 $l \cdot min^{-1}$ and the mean power output corresponding to 30%Δ was 200 ± 11 watts. During the three minutes of unloaded pedalling at 85 rpm, $\dot{V}O_{2b}$ reached a value of 0.85 ± 0.19 $l \cdot min^{-1}$. Asymptotic amplitudes of the primary phase attained 1.85 ± 0.38 $l \cdot min^{-1}$ with a time constant of 27.1 ± 15.0 s and a time delay of 12.8 ± 2.3 s. Amplitude of the slow component at time 2, 6, 10, 20, and 30 minutes are presented in Table 1. Values of $\dot{V}O_2sc$ as a percentage of the primary component are also described.

Table 1 Time course of slow component amplitude in absolute, and in percentage of the primary component. *AS_2, AS_6, AS_{10}, AS_{20}, and AS_{30} are the amplitude of the slow component at time 2, 6, 10, 20, 30 min respectively. Ap is the amplitude of primary component. Data are presented as mean ± SD*

	Amplitude ($l \cdot min^{-1}$)	Amplitude (% of Ap)
AS_2	0.037 ± 0.056	1.9 ± 2.5
AS_6	0.298 ± 0.130	16.6 ± 8.6
AS_{10}	0.373 ± 0.150	20.9 ± 10.2
AS_{20}	0.450 ± 0.202	25.3 ± 13.2
AS_{30}	0.515 ± 0.246	29.1 ± 16.3

Neuromuscular function

MVC measurement showed alteration over the course of exercise (Table 2). Post-hoc test revealed a significant reduction after 30 minutes of cycling compared to before exercise, 2, 6, and 10 minutes of exercise. After 20 minutes of exercise, a trend towards significance was observed compared to before exercise ($p = 0.1$) No effect of exercise duration was detected for VA (Table 2; $p > 0.05$).

No effect of exercise duration was detected for the M-wave amplitude of the VM and VL muscles (Table 2).

Table 2 Changes in neuromuscular function over the time course of the slow component. *MVC: maximal voluntary contraction, VA: voluntary activation, MWVM: M-wave amplitude of vastus medialis, and MWVL: M-wave amplitude of vastus lateralis. * Different from base line, 2min, 6 min, and 10 min ($p < 0.05$). Data are presented as mean ± SD*

	2 min	6 min	10 min	20 min	30 min
MVC [%]	-2.81 ± 5.39	-2.32 ± 4.19	-2.87 ± 5.31	-9.26 ± 9.67	-17.01 ± 13.09*
VA [%]	-1.81 ± 3.14	0.18 ± 3.90	-1.59 ± 3.4	-2.72 ± 3.92	-4.32 ± 5.66
MWVM [%]	-1.17 ± 5.72	-2.86 ± 8.28	-0.52 ± 8.50	-4.21 ± 8.05	-8.23 ± 7.59
MWVL [%]	4.07 ± 10.33	6.91 ± 6.39	5.07 ± 8.43	8.32 ± 15.07	6.9 ± 28.92

Twitch amplitude (Figure 32A) showed a significant reduction at 30 minutes of exercise compared to before, 2, 6, and 10 minutes of exercise; ($p < 0.05$). A significant reduction was also observed after 20 minutes of exercise compared to before, 2, and 6 minutes of exercise ($P < 0.05$). Finally, significant differences were observed for 10 minutes of exercise compared to 2 and 6 minutes of exercise ($p < 0.05$). P10 (Figure 32B) and P100 (Figure 32C) evolved in a similar manner. Specifically, significant differences were observed for 20 and 30 minutes compared to before, 2, 6, and 10 minutes of exercise ($p < 0.05$). Furthermore, significant differences were observed at 10 minutes compared to 2 and 6 minutes ($p < 0.05$).

Significant differences for the P10/P100 ratio (Figure 32D) were found for most exercise durations. A significantly lower P10/P100 ratio was observed at 30 minutes compared to before, 2, 6, and 10 minutes of exercise ($P < 0.05$). After 20 minutes of exercise, differences were observed compared to before, 2,

and 6 minutes of exercise ($p < 0.05$). Furthermore, significant differences were observed at 10 minutes compared to before and 2 minutes of exercise ($p < 0.05$).

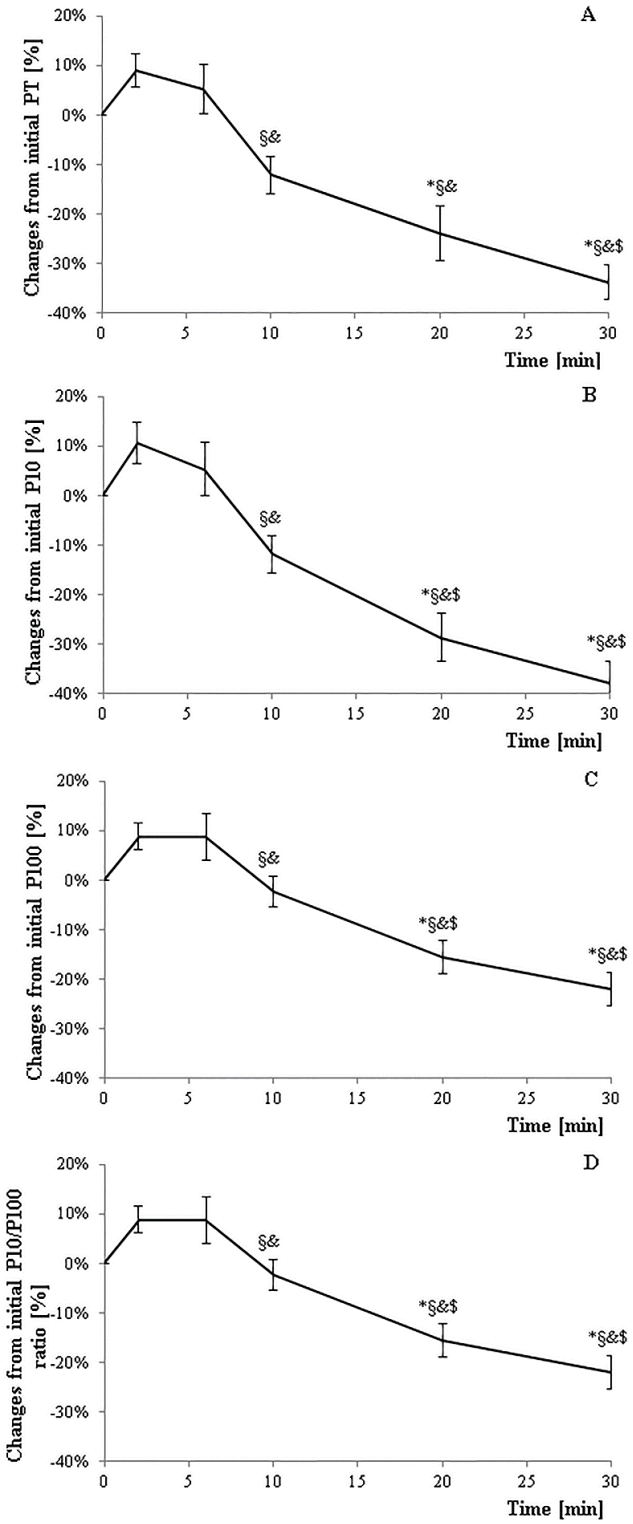

Figure 32 Neuromuscular alterations for peak twitch amplitude (Figure 2A), 10 Hz paired (P10) stimulation (Figure 2B), 100 Hz paired (P100) stimulation (Figure 2C), and P10/P100 (Figure 2D) over the course of exercise*. * Significant difference from baseline ($p < 0.05$); § Significant difference from 2 minutes ($p < 0.05$). & Significant difference from 6 minutes ($p < 0.05$). $ Significant difference from 10 minutes ($p < 0.05$). Error bars are SE.*

The $\dot{V}O_2$sc and Fatigue

Correlation analysis was used to investigate relationships between the $\dot{V}O_2$sc % and neuromuscular parameters (Table 3). Changes in M-wave amplitude for either of VL and VM, VA and MVC did not correlate with changes of the $\dot{V}O_2$sc relative to the primary phase. However, significant correlations were found between the $\dot{V}O_2$sc % and PT, P10, P100 (tendency), and P10/P100 ratio. For these neuromuscular parameters, P10/P100 showed the strongest correlation with $\dot{V}O_2$sc % (R^2 = 0.88), followed by P10 (R^2 = 0.81), PT (R^2 = 0.81), and P100 ratio (R^2 = 0.72). In contrast, the Box and Tidwell's test was smaller than 0.05 (see Table 3) for correlation relationships suggesting that the relationship is non-linear and therefore unrelated over time. In addition, Theil's line (see figure 32) showed that during the first phase, only the slow component grew (the points of this phase are distant from Theil's line); while during the second phase, the slow component continued to grow but fatigue also grew (the points of this phase then line up with Theil's line).

Table 3 Correlation coefficient between the slow component amplitude, as a percentage of the primary phase, and neuromuscular function. *MWVM: M-wave amplitude of vastus medialis, MWVL: M-wave amplitude of vastus lateralis, PT: Peak Torque of the single twitch, MVC: maximal voluntary contraction, VA: voluntary activation, P10: peak torque at 10Hz doublet stimulation, P100: peak torque at 100Hz doublet stimulation, P10/P100: ratio of peak torque between 10hz and 100hz doublet stimulation, R: correlation coefficient, p: significance.*

	Correlation		Box-Tidwell test	
	R	P	Z	P
MWVM	-0.69	0.196	-1.70	0.089
MWVL	0.76	0.137	-0.20	0.841
PT	-0.90	0.038	-3.06	0.002
MVC	-0.72	0.172	-5.57	<0.001
VA	-0.52	0.370	-5.48	<0.001
P10	-0.90	0.036	-3.39	<0.001
P100	-0.85	0.065	-3.97	<0.001
P10/P100	-0.94	0.019	-4.50	<0.001

9.2 Discussion

The main finding from the present study was that alterations to force production by the knee extensor muscles were present during exercise at an intensity of 30%Δ, which correlated with the development of the $\dot{V}O_2sc$, however, a temporal relationship between the development of the $\dot{V}O_2sc$ and fatigue does not appear to exist.

Origin of fatigue observed during exercise

With neuromuscular fatigue defined as a reduction in force generating capacity (Gandevia 2001), loss of MVC torque is used as a general index for evaluating the extent of neuromuscular fatigue. In the present study, MVC torque, compared to the beginning of exercise, was found to be significantly reduced only after 30 minutes of cycling at 30%Δ. However, while loss of MVC torque is a general index of fatigue, it does not provide information regarding the site of alterations (i.e. neuromuscular fatigue etiology). To determine the origin of the neuromuscular fatigue caused by various durations of cycling at 30%Δ, electrical stimulations were delivered at rest, as well as during MVC, allowing for the evaluation of VA, action potential transmission and propagation, and neuromuscular properties. VA, which is commonly used to evaluate central fatigue (Vollestad 1997), was not significantly affected by any exercise durations in the present study. The absence of significant central fatigue suggests that declines in motivation, afferent feedback, or central drive were not present, or that declines in central drive was countered by increased motivation(Bigland-Ritchie, Dawson et al. 1986, Ament and Verkerke 2009). It subsequently suggests a peripheral origin for the induced neuromuscular fatigue. Muscle membrane excitability and neuromuscular propagation appeared to be well preserved, as highlighted by the lack of alterations in VL and VM M-wave amplitudes. In contrast, reductions in evoked forces suggests the presence of peripheral fatigue. Interestingly, signs of peripheral fatigue were observed following shorter exercise durations, suggesting that evoked forces might be more sensitive than MVC for detecting fatigue when it is of peripheral origin. Indeed, PT, P10 and P100 were already reduced after 20 minutes of cycling compared to the beginning of exercise. As M-wave amplitudes were unaltered at all-time points, reductions in evoked forces can highlight either alterations in sarcoplasmic reticulum Ca^{2+} handling (Allen, Lamb et al. 2008) or alterations occurring at the cross-bridge level such

as reduced myofibrillar Ca^{2+} sensitivity, and/or reduced capacity for cross-bridge to produce force (Metzger and Moss 1990, Place, Yamada et al. 2010). Further supporting excitation-contraction failure, the P10/P100 ratio was found to be reduced following 10, 20, and 30 minutes of exercise compared to the start of exercise suggesting the presence of low frequency fatigue (Jones 1996). A study completed on rat *gastrocnemius* muscle ascribed low-frequency fatigue to Ca^{2+} handling alterations rather than to processes occurring at the cross-bridge level (Watanabe, Kanzaki et al. 2015). Indeed, altered Ca^{2+} handling is believed to occur with P_i accumulation during the development of fatigue and its subsequent precipitation with Ca^{2+} within the sarcoplasmic reticulum (Westerblad and Allen 1996). However, the exact mechanisms responsible for low-frequency fatigue remain unclear as previous results, also obtained using rodents, showed that the site (i.e. Ca^{2+} handling *vs.* cross-bridge level) responsible for this low-frequency fatigue is dependent on the antioxidant status of the individual (Bruton, Place et al. 2008). Therefore, based on the measures in the present study, it is likely that the observed neuromuscular fatigue following 20 and 30 minutes of cycling at 30%Δ is a result of peripheral rather than central fatigue. Based on the literature, while speculative, it suggests that fatigue it is from either impaired Ca handling or reduced cross-bridge kinetics. (Metzger and Moss 1990, Place, Yamada et al. 2010).

The $\dot{V}O_2$sc and Fatigue

Significant correlations were found between the $\dot{V}O_2$sc % and PT, P10, P100, and P10/P100. This finding is supportive of the theory regarding the presence of fatigue required to elicit the $\dot{V}O_2$sc. In contrast, for these parameters, the Box and Tidwell's test showed that the relationship between the development of the $\dot{V}O_2$sc and the alterations of the neuromuscular properties of knee extensor muscles were non-linear and therefore unrelated over time. In addition, Theil's line (see figure 33) showed two distinct phases; the first phase where only the slow component grew (the points of this phase are away from Theil's line); while during the second phase, the slow component continued to grow but fatigue also grew (the points of this phase then line up with Theil's line). (see figure 33). In other words, the development of the $\dot{V}O_2$sc, in fact, occurred mainly between 2-10 minutes during which neuromuscular properties were relatively stable (only a reduction in the P10/100 ratio was observed after 10 minutes of cycling). In contrast, PT, P10 and P100 were significantly reduced only after 20-30 minutes of exercise

compared to baseline values. These results suggest that the development of fatigue due to alterations of neuromuscular properties is not an essential requirement to elicit the $\dot{V}O_2sc$ at least during the first 10 minutes of exercises.

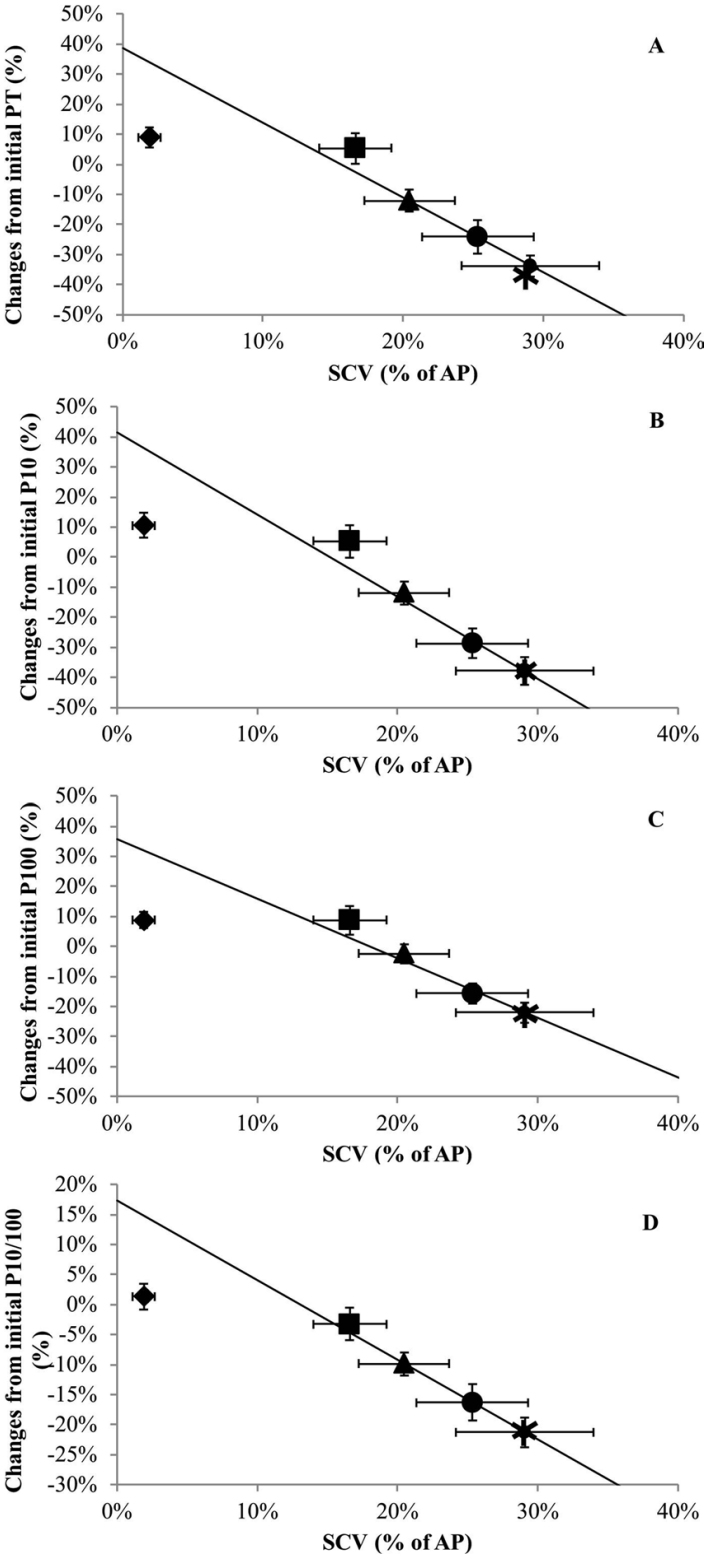

Figure 33 The relationship between peak twitch amplitude (Figure 33A), 10 Hz paired (P10) stimulation (Figure 33B), 100 Hz paired (P100) stimulation (Figure 33C), and P10/P100 (Figure 33D) and the change in of $\dot{V}O_2sc$ ***relative to the primary phase.*** *◆ 2 minutes; ■ 6 minutes; ▲ 10 minutes; ● 20 minutes; and ✱ 30 minutes represent average values. Theil's line is characterised by the dashed line. Error bars are SE. Error bars in the figures are presented as SE for more clarity.*

This finding is in line with those from Thistlethwaite and colleagues (Thistlethwaite, Thompson et al. 2008). They showed that, during heavy cycling exercise, when preceded either by heavy exercise or by heavy knee extensions (requiring twofold greater muscle activation relative to heavy exercise), τ_p, gain of the primary response, and the amplitude of the $\dot{V}O_2sc$ were similar between protocols. The authors concluded that muscle fatigue is not a determining factor for the development of the $\dot{V}O_2sc$. Hopker and colleagues attested similar results. Participants completed either a non-metabolically stressful 100 intermittent drop-jumps protocol (pre-fatigue condition) or rest (control) for 33 minutes. The results of their study showed that locomotor muscle fatigue, tested by the reduction in power in the maximal voluntary cycling power test, was not associated with the development of the of $\dot{V}O_2sc$ (Hopker, Caporaso et al. 2016). Interestingly, the magnitude of the $\dot{V}O_2sc$ was not significantly different between the two conditions despite significant differences in locomotor muscle fatigue. Recently, Dos Nascimento Salvador and colleagues published a study looking at the cause–effect relationship between the $\dot{V}O_2sc$ and fatigue. They switched from constant work rate to isokinetic pedaling to quantify reductions in peak torque at three and eight minutes, with and without priming exercise. Results showed that the $\dot{V}O_2sc$ after priming was reduced but there were no significant differences between conditions regarding the magnitude of the reduction of maximal isokinetic force and power at three and eight minutes (do Nascimento Salvador, Souza et al. 2018). This observation refutes a cause-effect relationship between fatigue and the development of the $\dot{V}O_2sc$.

However, the findings from this study are in contrast with the results by Keir and colleagues. Correlations were shown in both studies between some measures of fatigue and the $\dot{V}O_2sc$, however, a temporal association was only found in one study (Keir, Benson et al. 2016). In one perspective, this difference highlights the importance of exercise intensity. Indeed, in the present study, step transition exercise was in the heavy domain, while the study by Keir and colleagues was in the severe domain (Keir, Benson et al. 2016). In addition, the amplitude and type of fatigue was potentially different, as assessed by the difference in reduction of MVC after 18-20 minutes (9% for the present study vs 22% in the study by Keir and colleagues). If the $\dot{V}O_2sc$ is related to fatigue parameters, it should be present

in both exercise intensity domains. However, this was not the case, which suggests that the $\dot{V}O_2sc$ may not be related to fatigue parameters.

The results in the present study are in agreement with results from a previous study regarding changes to velocity-specific peak power during cycling. Cannon *et al.* (2011) (Cannon, White et al. 2011) observed a reduction in velocity-specific peak power, which correlated with the $\dot{V}O_2sc$. However, as was observed in the present study, the reduction they observed was not temporally related to the development of the $\dot{V}O_2sc$. The reduction in velocity-specific peak power occurred prior to the $\dot{V}O_2sc$ in their study, while excitation-contraction coupling was altered after the development of $\dot{V}O_2sc$ in the present study. Nevertheless, both reported no changes during the development of the $\dot{V}O_2sc$ which suggests that those alterations are likely not essential for the development of the $\dot{V}O_2sc$. If alterations to neuromuscular properties are not involved during the development of the $\dot{V}O_2sc$, at least during exercise in the heavy domain, it may be possible that the $\dot{V}O_2$ cost of force production may increase within a given fiber population. A progressive inhibition of ATP supply by anaerobic glycolysis, an increase in ATP usage per power output, and/or a reduction of ATP production per mole of oxygen (P/O_2 ratio) are probably implicated in the $\dot{V}O_2sc$ (Korzeniewski and Zoladz 2015). However, the documentation of a cause-effect relationship during exercise between muscle fatigue and reduced efficiency remains unknown.

Experimental consideration

As with the study by Keir and colleagues (2016), at the end of exercise, the time to transfer the subject from the ergometer to the Biodex before the start of neuromuscular testing was less than one minute. One could argue that fatigue was already modified, and consequently the interpretation of the data in relation to fatigue during exercise is limited. Simply, fatigue is likely to have been underestimated in the present study and the measurement of fatigue during exercise would have been more appropriate. However, neuromuscular measurements were taken after a similar amount of time after each exercise, for each participant, and consequently, the change of the robustness of the relationship between fatigue and the $\dot{V}O_2sc$ is likely to have been marginal, which should not change the general conclusions of the

present study. Furthermore, the cause (fatigue) has to precede the effect ($\dot{V}O_2sc$); however, the data from the present study indicates that this was not the case. A further limitation is the fact that fatigue was measured during static contractions whereas cycling is a dynamic movement.

9.3 Conclusion

Fatigue in the present study was observed during exercise completed at 30%Δ and which was at least 20 minutes in duration. Indirectly, these results suggest that the observed fatigue appears to be a result of impaired Ca^{2+} handling and/or reduced capability of cross-bridges to produce force. While significant correlations between the $\dot{V}O_2sc$ relative to the primary phase and neuromuscular parameters were found, a temporal relationship between the development of the $\dot{V}O_2sc$ and fatigue does not appear to exist. Therefore, it would seem that the alteration of neuromuscular properties in muscle is not required for the development of the $\dot{V}O_2sc$.

Data availability The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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Authors contributions
FB conceived and designed the experiments. Data collection was completed by FB and TG. FB, TG, SCA, JR and JA analysed, interpreted, revisited, and approved the final version of the manuscript.

Conflict of interest
The authors declare no conflicts of interest. No financial support was received from any organisation.

Ethical Approval
The University of Auckland Human Participants Ethics Committee approved this study. Written informed consent was provided by all participants prior to participation. All procedures conformed to the latest revision (2013) of the Declaration of Helsinki.

10 Article II : The Relationship between the Slow Component of Oxygen Uptake Gain and Changes to the Contractile Properties of the Knee extensors

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ABSTRACT

To better understand fatigue at different exercise intensities and investigate the concordance between fatigue appearance and the development of the oxygen uptake slow component ($\dot{V}O_{2SC}$), eleven recreational active men completed an incremental step test and three knee extension exercises at different intensities. Neuromuscular function was assessed through 100 Hz doublets before, during and after exercise in isometric mode. In Dynamic mode, before and after exercise, the force–velocity and power-velocity relationship was built by increasing the weight to be lifted by 6 kg until it was not possible to lift it anymore.

Results showed no significant differences in voluntary activation between the three exercise intensities. On the contrary, there were significant differences through maximal voluntary contraction (MVC), and potentiated doublet (PDb100) pre to post exercise and between heavy and severe intensities. Additionally significant differences pre to post exercise were found for dynamic variables, estimated force (ΔF_0) and power (ΔP_{max}) in both domains. During the time course of exercise a significant decline in contractile function in severe domain through doublet peak twitch force (Db100) and maximal rate of force development (MRFD) variables were found in isometric exercise. On the contrary, there was no sign of fatigue in heavy domain during the time course of the exercise in Db100 or MRFD and no correlation was found with the development of the $\dot{V}O_{2sc,}$ suggesting no relationship between them. Contrarily, in the severe domain, the alteration of neuromuscular function (i.e. decreases in Db100, and MRFD) was significantly correlated (r = -0.72 and -0.05, respectively) with the development of $\dot{V}O_{2sc}$, in addition, changes in the heavy and severe domains were statistically different for all variables representing contractile properties**.** Therefore, the present results suggest that the $\dot{V}O_{2SC}$ in the heavy and severe domains does not share the same origin.

Key Words:

Muscle fatigue, peripheral nerve stimulation, oxygen consumption, cycling, slow component, intensity domains.

