
GRADUATE PROGRAM IN MOVEMENT SCIENCES

Rodrigo de Araujo Bonetti de Poli

**Sprint Interval Training on Constant Work Rate High Intensity
Exercise: Time to task failure, Biomolecular Glycolytic Pathway
and Neuromuscular Function Adaptations**

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**Sprint Interval Training on Constant Work Rate High Intensity
Exercise: Time to task failure, Biomolecular Glycolytic Pathway
and Neuromuscular Function Adaptations**

Thesis presented to the Campus Unit of Bauru, São Paulo State University, as part of the requirements for obtaining the title of Doctor of Philosophy in Movement Sciences.

Supervisor: Alessandro Moura Zagatto

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The present research contributes to advancing the understanding of how glycolytic non-oxidative metabolism behaves during a constant effort performed above the critical intensity, following a period of high-intensity training. Additionally, it also brings advancements regarding the neuromuscular apparatus's behavior (at rest and during exercise) after this training model.

ATA DA DEFESA PÚBLICA DA TESE DE DOUTORADO DE RODRIGO DE ARAUJO BONETTI DE POLI, DISCENTE DO PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DO MOVIMENTO, DA FACULDADE DE CIÊNCIAS - CÂMPUS DE BAURU.

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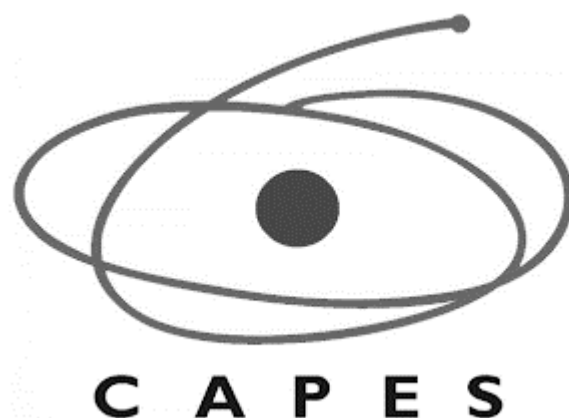
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“Irmão, você não percebeu que você é o único representante do seu sonho na face da terra? Se isso não fizer você correr, chapa, eu não sei o que vai.”

(Leandro Roque de Oliveira –, *Levanta e Anda*)

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Resumo

A presente tese foi dividida em dois estudos independentes. O **primeiro estudo** teve como objetivo examinar o impacto de um treinamento intervalado de sprint (SIT) de 5 semanas na resposta glicolítica (ou seja, conteúdo e atividade enzimática glicolítica e conteúdo de glicogênio) e sua relação com o tempo até a falha na tarefa (TTF) durante o exercício de carga constante (CWR) realizado acima da intensidade crítica. **MÉTODOS**: Neste estudo, 14 homens fisicamente ativos foram randomizados no grupo SIT (SIT_G; n=8; 3x semanais de 4-6 esforços de 30-s Wingate) ao longo de 5 semanas, e um grupo de controle (n=6). No início, os participantes foram submetidos a: i) um teste incremental de rampa para medir a função cardiorrespiratória; ii) CWR de ciclismo TTF a 150% da potência associada ao ponto de compensação respiratória (RCP-PO) com biópsias musculares em repouso e imediatamente após a falha na tarefa. Após as 5 semanas, as mesmas avaliações foram repetidas. O conteúdo e as atividades enzimáticas de glicogênio fosforilase (GPhos), hexoquinase (HK), fosfofrutoquinase (PFK) e lactato desidrogenase (LDH), assim como o conteúdo de glicogênio, foram analisados. **RESULTADOS**: O conteúdo e atividade das enzimas GPhos, HK, PFK, LDH, bem como o conteúdo de glicogênio também melhoraram pós-treinamento apenas no SIT_G, entretanto, nenhuma diferença foi verificada na utilização de glicogênio foi observada entre os grupos ou ao longo do tempo. Além disso, nenhuma diferença no TTF foi verificada quando o CWR foi realizado ajustado à condição física pós-treinamento. O **segundo estudo** teve como objetivo investigar se 5 semanas de SIT poderiam melhorar a função neuromuscular [NMF; contração voluntária máxima isométrica (iMVC) + estimulações de nervos periféricos (PNS) com avaliação de força evocada e sinal de eletromiografia (EMG)] e TTF durante o CWR realizado acima da intensidade crítica. **MÉTODOS**: participaram do estudo 21 homens fisicamente ativos, sendo randomicamente alocados no SIT_G (n=11) e no grupo de controle (n=10). O desenho experimental foi realizado de forma semelhante ao primeiro estudo; no entanto, em vez de uma biópsia muscular, foi realizado iMVC + PNS + EMG, para verificação da função neuromuscular. **RESULTADOS**: O SIT não induziu alterações na NMF medida pelo iMVC, assim como na fadigabilidade e no sinal de EMG durante o CWR. Também não foram verificadas alterações significativas no TTF correspondente aos atuais 150% de RCP-PO. Entretanto, o SIT_G mostrou uma melhoria significativa ao longo do tempo nos resultados dos treinos (função neuromuscular dinâmica; potência máxima e potência pico). **CONCLUSÃO DA TESE**: 5 semanas de SIT foram eficazes em causar alterações nas variáveis glicolíticas analisadas e na função neuromuscular dinâmica, entretanto essas alterações parecem não fundamentar a manutenção da tolerância ao exercício em esforços de intensidade severa.

