Paulo Cesar do Nascimento Salvador

EFFECTS OF PRIMING EXERCISE ON THE OXYGEN UPTAKE KINETICS AND MUSCLE FATIGUE DURING HIGH-INTENSITY CYCLING

Tese submetida ao Programa de Pós Graduação em Educação Física da Universidade Federal de Santa Catarina para a obtenção do Grau de Doutor em Educação Física.
Orientador: Prof. Dr. Benedito Sérgio Denadai.
Coorientador: Prof. Dr. Luiz Guilherme Antonacci Guglielmo.

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Esta Tese foi julgada adequada para obtenção do Título de "doutor" e aprovada em sua forma final pelo Programa de Pós Graduação em Educação Física da Universidade Federal de Santa Catarina.

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_____
Prof[a]. Kelly Samara da Silva, Dr[a].
Coordenadora do Curso

Banca Examinadora:

_____
Prof. Benedito Sérgio Denadai, Dr.
Orientador
Universidade Estadual Paulista

_____
Prof. Luiz Guilherme Antonacci Guglielmo, Dr.
Corientador
Universidade Federal de Santa Catarina

_____
Prof. Fabrizio Caputo, Dr.
Universidade do estado de Santa Catarina

_____
Prof. Renato Aparecido Corrêa Caritá, Dr.
Universidade Estadual Paulista
(Vídeo conferência)

_____
Prof. Juliano Dal Pupo, Dr.
Universidade Federal de Santa Catarina

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Every heartbeat is a miracle.
(Bruno Grassi, 2018)

RESUMO

Acredita-se que os mecanismos que determinam a cinética do consumo de oxigênio ($\dot{V}O_2$) refletem a fadiga muscular esquelética e uma consequente redução na eficiência muscular. Assim, o principal objetivo deste estudo foi verificar a relação da fadiga muscular com a cinética de $\dot{V}O_2$, analisando se o exercício prévio influenciaria essa relação durante o ciclismo de alta intensidade em indivíduos fisicamente ativos. Este trabalho foi dividido em três estudos, cada um buscando responder uma questão específica. No estudo um e dois, 13 homens saudáveis desempenharam testes para determinar o máximo consumo de $\dot{V}O_2$ ($\dot{V}O_{2peak}$), a potência crítica (CP) e a W′ (capacidade finita de trabalho acima da CP) e seis cargas constantes até três ou oito min, ou até a exaustão (estudo dois). Cada uma destas numa taxa de trabalho para depletar 70%W′ em 8 min, com um esforço isocinético máximo (MIE) realizado antes e depois das condições sem (VH_{CON}) e com exercício prévio (VH_{EXP}), para medir o decréscimo no pico de torque. No estudo três, 20 sujeitos (10 mulheres) saudáveis desempenharam quatro transições de exercício de intensidade muito pesada (VH) em ordem randomizada depois do exercício sem carga (U-VH) ou moderado (M-VH). O MIE foi desempenhado antes e depois de cada condição em duas diferentes intensidades (60 ou 120 rpm). No estudo um a magnitude do componente lento de $\dot{V}O_2$ ($\dot{V}O_{2SC}$) (VH_{CON} = 0,280 ± 0,234, VH_{EXP} = 0,116 ± 0,109 $L.min^{-1}$; p=0,04) no 3º min e a trajetória do $\dot{V}O_{2SC}$ foram significativamente menores na condição VH_{EXP} (VH_{CON} = 0,108 ± 0,042, VH_{EXP} = 0,063 ± 0,031 L,min^{-2}; p<0,01) levando a uma amplitude do $\dot{V}O_{2SC}$ no 8º min significativamente menor que na condição VH_{CON} (VH_{CON} = 0,626 ± 0,296 L,min^{-1}, VH_{EXP} = 0,337 ± 0,179; p<0,01). O pico de torque diminuiu progressivamente do pré-exercício para o 3º min (ΔTorque = 21,5 ± 7,7 *vs*. 19,6 ± 9,2 Nm) e para o 8º min (ΔTorque = 29,4 ± 15,8 *vs*. 27,5 ± 12,0 Nm) no VH_{CON} e VH_{EXP} respectivamente, sem diferenças significativas entre as condições. Entre os principais resultados do estudo dois, o tempo de exaustão (TTE) e o Δ da concentração de lactato sanguíneo ([La]) foram significativamente menores na condição VH_{EXP} comparada com a VH_{CON} (595 ± 118 s *vs*. 683 ± 148 s; 3,5 ± 1,2 $mmol.L^{-1}$ *vs*. 8,8 ± 2,3 $mmol.L^{-1}$; p<0,05, respectivamente). O exercício prévio VH induziu uma depleção de 60% da W′. Entretanto, depois de 10 min de recuperação, a reconstituição da W′ era ainda incompleta (92%). No estudo três, a constante de tempo (τ) aumentou significativamente

enquanto o $\dot{V}O_{2SC}$ diminuiu significativamente no M-VH ($p < 0,05$) em ambos os sexos. O pico de torque depois do exercício não foi significativamente influenciado pelas condições em ambos os sexos e velocidades (Male Δtorque 60 rpm in U-VH = 13 ± 10 Nm, in M-VH = 13 ± 9 Nm; 120 rpm in U-VH = 22 ± 14 Nm, in M-VH = 21 ± 12 Nm; Female 120 rpm in U-VH = 10 ± 9 Nm, in M-VH = 12 ± 8 Nm, $p > 0,05$). Em conclusão, considerando que os efeitos do exercício prévio no $\dot{V}O_{2SC}$ não foram acompanhados pelo padrão de força muscular, esses achados não evidenciam a hipótese de uma relação de causa-efeito entre o curso de tempo da fadiga muscular e o $\dot{V}O_{2SC}$. O exercício prévio VH diminui a performance de ciclismo de alta intensidade. Sugere-se que esse prejuízo é influenciado por distúrbios fisiológicos ligados a depleção da W′. As alterações na cinética de $\dot{V}O_2$ depois do exercício de baseline elevado não refletem alterações na produção de força muscular em ambos os sexos.

Palavras-chave: Cinética do consumo de oxigênio. Fadiga muscular. Diferenças sexuais.

ABSTRACT

It is believed that the mechanisms that determine the oxygen uptake ($\dot{V}O_2$) kinetics reflect skeletal muscle fatigue and a consequent reduction in muscle efficiency. Thus, the main purpose of this study was to verify the relation of muscular fatigue with the $\dot{V}O_2$ kinetics, analyzing whether prior exercise would influence this relationship during high intensity cycling in physically active individuals. This work was divided into three studies, each one seeking to answer one specific question. In the study one and two 13 healthy male subjects performed tests to determine the maximal $\dot{V}O_2$ ($\dot{V}O_{2peak}$), critical power (CP) and W′ (fixed amount of work above CP) and six square-wave bouts until 3 or 8 min, or until exhaustion (study two). Each of them at a work rate set to deplete 70%W′ in 8 min, with an maximal isokinetic effort (MIE) before and after the conditions without (VH_{CON}) and with prior exercise (VH_{EXP}), to measure the cycling peak torque decrement. In the study three 20 healthy subjects (10 female) performed 4 transitions of very-heavy (VH) intensity cycling in a randomized order after unloaded (U-VH) or moderate (M-VH) exercise. The MIE was performed before and after every condition at two different cadences (60 or 120 rpm). In the study one the $\dot{V}O_2$ slow component ($\dot{V}O_{2SC}$) magnitude at 3 min (VH_{CON} = 0.280 ± 0.234, VH_{EXP} = 0.116 ± 0.109 L.min^{-1}; p=0.04) and the $\dot{V}O_{2SC}$ trajectory were significantly lower for VH_{EXP} (VH_{CON} = 0.108 ± 0.042, VH_{EXP} = 0.063 ± 0.031 L.min^{-2}; p<0.01) leading to a $\dot{V}O_{2SC}$ magnitude at 8 min significantly lower than VH_{CON} (VH_{CON} = 0.626 ± 0.296 L.min^{-1}, VH_{EXP} = 0.337 ± 0.179; p<0.01). The peak torque progressively decreased from pre-exercise to 3 min (ΔTorque = 21.5 ± 7.7 *vs*. 19.6 ± 9.2 Nm) and to 8 min (ΔTorque = 29.4 ± 15.8 *vs*. 27.5 ± 12.0 Nm) at VH_{CON} and VH_{EXP} respectively, without significant differences between conditions. Between the main results of study two, the time to exhaustion (TTE) and Δ blood lactate concentration ([La]) were significantly lower in VH_{EXP} compared to VH_{CON} (595 ± 118 s *vs*. 683 ± 148 s; 3.5 ± 1.2 mmol.L^{-1} *vs*. 8.8 ± 2.3 mmol.L^{-1}; p<0.05, respectively). The prior VH intensity exercise bout induced a W′ depletion of 60%. However, after 10 min of recovery, W′ reconstitution was still incomplete (92%). In the study three, the time constant (τ) was significantly higher whereas the $\dot{V}O_{2SC}$ magnitude was significantly lower in M-VH (p <0.05) in both sexes. The peak torque after exercise was not significantly influenced by conditions in both sexes and velocities (Male Δtorque 60 rpm in U-VH = 13 ± 10 Nm, in M-VH = 13

± 9 Nm; 120 rpm in U-VH = 22 ± 14 Nm, in M-VH = 21 ± 12 Nm; Female 120 rpm in U-VH = 10 ± 9 Nm, in M-VH = 12 ± 8 Nm, $p>0.05$). In conclusion, considering that "priming" effects on the $\dot{V}O_{2SC}$ were not accompanied by the muscle force behavior, these findings do not support the hypothesis of a "causal" relationship between the time-course of muscle fatigue and $\dot{V}O_{2SC}$. Prior VH intensity exercise impairs high-intensity cycling performance. It is suggested that these impairments are mediated by physiological disturbances linked to W′ depletion. The alterations in $\dot{V}O_2$ kinetics after elevated baseline did not reflect alterations in muscle force production in both sexes.

Keywords: Oxygen uptake kinetics. Muscle impairments. Sex differences.

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LIST OF ABBREVIATIONS AND SYMBOLS

A	Amplitude for the fundamental phase
A_{TOTAL}	$A + VO_{2baseline}$
CP	Critical power
CO_2	Carbone dioxide
CON	Control condition
EXP	Experimental condition
GET	Gas-exchange threshold
HR	Heart rate
HR_{max}	HR maximal values
[La]	Blood lactate concentration
MIE	Maximal isokinetic effort
P_{peak}	Peak power output
RER	respiratory exchange ratio
$\tau W'$	time constant of the reconstitution of W′
TTE	Time to exhaustion
TD	Time delay
TD_s	TD of VO_{2SC} phase
τ	VO_2 time constant
TTE	Time to exhaustion
VE	Minute ventilation
VO_2	O_2 uptake
VCO_2	CO_2 expired
VO_{2peak}	Peak of oxygen uptake
VH	Very heavy-intensity exercise
VH_{CON}	Control condition
VH_{EXP}	Experimental condition
$VO_{2baseline}$	Average value over one min of resting baseline
VO_{2SC}	Slow component of VO_2
VO_{2SC} trajectory	slow component trajectory
VO_{2END}	The average VO_2 value over the last 15 s
W′	finite amount of work above critical power
70% Δ	70% of the difference between GET and VO_{2peak}
70% W’	Square-wave exercise intensity

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1 CHAPTER ONE

1.1 INTRODUCTION – Current perspectives on VO_2 kinetics and muscle fatigue

The response of oxygen uptake (VO_2) measured at a pulmonary level reflects changes in the oxidative metabolism in active tissues. Thus, the VO_2 response at the beginning of the exercise reflects adjustments of both, O_2 systemic transport and muscle metabolism (XU; RHODES, 1999). Therefore, the VO_2 kinetics can be considered when studying/investigating the physiological mechanisms underlying the VO_2 response to exercise and its subsequent recovery (JONES; POOLE, 2005). After the beginning of a square wave exercise bout at an intensity below the gas exchange threshold (GET) (e.g. moderate intensity), the VO_2 increases rapidly until it reaches a steady state within the first few minutes of exercise (XU; RHODES, 1999; DENADAI; CAPUTO, 2003). In contrast, during exercise above GET (heavy or very heavy intensities), the attainment of a steady state is delayed and the development of the so-called slow component of the VO_2 response (VO_{2SC}) can be observed/found (JONES et al., 2011).The VO_{2SC} can be explain as a higher VO_2 than that predicted for the work rate-VO_2 ratio below the GET. That is, it represents an additional VO_2 to that expected for a given intensity (GRASSI; ROSSITER; ZOLADZ, 2015; JONES et al., 2011).

The time to tolerance during high intensity exercise is characterized by a hyperbolic function of the produced external power (P), in which the asymptote describes/represents the critical power (CP). The constant curvature of this hyperbola (W') is mathematically equivalent to the amount of work that can be performed above the CP. The CP represents an aerobic function threshold and corresponds to the highest work rate during which a steady state in VO_2, arterial blood acid-base state (lactate - [La]), bicarbonate, hydrogen ions (H^+) and intramuscular phosphate (phosphocreatine - [PCr] and inorganic phosphate - [P_i]), can be attained (MURGATROYD et al., 2011; POOLE; JONES, 2012). Thus, CP refers to a metabolic threshold in which the majority energy production is provided in an aerobic form with minimum participation of the anaerobic metabolism. W' was first considered as a finite energy store, consisting of [ATP], [PCr], intramuscular glycogen and O_2 store (e.g. at the blood and other tissues) (FUKUBA; WHIPP, 1999; POOLE et al., 1988; POOLE; WARD; WHIPP, 1990). However, recently the W' has been related to substantial

substrate depletion (e.g. [PCr]) and the accumulation of metabolites up to a critical level (e.g., [Pi], [ADP], [H_2PO_4-] and [H +]) beyond which exhaustion occurs (BURNLEY et al., 2010; POOLE et al., 2008; VANHATALO et al., 2010).

As previously reported, during exercise below the CP (heavy intensity) but above GET, the VO_2 steady state is higher than predicted, but when the work rate is above the CP (very heavy intensity) this steady state it is not reached and the VO_2 continues to increase until the peak values (VO_{2peak}) are reached and therefore, the imminent end of exercise (MURGATROYD et al., 2011; VANHATALO et al., 2011). These characteristics of VO_2 during exercise have been used to classify several exercise intensity domains, namely: "moderate" below GET, "heavy" between GET and CP, "very heavy" between CP and VO_{2peak}, and "severe" for all intensities performed above VO_{2peak} (CANNON et al., 2011; OZYENER et al., 2001; ROSSITER, 2011).

To date, only a few studies sought to investigate the differences in VO_2 kinetics between men and women. despite physiological and anatomical differences between the sexes (HARMS, 2006). Men have higher pulmonary volumes, greater capacity for pulmonary diffusion at rest, and greater ability to supply and deliver O_2 to the muscles, which can alter the VO_2 kinetics (DOMINELLI et al., 2015; HARMS, 2006; MURIAS et al., 2013). Previous investigations found that female adolescents show slower kinetics and lower VO_{2SC} compared to male adolescents (FAWKNER; ARMSTRONG, 2003; LAI et al., 2016). Besides, no differences were observed between men and women in the VO_2 kinetics in swimming (REIS et al., 2017). To our knowledge, no study has sought to verify the differences between men and women in VO_2 kinetics relating to muscle fatigue. It is known that differences between men and women in the pattern of neuromuscular activation (CLARK et al., 2005), in activated muscle mass (ENOKA; DUCHATEAU, 2008), in fiber type distribution (higher percentage of type II fibers in men) (LIU et al., 2010; MAHER et al., 2009) and in muscle fatigue during dynamic exercise (less pronounced in women) exist (HUNTER, 2014). Thus, the question: How do the differences between sexes influence on the muscle force production and on the VO_2 kinetics? It is still unanswered.

The VO_2 response during exercise performed in the heavy and / or very heavy intensity domains have been characterized by bi-exponential mathematical adjustments with a second term having a delayed start (BARSTOW; MOLÉ, 1991). This suggests a dependence on time as well as a dependence on intensity, with loss of muscle

efficiency when exercise proceeds in the heavy and very heavy intensities (JONES et al., 2011). To date, the mechanisms controlling VO_{2SC} remains unclear and is controversially debated. Rossiter et al. (2002) demonstrated that ~ 88% of VO_{2SC} is manifested in the exercising muscles and in the hydrolysis profile of PCr. Yet, Pringle et al. (2003) indicated that the % of distribution of type I fibers was significantly correlated with the relative amplitude of the VO_{2SC} during heavy ($r = -0.74$) and very heavy exercise ($r = -0.64$). This is in line with previous findings of Barstow et al. (1996) and was later confirmed by further studies (BURNLEY; DOUST; JONES, 2002; LANZI et al., 2012). Thus, the concept that the mechanisms responsible for VO_{2SC} is originated within the muscle and may be related to the recruitment profile of motor units is well accepted in the literature (JONES; GRASSI; CHRISTENSEN, 2011).

Skeletal muscle fatigue can be characterized as a reduction in muscle strength/power over time (ENOKA; DUCHATEAU, 2008) being associated with or leading to a reduction in the efficiency of muscle contractions (the ratio between the production of mechanical energy and the demand for metabolic energy) (GRASSI; ROSSITER; ZOLADZ, 2015). During exercise involving large muscle groups, fatigue and decreased efficiency have been described as the major causes of exercise intolerance (defined as the inability to produce adequate strength or power to maintain a task) (GRASSI; ROSSITER; ZOLADZ, 2015). It has recently been suggested that muscle fatigue during very heavy intensity exercise is related to the use of W' and VO_{2SC} (CANNON et al., 2011; GRASSI; ROSSITER; ZOLADZ, 2015; MURGATROYD et al., 2011). In addition, VO_{2SC} was correlated with both fatigue (peak torque decline during maximal isokinetic cycling) (CANNON et al., 2011) and W' (MURGATROYD et al., 2011). Additionally, VO_{2SC} has been shown to follow peripheral muscle fatigue (KEIR et al., 2016a) during high intensity cycling and individuals with a slower VO_2 kinetics presented higher peripheral fatigue (TEMESI et al., 2017). Although the relationship between VO_2 kinetics and fatigue is still controversially debated, with some researchers demonstrating that muscle fatigue is not a necessity for the development of VO_{2SC} (DE SOUZA et al., 2016; DELEY et al., 2006; HOPKER et al., 2016; THISTLETHWAITE et al., 2008). It is believed that the mechanisms that determines VO_{2SC} reflect skeletal muscle fatigue and a consequent reduction in muscle efficiency (CANNON et al., 2011; GRASSI; ROSSITER; ZOLADZ, 2015; KEIR et al., 2016a) with or without a

recruitment of supplementary motor units (BARSTOW et al., 1996; ENDO et al., 2007; VANHATALO et al., 2011).

The mechanism proposed for this relationship is the activation of a "cascade" of events involving the depletion of substrates and the accumulation of fatigue-related metabolites that directly influence the production of muscle strength and the efficiency in high-intensity exercises (MURGATROYD et al., 2011). The decline in muscle efficiency during constant exercise above the CP (i.e. very heavy domain) can be explained by a higher energy demand in order to produce the same mechanical power. Therefore, the energy production of the muscular system is by definition limited and the rate of progression of this inefficiency is the major determinant of exercise intolerance (GRASSI; ROSSITER; ZOLADZ, 2015; MURGATROYD et al., 2011).

Several studies in the literature have shown that prior high intensity cycling exercise does not change the time constant (τ) of the primary component, but results in an acceleration of the total response of VO_2 kinetics (increase of absolute primary amplitude [Atotal] - that is the baseline VO_2 plus the amplitude of the primary component and a consequent reduction of VO_{2SC}) (BAILEY et al., 2009; BURNLEY et al., 2000, 2002; GERBINO; WARD; WHIPP, 1996; KOPPO; BOUCKAERT, 2002; LANZI et al., 2012). These findings were also confirmed for running exercise (NASCIMENTO et al., 2015). Among the mechanisms that explain these effects of prior exercise, the increase in motor recruitment for the same external load may represent a favorable adaptation to sustain the exercise, whereby, the tension that each fiber needs to generate and so the metabolic disturbance imposed on each of them can be reduced. Therefore, it seems reasonable to suggest that the effect of prior exercise (reducing VO_{2SC}) may result from an increase in the primary amplitude as a consequence of additional muscle fiber recruitment, resulting in a low metabolic disorder at the individual level on the fiber (BURNLEY; DOUST; JONES, 2002).

However, it is likely that improvements in both volume of muscle blood flow and local blood flow (hence increasing the supply and availability of O_2), as well as an increase in mitochondrial enzymatic activities after prior exercise, delay the rate of fatigue development and the requirement for additional motor units recruitment, which helps to maintain power output during the subsequent exercise (BAILEY et al., 2009; BURNLEY et al., 2002). Some of these mechanisms can be seen, for example, considering the effect of prior exercise on reducing the

slow component of [PCr] (ROSSITER et al., 2001), although contradictory data on this subject also exist (JONES; FULFORD; WILKERSON, 2008). While the potential of prior exercise to decrease VO_{2SC} is clearly reported in the literature (BAILEY et al., 2009; BURNLEY et al., 2000; DO NASCIMENTO et al., 2015; KOPPO; BOUCKAERT, 2002; LANZI et al., 2012; ROSSITER et al., 2001), the effect on exercise tolerance is rather controversial, with some studies reporting improvements (BAILEY et al., 2009; BURNLEY; DOUST; JONES, 2005), and others declines in subsequent performance (FERGUSON et al., 2007; WILKERSON et al., 2004). Further research is needed to clarify whether changes in VO_2 dynamics reflect improvements in exercise tolerance (BAILEY et al., 2009; BURNLEY; DAVISON; BAKER, 2011). Furthermore, one of the questions related to this topic is how does prior exercise influence the recovery of *W'* and the exercise tolerance during cycling?

Another acute intervention to alter VO_2 kinetics has been the elevated baseline. When an immediate transition from an exercise with a higher work rate than rest to a heavy or very heavy exercise (work-to-work transition) occurs, the VO_2 kinetics is slower, i.e. higher τ and the VO_{2SC} smaller (BEARDEN; MOFFATT, 2001; DA BOIT et al., 2014; DIMENNA et al., 2010a, 2010b; NEDERVEEN et al., 2017; WILKERSON; JONES, 2006; WÜST et al., 2014). Further mechanisms attributed to the changes in VO_2 kinetics include exercise starting from a less favorable intramuscular energy state (GRASSI et al., 2011; WÜST et al., 2014); slower adjustments in supply and delivery of O_2 to the muscles (KEIR et al., 2016b; NEDERVEEN et al., 2017) and recruitment of motor units composed of muscle fibers with a lower metabolic efficiency and a slower adjustment dynamics (DIMENNA et al., 2010b; WILKERSON; JONES, 2006).