Abbreviations:

GET	Gas exchange threshold
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_{2SC}$	$\dot{V}O_2$ Slow component
P/O ratio	Reduction of ATP production per mole of oxygen
$\dot{V}O_2$peak	Peak oxygen consumption
UCP3	Mitochondrial uncoupling protein3
MOD	Moderate domain
HVY	Heavy domain
SEV	Severe domain
IMVC	Isometric maximal voluntary contraction
$\dot{V}_E$	Minute ventilation
PT	Peak twitch force
$\dot{V}O_2(t)$	Oxygen consumption at a given time *t*
$\dot{V}O_{2rest}$	$\dot{V}O_2$ at rest
A_p	Amplitude of the primary phase
TD_p	Time delay of the primary phase
τ_p	Time constant of the primary phase
τ_s	Time constant of the slow component
A_s	Amplitude of the slow component
TD_s	Time delay of the slow component
A_s'	Real value of the amplitude of the slow component
MRFD	Maximal rate of force development
MRFR	Maximal rate of force relaxation
VA	Voluntary activation
PT_{MVC}	Amplitude of peak torque
PNS	Peripheral nerve stimulation
F_0	Estimated maximal force
V_0	Estimated velocity of unloaded shortening
SFV	Slope of force-velocity relationship
P_{max}	Peak power
V_{opt} & F_{opt}	Force and velocity at peak power

10.1 Introduction

Below gas exchange threshold (GET), oxygen uptake ($\dot{V}O_2$) rises mono-exponentially reaching a steady-state after a few minutes (Xu and Rhodes 1999, Jones and Poole 2005). The end-exercise $\dot{V}O_2$ increases linearly in function of exercise intensity. However, at intensities above the GET, the $\dot{V}O_2$ response becomes more complex, with a second rise in $\dot{V}O_2$ slowly developed and superimposed onto the initial $\dot{V}O_2$ response (Whipp and Wasserman 1972). This slowly developing rise in $\dot{V}O_2$, termed the slow component of $\dot{V}O_2$ ($\dot{V}O_{2SC}$), results in a greater end-exercise $\dot{V}O_2$ than that predicted by the $\dot{V}O_2$-intensity relationship suggesting a reduction in muscle efficiency (Poole, Gaesser et al. 1992).

Several mechanisms inside the muscle have been explored to find the putative cause of this phenomenon:

a) a reduction of ATP production per mole of oxygen (P/O ratio), b) a diminution of the energy yield per unit of hydrolyzed ATP c) an alteration of contractile properties of muscle filament to produce force, and/or d) a deterioration of the motor pattern of the motion.

The reduction of ATP production per mole of oxygen claims that supra GET exercise is associated with reductions in mitochondrial coupling, i.e. ratio ATP resynthesized per molecule of O_2 (P/O). The long uncoupling protein3 is exclusively and abundantly expressed in the skeletal muscle and would dissipate energy in the form of heat instead of being converted in ATP. It has been shown that the $\dot{V}O_{2SC}$ amplitude during cycling was significantly related to mitochondrial uncoupling protein3 (UCP3) RNA expression in vastus lateralis (Russell, Wadley et al. 2002).

A diminution of the energy yield per unit of hydrolyzed ATP is sustained by research from de Meis and colleagues (Meis 2000) who showed that during the hydrolysis of ATP, the amount of heat dissipated may vary between 7 and 32 Kcal/mol depending on whether or not a transmembrane Ca^{2+} gradient is formed across the sarcoplasmic reticulum membrane. It has also been estimated that half of the $\dot{V}O_{2SC}$ may be explained by a change of 10% of Gibbs free energy of ATP hydrolysis between the start and the end of the exercise (Borrani, Malatesta et al. 2009).

Alteration of contractile proprieties of muscle filaments may arise from metabolic processes occurring within the fibers already recruited. High [ADP] (Abbott and Mannherz 1970), accumulation of Pi

(Potma, Van Graas et al. 1995), high [H^+] (Cooke, Franks et al. 1988), and accumulations of NH_4 (Stephenson and Stephenson 1996) have a significant effect on muscle contractile properties. Because the $\dot{V}O_{2SC}$ and the accumulation of these muscles metabolites occur during high intensity exercise, the alterations of contractile properties have been considered a putative mediator of its emergence (Rossiter 2011, Poole and Jones 2012, Cannon, Bimson et al. 2014, Grassi, Rossiter et al. 2015, Keir, Copithorne et al. 2016).

Deterioration of the motor pattern is the mechanism that received the least attention. One the one hand it has been shown that under the influence of fatigue and despite a constant running speed, runners increased the external mechanical work between the beginning and the end of the $\dot{V}O_{2SC}$ period (Candau, Belli et al. 1998). On the other hand, it was argued that the $\dot{V}O_{2SC}$ during running might be due to the cost of generating force or to alterations in the storage and recoil of elastic energy (Borrani, Candau et al. 2003).

Others have shown that fatigue is not required to elicit the $\dot{V}O_{2SC}$. For example, during heavy exercise, when preceded either by heavy exercise or by heavy knee extensions, τ_p, gain of the primary response, and the amplitude of the $\dot{V}O_{2SC}$ were similar between protocols (Thistlethwaite, Thompson et al. 2008). Hopker and colleagues (Hopker, Caporaso et al. 2016) attested similar results with a pre-fatigue protocol vs a rest protocol. In 2018, a study looking at the cause–effect relationship between the $\dot{V}O_{2SC}$ and fatigue found no significant differences between pre fatigue and control conditions (do Nascimento Salvador, Souza et al. 2018). Finally, it has also been very recently shown the absence of a temporal relationship between the development of the $\dot{V}O_{2SC}$ and the alteration of neuromuscular properties due to the fatigue during a heavy exercise, concluding that fatigue is not an essential requirement to elicit its appearance (O'connell, Weir et al. 2017, Colosio, Caen et al. 2020, Gajanand, Alonso et al. 2020). These apparent discrepancies regarding the involvement or not of fatigue may be related to its measurement according to the stimulation methods (Jones, Bigland-Ritchie et al. 1979), to the protocol used (isometric vs. dynamic (Krüger, Aboodarda et al. 2019)), and to the time of data acquisition (during exercise, or with or without delay after exercise (Froyd, Millet et al. 2013)).

The present study was therefore designed to better understand the changes in fatigue during and after different exercise intensities. In this descriptive study, two questions were addressed. Firstly, what are the time course and the nature of the changes in fatigue that develop during moderate (MOD), heavy (HVY), severe (SEV) boots of dynamic exercise? Secondly, what is the concordance between muscle fatigue and the development of the $\dot{V}O_{2SC}$ during different exercise intensities?

To address these questions a specific protocol was designed. It has been shown that the contribution of the $\dot{V}O_{2SC}$ to the overall response, during HVY knee extensions, was significantly greater than for cycling exercise (Koga, Poole et al. 2005). Therefore, constant knee extension exercises, at different intensities, during 10 min, were performed in a home-made machine specially designed. In order to evaluate fatigue, neuromuscular function was evaluated in a static and dynamic way before, after and during the course of the exercise. This allowed to reveal the time course and nature of fatigue and the relationship with the development of the $\dot{V}O_{2SC}$.

10.2 Methods

Ethical approval

The University of Auckland Human Participants Ethics Committee approved this study, and all procedures compiled with the latest version of the declaration of Helsinki (2013). All subjects were informed about the requirements and potential risks involved in the study in both written and verbal forms, previous participation in the study.

Participants

Eleven healthy recreationally active males (mean ± SD, age 23 ± 2 years, body mass 78 ± 11 kg, and height 173 ± 7 cm) participated in this study. The subjects also completed a Q-AAP questionnaire to exclude all potential cardiorespiratory and injury risks. Participants were asked to avoid strenuous physical activity, alcohol, tobacco, and caffeine, in the 24h previous to the testing session. Furthermore, participants were asked to not consume any food for the 3h preceding a test and to arrive fully hydrated.

Study Design

This study involved each participant attending four separate laboratory sessions, with at least a 48h interval between tests, over a three-week period. All tests were completed in an air-conditioned (21ºC ±

1ºC) exercise physiology laboratory. The first session involved an incremental step test on leg extension home-made machine (Figure 34). This test was used to assign the specific intensity a work-rate for the subsequent three experimental sessions. Following the incremental step test, participants were familiarized with the procedure to be used to evaluate neuromuscular function. The three experimental sessions involved participants practicing leg extension at different intensities MOD, HVY, SEV domains respectively, and neuromuscular evaluation. During all test, pulmonary gas exchange was measured.

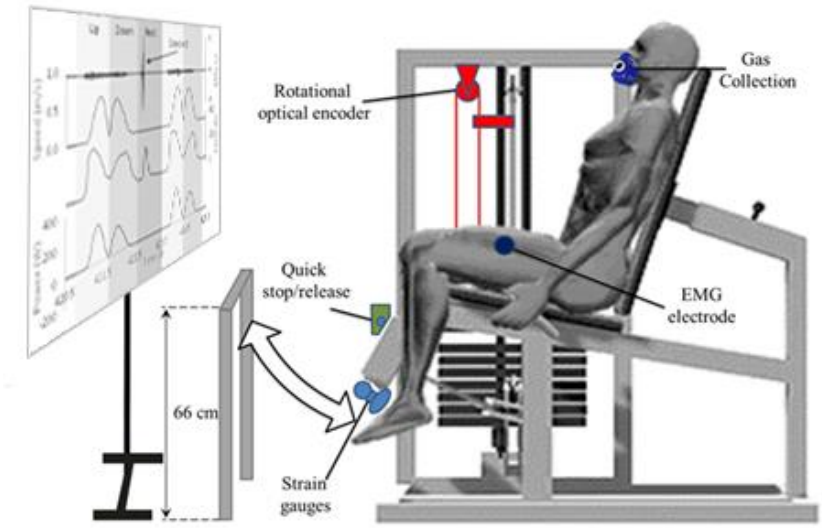

Figure 34 Leg extension home-made dynamometer*. The strain gauge was located between the mobile part of the machine and the participant. The rotational optical encoder was placed on the vertical column to measure linear movements. A horizontal target bar 66 cm above the ground indicated the range of motion. On the fixed part of the ergometer, a quick stop/release manually operated by a pedal allowed the stop/release of the mobile part to provide isometric neuromuscular function measurements during the exercise. Instantaneous visual feedback of the speed, force and power were provided as a real time signal displayed on a computer screen at the front.*

Testing procedures

Incremental test

Participants were subjected to an incremental test on the knee extensor ergometer to determine GET and peak oxygen consumption ($\dot{V}O_2$peak). Prior to the test, subjects were familiarized to the procedures and rhythm (metronome set at 70 beats per minute) of the test lightly loaded. Each beat was equivalent to a sequence of the verbal cue ‘up-down-rest’ (knee extension-knee flexion-rest) given by the experimenter throughout the duration of exercise. Following 3 minutes of seated rest, participants performed knee-extensions at a baseline load of 6 kg. Each exercise bout required the participants to extend both knees from a 90 degree angle to a height extension set at 66 cm. Additionally, a visual cue representing the

force, velocity, and power was also provided in order to allow subjects to keep the knee-extension and flexion intensity as constant as possible. Thereafter, the load was increased by 3 kg per minute until volitional exhaustion or until the set height and velocity of the extensions could no longer be maintained.

Step transition exercise

The three experimental sessions consisted of a combination of two constant-load knee-extension exercise separated by one-hour rest. The different constant-load exercises were performed twice at MOD (80% GET), HVY (30% of the difference in power between, GET and $\dot{V}O_{2peak}$, 30%Δ), and SEV (60%Δ) intensities for a duration of 10 minutes. The order and the intensity were randomized, but each intensity was performed in first place at least one time. Neuromuscular function of the knee-extensor muscle of the left leg was evaluated before, during, and after each test.

Neuromuscular function evaluation

Neuromuscular function was assessed in both isometric (associated with electrical nerve stimulation) and dynamic (through the force-velocity relationship) modes. Participants were first instructed to perform three 5-s isometric maximal voluntary contraction (IMVC) with one leg (left leg), separated by 60 s to establish their maximum torque. During every IMVC, a 100 Hz doublet was delivered on the IMVC plate (superimposed doublet), and 2 s after the IMVC (potentiated doublet) (Merton 1954).
During the 10-minute exercises, a doublet stimulation was also delivered to the quadriceps every 30 s during the rest period of 'up-down-rest' sequence. At post exercise, IMVC was repeated only one time in order to minimize the effect of recovery. In dynamic condition, the force–velocity relationship was built in a pyramidal way: starting with a light weight (6 kg). Participants were instructed to perform the knee extension as fast as possible. After about 7 s, a new knee extension was requested, and the weight to be lifted was increased by 6 kg until it was not possible to lift it anymore. The pyramid protocol was repeated in an inverted way and performed after the three IMVC pre and the IMVC post. Figure 35 summarizes the time course of neuromuscular function evaluation.

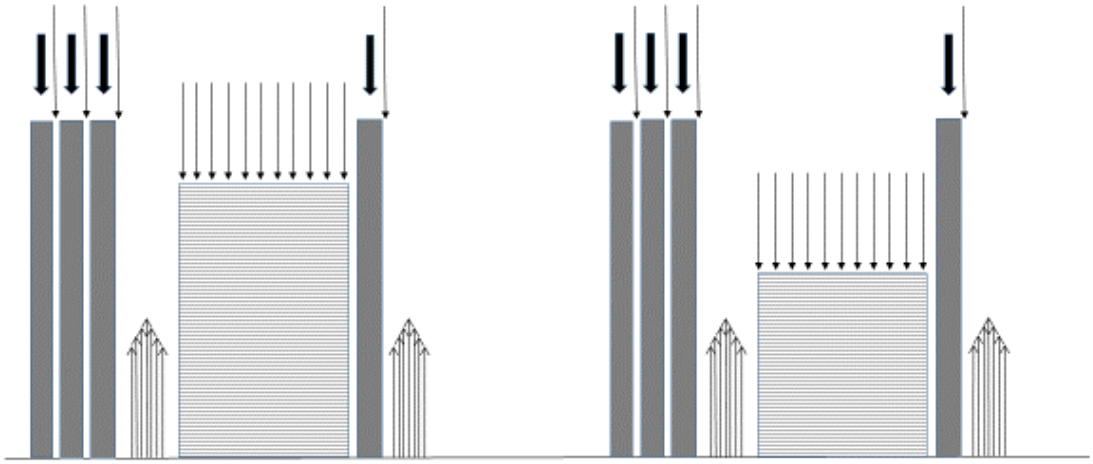

Figure 35 Schematic illustration of the time course of neuromuscular function evaluation. *The protocol depicted above involved a 5SIMVC with 60 s recovery between, represented with grey rectangles. Black thick arrows represent superimposed 100Hz doublets; thin arrows represent potentiated 100Hz doublets delivered 2s after each 5SIMVC and small pyramid arrows represent force-velocity test performed after the correspondent potentiated doublet. Rectangles with horizontal bars represent the 10 min step transition exercise.*

Measurements

Pulmonary gas exchange

Breath-by-breath pulmonary gas exchange (O_2 and CO_2) and ventilation ($\dot{V}_E$) were measured continuously throughout all exercise using a MetaMax 3B computerized system (Cortex, Cologne, Germany), and $\dot{V}O_2$ and $\dot{V}CO_2$ data were calculated and displayed breath-by-breath using metasoft software (Cortex, Cologne, Germany). Immediately prior each exercise, the gas analyzers and flowmeter turbine were calibrated with known concentrations of gases (O_2=14.01% and CO_2=6.03%) and a 3-litre Rudolph syringe (Hans Rudolph, Kansas City, MO), respectively.

Dynamometry

The seat and the lever arm positions of the knee-extension ergometer were adjusted horizontally and vertically, to align lateral condyle of the subject's femur with the rotational axis of the knee-extensor homemade machine (Figure 34). All these adjustments were made to establish both angles, hip and knee joints, at 90 degrees at rest and record them for further experimental testing. Straps were placed across

the chest, hips and thighs to stabilize the subject through exercise. A broad non-elastic strap over the left ankle served to maintain the leg against the strain gauge located between the mobile part of the machine and the participant. The rotational optical encoder was placed on the vertical column to measure linear movements. A horizontal target bar 66 cm above the ground indicated the range of motion. On the fixed part of the ergometer, a quick stop/release manually operated by a pedal allowed the stop/release of the mobile part to provide isometric neuromuscular function measurements during the exercise. Instantaneous visual feedback of the speed, force and power were provided as a real time signal displayed on a computer screen at the front.

Electrical nerve stimulation

Electrical stimulation was delivered using a high-voltage stimulator a constant current (DS7A, Digitimer, UK). In order to localize the femoral nerve, the anode electrode (18 x 7 cm American Imex, CA) was located over the gluteal fold while the cathode ball (0.5cm diameter) was manually pressed and maneuvered along the femoral triangle whilst intermittently applying a low electrical stimulation (20mA). The location of the femoral nerve was determined with the largest twitch and the greatest peak-to-peak amplitude of the M-wave of the knee-extensor muscle. Once the stimulation point was found, a cathode electrode (American Imex, CA) was placed over the alcohol wiped skin directly above it. To determine the optimal stimulation intensity, single electrical stimulations (400V, 1ms duration, rectangular pulse) with progressively increasing current intensity (from a baseline of 20mA) were applied upon the femoral nerve until a plateau in twitch force and M-wave amplitude were attained. The stimulation intensity was then further increased by 25% to ensure all muscle fibers of the knee extensors were recruited. Paired stimulation (doublet with 10ms interval between stimuli) of the established supramaximal intensity was used to examine the isometric neuromuscular function of the knee-extensor muscles before, during, and after exercise. During all electrical stimulations, pressure was applied upon the cathode electrode using a wooden device with a rubber end.

Data Analysis

Oxygen Consumption Analysis

The breath-by-breath $\dot{V}O_2$ data from each test was initially examined in order to exclude errant breaths that may have arisen from swallowing, sighing, or coughing. Values lying more than three SDs from residue were removed. The breath-by-breath data was then subsequently linearly interpolated to produce second-by-second values and identical repetitions for each individual were time aligned to the start of exercise and ensemble averaged. The first 20s of the data after the onset of exercise (cardiodynamic phase) was not considered (Weissman, Jones et al. 1982, Paterson and Whipp 1991). The following equations of a single, and bi-exponential model, were used to characterize the $\dot{V}O_2$ responses to moderate, heavy, and severe intensity exercise:

$$\dot{V}O_2(t) = \dot{V}O_{2rest} + A_p(1 - e^{-(t-TD_p)/\sigma_p}) \quad \text{(moderate)} \quad \text{Eq 13}$$

$$\dot{V}O_2(t) = \dot{V}O_{2rest} + A_p(1 - e^{-(t-TD_p)/\sigma_p}) + A_s(1 - e^{-(t-TD_s)/\sigma_s}) \quad \text{(heavy and severe)} \quad \text{Eq 14}$$

$\dot{V}O_2(t)$represents the oxygen consumption at a given time *t*; $\dot{V}O_{2rest}$ the $\dot{V}O_2$ at rest, A_p, TD_p, and τ_p represent the amplitude, time delay, and time constant, respectively describing the primary phase; and A_s, TD_s, and τ_s represent the amplitude of, the time delay before the onset of, and τ describing the time constant of the $\dot{V}O_2$slow component, respectively. A nonlinear least squares algorithm was used to minimize the error between model and data. Since the asymptotic values (A_s) of the exponential terms describing the $\dot{V}O_{2SC}$ may represent a higher value than the actually reached, the amplitude of $\dot{V}O_{2SC}$ were defined as A_s' and calculated as followed:

$$A_s{}' = A_s(1 - e^{-(t_{end}-TD_s)/\sigma_s}) \quad \text{Eq 15}$$

were t_{end} is the time at the end of exercise. The amplitude of the slow component was also described relative to the amplitude to the primary phase (As'/Ap).

Isometric Neuromuscular Function Analysis

From the traces associated to the potentiated doublets the following mechanical variables were measured: peak twitch force (Db100), maximal rate of force development (MRFD), as well as the

maximal rate of force relaxation (MRFR). For the IMVC**,** the maximal amplitude of peak torque achieved (MVC) and the amplitude of the superimposed twitch torque were recorded. If there was not a noticeable difference (~5-10%), MVC performed prior to exercise were taken and averaged, otherwise a third measure was performed, and the two best were selected.

Voluntary activation (VA) was calculated by expressing the superimposed twitch evoked during the IMVC as a fraction of the amplitude of the potentiated doublet evoked after the IMVC (PDb100). Since the twitch stimulation was not always perfectly timed with MVC, a correction factor was included in the equation used to calculate VA as portrayed below (Strojnik and Komi 1998).

$$VA = [1-((PT_{Baseline}/MVC) \times (\text{superimposed twitch}/\ PDb100))] \times 100 \quad \text{equ. 4}$$

Where $PT_{Baseline}$ is the torque just before the superimposed doublet.

Dynamic Neuromuscular Function Analysis

As suggested for a knee extension exercise, the force-velocity relationship was adjusted by a linear model (Iglesias-Soler, Fernández-del-Olmo et al. 2017). Estimated force when contraction velocity is null (F_0), estimated velocity of unloaded shortening (V_0), and slope of force-velocity (SFV) relationship were defined. Power-velocity relationship was adjusted by a quadratic equation. Pmax is the maximal value of Power-velocity relationship. The optimal velocity (V_{opt}) was deduced by setting the derivative of the quadratic function to zero. Finally, the optimal force (F_{opt}) was defined as the ratio between Pmax and Vopt. For both models, to minimize the sum of the squared errors between the fitted functions (linear and quadratic) and the calculated values, an iterative process was used.

As a mean to evaluate fatigue in isometric and dynamic neuromuscular function, the variables (MVC, PDb100, VA, F_0, V_0, SFV, P_{max}, V_{opt}, F_{opt}) were expressed as a function of their change from their pre values. Db100, MRFD, MRFR during the time course of exercise were expressed in percent of values obtained during the potentiated doublet.

Statistical Analysis

To determine if the change between pre and post was significant, one simple T-test was used for all variables. After inspecting residual plots, and no obvious deviations from homoscedasticity or normality were present in the data set, Linear mixed effects analyses were performed to compare the $\dot{V}O_2$ kinetics, and the neuromuscular parameters between intensities. The fixed effect was the intensities (MOD, HVY, SEV), were participant was the random effect. To obtain contrasts, Holm procedures were used. Since the contractile properties of the muscle evolved linearly during the time course of exercise, their behavior was estimated using the slope of the relationship. The relationships between the change in $\dot{V}O_{2sc}$ in percent of AP (As'/Ap) and the change in parameters of contractile properties of muscle (ΔDb100, ΔMRFD and ΔMRFR) during the time course of exercise were tested with the Pearson correlation coefficient. The participants' Pearson correlation coefficients were first transformed into z-values, then a mixed linear model was used to compare the mean z-value of modalities, and finally the mean z-value was converted back to an r-value (Fisher 1921). Values are expressed as mean ± SD, and the significance level was set at $p<0.05$.

10.3 Results

Pulmonary oxygen response.

The participants' average $\dot{V}O_{2peak}$ was 1.30 ± 0.21 L/min. Figure 36 shows the $\dot{V}O_2$ kinetics time course during the three exercise modalities. No significant differences were observed between MOD, HVY, SEV intensities for $\dot{V}O_{2rest}$ (0.42 ± 0.10 vs. 0.40 ± 0.11 vs. 0.40 ± 0.09 $L \cdot min^{-1}$), TDp (9.1 ± 5.9 vs. 10.4 ± 6.2 vs. 7.8 ± 8.7 s) and τp (38.5 ± 13.5 vs. 38.9 ± 11.0 vs 41.6 ± 20.5 s) parameters. Ap was significantly higher in HVY (0.59 ± 0.11 $L \cdot min^{-1}$, $p < 0.001$) and SEV (0.61 ± 0.11 $L \cdot min^{-1}$, $p < 0.001$) compared to MOD (0.45 ± 0.11 $L \cdot min^{-1}$). Likewise, As' (0.23 ± 0.10 vs 0.14 ± 0.08 $L \cdot min^{-1}$, p= 0.004), As'/Ap (39±16% vs 23±14%, p=0.007) and TDs (217 ± 71 vs 132 ± 62 s, p= 0.009) were higher in SEV compared to HVY.

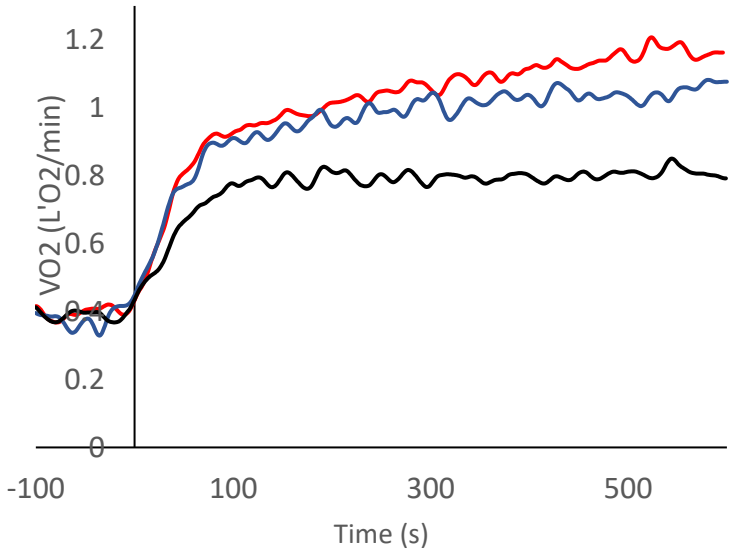

Figure 36 Illustration of mean VO_2 Kinetics during the 10min exercise from different intensities.
Black, blue and red colors represent moderate, heavy and severe intensities respectively.