Palavras chave: Exercício de alta intensidade; Tolerância ao exercício; Via glicolítica; Fadigabilidade.

Abstract

The present thesis has been divided into two independent studies. The **first study** aimed to examine the impact of a 5-week sprint interval training (SIT) on glycolytic response (i.e., glycolytic enzyme content and activity, and glycogen content) and its relationship with time to task failure (TTF) during constant load exercise (CWR) performed above critical intensity. **METHODS**: In this study, 14 physically active men were randomized into the SIT group (SIT_G; n=8; 3 times a week, 4-6 efforts of 30-second Wingate) over 5 weeks, and a control group (n=6). Initially, participants underwent: i) an incremental ramp test to measure cardiorespiratory function; ii) CWR cycling at 150% of power output associated with the respiratory compensation point (RCP-PO) with muscle biopsies at rest and immediately after task failure. After 5 weeks, the same assessments were repeated. Glycogen phosphorylase (GPhos), hexokinase (HK), phosphofructokinase (PFK), lactate dehydrogenase (LDH) enzyme content and activities, as well as glycogen content, were analyzed. **RESULTS**: Content and activity of GPhos, HK, PFK, LDH enzymes, as well as glycogen content, improved post-training only in SIT_G; however, no difference in glycogen utilization was observed between groups or over time. Additionally, no difference in TTF was observed when CWR was adjusted to post-training physical condition. The **second study** aimed to investigate whether 5 weeks of SIT could improve neuromuscular function [NMF; maximal voluntary isometric contraction (iMVC) + peripheral nerve stimulation (PNS) with evoked force assessment and electromyography (EMG)] and TTF during CWR performed above critical intensity. **METHODS**: 21 physically active men participated in the study, randomly allocated to SIT_G (n=11) and control group (n=10). The experimental design was similar to the first study; however, instead of muscle biopsy, iMVC + PNS + EMG were performed to verify neuromuscular function. **RESULTS**: SIT did not induce changes in NMF measured by iMVC, as well as in fatigue and EMG signal during CWR. No significant changes in TTF corresponding to the current 150% of RCP-PO were observed. However, SIT_G showed a significant improvement over time in training outcomes (dynamic neuromuscular function; maximum power and peak power). **THESIS CONCLUSION**: 5 Weeks of SIT were effective in causing changes in the analyzed glycolytic variables and dynamic neuromuscular function; however, these changes do not seem to underpin the maintenance of exercise tolerance during severe-intensity efforts.

Key words: High intensity exercise; Exercise tolerance; Glycolytic pathway; Fatiguability.