It is well known that the reduction in performance that results from muscle fatigue differs according to the type of contraction, the tested muscle groups and the duration and intensity of exercise (MILLET; LEPERS, 2004). The existence of a cycle ergometer that allows the external torque / power control to be performed in different modes (linear, hyperbolic or isokinetic) (*Lode Excalibur Sport, Lode BC, Groningen, Netherlands*), allows to evaluate/assess neuromuscular function and, thus, muscle fatigue, in a fast, reliable and specific way. Cannon et al. (2011) demonstrated that maximal isokinetic exercise before and after a constant cycling exercise is an useful technique for measuring fatigue (e.g. transition above the heavy intensity to observe the influence of muscle fatigue on VO_{2SC}).

The "fatigue and recruitment" hypothesis therefore suggests that the cost of ATP and O_2 for a given square-wave exercise bout increases during fatigue and this is manifested in the VO_{2SC} (CANNON et al., 2011). However, studies that support the evidence of muscle fatigue or progressive recruitment using electromyography techniques are controversial since some research failed to demonstrate changes in motor recruitment during exercise that presented the VO_{2SC} (LUCIA et al., 2000; SCHEUERMANN et al., 2001; PRINGLE; JONES, 2002; AVOGADRO; DOLENEC; BELLI, 2003; CLEUZIOU et al., 2004; GARLAND; WANG; WARD, 2006; MIGITA; HIRAKOBA, 2006). Cannon et al. (2011) showed that muscle fatigue is a requirement for VO_{2SC}. However, the maintenance of the peak isokinetic torque between the time VO_{2SC} started and at the end of exercise (i.e. minute 3 and 8 respectively) suggests that a progressive muscle recruitment is not mandatory, but the reduction in mechanical efficiency in the fatigued fibers is implicit.

Thus, considering these assumptions, the following questions were investigated: Is there a relationship between muscle fatigue (measured as the decline in peak isokinetic torque) and the VO_2 kinetics (e.g. VO_{2SC} and τ) during high-intensity cycling exercise? Does an acute intervention, such as very heavy prior exercise or elevated baseline that attenuates the $VO_{2SC,}$ demonstrate a cause-effect relationship? Therefore, this work was divided into three studies, each one seeking to answer the aforementioned questions within this introduction section.

1.2 OBJECTIVES

1.2.1 Main objective

To verify the relation of muscular fatigue with the VO_2 kinetics, analyzing whether prior exercise (which should change the VO_2 kinetics) would influence this relationship during high intensity cycling in physically active individuals.

1.2.2 Specific objectives

1.2.2.1 (Study 1)

To verify whether a prior very-heavy intensity exercise attenuates the muscle fatigue accompanying the reductions on the VO_{2SC} dynamic in a subsequent very-heavy intensity exercise;

1.2.2.2 (Study 2)

To link respiratory and cardiovascular responses following a prior exercise bout to the depletion and reconstitution of W' in order to better understand exercise tolerance during high-intensity cycling;

1.2.2.3 (Study 3)

To analyze the effects of elevated baseline (work-to-work transition) on the VO_2 kinetics and muscle force production behavior during maximal isokinetic efforts at different cadences (60 and 120 rpm) in male and female subjects.

1.3 HIPOTHESIS

The hypothesis of the present study is that VO_{2SC} will demonstrate a relationship with muscle fatigue and this relationship will be influenced by prior exercise.

1.3.1.1 Hypothesis Study 1

H1 The reductions in VO_{2SC} amplitude would be accompanied by attenuation of falls in peak torque during the experimental condition.

H2 The magnitude and time course of muscle fatigue would be related to the development of the VO_{2SC} in both conditions (i.e., control and experimental condition).

1.3.1.2 Hypothesis Study 2

H1 The prior very-heavy intensity exercise would reduce a subsequent time to exhaustion if W' reconstitution remains incomplete between the bouts.

1.3.1.3 Hypothesis Study 3

H1: The work-to-work transitions lead to a lower VO_{2SC} amplitude and a slower time constant in both sexes;

H2: female subjects present a lower amplitude of fundamental and slow phases compared to male counterparts.

H3: Alterations in muscle force production behaviour are accompanied by changes in VO_{2SC} and τ for both conditions, both velocities and both sexes.

2 CHAPTER TWO

2.1 STUDY ONE: The effects of priming exercise on the VO_2 slow component and the time-course of muscle fatigue during very heavy intensity exercise in humans.

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Original research

Title

The effects of priming exercise on the VO_2 slow component and the time-course of muscle fatigue during very heavy intensity exercise in humans.

Running title

VO_2 slow component and time-course of muscle fatigue during exercise

Authors:

Paulo Cesar do Nascimento Salvador[1], Kristopher Mendes de Souza[1], Ricardo Dantas De Lucas[1], Luiz Guilherme Antonacci Guglielmo[1], Benedito Sérgio Denadai[2].

[1]Physical effort Laboratory, Sports Center, Federal University of Santa Catarina, Florianópolis, Brazil

[2]Human Performance Laboratory, São Paulo State University, Rio Claro, Brazil

Corresponding author:

Paulo Cesar do Nascimento Salvador

ORCID iD 0000-0001-8228-5115

Adress: Rua Silvio Possobon, 70, apartamento 1009, Abraão, CEP: 88085-190, Florianópolis, Santa Catarina, Brasil.

Phone: +55 48 9949-6762, Fax: +55 48 3721-6248

Email: nascimentopc84@hotmail.com

Kristopher Mendes de Souza - kristophersouza@yahoo.com.br
Ricardo Dantas De Lucas - tridantas@hotmail.com
Luiz Guilherme Antonacci Guglielmo - luiz.guilherme@ufsc.br
Benedito Sérgio Denadai - bdenadai@hotmail.com
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Abstract

We hypothesized that prior exercise would attenuate the muscle fatigue accompanied by the VO_{2SC} behavior during a subsequent very heavy (VH) intensity cycling exercise. Thirteen healthy male subjects performed tests to determine the critical power (CP) and W′ and six square-wave bouts until 3 or 8 min, each at a work rate set to deplete 70 % W′ in 8 min, with an maximal isokinetic effort (MIE) before and after the conditions without (VH_{CON}) and with prior exercise (VH_{EXP}), to measure the cycling peak torque decrement. The VO_{2SC} magnitude at 3 min (VH_{CON} = 0.280 ± 0.234, VH_{EXP} = 0.116 ± 0.109 $L.min^{-1}$; p=0.04) and the VO_{2SC} trajectory were significantly lower for VH_{EXP} (VH_{CON} = 0.108 ± 0.042, VH_{EXP} = 0.063 ± 0.031 $L.min^{-2}$; p<0.01) leading to a VO_{2SC} magnitude at 8 min significantly lower than VH_{CON} (VH_{CON} = 0.626 ± 0.296 $L.min^{-1}$, VH_{EXP} = 0.337 ± 0.179; p<0.01). Conversely, peak torque progressively decreased from pre-exercise to 3 min (ΔTorque = 21.5 ± 7.7 *vs*. 19.6 ± 9.2 Nm) and to 8 min (ΔTorque = 29.4 ± 15.8 *vs*. 27.5 ± 12.0 Nm) at VH_{CON} and VH_{EXP} respectively, without significant differences between conditions. Regardless of the condition, there was a significant relationship between Δtorque and the VO_{2SC} (R^2 VH_{CON} = 0.23, VH_{EXP} = 0.25; p=0.01). Considering that "priming" effects on the VO_{2SC} were not accompanied by the muscle force behavior, these findings do not support the hypothesis of a "causal" relationship between the time-course of muscle fatigue and VO_{2SC}.

Key words priming exercise; slow component of oxygen uptake; VO_2 kinetics, muscle force production; high-intensity exercise

2.1.1 Introduction

During exercise performed above the gas exchange threshold (GET), the fundamental oxygen uptake (VO_2) kinetics is supplemented by a delayed-onset VO_2 slow component (VO_{2SC}). The VO_{2SC} delays the attainment of a steady state during heavy-intensity exercise [below the critical power (CP)] or drives the VO_2 toward to its maximum (VO_{2peak}) during VH-intensity exercise (above the CP) (JONES et al., 2011; ROSSITER, 2011). Although the development of the VO_{2SC} indicates a progressive loss of muscle efficiency as supra-GET exercise proceeds (GRASSI; ROSSITER; ZOLADZ, 2015; JONES et al., 2010; POOLE et al., 2016), the putative mechanisms determining the VO_{2SC} are still controversial and are not entirely understood (GRASSI; ROSSITER; ZOLADZ, 2015; JONES et al., 2011). The majority of the VO_{2SC} (>85%) originates in the locomotor muscles (POOLE et al., 1991; ROSSITER et al., 2002a). Indeed, previous studies using different techniques such as electromyography (BORRANI et al., 2001; BURNLEY et al., 2002; LANZI et al., 2012), magnetic resonance imaging (ENDO et al., 2007; SAUNDERS et al., 2000, 2003), glycogen depletion (CARTER et al., 2004; KRUSTRUP et al., 2004a), muscle biopsy (KRUSTRUP et al., 2004b) and neural blockade of type I muscle fibers (KRUSTRUP et al., 2008), confirm that the dominant portion of the VO_{2SC} derives from intramuscular sites (JONES et al., 2011). Thus, the VO_{2SC} might be caused by an additional recruitment of less-efficient type II muscle fibers in order to compensate for progressive fatigue in the early recruited fibers and/or by an increased metabolic demand within those already recruited fibers (JONES et al., 2011).

In this context, the VO_{2SC} is related to muscle fatigue (defined as an exercise-induced reduction in maximal muscle force, torque or power-generating capacity) during supra-GET exercise (CANNON et al., 2011; GRASSI; ROSSITER; ZOLADZ, 2015; KEIR et al., 2016a). Cannon et al. (2011), analyzed three different work rates (below the GET, between GET and CP, and above the CP) and three different pedal cadences (60, 90 and 120 rpm) in the cycling exercise, and demonstrated a significant correlation between VO_{2SC} and muscle fatigue (quantified by reductions in peak torque and power during an all-out isokinetic sprint cycling). However, the authors observed that the time course of muscle fatigue was unrelated to the VO_{2SC} progression, because the reductions in peak torque and power were unchanged between 3 and 8 min of heavy- and VH-intensity exercise (CANNON et al., 2011). This finding suggests that loss of muscle efficiency during supra-GET

exercise might occur without progressive fatigue development. Moreover, the development of the VO_{2SC} seems to be independent of an additional recruitment of less-efficient type II muscle fibers (CANNON et al., 2011; GRASSI; ROSSITER; ZOLADZ, 2015; JONES et al., 2011).

On the other hand, Keir et al. (2016) reported that the time course of peripheral muscle fatigue (as determined by pre- vs. post-exercise differences in electrically stimulated quadriceps muscle torque) was associated with the development of the VO_{2SC} during 18 min of a VH-intensity cycling exercise. This quantitative and temporal relationship between VO_{2SC} and peripheral muscle fatigue suggests, therefore, that both phenomena share common mechanisms. Indeed, above the CP, the trajectory of the VO_{2SC} mirrors those of depletion of substrates (e.g., [PCr] and glycogen) and the accumulation of fatigue-related metabolites (e.g., [ADP], [P_i], [H^+] and [K^+]) (BLACK et al., 2017; BURNLEY; JONES, 2016; CARTER et al., 2004; JONES et al., 2011, 2010; POOLE et al., 2016). In addition, the development of the VO_{2SC} is limited by the attainment of the VO_{2peak}, and the level of metabolic muscle perturbation is also restricted to the attainment of some critical threshold (BLACK et al., 2017; BURNLEY et al., 2010; BURNLEY; JONES, 2016; VANHATALO et al., 2016). This is consistent with the 'critical threshold of peripheral muscle fatigue' proposed for high-intensity exercise (AMANN et al., 2011).

Although the relationship between VO_{2SC} and muscle fatigue seems to be a 'cause-effect relationship', there is not enough evidence regarding this issue. There are no studies that have investigated the influence of an intervention changing the VO_{2SC} in this relationship. It has been demonstrated that the performance of a prior heavy- or VH-intensity exercise results in a reduction of the VO_{2SC} during a subsequent VH-intensity exercise (BAILEY et al., 2009; BURNLEY; DOUST; JONES, 2002, 2006; DO NASCIMENTO et al., 2015; JONES et al., 2004; KOPPO; BOUCKAERT, 2002; SAHLIN et al., 2005). The overall effects of prior high-intensity exercise on the VO_{2SC} of a subsequent supra-CP exercise are attributed to an increase in the muscle blood flow, O_2 extraction, and mitochondrial enzyme activity, as well as reductions in additional motor unit recruitment (BURNLEY et al., 2002; JONES et al., 2011). Moreover, decreasing the substrate-level phosphorylation and an accumulation of metabolites by reducing the muscle fatigue during supra-CP exercise, would therefore be expected to result in an attenuated VO_{2SC} magnitude (ROSSITER et al., 2001, 2002b).

Thus, the aim of this study was to verify whether a prior VH-intensity exercise attenuates the muscle fatigue accompanying the reductions on the VO_{2SC} dynamic in a subsequent VH-intensity exercise. In the same way as previous studies (CANNON et al., 2011; DE SOUZA et al., 2016), we used instantaneous switching from constant work rate to isokinetic (constant pedal cadence) cycling to quantify reductions in peak torque at specific timings (pre-exercise, 3 and 8 min) during a VH-intensity exercise without (control condition - VH_{CON}) and with (experimental condition - VH_{EXP}) 'priming' influence. We hypothesized that reductions in VO_{2SC} amplitude would be accompanied by attenuation of falls in peak torque during VH_{EXP}. In addition, we hypothesized that the magnitude and time course of muscle fatigue would be related to the development of the VO_{2SC} in both conditions (i.e., VH_{CON} and VH_{EXP}).

2.1.2 Methods

Ethical approval

The experimental protocol was approved by the Research Ethics Committee of the Federal University of Santa Catarina and was conducted in accordance with the Declaration of Helsinki. After being fully informed of the risks and stresses associated with the study, the subjects gave their written informed consent to participate.

Participants

Sample size was calculated *a priori* based on the effect size (ES) = 0.5 (moderate effect) and p = 0.05, set at a minimum power of 80 % of the statistical analysis. Based on these parameters, a minimum of 12 participants was required. Thus, thirteen healthy male subjects (age 28 ± 7 years; mass 82.6 ± 12.7 kg; height 180.8 ± 6.9 cm; VO_{2peak} 51.5 ± 7.4 $ml.kg.min^{-1}$) volunteered to participate in the study. They participated in any exercise at a recreational level (3-4 sessions per week; 150-300 min per week), but were not highly trained, and were familiar with laboratory exercise testing procedures.

Overview of study design

Subjects were required to visit the laboratory on 10-12 occasions. Breath-by-breath pulmonary gas exchange and heart rate (HR) data were measured continuously during all tests. On the first visit, each subject performed a maximal incremental ramp test for the determination of the GET, VO_{2peak} and maximal power output (P_{max}). On subsequent visits, subjects performed 3-5 maximal constant work rate prediction tests for the determination of the CP and W' (fixed

amount of work above CP). After that, a series of six VH-intensity exercises without (VH_{CON}) and with (VH_{EXP}) 'priming' effect was completed to verify the relationship between VO_{2SC} and muscle fatigue above the CP (Figure 2.1). Subjects were instructed to avoid any intake of caffeine for 3 h, or alcohol and strenuous exercise in the 24 h preceding the test sessions and to arrive at the laboratory in a rested and fully hydrated state, at least 2 h postprandial. All tests were performed at the same time of day in a controlled environmental laboratory condition (19-22°C; 50-60%RH) to minimize the effects of diurnal biological variation on the results. Subjects performed only one test on any given day, and each test was separated by 24-72 h but completed within a period of five weeks.

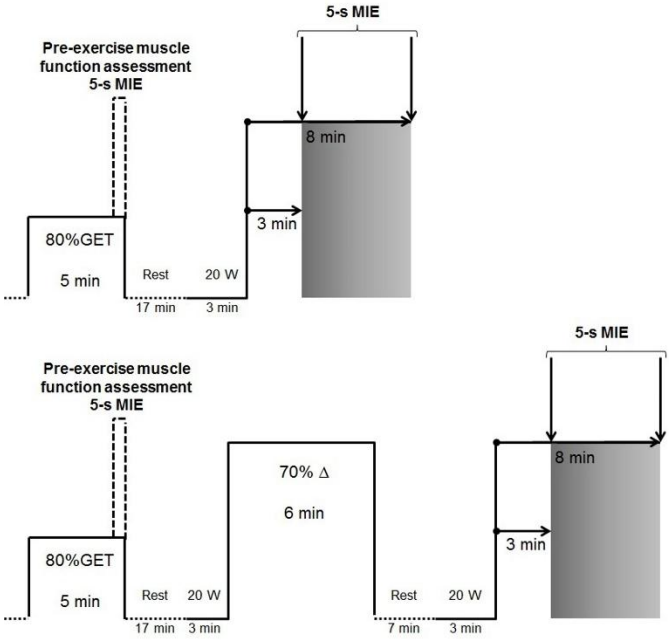

Figure 2.1
Experimental design of study. GET = Gas exchange threshold. MIE = Maximal isokinetic effort. Upper panel = control exercise condition. Lower Panel = Prior exercise condition

Equipment

All tests were performed on an electromagnetically braked cycle ergometer (Excalibur Sport PFM, Lode BV, Groningen, Netherlands). Respiratory and pulmonary gas exchange variables were measured using a breath-by-breath analyzer (Quark PFTergo, Cosmed, Rome, Italy). Before each test, the O_2 and CO_2 analysis systems were calibrated using ambient air (20.94% O_2 and 0.03% CO_2) and a gas of a known O_2 and CO_2 concentration (16.00% O_2 and 5.00% CO_2) according to the manufacturer's instructions. Likewise, the turbine flow meter was calibrated before each test using a 3 L syringe (Quark PFTergo, Cosmed, Rome, Italy). A monitor coupled to the gas analyzer was used to measure the HR. Capillary blood samples (25 μl) were obtained from the earlobe of each subject and the blood lactate concentration ([La]) was measured using an electrochemical analyzer (YSL 2700 STAT, Yellow Springs, Ohio, USA). The cycle ergometer, the breath-by-breath analyzer and the electrochemical analyzer were calibrated in accordance with specific manufacturer's recommended procedures.

Determination of GET and VO_{2peak}

On the first laboratory visit, 15 min after the isokinetic sprint familiarization, subjects performed an incremental ramp test for the determination of the GET, VO_{2peak} and P_{max}. After a 4-min period of cycling at 20 W (baseline), an incremental ramp test to exhaustion was undertaken with power output increasing by a rate of 30 $W.min^{-1}$. Subjects were instructed to maintain their preferred cadence (83 ± 6 rpm) throughout the test. The preferred cadence along with saddle and handle bar height and configuration was recorded and reproduced in subsequent tests. Each subject was verbally encouraged to undertake maximal effort. The test was terminated when the cadence fell by more than 10 rpm below the preferred cadence for more than 5 s despite strong verbal encouragement (BLACK et al., 2015). The VO_2 values were averaged over 15-s periods. The VO_{2peak} was defined as the highest value obtained in a 15-s interval, or if a VO_2 plateau observed, the VO_{2peak} was considered as the average of the final minute of exercise (DAY et al., 2003). The attainment of VO_{2peak} was defined using the criteria proposed by Bassett and Howley (2000). The P_{max} was considered as the highest power output attained during the test.

The GET was determined using a cluster of measurements as the V-slope method and the ventilatory equivalent method (BEAVER; WASSERMAN; WHIPP, 1986). The data from the ramp test were used

to calculate the work rate corresponding to 70 % Δ (i.e., GET plus 70 % of the difference between the work rate at the GET and VO_{2peak}), with the lag in VO_2 during incremental exercise taken into account by a deduction of two-thirds of the ramp rate from the work rate at the GET (BURNLEY; DAVISON; BAKER, 2011). The work rate at 70 % Δ was calculated by interpolation of the linear regression of VO_2 with respect to work rate during the incremental test. This excluded the first few minutes (2 – 3 min), as well as the time following the attainment of any plateau in VO_2.

Determination of CP and W'

The participants performed a series of 3-5 constant work rate prediction trials in a randomized order on separate days for the determination of the CP and *W'*. The work rates were chosen to elicit exhaustion between ~2 and ~15 min (e.g., 75% to 105% of P_{max}), which is the range recommended for the determination of the CP and *W'* (BURNLEY; DAVISON; BAKER, 2011; JONES et al., 2010). After the performance of the first two trials, a linear regression was used to estimate the subsequent trials. The work rates were estimated using CP and W′ parameters derived from equation 1. Each test started with a 5 min warm-up at GET followed by 5 min of passive rest. Furthermore, after 3 min of cycling at 20 W, the work rate was adjusted to one of the previously established work rates and subjects were instructed to perform until exhaustion. Subjects were instructed to remain seated and to maintain their preferred cadence for as long as possible with the test being terminated when the cadence fell by more than 10 rpm below the preferred cadence for more than 5 s despite strong verbal encouragement (BLACK et al., 2015). The time to exhaustion (T_{lim}) was recorded to the nearest second. For each subject, the following three equivalents of the 2-parameter model were used to fit the data and to estimate CP and *W'*:

The linear power output (P) *versus* 1/time to exhaustion:

$$P = (\frac{W'}{T_{lim}}) + CP \qquad \text{Equation 2.1}$$

The linear work (W) *versus* time to exhaustion:

$$W = (CP\ x\ T_{lim}) + W' \qquad \text{Equation 2.2}$$

Non-linear power output (P) *versus* time to exhaustion:

$$T_{lim} = (\frac{W'}{P-CP}) \qquad \text{Equation 2.3}$$

As a quality control measure of the mathematical modeling of power-time parameters, a priori criteria were set for the standard errors of estimate (SEE) associated with the CP and *W′*, such that if the SEE exceeded 1% and 5%, respectively, after 3 predictions trials had been performed, a fourth prediction trial was then completed. If required a fifth prediction trial would be performed and inserted in the models to bring the SEE of the CP and *W′* below the criteria. The SEE values from the equations 1-3 were compared in order to select the CP and *W′* estimates from the best fit (DE SOUZA et al., 2016; VANHATALO et al., 2010).