Isometric and Dynamic Neuromuscular Function

The changes in isometric and dynamic neuromuscular variables between pre and post exercise modalities are presented in Table 4. The decrease on ΔMVC between pre and post was significant in the three modalities ($p<0.016$) and was higher in HVY compared to MOD ($p<0.001$), and in SEV compared to MOD ($p<0.001$) and HVY ($p=0.02$). The decreases in ΔPDb100 were significantly bigger in SEV and HVY compared with MOD ($p<0.001$; $p=0.003$ respectively), and in SEV compared with HVY ($p=0.049$), in addition the decrease between pre and post was significant for HVY and SEV ($p<0.01$). No difference was observed for ΔVA between intensities ($p=0.41$) while the decrease between pre and post was significant for MOD ($p=0.006$) and HVY ($p=0.038$) modalities. Among the dynamic properties, ΔF_0, ΔP_{max}, and ΔF_{opt} followed the same trend, namely a significant differences between pre and post during HVY ($P<0.043$) and SEV ($P=0.001$) modalities. In addition, for those three parameters, the decrease was higher in HVY compared to MOD ($p<0.039$), and in SEV compared to MOD ($p=0.001$) and HVY ($p<0.016$). Concerning ΔV_0, only a significative decrease in SEV between pre and post was observed ($p=0.04$). ΔSFV were more pronounced for SEV compared to MOD ($p=0.018$) with no significant differences between pre vs post. Neither differences were found for ΔV_{opt} parameters ($p>0.51$).

Table 4 Change (Δ) between pre and post exercise modalities for isometric and dynamic neuromuscular variables . MVC, maximal voluntary contraction; VA, voluntary activation; V_0, estimated velocity of unloaded shortening; F_0, estimated force when contraction velocity is null; SFV Slope of force–velocity relationship; Pmax, maximal power; F_{opt}, force at the maximum power; V_{opt}, velocity at the maximum power. $ Significantly different between pre-post; * Significantly different from moderate (p <= 0.05); # Significantly different from heavy. (p <= 0.05)

	Moderate	Heavy	Severe
ΔMVC (%)	-8.0±9.2 **$**	-22.7±7.3 **$***	-30.4±12.6 **$*#**
ΔPDb100 (%)	-2.4±5.2	-17.6±12.05 **$***	-26.0±13.1 **$*#**
ΔVA (%)	-6.7±6.3 **$**	-4.3±5.9 **$**	-4.5±8.7
ΔV_0 (%)	-2.2±5.1	-2.5±4.8	-5.1±7.3 $
ΔF_0 (%)	-1.3±6.3	-5.7±9.4 **$***	-17.1±12.9 **$*#**
ΔSFV (%)	-1.5±3.6	-3.7±12.9	-11.6±17.9 ****
ΔP_{max} (%)	-1.5±5.8	-9.3±7.3 **$***	-21.9±10.1 **$*#**
ΔF_{opt} (%)	1.0±8.7	-6.6±9.2 **$***	-17.5±12.0 **$*#**
ΔV_{opt} (%)	0.3±7.5	-2.6±4.5	-4.8±7.9

Time course of neuromuscular variables

The development of the relative $\dot{V}O_{2sc}$ (As'/Ap) in function of the change in neuromuscular parameters during the time course of exercise is depicted in Figure 37: in the frontal plane is showed the evolution of the relative $\dot{V}O_{2sc}$ as a function of time, in the transverse plane the time course of neuromuscular variables change representing fatigue and, in the sagittal plane, the evolution of relative $\dot{V}O_{2sc}$ as a function of these variables. The slope of each neuromuscular variable during the time course of the exercise (sagittal plane) are showed in Table 5A. At MOD intensity, the slope of ΔDb100 (p = 0.028), ΔMRFD (p<0.001), and ΔMRFR (p <0.001), were significantly positives. During HVY intensity, only ΔMRFR parameter showed a positive significant slope (p= 0.022). On the contrary, during SEV intensity, the slope of ΔMRFD (p = 0.036) and ΔDb100 (p = 0.003) were significantly negative. Interestingly, the slopes were significantly different between MOD and SEV, as well as between HVY and SEV for ΔDb100 (p<0.02), and ΔMRFD (p<0.003).

Table 5 A) Slope changes in contractile properties during the time course of exercise (transversal plane in figure 37) ; B) Correlations and z-values between the As'/Ap and the change in contractile properties relationship (sagittal plane in figure 37)*. ΔDb100, change in peak twitch force; ΔMRFD, change in maximal rate of force development; ΔMRFR, change in maximal rate of force relaxation. * Significantly different from moderate (p< 0.05). # Significantly different from heavy. (p<0.05). § Slope Significantly different from 0t. (p<0.05). $ Significant correlation. (p>0.05)*

A		**Moderate**	**Heavy**	**Severe**
ΔDb100 ($N/s \cdot 10^{-3}$)	Slope	5.6±7.3 §	-2.2±16.5	-26.1±22.2 §***#**
ΔMRFD ($N/s^2 \cdot 10^{-3}$)	Slope	11.4±7.4 §	4.6±14.7	-19.2±26.2 § *#
ΔMRFR ($N/s^2 \cdot 10^{-3}$)	Slope	-28.7±20.6 §	16.3±20.0 §	-1.9±24.3 *
B		**Moderate**	**Heavy**	**Severe**
ΔDb100	Z		0.02±0.63	-0.88±0.70 $#
	r		0.02	-0.7
ΔMRFD	Z		0.28±0.64	-0.71±0.95 $#
	r		0.28	-0.61
ΔMRFR	Z		0.36±0.33 $	-0.01±0.50 #
	r		0.35	-0.01

$\dot{V}O_{2sc}$-*Neuromuscular Function relationships.*

The relationship between relative $\dot{V}O_{2sc}$ and the change in contractile properties are summarized in Table 5B. As'/Ap was significantly correlated with ΔMRFR (r=0.35, p=0.005) in HVY intensity, on the contrary, at SEV intensity, Db100 (r= -0.7, p=0.002) and ΔMRFD (r=-0.61, p=0.032) were negatively correlated with As'/Ap . It is also worth noting that the strength of the link was significantly different between HVY and SEV, for ΔDb100 (p < 0.001), ΔMRFD (p= 0.001), and ΔMRFR (p = 0.008).

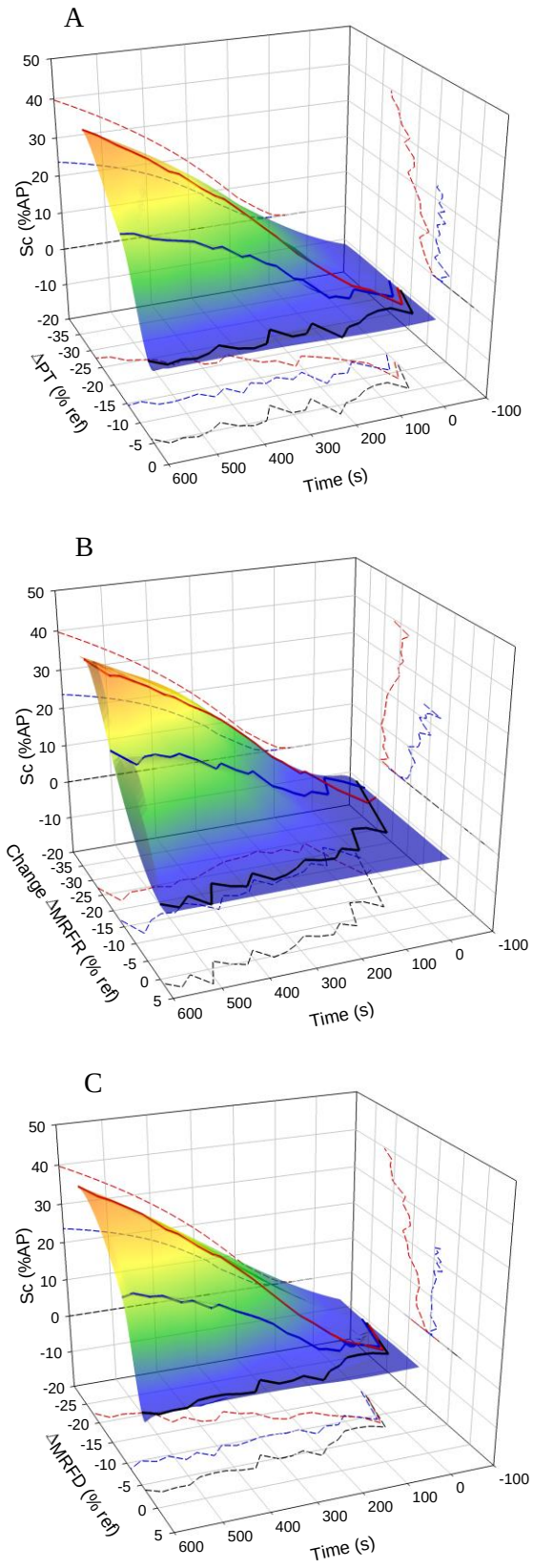

Figure 37 Development of the relative $\dot{V}O_2sc$ in function of the change in neuromuscular parameters during the time course of exercise. Black, blue and red lines represent moderate, heavy and severe intensity respectively. In the frontal plane is represented the evolution of the relative $\dot{V}O_2sc$ (As'/Ap) in function of time, in the transverse plane the time course of fatigue for the change of different neuromuscular variables representing the fatigue, and in the sagittal plane the evolution of relative $\dot{V}O_2sc$ as a function of these same variables. Plot A, Db100 (PT) ; B maximal rate of force relaxation (MRFR) and C maximal rate of force development (MRFD).

10.4 Discussion

Correlation between relative $\dot{V}O_{2sc}$ and contractile properties during the time course of the exercise showed that the slow component is not related to fatigue in the HVY domain, and only partially related in the SEV domain during constant load cycling exercise.

Isometric and dynamic neuromuscular function

In the present study, MVC force decreased significantly in all modalities between Pre and Post exercise and was significantly more reduced after exercise in HVY and SEV domain compared with MOD, and in SEV compared to HYV. However, while a decrease in MVC force shows the existence of fatigue, it does not provide information regarding its etiology (central vs peripheral). Central fatigue, commonly assessed by VA was not significantly different between the three exercise intensities, suggesting that there was no significant difference in decline in central drive. The fact that the change from pre- to post-exercise in potentiated doublet followed the same trend as MVC suggested the peripheral origin of neuromuscular fatigue. This may seem to be in contradiction with the changes observed in Db100, MRFD, and MRFR during the time course of exercise. Indeed, whereas Db100 increased during MOD exercise it remained constant during HVY activity, and decreased significantly during SEV exercise. However, it is forgotten that the contractile response of the muscle is related to the history of its activation and that, its response is the balance between potentiation and fatigue (Kufel, Pineda et al. 2002). Indeed, for the potentiated doublet after MVC, the potentiation is supposed to be maximal and only fatigue makes the response vary, while during a sub-maximal exercise, i.e. during MOD, HVY and SEV intensities, the potentiation increases with the intensity of the exercise as well as fatigue, so the response is a subtle combination of both (Rassier and Macintosh 2000). Thus, during MOD, even if fatigue exists the potentiation effect (due to the precedent contractions during the knee extension exercise) is more pronounced, giving as a result a positive slope. In HVY, even if there is fatigue, the two quantities compensate each other showing null slope. On the contrary, the fatigue in the severe domain is so evident that not even potentiation is able to compensate it, resulting in a negative slope. Interestingly, the decrease through the time course of ΔDb100 (slope) was significantly different during

all conditions, being greater as the intensity increases. Together these results suggest different fatigue regulation into these three domains or intensities.

Decrements in Db100 have been linked with accumulations of Pi (Westerblad, Allen et al. 2002), especially in presence of H^+ and its effects inhibiting Ca^{2+} binding Troponin C (Fitts 1994). Similarly, the decrease of ΔMRFD observed in the SEV domain is linked to the reduced [Ca^{2+}] concentration and has been associated with high concentrations of Pi and H^+ (Kent-Braun, Fitts et al. 2011). ΔMRFR increases considerably more in SEV domain, suggesting a slower dissociation of actin from myosin in fatigued muscle cells, which could be explained by an impairment on the cross-bridge detachment rate or a reduced rate of Ca^{2+} reuptake by the reticulum sarcoplasmic pumps (Westerblad and Allen 1993). Reduction in the rate of force relaxation has also been related with Pi accumulation(Allen, Lamb et al. 2008) and acidosis(Westerblad, Lännergren et al. 1997).

In dynamic exercise, power is the product of force and velocity. In the present study, V_0 was significantly decreased only after SEV intensity, in contrast V_{opt} did not change after any of the modalities and neither changed during the course of the exercise. From these observations, it could be concluded that changes in P_{max} could be explained mostly from the decreases in force. Indeed, the decreases on F_0 in HVY and SEV modalities after the exercise, and the greater fall in SEV compared to HVY and MOD intensity are in line with results of Jones et al. (Jones, De Ruiter et al. 2006). They showed that the changes in V_0 appeared late in the development of fatigue and played a relatively small role in the loss of power, contrarily to the loss of force. They speculated that this could be due to a decrease in the rate constant for attachment (the first bond of myosin to actin and the subsequent high force state with the release of Pi) which is normally slowed in the presence of Pi (Cooke, Franks et al. 1988, Godt and Nosek 1989).

Taken together, the loss of contractile function observed through Db100 and MRFD variables in isometric exercise, and the decrease of F_0 and P_{max} in dynamic exercise during SEV work, suggests the accumulation of metabolites. On the other hand, since metabolites do not supposed to accumulate, but rather to reach a steady state as exercise proceeds in the HVY domain, it is not surprising to observe that contractile properties during the time course of exercise were not deteriorated. Critical power (CP), would therefore, act as a continuous transition phase threshold (Pethick, Winter et al. 2020) separating domains with a different neuromuscular fatigue profile (Burnley, Vanhatalo et al. 2012, Burnley and

Jones 2018). Consequently, all the activity performed beyond will results in substantial changes in Pi, pH, and muscle metabolites that cannot be stabilized, with the consequent progressive development of peripheral fatigue (Jones, Wilkerson et al. 2008).

$\dot{V}O_2$sc neuromuscular function relationships

During the exercise in the HVY domain, there were no significant relationship between change in contractile properties (ΔDb100 and ΔMRFD) and the development of the $\dot{V}O_2$sc expressed as a percentage of the amplitude of primary phase. Although the correlation coefficient was significant between relative $\dot{V}O_2$sc and ΔMRFR the fact that the coefficient of correlation was low (r= 0.35), the strength of the relationship between the two variables is considered as weak. These findings are in line with previous papers, where it was found no temporal relationship between the development of the $\dot{V}O_2$sc and fatigue parameters during HVY exercise (Cannon, White et al. 2011, Gajanand, Alonso et al. 2020).

On the contrary, during the exercise performed in the SEV domain, significant relationships between relative $\dot{V}O_2$sc and ΔDb100 (r = -0.70, moderate link) as well as ΔMRFD (r =-0.61, moderate link) were observed, showing a partial link with the $\dot{V}O_2$sc. Similarly, the correlations in the HVY and SEV domains were statistically different for all variables representing contractile properties. Taken as a whole, these results suggest that the development of the slow component have not the same origin in the two intensity domains, and that fatigue, even if exist, is not a *sine qua non* for the development of the $\dot{V}O_2$sc, at least in the heavy domain.

As mentioned above, the two parameters (Db100 & MRFD) that were affected during the time course of exercise suggested that contraction function failure is expressed only in SEV domain. Accumulation of Pi is considered the largest contributor to fatigue(Allen, Lamb et al. 2008). Similarly, the rate-limiting step for a power stroke to occur and the transition to high-force states between actin and myosin is the detachment of Pi from the myosin head; an increased Pi concentration, could therefore, enable a decrease in force production or MRFD per cross bridge (Fitts 2008). The fact that, neither of these two parameters

were affected in the HVY domain could be explained by the absence of deleterious effects of metabolites accumulation at this intensity and the consequent absence of contraction function failure.

The $\dot{V}O_{2sc}$ is measured in the HVY and in the SEV exercise domains as the expression of a single phenomenon, nevertheless, in exercise physiology there is a clear distinction between exercises conducted above and below the CP (Wasserman, Kessel et al. 1967, Jones and Poole 2013). Traditionally, the $\dot{V}O_2$sc has been attributed to an increase cost of locomotion whatever the domain of intensity. However, recent studies have refuted this assumption. Indeed, Barret and colleagues (Bartlett, Fitzgerald et al. 2021) have showed with an incremental ramp test protocol at 6-10% of the participant's MVIC force that, for workloads above the GET there was no change in ATP cost through the exercise. They suggested that the greater O_2 cost of contractions above the GET was not caused by an increase in ATP cost but rather by an alteration in the mitochondrial function capacity. O'Connell and colleagues (O'connell, Weir et al. 2017) have shown that there were no change in O_2 cost of locomotors muscles during a constant power-cycling ride in the HVY domain, other than the additional O_2 uptake for the increased cost of ventilation. Very recently, Colosio and colleagues (Colosio, Caen et al. 2020) performed the same experiments in the three different domains, concluding that in the HVY domain the $\dot{V}O_{2sc}$ was ascribable to a "metabolic shift" between aerobic and anaerobic metabolisms and an increase, even if small, cost of ventilation, rather than an increase cost of locomotion. Contrarily, in the SEV domain, the cost of the $\dot{V}O_{2sc}$ minus the ventilatory cost could not completely be explained by a prolonged metabolic shift, a possible indication that, in this domain there is a true loss of efficiency as shown in the present study.

Conclusion

For the first time, fatigue was measured throughout the exercise, revealing that the slow component in the HVY and SEV domains is not the product of an identical mechanism. Indeed, results suggested that its development in the HVY domain is not necessarily related to fatigue, reason why no significant changes are observed in the isometric contractile properties (Db100 and MRFD) over exercise and no relationship is found with the relative $\dot{V}O_2$sc.

Contrarily, in the SEV domain, the significant decrements in the slope of the neuromuscular-time relationship (Db100 and MRFD) during the time course of exercise and the diminution in F_0, reflected fatigue processes that, in addition, were partially correlated with the development of the relative $\dot{V}O_{2sc}$. The literature in the field suggests that degradation of PCr with the consequent accumulation of Pi and H^+ over a threshold at intensities above CP are behind the origin of muscle fatigue (Korzeniewski and Rossiter 2020). Therefore, the former could be one of the explanations for the appearance of $\dot{V}O_{2sc}$ in the severe domain.

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11 Article III : The metabolic profiles of different fiber type populations under the emergence of the slow component of oxygen uptake.

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Abstract

To investigate the influence of different metabolic muscle fiber profiles on the emergence of the slow component of oxygen uptake ($\dot{V}O_{2SC}$),,twelve habitually active males completed four sessions of different combinations of work-to-work transition exercises up to severe intensity. Each transition was modeled to analyze the different kinetic parameters. Using a new approach, combining Henneman's principle and superposition principle, a reconstructed kinetics was built by temporally aligning the start of each new transition and summing them. The primary phase time constant significantly slowed and the gain at the end (GainEnd) significantly increased when transitions started from a higher intensity ($p<0.001$). Kinetic parameters from the reconstructed curve ($\dot{V}O_2$baseline, time delay of primary phase, $\dot{V}O_2$End and GainEnd) were not significantly different from one transition to severe exercise. These results suggest that the appearance of the $\dot{V}O_{2SC}$ is at least related to, if not the result of, the different metabolic properties of muscle fibers.

Key words: oxygen consumption kinetics, slow component, muscle fatigue, muscle fibers metabolic properties.

11.1 Introduction

The fundamental response of muscle oxygen consumption ($\dot{V}O_2$) kinetics, during moderate transition, may closely reflect the kinetics of $\dot{V}O_2$in the contracting muscles(Grassi, Poole et al. 1996). At a constant work rate exceeding the gas exchange threshold (GET), this response is characterized by a delayed-onset of the new metabolic requirements, defined as the '$\dot{V}O_2$ slow component' ($\dot{V}O_2$sc), elevating $\dot{V}O_2$ above the 'steady-state' value predicted for this work rate(Whipp and Wasserman 1972, Linnarsson 1974, Barstow and Mole 1987). This excess $\dot{V}O_2$ is a reflection of a loss of muscle efficiency(Poole, Schaffartzik et al. 1991). To date, the putative mechanisms of $\dot{V}O_2$sc are poorly understood, but several hypotheses have been proposed. Among these is the potential influence of the different metabolic response profiles of different fiber type populations during the development of the $\dot{V}O_{2SC}$. Indeed, it has been shown that the mammalian skeletal muscle is composed of different cell populations, with different metabolic and mechanical characteristics, mitochondrial content, and contractile proteins (Pette and Staron 1997). Kushmerick and colleagues (Kushmerick, Meyer et al. 1992) compared the $\dot{V}O_2$ of the two different muscle fiber types, demonstrating that the mechanism of control of cellular respiration is quantitatively and qualitatively different in fast and slow muscle fibers. Also, Stienen and colleagues (Stienen, Kiers et al. 1996) showed in their study using single muscle human fibers during isometric contraction, that the ATP consumption depends on the myosin isoform composition. Specifically, the ATP consumption in fast IIb fibers was four fold larger than in slow type I. In addition, it's been shown in animals that respiration of mitochondria (Willis and Jackman 1994), the mitochondrial volume density(Hoppeler, Hudlicka et al. 1987) and the mitochondrial rate of O_2 consumption (Blanchaer 1964) are greater in type I compared with type II muscle fibers. These differences between the slow and fast switch motor units may have an impact on the kinetics of mitochondrial oxidative phosphorylation during exercise above the gas exchange threshold (GET) and thus, contribute to the appearance of the $\dot{V}O_{2SC}$.

The size principle(Henneman E 1981) posits that skeletal muscle fibers are recruited in a hierarchical manner during exercise according to intensity. In order to manipulate motor unit recruitment and reveal the metabolic response profiles of different fiber type populations, "work-to-work" step

exercise has been used(Hughson and Morrissey 1982, Brittain, Rossiter et al. 2001, DiMenna, Wilkerson et al. 2008). For instance, transitions between low work rate intensities would be expected to solicit the recruitment of muscle fibers that are positioned lower in the recruitment hierarchy (i.e., slow type fibers), whereas a transitions between high work rate intensities would be expected to involve the recruitment of muscle fibers positioned higher in the recruitment hierarchy (i.e., fast type fibers)(Krustrup, Söderlund et al. 2004). Thus, it should be possible to distinguish the effect of the recruitment of new motor units residing higher in the recruitment hierarchy during the $\dot{V}O_2$ kinetics while completing transitions between different exercise intensities (moderate, heavy and severe). In addition, because each fiber that contributes to tension development is as a unique system unto itself, and because the pulmonary $\dot{V}O_2$ signal homogenizes any oxidative response diversity within the activated pool of motor unit, the principle of superposition might be applied.

Therefore, in accordance with the Henneman and the superposition principle, and considering that the appearance of the $\dot{V}O_{2SC}$ is due to the difference in mitochondrial oxidative phosphorylation kinetics between fibers types, the differences in $\dot{V}O_2$ kinetics between a single work transition and a work-to-work transitions of an equal final power, may be due to the temporally shift of MU activation residing higher in the recruitment hierarchy. In keeping with this, temporally aligning the beginning of each new transition (activation of new fibers positioned higher in the recruitment hierarchy (Henneman principle)) and summing them (superposition principle) to form new reconstructed kinetics should not give a different $\dot{V}O_2$ kinetics with that measured in a simple transition to equal final power.

The purpose of this study was to add novel evidence to the debate of the origins of the $\dot{V}O_{2SC,}$ specifically, if the different metabolic response profiles of different fiber type populations are one of the culprits in the development of the $\dot{V}O_{2SC}$.

The hypotheses were:

The time constant (τ) would be significantly smaller between low intensity work rate transitions compared with transitions between high work rate intensities.

The reconstructed $\dot{V}O_2$ kinetics from multiple transitions would, in fact, have an identical kinetic to a simple transition at the same final intensity.

11.2 Methods

Twelve healthy habitually active males aged 18-50 years (mean ± SD: age 24.33 ± 0.72 year, height 178.41 ± 7.76 cm, weight 76.31 ± 11.62 kg) were recruited to participate in this study. Participants were excluded if they, or their family, suffered from any heart or cardiovascular condition, bleeding disorder, or were taking prescribed medication. Participants were instructed to refrain from training and other vigorous physical activity, alcohol consumption, caffeine intake and tobacco for a minimum of 24h before experiments. Participants were advised to arrive at the laboratory in a rested, fully hydrated, and at least 3h postprandial state. The research protocol was accepted by local Human Participants Ethics Committee, and completed according with the seventh Declaration of Helsinki (2013). Prior to participation in the study, the protocol and possible risks involved were explained to all participants before written informed consent was collected. All participants were advised of their right to withdraw from the study at any time without prejudice.

The experimentation required five visits to the laboratory. The tests included a first session of a ramp incremental test on cycle ergometer (Velotron racemate Inc Spearfish, USA) in order to assess GET and peak oxygen uptake ($\dot{V}O_{2peak}$). The ergometer seat and handlebars were adjusted for comfort, and the measurements were recorded to reproduce a consistent set up for the subsequent tests. On subsequent days, participants completed an additional four sessions with various combinations of work-to-work transitions across a wider range of square wave exercises. Heart rate (RS800, Polar, Finland) and pulmonary gas exchange were continuously measured using a computerized system (Metamax 3B, Cortex GmbH, Leipzig, Germany) during all sessions. Testing took place at a similar time of the day (± 2 h), conducted in a temperature-controlled laboratory (maintained at 18 ± 1°C).

During the ramp incremental test, participants rested for 3min on the cycle ergometer before cycling for 6min with a load of 60 watts (W) at a comfortable self-selected pedal rate between 70–90 rpm, what was reproduced for subsequent tests. The power was then increased in a ramp fashion of 30w/min until volitional exhaustion or till one of the American College of Sports Medicine established criteria for maximal testing was reached(Medicine 2013). Verbal encouragement was given to the participants during the test. GET was determined by 1) the first disproportionate increase in carbon dioxide output ($\dot{V}CO_2$) from visual inspection of individual plots of $\dot{V}CO_2$ vs. $\dot{V}O_2$; 2) an increase in ventilatory

equivalents for oxygen and not in carbon dioxide; and 3) an increase in end-tidal O_2 tension with no fall in end-tidal CO_2 tension. $\dot{V}O_{2peak}$ was determined as the highest value in a 30s range recorded before the participant volitional exhaustion

On subsequent visits, participants performed a various combinations of work-to-work transitions between moderate (M, six minutes at the 80% of the GET), heavy (H, six minutes at 20% of the difference in power between GET and the $\dot{V}O_{2peak}$) and severe (S, six minutes at 60% of the difference in power between GET and the $\dot{V}O_{2peak}$) intensity exercises. After three minutes at rest and three minutes "unloaded" baseline cycling, participants started one of the following four protocols: 1) M followed by S (M→S); 2) H followed by S (H→S); 3) M followed by H and by S (MH→S); 4) S followed by 3min rest (S) followed by 3min "unloaded" baseline and by S (SPost). Note that the last protocol provided data for S and SPost.