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List of Abbreviations and Acronyms

Ag/AgCl – Silver / silver chloride

BF - Biceps femoris muscle

BL – Baseline period (pre intervention)

Ca⁺⁺ - Calcium

CARNS1 - Carnosine synthase 1

CON – Control Group

CP – Critical Power

CWR – Constant Workload rate exercise

Db10 - Force level at doublets low-frequency stimulus (10Hz) stimulus

Db10 / Db100 - Ratio between force level at doublets low-frequency stimulus and Force level at doublets high-frequency stimulus

Db100 - Force level at doublets high-frequency (100Hz) stimulus

Db100-superimposed – Superimposed force of doublet high-frequency (100Hz) stimulus

EMG – Surface Electromyography signal

GM - Gluteus maximus muscle

GPhos - Glycogen phosphorylase

H⁺ - Hydrogen ion

H₂SO₄ - Sulfuric acid

HCl - Hydrochloric acid

HIF-1 α - Hypoxia-inducible factor-1 α

HK – Hexokinase

iMVC - Isometric maximal voluntary contraction

iMVCForce - peak force peak measured during isometric maximal voluntary contraction

KOH - Potassium hydroxide

LDH - Lactate dehydrogenase

MCT4 - Monocarboxylate transporter isoform 4

MP – Wingate Mean Power

Na₂SO₄ - Sodium sulfate

NAD - Nicotinamide adenine dinucleotide

NaF - Sodium fluoride

NMF - Neuromuscular function

PCO₂ - End-tidal partial pressure of CO₂

PNS - Peripheral nerve stimulations

PO – Power Output

PO/RMS – Power output normalized by roots mean square (neuromuscular efficiency)

Post 5-weeks – After 5 weeks of intervention period

PostBL-RCP - Constant Workload rate exercise using the same baseline power output

PP – Wingate Peak Power

PPO - Peak power output

PPP1R3C - Protein phosphatase 1, regulatory subunit 3C

Q_{tw} – Force level at single stimulus

RCP - Respiratory compensation point

RCP-PO - Power output at respiratory compensation point

RF - Rectus femoris muscle

RMS - Roots mean square

RMS% - Roots mean square normalized by first 5-s

RMSiMVC – Roots mean square at isometric maximal voluntary contraction

RMSiMVC/M-Wave - Ratio between roots mean square at isometric maximal voluntary contraction and M-Wave amplitude

SENIAM - Surface EMG for a non-invasive assessment of muscles

SGK1 - Serum/glucocorticoid regulated kinase 1

SIT – Sprint Interval Training

SITG – Group underwent to Sprint Interval Training

TTF – Time to task failure

$\dot{V}_E$ – Ventilation

$\dot{V}_E/\dot{V}_{CO_2}$ - Ventilation/carbon dioxide production

VL - Vastus lateralis muscle

$\dot{V}O_2$ - Oxygen consumption

$\dot{V}O_{2max}$ - Maximal oxygen consumption

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CHAPTER 1

General Introduction and Program of Research

1. General Introduction

1.1. Exercise above of the respiratory compensation point intensity

1.1.1 Intensity definitions

Exercise triggers a range of integrated responses, encompassing both energetic and neuromuscular aspects, to effectively manage the execution of external tasks (HAWLEY et al., 2014). As effort intensity increases and the demand for task execution rises, there is a corresponding enhancement in the ATP resynthesis process to meet this heightened demand. However, as the effort load continues to escalate, a disruption in metabolic homeostasis becomes evident, leading to alterations in systemic behavior. This is notably reflected in the dynamics of oxygen consumption and other cardiorespiratory variables, such as heightened ventilation and increased carbon dioxide production, evidencing some “break points” that represents metabolic boundaries (KEIR et al., 2022).

The first metabolic boundary, can be referred to as the gas exchange threshold [GET; the gas exchange analogue of the lactate threshold (KEIR et al., 2022)], indicating the highest metabolic rate not associated with metabolic acidosis and the decline in efficiency, resulting in the slow component in oxygen uptake (BROWNSTEIN et al., 2022; POOLE et al., 2021). The second is the respiratory compensation point [RCP; analogue to the maximal metabolic steady state (KEIR et al., 2022)] and is characterized as the point when the body can no longer sustain the accumulation of hydrogen ions (H^+) production through oxidative and non-oxidative glycolytic pathways with concomitant increase of carbon dioxide (CO_2) production (WASSERMAN; MCILROY, 1964), representing the oxidative-non-oxidative "transition" (KINDERMANN; SIMON; KEUL, 1979). In this way, considering the magnitude of metabolic stress (i.e., delineated previously cited discernible metabolic boundaries), the exercise intensity domains can be classified into four: moderate, heavy, very heavy/severe and extreme domain (JAMNICK et al., 2020).