Maximal isokinetic effort

The muscle fatigue was assessed for each subject before and after square-wave bouts (VH_{CON} and VH_{EXP}) by a 5-s maximal isokinetic effort (MIE) at 120 rpm, in order to quantify peak torque and power. This protocol was similar to the previously used by Cannon et al. (2011) and de Souza et al. (2016). In the pre-exercise muscle function assessment subjects performed a 5 min warm-up at 80 % GET immediately followed by the MIE. After this, the subjects performed 5 min of active recovery at 80 % GET and a period of 12 min rest before the square-wave bouts. In post-exercise muscle fatigue assessment, the MIE was performed immediately after the VH_{CON} and VH_{EXP} (see figure 1 for more details). Subjects were given an auditory cue to begin the all-out effort in the seated position and strong verbal encouragement was given throughout the 10 crank revolutions (5 s at 120 rpm). The cycle ergometer was instrumented with pedal force measurement (Excalibur Sport PFM, Lode BV, Groningen, Netherlands). For the muscle fatigue measurements, the torque and power data were recorded continuously during all the MIE. As described by Altenburg et al. (2007), the peak torque and power in each crank arm were determined by visual inspection as the average of the four consecutive highest torque and power values (2 s) excluding any occasional overshoot. Thus, the peak torque and power during the MIE were then considered as the average of the peak values of both left and right crank arms.

Square-wave bouts

In the main part of this investigation, six square-wave bouts set to deplete 70 % *W′* in 8 min were performed in a randomized order until 3 min or 8 min (twice), three with (VH_{EXP}) and three without (VH_{CON}) prior VH exercise. In VH_{CON}, 17 min after the pre-exercise measurements of MIE the exercise protocol started with 3-min of

baseline (cycling at 20 W), immediately followed by an abrupt transition to the target work rates. Considering that the muscle fatigue is related to the total amount of work that can be performed above the CP (i.e., *W′*) (MURGATROYD et al., 2011; VANHATALO et al., 2016), the work rates were calculated to induce a depletion 70 % W′ at 8 min (70 % *W′*) according to equation 1.

In VH_{EXP}, in the same way to control, 17 min after the pre-exercise MIE and 3 min of baseline cycling, an abrupt transition to a 70 % Δ during 6 min was set. After 7 min of passive recovery and 3 min of baseline an abrupt transition to a 70 % *W′* work rate until 3 min or 8 min were set. This intensity and this duration of time to recovery were shown by Bailey et al. (2009) as an efficient prior exercise strategy that attenuates the VO_{2SC} and improves T_{lim}. Capillary blood samples were collected from the ear lobe 30 s before the beginning, as well as 30 s before the 6th min at prior exercise bouts, 3rd min or 8th min of exercise during the square-wave bouts. The Δ [La] was defined as the difference between the [La] at the end, and the [La] at the beginning of each exercise bout. To ensure that the pre-exercise muscle assessments performed before VH priming would be reflective of those that could be measured after priming, all participants performed a constant work rate exercise bout to exhaustion at a VH intensity (~70 % Δ) with the MIE performed before (as in the same way that the pre-measurements) and 1 min after the intolerance.

Data analysis

Breath-by-breath data for each test were initially examined to exclude outlier values caused by sighs, swallowing and coughs (LAMARRA et al., 1987). For each exercise transition, the breath-by-breath data was linearly interpolated to 1-s intervals. Each condition was then time-aligned to the start of exercise, and ensemble-averaged (three trials until 3 min and two trials until 8 min for each condition) to yield a single profile for each subject. The single profile data was reduced to a 5-s stationary average to decrease the influence of "signal error" and improve parameter estimation (WHIPP; ROSSITER, 2005). The first 20-s of data after the onset of exercise (i.e., the "cardiodynamic" phase) were not included in the analysis (ROSSITER; HOWE; WARD, 2005). Non-linear regression techniques were used to fit the data after the onset of a fundamental phase with an exponential function (OriginPro 8; OriginLab). An iterative process ensured that the sum of squared errors was minimized. The mathematical model consisted of an exponential term (equation 4) as described previously (BARSTOW et al., 1996). The

fundamental VO_2 kinetics (phase II) was isolated following the iterative method to identify the exponential region (Murgatroyd et al., 2011; Rossiter et al., 2002; Rossiter et al., 2001). Based on previous study (BARSTOW et al., 1996), the model was constrained in $VO_{2baseline}$ to aid in the identification of the key parameters according to the following equation:

$$VO_2(t) = VO_{2baseline} + A\,x\,[1 - e^{-\left(\frac{t-TD}{\tau}\right)}] \qquad \text{Equation 2.4}$$

where: $VO_2(t)$ represents the value of VO_2 at a given time (t); $VO_{2baseline}$ is the average value over the last minute of baseline cycling; A is the asymptotic amplitude for the exponential term describing changes in VO_2 from baseline to its asymptote; τ is the time constant; and the TD is the time delay.

The identification of VO_{2SC} during the VH intensity exercise, in which the exponential region varies in duration among the subjects due to the variably delayed appearance of the VO_{2SC} (MURGATROYD et al., 2011) was performed individually. The identification at the end of fundamental phase (i.e., TD_s) was performed by fitting a window from the start of the fundamental phase (i.e., after 20 s cardio-dynamic phase) initially set at 60 s. The window was lengthened iteratively until the exponential model fit demonstrated a discernible and consistent departure from the measured VO_2 values by considering the following criteria: 1) the narrowest confidence interval for τ; 2) a breakpoint and systematic increase in both τ and A, with a decrease in TD; 3) a breakpoint and systematic rise in the χ^2 for the fitted model; and 4) a departure from an even distribution of residuals around zero (as judged from the visual inspection of a plot of the residuals of the fit) (MURGATROYD et al., 2011; ROSSITER et al., 2002; ROSSITER et al., 2001). Thus, the fitting window was constrained to this time point and a single-exponential fitting was performed only on the fundamental phase to identify the kinetics parameters. The VO_{2SC} was calculated according to the following equation:

$$VO_{2SC} = VO_{2END} - (VO_{2baseline} + A) \qquad \text{Equation 2.5}$$

Where: VO_{2END} is the average VO_2 value over the last 15 s at 3 or 8 min of exercise. The functional gain of the fundamental phase with respect to work rate (in $ml.W^{-1}.min^{-1}$) was calculated according to equation 2.6:

$$\text{Gain} = \frac{A}{W - \text{unloaded } baseline} \qquad \text{Equation 2.6}$$

To analyze the rate of increase in VO_2 during the second phase (i.e., the slow component trajectory - VO_{2SC} trajectory), an index of efficiency that does not depend on the value of the primary amplitude or the VO_{2END} and demonstrates the VO_{2SC} behavior without the influence of the other parameters (BURNLEY; DAVISON; BAKER, 2011). The VO_{2SC} trajectory was calculated using the following equation (BURNLEY; DAVISON; BAKER, 2011):

$$VO_{2SC}\,\text{trajectory} = \frac{VO_{2SC}}{(t - TDs)} \qquad \text{Equation 2.7}$$

Statistical analysis

Descriptive statistics are expressed as mean ± standard deviation. The Shapiro-Wilk test was applied to ensure a Gaussian distribution of the data ($n < 50$). The VO_2 kinetics parameters obtained from the square-wave bouts were compared between the conditions (VH_{CON} *vs*. VH_{EXP}) using the paired "t" test. Relationships between variables were assessed using Pearson's product-moment correlation coefficients. A two-way ANOVA with repeated measures was used to analyze the interaction over time and conditions for torque and power output parameters, [La] and as well for VO_{2SC} at 3 or 8 min. Assumptions of sphericity were assessed using the Mauchly test, and any violations were corrected using the Greenhouse-Geisser correction factor. The Shapiro-Wilk test was used to verify the normality of residuals. When significant effects were observed the Bonferroni post hoc test was used for comparisons. Analyzes were performed using the Statistical Package for Social Sciences Windows (SPSS Inc. version 17.0; Chicago, IL, USA) and Graph-pad Prism software package for Windows (version 5.0; Graph-Pad Prism Software Inc., San Diego, California, USA). The level of significance adopted was set at $p < 0.05$.

2.1.3 Results

Incremental ramp test and predictive tests of CP and W'

The results of selected peak variables obtained during incremental tests were presented in **Erro! Fonte de referência não encontrada.**.

Table 2.1 Peak exercise values obtained during ramp incremental cycling.

		CI 95%	
Variables	Mean ± SD	Lower bound	Upper bound
VO_{2peak} ($L.min^{-1}$)	4.2 ± 0.5	3.9	4.5
VO_{2peak} ($ml.kg.min^{-1}$)	51.5 ± 7.4	47.0	56.0
HR_{max} (bpm)	181 ± 12	174	188
P_{max} (W)	378 ± 39	355	402
[La] peak ($mmol.L^{-1}$)	10.6 ± 2.3	9.2	11.9

CI = confidence interval. SD = standard deviation. VO_{2peak} = peak of oxygen uptake. HR_{max}, = heart rate maximal values. P_{max} = maximal power output. [La] peak = blood lactate peak values.

The GET occurred at 25.8 ± 5.5 $ml.kg.min^{-1}$ (50 ± 5 % VO_{2peak}) and at a power of 131 ± 25 W. The [La] at the beginning and the end of the ramp tests were 1.4 ± 0.3 and 10.6 ± 2.3 $mmol.L.^{-1}$, respectively. The CP and *W'* were 249 ± 31 W (54 ± 10 % Δ) and 21.9 ± 7.6 kJ, respectively. The SEE associated with the estimated CP and *W'* were 0.6 % and 2.6 % with 95 % confidence intervals between 0.6 to 2.8 W and 0.3 to 1.0 kJ for CP and *W'*, respectively. The CP and *W'* were not correlated with τ or VO_{2SC}, respectively. The prior exercise work rate corresponding to 70 % Δ was 284 ± 31 W (75 ± 3 % P_{max}; 115 ± 9 % CP) and according to equation 1, depleted ~62 % *W'* in 6 min. The square-wave bouts set to deplete 70 % *W'* (15.3 ± 5.3 kJ) in 8 min were performed at 281 ± 34 W (74 ± 3 % P_{max}; 69 ± 8 % Δ).

Square-wave bouts and VO_2 kinetics responses

The τ (p = 0.19), TD for the slow component phase (i.e., TD_s; p = 0.26) and the VO_{2END} (i.e., VO_2 at 8 min; p = 0.51) were not significantly different between the conditions (Table 2.2). VO_{2END} in both conditions was not significantly different from VO_{2peak} in the ramp test (F = 0.39, p = 0.64). Conversely, in the $VH_{EXP,}$ higher total amplitude of phase II was observed (A_{TOTAL}; i.e., the $VO_{2baseline}$ + A_2; p < 0.01). In this condition VO_{2SC} magnitude at 3 min was significantly lower (p= 0.04). Despite the significant increase in both conditions, the VO_{2SC} trajectory was significantly lower for VH_{EXP} (p <0.01) leading to a VO_{2SC} magnitude at 8 min significantly lower than VH_{CON} (p <0.01) with a decrease of 37 ± 34 % (table 2.2 and figure 2.2).

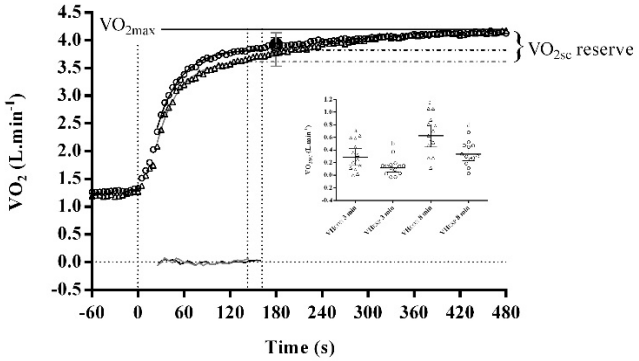

Figure 2.2
Mean group values of oxygen uptake (VO_2) in square-wave exercise transitions set to deplete 70% W′. Triangle symbols represent the control condition (VH_{CON}). Open circle symbols represent the prior exercise condition (VH_{EXP}). The gray triangle and the black circle represent the slow component of VO_2 (VO_{2SC}) at 3 min during VH_{CON} and VH_{EXP} condition, respectively. The dotted vertical lines represent begin of exercise and the start of VO_{2SC}. The continued lines showed the fit and residuals (gray for VH_{CON} and black for VH_{EXP}). Note that the VO_{2SC} was significant different between conditions and the VO_2 maximal values (VO_{2peak}) were attained

Table 2.2 VO_2 parameters during the square-wave bouts with and without prior exercise.

	VH_{CON}	VH_{EXP}	t value	p value
$VO_{2baseline}$ (L.min^{-1})	1.27 ± 0.17 (95%CI: 1.17-1.38)	1.33 ± 0.17* (95%CI: 1.23-1.43)	2.99	0.02
A (L.min^{-1})	2.28 ± 0.27 (95%CI: 2.12-2.44)	2.48 ± 0.39* (95%CI: 2.25-2.72)	2.82	0.02
Gain (ml.W^{-1}.min^{-1})	8.8 ± 0.7 (95%CI: 8.4-9.2)	9.5 ± 0.6* (95%CI: 9.2-9.9)	2.65	0.02
A_{TOTAL} (L.min^{-1})	3.55 ± 0.38 (95%CI: 3.33-3.78)	3.81 ± 0.48* (95%CI: 3.52-4.10)	4.33	<0.01
τ (s)	24.5 ± 7.9 (95%CI: 19.8-29.3)	27.8 ± 6.8 (95%CI: 23.8-31.9)	1.40	0.19
TD (s)	13.4 ± 4.8 (95%CI: 10.5-16.2)	9.6 ± 4.3* (95%CI: 7.1-12.2)	2.25	0.04
VO_{2SC} 3 min (L.min^{-1})	0.28 ± 0.23 (95%CI: 0.14-0.42)	0.12 ± 0.11* (95%CI: 0.05-0.18)	Anova 4.78	0.04
VO_{2SC} 8 min (L.min^{-1})	0.63 ± 0.30 (95%CI: 0.45-0.80)	0.34 ± 0.18* (95%CI: 0.23-0.45)	Anova 4.78	<0.01
VO_{2SC}trajectory (L.min^{-2})	0.11 ± 0.04 (95%CI: 0.08-0.13)	0.06 ± 0.03* (95%CI: 0.04-0.08)	4.66	<0.01
TD_s (s)	144 ± 46 (95%CI: 116-171)	162 ± 33 (95%CI: 142-182)	1.19	0.26
VO_{2END} (L.min^{-1})	4.18 ± 0.50 (95%CI: 3.88-4.48)	4.15 ± 0.52 (95%CI: 3.83-4.46)	0.67	0.51

VH_{CON}: control condition. VH_{EXP}: experimental condition. $VO_{2baseline}$ = the average value over the one min of resting baseline; A = the amplitude for the fundamental phase; A_{TOTAL} = A + $VO_{2baseline}$; τ = the time constant; TD = the time delay; VO_{2SC} = slow component of VO_2; TD_s = TD of VO_{2SC} phase; VO_{2END} = the average VO_2 value over the last 15 s at 3 or 8 min of exercise.

The [La] increased from rest to 3 min and to 8 min in VH_{CON}, whereas in VH_{EXP} [La] at rest was markedly augmented ($p < 0.05$), and it did not increase further after the first 3 min but increased from 3 min to 8 min. There were observed differences ($p < 0.05$) between conditions for the baseline values, at 3 min, but no differences were found between the conditions at 8 min of exercise (Figure 2.3). There was no significant correlation ($p > 0.05$) between the VO_{2SC} and [La] at any time in both conditions. The HR was slightly higher ($p < 0.05$) during VH_{EXP} protocol throughout most of the test, but that difference gradually decreased and at the end of the exercise no differences were observed between the two conditions ($p = 0.19$). The HR at the end of VH_{CON} showed a significant difference in relation to the HR_{max} obtained during the ramp test ($F = 7.06$; $p < 0.01$) but HR at the end of VH_{EXP} and HR_{max} were similar ($p = 0.34$).

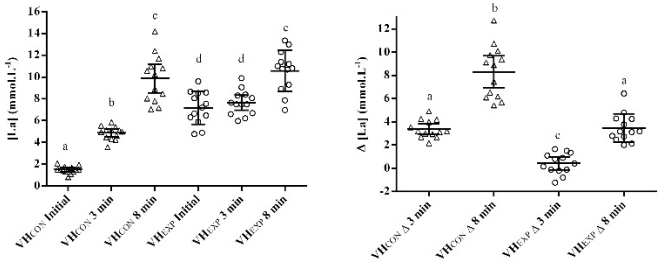

Figure 2.3
VH_{CON}: control condition. VH_{EXP}: experimental condition. There was a significant effect condition x time for blood lactate concentration ([La]) and Δ[La] ($F = 68.33$, $p < 0.01$; $F = 14.41$, $p < 0.01$, respectively). Different letters showed significant differences $p < 0.05$. Open triangles represent the VH_{CON}; open circles represent the VH_{EXP}

Peak torque and peak power output during MIE

No differences were observed for both peak torque (intraclass correlation coefficient = 0.99; typical error = 3.7 %) and peak power output (intraclass correlation coefficient = 0.99; typical error = 4.2 %) obtained during the pre-exercise assessments. The average of the three pre-exercise assessments was taken for each condition. Peak torque and power output progressively decreased from pre-exercise to 3 min and to

8 min ($p < 0.05$) in both conditions. At all assessment times, no differences between VH_{CON} and VH_{EXP} were observed ($p > 0.05$). Thus, prior exercise had no effect on muscle force production (Table 2.3 and figure 2.4). The peak torque and power output decreases were recovered 1 min after the intolerance (Table 2.4). Regardless of the condition, there was a significant relationship between muscle force behavior and the changes in the VO_{2SC}. When the peak torque was analyzed in each separate condition, a significant temporal relationship between VO_{2SC} magnitude and Δ torque at 3rd and 8th min (VH_{CON}: $R^2 = 0.23$, $p = 0.01$ and VH_{EXP}: $R^2 = 0.25$, $p = 0.01$; figure 2.5) was found. The Δ power was significantly associated with the VO_{2SC} magnitude in a similar way (VH_{CON}: $R^2 = 0.24$, $p = 0.01$ and VH_{EXP}: $R^2 = 0.34$, $p < 0.01$; figure 2.5, B and D). There was no significant association between the decrease in muscle performance indices and τ of VO_2 kinetics ($p = 0.40$).

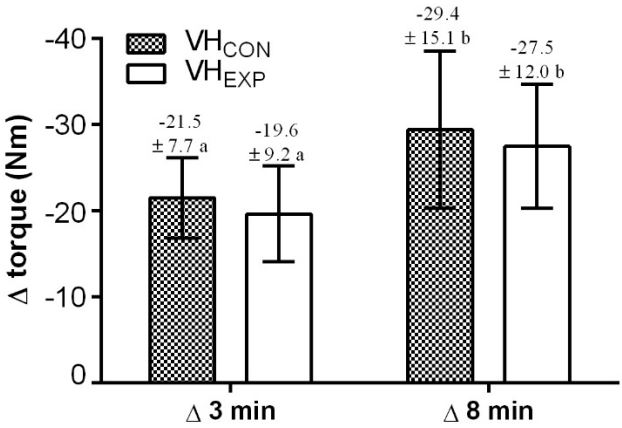

Figure 2.4
VH_{CON}: control condition. VH_{EXP}: experimental condition. There was no significant interaction condition x time for Δtorque $F = 1.09$, $p = 0.35$; but a significant effect on time within subjects; $F = 83.4$, $p < 0.01$. Different letters showed significant differences

Table 2.3 Peak torque and peak power output measured during the maximal isokinetic effort performed previous or following square-wave bouts with and without prior exercise.

Condition	**VH_{CON}**			**VH_{EXP}**		
Time	Pre-exercise	3min	8min	Pre-exercise	3min	8min
Peak Torque (Nm)	136 ± 26 (95%CI: 120-151)[a]	114 ± 28 (95%CI: 97-131)[b]	106 ± 30 (95%CI: 89-124)[c]	135 ± 25 (95%CI: 120-150)[a]	115 ± 25 (95%CI: 100-131)[b]	107 ± 29 (95%CI: 90-125)[c]
Peak Torque (%)	100	84 ± 6 (95%CI: 80-87)	78 ± 10 (95%CI: 72-84)	100	85 ± 7 (95%CI: 81-89)	79 ± 9 (95%CI: 73-84)
Peak Power output (W)	1705 ± 344 (95%CI: 1497-1913)[a]	1347 ± 393 (95%CI: 1110-1585)[b]	1233 ± 388 (95%CI: 998-1467)[c]	1689 ± 326 (95%CI: 1493-1886)[a]	1385 ± 366 (95%CI: 1164-1606)[b]	1258 ± 363 (95%CI: 1038-1477)[c]
Peak Power output (%)	100	78 ± 8 (95%CI: 73-82)	72 ± 12 (95%CI: 65-79)	100	81 ± 9 (95%CI: 76-86)	74 ± 9 (95%CI: 68-79)

VH_{CON}: control condition. VH_{EXP}: experimental condition. There was a significant effect on time within subjects for both variables. Different letters showed significant differences $p< 0.01$.

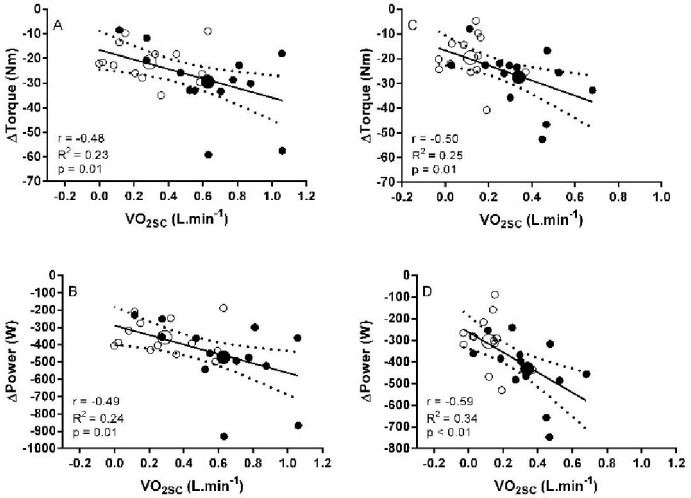

Figure 2.5
VO_{2SC} = slow component of oxygen uptake. *Panels A and B*: Control condition. *Panels C and D*: prior exercise condition. There was significant temporal relationship between VO_{2SC} magnitude at 3 min (white circles) or 8 min (black circles) and Δ torque or Δ power at 3rd and 8th min. The bigger white and black circles represent mean values at 3 min and 8 min of exercise, respectively

Table 2.4 Peak torque and peak power output measured during the maximal isokinetic effort performed previous or 1 min after very heavy-intensity exercise until exhaustion (pilot study).