During the session, participants completed exercise twice separated by 1h brake in pseudo-randomized manner as each exercise was performed once at first place and once in second place.

$\dot{V}O_2$, pulmonary gas exchange and ventilation were computed breath-by-breath. Prior to each test, the calorimeter and turbine were calibrated using ambient air and gases of known concentration (O_2=14.01%, CO_2=6.03%) and 3L calibration Rudolf syringe (cortex, Leipzig, Germany), respectively. The breath-by-breath $\dot{V}O_2$ data was initially examined to eliminate errant values caused by coughing, swallowing, etc., and values laying more than three 3 SDs from the local mean. Linear interpolation was used to provide second-by-second data, and, for each individual, identical repetitions were time aligned to the start of exercise and the ensemble averaged. Mono-exponential equation was computed to isolate the primary component of the $\dot{V}O_2$ kinetics using the iterative method proposed by Rossiter et al(Rossiter, Ward et al. 2002).

$$\dot{V}O_2(t) = \dot{V}O_2\text{baseline} + A_p\,(1\text{-}\exp^{-(t-TDp)/\tau p}) \qquad \text{Eq 1}$$

Where $\dot{V}O_2(t)$ is the time course of $\dot{V}O_2$, $\dot{V}O_2$baseline is the oxygen consumption at the beginning of exercise, Ap is the amplitude, TDp is the time delay, and τp is the time constant of the primary phase, respectively. The first 20s of the pulmonary $\dot{V}O_2$ signal were removed from analysis since it has been demonstrated that the cardiodynamic phase of the $\dot{V}O_2$ kinetics does not represent an increased muscle

O_2 consumption(Whipp, Ward et al. 1982). Identification of the end of the primary phase was made by criteria consideration as recommended by Rossiter et al(Rossiter, Ward et al. 2001) and Murgartroyd et al(Murgatroyd, Ferguson et al. 2011). The magnitude of the slow component (A_S) was defined as the difference between the $\dot{V}O_2$ projected primary phase, and the averaged amplitude from the last 30 s of the response (termed $\dot{V}O_2$end). The Gain Amplitude (GainAp) was defined as the increase in $\dot{V}O_2$ above baseline per unit increase in external work rate above baseline, $\dot{V}O_2$/**Δ**WR. The Gain End (GainEnd) as the sum of the Ap and A_S per unit increase in external work rate above baseline (Ap + As/**Δ**WR). The mean response time (MRT) was calculated as the sum of the Tdp and τp.

The superposition principle was applied to build the reconstructed curve. The start of each transition was time aligned and baseline was set at zero in order to sum the different kinetics curves as represented in Figure 38. The parameters of the reconstructed curve were therefore calculated using the iterative method proposed by Rossiter et al(Rossiter, Ward et al. 2002) (see above). Therefore, the reconstructed curve of M→S turn into MS; H→S turn into HS and MH→S turn into MHS.

Data statistical analysis

Analyses were performed using Jamovi ((Version 0.9.5.17) [Computer Software]), retrieved from https://www.jamovi.org). Linear mixed model was the statistical test used to compare the $\dot{V}O_2$ kinetics parameters between the different conditions. Condition was the fixed effects and participant as the random effect. After inspecting residual plots, no obvious deviations from homoscedasticity or normality were present.

Linear mixed model was also used to temporally analyze data of the overall $\dot{V}O_2$ constructed kinetics, in order to compare the different conditions. The fit between the $\dot{V}O_2$ constructed kinetics was assessed by summing percent of time of $\dot{V}O_2$ constructed kinetics where differences were not significant. For all tests, the level of significance was set at 0.05 and dispersion about the mean expressed as SD.

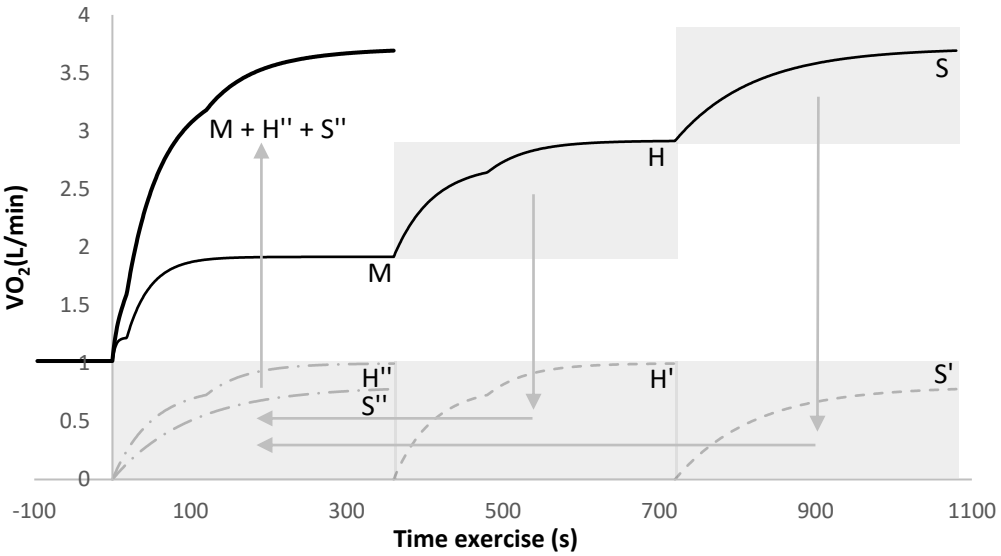

Figure 38 Illustration of reconstructed method used to analyze the work-to-work transitions protocol kinetics. *Letters M, H and S represent the model for Moderate, Heavy and Severe intensities, respectively; H' and S' represents model for Heavy and Severe kinetic curves when $\dot{V}O_2$ baseline was set at zero; H" and S" represents Heavy and Severe kinetic curves when time was aligned to zero; M+H"+S" represent the reconstructed curve with the sum of the three different intensities.*

11.3 Results

Relative $\dot{V}O_2$ was 55.77±5.21ml/kg/min. The parameters of the $\dot{V}O_2$ response for each different transition are reported in Table 6. $\dot{V}O_2$baseline was significantly different ($p<0.001$) between conditions except between S and Spost, and between H→S and MH→S condition. Concerning Ap, only the comparison between H→S or MH→S was not significantly different. All other comparisons had a p value below 0.001, other than between S and Spost ($p=0.031$). GainAp was significantly different between conditions ($p<0.003$), other than between Spost and both S and M→S. TDp was not significantly different between any of the conditions. As for $\dot{V}O_2$baseline, τp was significantly different ($p<0.002$) between conditions except between S and Spost, and between H→S and MH→S. Regarding MRT, H→S and MH→S were significantly slower ($p<0.001$) compared with S, Spost, and M→S, respectively. As was different between all conditions ($p<0.004$), apart from between H→S and MH→S. Concerning $\dot{V}O_2$End, H→S was significantly different to both M→S ($p=0.007$) and Spost ($p=0.037$).

Table 6 Comparation of the parameters of the $\dot{V}O_2$ response kinetics in the different transitions. *S, Severe Intensity; S Post, Severe Intensity after Prior Severe Intensity; M, Moderate Intensity; H, Heavy intensity. $\dot{V}O_2$ baseline, Oxygen consumption at the beginning of exercise; Ap, Amplitude of the primary phase; GainAp, increase in $\dot{V}O_2$ above baseline per unit increase in external work rate above baseline; TDp, Time Delay of the primary phase; ; τp, Time constant of the primary phase; MRT, the sum of the TDp and τp; As, Amplitude of the secondary phase; $\dot{V}O_2$ end, averaged amplitude from the last 30 s of the response; GainEnd, sum of the Ap and As per unit increase in external work rate above baseline. Values are presented as the mean SD. *Significant differences with S; § Significant differences with SPost; £ Significant differences with M→S; $ Significant differences with H→S (P < 0.05).*

	S	SPost	M →S	H →S	MH →S
$\dot{V}O_2$ Baseline (L.min^{-1})	1.07 ± 0.16	1.04 ± 0.21	1.85 ± 0.21*§	2.89 ± 0.23*§£	2.90 ± 0.25*§£
Ap (L.min^{-1})	2.02 ± 0.27	2.16 ± 0.30*	1.43 ± 0.26*§	0.77 ± 0.15*§£	0.70 ± 0.15*§£
Gain Ap (mL.min-1.W^{-1})	8.96 ± 0.73	9.56 ± 0.78	10.23 ± 0.60*	13.70 ± 1.50*§£	12.27 ± 1.16*§£$
TDp (s)	10.21 ± 5.54	11.76 ± 4.72	6.17 ± 5.37	6.06 ± 8.81	7.16 ± 9.83
τp (s)	29.31 ± 9.46	28.27 ± 6.79	46.51 ± 11.00*§	95.94 ± 19.61*§£	93.43 ± 13.83*§£
MRT (s)	39.52 ± 5.28	40.03 ± 4.21	52.67 ± 12.46	102.00 ± 27.57*§£	100.59 ± 18.17*§£
As (L.min^{-1})	0.56 ± 0.13	0.44 ± 0.10*	0.33 ± 0.10*§	0.07 ± 0.07*§£	0.10 ± 0.07*§£
$\dot{V}O_2$ End (L.min^{-1})	3.6 5± 0.38	3.63 ± 0.35	3.61 ± 0.37	3.73 ± 0.33§£	3.70 ± 0.37
Gain End (mL.min-1.W^{-1})	11.47 ± 0.78	11.56 ± 0.67	12.62 ± 0.78*§	15.00 ± 1.96*§£	14.11 ± 1.55*§£

GainEnd was not significantly different between S and Spost, and between H→S and MH→S conditions. All others comparison were significantly different (p value range: <0.001 to 0.047).

The parameters of the results of the reconstructed method reported in Table 7 showed that $\dot{V}O_2$baseline, TDp, VO_2End, and GainEnd were not significantly different between conditions. Ap was significantly different between S and both MS (p=0.011) and MHS (p=0.010). Furthermore, GainAp was significantly different between S and both MS (p=0.007) and MHS (p=0.007). Concerning τp, MHS was significantly slower compared with S (p=0.011), Spost (p=0.004), MS (p=0.041), and HS (p=0.038). Similarly, MRT from MHS was significantly slower compared with S (p<0.001), Spost (p<0.001), MS (p=0.002), and HS (p=0.016). Regarding As, S was slightly different (p=0.043) from MS. Reconstructed kinetics of the different conditions are depicted at the bottom of figure 39. Fit calculation indicated similarity between the reconstructed curve, indeed, the similitude average was 96.38% with a maximum of 100% (MS

vs.Spost). The largest differences were observed only during 11.94% of exercise duration (MHS vs. Spost), at the beginning of primary phase.

Table 7 Comparison of the parameters of the $\dot{V}O_2$ response kinetics of the reconstructed curve for the different transitions protocols.

*MS, Kinetics sum of M+S; HS, Kinetics sum of H and S; MHS, Kinetics sum of M+H+S. S, Severe Intensity; S Post, Severe Intensity after Prior Severe Intensity; M, Moderate Intensity; H, Heavy intensity. $\dot{V}O_2$ baseline, Oxygen consumption at the beginning of exercise; Ap, Amplitude of the primary phase; GainAp, increase in $\dot{V}O_2$ above baseline per unit increase in external work rate above baseline; TDp, Time Delay of the primary phase; ; τp, Time constant of the primary phase; MRT, the sum of the TDp and τp; As, Amplitude of the secondary phase; $\dot{V}O_2$ end, averaged amplitude from the last 30 s of the response; GainEnd, sum of the Ap and As per unit increase in external work rate above baseline. Values are presented as the mean SD. *Significant differences with S; § Significant differences with SPost; £ Significant differences with MS; $ Significant differences with HS (P < 0.05)*

	S	SPost	MS	HS	MHS
$\dot{V}O_2$ Baseline (L.min^{-1})	1.07 ± 0.16	1.04 ± 0.21	0.97± 0.19	1.02 ± 0.23	1.01 ± 0.21
Ap (L.min^{-1})	2.02 ± 0.27	2.16 ± 0.30	2.24 ± 0.29*	2.15 ± 0.30	2.24 ± 0.30*
Gain Ap (mL.min-1.W^{-1})	8.96 ± 0.73	9.56 ± 0.78	9.95 ± 1.02*	9.55 ± 1.20	9.95 ± 1.12*
TDp (s)	10.21 ± 5.54	11.76 ± 4.72	11.41 ± 6.34	13.19 ± 4.73	10.64 ± 5.69
τp (s)	29.31 ± 9.46	28.27 ± 6.79	31.25 ±7.51	31.00 ± 6.69	40.58±11.68*§£$
MRT (s)	39.52 ± 5.28	40.03 ± 4.21	42.65 ± 5.86	44.19 ± 4.25	51.22±7.07*§£$
As (L.min^{-1})	0.56 ± 0.13	0.44 ± 0.10	0.41 ± 0.15*	0.53 ± 0.11	0.46 ± 0.17
$\dot{V}O_2$ End (L.min^{-1})	3.65 ± 0.38	3.63±0.35	3.62 ± 0.41	3.70 ± 0.34	3.71 ± 0.38
Gain End (mL.min-1.W^{-1})	11.47 ± 0.78	11.56 ± 0.67	11.77 ± 0.92	11.90 ± 1.20	11.99 ± 0.84

11.4 Discussion

The main finding was that the reconstructed $\dot{V}O_2$ kinetics, using a novel approach of combining Henneman's principle with the principle of superposition, had a similar kinetic curve (96.4 ± 3.6% of similarity between conditions) to a simple transition at the same final severe intensity.

As hypothesized, when transitions started from a higher intensity, τ and Gain model parameters increased while amplitude parameters decreased, although, $\dot{V}O_2$end at the final transition was similar. These results are in line with previous studies(Brittain, Rossiter et al. 2001, Wilkerson and Jones 2006), which were interpreted as a reflection of metabolic differences in the pool of muscle fibers recruited

under these specific circumstances. Indeed, the elevated baseline in the work-to-work protocols implies that type I MU are already recruited according to the well-established size principle of MU recruitment(Henneman 1957)consequently, only a percentage fibers residing higher in the recruitment hierarchy would be activated during the second part of the protocol(Brittain, Rossiter et al. 2001). Type II muscle fibers are characterized by reduced mitochondrial content , lower oxidative enzyme activity(Meyer, Brown et al. 1985) and greater ATP cost for force production(Han, Proctor et al. 2001), therefore, slower $\dot{V}O_2$ kinetics and lower efficiency(Crow and Kushmerick 1982). Consistent with the increased contribution of fibers with lower oxidative efficiency, the Gain amplitude of the primary phase was progressively increased when exercise was initiated from an elevated baseline(Brittain, Rossiter et al. 2001, Wilkerson and Jones 2006).

The second hypothesis was also validate since there were only scarce differences between the reconstructed kinetics and the work-to-work transitions (Figure 39). The disparities were mainly in the first 40s of the exercise due to a significant slower τp in MHS compared with the other conditions. However, during the time course of $\dot{V}O_{2SC}$ and at the end of exercise, only sporadic differences were observed.

The fact that each severe exercise, preceded by a different modality producing a different fatigue, had similar reconstructed kinetics, suggests that: a) fatigue was not the main process involved in the $\dot{V}O_2$sc; b) the progressive fiber recruitment, due to fatigue, was consequently not required for the development of the $\dot{V}O_2$sc. During work-to-work exercise, new fibers are activated at the beginning of each transition, modeling the $\dot{V}O_2$ kinetics response. The result of temporally aligning the kinetics of $\dot{V}O_2$ at the beginning of each transition and summing them, seems to be similar to the result of a complete stimulation of the different fibers involved in a single transition of severe intensity exercise. This is consistent with the fact that the kinetics shape is mainly driven by the metabolic response profiles of different fibers populations. Several studies have demonstrate the link between the different profiles of fibers and the appearance of the $\dot{V}O_2$sc. The first authors to demonstrate that type I muscle fibers were significantly correlated with the $\dot{V}O_2$sc were Barstow and colleagues(Barstow, Jones et al. 1996). They exercised participants at **Δ**50% and took muscle biopsies of the vastus lateralis for determination of fiber

type. Participants with a higher percentage of type I muscle fibers had a higher primary phase and this was significantly correlated with the amplitude of the $\dot{V}O_2$sc (r=-0.83). Previous findings indicate significant correlations between the percentage of type II muscle fibers and markers of aerobic fitness and the relative magnitude of the $\dot{V}O_2$sc (r=0.60; P<0.01) and (r=−0.73; P<0.01), respectively)(Russell, Wadley et al. 2002). These findings are in line with other studies relating to the percentage of type I muscle fibers with an improved efficiency, or reduced $\dot{V}O_2$, for the same work rate in cycling(Coyle, Sidossis et al. 1992) or running(Bosco, Montanari et al. 1987). Pringle et al.(Pringle, Doust et al. 2003) took muscle biopsies from fourteen participants for histochemical determination and made them complete square-wave cycling tests at moderate, heavy and severe intensities. Percent of type I muscle fibers were correlated with the amplitude of the $\dot{V}O_2$sc for heavy (r=-0.74; P<0.01) and severe (r=-64; P<0.05) exercises and with τ of the primary component (r=-68; P<0.01) in heavy intensity. Indeed, after a protocol aiming for the depletion of glycogen from type II muscle fibers, there was a decrease in the amplitude of the $\dot{V}O_2$sc(Carter, Pringle et al. 2004). Deley and colleagues(Deley, Millet et al. 2006) showed that after pre-fatiguing type II muscle fibers, the amplitude of the $\dot{V}O_2$sc was significantly reduced, concluding that the recruitment of type II may be involved in the $\dot{V}O_2$sc phenomenon. Krustrup and colleagues(Krustrup, Secher et al. 2008) confirmed the idea that the energy turnover and ATP cost, was higher for type II fibers when a neuromuscular blockage of type I was performed. Certainly, muscle O_2 uptake was 20% higher and MRT was longer in type II muscle fibers, supporting the idea that type II fibers had slower kinetics and greater ATP cost than type I during dynamic exercise.

Finally, if the metabolic characteristics of the different fibers shape the $\dot{V}O_2$ kinetic curve and fatigue does not play a role in the development of $\dot{V}O_{2SC}$, neither will the progressive recruitment of these fibers. This result has been seen in isolated gastrocnemius dogs(Zoladz, Gladden et al. 2008) and in the vastus lateralis in humans(Vanhatalo, Poole et al. 2011) when all muscle fibers were activated or when a systematic increase in the cost of O_2 per unit of external power was concomitant with no changes in iEMG, respectively. Taken together, these results suggest a lack of progressive muscle fiber recruitment during $\dot{V}O_{2SC}$.

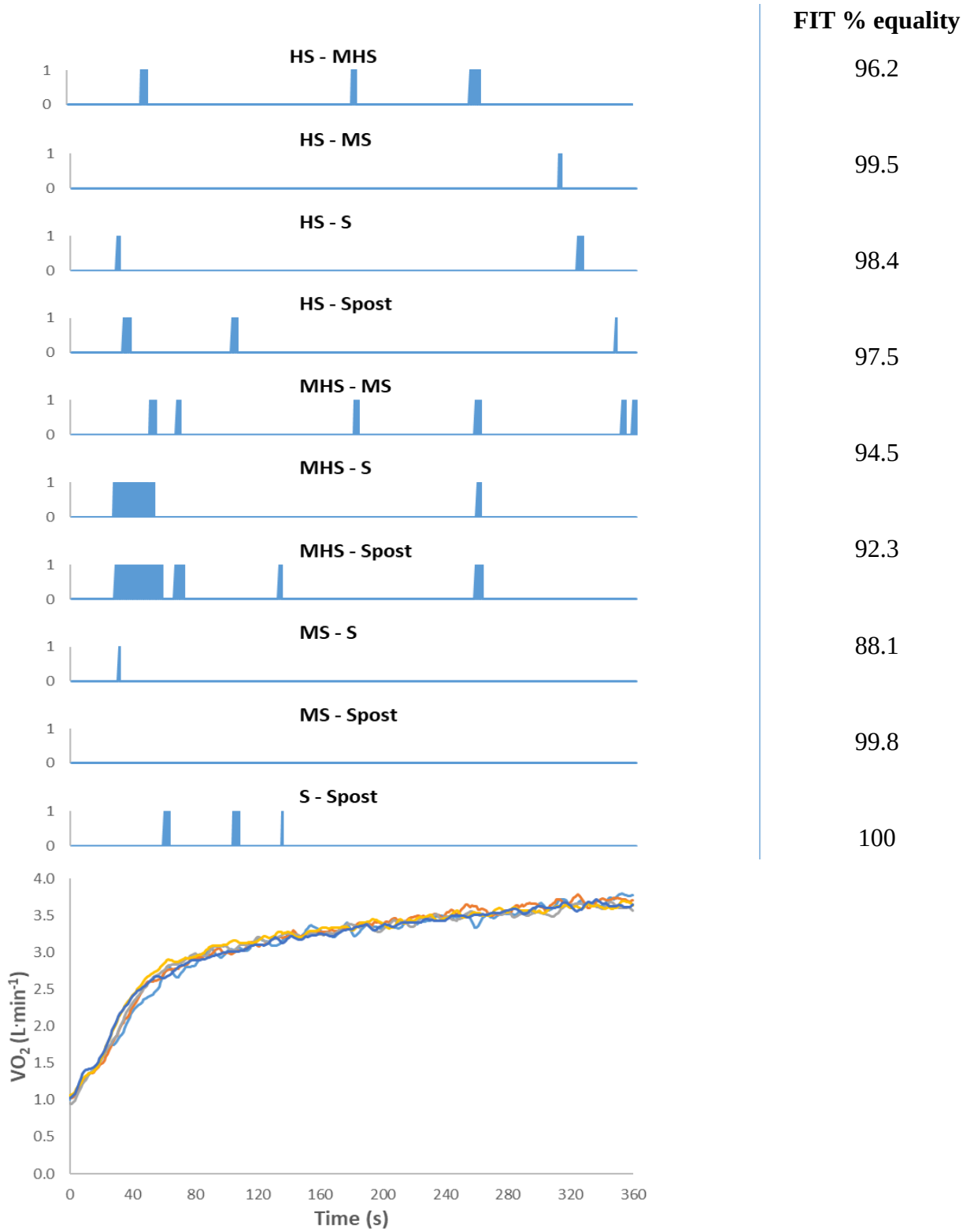

Figure 39. Pulmonary oxygen response ($\dot{V}O_2$) of reconstructed curve. *Upper panel illustrates time course comparison of the different reconstructed curves. Blue thick vertical squares represent the differences between protocols. On the right, Fit calculations represent the percentage of equality between protocols. Lower panel shows the time course of all reconstructed curves. Light blue color represents MHS; Red color represents HS; Grey color represents MS; Yellow color represents SPost; Dark blue color represents S. HS, reconstructed curve for Heavy and Severe intensities; MHS, reconstructed curve for Moderate, Heavy and Severe intensities; MS, reconstructed curve for Moderate and Severe intensities; S, reconstructed for Severe intensity; SPost, reconstructed curve for Severe intensity after prior Severe intensity.*

11.5 Conclusion

These results confirm that τ is significantly smaller between low work rate transitions compared with transitions between high work rate intensities. In addition, the $\dot{V}O_2$ severe intensity kinetic curve is similar to the reconstructed kinetics curve resulting, from combining Henneman's and superposition principle's. These finding are consistent with the appearance of the $\dot{V}O_2$sc and maybe linked to the intrinsic differences in metabolic properties of different fiber types.

Declarations

Ethics approval and consent to participate

The research protocol was accepted by University of Auckland Human Participants Ethics Committee (UAHPEC), and completed in accordance with the seventh version of the Declaration of Helsinki (2013).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analised during the current study are available from the corresponding author on reasonable request.

Competing interest

The authors declare that they have no competing interest.

Funding

Not applicable.

Authors contributions

FB conceived and designed the experiments. FB and TG completed data collection. FB, TG, SCA, JR and JA analyzed, interpreted, revisited, and approved the final version of the manuscript.

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12 General discussion

Overall, these results suggest that discrepancies regarding the relationship between fatigue processes and the $\dot{V}O_2sc$ may be because researchers were not intensity-domain-specific in their analyses.

The slow component arises at intensities above the GET in the heavy and severe domains; nevertheless, the mechanistic causes of $\dot{V}O_2sc$ development may be domain-dependent. Indeed, in exercise physiology, there is a clear distinction between exercises performed below and above the CP, although the $\dot{V}O_2sc$ has been interpreted as an expression of a single phenomenon, when perhaps it is not (Jones, Wilkerson et al. 2008, Burnley, Vanhatalo et al. 2012, Burnley and Jones 2018, Colosio, Caen et al. 2020).

Recently, several authors have discussed the differences in fatigue development in the heavy and severe domains.

- Heavy domain

Below the CP, at heavy intensity, when peripheral fatigue developed during exhaustive exercise (1-h duration), the torque, average rectified EMG, and potentiated doublet changed modestly as exercise progressed (Burnley, Vanhatalo et al. 2012).

The origin of this fatigue was not mediated by muscle metabolites, as an SS in PCr and pH was attained after 3 min, and Pi was stabilized 1 min after exercise onset with no further significant changes in these variables (Jones, Wilkerson et al. 2008).