The moderate-intensity domain is assumed as exercise performed above GET, and is characterized by a swift attainment of a steady state in oxygen uptake (KEIR et al., 2016). Additionally, within this intensity range, there is a low to moderate accumulation of metabolites [e.g., H^+ and inorganic phosphate (P_i)], promoting the sustained maintenance of effort for an extended period (BLACK et al., 2017). Within the heavy-intensity domain, exercise intensity assumed with boundaries between GET and RCP, it is possible to observe a greater metabolic

disturbance (e.g., higher concentrations of H^+ and P_i) (CANNON et al., 2014), although stabilized over time, which reflects in the emergence of a slow component in the kinetics of oxygen consumption (BARSTOW; MOLE, 1991), greater fatigue accumulation, and a reduction in exercise tolerance (BARON et al., 2008; BLACK et al., 2017). Within severe-intensity domain and extreme-intensity domain (both above RCP) had some similarities, as the hyperbolic ATP resynthesis rises (e.g., more intensity leading to a hyperbolic participation of non-oxidative glycolytic pathway), leading to a higher metabolic disturbance with consequent decreasing exercise tolerance, and higher slow component in the kinetics of oxygen consumption (HILL; POOLE; SMITH, 2002; HUREAU et al., 2022). However, the main difference between these two domains is the attainment of maximal oxygen uptake ($\dot{V}O_{2max}$). In the severe-intensity domain, $\dot{V}O_{2max}$ is reached, while in the extreme-intensity domain, the development of fatigue hinders the attainment of $\dot{V}O_{2max}$ (HILL; POOLE; SMITH, 2002) (Figure 1).