	Very heavy exercise	
	Pre-exercise	Post-exercise
Peak Torque (Nm)	136 ± 25 (95%CI: 121-152)	132 ± 31 (95%CI: 113-151)
Peak Power output (W)	1722 ± 335 (95%CI: 1520-1925)	1616 ± 399 (95%CI: 1375-1857)

There was no significant effect on time within subjects for both variables.

2.1.4 Discussion

This is the first study to investigate the effects of prior exercise on the relationship between muscle fatigue and VO_{2SC}. Some recent papers suggested a direct association between muscle fatigue and the VO_{2SC} (CANNON et al., 2011; KEIR et al., 2016a). The present work aimed to test whether the time course of muscle fatigue follows a "cause-effect" relationship by manipulating the VO_{2SC} with "priming" exercise. Compared to a non-priming control (VH_{CON}), the VO_{2SC} during VH exercise was attenuated with priming (VH_{EXP} protocol) but there were no between-condition differences in the magnitude of the reduction (from pre-exercise) of maximal isokinetic force and power at the third and eighth minutes of VH exercise. Therefore, the within-condition results confirm a relationship between the magnitude (CANNON et al., 2011) and time-course (KEIR et al., 2016a) of muscle fatigue and the VO_{2sc} during VH exercise but the between-condition findings refute a "cause-effect" relationship between VO_{2sc} and muscle fatigue."

Relation between muscle fatigue and VO_{2sc}

Cannon et al. (2011) observed that when the exercise was performed at heavy domain the force production decreased significantly within 3 min of exercise with no additional reduction in peak torque between 3 and 8 min of exercise. Even more, at VH exercise which VO_{2sc} magnitude is higher than other intensities the muscle fatigue was similar between 3 and 8 min of exercise. This data suggests that additional recruitment of motor units was not obligatory for VO_{2SC} appearance, and besides that a reduction in mechanical efficiency in fatigued fibers was implicated and precedes VO_{2SC}. The authors affirmed that an increased ATP and/or O_2 cost of power production in fatigued fibers, that is a precedent fatigue, would be responsible for the VO_{2SC}. In the present study, it was observed that a significant decrease in torque production from pre-exercise to 3 min (VH_{CON} = 16.4 ± 6.4 %; VH_{EXP} = 14.9 ± 7.1 %), and that a continued to decrease significantly from 3rd to 8th min for both conditions (VH_{CON} = 5.8 ± 7.6 %; VH_{EXP} = 6.2 ± 8.3 %). Likewise, the beginning of VO_{2SC} on the 3rd min of exercise (VH_{CON} = 7.8 ± 6.4 %; VH_{EXP} = 3.2 ± 3.4 % A_{TOTAL}) significant increases to the 8th min (VH_{CON} = 17.8 ± 8.3 %; VH_{EXP} = 9.0 ± 4.9 % A_{TOTAL}) with similar values of TD_s (VH_{CON} ~140 s and VH_{EXP} ~160 s; Table 2). Contrary to the findings of Cannon et al. (2011), this study found a significant temporal relationship between VO_{2SC}

magnitude and the time-course of force production for both conditions (figure 5). However, it is important to note that the bigger reductions in torque in both conditions occurred within 3 min of exercise (~15 % vs. ~6 %) agreeing with Cannon et al. (2011) showing a muscle fatigue that precedes the emergence of the VO_{2sc}. However, Keir et al. (2016) showed a mirror image between the temporal pattern of VO_{2SC} and the decrements in muscle torque production as well as significant correlation between the VO_{2SC} with the peripheral muscle fatigue ($p<0.01$; $r^2 = 0.69$) in a constant power-output cycling exercise (i.e., 60 % Δ) during 18 min. The authors observed that VO_{2sc} increases 25, 59, 71, 77 % of VO_{2sc} reserve (VO_{2peak} – extrapolated A) in the 3, 8, 13 and 18 min during exercise, respectively. In the same way, the percentages of decreases in the maximal voluntary contraction (MVC) were 7, 9, 19 and 22 % in the cited time intervals. The present findings confirmed the study cited above to an exercise performed above the CP at a higher intensity (69.4 % Δ with the attainment of VO_{2peak} vs. 60 % Δ no attainment of VO_{2peak}) and a protocol to measure the force production immediately in a specific mode of exercise.

However, the likely 'causal' relationship between muscle fatigue and VO_{2SC} is still under debate in the literature. To test the hypothesis of a cause-effect relation between muscle fatigue and VO_{2SC}, this relationship should be influenced by an intervention changing one of the factors. The study of de Souza et al. (2016) verified that two different conditions set to deplete 70 % *W'* within 3 or 10 min triggered different VO_{2SC} magnitudes (0.12 vs. 0.44 $L.min^{-1}$, respectively), although the peak torque (assessed with the same technique in the present study) at the end of exercise was the same. Deley et al. (2006) verified a greater muscle fatigue of type II fibers and lower VO_{2SC} during cycling exercise after a fatiguing electromyostimulation protocol. It is interesting that in this case, the VO_{2SC} showed an inverse behavior compared to the muscle fatigue and the muscle fatigue after voluntary contractions did not alter the VO_{2sc}. Furthermore, Thistlethwaite et al. (2008) showed that a 2-fold increase in the activation of the additional motor units (~38 % vs. ~21 % MVC) had a similar VO_{2SC} during a subsequent heavy-intensity cycling exercise in relation to exercise bout after a priming condition at the same intensity, indicating that muscle fatigue is not a primary determinant of the VO_{2SC}. Moreover, Hopker et al. (2016) argued that progressive muscle fatigue per se, may not be associated with the development of the VO_{2SC} and suggested that the correlations presented by Cannon et al (2011) across exercise intensities may be misbegotten. Therefore, our results corroborate with these

studies (DE SOUZA et al., 2016; DELEY et al., 2006; HOPKER et al., 2016; THISTLETHWAITE et al., 2008) showing that an acute intervention changing the VO_{2SC} dynamic does not change the post force production behavior at least during cycling exercise. The relation between VO_{2SC} and muscle fatigue could not be causal.

Physiological Mechanisms that could explain the attenuation of VO_{2SC}

When the prior exercise is performed above the CP the *W′* depletion could attain critical values or a total depletion in the some cases (FERGUSON et al., 2010). This triggers a metabolic disturbance such as impairment on Ca^{+} regulation, an increase in H^{+} concentrations, ADP and Pi inside the sarcoplasm, and a critical decrease in PCr concentrations. In the present study, according to equation 1 the *W′* depletion at prior bout during the VH_{EXP} was 62 ± 17 % with a 10 min recovery interval. After a prior VH exercise that depleted 100 % *W′* in 6 min, Ferguson et al. (2010) found a *W′* restoration of 85 % with a 15 min recovery interval. Thus, it is possible that *W′* had not totally recovered at the beginning of second bout of exercise and influenced the metabolic efficiency. Consequently, the recovery between the prior and subsequent exercises need to be enough to recover totally *W′* (BAILEY et al., 2009).

Furthermore, our results showed a higher primary gain in VH_{EXP}, more specifically a higher cost of O_2 for the same work rate. Bailey et al. (2009) observed that longer recovery intervals could be needed (e.g., 20 min) to associate a reduced VO_{2SC} with a better metabolic efficiency. The results of the present study showed a significant attenuation of the VO_{2SC} ~40 % in VH_{EXP} (with a 10 min recovery interval), with the same force production in relation to VH_{CON} and with higher HR values for the same power output. These results confirm that accelerating the overall VO_2 kinetics, per se, is not necessarily ergogenic and indicates a dissociation between changes in HR kinetics (a possible enhancement of muscle O_2 supply), and VO_2 kinetics (BAILEY et al., 2009). Additionally, if the primary amplitude is increased and the VO_{2END} does not change, the VO_{2SC} amplitude must decrease, even without mechanistic significance (BURNLEY; DAVISON; BAKER, 2011). Nonetheless, the VO_{2SC} trajectory was lower after priming and could be more meaningful than the VO_{2SC} amplitude.

Grassi et al. (2015) suggest that muscle inefficiency may be a consequence of higher ATP cost to determine force production, specifically a progressive increase in ATP turnover instead of a decrease

in ATP production (Grassi et al., 2015; Jones et al., 2011; Rossiter et al., 2002). Rossiter et al. (2001) showed that prior high-intensity exercise decreases the VO_{2SC}, the "slow component" of PCr and the phase II τ (lower O_2 deficit). Considering the significant attenuation of VO_{2SC} during VH_{EXP}, as well as an increase of A_{TOTAL}, it is possible to consider that these putative mechanisms happened in this work. Thus, a better equilibrium on ATP turnover in the first minutes of exercise would be more evident after VH_{EXP}. On the other hand, the [La] was markedly augmented (~7 $mmol.L^{-1}$) at the start of VH_{EXP} main bout and remained stable during 3 min (Δ [La] 0.4 ± 0.9 $mmol.L^{-1}$) in spite of a significant increase ~3 $mmol.L^{-1}$ in VH_{CON}. This difference at the start of the exercise could be indicating a higher oxidative production of ATP to maintain the work rate since that glycolytic production could be limited to "critical levels". The VO_{2SC} could be caused by inhibition of ATP production by anaerobic glycolysis by progressive cytosol acidification (KORZENIEWSKI; ZOLADZ, 2015; ZOLADZ et al., 2016) and, this scenario slows down further progress on the VO_{2sc} (ZOLADZ et al., 2016). Considering that Δ [La] 3min at VH_{EXP} was very close to zero because the higher acidosis caused by prior exercise, it is reasonable to assume these reasons in light of the present results.

Lastly, Hopker et al. (2016) observed significant muscle fatigue assessed by 6 s MIE (90 $rev \cdot min^{-1}$) after a muscle damage protocol but, the metabolic acidosis and VO_{2SC} (464 ± 302 $mL.min^{-1}$ in fatigue situation vs. 556 ± 223 $mL.min^{-1}$ in control) were not significantly different during the cycling exercise. These authors concluded that a locomotor muscle fatigue without inducing metabolic stress could be dissociated from the VO_{2SC} and, an additional recruitment of motor units to compensate for muscle fatigue might not be the main driver of the VO_{2SC} during high-intensity cycling exercise. Unfortunately, in the present work it is not possible to dissociate a possible decrease within muscle contractile capacity from metabolic acidosis induced by VH_{EXP}. Nevertheless, agreeing to Hopker et al. (2016) it is noteworthy that changes caused by priming on the VO_{2SC} does not mean a "mirror effect" on the muscle force production behavior. Thus, these results, do not confirm the cause-effect relationship between muscle fatigue and VO_{2SC}.

Limitations

The present study used a 5-s MIE to quantify muscle fatigue during VH intensity cycling. With this technique, the fatigue measured is an "overall" picture of fatigue and cannot discriminate its central and

peripheral components. Thus, it is not possible to ascertain whether the proportional contributions of central and peripheral factors to the reductions in maximal isokinetic torque were consistent across time or between conditions. For example, a significant reduction in central drive may have influenced the MIE differently in VH_{EXP} relative to VH_{CON}. In addition, the pre-exercise MIE in VH_{EXP} was performed before the bout of VH priming (rather than after) and therefore it was assumed that the pre-exercise muscle torque and power values were the same before versus after the bout of VH priming in VH_{EXP}. In support of this assumption, our pilot data showed that torque production returned to pre-exercise values as early as 1 minute after a bout of exhaustive priming (Table 4). Therefore, it was assumed that the pre-exercise values in VH_{EXP} would have been the same regardless of whether they had been measured before or after priming.

2.1.5 Conclusion

This study observed a decrease on the VO_{2SC} after a priming VH intensity exercise that was accompanied by higher metabolic acidosis and cardiovascular responses. Besides, the time-course of muscle force production was the same in the control and prior exercise conditions. It was observed that there was a temporal relationship between muscle fatigue and VO_{2SC} regardless of the condition. Therefore, it seems that the mechanisms that explain muscle fatigue in part could explain the VO_{2SC}. Considering that VO_{2SC} attenuation was not accompanied by the muscle force production behavior, these findings do not convincingly support the hypothesis of a causal relationship between time-course of muscle fatigue and VO_{2SC} during VH intensity exercise.

Conflict of Interest

None of the authors of this paper has a competing interest.

References

The references of the paper are at “references section” page 107.

Author contributions

Conception and design of the work: B.S.D. and P.C.N.S. Acquisition, analysis and interpretation of data for the work: P.C.N.S, K.M.S, R.D.L and B.S.D. Drafting the work or revising it critically for important intellectual content: P.C.N.S, K.M.S, R.D.L, L.G.A.G and B.S.D. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

3CHAPTER THREE

3.1 STUDY TWO: The effects of depletion and recovery of *W′* on cycling exercise tolerance above critical power

This second paper was submitted online in its first version on the European Journal of Applied Physiology. The current status is under review.

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Original article

Title

The effects of depletion and recovery of *W′* on cycling exercise tolerance above critical power

Running title

Recovery of W′ and cycling exercise tolerance

Key words W′ reconstitution; exhaustion; very-heavy intensity cycling; VO2 dynamic.

Authors:

Paulo Cesar do Nascimento Salvador[1], Lisa Schaffer[2], Luiz Guilherme Antonacci Guglielmo[1], Bruno Grassi[3], Benedito Sérgio Denadai[4].

[1]Physical effort Laboratory, Sports Center, Federal University of Santa Catarina, Florianópolis, Brazil

[2]Centre for Sport and Exercise Science and Medicine, Brighton University, United Kingdom

[3]Exercise Physiology Laboratory, Department of Medicine, Università Degli Studi di Udine, Italy

[4]Human Performance Laboratory, São Paulo State University, Rio Claro, Brazil

Corresponding author:

Paulo Cesar do Nascimento Salvador
ORCID iD 0000-0001-8228-5115

Adress: Rua Silvio Possobon, 70, apartamento 1009, Abraão, CEP: 88085-190, Florianópolis, Santa Catarina, Brasil.

Phone: +55 489 99045700, Fax: +55 48 3721-6248
Email: nascimentopc84@hotmail.com

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Author contributions

Conception and design of the work: B.S.D. and P.C.N.S. Acquisition, analysis and interpretation of data for the work: P.C.N.S, B.G and B.S.D. Drafting the work or revising it critically for important intellectual content: P.C.N.S, L.S, B.G, L.G.A.G and B.S.D. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Abstract

Purpose We hypothesized that the W′ (finite amount of work above critical power - CP) depletion and insufficient reconstitution at experimental condition influenced by the prior very-heavy intensity exercise would influence the tolerance during a high-intensity cycling exercise until exhaustion. **Methods** Thirteen male subjects (age 28 ± 7 years; mass 83 ± 13 kg; height 181 ± 7 cm) performed 3-5 constant work rate tests until exhaustion (range: 2 ± 1 to 13 ± 2 min) for CP and W′ determination followed by two square-wave bouts until exhaustion either with prior very-heavy intensity cycling (EXP) or without (CON). In both trials, work rate was set to deplete 70% W′ in 8 min. **Results** Time to exhaustion (TTE) and Δ blood lactate concentration ([La]) were significantly lower in EXP compared to CON (595 ± 118 s vs. 683 ± 148 s; 3.5 ± 1.2 mmol.L^{-1} vs. 8.8 ± 2.3 mmol.L^{-1}; $p<0.05$, respectively) leading to a significant slower VCO_2 kinetics in EXP (mean response time = 87.8 ± 17.8 s vs. 73.7 ± 16.6 s in CON; $p<0.05$). VO_2 and heart rate (HR) were significantly higher during the first 150 s of the square-wave bouts in EXP ($p < 0.05$). The prior very-heavy intensity exercise bout induced a W′ depletion of 60%. However, after 10 min of recovery, W′ reconstitution was still incomplete (92%). **Conclusions** Prior very-heavy intensity exercise impairs high-intensity cycling performance. It is suggested that these impairments are mediated by physiological disturbances linked to W′ depletion.

Key words W′ reconstitution; exhaustion; very-heavy intensity cycling; VO_2 dynamic

Abbreviations

CP	Critical power
GET	Gas-exchange threshold
HR	Heart rate
HR_{max}	HR maximal values
[La]	Blood lactate concentration
P_{peak}	Peak power output
RER	respiratory exchange ratio
τW′	time constant of the reconstitution of W′
TTE	Time to exhaustion
VE	Minute ventilation
VO_2	O_2 uptake

CON	Control condition
EXP	Experimental condition
VO_{2peak}	Peak of oxygen uptake
W′	finite amount of work above critical power
70% Δ	70% of the difference between GET and VO_{2peak}
70% W’	Square-wave exercise intensity

3.1.1 Introduction

Exercise places an increased stress upon the integrated coupling of the physiological mechanisms controlling the cardiopulmonary and neuromuscular systems (HUREAU; ROMER; AMANN, 2016; ROSSITER, 2011). In this context, critical power (CP), represented by the asymptote of the power-time relationship, corresponds to the highest sustainable rate of oxidative metabolism (JONES et al., 2010; VANHATALO et al., 2016) and could represents a key determinant of high-intensity cycling performance (POOLE et al., 2016). At exercise intensities performed below CP, muscle lactate, pH, phosphocreatine (PCr) and inorganic phosphate (Pi) attained a steady-state within few minutes following the onset of exercise. In contrast, above CP, no steady-state in these variables can be observed (VANHATALO et al., 2010, 2016). The curvature constant of the hyperbolic P-t relationship (W') has been suggested to represent the total amount of work that can be performed above CP before exhaustion occurs (DE SOUZA et al., 2016; MORTON, 2006). The mechanistic basis of W' is rather complex and remains equivocal. However, there is evidence that W' dictates the time to exhaustion (TTE) during high-intensity exercise and that exercise intolerance corresponds to a critical level of metabolite accumulation (i.e., PCr, Pi and H^+) that is linked to the process of muscle fatigue (BLACK et al., 2016; MURGATROYD et al., 2011; POOLE et al., 2016; VANHATALO et al., 2010).

The mechanisms contributing to muscle fatigue also contributes to an increased O_2 cost of exercise (GRASSI; ROSSITER; ZOLADZ, 2015). A series of events ("fatigue cascade") happens when exercise is performed above CP that directly link W' to a progressive increase in the O_2 cost of power production leading to attainment of maximal VO_2 values (VO_{2peak}) and the exercise intolerance (DE SOUZA et al., 2016; GRASSI; ROSSITER; ZOLADZ, 2015; KRUSTRUP et al., 2004a; MURGATROYD et al., 2011; VANHATALO et al., 2016). In addition, impairments in ATP utilization and/or ATP production may be induced during exercise to exhaustion (BLACK et al., 2017). This suggests that muscle inefficiency may be a consequence to impairments in both ATP turnover and ATP production (GRASSI; ROSSITER; ZOLADZ, 2015). In contrast, attenuation of either metabolic acidosis or additional ATP usage during high-intensity exercise may improve exercise tolerance (ZOLADZ et al., 2016).

An insufficient recovery following exercise in the very-heavy intensity domain may impair subsequent exercise tolerance. The prior

very-heavy intensity exercise could deplete partially or totally the *W′* and it depends on the time of exercise and recovery. (BAILEY et al., 2009; BURNLEY; DAVISON; BAKER, 2011; FERGUSON et al., 2007, 2010). It has been suggested that these impairments are linked to the depletion and reconstitution of *W′* (BURNLEY; DAVISON; BAKER, 2011; FERGUSON et al., 2010; JONES et al., 2003; MIURA et al., 2009). In practical terms, the depletion and reconstitution of *W′* during and following exercise could be of great relevance for athletes to improve performance. The balance of *W′* remaining (W'_{BAL}) at any point during a competition determines the pace that can be done and/or the exercise intensity above CP that can be performed at before exhaustion will occur (SKIBA et al., 2014a). The W'_{BAL} model proposed by Skiba et al. (2012) allows to make inferences between *W′* and the metabolic disturbance inside the muscle cell that could explain the high-intensity exercise tolerance. However, to the best of our knowledge, no study has used the W'_{BAL} model to analyze the effects of prior exercise on the performance during whole-body constant work rate exercise. Thus, the present study aims to fill this gap and investigate the effect of depletion and reconstitution of *W′* on high-intensity cycling exercise. Therefore, the aim of the present study was to link respiratory and cardiovascular responses following a prior exercise bout to the depletion and reconstitution of *W′* in order to better understand exercise tolerance during high-intensity cycling. We hypothesized that prior very-heavy intensity exercise would reduce a subsequent time to exhaustion if *W′* reconstitution remains incomplete between the bouts.

3.1.2 Methods

Participants

Sample size was calculated *a priori* based on an effect size (ES) of 0.5 (moderate effect), an alpha level of 0.05 and power of 0.8. Thus, thirteen recreationally active (3-4 sessions.week^{-1}; 150-300 min.week^{-1}) male subjects (age 28 ± 7 years; mass 82.6 ± 12.7 kg; height 180.8 ± 6.9 cm; VO_{2peak} 51.5 ± 7.4 ml.kg.$^{-1}$min^{-1}) volunteered to participate in the study. All testing was conducted in accordance with the Declaration of Helsinki and was approved by the Research Ethics Committee of the Federal University of Santa Catarina.

Overview

Participants were required to visit the laboratory on 6-8 occasions. On the first visit, each participant performed a maximal ramp

incremental test for the determination of the gas exchange threshold (GET), VO_{2peak} and maximal power output (P_{peak}). On subsequent visits, subjects performed 3-5 maximal constant work rate prediction tests for the determination of CP and *W'*, followed by two very heavy-intensity exercise bouts performed until exhaustion without (CON) or with (EXP) prior exercise (Figure 3.1). Participants were instructed to avoid any intake of caffeine or alcohol and strenuous exercise in the 24 h preceding the test sessions and to arrive at the laboratory in a rested and fully hydrated state, at least 2 h postprandial. All tests were performed at the same time of day in a controlled environmental laboratory condition (19-22°C; 50-60%RH) to minimize the effects of diurnal biological variation. Each test was separated by 24-72 h and all testing was completed within a period of five weeks.