The authors suggested that the mechanism after this fatigue in the heavy domain could be the result of muscle glycogen depletion (Bergström, Hermansen et al. 1967, Jones, Wilkerson et al. 2008). Indeed, with electron microscopy techniques, three different pools of glycogen have been identified: subsarcolemmal, intermyofibrillar and intramyofibrillar glycogen stores. Interestingly, intramyofibrillar glycogen stores served the triad junctions. In rats (Nielsen, Schrøder et al. 2009) and humans (Nielsen, Holmberg et al. 2011) during exercise, intramyofibrillar stores are the first to be depleted, and this depletion correlates with a decreased SR Ca^{2+} release rate. Therefore, when this particular store is

depleted, it will cause excitation-contraction coupling failure without the need for a significant impact on total myocyte ATP.

However, the result of muscle glycogen depletion when exercise is extended (1 h) is not the origin of the $\dot{V}O_2sc$ since the latter starts after approximately 2-3 min, suggesting that other mechanisms are at the origin of the slow component in heavy exercise.

Fatigue has been one of the candidate mechanisms; nevertheless, several studies have found a lack of an association between fatigue in the heavy domain and the slow component and therefore its origin. For instance, Thistlethwaite and colleagues (Thistlethwaite, Thompson et al. 2008) showed that muscle fatigue was not a determining factor for the development of the $\dot{V}O_2sc$ after cycling or KE-fatiguing exercises; τ_p, the gain in the primary response, and the amplitude of the $\dot{V}O_2sc$ were similar in the subsequent bout of heavy exercise.

Scheuerman and colleagues (Scheuermann, Hoelting et al. 2001) performed their experiments in the heavy domain and found a lack of association between the $\dot{V}O_{2SC}$ and the changes in the iEMG or MPF. In addition, the results of the first and second studies of this manuscript (chapters 9&10) (Gajanand, Alonso et al. 2020) showed that the relationship between the development of the $\dot{V}O_{2sc}$ and the alteration in the neuromuscular properties of knee extensor muscles was nonlinear; therefore, these parameters were unrelated over time. Similarly, Cannon and colleagues (Cannon, Bimson et al. 2014) observed a reduction in velocity-specific peak power that correlated with the $\dot{V}O_{2sc}$; however, this reduction was not temporally related to $\dot{V}O_{2SC}$ development.

Thus, despite the controversy in the literature, the results suggest that fatigue is not the main process involved in the development of the $\dot{V}O_2sc$, and, if it is involved, the time course of fatigue and $\dot{V}O_{2SC}$ development is unrelated over time in the heavy domain.

However, the fundamental mechanism involved in $\dot{V}O_2sc$ appearance is still obscure.

Very recently, Colosio and colleagues (Colosio, Caen et al. 2020), in an attempt to answer this question, tested whether and to what extent a true loss of efficiency during cycling explains the emergence of the slow component of $\dot{V}O_2$ in different intensity domains. They answered these questions by calculating the energy cost of ventilation and the glycolytic contribution to exercise and directly

measuring the aerobic cost of locomotion over time. The results showed that in the heavy domain, the emergence of the slow component was attributable to a metabolic shift between aerobic and anaerobic metabolism and a significant increase in the $\dot{V}O_2$ cost of ventilation. There was no increase in the cost of locomotion.

Taken together, regarding the heavy domain, recent publications do not provide evidence of an increase in O_2 cost and explain its increase as a metabolic shift. Therefore, the slow component in this domain could be illustrated by the speculation put forward by Colosio et al. (Colosio, Caen et al. 2020); that is, the emergence of the slow component is attributable to a metabolic shift between aerobic and anaerobic metabolism and a significant increase in the $\dot{V}O_2$ cost of ventilation. An imaginary model reflecting these results is constructed in figure 40.

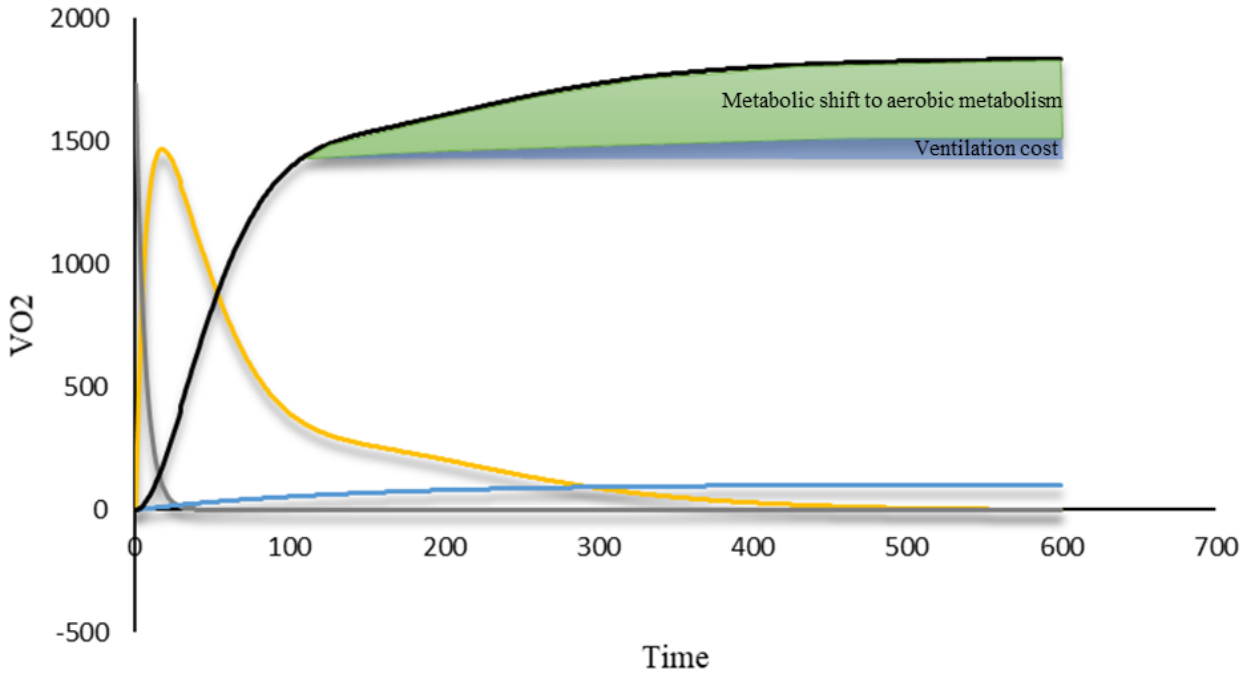

Figure 40 Hypothetical model of the VO_{2sc} in the heavy domain. *The gray line represents the energy cost of the anaerobic alactic metabolism; the yellow line represents the energy cost of anaerobic lactate metabolism; the blue line represents the aerobic energy cost of ventilation; and the black line represents the aerobic muscular energy cost + the cost of ventilation. The blue and green zones represent an increase in the VO_2 cost of ventilation and the metabolic shift from anaerobic to aerobic metabolism, respectively, explaining the appearance of the VO_{2Sc}.*

- Severe domain

When the CP is exceeded, the rate of fatigue development has been shown to increase suddenly rather than being proportional to the torque required (Burnley, Vanhatalo et al. 2012). Certainly, during KE exercises executed above the CP, pH and Pi changed precipitously during the first 3-6 min, and PCr dropped progressively until the subjects were no longer able to sustain the WR (Jones, Wilkerson et al. 2008). These observations highlight the metabolic instability characteristic of exercises above the CP and the acute stress that the body experiences at these intensities, leading to the development of fatigue. Indeed, in an attempt to prevent the imminent drop in pH, the respiratory system dramatically increases its respiratory rate. This rise in the frequency of breathing could consequently cause an increase in the O_2 cost of ventilation and respiratory muscle fatigue, both contributing to the development of the $\dot{V}O_2sc$ (Cross, Sabapathy et al. 2010).

In line with this latter study, in a very interesting paper, O'Connell and colleagues (O'connell, Weir et al. 2017) showed that the extra O_2 cost of a CP cycling ride at Δ60% could be explained as the O_2 cost required to maintain the progressive increase in ventilation (figure 41).

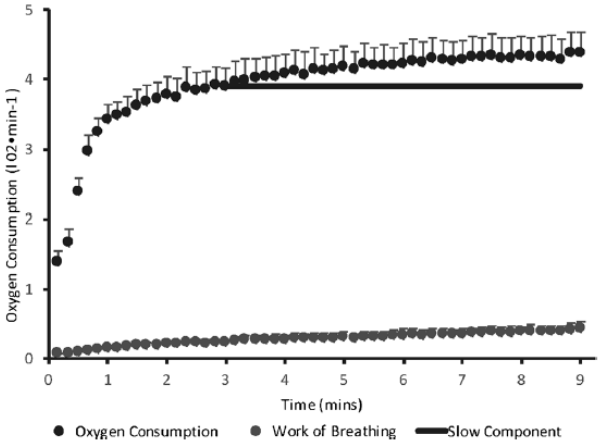

Figure 41 Mean (± SD) oxygen consumption (black) and oxygen consumption allocated to the work of breathing (gray) during 9 min of cycling exercise at Δ60%. *The VO_{2SC} is indicated by the area between the horizontal black line and the data indicated by the black symbols. From O'Connell 2017.*

They also argued that if the $\dot{V}O_2sc$ is associated with fatigue processes and a decrease in exercise efficiency, then it would be represented by an increase in lactate accumulation, since the $\dot{V}O_2sc$ develops above the GET. They therefore measured lactate accumulation at 3, 6 and 9 min of constant severe cycling exercise, observing that the lactate concentration diminished and the aerobic contribution increased. The overall cost of locomotion did not increase over time. The authors concluded that approximately 20% of the $\dot{V}O_2sc$ could be explained by the increased cost of ventilation, while the remaining 80% could be due to an

increase in O_2 uptake to compensate for the decrease in anaerobic contribution refuting the loss of efficiency. Additionally, they argued that as humans have a finite anaerobic system capacity, decreasing the energy rate provided by this system could prolong the duration of exercise.

These results are in accord with those of Colosio (Colosio, Caen et al. 2020), who reported no increase in the cost of locomotion in the heavy domain.

In contrast, in the severe domain (which they delimited by methods other than lactate measures), they (Colosio, Caen et al. 2020) found that the $\dot{V}O_2sc$ deprived of its ventilatory cost could not be explained totally by a prolonged metabolic shift, a possible indication that could exhibit a true loss of efficiency. They speculated that as intensity increases, the recruitment of higher-order, type II glycolytic fibers could explain the higher contribution of glycolysis and the true loss of efficiency manifested over time in the severe domain (figure 42).

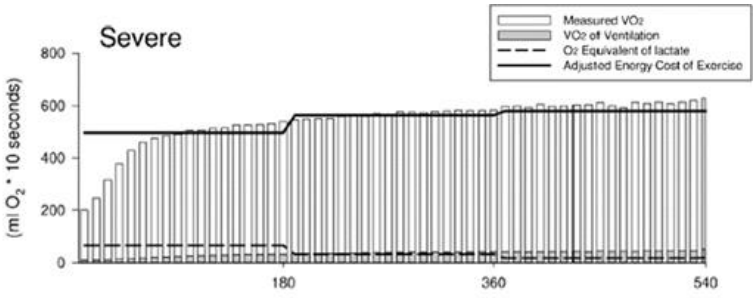

Figure 42 Overview of the energetic contribution to exercise at severe intensity. *The white columns represent directly measured VO_2. The gray columns indicate the O_2 cost required by ventilation. The black dashed line displays the energy provided by glycolytic sources over 3-min segments and the black solid line represents the adjusted cost of exercise accounting for both aerobic and glycolytic energy sources. Modified from Colosio, Caen et al.2020.*

Indeed, several studies have demonstrated the link between the $\dot{V}O_2sc$ rise and the different fiber profiles. Authors such as Barstow (Barstow, Jones et al. 1996), Pringle (Pringle, Doust et al. 2003) and Deley (Deley, Millet et al. 2006) have shown that type I muscle fibers are significantly positively correlated with the amplitude of the primary phase, while type II muscle fibers are related to the $\dot{V}O_2sc$. The proposed hypothesis is that the potential influence of the different metabolic response profiles of different fiber-type populations may have an impact on the kinetics of mitochondrial oxidative phosphorylation during exercise above the GET and thus contribute to the appearance of the $\dot{V}O_2sc$. The results of chapter 11 of this manuscript (Conde Alonso, Gajanand et al. 2020) strongly agree with this hypothesis, as the reconstructed $\dot{V}O_2sc$ kinetic curve (the result of summing each transition) had similar kinetics to a simple transition at the same final severe intensity. Further, when transitions started from higher intensity, τp and gain model parameters increased while amplitude parameters decreased,

although the end $\dot{V}O_2$ at the final transition was similar, reflecting the metabolic differences in the pool of muscle fibers recruited (Brittain, Rossiter et al. 2001, Wilkerson and Jones 2006).

Additionally, Saunders and colleagues (Saunders, Evans et al. 2003) published a paper showing the link between the diminution of $\dot{V}O_2$sc magnitude and a smaller reliance on fast-twitch MUs during severe constant-load cycling. After training subjects for 4 weeks with severe cycling exercise, the authors observed a reduction in end-exercise $\dot{V}O_2$ of 58 ± 172 ml/min in parallel with a significant reduction in the T2 of the vastus lateralis. Saunders and colleagues explained this finding by considering the possibility that reductions in end-exercise $\dot{V}O_2$ and therefore the $\dot{V}O_2$sc after training were due to a reduced reliance on fast-twitch muscle fiber MUs. Training would have improved the muscle mitochondrial content and oxidative capacity of type I muscle fibers; therefore, they would be more efficient, resulting in fewer additional fast-twitch muscle fibers recruited over time to replace fatigued fibers.

Regarding the relation between fatigue and the $\dot{V}O_2$sc, it is worth showing that most of the studies that related $\dot{V}O_2$sc development to fatigue parameters were performed in the severe domain. For instance, Borrani et al. (Borrani, Candau et al. 2001) found in subjects exercising at 95% of $\dot{V}O_2$max a concomitance between the beginning of the $\dot{V}O_{2SC}$ and the increase in the MPF. Vanhatalo et al. (Vanhatalo, Poole et al. 2011) performed experiments during all-out exercises, considering a dramatic increase in the O_2 cost of power production to be a slow-component-like phenomenon. Zoladz et al. (Zoladz, Gladden et al. 2008) fully activated dog gastrocnemius muscle fibers. During this maximal activation, the metabolic environment could be interpreted as being similar to that during severe-intensity exercise. They observed a constant $\dot{V}O_2$ value in the presence of a diminution of force output reflected in the increase in the $\dot{V}O_2$/force, calling that a "slow-component-like response". Keir et al. (Keir, Benson et al. 2016) also performed their experiments in the severe domain. The finding of a strong association between peripheral fatigue and $\dot{V}O_2$sc amplitude suggests that the fatigued muscle fiber pool remained capable of generating the required power to continue exercise but with a greater O_2 cost. Although the aforementioned evidence suggests a link between fatigue and the $\dot{V}O_2$sc, others have found different outcomes in the severe domain. For instance, Hopker and colleagues prefatigued the locomotor

muscles used during subsequent severe-intensity exercise. Participants completed either a nonmetabolically stressful 100 intermittent drop-jump protocol (prefatigue condition) or rested (control) for 33 min, and the results showed that locomotor muscle fatigue (reduction in power in the maximal voluntary cycling power test) was not associated with the development of the $\dot{V}O_2sc$ (Hopker, Caporaso et al. 2016). Furthermore, the magnitude of the $\dot{V}O_2sc$ was not significantly different between the two conditions despite significant differences in locomotor muscle fatigue. Looking for the cause-effect relationship between the $\dot{V}O_2sc$ and fatigue, Dos Nascimento and colleagues (do Nascimento Salvador, Souza et al. 2018) switched from constant-WR to isokinetic pedaling to quantify reductions in Pt at 3 and 8 min, with and without priming exercise. The results showed that the $\dot{V}O_2sc$ after priming was reduced, but there were no significant differences between conditions regarding the magnitude of the reduction in the maximal isokinetic force and power at 3 and 8 min. The authors concluded that this observation refutes a cause-effect relationship between fatigue and $\dot{V}O_2sc$ development (do Nascimento Salvador, Souza et al. 2018). The same group (do Nascimento Salvador, Schäfer et al. 2019) found similar results one year later. In their study, subjects performed transitions to severe intensity after unloaded or moderate-intensity cycling exercises. Maximal kinetic efforts were performed before and after both conditions. The results showed that the elevated WR led to significantly lower values of $\dot{V}O_2sc$ amplitude and slower values of τ in both sexes, reflecting type II muscle fiber characteristics. However, these alterations in VO_2 kinetics did not reflect alterations in muscle force production, challenging a cause-effect relationship between the $\dot{V}O_2sc$ and muscle fatigue.

Hence, although there is still controversy regarding the severe domain, the data redundantly show that when the CP threshold is surpassed, the rate of fatigue development increases suddenly. This fatigue appears to be the product of metabolic instability, especially the accumulation of Pi and its role in SR precipitation with Ca^{2+} and the limitation of Ca^{2+} release after excitation (Allen, Lamb et al. 2008). Further, the data suggest that this fatigue evolves in accordance with the $\dot{V}O_2sc$ and represents an increased cost of locomotion.

In line with these assumptions are the results of the second study of this thesis (chapter 10). During exercise performed in the severe domain, a significant relationship with ΔPT (r = -0.70) and ΔMRFD (r

=-0.61) was observed, showing a partial link with the $\dot{V}O_2$sc. The alteration of these parameters suggested that alterations of excitation-contraction failure were expressed in the severe domain.

Taking into account that Pi is considered the largest contributor to fatigue and that the rate-limiting step for a power stroke to occur is the detachment of Pi from the myosin head, an increase in the Pi concentration could therefore enable a decrease in force production or ΔMRFD per cross-bridge (Fitts 2008).

Certainly, the effect of the accumulation of metabolites, in particular the accumulation of Pi, has been very recently illustrated by the theoretical study of Korzeniewsky and Rossiter (Korzeniewski and Rossiter 2020). The "Pi double threshold hypothesis" established Pi accumulation as the major factor involved in fatigue processes. But also for the beginning, the magnitude and the evolution of the $\dot{V}O_2$sc and the extra ATP usage. The logic of the theory is that the additional ATP usage (P/ATP ratio) that underlies the $\dot{V}O_2$sc and muscle inefficiency is initiated when Pi exceeds a certain critical Pi concentration termed Pi_{critic}. Exercise intolerance results when another, larger, peak value is reached, termed $Pi_{peak.}$ Finally, mutual stimulation gives rise to the additional ATP that elevates Pi_{critic} to Pi_{peak} through self-driving feedback as a function of Pi-Pi_{critic}. In other words, when a certain critical value of Pi is exceeded, a series of metabolic events occur that determine fatigue and with it the end of muscular activity. The sooner this threshold is reached, the earlier the exercise will be compromised and the larger the $\dot{V}O_2$sc magnitude will be (Korzeniewski and Rossiter 2020). The same authors also demonstrated through computer simulations that changes such as increases in the $\dot{V}O_{2max}$ and CP, reductions in Tao, $\dot{V}O_2$sc and Pi peak accumulations induced by long-term endurance training can be caused by an increase in oxidative phosphorylation and a decrease in the Pi peak (Korzeniewski and Rossiter). This theory justifies the development of fatigue and partly the slow component in the severe domain.

According to the results of Rossiter (Korzeniewski and Rossiter 2020), the accumulation of Pi would be one of the final perpetrators of VO_{2sc} development. Type II muscle fibers have a higher concentration of Cp at rest (Meyer, Brown et al. 1985, Kushmerick, Moerland et al. 1992), and as previously mentioned, other authors (Barstow, Jones et al. 1996, Pringle, Doust et al. 2003, Deley, Millet et al.

2006, Conde Alonso, Gajanand et al. 2020) have proposed a link between the $\dot{V}O_2$sc rise and glycolytic type II muscle fibers.

Thus, taking all results together, studies show that the $\dot{V}O_2$sc in the severe domain is the superimposition of three quite distinct phenomena. That is, the emergence of the slow component is attributable to a significant increase in the $\dot{V}O_2$ of ventilation to keep homeostasis as stable as possible; a metabolic shift between anaerobic and aerobic metabolism; and finally the accumulation of Pi above a critical threshold (Pi critic) increases the rise in ATP demand, possibly under the influence of type II muscle fiber metabolism. This scenario is illustrated in figure 43.

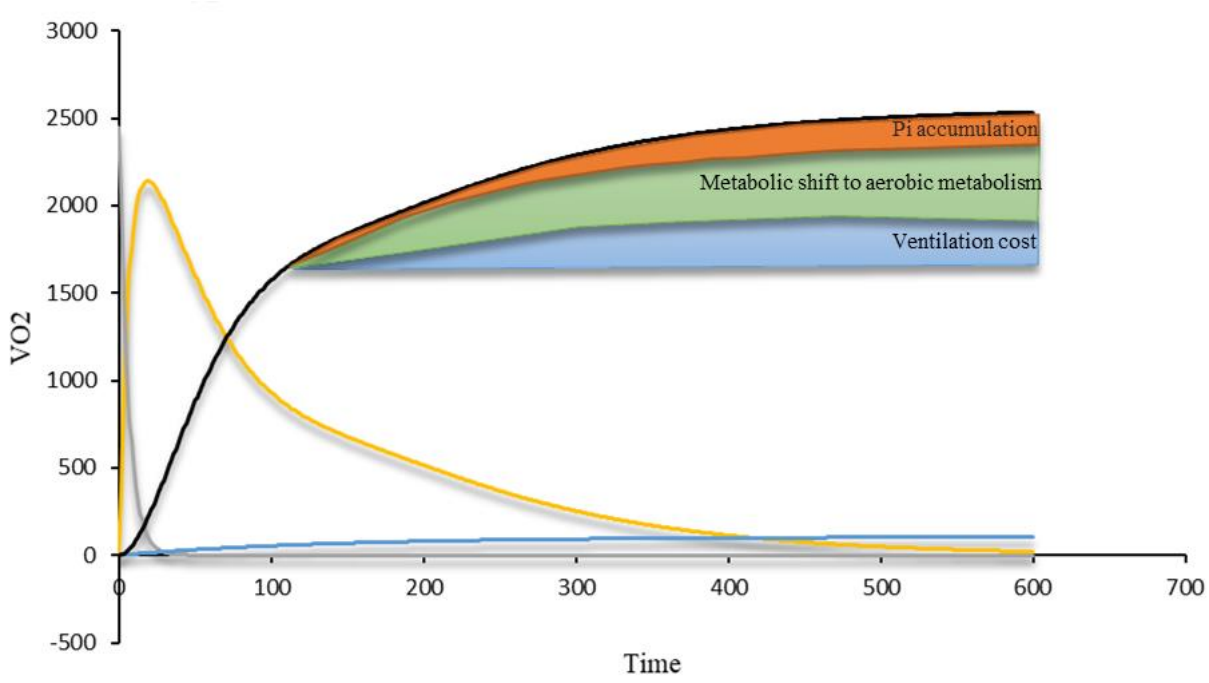

Figure 43 Hypothetical model of VO_{2sc} in the severe domain. *The gray line represents the energy cost of the anaerobic alactic metabolism; the yellow line represents the energy cost of the anaerobic lactic metabolism; the blue line represents the aerobic energy cost of ventilation and the black line represents the aerobic muscular energy cost + the cost of ventilation. The blue, green and red zones represent an increase in the VO_2 cost of ventilation, the metabolic shift from anaerobic to aerobic metabolism and the Pi accumulation, respectively, explaining the appearance of the VO_{2Sc}.*

13 Conclusion

Based on the findings of the original studies included in this manuscript and in view of what has been published in the literature, fatigue in the heavy domain does not appear to be the cause of the appearance and development of the $\dot{V}O_{2sc.}$ Further, even if there is fatigue in the heavy domain, a temporal relationship between $\dot{V}O_{2sc}$ development and fatigue does not appear to exist. In the severe domain, there is still controversy, even though significant correlations have been found between fatigue processes, such as diminution in Pt and MRFD, and the progress of the $\dot{V}O_{2sc.}$ The largest contributors seem to be alterations in the metabolic environment at these severe intensities, especially the accumulation of Pi and its role in entering the SR to bind with Ca^{2+}-insoluble compounds.

The latest publications point to a new hypothesis, suggesting that the slow component in the heavy and severe domains is not the product of an identical mechanism and therefore must be evaluated by taking into account the intensities at which it emerges. For instance, in the heavy domain, an increase in the $\dot{V}O_{2sc}$ has been ascribed to a metabolic shift rather than an increase in the O_2 cost of locomotion. In contrast, in the severe domain, an increase in the $\dot{V}O_{2sc}$ could not be explained totally by a prolonged metabolic shift, a possible indication that a true loss of efficiency may exist. Indeed, several publications point towards the role that type II muscle fibers (characterized by greater O_2 cost, PCr content and lower efficiency) play in the kinetics of $\dot{V}O_2$ and therefore in the increase in the $\dot{V}O_{2sc}$. This thesis provides additional evidence supporting the idea that the kinetics of $\dot{V}O_2$ are mainly driven by the metabolic response profiles of different fiber-type populations.

14 Perspectives

Future research should expand the current knowledge obtained by combining high-density surface integrated electromyography (HDEMG) and work-to-work protocols to unveil the MU recruitment pattern in the different domains of exercise.

As mentioned in this manuscript, one of the most prevalent hypotheses explaining the appearance of the $\dot{V}O_{2sc}$ is the progressive increase in MU recruitment during supra-LT exercise (Whipp 1994).

This increase in recruitment would be necessary to compensate for the deficiency of type I slow-twitch fibers during heavy-intensity exercise and maintain force production to counter this muscle fatigue (Gaesser and poole 1996). Some authors have found fatigue as a candidate for the appearance of the $\dot{V}O_{2SC}$ when the decrease in efficiency, reflected as an increase in $\dot{V}O_2$ during constant power output, was correlated with an increase in the iEMG (Moritani, Sherman et al. 1992). However, others have found different results with $\dot{V}O_{2SC}$ evident with no corresponding changes in the iEMG or MPF (Scheuermann, Hoelting et al. 2001).