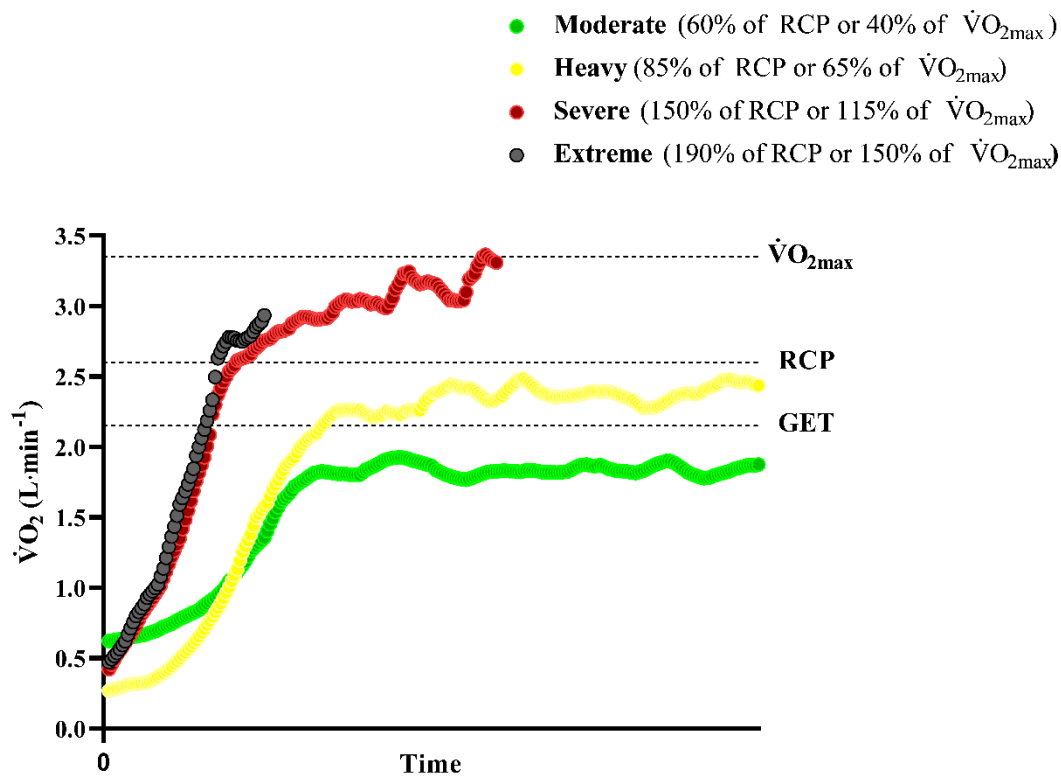


Figure 1. Real data of oxygen uptake consumption during moderate, heavy, severe and extreme domains. The moderate domain represents 60% and 40% of respiratory compensation point (RCP) and maximal oxygen uptake ($\dot{V}O_{2max}$), respectively. Heavy-intensity domain, 85% of

RCP and 65% of $\dot{V}O_{2\max}$. Severe-intensity domain, 150% of RCP and 115% of $\dot{V}O_{2\max}$. Extreme-intensity domain, 190% of RCP and 150% of $\dot{V}O_{2\max}$.

1.1.2. Severe intensity-domain exercise: Metabolic and Neuromuscular characteristics

Specifically, in the context of severe domain exercise, the adenosine tri-phosphate (ATP) resynthesis through non-oxidative pathways (HARGREAVES; SPRIET, 2020), with the glycolytic pathway emerging as a crucial determinant that rapidly meets the energy demand for high-intensity muscular contractions (HEARRIS et al., 2018; VIGH-LARSEN et al., 2021). Consequently, maintaining a constant work rate (CWR) above critical intensity is likely associated with limitations within the glycolytic non-oxidative pathway to meet the imposed metabolic demand (ALLEN; LAMB; WESTERBLAD, 2008; VIGH-LARSEN et al., 2021).

The activation of the glycolytic pathway initiates as soon as exercise above critical intensity begins, depleting intracellular ATP rapidly ($\approx 2s^{-1}$), along with ATP resynthesized by the phosphagen pathway (HARGREAVES; SPRIET, 2020). Additionally, in a general overview the high intensity characteristic of exercise elevates the concentration of catecholamines (e.g., adrenaline) (JENSEN; LAI, 2009), which bind to adrenoreceptors in the muscle, inducing the activation of phosphorylase kinase (PhK) (facilitated by the formation of cyclic adenosine monophosphate by adenylate cyclase activity). This, in turn, alters the state of glycogen phosphorylase (GPhos) (JENSEN; LAI, 2009). In this process, muscle glycogen breaks down to glucose-1-phosphate by GPhos, requiring inorganic phosphate (P_i) for the reaction. This breakdown results in the formation of glucose-6-phosphate, a molecule provided through the phosphorylation of cytosol glucose by hexokinase (HK) in a reversible reaction (HARGREAVES; SPRIET, 2020; HEARRIS et al., 2018). Subsequently, the rate-limiting enzyme phosphofructokinase (PFK) is allosterically activated, converting glucose-6-phosphate into pyruvate through a complex reaction. The pyruvate is then converted into lactate and nicotinamide adenine dinucleotide (NAD) through lactate dehydrogenase (LDH) in a reversible reaction, providing a "fast" ATP source (SPRIET; HOWLETT; HEIGENHAUSER, 2000) (Figure 2).