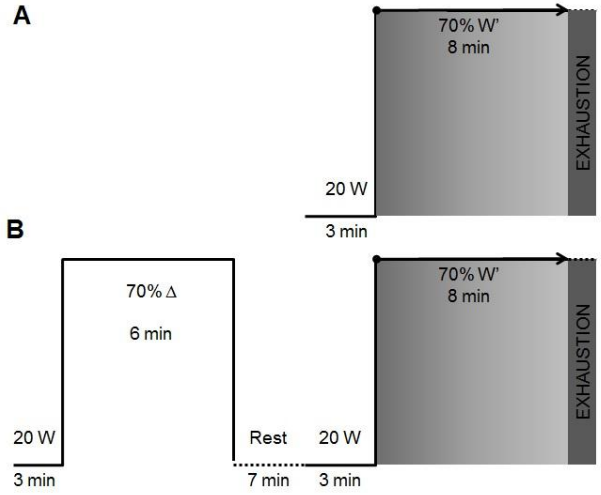

Figure 3.1
Experimental design of study. Above the control situation. Below the experimental situation.

Equipment

All tests were performed on an electromagnetically braked cycle ergometer (Excalibur Sport PFM, Lode BV, Groningen, Netherlands). Respiratory and pulmonary gas exchange variables were measured continuously throughout all testing using a breath-by-breath analyzer (Quark PFTergo, Cosmed, Rome, Italy). Before each test, the

O_2 and CO_2 analysis systems were calibrated using ambient air (20.94% O_2 and 0.03% CO_2) and a gas of a known O_2 and CO_2 concentration (16.00% O_2 and 5.00% CO_2) according to the manufacturer's instructions. Likewise, the turbine flow meter was calibrated before each test using a 3 L syringe (Quark PFTergo, Cosmed, Rome, Italy). A monitor coupled to the gas analyzer was used to measure heart rate (HR). Capillary blood samples (25 μl) were obtained from the earlobe of each subject and the blood lactate concentration ([La]) was measured using an electrochemical analyzer (YSL 2700 STAT, Yellow Springs, Ohio, USA). The cycle ergometer and the electrochemical analyzer were calibrated in accordance with specific manufacturer's recommended procedures.

Determination of GET and VO_{2peak}

On the first visit participants performed a ramp incremental test for the determination of the GET, VO_{2peak} and P_{peak}. After a 4-min period of cycling at 20 W (baseline), a ramp incremental test to exhaustion was undertaken with power output increasing by a rate of 30 $W.min^{-1}$. Subjects were instructed to maintain their preferred cadence (~80 rpm) throughout the test. The preferred cadence along with saddle and handle bar height and configuration was recorded and replicated in subsequent tests. Each participant was verbally encouraged to undertake maximal effort. The test was terminated when the cadence fell by more than 10 rpm below the preferred cadence for more than 5 s despite strong verbal encouragement (BLACK et al., 2015). Breath-by-breath pulmonary gas exchange and HR data were measured continuously throughout testing and averaged over 15-s periods. VO_{2peak} was defined as the highest 15-s average, or if a VO_2 plateau observed, it was considered as the average of the final minute of exercise (DAY et al., 2003). The P_{peak} was considered as the highest power output attained during the test.

The GET was determined using a cluster of measurements as the V-slope method and the ventilatory equivalent method (BEAVER; WASSERMAN; WHIPP, 1986). The results from the ramp test were used to calculate the work rate corresponding to 70% Δ (i.e., GET plus 70% of the difference between the work rate at the GET and VO_{2peak}), with the lag in VO_2 during incremental exercise taken into account by deduction of two-thirds of the ramp rate from the work rate at the GET (BURNLEY; DAVISON; BAKER, 2011). The work rate at 70% Δ was calculated by interpolation of the linear regression of VO_2 with respect to work rate during the incremental test. This excluded the first few

minutes (2 – 3 min), as well as the time following the attainment of a plateau in VO_2.

Determination of CP and W′

Participants performed a series of 3-5 maximal constant work rate tests in a randomized order for the determination of CP and *W′*. The work rate was selected to elicit exhaustion between ~2 and ~15 min, which is the range recommended for the determination of the CP and *W′* (BURNLEY; DAVISON; BAKER, 2011; JONES et al., 2010). Following the performance of the first two bouts, a linear regression was used to estimate the subsequent trials. The work rates were estimated using CP and *W′* parameters through equation 1. Each test started with a 5 min warm-up at GET followed by 5 min of passive rest. Furthermore, after 3 min of cycling at 20 W, the work rate was adjusted to the previously established work rate and subjects were instructed to to remain seated perform until they were unable to maintain their preferred cadence. The test was terminated when cadence fell by more than 10 rpm below the preferred cadence for more than 5 s despite strong verbal encouragement (BLACK et al., 2015). The time to exhaustion (TTE) was recorded to the nearest second. For each subject, the following three equivalents of the two-parameters model were used to fit the data and estimate CP and *W′*:

The linear power output (P) *versus* 1/time to exhaustion (1/TTE):

$$P = (W'/1/TTE) + CP \quad \text{Equation 3.1}$$

The linear work (W) *versus* time to exhaustion (TTE):

$$W = (CP \times TTE) + W' \quad \text{Equation 3.2}$$

Non-linear power output (P) *versus* time to exhaustion (TTE):

$$TTE = W'/(P - CP) \quad \text{Equation 3.3}$$

If the SEE for CP and *W′* exceeded 1% and 5%, respectively a fourth or fifth prediction trials was performed. The SEE values from the equations 1-3 were compared in order to select the CP and *W′* estimates from the best fit (DE SOUZA et al., 2016; VANHATALO et al., 2010).

Square-wave bouts

Two square-wave bouts were performed to exhaustion under CON or EXP conditions. The Tlim was estimated using the power-time relationship as described above. During CON, the exercise protocol started with 3 min of baseline pedaling at 20 W, followed by an abrupt

transition to the target work rate. Work rates were calculated to induce a depletion of 70% *W′* within 8 min (70% *W′*) using equation 1. In EXP, participants cycled for 3 min at 20 W followed by an abrupt transition to 70% Δ for 6 min. After 7 min of passive recovery and 3 min of baseline pedaling, work rate was again abruptly increased to deplete 70% *W′* within 8 min. Capillary blood samples were collected from the ear lobe at rest, in the 6th during the prior exercise bout and in the 8th min of exercise during the square-wave bout. The data from both conditions were fit by the *W′* balance model (W'_{BAL}), an equation (3.4) proposed by Skiba et al. (2012). This model allows the calculation of the remaining *W′* at any time during exercise.

Equation 3.4

$$W'_{BAL} = W' - \int_{0}^{t} W' exp \,.\, e^{\frac{-(t-u)}{\tau W'}} . du$$

where *W′* represents the subject's known *W′* as calculated using the two-parameter CP model, *W′*exp the expended *W′*, (t - u) the time in seconds between segments of the exercise session that resulted in a depletion of *W′*, and $\tau W'$ is the time constant of the reconstitution of *W′* (SKIBA et al., 2012). The $\tau W'$ parameter was calculated according the following equation:

Equation 3.5

$$\tau_{W'} = 546\, e(-0.01 Dcp) + 316$$

Considering that Dcp is equal to the difference between the power output during recovery and CP. Breath-by-breath data of each condition were examined to exclude outliers according our previous studies (DE SOUZA et al., 2016; DO NASCIMENTO SALVADOR et al., 2016) and aiming to analyze the VCO_2 kinetics mean response time (MRT) using the equation 3.6:

Equation 3.6

$$\mathrm{VCO_2(t)} = \mathrm{VCO_{2Baseline}} + \mathrm{Amplitude} \,.\, \left[1 - \mathrm{e}^{\left(\frac{\mathrm{t}}{MRT}\right)}\right]$$

Statistical analysis

Descriptive statistics are expressed as mean ± standard deviation. The Shapiro-Wilk test was applied to ensure a Gaussian distribution of the data (n < 50). The paired “t” test was used to compare some parameters obtained i.e., TTE between conditions. A two-way

repeated measures ANOVA was performed to identify time and condition (EXP vs CON) differences for VO_2, VCO_2, RER, HR, VE and [La]. The assumption of sphericity was assessed using the Mauchly`s test and any violations were corrected using the Greenhouse-Geisser correction factor. The Shapiro-Wilk test was used to verify the normality of residuals. When significant effects were observed the Bonferroni post hoc test was used for pair-wise comparisons. All statistical analysis was performed using the Statistical Package for Social Sciences Windows (SPSS Inc. version 17.0; Chicago, IL, USA). The level of significance adopted was set at $p < 0.05$.

3.1.3 Results

Ramp incremental and power-duration relationship tests

Maximum HR (HR_{max}), VO_{2max}, and P_{peak} were 181 ± 11 bpm, 4.2 ± 0.5 $L.min^{-1}$ and 378 ± 39 W, respectively. The minute ventilation (VE) and the respiratory exchange ratio (RER) were 170.2 ± 24.7 $L.min^{-1}$ and 1.23 ± 0.04, respectively. The GET was 25.8 ± 5.5 $ml.kg.min^{-1}$ (50 ± 5% VO_{2max}) and occurred at a power of 131 ± 25 W. The [La] in the beginning and at the end of ramp test were 1.4 ± 0.3 and 10.6 ± 2.3 $mmol.L.^{-1}$, respectively. The CP and *W′* were 249 ± 31 W (66 ± 5% P_{peak}) and 21.9 ± 7.6 kJ, respectively. The 95 % confidence intervals of the standard error of estimate (SEE) were 0.6 to 2.8 W and 0.3 to 1.0 kJ for CP and *W′*, respectively. Prior exercise at 70% Δ was on average 284 ± 31 W (75 ± 3% P_{peak}; 115 ± 9 % CP) and the square-wave bouts were performed at 281 ± 34 W (74 ± 3% P_{peak}; 69 ± 8% Δ).

Tables

Table 3.1 Physiological parameters during the main bout of exercise in the control (CON) and experimental (EXP) condition.

	[La] (mmol.L.$^{-1}$)		**VO_2 (L.min^{-1})**		**VCO_2 (L.min^{-1})**		**VE (L.min^{-1})**		**RER**		**HR (bpm)**	
	CON	EXP	CON	EXP	CON	EXP	CON	EXP	CON	EXP	CON	EXP
Start	1.4 ± 0.5	7.2 ± 1.7*	1.24 ± 0.15	1.30 ± 0.23	0.99 ± 0.13	1.06 ± 0.24	29.4 ± 4.4	33.9 ± 5.6*	0.81 ± 0.03	0.81 ± 0.04	96 ± 12	109 ± 11*
END	10.3 ± 2.4	10.6 ± 1.9	4.10 ± 0.50	4.07 ± 0.53	4.34 ± 0.53$^{\#}$	4.32 ± 0.63$^{\#}$	160.4 ± 20.0	163.7 ± 22.4	1.07 ± 0.05$^{\#}$	1.08 ± 0.06$^{\#}$	178 ± 12	178 ± 11
MAX	10.6 ± 2.3		4.19 ± 0.52		5.29 ± 0.56		170.2 ± 24.7		1.23 ± 0.04		181 ± 12	

Start = Start of exercise, END = final values after the main bout of exercise; MAX = maximal values during the ramp test. * Differences between conditions $p<0.05$; # Differences between END and MAX values $p.<0.05$.

Square-wave bouts exercise

According to equation 3.5, $\tau W'$ were 707 ± 78 s at the first bout exercise at EXP and 715 ± 43 s at CON and the second bout exercise at EXP, respectively. During the recovery in EXP, $\tau W'$ was 364 ± 14 s and 374 ± 17 s for the 7 min of passive recovery and 3 min of baseline pedaling, respectively. The W' remaining after 6 min of prior exercise at EXP was 40 ± 3 %. Reconstitution of W' during recovery followed an exponential behavior with greater W' recovery within the first min (69%). From the second min until 10th min the W' reconstitution was 73%, 78%, 81%, 84, 86%, 88%, 90%, 91% and 92% every min, respectively (Figure 3.2). The Tlim for EXP (595 ± 118 s) was significantly lower than CON (683 ± 148 s) and Tlim estimated by the 2-parameters model (686 ± 1 s; $p < 0.01$; figure 3.3). Tlim for CON was not significant different from the estimated Tlim ($p > 0.05$). The Tlim estimated after the recovery in EXP (i.e., with ~92 % of W') was not significantly different from the Tlim measured (625 ± 6 vs. 595 ± 118 s; $p > 0.05$).

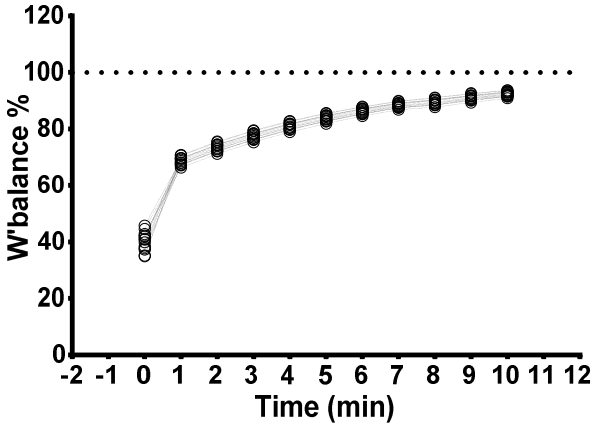

Figure 3.2
The behavior of The W' reconstitution during the recovery for every participant (see details in the text).

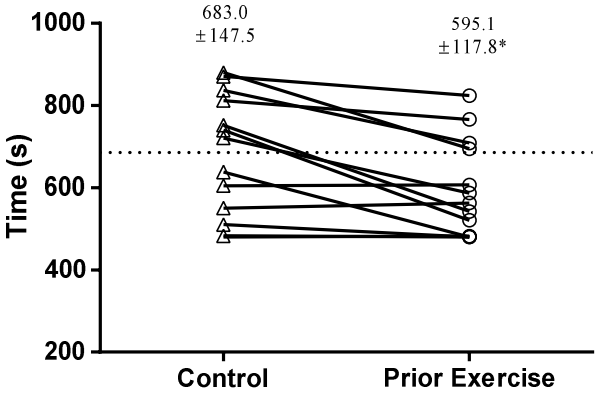

Figure 3.3
* There was a significant difference for Time to exhaustion (TTE) between conditions ($p < 0.01$). Triangle symbols represent the control condition. Open circle symbols represent the prior exercise condition. The dotted line represents the Tlim mean values estimated by the two parameters model.

The [La] during CON increased from 1.4 ± 0.5 mmol.L^{-1} to 10.3 ± 2.4 mmol.L^{-1}. During the prior exercise, [La] increased from 1.4 ± 0.4 mmol.L^{-1} to 8.4 ± 1.9 mmol.L^{-1} and from 7.2 ± 1.7 mmol.L^{-1} to 10.6 ± 1.9 mmol.L^{-1} during the second bout in EXP. The [La] before the second bout in EXP was significantly higher than CON at baseline ($p < 0.05$), but there was not significant difference between conditions at end of exercise ($p > 0.05$). The VO_2 values were significantly higher during the first 150 s at EXP compared to CON ($p < 0.01$; figure 3.4) and showed no significant differences ($p > 0.05$) with the VO_{2peak} values after 240 s of exercise. The VO_{2peak} was not significantly different from VO_2 values in CON only after 300 s ($p < 0.05$). The VE increased with time ($p < 0.05$) in both conditions and attained the maximum values at exhaustion ($p > 0.05$; see table 3.1 for more details). The higher metabolic acidosis caused by prior exercise leaded to a significantly slower VCO_2 kinetics (MRT = 87.8 ± 17.8 s at EXP *vs.* 73.7 ± 16.6 s at CON, $p < 0.05$) and a lower RER during the first 6 min of the second exercise bout (figure 3.4). In both conditions, RER at exhaustion was significantly different compared to the maximal RER observed during the incremental test ($p < 0.05$). Heart rate was slightly higher during EXP throughout most part of the test ($p < 0.05$), but differences

gradually decreased, so that after 7 min of exercise no differences were observed between the two conditions ($p > 0.05$; figure 3.4). It is worthy to note that, the HR_{max} obtained during the incremental test was not different from the final HR values in both conditions ($p > 0.05$). However, similar HR values compared to HR_{max} were found for EXP after 300 s and for CON just after 420 s ($p > 0.05$).

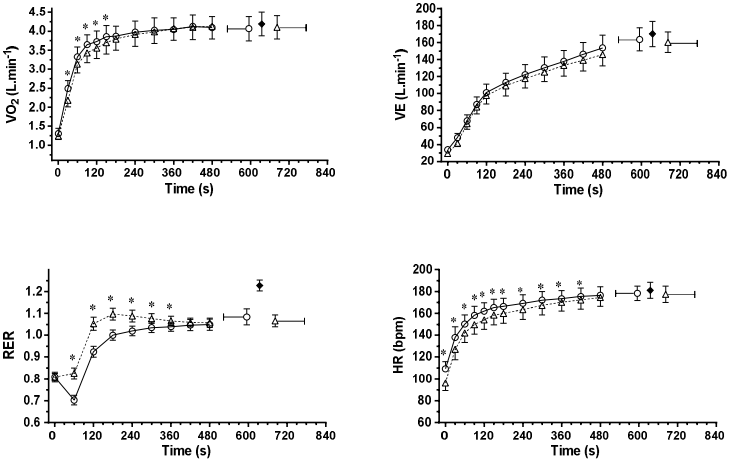

Figure 3.4
The behavior of respiratory and cardiac responses during the square-wave bouts. *Upper left panel*: oxygen uptake (VO_2). *Upper right panel*: minute ventilation (VE). *Lower left panel*: respiratory exchange ratio (RER). *Lower right panel*: heart rate (HR). There was a significant effect on time for all variables. With the exception of VE there was a significant effect condition x time for the other 3 variables. Triangle symbols represent the control condition. Open circle symbols represent the prior exercise condition. * showed significant differences between conditions.

3.1.4 Discussion

To the best of our knowledge, this is the first study to analyze the effect of prior constant work rate exercise on *W′* depletion and reconstitution during a prolonged recovery period. Further, this is the first investigation to demonstrate how *W′* recovery may influence high-intensity cycling exercise performed until task failure. The present study aimed to verify physiological responses associated with exercise tolerance during high-intensity cycling and attempted to relate these with the depletion and reconstitution of *W′*. We hypothesized that an insufficient reconstitution of *W′* during EXP caused by a prior exercise bout would influence exercise tolerance during a second exercise bout performed until exhaustion.

The main finding of the present study was that *W′* reconstitution was 92 ± 1% after 10 min of recovery. However, although less than 8% of *W′* remained depleted, Tlim during EXP was shorter compared to CON (average Δ 88 s). Thus, the presented hypothesis was confirmed. Consequently, it is suggested that the lower Tlim found in EXP was related to an incomplete *W′* reconstitution and the associated physiological disturbances caused by the prior exercise bout. The Tlim during high-intensity cycling exercise is dependent on the interaction between CP and *W′* (MURGATROYD et al., 2011). Murgatroyd et al. (2011) confirmed that the power-duration relationship model is reliable to predict cycling exercise above CP in healthy young subjects. The small discrepancies between the estimated Tlim in CON and EXP considering 92% of *W′* recovered and measured Tlim found in the present study confirm the robustness of the model.

There is evidence that *W′* recovers exponentially and is restored when power drops below CP (FERGUSON et al., 2010; SKIBA et al., 2012). Furthermore, a larger Dcp may speed up the $\tau W'$, irrespective of whether larger Dcp was a result of a lower recovery power or a higher CP (SKIBA et al., 2012, 2014a) facilitating the *W′* reconstitution to a following task. Several investigations tried to analyze the effect of very-heavy prior exercise effects on TTE (BAILEY et al., 2009; BURNLEY; DAVISON; BAKER, 2011; FERGUSON et al., 2007, 2010). It has been suggested that an improvement in TTE can be achieved when the prior exercise either increases or not influences *W′* (BURNLEY; DAVISON; BAKER, 2011). Critical power is commonly not altered by prior exercise (FERGUSON et al. 2007; BAILEY et al. 2009; FERGUSON et al. 2010; BURNLEY et al. 2011; SIMPSON et al. 2012). As well, Simpson et al. (2012) affirmed that the *W′* depletion just

happens in exercise above CP and the mechanisms that deplete the *W′* do not influence CP.

Ferguson et al. (2010) aimed to estimate the effect of different recovery durations on *W′* reconsitution. Participants performed a supra-CP exercise bout followed by either 2, 6 or 15 min of baseline pedalling at 20 W and a constant-load supra-CP exercise until exhaustion. These authors observed a *W′* restoration of ~36%, 65% and 85 %, respectively. In contrast, this study showed a ~92% restoration of *W′* after 10 min of passive (7 min) and active (3 min) recovery using the W'_{BAL} model to estimate *W′* restoration. In addition, Ferguson et al. (2010) used a 2-parameters linear model to estimate *W′* reconstitution. However, the W'_{BAL} model could led to a relative underprediction of TTE and *W′* actually measured (SKIBA et al., 2014). Futhermore, the present study used a prior exercise bout that depleted ~60% of *W′* in 6 min while Ferguson et al. (2010) used a work rate predicted to induce exhaustion within 6 min. Therefore, the prior exercise intensity, mathematical aproach and recovery duration may explain the differences found between the present investigation and previous literature. It is important to note that the recovery of *W′* is highly variable both within and between subjects (SKIBA et al., 2014b). Skiba et al. (2012) stated that despite of the restauration of *W′* recovers in an exponential pattern, the depletion of *W′* during exercise could be linear. Considering these aspects, the present study aimed to set the exercise intesity after a prior very heavy exercise looking to control the *W′* depletion rate (i.e., 70% *W′* in 8 min) during the second exercise bout. It was made trying to avoid the influence of individual depletion rate of *W′* on the exhaustion.

Burnley et al. (2011) showed that prior heavy-intensity exercise (25% Δ) increases TTE (315 s to 370 s) and improves *W′* (16.0 kJ to 18.7 kJ) during a subsequent high-intensity exercise bout (i.e., 70 % Δ very similar with present study 69.4 % Δ) with an interval recovery of 10 min. However, the very heavy prior exercise (set to deplete 100% *W′* in 8 min maintained for 6 min) did not significantly change TTE or *W′* during the subsequent high-intensity cycling exercise. Furthermore, Bailey et al. (2009) investigated different prior exercise intensities and recovery intervals (3, 9 or 20 min). These authors observed that prior heavy-intensity exercise (40% Δ) did not influence TTE during subsequent high-intensity exercise (control: ~440 s) regardless of the recovery duration. Conversely, exercise tolerance was reduced by ~16% 3 min after very-heavy intensity exercise (70% Δ). However, when individuals recovered for 9 or 20 min, TTE on the second bout was significantly increased by ~15 % or ~30 %, respectively. Interestingly,

prior exercise was sufficiently intense to accelerate the VO_2 response and the recovery duration was long enough for VO_2 and [La] to return back to control values (BAILEY et al., 2009).