Therefore, a manner to decipher if there is or not increase in MU recruitment above the GET reflecting fatigue, would be indeed, the combination of the work-to-work protocols and a technique to unveil MU recruitment.

The final iEMG signal is no other thing than the summation and time-course of the pool of MUAP, and gives global and rarely individual information about MUs activity (Cavalcanti Garcia and Vieira 2011). The advantage of the HDEMG is that it gives information of each MU.

Figure 44, illustrate how when a motoneurons discharge an axonal action potential, it arrives to the muscle and the MU (composed with that motoneurons axon and the fibers that innervates) generates an action potential in the muscle. The action potential of each MU discharge is unique! (Del Vecchio, Holobar et al. 2020).

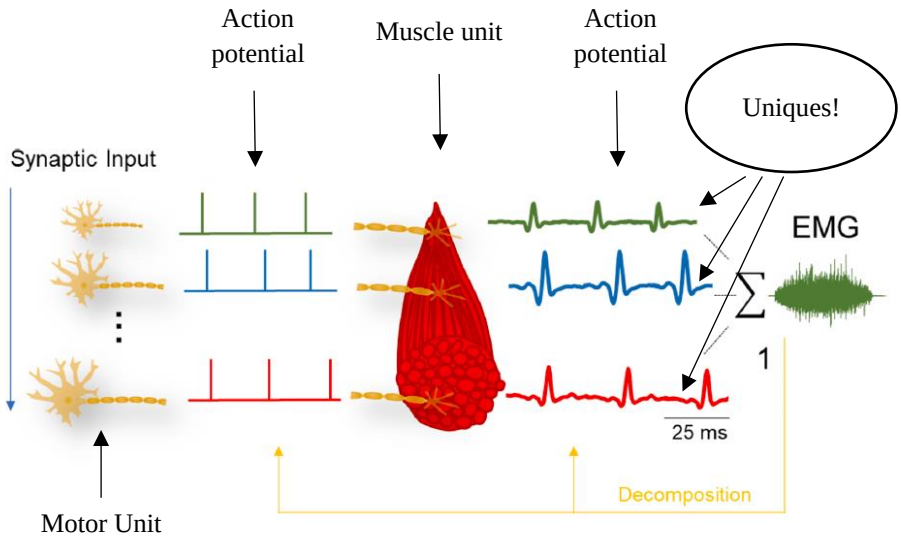

Figure 44 The one-to-one correspondence between axonal action potentials and MUAP. *Adapted from del Vecchio 2020.*

HDEMG, based on blind source separation methods, allows decomposing from the pull of the iEMG each one of the action potentials, going back to the spinal cord and identifying unique discharge patterns of each MU. With this technology is possible to identify the discharge times of individual MU during voluntary contractions and therefore compare the MU properties through subjects and time (Del Vecchio, Holobar et al. 2020). More important in this case, it is possible to direct estimate the neural drive to the muscle. i.e, neural drive would increase in presence of fatigue.

For instance, as illustrated in figure 45, and following with the hypothesis of this thesis, with HDEMG it would be possible to unveil the MU implicated in each domain.

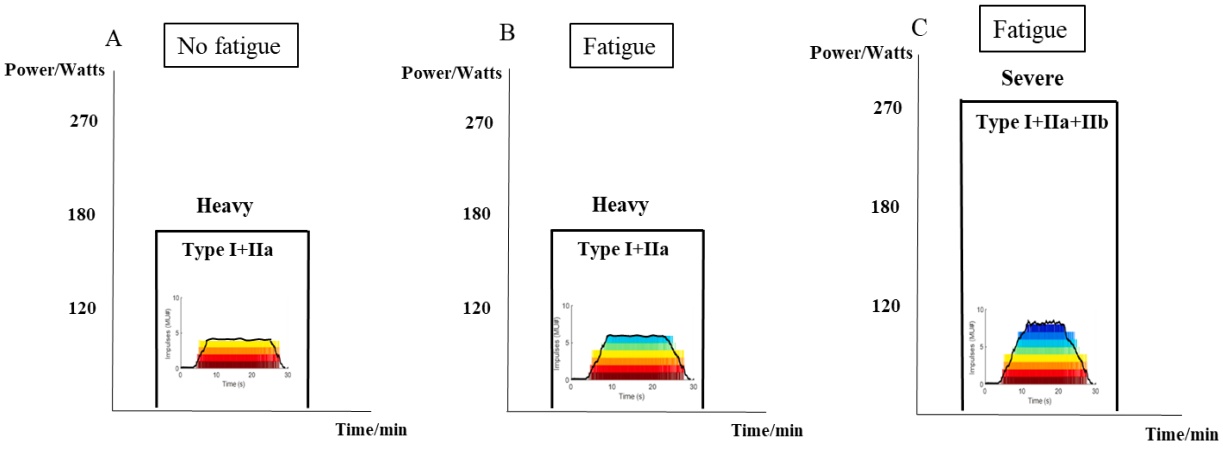

Figure 45 Hypothetical description of the experimentation.

That it to say, scenario 45A shows a constant work rate exercise in the heavy intensity domain with no fatigue, the recruited MU are the correspondent to the intensity following Henneman principle. Scenario 45B shows a constant work rate exercise in the heavy intensity domain with fatigue, and therefore with an increase in the number of the MU recruited during the course of the exercise. Finally, scenario 45C shows a constant work rate exercise in the severe domain in presence of fatigue, i.e, with an increase of MU recruited.

From this experimentation could rise another question: Which is the real cause of the appearance of this fatigue in severe domain?

Using 31-phosphorus magnetic resonance spectroscopy (31P-MRS) it could be possible to measure de decrease and resynthesis of PCr during the exercise performed in the severe domain (Bartlett, Fitzgerald et al. 2021). In this way, it could be possible to confirm the fatigue hypothesis based on Korzeniewski publication stablished on the "Pi double threshold hypothesis" (Korzeniewski and Rossiter 2020).

Furthermore, other hypothesis trying to explain the phenomenon of the $\dot{V}O_{2sc}$ is the increase in ATP cost of force production for a given increase in muscle workload (Rossiter, Ward et al. 2002, Whipp, Rossiter et al. 2002). Therefore with these techniques it could be also possible to decipher if there is a real increase in ATP cost of force production during heavy and severe intensities concomitant with the appearance or development of the $\dot{V}O_{2sc}$ (Valkovič, Chmelík et al. 2017).

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(Stienen, Kiers et al. 1996)

16 Appendix. Articles published

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SHORT COMMUNICATION Open Access

The metabolic profiles of different fiber type populations under the emergence of the slow component of oxygen uptake

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Abstract

To investigate the influence of different metabolic muscle fiber profiles on the emergence of the slow component of oxygen uptake ($\dot{V}O_{2\,SC}$), 12 habitually active males completed four sessions of different combinations of work-to-work transition exercises up to severe intensity. Each transition was modeled to analyze the different kinetic parameters. Using a new approach, combining Henneman's principle and superposition principle, a reconstructed kinetics was built by temporally aligning the start of each new transition and summing them. The primary phase time constant significantly slowed and the gain at the end (GainEnd) significantly increased when transitions started from a higher intensity ($p < 0.001$). Kinetic parameters from the reconstructed curve ($\dot{V}O_2$baseline, time delay of primary phase, $\dot{V}O_2$ End and GainEnd) were not significantly different from one transition to severe exercise. These results suggest that the appearance of the $\dot{V}O_{2SC}$ is at least related to, if not the result of, the different metabolic properties of muscle fibers.

Keywords: Oxygen consumption kinetics, Slow component, Muscle fatigue, Muscle fibers' metabolic properties

Background

The fundamental response of muscle oxygen consumption ($\dot{V}O_2$) kinetics, during moderate transition, may closely reflect the kinetics of $\dot{V}O_2$ in the contracting muscles [1]. At a constant work rate exceeding the gas exchange threshold (GET), this response is characterized by a delayed-onset of the new metabolic requirements, defined as the '$\dot{V}O_2$ slow component' ($\dot{V}O_{2SC}$), elevating $\dot{V}O_2$ above the 'steady-state' value predicted for this work rate [2–4]. This excess $\dot{V}O_2$ is a reflection of a loss of muscle efficiency [5]. To date, the putative mechanisms of $\dot{V}O_{2SC}$ are poorly understood, but several hypotheses have been proposed. Among these is the potential influence of the different metabolic response profiles of different fiber type populations during the development of the $\dot{V}O_{2SC}$. Indeed, it has been shown that the mammalian skeletal muscle is composed of different cell populations, with different metabolic and mechanical characteristics, mitochondrial content, and contractile proteins [6]. Kushmerick et al. [7] compared the $\dot{V}O_2$ of the two different muscle fiber types, demonstrating that the mechanism of control of cellular respiration is quantitatively and qualitatively different in fast and slow muscle fibers. Also, Stienen et al. [8] showed in their study using single muscle human fibers during isometric contraction, that the ATP consumption depends on the myosin isoform composition. In specific, the ATP consumption in fast IIb fibers was fourfold larger than in slow type I. In addition, it has been shown in animals that respiration of mitochondria [9], the mitochondrial volume density [10] and the mitochondrial rate of O_2 consumption [11] are greater in type I compared with type II muscle fibers. These differences between the slow and fast switch motor units may have an impact on the kinetics of mitochondrial oxidative phosphorylation during

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exercise above the gas exchange threshold (GET) and thus, contribute to the appearance of the $\dot{V}O_{2SC}$.

The size principle [12] posits that skeletal muscle fibers are recruited in a hierarchical manner during exercise according to intensity. To manipulate motor unit recruitment and reveal the metabolic response profiles of different fiber type populations, "work-to-work" step exercise has been used [13–15]. For instance, transitions between low work rate intensities would be expected to solicit the recruitment of muscle fibers that are positioned lower in the recruitment hierarchy (i.e., slow type fibers), whereas a transition between high work rate intensities would be expected to involve the recruitment of muscle fibers positioned higher in the recruitment hierarchy (i.e., fast type fibers) [16]. Thus, it should be possible to distinguish the effect of the recruitment of new motor units residing higher in the recruitment hierarchy during the $\dot{V}O_2$ kinetics while completing transitions between different exercise intensities (moderate, heavy and severe). In addition, because each fiber that contributes to tension development is as a unique system unto itself, and because the pulmonary $\dot{V}O_2$ signal homogenizes any oxidative response diversity within the activated pool of motor unit, the principle of superposition might be applied.

Therefore, in accordance with the Henneman and the superposition principle, and considering that the appearance of the $\dot{V}O_{2SC}$ is due to the difference in mitochondrial oxidative phosphorylation kinetics between fibers' types, the differences in $\dot{V}O_2$ kinetics between a single work transition and a work-to-work transition of an equal final power may be due to the temporally shift of MU activation residing higher in the recruitment hierarchy. In keeping with this, temporally aligning the beginning of each new transition [activation of new fibers positioned higher in the recruitment hierarchy (Henneman principle)] and summing them (superposition principle) to form new reconstructed kinetics should not give a different $\dot{V}O_2$ kinetics with that measured in a simple transition to equal final power.

The purpose of this study was to add novel evidence to the debate of the origins of the $\dot{V}O_{2SC}$, specifically, if the different metabolic response profiles of different fiber type populations are one of the culprits in the development of the $\dot{V}O_{2SC}$.

The hypotheses were

The time constant (τ) would be significantly smaller between low-intensity work rate transitions compared with transitions between high work rate intensities.

The reconstructed $\dot{V}O_2$ kinetics from multiple transitions would, in fact, have an identical kinetic to a simple transition at the same final intensity.

Methods

Twelve healthy habitually active males aged 18–50 years (mean ± SD: age 24.33 ± 0.72 years, height 178.41 ± 7.76 cm, weight 76.31 ± 11.62 kg) were recruited to participate in this study. Participants were excluded if they, or their family, suffered from any heart or cardiovascular condition, bleeding disorder, or were taking prescribed medication. Participants were instructed to refrain from training and other vigorous physical activities, alcohol consumption, caffeine intake and tobacco for a minimum of 24 h before experiments. Participants were advised to arrive at the laboratory in a rested, fully hydrated, and at least 3 h postprandial state. The research protocol was accepted by local Human Participants Ethics Committee, and completed according with the seventh Declaration of Helsinki (2013). Prior to participation in the study, the protocol and possible risks involved were explained to all participants before written informed consent was collected. All participants were advised of their right to withdraw from the study at any time without prejudice.

The experimentation required five visits to the laboratory. The tests included a first session of a ramp incremental test on cycle ergometer (Velotron racemate Inc Spearfish, USA) to assess GET and peak oxygen uptake ($\dot{V}O_{2peak}$). The ergometer seat and handlebars were adjusted for comfort, and the measurements were recorded to reproduce a consistent setup for the subsequent tests. On subsequent days, participants completed an additional four sessions with various combinations of work-to-work transitions across a wider range of square-wave exercises. Heart rate (RS800, Polar, Finland) and pulmonary gas exchange were continuously measured using a computerized system (Metamax 3B, Cortex GmbH, Leipzig, Germany) during all sessions. Testing took place at a similar time of the day (± 2 h), conducted in a temperature-controlled laboratory (maintained at 18 ± 1 °C).

During the ramp incremental test, participants rested for 3 min on the cycle ergometer before cycling for 6 min with a load of 60 W at a comfortable self-selected pedal rate between 70 and 90 rpm, what was reproduced for subsequent tests. The power was then increased in a ramp fashion of 30 W/min until volitional exhaustion or till one of the American College of Sports Medicine established criteria for maximal testing was reached [17]. Verbal encouragement was given to the participants during the test. GET was determined by (1) the first disproportionate increase in carbon dioxide output ($\dot{V}O_2$) from visual inspection of individual plots of $\dot{V}CO_2$ vs. $\dot{V}O_2$; (2) an increase in ventilatory equivalents for oxygen and not in carbon dioxide; and (3) an increase in end-tidal O_2 tension with no fall in end-tidal CO_2 tension. $\dot{V}O_{2peak}$ was determined as the highest

value in a 30-s range recorded before the participant volitional exhaustion.

On subsequent visits, participants performed a various combinations of work-to-work transitions between moderate (M, 6 min at the 80% of the GET), heavy (H, 6 min at 20% of the difference in power between GET and the $\dot{V}O_{2peak}$) and severe (S, 6 min at 60% of the difference in power between GET and the $\dot{V}O_{2peak}$) intensity exercises. After 3 min at rest and 3-min "unloaded" baseline cycling, participants started one of the following four protocols: (1) M followed by S (M → S); (2) H followed by S (H → S); (3) M followed by H and by S (MH → S); (4) S followed by 3-min rest (S) followed by 3-min "unloaded" baseline and by S (SPost). Note that the last protocol provided data for S and SPost.

During the session, participants completed exercise twice separated by 1-h break in pseudo-randomized manner as each exercise was performed once at first place and once in second place.

$\dot{V}O_2$, pulmonary gas exchange and ventilation were computed breath-by-breath. Prior to each test, the calorimeter and turbine were calibrated using ambient air and gases of known concentration (O_2 = 14.01%, CO_2 = 6.03%) and 3-L calibration Rudolf syringe (cortex, Leipzig, Germany), respectively.

The breath-by-breath $\dot{V}O_2$ data were initially examined to eliminate errant values caused by coughing, swallowing, etc., and values laying more than three 3 SDs from the local mean. Linear interpolation was used to provide second-by-second data, and for each individual, identical repetitions were time aligned to the start of exercise and the ensemble averaged. Mono-exponential equation was computed to isolate the primary component of the $\dot{V}O_2$ kinetics using the iterative method proposed by Rossiter et al. [18]

$$\dot{V}O_2(t) = \dot{V}O_2\text{baseline} + A_p\left(1 - \exp^{-(t - TDp)/\tau p}\right)$$

where $\dot{V}O_2(t)$ is the time course of $\dot{V}O_2$, $\dot{V}O_2$baseline is the oxygen consumption at the beginning of exercise, A_p is the amplitude, TD_p is the time delay, and τ_p is the time constant of the primary phase, respectively. The first 20 s of the pulmonary $\dot{V}O_2$ signal was removed from analysis since it has been demonstrated that the cardiodynamic phase of the $\dot{V}O_2$ kinetics does not represent an increased muscle O_2 consumption [19]. Identification of the end of the primary phase was made by criteria consideration as recommended by Rossiter et al. [20] and Murgartroyd et al. [21]. The magnitude of the slow component (As) was defined as the difference between the $\dot{V}O_2$ projected primary phase and the averaged amplitude from the last 30 s of the response (termed $\dot{V}O_2$end). The Gain Amplitude (GainAp) was defined as the increase in $\dot{V}O_2$ above baseline per unit increase in external work rate above baseline, $\dot{V}O_2/\Delta WR$. The Gain End (GainEnd) as the sum of the A_p and As per unit increase in external work rate above baseline ($A_p + As/\Delta WR$). The mean response time (MRT) was calculated as the sum of the Td_p and τ_p.

The superposition principle was applied to build the reconstructed curve. The start of each transition was time aligned and baseline was set at zero to sum the different kinetics curves as represented in Fig. 1. The parameters of the reconstructed curve were therefore calculated using the iterative method proposed by Rossiter et al. [18] (see above). Therefore, the reconstructed curve of M → S turns into MS; H → S turns into HS and MH → S turns into MHS.

Data statistical analysis

Analyses were performed using Jamovi (Version 0.9.5.17 [Computer Software], retrieved from https://www.jamovi.org). Linear mixed model was the statistical test used to compare the $\dot{V}O_2$ kinetics parameters between the different conditions. Condition was the fixed effects and participant as the random effect. After inspecting residual plots, no obvious deviations from homoscedasticity or normality were present.

Linear mixed model was also used to temporally analyze data of the overall $\dot{V}O_2$ constructed kinetics, to compare the different conditions. The fit between the $\dot{V}O_2$ constructed kinetics was assessed by summing percent of time of $\dot{V}O_2$ constructed kinetics where differences were not significant. For all tests, the level of significance was set at 0.05 and dispersion about the mean expressed as SD.

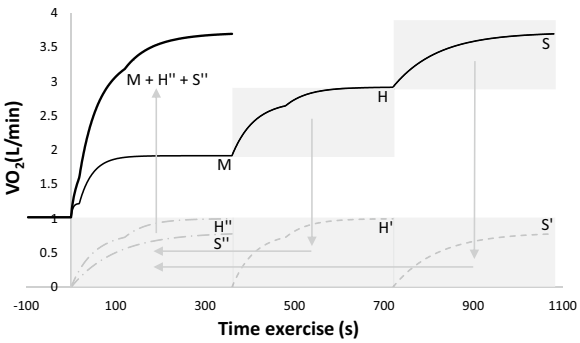


Fig. 1 Illustration of reconstructed method used to analyze the work-to-work transitions protocol kinetics. Letters M, H and S represent the model for Moderate, Heavy and Severe intensities, respectively; H′ and S′ represent model for Heavy and Severe kinetic curves when $\dot{V}O_2$ baseline was set at zero; H″ and S″ represent heavy and severe kinetic curves when time was aligned to zero; M + H″ + S″ represents the reconstructed curve with the sum of the three different intensities

Results

Relative $\dot{V}O_2$ was 55.77 ± 5.21 mL kg^{-1} min^{-1}. The parameters of the $\dot{V}O_2$ response for each different transition are reported in Table 1. $\dot{V}O_2$baseline was significantly different ($p < 0.001$) between conditions except between S and Spost, and between H → S and MH → S conditions. Concerning Ap, only the comparison between H → S or MH → S was not significantly different. All other comparisons had a p value below 0.001, other than between S and Spost ($p = 0.031$). GainAp was significantly different between conditions ($p < 0.003$), other than between SPost and both S and M → S. TDp was not significantly different between any of the conditions. As for $\dot{V}O_2$ baseline, τp was significantly different ($p < 0.002$) between conditions except between S and SPost, and between H → S and MH → S. Regarding MRT, H → S and MH → S were significantly slower ($p < 0.001$) compared with S, SPost, and M → S, respectively. As was different between all conditions ($p < 0.004$), apart from between H → S and MH → S. Concerning $\dot{V}O_2$End, H → S was significantly different to both M → S ($p = 0.007$) and SPost ($p = 0.037$). GainEnd was not significantly different between S and SPost, and between H → S and MH → S conditions. All other comparisons were significantly different (p value range < 0.001 to 0.047).

The parameters of the results of the reconstructed method reported in Table 2 showed that $\dot{V}O_2$baseline, TDp, $\dot{V}O_2$End, and GainEnd were not significantly

Table 1 Comparison of the parameters of the $\dot{V}O_2$ response kinetics in the different transitions

	S	SPost	M → S	H → S	MH → S
$\dot{V}O_2$ Baseline (L min^{-1})	1.07 ± 0.16	1.04 ± 0.21	1.85 ± 0.21*§	2.89 ± 0.23$^{*§£}$	2.90 ± 0.25$^{*§£}$
Ap (L min^{-1})	2.02 ± 0.27	2.16 ± 0.30*	1.43 ± 0.26*§	0.77 ± 0.15$^{*§£}$	0.70 ± 0.15$^{*§£}$
Gain Ap (mL min^{-1} W^{-1})	8.96 ± 0.73	9.56 ± 0.78	10.23 ± 0.60*	13.70 ± 1.50$^{*§£}$	12.27 ± 1.16$^{*§£$}$
TDp (s)	10.21 ± 5.54	11.76 ± 4.72	6.17 ± 5.37	6.06 ± 8.81	7.16 ± 9.83
τp (s)	29.31 ± 9.46	28.27 ± 6.79	46.51 ± 11.00*§	95.94 ± 19.61$^{*§£}$	93.43 ± 13.83$^{*§£}$
MRT (s)	39.52 ± 5.28	40.03 ± 4.21	52.67 ± 12.46	102.00 ± 27.57$^{*§£}$	100.59 ± 18.17$^{*§£}$
As (L min^{-1})	0.56 ± 0.13	0.44 ± 0.10*	0.33 ± 0.10*§	0.07 ± 0.07$^{*§£}$	0.10 ± 0.07$^{*§£}$
$\dot{V}O_2$ End (L min^{-1})	3.65 ± 0.38	3.63 ± 0.35	3.61 ± 0.37	3.73 ± 0.33$^{§£}$	3.70 ± 0.37
GainEnd (mL min^{-1} W^{-1})	11.47 ± 0.78	11.56 ± 0.67	12.62 ± 0.78*§	15.00 ± 1.96$^{*§£}$	14.11 ± 1.55$^{*§£}$

S: severe intensity; SPost: severe intensity after prior severe intensity; M: moderate intensity; H: heavy intensity. $\dot{V}O_2$ baseline: oxygen consumption at the beginning of exercise; Ap: amplitude of the primary phase; GainAp: increase in $\dot{V}O_2$ above baseline per unit increase in external work rate above baseline; TDp: time delay of the primary phase; τp: time constant of the primary phase; MRT: the sum of the TDp and τp; As: amplitude of the secondary phase; $\dot{V}O_2$ end, averaged amplitude from the last 30 s of the response; GainEnd: sum of the Ap and As per unit increase in external work rate above baseline. Values are presented as the mean SD

*Significant differences with S; §significant differences with SPost; $^{£}$significant differences with M → S; $^{$}$significant differences with H → S ($p < 0.05$)

Table 2 Comparison of the parameters of the $\dot{V}O_2$ response kinetics of the reconstructed curve for the different transitions' protocols

	S	SPost	MS	HS	MHS
$\dot{V}O_2$ Baseline (L min^{-1})	1.07 ± 0.16	1.04 ± 0.21	0.97 ± 0.19	1.02 ± 0.23	1.01 ± 0.21
Ap (L min^{-1})	2.02 ± 0.27	2.16 ± 0.30	2.24 ± 0.29*	2.15 ± 0.30	2.24 ± 0.30*
Gain Ap (mL min^{-1} W^{-1})	8.96 ± 0.73	9.56 ± 0.78	9.95 ± 1.02*	9.55 ± 1.20	9.95 ± 1.12*
TDp (s)	10.21 ± 5.54	11.76 ± 4.72	11.41 ± 6.34	13.19 ± 4.73	10.64 ± 5.69
τp (s)	29.31 ± 9.46	28.27 ± 6.79	31.25 ± 7.51	31.00 ± 6.69	40.58 ± 11.68$^{*§£$}$
MRT (s)	39.52 ± 5.28	40.03 ± 4.21	42.65 ± 5.86	44.19 ± 4.25	51.22 ± 7.07$^{*§£$}$
As (L min^{-1})	0.56 ± 0.13	0.44 ± 0.10	0.41 ± 0.15*	0.53 ± 0.11	0.46 ± 0.17
$\dot{V}O_2$ End (L min^{-1})	3.65 ± 0.38	3.63 ± 0.35	3.62 ± 0.41	3.70 ± 0.34	3.71 ± 0.38
Gain End (mL min^{-1} W^{-1})	11.47 ± 0.78	11.56 ± 0.67	11.77 ± 0.92	11.90 ± 1.20	11.99 ± 0.84

MS: kinetics sum of M + S; HS: Kinetics sum of H and S; MHS: kinetics sum of M + H+S. S: severe intensity; SPost: severe intensity after prior severe intensity; M: moderate intensity; H: heavy intensity. $\dot{V}O_2$ baseline: oxygen consumption at the beginning of exercise; Ap: amplitude of the primary phase; GainAp: increase in $\dot{V}O_2$ above baseline per unit increase in external work rate above baseline; TDp: time delay of the primary phase; τp: Time constant of the primary phase; MRT: the sum of the TDp and τp; As: amplitude of the secondary phase; $\dot{V}O_2$ end: averaged amplitude from the last 30 s of the response; GainEnd: sum of the Ap and As per unit increase in external work rate above baseline. Values are presented as the mean SD

*Significant differences with S; §significant differences with SPost; $^{£}$significant differences with MS; $^{$}$significant differences with HS ($p < 0.05$)

different between conditions. Ap was significantly different between S and both MS ($p=0.011$) and MHS ($p=0.010$). Furthermore, GainAp was significantly different between S and both MS ($p=0.007$) and MHS ($p=0.007$). Concerning τp, MHS was significantly slower compared with S ($p=0.011$), SPost ($p=0.004$), MS ($p=0.041$), and HS ($p=0.038$). Similarly, MRT from MHS was significantly slower compared with S ($p<0.001$), SPost ($p<0.001$), MS ($p=0.002$), and HS ($p=0.016$). Regarding As, S was slightly different ($p=0.043$) from MS. Reconstructed kinetics of the different conditions are depicted at the bottom of Fig. 2. Fit calculation indicated similarity between the reconstructed curves; indeed, the similitude average was 96.38% with a maximum of 100% (MS vs. SPost). The largest differences were observed only during 11.94% of exercise duration (MHS vs. SPost), at the beginning of primary phase.

Discussion

The main finding was that the reconstructed $\dot{V}O_2$ kinetics, using a novel approach of combining Henneman's principle with the principle of superposition, had a similar kinetic curve (96.4 ± 3.6% of similarity between conditions) to a simple transition at the same final severe intensity.

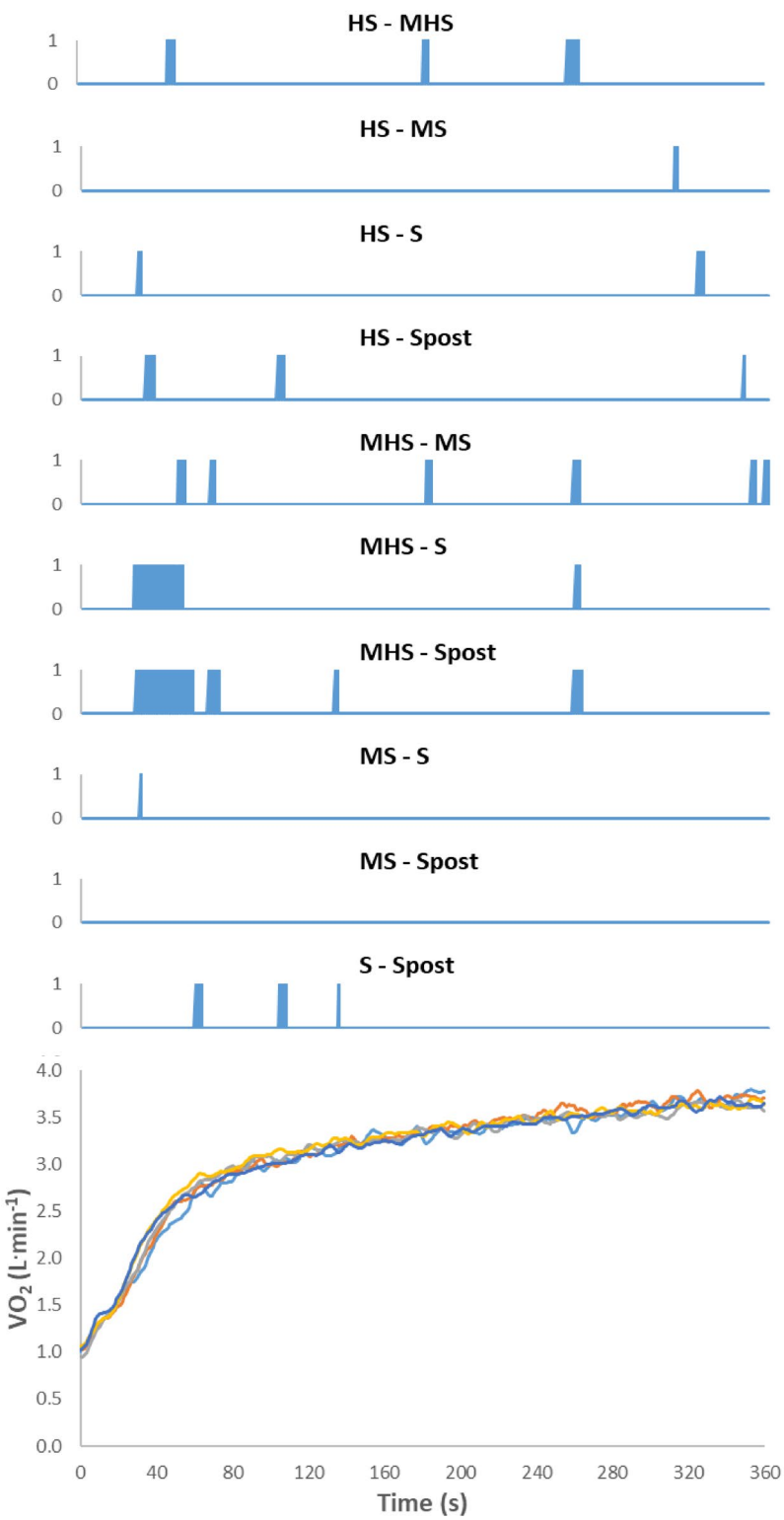
As hypothesized, when transitions started from a higher intensity, τ and Gain model parameters increased while amplitude parameters decreased, although $\dot{V}O_2$ end at the final transition was similar. These results are in line with previous studies [13, 22], which were interpreted as a reflection of metabolic differences in the pool of muscle fibers recruited under these specific circumstances. Indeed, the elevated baseline in the work-to-work protocols implies that type I MU are already recruited according to the well-established size principle of MU recruitment [23]; consequently, only a percentage fibers residing higher in the recruitment hierarchy would be activated during the second part of the protocol [13]. Type II muscle fibers are characterized by reduced mitochondrial content [24], lower oxidative enzyme activity [25] and greater ATP cost for force production [26]; therefore, slower $\dot{V}O_2$ kinetics and lower efficiency [27]. Consistent with the increased contribution of fibers with lower oxidative efficiency, the Gain amplitude of the primary phase was progressively increased when exercise was initiated from an elevated baseline [13, 22].

The second hypothesis was also validated since there were only scarce differences between the reconstructed kinetics and the work-to-work transitions (Fig. 2). The disparities were mainly in the first 40 s of the exercise due to a significant slower τp in MHS compared with the other conditions. However, during the time course of $\dot{V}O_{2SC}$ and at the end of exercise, only sporadic differences were observed.

The fact that each severe exercise, preceded by a different modality producing a different fatigue, had similar reconstructed kinetics, suggesting that (a) fatigue was not the main process involved in the $\dot{V}O_{2SC}$; (b) the progressive fiber recruitment, due to fatigue, was consequently not required for the development of the $\dot{V}O_{2SC}$. During work-to-work exercise, new fibers are activated at the beginning of each transition, modeling the $\dot{V}O_2$ kinetics response. The result of temporally aligning the kinetics of $\dot{V}O_2$ at the beginning of each transition and summing them seems to be similar to the result of a complete stimulation of the different fibers involved in a single transition of severe intensity exercise. This is consistent with the fact that the kinetics shape is mainly driven by the metabolic response profiles of different fibers populations. Several studies have demonstrated the link between the different profiles of fibers and the appearance of the $\dot{V}O_{2SC}$. The first authors to demonstrate that type I muscle fibers were significantly correlated with the $\dot{V}O_{2SC}$ were Barstow and colleagues [28]. They exercised participants at Δ50% and took muscle biopsies of the vastus lateralis for determination of fiber type. Participants with a higher percentage of type I muscle fibers had a higher primary phase and this was significantly correlated with the amplitude of the $\dot{V}O_{2SC}$ ($r=-0.83$). Previous findings indicate significant correlations between the percentage of type II muscle fibers and markers of aerobic fitness and the relative magnitude of the $\dot{V}O_{2SC}$ ($r=0.60$; $p<0.01$) and ($r=-0.73$; $p<0.01$), respectively [29]. These findings are in line with other studies relating to the percentage of type I muscle fibers with an improved efficiency, or reduced $\dot{V}O_2$, for the same work rate in cycling [30] or running [31]. Pringle et al. [32] took muscle biopsies from 14 participants for histochemical determination and made them complete square-wave

(See figure on next page.)

Fig. 2 Pulmonary oxygen response ($\dot{V}O_2$) of reconstructed curve. Upper panel illustrates time course comparison of the different reconstructed curves. Blue thick vertical squares represent the differences between protocols. On the right, fit calculations represent the percentage of equality between protocols. Lower panel shows the time course of all reconstructed curves. Light blue color represents MHS; red color represents HS; grey color represents MS; yellow color represents SPost; and dark blue color represents S. HS: reconstructed curve for Heavy and Severe intensities; MHS: reconstructed curve for moderate, heavy and severe intensities; MS: reconstructed curve for moderate and severe intensities; S, reconstructed for Severe intensity; SPost, reconstructed curve for severe intensity after prior severe intensity



FIT % equality
96.2
99.5
98.4
97.5
94.5
92.3
88.1
99.8
100
97.5

cycling tests at moderate, heavy and severe intensities. Percent of type I muscle fibers was correlated with the amplitude of the $\dot{V}O_{2SC}$ for heavy ($r = -0.74$; $p < 0.01$) and severe ($r = -64$; $p < 0.05$) exercises and with τ of the primary component ($r = -68$; $p < 0.01$) in heavy intensity. Indeed, after a protocol aiming for the depletion of glycogen from type II muscle fibers, there was a decrease in the amplitude of the $\dot{V}O_{2SC}$ [33]. Deley et al. [34] showed that after pre-fatiguing type II muscle fibers, the amplitude of the $\dot{V}O_{2SC}$ was significantly reduced, concluding that the recruitment of type II may be involved in the $\dot{V}O_{2SC}$ phenomenon. Krustrup et al. [35] confirmed the idea that the energy turnover and ATP cost were higher for type II fibers when a neuromuscular blockage of type I was performed. Certainly, muscle O_2 uptake was 20% higher and MRT was longer in type II muscle fibers, supporting the idea that type II fibers had slower kinetics and greater ATP cost than type I during dynamic exercise.

Finally, if the metabolic characteristics of the different fibers shape the $\dot{V}O_2$ kinetic curve and fatigue does not play a role in the development of $\dot{V}O_{2SC}$, neither will the progressive recruitment of these fibers. This result has been seen in isolated gastrocnemius dogs [36] and in the vastus lateralis in humans [37] when all muscle fibers were activated or when a systematic increase in the cost of O_2 per unit of external power was concomitant with no changes in iEMG, respectively. Taken together, these results suggest a lack of progressive muscle fiber recruitment during $\dot{V}O_{2SC}$.

Conclusion

These results confirm that τ is significantly smaller between low work rate transitions compared with transitions between high work rate intensities. In addition, the $\dot{V}O_2$ severe intensity kinetic curve is similar to the reconstructed kinetics curve resulting from combining Henneman's and superposition principles. These findings are consistent with the appearance of the $\dot{V}O_{2sc}$ and maybe linked to the intrinsic differences in metabolic properties of different fiber types.

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Authors' contributions
FB conceived and designed the experiments. FB and TG completed data collection. FB, TG, SCA, JSR and JA analyzed, interpreted, and revisited the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
The research protocol was accepted by University of Auckland Human Participants Ethics Committee (UAHPEC), and completed in accordance with the seventh version of the Declaration of Helsinki (2013).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interest.

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Alterations to neuromuscular properties of skeletal muscle are temporally dissociated from the oxygen uptake slow component

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To assess if the alteration of neuromuscular properties of knee extensors muscles during heavy exercise co-vary with the SCV ($\dot{V}O_2$ slow component), eleven healthy male participants completed an incremental ramp test to exhaustion and five constant heavy intensity cycling bouts of 2, 6, 10, 20 and 30 minutes. Neuromuscular testing of the knee extensor muscles were completed before and after exercise. Results showed a significant decline in maximal voluntary contraction (MVC) torque only after 30 minutes of exercise ($-17.01\% \pm 13.09\%$; $p < 0.05$) while single twitch (PT), 10 Hz (P10), and 100 Hz (P100) doublet peak torque amplitudes were reduced after 20 and 30 minutes ($p < 0.05$). Voluntary activation (VA) and M-wave were not affected by exercise, but significant correlation was found between the SCV and PT, MVC, VA, P10, P100, and P10/P100 ratio, respectively ($p < 0.015$). Therefore, because the development of the SCV occurred mainly between 2–10 minutes, during which neuromuscular properties were relatively stable, and because PT, P10 and P100 were significantly reduced only after 20-30 minutes of exercise while SCV is stable, a temporal relationship between them does not appear to exist. These results suggest that the development of fatigue due to alterations of neuromuscular properties is not an essential requirement to elicit the SCV.

At the onset of constant power exercise, the muscles requirements for ATP re-synthesis increase immediately following exercise onset. The same cannot be said about the oxygen uptake ($\dot{V}O_2$) response that instead, displays a sluggishness to fully activate metabolism[1–3]. During exercise below the lactate threshold, $\dot{V}O_2$ rises mono-exponentially to a new steady-state[3,4] and from unload pedalling, the rise of $\dot{V}O_2$ increases as a linear function of work-rate[5]. However, during constant-load exercise completed at intensities above the lactate threshold, the $\dot{V}O_2$ response becomes more complex with a second rise in $\dot{V}O_2$, developing slowly, which is superimposed onto the initial $\dot{V}O_2$ response[6]. This slowly developing rise in $\dot{V}O_2$, termed the slow component of $\dot{V}O_2$ (SCV), results in a greater end-exercise $\dot{V}O_2$ than that predicted by the sub-LT $\dot{V}O_2$-power output relationship. It has been proposed that the inefficiency which leads to the SCV originates primarily from the active muscles[7]. However, the reason for this observed inefficiency in the muscle is not clear and may potentially result from reduction of ATP production per mole of oxygen (P/O ratio), diminution of the energy yield per unit of hydrolysed ATP, alteration of neuromuscular properties of muscle filament to produce force, and/or deterioration of the motor pattern of the motion[8]. However, the potential link between the alteration of neuromuscular properties of muscle filament and progressive muscle inefficiency, and therefore the SCV, is not well explored. The capability of muscle to produce force progressively declines during high-intensity exercise when fatigue gradually develops[9]. It is widely accepted that alterations of the metabolic milieu of locomotor muscles are mainly responsible for the

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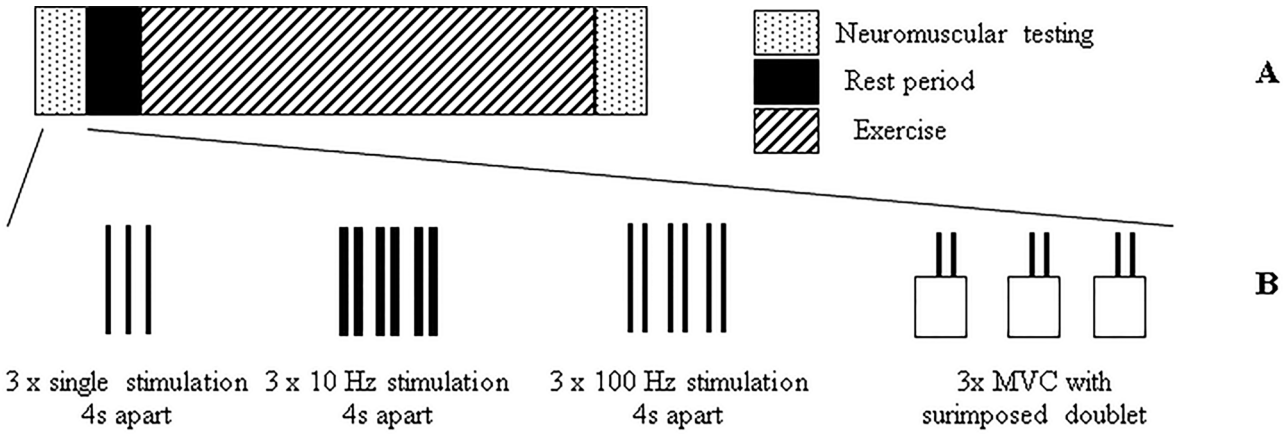

Figure 1. Description of events completed during experimental testing (**A**) and during neuromuscular testing (**B**). Neuromuscular tests (dotted box) were completed prior to the rest period (filled box) and after exercise (box with diagonal lines). Neuromuscular testing involved three single stimulations (single solid lines) followed by three stimulations at 10 Hz (thick-double solid lines) and then three stimulations at 100 Hz (thin-double solid lines). Each stimulation had a four second separation. Finally, three MVCs were completed with superimposed 100 Hz doublets applied (empty box with thin-double solid lines), each separated by a minute rest period.

decline in force. Indeed, neuromuscular properties of knee extensor muscles are sensitive to the accumulation of muscle metabolites such as adenosine diphosphate (ADP), inorganic phosphate (P_i), hydrogen ion (H^+), and magnesium ion (Mg^{2+}). Muscular force production is reduced by increases in [P_i], [Mg^{2+}], and [H^+] while augmented by an increase in [ADP][10]. Additionally, increased [ADP] reduces cross-bridge cycling rate[10].

Since the SCV occurs during high-intensity exercise, and because high-intensity exercise is always associated with changes in metabolite concentration that may produce an alteration in neuromuscular properties of muscle filament, the latter may be considered a putative mediator of the SCV (in line with current views;[11–14]).

Standardised investigative methods of neuromuscular function, such as peripheral nerve stimulation (PNS), have been extensively used to explore the complex relationship between exercise and fatigue. For instance, using PNS, Decorte and colleagues showed that during exhaustive constant-load cycling at 80% of maximum aerobic power output, neuromuscular properties were significantly reduced as early as 20% of the total duration of cycling, indicating a potential link with the SCV[15]. Although, little is known about the possible relationship between the SCV and the alteration of neuromuscular properties of knee extensors, Keir and colleagues[16] in 2016 showed a significant association between the decrements in muscle torque and the SCV, without changes in muscle activation over the course of the exercise. Also in an *in vivo* study using cycle ergometry, Cannon and colleagues have shown that changes in velocity-specific peak power generated in the initial minutes of exercise were correlated to the SCV measured between three and eight minutes of heavy and severe exercise[17]. Results from the same working group suggest that the SCV during heavy exercise arises from both contractile and mitochondrial sources[17]. Furthermore, using self-paced dynamic concentric extension/flexion of the knee and interleaving voluntary and electrically evoked contractions, Froyd and colleagues have shown, even without measuring directly VO_2 kinetics, that fatigue progresses with similar dynamics to those expected of the SCV during an approximately 6-min time trial[18]. However, these findings do not show the mechanism linking the alteration of neuromuscular properties of knee extensors, per se, and the SCV.

The aim of the present study was to quantify the alteration of neuromuscular properties of knee extensors during heavy exercise and to see if these impairments co-vary, as function of time, with the SCV amplitude. The hypothesis was that the SCV amplitude correlates with the change in neuromuscular properties of knee extensor muscles, depicted by a decrease in evoked peak torque.

Methods

Participants. Eleven healthy, recreationally active, male participants (mean ± SD, age 27 ± 6.6 years, body mass 76 ± 7.6 kg, and height 179 ± 8.1 cm) were recruited for this study. The participants were provided with a participant information sheet outlining the procedures involved, time commitment, and requirements of the study. Participants were screened using a self-administrated pre-exercise health questionnaire designed to identify those who may be at risk of an adverse event during exercise. Participants were advised of their right to withdraw from the study at any time without disadvantage.

Participants were asked to avoid, in the 24 h preceding a testing session, strenuous physical activity, alcohol, tobacco, and caffeine. Furthermore, participants were asked not to consume any food for the 3 h preceding a test and to arrive fully hydrated. All tests were completed at a similar time of day (±1 h). The study was approved by the local humans Ethics Committee and conformed to the latest revision (2013) of the Declaration of Helsinki. All participants provided written informed consent prior to participation.

Experimental design. This study involved each participant attending six separate laboratory sessions, with at least a 48 h interval between tests, over a three-week period. All tests were completed in an air-conditioned

(21 °C ± 1 °C) exercise physiology laboratory. The first session involved an incremental ramp test on a cycle ergometer (Velotron, RacerMate, Seattle, WA, USA). This test was used to assign a work-rate for the subsequent five experimental sessions during which constant work-rate exercise was completed. Following the incremental ramp test, participants were familiarised with the procedure to be used to evaluate neuromuscular function. The five experimental sessions (Fig. 1A) involved participants cycling for different durations of time in a random order at an identical power (heavy domain, see below). Neuromuscular evaluation was performed before exercise, and within 1-minute of completing constant work-rate exercise. This was completed to determine the central and peripheral fatigue through neural and neuromuscular properties of the knee extensor muscles.

Testing procedures. *Incremental ramp test.* Incremental ramp exercise test was completed in order to determine the gas exchange threshold (GET) and peak oxygen consumption ($\dot{V}O_{2peak}$). After a three-minute rest period seated on the cycle ergometer, participants performed six minutes of baseline cycling at 60 watts, after which, the work rate was increased by the rate of 30 watts each minute until reaching the limit of tolerance. The ergometer allows participants to cycle at a constant power output independent of pedal rate, though participants were asked to maintain a pedal rate of 85 revolutions per minute (rpm). Verbal encouragement was provided throughout the test. The test was terminated when the pedal rate dropped by more than 10 rpm (i.e. 75 rpm). All cycle tests were completed on an electromagnetically braked cycle ergometer where the seat and handlebars were fully adjustable both vertically and horizontally. The horizontal and vertical direction of both the seat and handlebars were adjusted to suit each participant and were recorded following the ramp test and replicated for subsequent tests. Pulmonary gas exchange and ventilation were measured from the beginning of the rest period until cessation of the test.

Step transition tests. Each participant attended a total of five experimental sessions during which cycling at a constant-load were completed. The test began with a 5-minute rest period before participants completed three minutes of unloaded cycling (20 watts). At the end of the three minutes, an immediate transition to the work rate equal to 30%Δ (GET plus 30% of the difference between the work rate at the GET and $\dot{V}O_{2peak}$; heavy exercise) was imposed with the duration altered at each session (2, 6, 10, 20, 30 minutes). Constant power was maintained at 85 rpm and was maintained for the duration specified for each of the tests.

Neuromuscular evaluation. Neuromuscular evaluation (Fig. 1B) consisted of (1) 3 x single supra maximal electrical stimulations, each separated by four seconds, (2) 3 x paired at 10 Hz (two stimulation pulses separated by 100 ms) and 3 x paired at 100 Hz (two stimulation pulses separated by 10 ms) electrical stimulations, each separated by four seconds, and (3) 3 x five-second isometric maximal voluntary contraction (MVC) tests of the knee extensor muscles during which a 100 Hz doublet was superimposed to the MVC. A one-minute rest period separated each MVC. Strong, standardised, verbal encouragement was provided throughout the MVC. In order to increase the contact between the electrode and the skin during all electrical stimulations, a pressure was applied to the cathode electrode using a wooden handle with a rubber end. Note that during post exercise, each sequence was repeated only one time in order to diminish the possible effect of recovery time. Less than one minute was required to position the participant for testing after exercise.

Measurements. *Pulmonary gas exchange.* During all tests, pulmonary gas exchange was continuously measured using a computerised system (MetaMax 3b, Cortex, Leipzig, Germany). The system used an infrared sensor and an electrochemical cell to measure fractional concentrations of CO_2 and O_2 in expired gas. A digital transducer turbine assessed inspired and expired gas volume. A capillary line was used to continuously sample gas concentration. The transducer and the capillary line were securely attached to the facemask, which was firmly fitted to the participants face using Velcro straps. Immediately before each exercise test, the gas analysers were calibrated with gases of known concentration ($O_2 = 14.01\%$, $CO_2 = 6.03\%$), and the turbine volume transducer was calibrated using a three-litre Rudolph syringe (Cortex, Leipzig, Germany).

$\dot{V}O_{2peak}$ was noted as the highest 30-second average value attained during the incremental ramp test. The GET was determined using a number of measurements: (1) visual examination for the first disproportionate increase in CO_2 production ($\dot{V}CO_2$) from $\dot{V}CO_2$ versus $\dot{V}O_2$ graph, (2) an increase in ventilatory equivalent of oxygen ($\dot{V}_E/\dot{V}O_2$) without increase in ventilatory equivalent of carbon dioxide ($\dot{V}_E/\dot{V}CO_2$), and (3) increase in partial pressure of end-tidal oxygen with no decrease in partial pressure of end-tidal carbon dioxide. Subsequently, the work rate that would require 30%Δ was calculated and assigned for the experimental tests after accounting for the mean response time for $\dot{V}O_2$ during ramp exercise (2/3 of the ramp rate was subtracted from the work rate at the gas exchange threshold and $\dot{V}O_{2peak}$, i.e. 20 watts)[19,20].

PNS. Electrical stimulation was delivered using a high-voltage stimulator (model DS7, Digitimer Stimulator, Hertfordshire, UK). Low intensity stimulation (~ 20 mA) was used to locate the femoral nerve by means of a cathode ball electrode (0.5 cm diameter) which was manually pressed into the femoral triangle and maneuvered until the femoral nerve was properly located (determined by observing contraction of the leg). A 5 cm diameter cathode electrode (American Imex, CA, USA) was then placed on the site after it was cleaned with an alcohol wipe. The anode, a rectangular electrode (18 × 7 cm, American Imex, CA, USA), was placed opposite the cathode in the gluteal fold. Both the cathode and anode electrodes were worn during exercise and therefore both were taped to the skin using micropore tape (3 M Micropore, St. Paul, MN, USA) to limit movement. To determine maximal stimulation, single electrical stimulations (rectangular pulse, 1 ms duration, 400 V) were delivered to the nerve and progressively increased until a plateau in the twitch torque and M-wave amplitude were achieved. The current that achieved plateau was increased by 20%, which was then used for subsequent tests.

Torque measurement. The evaluation of neuromuscular function was conducted on the right knee extensor muscles with participants seated in a Biodex isokinetic dynamometer (Biodex Medical Systems Inc., Shirley, NY, USA). The hip and knee angles were fixed at 90° (0° = full knee extension) with the ankle strapped to the lever arm of the Biodex. The rotational axis of the dynamometer was aligned with the lateral epicondyle of the femur, found after palpation. Two crossover straps were placed firmly across the shoulders to limit upper body movement and one strap was placed midway across the thigh of the right leg. Participants were asked to cross their arms across their chest during testing. Adjustments made to the seat position and to the lever arm of the Biodex were recorded for each participant during familiarisation and reproduced for subsequent tests.