It is important to note, that TTE was 11.7% lower in EXP in the present study. This has been associated with a baseline [La] of ~7 $m.mol.L^{-1}$, which is in line with the values reported by Bailey et al. (2009) after 3 min of very-heavy intensity exercise (~7 $m.mol.L^{-1}$). These authors found lower [La] in the conditions that improved exercise tolerance (~5 and ~3 $m.mol.L^{-1}$ after 9 and 20 min, respectively). Therefore, the effects of prior exercise to reflect a better metabolic efficiency appear to be related to exercise under conditions of residual blood acidosis. Although there seems to be a "threshold" (~4 $mmol.L^{-1}$) and after this point the prior exercise has no more additional effect on the metabolic efficiency (BAILEY et al., 2009; BURNLEY; DOUST; JONES, 2002; DO NASCIMENTO et al., 2015). The prior exercise bouts that seem to improve TTE are those combining a heavy-intensity (BURNLEY; DAVISON; BAKER, 2011) or very-heavy intensity exercise bout with longer recovery durations (e.g., 20 min) (BAILEY et al., 2009). The present study confirms that prior exercise does not always have ergogenic effects.

Skiba et al. (2014a) found that W' recovery was in good agreement with the expected recovery kinetics of [PCr] and muscle carnosine concentration. Furthermore, [PCr] and VO_2 showed faster, but [La] slower recovery kinetics than W' (FERGUSON et al., 2010; SKIBA et al., 2014b). In this context, at the onset of the second exercise bout [La] described still very levels (~7 $mmol.L^{-1}$) although W' was substantially recovered. This is in agreement with findings from Ferguson et al. (2010) showing that it seems rather unlikely that intramuscular [La] clearance is an exclusive mediator of W' restoration. Therefore, a decreased exercise tolerance (TTE) in EXP may have occurred as a consequence, at least in part, of metabolic acidosis and less reliance on non-oxidative metabolism (GRASSI, 2005).

A better equilibrium of oxidative ATP turnover in the first minutes of exercise would be more evident in EXP as a consequence of higher ATP cost instead of a decrease in ATP production (GRASSI et al., 2015; JONES et al., 2011; ROSSITER et al., 2002) which is suggested to be in line with the present study. A higher blood [La] during exercise is predominantly a result of muscle glycogenolysis (EMHOFF et al., 2013). The significant increase in [La] during EXP may indicate a higher oxidative ATP production in order to maintain the required work rate, assuming the glycolytic production is limited to a

"critical level". Furthermore, the lower RER values during the first 6 min in EXP (figure 4) support a higher oxidative participation considering that the anaerobic sources were already in a critical level of depletion.

A consequence of the blood [La] buffering mechanisms is the VCO_2 production (RIBEIRO et al., 1986). Thus, during EXP, VCO_2 kinetics was slower likely due to the blood acidosis buffering. In this sense, VO_2 was elevated during the first 150 s suggesting that oxidative phosphorylation was more activated. The oxygen deficit was lower in EXP, but did not result in an improved exercise tolerance. Considering that VO_{2peak} was attained earlier in EXP than CON, it may be suggested that the anaerobic energy sources that would allow to sustain exercise close to exhaustion, were already depleted during the prior exercise bout and consequently decrease the TTE. Although a similar respiratory work (analyzed by VE) was found between conditions, the cardiac work was higher at EXP leading to attainment of HR values close to maximum earlier than control (300 *vs.* 420 s). The attainment of a maximal cardiac and respiratory work, as well a high metabolic acidosis at the end of exercise resulted in an impaired exercise tolerance in EXP. The maximal VE values may represent respiratory muscle fatigue (CROSS et al., 2010) which can increase neural feedback from the muscles and trigger a sympathetically mediated restriction of locomotor muscle blood flow due to vasoconstriction in the lower limbs. Afferent feedback from these fatigued muscles may therefore accelerate the attainment of the "sensory tolerance limit" (AMANN, 2012; HUREAU; ROMER; AMANN, 2016). These mechanisms could explain the decreased exercise tolerance in EXP.

Skiba et al. (2012) suggested that CP and W' are interrelated and the nature of the W' might need to be reconsidered (VANHATALO et al., 2010). According to these authors, the smaller the difference between the VO_2 required to maintain the recovery and the VO_2 at CP, the smaller the capacity to reconstitute W'. Thus, it seems that there is an "oxidative reserve" related to the difference between the recovery work rate and CP (i.e., Dcp) (SKIBA et al., 2012). There was an inverse exponential relationship between $\tau W'$ and CP in the present study (R^2 = 0.98). This relation is consistent with previous findings for hand-grip exercise (BROXTERMAN et al., 2016) and the relationship between $\tau W'$ and Dcp during intermittent exercise (SKIBA et al., 2012, 2014b). According to Broxterman et al. (2016) this relationship suggests that the physiological variables associated with the oxidative metabolism (e.g., mitochondrial function and blood flow) also influence the mechanisms

determining $\tau W'$ (e.g., the rate of PCr resynthesis and the removal of fatigue inducing metabolites).

Limitations

The W'_{BAL} model was initially proposed to intermittent exercise. To date, only one paper used this model for a continuous exercise. The results of W' restauration are limit to the equation power to estimate the W' recovery after a constant work-rate exercise and during a long period of recovery. Although an experimental design with two bouts of exercise and a period of recovery between seems like a "big portion" of an intermittent exercise. Therefore, we extending the initial purpose of the model. Another limitation was that the present work used the formula proposed by Skiba et al. (2012) to estimate $\tau W'$ values in healthy subjects. The power to estimate a "real" $\tau W'$ value is restricted to the mathematical asymptote of the equation. It remains unknown whether this may lead to an overestimation or underestimation of $\tau W'$.

3.1.5 Conclusion

The present work showed an exponential behavior of W' balance during the 10 min of recovery following a prior high-intensity cycling exercise, leading to a W' restoration of 92%. Based on our findings, it is suggested that the tolerance to high-intensity exercise was compromised in part by the unrecovered W'. Therefore, estimated TTE for CON and EXP confirmed the robustness of the power-duration relationship and of the W' balance to predict the high-intensity exercise tolerance. It is suggested that the higher metabolic acidosis after the prior bout of exercise, verified by the higher [La], leaded to slower VCO_2 kinetics and a possibly higher oxidative ATP production, verified by the changes in RER during the experimental condition. A speeding of the VO_2 response in EXP did not lead to improvements in exercise tolerance. Additionally, a higher cardiac work was observed during the experimental condition for the same absolute amount of work. These results provide evidence for a link between the physiological disturbances caused by prior exercise, the restauration of W' and the tolerance to a constant high-intensity cycling exercise in healthy subjects. In a practical way, the use of 2-parameters model or the W' balance model could be useful for athletes and coaches to set intensity and recovery time for high-intensity trainings. Moreover, our results showed that to use prior high-intensity strategy as warm-up, should be better to consider a period of recovery longer than 10 min or lower intensities (e.g. heavy) in order to improve the exercise tolerance.

References

The references of the paper are at “references section” page 107.

4CHAPTER FOUR

4.1 STUDY THREE: Changes on the VO_2 kinetics after elevated baseline do not necessarily reflect alterations in muscle force production in both sexes

This third paper have been submitted online in its first version on the Journal of Applied Physiology. The current status is under review.

Manuscript number JAPPL-00418-2018, initial date submitted May 11th, 2018.

Original article

Title

Changes on the VO_2 kinetics after elevated baseline do not necessarily reflect alterations in muscle force production in both sexes

Running title

VO_2 kinetics and muscle force in both sexes

Key words motor unit recruitment; muscle fatigue; O_2 delivery; oxidative phosphorylation; VO_2 dynamic.

Authors:

Paulo Cesar do Nascimento Salvador[1], Lisa Schäfer[2], Bruno Grassi[3], Luiz Guilherme Antonacci Guglielmo[1], Benedito Sérgio Denadai[4].

[1]Physical effort Laboratory, Sports Center, Federal University of Santa Catarina, Florianópolis, Brazil

[2]Centre for Sport and Exercise Science and Medicine, Brighton University, United Kingdom

[3]Exercise Physiology Laboratory, Department of Medicine, Università Degli Studi di Udine, Italy

[4]Human Performance Laboratory, São Paulo State University, Rio Claro, Brazil

Corresponding author:

Paulo Cesar do Nascimento Salvador
ORCID iD 0000-0001-8228-5115

Adress: Rua Silvio Possobon, 70, apartamento 1009, Abraão, CEP: 88085-190, Florianópolis, Santa Catarina, Brasil.

Phone: +55 489 99045700, Fax: +55 48 3721-6248
Email: nascimentopc84@hotmail.com

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Author contributions

Conception and design of the work: B.S.D. and P.C.N.S. Acquisition, analysis and interpretation of data for the work: P.C.N.S, B.G and B.S.D. Drafting the work or revising it critically for important intellectual content: P.C.N.S, L.S, B.G, L.G.A.G and B.S.D. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Abstract

This study verified the effects of elevated baseline on the oxygen uptake (VO_2) kinetics and muscle force production behavior (MFP) on 10 male and 10 female healthy subjects. Subjects performed 4 transitions of very-heavy (VH) intensity cycling in a randomized order after unloaded (U-VH) or moderate (M-VH) exercise. Maximal isokinetic efforts (MIE) were performed before and after each condition at two different cadences (60 or 120 rpm). VO_2 baseline and time constant (τ) were significantly higher whereas the fundamental amplitude and VO_2 slow component (VO_{2SC}) were significantly lower in M-VH ($p < 0.05$) in both sexes. The blood lactate concentration ([La]) and rate of perceived exertion (RPE) were not influenced by condition or sex ($p > 0.05$). The MFP post-exercise was not significantly influenced by conditions in both sexes and velocities (Male Δtorque 60 rpm in U-VH = 13 ± 10 Nm, in M-VH = 13 ± 9 Nm; 120 rpm in U-VH = 22 ± 14 Nm, in M-VH = 21 ± 12 Nm; Female 120 rpm in U-VH = 10 ± 9 Nm, in M-VH = 12 ± 8 Nm, $p > 0.05$) with exception that female subjects presented smaller decreases in M-UH at 60 rpm (18 ± 14 vs. 11 ± 13 Nm; $p < 0.05$). There was no correlation between the decrease in torque production and VO_2 kinetics parameters ($p > 0.05$). The alterations in VO_2 kinetics which have been suggested to be linked to changes in motor unit recruitment after elevated baseline did not reflect alterations in muscle force production and fatigue in both sexes.

Key words: motor unit recruitment; muscle fatigue; O_2 delivery; oxidative phosphorylation; VO_2 dynamic.

4.1.1 Introduction

Over the last decades, numerous studies have aimed to understand the physiological mechanisms underlying a loss of work efficiency or an increase in the O_2 cost per unit of work during constant-load exercise above the gas exchange threshold (GET), i.e. the slow component of O_2 uptake kinetics (VO_{2SC}). Ever since, Poole et al. (1988, 1991) demonstrated that the mechanism explaining the VO_{2SC} is likely within the exercising muscle. Previous literature investigated the motor unit recruitment and/or muscle fatigue discarding the lactate metabolism or O_2 consuming process outside the exercising limbs as dominant mediators (JONES et al., 2011). Cannon et al. (2011) proposed that the muscle fatigue and an increased ATP cost of already recruited motor units instead of the recruitment of less efficient muscle fibers as the

main mechanisms explaining the VO_{2SC}. Notwithstanding, Keir et al. (2016) showed a relationship between the VO_{2SC} and time course of peripheral muscle fatigue during high-intensity exercise. Moreover, Temesi et al. (2017) suggested that subjects with slow VO_2 kinetics (i.e., higher τ values) experienced more peripheral fatigue during very-heavy cycling exercise. However, this relationship between VO_2 kinetics and muscle fatigue remains to be established. Hopker et al. (2016) analyzed the effects of muscle damage on VO_2 kinetics and suggested that locomotor muscle fatigue does not influence either the kinetic response i.e τ or the VO_{2SC}. Furthermore, de Souza et al. (2016) showed differences in the VO_{2SC} and τ for the same muscle fatigue during very-heavy cycling exercise. Do Nascimento Salvador et al. (2018) demonstrated that prior cycling exercise decreased the VO_{2SC} behavior, but did not modify the time-course of muscle torque production in a subsequent very-heavy cycling bout. Furthermore, the studies from Deley et al. (2006) and Thistlethwaite et al. (2008) did not find the same behavior of muscle fatigue and VO_2 kinetics.

To the best of our knowledge, no study investigated the relationship between VO_2 kinetics and muscle fatigue with respect to the differences in pulmonary and neuromuscular capacity between male and female subjects. Harms (2006) suggested different anatomical and physiological characteristics between females and males which may have implications for the pulmonary gas exchange during exercise. Females present smaller lung volumes, lower resting lung diffusion capacities and differences in O_2 delivery, O_2 extraction and blood flow (DOMINELLI et al., 2015; HARMS, 2006; MURIAS et al., 2013). Reis et al. (2017) stated that the lower cardiac and respiratory capacities during exercise could reduce O_2 delivery and utilization to the muscle, and consequently, lead to slower VO_2 kinetics in females. Up to date, surprisingly little is known about the gender differences in VO_2 kinetics. Studies showed that adolescent females had slower VO_2 kinetics and lower VO_2sc during heavy-intensity exercise compared to adolescent males (FAWKNER; ARMSTRONG, 2003; LAI et al., 2016). Middle aged males and females presented similar τ values during moderate exercise (O'CONNOR et al., 2012). Furthermore, Reis et al. (2017) did not find differences in the VO_2sc and τ between women and men during heavy-intensity swimming. Hunter (2014) indicated that women exhibited less fatigue than men for dynamic fatiguing contractions (loss of maximal torque) when the velocity of contraction was controlled. Moreover, there are differences between sexes in neuromuscular activation pattern of the quadriceps muscle (CLARK et al., 2005), in

muscle mass activated (greater in men) (ENOKA; DUCHATEAU, 2008; HUNTER, 2014), muscle fiber type (greater proportional of type II in men) and gene expression and interactions with sex-specific hormones (LIU et al., 2010; MAHER et al., 2009).

Previous literature showed alterations in VO_2 dynamic when high-intensity exercise was immediately preceded by an elevated baseline (elevated VO_2 values and/or elevated work-rate) (DA BOIT et al., 2014; HUGHSON; MORRISSEY, 1982; JONES; WILKERSON; FULFORD, 2008; WILKERSON; JONES, 2006; WÜST et al., 2014). Lower amplitudes of the fundamental component and VO_{2SC} and a slower time response (i.e., lower τ values) can be found during high-intensity exercise when preceded by an elevated baseline compared to unloaded pedaling. The main mechanisms are yet to be completely understood. However, it has been suggested that these changes may represent adjustments in the muscle O_2 delivery and/or the muscle recruitment of motor units which are characterized by less mitochondrial content and lower metabolic efficiency and are positioned higher in the muscle recruitment hierarchy (BEARDEN; MOFFATT, 2001; WILKERSON; JONES, 2006). Dimenna et al. (2010b) stated that it is not the elevated baseline VO_2 per se that explains a slower VO_2 kinetics, but the proportionally greater contribution of higher-order fibers to power production during transitions from an elevated baseline work-rate. To the best of our knowledge, no study has investigated the differences between sexes in VO_2 kinetics during very-heavy cycling exercise. Notably, the effects of elevated baseline on muscle force production and its association with VO_{2SC} remains to be established.

Thus, the main aim of the present study was to analyze the effects of elevated baseline (work-to-work transition) on the VO_2 kinetics and muscle force production behavior during maximal isokinetic efforts at different cadences (60 and 120 rpm) in male and female subjects. We hypothesized that (1) the work-to-work transitions lead to a lower VO_{2SC} amplitude and a slower time constant in both sexes; (2) female subjects present a lower amplitude of fundamental and slow phases compared to male counterparts; (3) alterations in MFP are accompanied by changes in $VO_{2\,SC}$ and τ for both conditions, both cadences and both sexes.

4.1.2 Materials and Methods

Ethical approval

The present work was approved by the Research Ethics Committee of the Federal University of Santa Catarina and was

conducted in accordance with the Declaration of Helsinki. After being fully informed of the risks and stresses associated with the study, the participants gave their written informed consent to participate.

Participants

Twenty healthy participants (10 females: age 28 ± 6 years; mass 58 ± 7 kg; height 161 ± 5 cm; 10 males: age 26 ± 5 years; mass 75 ± 7 kg; height 177 ± 5 cm) volunteered to participate in the study. Subjects undertook exercise at a recreational level (3-4 sessions per week^{-1}; 150-300 min per week^{-1}), and were familiar with laboratory exercise testing procedures. Women performed the constant work-rate tests in the follicular phase being performed 2-4 days after the menses.

Overview of study design

Subjects were required to visit the laboratory on 5 occasions. On the first visit, each subject performed a maximal ramp test for the determination of the GET, VO_{2peak} and peak power output (P_{peak}). On subsequent visits, subjects performed bouts (twice in each condition) of very heavy-intensity exercises immediately after unloaded (U-VH) or moderate (M-VH) baseline to verify the effects of work-to-work transitions on VO_2 kinetics and muscle force behavior (Figure 4.1). A maximal isokinetic effort (MIE; constant pedal cadence at 60 or 120 rpm) was performed following a standardized warm-up and immediately following the constant work-rate cycling bout to quantify reductions in peak torque. Subjects were instructed to avoid any intake of caffeine for 3 h, or alcohol and strenuous exercise in the 24 h preceding the test sessions and to arrive at the laboratory in a rested and fully hydrated state, at least 2 h postprandial. All tests were performed at the same time of day in a controlled environmental laboratory condition (19-22°C; 50-60% RH) to minimize the effects of diurnal biological variation. Subjects performed only one test on any given day, and each test was separated by at least 48 h but completed within a period of two weeks.

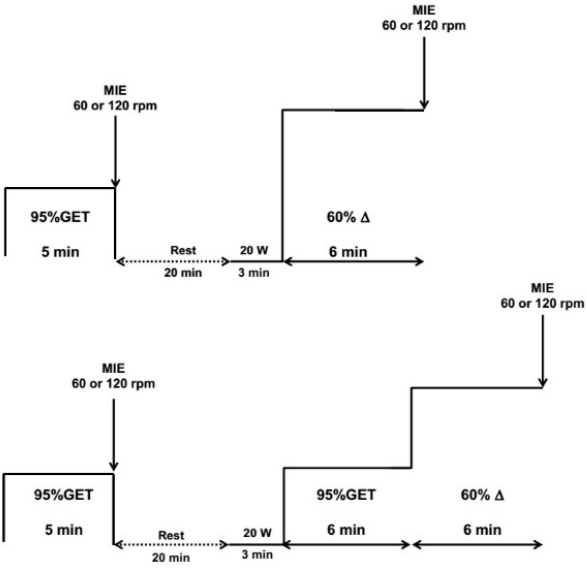

Figure 4.1
Experimental design of work. Top control condition (unloaded to very-heavy intensity exercise); bottom experimental condition (moderate to very-heavy intensity exercise). MIE = maximal isokinetic effort. GET = gas exchange threshold.

Equipment

All tests were performed on an electromagnetically braked cycle ergometer (Excalibur Sport PFM, Lode BV, Groningen, Netherlands). Respiratory and pulmonary gas exchange variables were measured using a breath-by-breath analyzer (Quark PFTergo, Cosmed, Rome, Italy). Before each test, the O_2 and CO_2 analysis systems were calibrated using ambient air (20.94% O_2 and 0.03% CO_2) and a gas of a known O_2 and CO_2 concentration (16.00% O_2 and 5.00% CO_2) according to the manufacturer's instructions. Likewise, the turbine flow meter was calibrated before each test using a 3 L syringe (Quark PFTergo, Cosmed, Rome, Italy). A monitor coupled to the gas analyzer was used to measure the heart rate (HR). Capillary blood samples (25 μl) were obtained from the earlobe of each subject and the blood lactate concentration ([La]) was measured using an electrochemical analyzer

(YSL 2700 STAT, Yellow Springs, Ohio, USA). The cycle ergometer, the breath-by-breath analyzer and the electrochemical analyzer were calibrated in accordance with specific manufacturer's recommended procedures.

Determination of GET and VO_{2peak}

On the first laboratory visit, 15 min after the isokinetic sprint familiarization subjects performed an incremental ramp test for the determination of the GET, VO_{2peak} and P_{peak}. After a 4-min period of cycling at 20 W (baseline), an incremental ramp test to exhaustion was undertaken with power output increasing by a rate of 30 $W.min^{-1}$. Subjects were instructed to maintain their preferred cadence (female 77 ± 6 rpm; male 82 ± 6 rpm) throughout the test. The preferred cadence along with saddle and handle bar height and configuration was recorded and replicated in subsequent tests. Each subject was verbally encouraged to undertake maximal effort. The test was terminated when the cadence fell by more than 10 rpm below the preferred cadence for more than 5 s despite strong verbal encouragement (BLACK et al., 2015). Breath-by-breath pulmonary gas exchange and HR data were measured continuously during the test and averaged over 15-s periods. VO_{2peak} was defined as the highest value obtained in a 15-s interval, or if a VO_2 plateau observed, it was considered as the average of the final minute of exercise (DAY et al., 2003). The attainment of VO_{2peak} was defined using the criteria proposed by Bassett and Howley (2000). The P_{peak} was considered as the highest power output attained during the test. The GET was determined using a cluster of measurements as the V-slope method and the ventilatory equivalent method (BEAVER; WASSERMAN; WHIPP, 1986). The data from the ramp test were used to calculate the work rate corresponding to 60% Δ (i.e., GET plus 60% of the difference between the work-rate at the GET and VO_{2peak}). The lag in VO_2 during incremental exercise taken into account by a deduction of two-thirds of the ramp rate from the work-rate at the GET (BURNLEY; DAVISON; BAKER, 2011).