Electromyography recordings. Once participants were seated, the right vastus medialis (VM) and vastus lateralis (VL) muscles were palpated and prepared for electromyogram signal (EMG) recording. To reduce impedance, the skin around the belly of the muscles was shaven, lightly abraded (3 M Red Dot Trace Prep, Ontario, Canada) and cleaned using 70% isopropyl alcohol wipes (Kendall Company, Mansfield, MA, USA). One pair of silver-chloride electrodes (3 M Red Dot, St. Paul, MN, USA) of 10 mm diameter with an interelectrode (center to center) distance of 2 cm were then placed lengthwise over the prepared muscle. The ground electrode was placed over the patella of the right leg. EMG and torque signals were recorded through chart software (v. 5.5.6, ADInstruments, Sydney, Australia). EMG signals were amplified with a bandwidth frequency ranging from 1.5 Hz to 2 kHz (common mode rejection = 90 dB; impedance = 100 MΩ; gain = 1000). The myoelectric and mechanical responses were digitised on-line at a sampling frequency of 2000 Hz and stored for off-line analysis.

Data analysis. *Oxygen uptake kinetic analysis.* The breath-by-breath $\dot{V}O_2$ data from each of the 30%Δ tests were initially examined to exclude errant breaths caused by coughing, swallowing, sighing, etc., and those values lying more than three standard deviations from the model $\dot{V}O_2$ were considered outliers and were removed. The breath-by-breath data from the different exercise durations were subsequently linearly interpolated to provide second-by-second values, and, for each individual, repetitions from different durations were time aligned to the start of exercise and the ensemble averaged. The primary component (phase 2) kinetics were isolated to identify the mono-exponential region and modelled by the following equation:

$$\dot{V}O_2(t) = \dot{V}O_{2b} + A_p \cdot \left(1 - exp^{\left(-\frac{t-td_p}{\tau_p}\right)}\right) \cdot U$$

Where $\dot{V}O_2(t)$ represents $\dot{V}O_2$ at a given time t; U= 0 for $t < td_1$ and U = 1 for $t \geq td_1$

$\dot{V}O_{2b}$ is the $\dot{V}O_2$ during unloaded cycling defined as the mean $\dot{V}O_2$ measured over the final 90 seconds of baseline pedaling; A_P is the asymptotic amplitudes for the primary phases; τ_P is the time constant, and td_P represents the time delay. Since the focus of this study was the SC, the cardiodynamic phase was removed from analysis[21,22], and therefore, not modelled, in order to ensure that the early initial component did not influence the results[23]. Initially, the fitting window extended from 20 seconds (i.e., at the end of phase I) to 80 seconds (only 60 s into the exercise). The window was lengthened iteratively in order to attain four series of the initial window length. For each window length, the parameters of the model were determined with an iterative procedure by minimising the sum of the mean squares of the differences between the model $\dot{V}O_2$ and actual $\dot{V}O_2$.

Identification of the end of the primary phase was completed using H.B. Rossiter criteria consideration[24,25].

As such, the amplitude of the slow component at time 2, 6, 10, 20, and 30 minutes were assigned the value (A_{SX}) and were defined as the difference between the value of $\dot{V}O_2$ at a given time and the sum of the primary phase and the $\dot{V}O_{2b}$ at the same given time.

SCV was also described as a percentage of the primary component (SCV%) since this ratio would provide information regarding the loss of efficiency.

Neuromuscular function analysis. From the EMG trace of single stimulations, peak-to-peak amplitude (M-waves) of the VL (MWVL) and VM (MWVM) were measured. Peak torque (PT) was determined from the torque signal of the single twitch. The highest torque achieved during MVC in their respective conditions were taken as the MVC torque. The PT of doublet stimulations were quantified and termed P10 and P100 for 10 Hz, and 100 Hz, respectively. In addition, the P10-to-P100 ratio (P10/P100) was calculated to assess for the occurrence of low or high frequency fatigue.

The voluntary activation (VA) level was calculated by expressing any increment in torque evoked during maximal isometric contractions (superimposed twitch) as a fraction of the amplitude of the response evoked by the potentiated doublet[26].

In agreement with the work by Strojnick and colleagues, the following correction factor (CF, the ratio between the torque just before the superimposed doublet divided by MVC peak torque) was used in order to take into account the possibility that the superimposed twitch was not necessarily applied when the torque level was at the true maximal voluntary force[27].

$$VA = 100 - \left[CF \cdot \left(\frac{superimposed\ doublet}{potentiated\ doublet}\right)\right] \cdot 100$$

All data presented are the average of three measurements in pre, and a single measurement on post.

	Amplitude ($l \cdot min^{-1}$)	Amplitude (% of Ap)
AS_2	0.037 ± 0.056	1.9 ± 2.5
AS_6	0.298 ± 0.130*	16.6 ± 8.6*
AS_{10}	0.373 ± 0.150*	20.9 ± 10.2*
AS_{20}	0.450 ± 0.202*&	25.3 ± 13.2*&
AS_{30}	0.452 ± 0.246*&	29.1 ± 16.3*&%

Table 1. Time course of slow component amplitude in absolute, and in percentage of the primary component. AS2, AS6, AS10, AS20, and AS30 are the amplitude of the slow component at time 2, 6, 10, 20, 30 min respectively. Ap is the amplitude of primary component. *Significantly different from 2 min. &: Significantly different from 6 min. %: Significantly different from 10 min. Data are presented as mean ± SD.

	2 min	6 min	10 min	20 min	30 min
MVC [%]	−2.81 ± 5.39	−2.32 ± 4.19	−2.87 ± 5.31	−9.26 ± 9.67	−17.01 ± 13.09*
VA [%]	−1.81 ± 3.14	0.18 ± 3.90	−1.59 ± 3.4	−2.72 ± 3.92	−4.32 ± 5.66
MWVM [%]	−1.17 ± 5.72	−2.86 ± 8.28	−0.52 ± 8.50	−4.21 ± 8.05	−8.23 ± 7.59
MWVL [%]	4.07 ± 10.33	6.91 ± 6.39	5.07 ± 8.43	8.32 ± 15.07	6.9 ± 28.92

Table 2. Changes in neuromuscular function over the time course of the slow component. MVC: maximal voluntary contraction, VA: voluntary activation, MWVM: M-wave amplitude of vastus medialis, and MWVL: M-wave amplitude of vastus lateralis. *Different from base line, 2 min, 6 min, and 10 min ($p < 0.05$). Data are presented as mean ± SD.

Data and statistical analysis. Data were normalised by expressing the measures taken immediately after exercise as a percentage change relative to before exercise. This was completed to avoid day-to-day variations in measures that may occur. Normality test (Kolmogorov-Smirnov) and F-test of equality of variances were completed to test for normal distribution and equality of variance. One-way repeated measures analysis of variance (ANOVA) was used to test the effect of exercise duration on measurers of neuromuscular function. When a significant main effect was found, significant differences were located using Tukey's post hoc analysis test. Pearson correlation coefficient was used to assess relationships between the change of SCV% and changes to neuromuscular parameters. Analyses were completed with Box and Tidwell tests, and the Theil method (Theil nonparametric regression technique). The Box and Tidwell test assesses whether the association between the slow component and fatigue is linear or not, and therefore related to time. In contrast, Theil's regression highlights, in a qualitative way, the points that are distant from the linear relationship. For all tests, significance was set at $p < 0.05$. Data are expressed as mean ± SD.

Ethical approval. The University of Auckland Human Participants Ethics Committee approved this study. Written informed consent was provided by all participants prior to participation. All procedures conformed to the latest revision (2013) of the Declaration of Helsinki.

Results

Oxygen uptake kinetics. Mean $\dot{V}O_{2peak}$ was 3.95 ± 0.18 $l \cdot min^{-1}$ and the mean power output corresponding to 30%Δ was 200 ± 11 watts. During the three minutes of unloaded pedalling at 85 rpm, $\dot{V}O_{2b}$ reached a value of 0.85 ± 0.19 $l \cdot min^{-1}$. Asymptotic amplitudes of the primary phase attained 1.85 ± 0.38 $l \cdot min^{-1}$ with a time constant of 27.1 ± 15.0 s and a time delay of 12.8 ± 2.3 s. Amplitude of the slow component at time 2, 6, 10, 20, and 30 minutes are presented in Table 1. Values of SCV as a percentage of the primary component are also described.

Neuromuscular function. MVC measurement showed alteration over the course of exercise (Table 2). Post-hoc test revealed a significant reduction after 30 minutes of cycling compared to before exercise, 2, 6, and 10 minutes of exercise. After 20 minutes of exercise, a trend towards significance was observed compared to before exercise ($p = 0.1$) No effect of exercise duration was detected for VA (Table 2; $p > 0.05$).

No effect of exercise duration was detected for the M-wave amplitude of the VM and VL muscles (Table 2).

Twitch amplitude (Fig. 2A) showed a significant reduction at 30 minutes of exercise compared to before, 2, 6, and 10 minutes of exercise; ($p < 0.05$). A significant reduction was also observed after 20 minutes of exercise compared to before, 2, and 6 minutes of exercise ($P < 0.05$). Finally, significant differences were observed for 10 minutes of exercise compared to 2 and 6 minutes of exercise ($p < 0.05$).

P10 (Fig. 2B) and P100 (Fig. 2C) evolved in a similar manner. Specifically, significant differences were observed for 20 and 30 minutes compared to before, 2, 6, and 10 minutes of exercise ($p < 0.05$). Furthermore, significant differences were observed at 10 minutes compared to 2 and 6 minutes ($p < 0.05$).

Significant differences for the P10/P100 ratio (Fig. 2D) were found for most exercise durations. A significantly lower P10/P100 ratio was observed at 30 minutes compared to before, 2, 6, and 10 minutes of exercise ($P < 0.05$). After 20 minutes of exercise, differences were observed compared to before, 2, and 6 minutes of exercise ($p < 0.05$). Furthermore, significant differences were observed at 10 minutes compared to before and 2 minutes of exercise ($p < 0.05$).

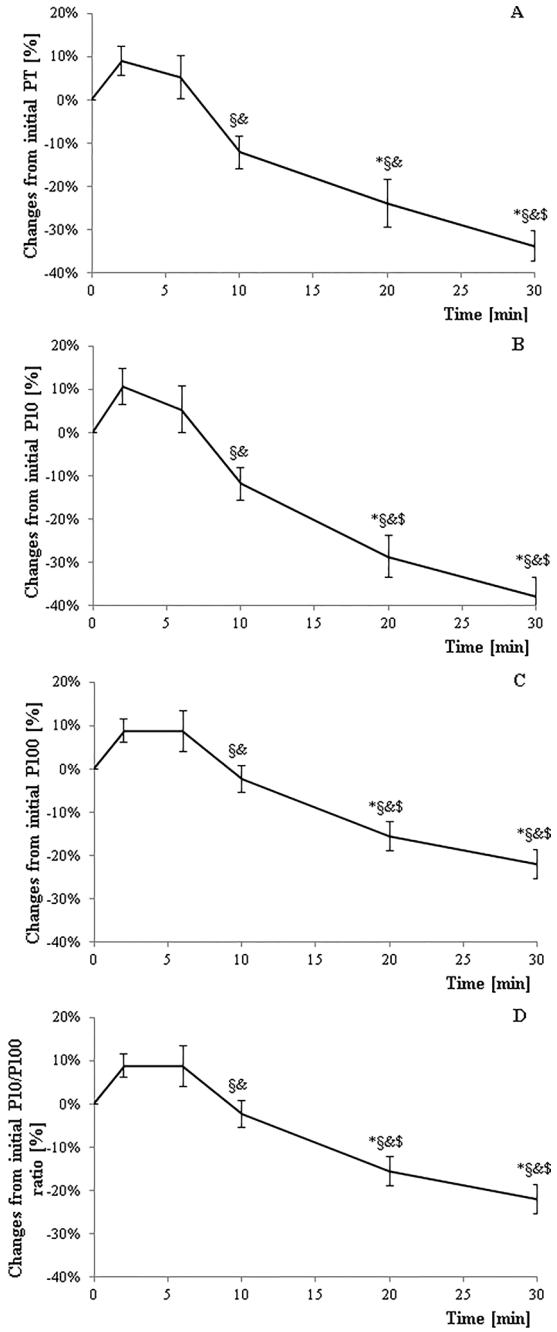

Figure 2. Neuromuscular alterations for peak twitch amplitude (**A**), 10 Hz paired (P10) stimulation (**B**), 100 Hz paired (P100) stimulation (**C**), and P10/P100 (**D**) over the course of exercise. *Significant difference from baseline ($p < 0.05$); $^{\$}$Significant difference from 2 minutes. ($p < 0.05$). $^{\&}$Significant difference from 6 minutes ($p < 0.05$). $^{\$}$Significant difference from 10 minutes ($p < 0.05$). Error bars are SE.

The SCV and fatigue. Correlation analysis was used to investigate relationships between the SCV% and neuromuscular parameters (Table 3). Changes in M-wave amplitude for either of VL and VM, VA and MVC did not correlate with changes of the SCV relative to the primary phase. However, significant correlations were found between the SCV% and PT, P10, P100 (tendency), and P10/P100 ratio. For these neuromuscular parameters, P10/P100 showed the strongest correlation with SCV% ($R^2 = 0.88$), followed by P10 ($R^2 = 0.81$), PT ($R^2 = 0.81$), and P100 ratio ($R^2 = 0.72$). In contrast, the Box and Tidwell's test was smaller than 0.05 (see Table 3) for correlation relationships suggesting that the relationship is non-linear and therefore unrelated over time. In addition, Theil's line (see Fig. 3) showed that during the first phase, only the slow component grew (the points of this phase are distant from Theil's line); while during the second phase, the slow component continued to grow but fatigue also grew (the points of this phase then line up with Theil's line).

	Correlation		Box-Tidwell test	
	R	P	Z	P
MWVM	−0.69	0.196	−1.70	0.089
MWVL	0.76	0.137	−0.20	0.841
PT	−0.90	0.038	−3.06	0.002
MVC	−0.72	0.172	−5.57	<0.001
VA	−0.52	0.370	−5.48	<0.001
P10	−0.90	0.036	−3.39	<0.001
P100	−0.85	0.065	−3.97	<0.001
P10/P100	−0.94	0.019	−4.50	<0.001

Table 3. Correlation coefficient and Box-Tidwell test between the slow component amplitude, as a percentage of the primary phase, and neuromuscular function. MWVM: M-wave amplitude of vastus medialis, MWVL: M-wave amplitude of vastus lateralis, PT: Peak Torque of the single twitch, MVC: maximal voluntary contraction, VA: voluntary activation, P10: peak torque at 10 Hz doublet stimulation, P100: peak torque at 100 Hz doublet stimulation, P10/P100: ratio of peak torque between 10hz and 100hz doublet stimulation, R: correlation coefficient, Z score statistic, P: significance.

Discussion

The main finding from the present study was that alterations to force production by the knee extensor muscles were present during exercise at an intensity of 30%Δ, which correlated with the development of the SCV, however, a temporal relationship between the development of the SCV and fatigue does not appear to exist.

Origin of fatigue observed during exercise. With neuromuscular fatigue defined as a reduction in force generating capacity[28], loss of MVC torque is used as a general index for evaluating the extent of neuromuscular fatigue. In the present study, MVC torque, compared to the beginning of exercise, was found to be significantly reduced only after 30 minutes of cycling at 30%Δ. However, while loss of MVC torque is a general index of fatigue, it does not provide information regarding the site of alterations (i.e. neuromuscular fatigue etiology). To determine the origin of the neuromuscular fatigue caused by various durations of cycling at 30%Δ, electrical stimulations were delivered at rest, as well as during MVC, allowing for the evaluation of VA, action potential transmission and propagation, and neuromuscular properties. VA, which is commonly used to evaluate central fatigue[29], was not significantly affected by any exercise durations in the present study. The absence of significant central fatigue suggests that declines in motivation, afferent feedback, or central drive were not present, or that declines in central drive was countered by increased motivation[10,30]. It subsequently suggests a peripheral origin for the induced neuromuscular fatigue. Muscle membrane excitability and neuromuscular propagation appeared to be well preserved, as highlighted by the lack of alterations in VL and VM M-wave amplitudes. In contrast, reductions in evoked forces suggests the presence of peripheral fatigue. Interestingly, signs of peripheral fatigue were observed following shorter exercise durations, suggesting that evoked forces might be more sensitive than MVC for detecting fatigue when it is of peripheral origin. Indeed, PT, P10 and P100 were already reduced after 20 minutes of cycling compared to the beginning of exercise. As M-wave amplitudes were unaltered at all-time points, reductions in evoked forces can highlight either alterations in sarcoplasmic reticulum Ca^{2+} handling[8] or alterations occurring at the cross-bridge level such as reduced myofibrillar Ca^{2+} sensitivity, and/or reduced capacity for cross-bridge to produce force[31,32]. Further supporting excitation-contraction failure, the P10/P100 ratio was found to be reduced following 10, 20, and 30 minutes of exercise compared to the start of exercise suggesting the presence of low frequency fatigue[33]. A study completed on rat *gastrocnemius* muscle ascribed low-frequency fatigue to Ca^{2+} handling alterations rather than to processes occurring at the cross-bridge level[34]. Indeed, altered Ca^{2+} handling is believed to occur with P_i accumulation during the development of fatigue and its subsequent precipitation with Ca^{2+} within the sarcoplasmic reticulum[35]. However, the exact mechanisms responsible for low-frequency fatigue remain unclear as previous results, also obtained using rodents, showed that the site (i.e. Ca^{2+} handling *vs*. cross-bridge level) responsible for this low-frequency fatigue is dependent on the antioxidant status of the individual[36]. Therefore, based on the measures in the present study, it is likely that the observed neuromuscular fatigue following 20 and 30 minutes of cycling at 30%Δ is a result of peripheral rather than central fatigue. Based on the literature, while speculative, it suggests that fatigue it is from either impaired Ca handling or reduced cross-bridge kinetics.[31,32].

The SCV and fatigue. Significant correlations were found between the SCV% and PT, P10, P100, and P10/P100. This finding is supportive of the theory regarding the presence of fatigue required to elicit the SCV. In contrast, for these parameters, the Box and Tidwell's test showed that the relationship between the development of the SCV and the alterations of the neuromuscular properties of knee extensor muscles were non-linear and therefore unrelated over time. In addition, Theil's line (see Fig. 3) showed two distinct phases; the first phase where only the slow component grew (the points of this phase are away from Theil's line); while during the second phase, the slow component continued to grow but fatigue also grew (the points of this phase then line up with Theil's line). (see Fig. 3). In other words, the development of the SCV, in fact, occurred mainly between 2–10 minutes during which neuromuscular properties were relatively stable (only a reduction in the P10/100 ratio was observed after 10 minutes of cycling). In contrast, PT, P10 and P100 were significantly reduced only after 20-30 minutes

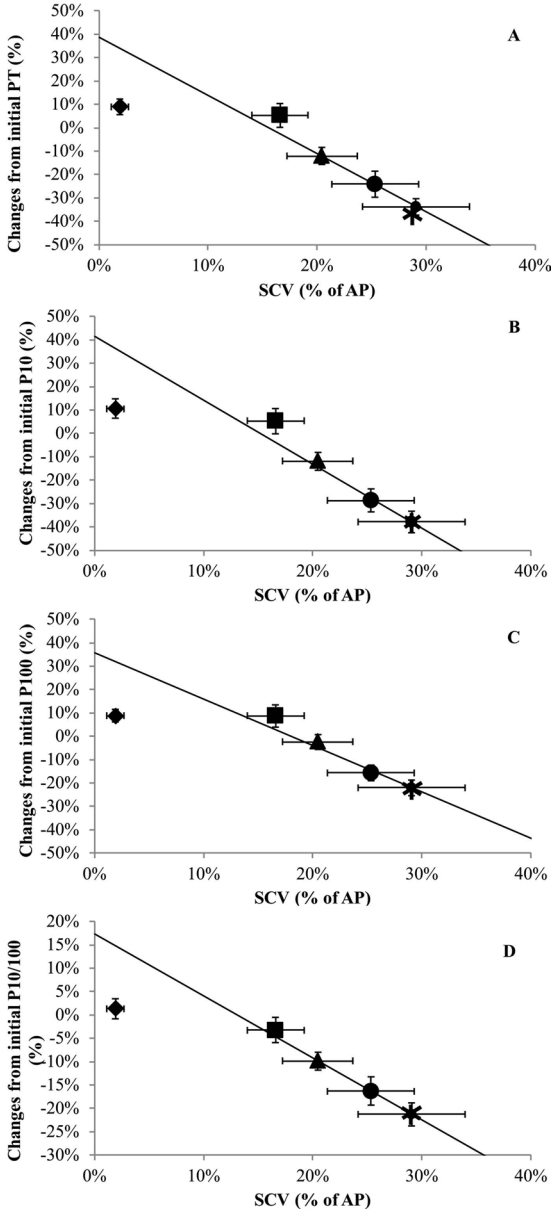

Figure 3. The relationship between peak twitch amplitude (**A**), 10 Hz paired (P10) stimulation (**B**), 100 Hz paired (P100) stimulation (**C**), and P10/P100 (**D**) and the change in SCV relative to the primary phase.◆ 2 minutes; ■ 6 minutes; ▲ 10 minutes; ● 20 minutes; and ✶ 30 minutes represent average values. Theil's line is characterised by the dashed line. Error bars are SE. Error bars in the figures are presented as SE for more clarity.

of exercise compared to baseline values. These results suggest that the development of fatigue due to alterations of neuromuscular properties is not an essential requirement to elicit the SCV at least during the first 10 minutes of exercises. This finding is in line with those from Thistlethwaite and colleagues[37]. They showed that, during heavy cycling exercise, when preceded either by heavy exercise or by heavy knee extensions (requiring twofold greater muscle activation relative to heavy exercise), τ_p, gain of the primary response, and the amplitude of the SCV were similar between protocols. The authors concluded that muscle fatigue is not a determining factor for the development of the SCV. Hopker and colleagues attested similar results. Participants completed either a non-metabolically stressful 100 intermittent drop-jumps protocol (pre-fatigue condition) or rest (control) for 33 minutes. The results of their study showed that locomotor muscle fatigue, tested by the reduction in power in the maximal voluntary cycling power test, was not associated with the development of the SCV[38]. Interestingly, the magnitude of the SCV was not significantly different between the two conditions despite significant differences in locomotor muscle fatigue. Recently, Dos Nascimento Salvador and colleagues published a study looking at the cause–effect relationship between the SCV and fatigue. They switched from constant work rate to isokinetic pedaling to quantify reductions in peak torque at three and eight minutes, with and without priming exercise. Results showed that the SCV after priming was reduced but there were no significant differences between

conditions regarding the magnitude of the reduction of maximal isokinetic force and power at three and eight minutes[39]. This observation refutes a cause-effect relationship between fatigue and the development of the SCV.

However, the findings from this study are in contrast with the results by Keir and colleagues. Correlations were shown in both studies between some measures of fatigue and the SCV, however, a temporal association was only found in one study[9]. In one perspective, this difference highlights the importance of exercise intensity. Indeed, in the present study, step transition exercise was in the heavy domain, while the study by Keir and colleagues was in the severe domain[9]. In addition, the amplitude and type of fatigue was potentially different, as assessed by the difference in reduction of MVC after 18–20 minutes (9% for the present study vs 22% in the study by Keir and colleagues). If the SCV is related to fatigue parameters, it should be present in both exercise intensity domains. However, this was not the case, which suggests that the SCV may not be related to fatigue parameters.

The results in the present study are in agreement with results from a previous study regarding changes to velocity-specific peak power during cycling. Cannon *et al.*[17] observed a reduction in velocity-specific peak power, which correlated with the SCV. However, as was observed in the present study, the reduction they observed was not temporally related to the development of the SCV. The reduction in velocity-specific peak power occurred prior to the SCV in their study, while excitation-contraction coupling was altered after the development of SCV in the present study. Nevertheless, both reported no changes during the development of the SCV which suggests that those alterations are likely not essential for the development of the SCV. If alterations to neuromuscular properties are not involved during the development of the SCV, at least during exercise in the heavy domain, it may be possible that the $\dot{V}O_2$ cost of force production may increase within a given fiber population. A progressive inhibition of ATP supply by anaerobic glycolysis, an increase in ATP usage per power output, and/or a reduction of ATP production per mole of oxygen (P/O_2 ratio) are probably implicated in the SCV[40]. However, the documentation of a cause-effect relationship during exercise between muscle fatigue and reduced efficiency remains unknown.

Experimental consideration. As with the study by Keir and colleagues (2016), at the end of exercise, the time to transfer the subject from the ergometer to the Biodex before the start of neuromuscular testing was less than one minute. One could argue that fatigue was already modified, and consequently the interpretation of the data in relation to fatigue during exercise is limited. Simply, fatigue is likely to have been underestimated in the present study and the measurement of fatigue during exercise would have been more appropriate. However, neuromuscular measurements were taken after a similar amount of time after each exercise, for each participant, and consequently, the change of the robustness of the relationship between fatigue and the SCV is likely to have been marginal, which should not change the general conclusions of the present study. Furthermore, the cause (fatigue) has to precede the effect (SCV); however, the data from the present study indicates that this was not the case. A further limitation is the fact that fatigue was measured during static contractions whereas cycling is a dynamic movement.

Conclusion

Fatigue in the present study was observed during exercise completed at 30%Δ and which was at least 20 minutes in duration. Indirectly, these results suggest that the observed fatigue appears to be a result of impaired Ca^{2+} handling and/or reduced capability of cross-bridges to produce force. While significant correlations between the SCV relative to the primary phase and neuromuscular parameters were found, a temporal relationship between the development of the SCV and fatigue does not appear to exist. Therefore, it would seem that the alteration of neuromuscular properties in muscle is not required for the development of the SCV.

Data availability

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions

F.B. conceived and designed the experiments. Data collection was completed by F.B. and T.G. F.B., T.G., S.C.A., J.R. and J.A. analysed, interpreted, revisited, and approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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