Maximal isokinetic effort measurement

The cycle ergometer was instrumented with pedal force measurement (Lode PFM, Groningen, Netherlands) to quantify muscle fatigue during the MIE. A switch from the hyperbolic mode to the isokinetic mode happened instantaneously when required. This protocol was similar to the previously used protocols (CANNON et al., 2011; DE SOUZA et al., 2016; DO NASCIMENTO SALVADOR et al., 2018;

HOPKER et al., 2016). The peak torque was assessed for each subject by a 5-s cycling sprint test in the isokinetic mode at 60 or 120 rpm. In the pre-exercise muscle function assessment, subjects performed a 5 min warm-up at 95 % GET immediately followed by the 5-s MIE. After this, the subjects performed 5 min of active recovery at 95 % GET and a period of 15 min rest before the main exercise bouts. The MIE was repeated immediately after the exercise in U-VH and M-VH (see figure 1). Subjects were given an auditory cue to begin the all-out effort in the seated position and strong verbal encouragement was given throughout the 5-s. The torque and power data were recorded continuously during the MIE. As described by Altenburg et al. (2007), the peak torque in each crank arm was determined as the average of the four consecutive highest torque values (2 s). Thus, the peak torque during the MIE was then considered as the average of the peak values of both left and right crank arms.

Data analysis

Breath-by-breath data for each test were initially examined to exclude outlier values caused by sighs, swallowing and coughs (LAMARRA et al., 1987). After that, for each exercise transition, the breath-by-breath data was processed and analyzed according to the methods used by our laboratory in previous papers (DE SOUZA et al., 2016; DO NASCIMENTO et al., 2015; DO NASCIMENTO SALVADOR et al., 2018). Non-linear regression techniques were used to fit the data after the onset of a fundamental phase with an exponential function (OriginPro 8; OriginLab). An iterative process ensured that the sum of squared errors was minimized. Due to large inter-individual differences in the duration of the exponential region (MURGATROYD et al., 2011), the identification of the VO_{2SC} during the VH intensity exercise was performed individually. The fundamental VO_2 kinetics (phase II) was isolated following the iterative method to identify the exponential region (Murgatroyd et al., 2011; Rossiter et al., 2002; Rossiter et al., 2001). The identification at the end of the fundamental phase (i.e., TD_s) was performed by fitting a window from the start of the fundamental phase (i.e., after 20 s cardio-dynamic phase) initially set at 60 s. The window was lengthened iteratively until the exponential model fit demonstrated a discernible and consistent departure from the measured VO_2 values by considering the criteria proposed in literature (Murgatroyd et al., 2011; Rossiter et al., 2002; Rossiter et al., 2001). Thus, the fitting window was constrained to this time point and a single-exponential fitting was performed only on the fundamental phase to

identify the kinetics parameters. The model was constrained in $VO_{2baseline}$ to aid in the identification of the key parameters according to the following equation:

$$VO_2(t) = VO_{2baseline} + A\,x\,[1 - e^{-\left(\frac{t-TD}{\tau}\right)}] \quad \text{Equation 4.1}$$

where: $VO_2(t)$ represents the value of VO_2 at a given time (t); $VO_{2baseline}$ is the average value over the last minute of baseline cycling; A is the asymptotic amplitude for the exponential term describing changes in VO_2 from baseline to its asymptote; τ is the time constant; and the TD is the time delay. The VO_{2SC} was calculated according to the equation 4.2:

$$VO_{2_{SC}} = VO_{2end} - (VO_{2baseline} + A) \quad \text{Equation 4.2}$$

Where: VO_{2end} is the average VO_2 value over the last 20 s at 6 min of exercise.

Statistical analysis

Descriptive statistics are expressed as mean ± standard deviation. The Shapiro-Wilk test was applied to ensure a Gaussian distribution of the data ($n < 50$). A two-way mixed-model ANOVA was used to analyze the interaction over time and condition. Assumptions of sphericity were assessed using the Mauchly test, and any violation was corrected using the Greenhouse-Geisser correction factor. The Shapiro-Wilk test was used to verify the normality of residuals. When significant effects were observed the Bonferroni post hoc test was used for pairwise comparisons. Analyzes were performed using the Statistical Package for Social Sciences Windows (SPSS Inc. version 17.0; Chicago, IL, USA). The level of significance adopted was set at $p < 0.05$.

4.1.3 Results

The HRmax, VO_{2peak}, and P_{peak} were 182 ± 8 bpm, 43.0 ± 8.9 $ml.kg^{-1}.min^{-1}$ and 293 ± 71 W, respectively. The [La] at the beginning and at the end of ramp test were 1.6 ± 0.5 and 10.1 ± 2.4 $mmol.L^{-1}$, respectively. The male subjects showed significantly higher values for power at GET (129 ± 20 vs. 95 ± 14 W, $p<0.01$), VO_{2peak} (46.2 ± 8.2 vs. 39.2 ± 8.3 $ml.kg^{-1}.min^{-1}$, $p<0.05$), minute ventilation (VE, 167.2 ± 35.7 vs. 94.4 ± 20.5 $L.min^{-1}$, $p<0.01$) and P_{peak} (351 ± 51 vs. 235 ± 26 W, $p<0.01$) than their female counterparts. The GET was 24.3 ± 4.7 and

25.8 ± 4.7 ml.kg^{-1}.min^{-1} for female and male subjects, respectively. The 60% Δ was performed at 157 ± 23 and 240 ± 33 W and represented 85 ± 3 and 82 ± 3% of VO_{2peak} for female and male subjects, respectively.

Square-wave exercise bouts

During M-VH, the $VO_{2baseline}$ was significantly higher, A, TD and VO_{2sc} lower and τ and TDs slower compared to U-VH for both sexes ($p<0.05$). There were no significant differences for Atotal (i.e., A + $VO_{2baseline}$) and VO_{2end} between conditions for both sexes ($p>0.05$; table 4.1). Female subjects presented lower amplitudes, VO_{2SC} and VO_{2end} than male counterparts ($p>0.05$; figure 4.2; table 4.1). The [La] post-exercise was not significantly different between conditions or sexes ($p>0.05$; figure 4.3). There was no difference between conditions ($p = 0.23$) in both sexes ($p = 0.31$) for rate of perceived exertion (RPE), thus, male and female subjects perceived the effort in a similar way after the exercise in both conditions (figure 4.4).

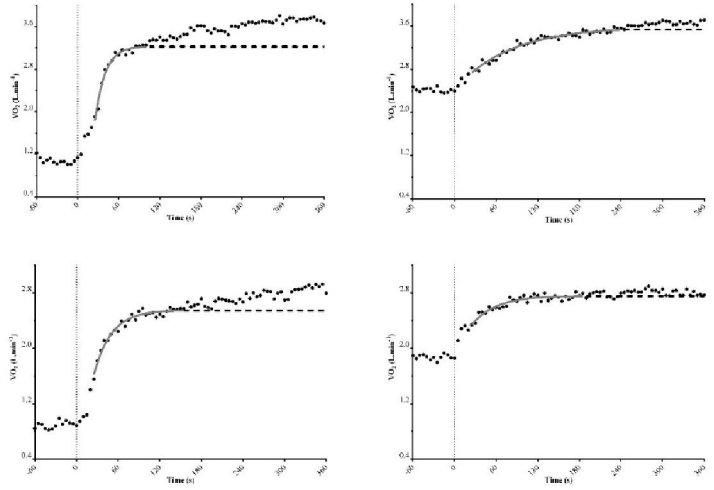

Figure 4.2
VO_2 kinetics response for representative male (*top*) and female (*bottom*) subjects during transitions from unloading (*left*) or moderate (*right*) exercise.

Table 4.1 VO_2 kinetics responses during rest-to-work and work-to-work exercise in male and female subjects.

	Male		Female	
Parameters	**U-VH**	**M-VH**	**U-VH**	**M-VH**
$VO_{2baseline}$ ($L.min^{-1}$)	1.0 ± 0.1 (CI95% 1.0-1.2)	2.1 ± 0.2 (CI95% 1.9-2.2)*	0.9 ± 0.1 (CI95% 0.8-1.0)	1.6 ± 0.2 (CI95% 1.5-1.7)*ɨ
A ($L.min^{-1}$)	2.0 ± 0.4 (CI95% 1.7-2.2)	1.1 ± 0.3 (CI95% 0.9-1.3)*	1.1 ± 0.3 (CI95% 0.9-1.4)ɨ	0.5 ± 0.2 (CI95% 0.4-0.7)*ɨ
A_{TOTAL} ($L.min^{-1}$)	3.1 ± 0.4 (CI95% 2.8-3.4)	3.2 ± 0.4 (CI95% 2.9-3.5)	2.0 ± 0.3 (CI95% 1.9-2.2)ɨ	2.1 ± 0.3 (CI95% 1.9-2.3)ɨ
VO_2sc ($L.min^{-1}$)	0.30 ± 0.14 (CI95% 0.20-0.40)	0.18 ± 0.14 (CI95% 0.08-0.29)*	0.18 ± 0.10 (CI95% 0.10-0.25)ɨ	0.09 ± 0.07 (CI95% 0.04-0.14)*ɨ
τ (s)	28.8 ± 8.5 (CI95% 22.7-34.9)	54.9 ± 22.4 (CI95% 38.8-71.0)*	27.4 ± 5.3 (CI95% 23.6-31.2)	48.3 ± 19.0 (CI95% 34.8-61.9)*
TD (s)	13.9 ± 4.2 (CI95% 10.9-16.9)	07.3 ± 6.1 (CI95% 02.9-11.7)*	11.0 ± 6.2 (CI95% 6.6-15.4)	04.8 ± 8.1 (CI95% 00.0-10.7)*
TD_S (s)	164 ± 27 (CI95% 144-183)	195 ± 44 (CI95% 164-227)*	154 ± 27 (CI95% 134-173)	179 ± 48 (CI95% 145-214)*
VO_{2END} ($L.min^{-1}$)	3.4 ± 0.5 (CI95% 3.0-3.7)	3.4 ± 0.5 (CI95% 3.0-3.8)	2.2 ± 0.3 (CI95% 2.0-2.5)ɨ	2.2 ± 0.3 (CI95% 2.0-2.4)ɨ

*Differences between conditions $p < 0.05$. ɨ Differences between sex within condition $p < 0.05$. U-VH = unloaded very-heavy; M-VH = moderate very-heavy**;** A = amplitude; Atotal = amplitude + $VO_{2baseline}$; VO_{2sc} = VO_2 slow component; τ = time constant; TD = time delay, TDs = TD of slow component phase; VO_{2end} = VO_2 at the end of exercise bout.

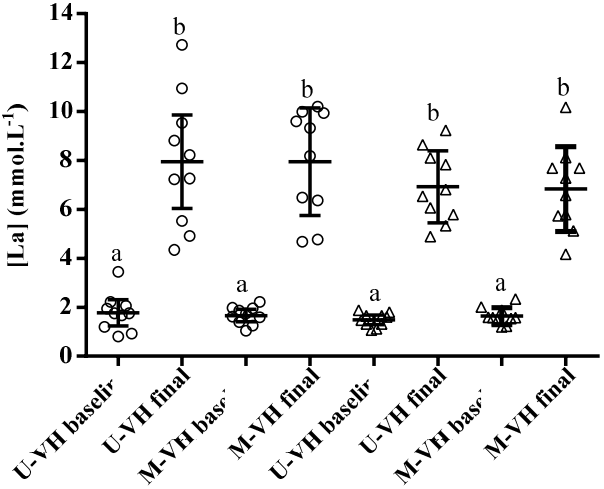

Figure 4.3
[La] = blood lactate concentration. Circle symbols show the female values. Triangle symbols show male values. U-VH = unloaded to very heavy intensity exercise. M-VH = moderate to very heavy intensity exercise.

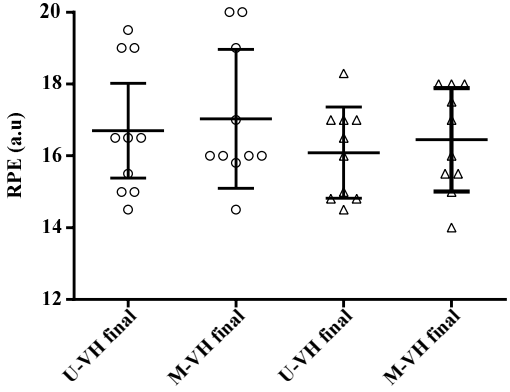

Figure 4.4
RPE = ratio of perceived exertion. Circle symbols show the female values. Triangle symbols show male values. U-VH = unloaded to very heavy intensity exercise. M-VH = moderate to very heavy intensity exercise.

Torque production behavior

There were no significant differences in MFP between conditions (U-VH vs. M-VH) for men in both velocities (60 rpm = main effect *condition vs. time* $F = 0.09$; $p = 0.77$; 120 rpm = main effect *condition vs. time* $F = 0.48$; $p = 0.50$). Female subjects showed no significant differences in MFP between conditions at 120 rpm (main effect *condition vs. time* $F = 0.36$; $p = 0.56$), but smaller decreases in torque production were observed following M-VH at 60 rpm compared to U-VH (main effect *condition vs. time* $F = 18.2$; $p = 0.01$). The torque decrement at 120 rpm was lower for female than male subjects ($p<0.05$, table 4.2). There were no significant correlations between Δ torque and VO_{2SC} or τ in the different conditions or velocities in both sexes.

Table 4.2 Torque production behavior before and after rest-to-work and work-to-work exercise in male and female subjects.

		Unloaded very-heavy			Moderate very-heavy		
		Initial	**Final**	**Δ torque**	**Initial**	**Final**	**Δ torque**
Male	*60 rpm*	163 ± 22 (CI95% 147-179)	150 ± 26 (CI95% 132-169)*	13 ± 10 (CI95% 05-20)	164 ± 22 (CI95% 148-180)	151 ± 27 (CI95% 132-170)*	13 ± 9 (CI95% 07-20)
	120 rpm	118 ± 20 (CI95% 104-132)	96 ± 23 (CI95% 80-112)*	22 ± 14 (CI95% 12-32)	122 ± 22 (CI95% 106-137)	101 ± 23 (CI95% 84-117)*	21 ± 12 (CI95% 12-30)
Female	*60 rpm*	108 ± 7 (CI95% 103-114)	91 ± 14 (CI95% 81-101)*	18 ± 14 (CI95% 08-27)	108 ± 10 (CI95% 101-115)	97 ± 14 (CI95% 87-107)*#	11 ± 13 (CI95% 01-20)
	120 rpm	71 ± 10 (CI95% 64-78)	60 ± 11 (CI95% 52-69)*	10 ± 9 (CI95% 04-17)ɨ	72 ± 9 (CI95% 65-78)	60 ± 11 (CI95% 52-68)*	12 ± 8 (CI95% 06-18)ɨ

ɨ Differences between sex within velocity $p < 0.05$. * Differences within condition $p < 0.05$. # Differences between conditions $p < 0.05$.

4.1.4 Discussion

We hypothesized that elevated baseline would lead to lower amplitudes of the VO_{2SC} and slower values for τ for both sexes despite of the differences between male and female counterparts. Our findings confirm these hypotheses showing that the VO_2 fundamental and slow phases were slower and the amplitude of each phase were lower at work-to-work transitions in both sexes. However, the hypothesis stating that reductions in MFP would be accompanied by changes in VO_2 kinetics for both conditions was not confirmed.

The main finding of this study was that alterations in VO_2 kinetics induced by preceding elevated baseline did not affect the MFP for both males and females. Thus, this work has demonstrated experimentally that isolated decreases in VO_{2SC} and fundamental phase or changes in TDs and τ are not linked to muscle force production during high-intensity cycling exercise in healthy male and female subjects. Considering that there were no differences between conditions (U-VH vs. M-VH) or sexes in RPE as well as in [La], it is suggested that the effects of work-to-work transitions on the VO_2 kinetics can be dissociated from differences in blood acidosis and the perception of effort.

When a high-intensity exercise transition is performed following an elevated baseline or work-rate, the VO_2 dynamics are changed with slower response (i.e., lower τ) or lower VO_{2SC} amplitudes. The putative mechanisms likely explaining these alterations may be represented by the balance between the parasympathetic and sympathetic control of the HR. Elevated work-rates seem to alter parasympathetic withdrawal leaving the slower sympathetic control to mediate increases in HR (BEARDEN; MOFFATT, 2001; DIMENNA et al., 2010b; HUGHSON; MORRISSEY, 1982). It has been suggested that a slowing of the HR kinetics may limit the O_2 delivery to cellular respiration (DIMENNA et al., 2010b; HUGHSON; MORRISSEY, 1982). Alternatively, cellular respiration might adjust more slowly in muscle fibers that are already active and/or recruitment of motor units that are believed to possess slower VO_2 kinetics and a higher VO_2 cost of tension (POOLE et al., 2008). Likewise, when a high-intensity exercise is preceded by an elevated work-rate the fundamental PCr τ is lengthened and the fall in PCr is greater compared to U-VH transitions (JONES; WILKERSON; FULFORD, 2008). These mechanisms are consistent with a greater proportional involvement of muscle fibers that are positioned higher in the recruitment hierarchy (e.g., type II muscle fibers) (DIMENNA et al., 2010a; HENNEMAN; SOMJEN; CARPENTER, 1965; JONES;

WILKERSON; FULFORD, 2008). However, Dimenna et al. (2010b) suggested that it is not elevated VO_2 baseline per se; rather, the elevated baseline work-rate, which would be expected to dictate the subsequent muscle fiber recruitment profile. Thus, changes in VO_2 kinetics that occur during work-to-work exercise may be linked to alterations in the muscle fiber recruitment profile. Although the present study does not allow to distinguish between the effect of elevated VO_2 and elevated work-rate, our results confirm findings from previous literature (DA BOIT et al., 2014; DIMENNA et al., 2010a; HUGHSON; MORRISSEY, 1982; JONES; WILKERSON; FULFORD, 2008; WILKERSON; JONES, 2006; WÜST et al., 2014).

Despite the differences in the amplitudes of the fundamental and slow phases between sexes in both U-VH and M-VH, τ was not significantly different between sexes in spite of the elevated baseline influence. These results are in contrast to previous findings reported for τ in adolescents (FAWKNER; ARMSTRONG, 2003; LAI et al., 2016), but in agreement in relation to VO_{2SC}. Besides, we extend to healthy subjects during cycling, the results of Reis et al. (2017) showing no differences in τ between women and men trained swimmers during heavy-intensity swimming. Moreover, our results are in accordance to what was reported for middle age subjects in cycling for moderate exercise (O'CONNOR et al., 2012). To date, no study has investigated the effects of work-to-work transition on the VO_2 kinetics and MFP comparing sexes. Although males present higher fundamental amplitudes and similar τ values compared to females, the gross rate of increase of oxygen uptake per second is higher in men, suggesting a quicker onset (REIS et al., 2017). According Reis et al. (2017) this could be due to the higher VO_{2peak} and anatomic differences presented by males. It has been reported that females could present lower O_2 delivery, O_2 extraction and blood flow due to smaller hearts, smaller lung volumes, lower diffusion capacities and cardiac output (DOMINELLI et al., 2015; HARMS, 2006). The present study found significant lower valued for VO_{2peak}, power at GET and VE for females compared to their male counterparts. This could be a result of the anatomic differences in the cardiorespiratory system. According to Murias et al. (2013) females presented less effective matching of the O_2 delivery and O_2 utilization, which seem to represent impairments in blood flow. However, a lower oxygen delivery to the muscles seems not to influence the VO_2 kinetics in high-intensity exercise (REIS et al., 2017). Olfert et al. (2004) affirmed that female subjects did not experience greater O_2 diffusion limitations during exercise, which may

emphasize the importance of absolute lung size or aerobic fitness in determining susceptibility to gas exchange impairment rather than sex per se.

Previous literature demonstrated a relation between VO_2 kinetics parameters (i.e., VO_{2SC}, τ) and the loss in torque/force production during high-intensity exercise (CANNON et al., 2011; KEIR et al., 2016; TEMESI et al., 2017). Cannon et al (2011) proposed that greater levels of muscle fatigue are reflected by a larger amplitude of the VO_{2SC} or vice-versa. The mechanisms contributing to peripheral muscle fatigue has been suggested to contribute to an increased O_2 cost of exercise (KEIR et al., 2016). Moreover, Temesi et al. (2017) suggested that subjects with slower VO_2 kinetics (higher τ values) experience a greater level of peripheral muscle fatigue. Thus, it was expected that alterations of the VO_2 kinetics would alter the MFP behavior. However, the decrease in torque production in both conditions and for both sexes were not related or linked with the VO_{2SC} or τ. Our results are in accordance with Hopker et al. (2016) who showed that exercise-induced muscle damage led to a significant locomotor muscle fatigue, but did not alter the VO_{2SC} or τ during subsequent high-intensity cycling. Hopker et al. (2016) also affirmed that the results from Cannon et al. (2011) could be misbegotten because the relationship presented by these authors was considering different intensity domains. Deley et al. (2006) demonstrated an inverse relation between the level of muscle fatigue in type II muscle fibers induced by an electromyostimulation protocol and the VO_{2SC} during subsequent high-intensity cycling. Additionally, τ was not altered by this intervention. Moreover, Thistlethwaite et al. (2008) investigated two types of prior exercise (knee extension vs. cycling) which caused different activation patterns/levels of additional motor units (~38% in knee extension vs. 21% in cycling) and found similar VO_2 responses (VO_{2SC} or τ) during the subsequent bout of heavy cycling exercise. The authors concluded that muscle fatigue is neither the primary determinant of the VO_{2SC} nor does it affect the τ of the fundamental phase.

The present study observed no differences in Δtorque at 60 rpm and 120 rpm following M-VH and U-VH for males and at 120 rpm for females despite of the differences in the VO_2 kinetics. No correlation between Δtorque and VO_{2SC} or τ was found in any condition or velocity in both sexes. These results do not support previous literature proposing a causal relation between muscle fatigue and VO_2 kinetics (CANNON et al., 2011; KEIR et al., 2016; TEMESI et al., 2017). However, female participants showed a lower decrement in torque production in M-VH at

MIE 60 rpm. This may be explained by a lower percentage of type II fibers and a greater fatigue resistance in females compared to males (HUNTER, 2014; LIU et al., 2010; MAHER et al., 2009). Further, a reduced proportion of type II muscle fibers may explain the smaller decrements in torque production by females observed at 120 rpm where the reliance on type II muscle fibers increases. Considering the higher percentage of type II muscle fibers in men (MAHER et al., 2009), it may be suggested that proportionally more type II fibers were activated at 120 rpm (SARGEANT, 2007) and consequently, lead to a greater level of muscle fatigue compared to 60 rpm and compared to women. When exercise is preceded by an elevated baseline, it may be suggested that the recruitment of additional motor units which are characterized by a lesser mitochondrial content and a higher VO_2 cost per unit of force (i.e., type II fibers) becomes inevitable in order to maintain the exercise intensity (WÜST et al., 2014). Thus, female subjects may have shown a lower Δtorque in M-VH at 60 rpm because the reliance on type II fibers was less. Recently, do Nascimento Salvador et al. (2018) showed that prior very-heavy cycling changed the VO_{2SC} and the trajectory of the VO_{2SC} in a subsequent very-heavy cycling exercise, but did not alter MFP behaviour. These results are in line with the present study and refute a "cause-effect" relationship between VO_{2SC} and muscle fatigue.

Furthermore, according to Hopker et al. (2016) the RPE measured supports indirectly the assumption that a level of central motor command was required to produce the power output. This study found that the RPE after the exercise was not different between conditions (U-VH vs. M-VH) or sexes. Based on these considerations, a dissociation between the changes in VO_2 kinetics (i.e. slower τ and the lower VO_{2SC}) and a decrease in central motor drive may be suggested. The performance of a maximal isokinetic cycling effort taken immediately post-exercise is a "global" but ecologically valid protocol to quantify the decrease in MFP in a specific way in cycling. However, the identification of the origin of fatigue (i.e. central or peripheral) is not possible. Although, Beelen et al. (1995) suggest that the fatigue in this type of dynamic isokinetic exercise may be due to changes in the muscle itself and not to failure of central drive. Considering that muscle fatigue measured by MIE recovers back to baseline values within 1-3 minutes (BEELEN et al. 1995, COELHO et al. 2015, DO NASCIMENTO SALVADOR et al. 2018), it represents a tendency to indicate more the peripheral fatigue.

4.1.5 Conclusion

In summary, this investigation has demonstrated that the fundamental and the VO_{2SC} amplitude were lower and the time constant and time delay of slow phase were longer during high-intensity cycling exercise from elevated baseline despite of the differences in both sexes. These alterations were dissociated from the changes in blood lactate concentration and from the perception of exertion. The same RPE for both sexes in both conditions could be indicating a similar level of fatigue in central motor drive. Further, muscle force production decreased after the exercise to a similar magnitude for both conditions (U-VH and M-VH) in males and females with exception that females demonstrated a smaller decreases after M-VH during isokinetic efforts at 60 rpm. This pattern in torque production could be related to the activation and distribution of muscle fiber type in men and women. The decrease in muscle force was not associated with the VO_2 kinetics parameters. Thus, isolated alterations in VO_2 kinetics after elevated baseline, which may be linked to changes in motor unit recruitment, do not reflect alterations in muscle force production and fatigue in healthy male and female subjects. These results refute a "cause-effect" relationship between VO_2sc or τ and muscle fatigue.

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The references of the paper are at “references section” page 107.

5 CONCLUSION

According to the specific objective of study 1, it was observed that the VO_{2SC} attenuation was not accompanied by the muscle force production behavior; these findings do not convincingly support the hypothesis of a causal relationship between time-course of muscle fatigue and VO_{2SC} during VH intensity exercise.

In relation to the main goal of study 2, the present work showed an exponential behavior of *W′* balance during the 10 min of recovery following a prior high-intensity cycling exercise, leading to a *W′* restoration of 92%. It is suggested that the tolerance to high-intensity exercise was compromised in part by the unrecovered *W′*. A higher metabolic acidosis after the prior bout of exercise, verified by the higher [La], leaded to slower VCO_2 kinetics and a possibly higher oxidative ATP production, verified by the changes in RER during the experimental condition. These results provide evidence for a link between the physiological disturbances caused by prior exercise, the restauration of *W′* and the tolerance to a constant high-intensity cycling exercise in healthy subjects.

Finally, answering the purpose of study 3, the decreases in muscle force were not associated with the VO_2 kinetics parameters. It means that, the isolated alterations in VO_2 kinetics after elevated baseline, that are possibly linked to changes in motor unit recruitment do not reflect alterations in muscle force production and fatigue in healthy male and female subjects. These results refute a “cause-effect” relationship between VO_2sc or τ and muscle fatigue.

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APPENDIX A – TCLE

UNIVERSIDADE FEDERAL DE SANTA CATARINA
CENTRO DE DESPORTOS
PROGRAMA DE PÓS-GRADUAÇÃO EM EDUCAÇÃO FÍSICA
ÁREA DE BIODINÂMICA DO DESEMPENHO HUMANO

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

De acordo com resolução 466/2012 do Conselho Nacional de Saúde, todas as pesquisas conduzidas com seres humanos necessitam do termo de Consentimento Livre e Esclarecido, devendo o participante estar ciente dos objetivos do estudo. Você está sendo convidado a participar do projeto que estamos conduzindo intitulado: O EFEITO DA FADIGA MUSCULAR SOBRE A CINÉTICA DO CONSUMO DE OXIGÊNIO, que tem como objetivo verificar a influência da fadiga muscular na cinética de consumo de oxigênio e investigar os mecanismos que podem estar relacionados ao componente lento.

O projeto envolve os professores Dr. Benedito Sérgio Denadai, Dr. Luiz Guilherme Antonacci Guglielmo e o doutorando do curso de pós-graduação em Educação Física Paulo Cesar do Nascimento Salvador. A participação no estudo é voluntária não envolve nenhum gasto com exceção daqueles advindos de deslocamentos do participante. Todos os materiais necessários serão providenciados pelos pesquisadores. As avaliações serão realizadas no Laboratório do Esforço Físico (LAEF) do Centro de Desportos (CDS); sendo necessárias de dez a onze visitas ao LAEF. Todo o período do experimento durará em torno de um mês e serão realizados testes laboratoriais como será descrito a seguir: 1) Familiarização e teste incremental; 2) três a quatro testes de carga constante até a exaustão voluntária; 3) testes de carga constante com diferentes tempos de exercício e execução de sprints isocinéticos de cinco segundos antes e após o exercício.

1ª etapa: Familiarização e teste incremental (duração aproximada de 60 minutos) – No primeiro momento será realizada a avaliação antropométrica - Medidas de peso corporal e estatura, nenhuma delas oferece riscos para o participante. Familiarização com os equipamentos, protocolos de testes e teste de esforço em cicloergômetro com cadência preferida e aumentos de 30 W por minuto até à exaustão voluntária (~10 min). A orientação básica ao participante é que ele deve alcançar o seu

limite de esforço o que pode gerar algum desconforto físico por causa do esforço mais intenso podendo este, mesmo assim, solicitar a interrupção do teste no momento em que se sentir cansado ou se desejar terminar o teste. Um avaliador experiente realizará coletas de sangue de 25 µl do lóbulo da orelha para dosagem do lactato sanguíneo em repouso e no final através de micro túbulos de polietileno e transferidos para vasos tipo Eppendorf e armazenados em gelo. A coleta de sangue pode gerar uma pequena dor ao participante dependendo da sensibilidade, mas ressalta-se que é apenas um furo bem pequeno na orelha (similar a uma picada de inseto) e a quantia coletada é bem baixa (uma ou duas gotas), além disso, o participante pode optar por não realizar este procedimento. No teste de esforço, o participante usará uma máscara no seu rosto para a análise dos gases de oxigênio e gás carbônico do ar expirado. O participante usará também um medidor da frequência cardíaca (FC) do tipo Polar. Estes últimos procedimentos podem não ser tão naturais a prática de exercícios mas não oferecem riscos.

2ª etapa: cargas constantes até a exaustão voluntária (duração aproximada de 30 minutos cada dia) – Após um intervalo de no mínimo 24 horas será realizado em cicloergômetro os testes de carga constante, um por dia sempre respeitando o intervalo de recuperação. O indivíduo utilizará a máscara para análise do O2 expirado e a fita do frequencímetro. Após um aquecimento e uma pausa passiva deverá realizar transições até a exaustão voluntária em intensidades submáximas e máximas (cargas com duração de 2 à 15 min), sendo que o participante poderá parar o exercício a qualquer momento que desejar. Haverá coleta de sangue do lóbulo da orelha como descrito anteriormente, mas o participante poderá optar por não realizar este procedimento se preferir. Serão de 3 a 4 visitas nesse formato com o objetivo de determinar através de ajustes matemáticos a intensidade referente ao limiar metabólico no qual ocorre a maior produção de energia de forma aeróbia com o mínimo de participação do metabolismo anaeróbio.

3ª etapa: cargas constantes e execução de sprints isocinéticos (duração aproximada de 60 minutos) - Após um intervalo de no mínimo 24 horas da última avaliação da segunda etapa serão realizados testes em cicloergômetro para determinação da fadiga muscular e do comportamento do consumo de O2 em intensidade submáxima de esforço. Após aquecimento o participante deverá realizar um sprint isocinético (cadência fixa de 120 rpm) por 5 s para determinação da força muscular sem fadiga. Após uma recuperação de 15 minutos o indivíduo realizará uma ou duas cargas constantes submáximas com

tempos distintos (3min, 8min ou até o limite de esforço) em diferentes combinações para cada situação, sendo uma por dia. No final de cada exercício repetirá o sprint isocinético para verificação do decaimento de força muscular. Haverá coleta de sangue do lóbulo da orelha antes e depois das cargas como descrito anteriormente. Os participantes utilizarão a máscara para análise do consumo de O2 e a fita do frequencímetro nestes testes. Ao todo esta terceira etapa são 6 visitas ao laboratório.

Esta pesquisa terá como principal benefício possibilitar um melhor entendimento do funcionamento do organismo durante o exercício e aprimorar avaliação e prescrição de atividades físicas orientadas. Todos os dias de testes serão acompanhados por no mínimo dois profissionais experientes e formados da área da saúde que realizaram disciplinas/cursos de emergências e primeiros socorros para qualquer eventualidade. Ressalta-se que todos os indivíduos selecionados para participar do estudo serão maiores de 18 anos, fisicamente ativos e acostumados com exercícios em cicloergômetro no modelo deste projeto. Todos os dados coletados neste estudo são estritamente confidenciais e serão utilizados para produção de artigos técnicos científicos. Apenas os pesquisadores terão acesso aos dados, que serão codificados e armazenados em banco de dados, de forma que a identificação por outras pessoas não seja possível. No entanto, essas informações poderão ser requisitadas pelo participante. Todos os participantes receberão um relatório detalhado de suas avaliações com orientações para o aprimoramento de exercícios físicos, além disso, os participantes poderão pedir aos pesquisadores a qualquer momento durante ou após a participação orientações de como entender e aplicar os resultados obtidos. Sua participação é voluntária e você poderá desistir dela em qualquer momento do estudo sem penalidade alguma, bastando apenas informar aos pesquisadores. Você receberá uma via do termo de consentimento para qualquer dúvida possível. Qualquer eventual dano que possa advir da participação nesta pesquisa será ressarcido totalmente pelos pesquisadores. Caso você tenha alguma dúvida, poderá entrar em contato pelo telefone do LAEF: (048) 3721-6248, com:

Prof. Dr. Luiz Guilherme Antonacci Guglielmo
Departamento de Educação Física – UFSC
e-mail: luiz.guilherme@ufsc.br

O Comitê de Ética em Pesquisa com Seres Humanos (CEPSH-UFSC) fica localizado na Rua Desembargador Vitor Lima, nº 222,

Prédio Reitoria II, 4º andar, sala 401, Trindade, Florianópolis. Telefone para contato: 3721-6094.

Desde já, agradecemos a sua colaboração.

UNIVERSIDADE FEDERAL DE SANTA CATARINA
CENTRO DE DESPORTOS

DECLARAÇÃO

Declaro para os devidos fins e efeitos legais que, objetivando atender as exigências do Comitê de Ética em Pesquisa com Seres Humanos, e como pesquisador responsável, afirmo que cumprirei os termos da Resolução CNS 466/2012 e suas complementares durante a realização do projeto de pesquisa: O EFEITO DA FADIGA MUSCULAR SOBRE A CINÉTICA DO CONSUMO DE OXIGÊNIO.

Florianópolis, / /201 .

Prof. Dr. Luiz Guilherme Antonacci Guglielmo
CPF: 134106818-84

UNIVERSIDADE FEDERAL DE SANTA CATARINA
CENTRO DE DESPORTOS
PROGRAMA DE PÓS-GRADUAÇÃO EM EDUCAÇÃO FÍSICA
AREA DE BIODINÂMICA DO DESEMPENHO HUMANO

TERMO DE CONSENTIMENTO

Declaro que fui informado, de forma clara e objetiva, sobre todos os procedimentos do projeto de pesquisa intitulado O EFEITO DA FADIGA MUSCULAR SOBRE A CINÉTICA DO CONSUMO DE OXIGÊNIO. Estou ciente que todos os dados a meu respeito serão sigilosos e que posso me retirar do estudo a qualquer momento. Assinando este termo, eu concordo em participar deste estudo.

Nome por extenso

_____

Assinatura

_____

Florianópolis (SC) _____/_____/_____

_____
Prof. Dr. Luiz Guilherme Antonacci Guglielmo
CPF: 134106818-84

ATTACHMENT A – Parecer consubstanciado do CEP

UNIVERSIDADE FEDERAL DE SANTA CATARINA - UFSC

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: O EFEITO DA FADIGA MUSCULAR SOBRE A CINÉTICA DO CONSUMO DE OXIGÊNIO

Pesquisador: Luiz Guilherme Antonacci Guglielmo

Área Temática:

Versão: 2

CAAE: 54179616.1.0000.0121

Instituição Proponente: Universidade Federal de Santa Catarina

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.621.448

Apresentação do Projeto:

Projeto preliminar apresentado ao programa de Pós-Graduação em Educação Física, da Universidade Federal de Santa Catarina, como requisito parcial para a qualificação de doutorado na área de Biodinâmica do Desempenho Humano. O projeto envolve os professores Dr. Benedito Sérgio Denadai, Dr. Luiz Guilherme Antonacci Guglielmo e o doutorando do curso de pós-graduação em Educação Física Paulo Cesar do Nascimento Salvador que apresenta esta proposta.

A seleção dos sujeitos será do tipo intencional não probabilística. Participarão deste estudo 30 indivíduos fisicamente ativos, de ambos os sexos, aparentemente saudáveis, não fumantes e que não façam uso regular de qualquer tipo de medicamento. Serão considerados fisicamente ativos os indivíduos que relatarem ter participado de alguma forma de atividade física nos últimos seis meses por um período maior do que 30 minutos por dia ou 150 minutos semanais e com frequência de pelo menos três vezes por semana (WHO, 2015). Os mesmos serão informados textual e verbalmente sobre os objetivos e métodos desse estudo, assinando posteriormente um termo de consentimento livre e esclarecido.Todos os indivíduos que participarão do estudo deverão comparecer ao laboratório em pelo menos 10 oportunidades diferentes, com um intervalo de um a três dias entre os testes num período de 5 semanas.

Endereço: Universidade Federal de Santa Catarina, Prédio Reitoria II, R: Desembargador Vitor Lima, nº 222, sala 401
Bairro: Trindade **CEP:** 88.040-400
UF: SC **Município:** FLORIANOPOLIS
Telefone: (48)3721-6094 **E-mail:** cep.propesq@contato.ufsc.br

Maiores de 18 anos, aparentemente saudáveis, não fumantes e que não façam uso regular de qualquer tipo de medicamento. Serão incluídos no
estudo qualquer indivíduo fisicamente ativo com idade entre 18 e 45 anos, moradores da grande Florianópolis.

O presente estudo pode ser classificado quanto a sua natureza como sendo uma pesquisa aplicada e, em relação à abordagem do problema, o estudo é considerado uma pesquisa quantitativa, pois segundo Silva et al. (2011) essa classificação pode ser empregada para estudos que tem como objetivos gerar conhecimentos para aplicação prática e dirigidos para solucionar problemas específicos. Ainda, em relação a abordagem para
os trabalhos que consideram que os dados serão quantificados para serem classificados e analisados.Quanto aos objetivos propostos, à pesquisa caracteriza-se como sendo descritiva e, em relação aos procedimentos técnicos o estudo se enquadra como uma pesquisa empírica exploratória, pois segundo Silva et al. (2011), a pesquisa exploratória permite o pesquisador escolher as melhores técnicas e métodos para conduzir sua pesquisa e tomar decisões necessárias para enfatizar e detalhar melhor seu objeto de estudo.

As hipóteses do presente estudo serão as seguintes:H1 O exercício prévio muito pesado irá atenuar o componente lento de VO2;H2 O exercício prévio muito pesado irá diminuir a fadiga muscular mensurada no pico de torque isocinético;H3 A atenuação do componente lento de VO2 por causa do exercício prévio muito pesado será relacionada à menor fadiga muscular;H4 O componente lento de VO2 durante o exercício de esforço muito pesado apresentará relação com a capacidade de trabalho anaeróbio;H5 A constante tempo da cinética de VO2 (t) no exercício de intensidade muito pesada apresentará relação inversa com a potência crítica;H6 O exercício prévio terá efeito sobre o tempo de exaustão durante o exercício muito pesado de esforço;H7 Haverá evidências de relação de causa-efeito entre o componente lento de VO2 e a fadiga muscular em indivíduos fisicamente ativos durante o exercício muito pesado.

Objetivo da Pesquisa:
Analisar a relação de causa e efeito entre a fadiga muscular e o componente lento no consumo de oxigênio de indivíduos fisicamente ativos durante o exercício no domínio muito pesado de esforço

em cicloergômetro.
Obj. Específicos?
1) Determinar os índices de capacidade (limiar ventilatório e potência crítica) e potência aeróbia (consumo máximo de O2 e potência máxima aeróbia) de indivíduos fisicamente ativos;
2) Determinar a capacidade de trabalho anaeróbio nos indivíduos fisicamente ativos;
3) Analisar o pico de torque isocinético antes, durante e após a fadiga muscular gerada pelo exercício muito pesado;
4) Caracterizar as variáveis da cinética de VO2 (linha de base, amplitudes e constantes de tempo) no exercício de esforço muito pesado;
5) Investigar a relação entre a fadiga muscular e o componente lento de VO2 no exercício realizado no domínio muito pesado de esforço;
6) Analisar o efeito do exercício prévio realizado no domínio muito pesado em cicloergômetro no componente lento de VO2, na fadiga muscular e no tempo de exaustão;
7) Verificar se o componente lento de VO2 durante o exercício de esforço muito pesado apresenta relação com a capacidade de trabalho anaeróbio.
8) Examinar se a constante tempo da cinética de VO2 (t) no exercício de

Avaliação dos Riscos e Benefícios:
Riscos:
Nos procedimentos de coleta de dados os sujeitos serão submetidos a testes físicos o que poderá gerar um nível da fadiga central e periférica. Além disso, durante os testes pode gerar certos desconfortos, possibilidade de tonturas e náuseas. Ressalta-se que geralmente poucos sujeitos apresentam esses sintomas e que os mesmos poderão parar a realização de qualquer um dos testes em qualquer momento. Para minimizar este tipo de risco, os avaliadores ficarão monitorando continuamente as variáveis cardíacas e respiratórias dos indivíduos e a percepção subjetiva de esforço, no momento que perceberem qualquer tipo de anormalidade os testes serão interrompidos. Todos os dias de testes serão acompanhados por no mínimo dois profissionais experientes e formados da área da saúde que realizaram disciplinas/cursos de emergências e primeiros socorros
para qualquer eventualidade.

Benefícios:

Além dos benefícios científicos óbvios, onde o estudo contribuirá para o aprimoramento do conhecimento da área sobre os aspectos relacionados a avaliação e prescrição do treinamento, os participantes receberão relatórios com seus resultados em cada teste, onde haverá informações pertinentes as capacidades e potencialidades de cada um, podendo assim, realizar o seu treinamento de forma mais adequada, bem como, para os possíveis grupos/clubes/equipes onde estes sujeitos possam estar envolvidos, que poderão aprimorar a prescrição e a metodologia do treinamento a partir dos resultados obtidos. Os relatórios dos testes que serão oferecidos poderão servir de motivação e critérios de planejamento de atividade física para o bem estar e a saúde dos indivíduos. Além disso, os participantes poderão pedir aos pesquisadores a qualquer momento durante ou após a participação orientações de como entender e aplicar os resultados obtidos.

Foram revistos riscos e benefícios de acordo com as considerações do parecer Número: 1.468.672

Comentários e Considerações sobre a Pesquisa:

A presente proposta possui relevância acadêmica e pode fomentar estudos e embasar dados de cunho acadêmico e profissional.

Considerações sobre os Termos de apresentação obrigatória:

Folha de Rosto assinada pelo Diretor do CDS UFSC.

Anuência do Diretor do CDS.

Anuência do responsável pelo laboratório que será utilizado, apresentada.

TCLE revisado de acordo com as recomendações do parecer número: 1.468.672;

Recomendações:

Seria interessante rever o deslocamento dos participantes e verificar possibilidade de arcar com esta despesa, são muitas as intervenções e momento de ida ao laboratório.

Conclusões ou Pendências e Lista de Inadequações:

Diante da Carta resposta apresentada e das alterações realizadas nos documentos consideramos o que se apresenta neste momento como aprovado, destacando a relevância de se encaminhar a este comitê os relatórios finas da pesquisa assim como manter-se atento aos preceitos éticos durante todos o processo.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

UNIVERSIDADE FEDERAL DE SANTA CATARINA - UFSC

Continuação do Parecer: 1.621.448

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_673490.pdf	15/06/2016 18:23:02		Aceito
Recurso Anexado pelo Pesquisador	resposta_pendencias.docx	15/06/2016 18:22:07	Paulo Cesar do Nascimento Salvador	Aceito
Projeto Detalhado / Brochura Investigador	projeto_cepsh_maio16.docx	26/05/2016 17:01:11	Paulo Cesar do Nascimento Salvador	Aceito
Declaração de Pesquisadores	Declaracao_do_Pesquisador.docx	26/05/2016 16:08:15	Paulo Cesar do Nascimento Salvador	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_tese.docx	26/05/2016 16:07:47	Paulo Cesar do Nascimento Salvador	Aceito
Declaração de Instituição e Infraestrutura	declaracao.pdf	14/03/2016 17:42:45	Paulo Cesar do Nascimento Salvador	Aceito
Folha de Rosto	folha_de_rosto.pdf	14/03/2016 17:39:44	Paulo Cesar do Nascimento Salvador	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

FLORIANOPOLIS, 04 de Julho de 2016

Assinado por:
Washington Portela de Souza
(Coordenador)

Endereço: Universidade Federal de Santa Catarina, Prédio Reitoria II, R: Desembargador Vitor Lima, nº 222, sala 401
Bairro: Trindade **CEP:** 88.040-400
UF: SC **Município:** FLORIANOPOLIS
Telefone: (48)3721-6094 **E-mail:** cep.propesq@contato.ufsc.br