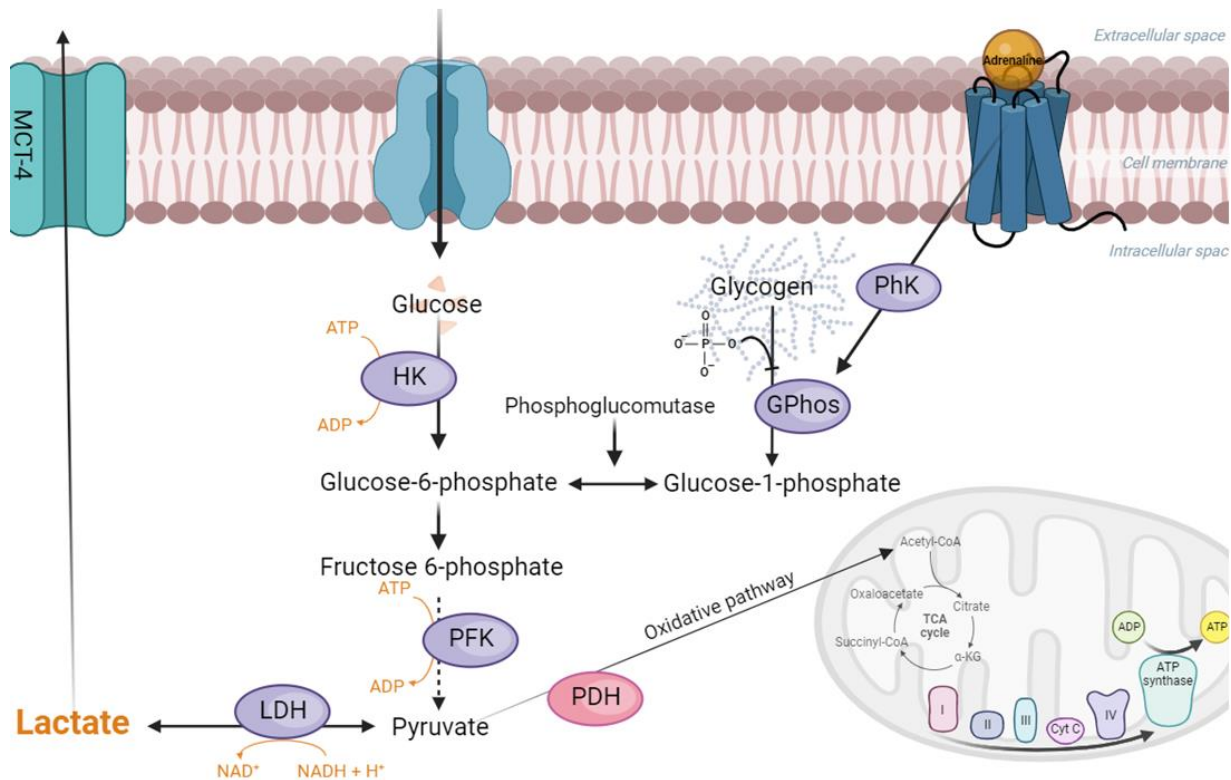


Figure 2. Overview of Glycolysis in skeletal muscle cell. PhK – phosphorylase kinase; GPhos – glycogen phosphorylase; HK – Hexokinase; PFK – phosphofructokinase; LDH – lactate dehydrogenase; GLUT-4 – glucose transporter 4; PDH – pyruvate dehydrogenase; MCT-4 – monocarboxylate transporter 4. [redrawn by Hargreaves & Spriet (2020)].

Despite that, the increased activity of the glycolytic pathway results in disruptions to intracellular homeostasis characterized by the accumulation of metabolites from ATP hydrolysis, including adenosine diphosphate (ADP), potassium (K^+), P_i , and H^+ (HUREAU et al., 2022; MOHR et al., 2004; SUNDBERG; FITTS, 2019). Significantly, the excessive buildup of P_i and H^+ seems to play a crucial role in the temporary reduction of the contractile process and subsequent task failure (ALLEN; LAMB; WESTERBLAD, 2008; HUREAU et al., 2022; ZAGATTO et al., 2022).

For instance, elevated P_i levels can infiltrate the sarcoplasmic reticulum, resulting in Ca^{++} precipitation and reduced Ca^{++} release during excitation-contraction coupling, likely contributing to impaired skeletal muscle contractility. Conversely, acidosis caused by H^+ accumulation appears to be more closely associated with increased muscle afference mediated by metabosensitive groups III/IV, leading to decreased central nervous system input and subsequent restriction of muscle activation (Figure 3) (AMANN et al., 2008). Also,

highlighting the significance of the neuromuscular apparatus in determining exercise tolerance above the critical intensity (BLACK et al., 2017).

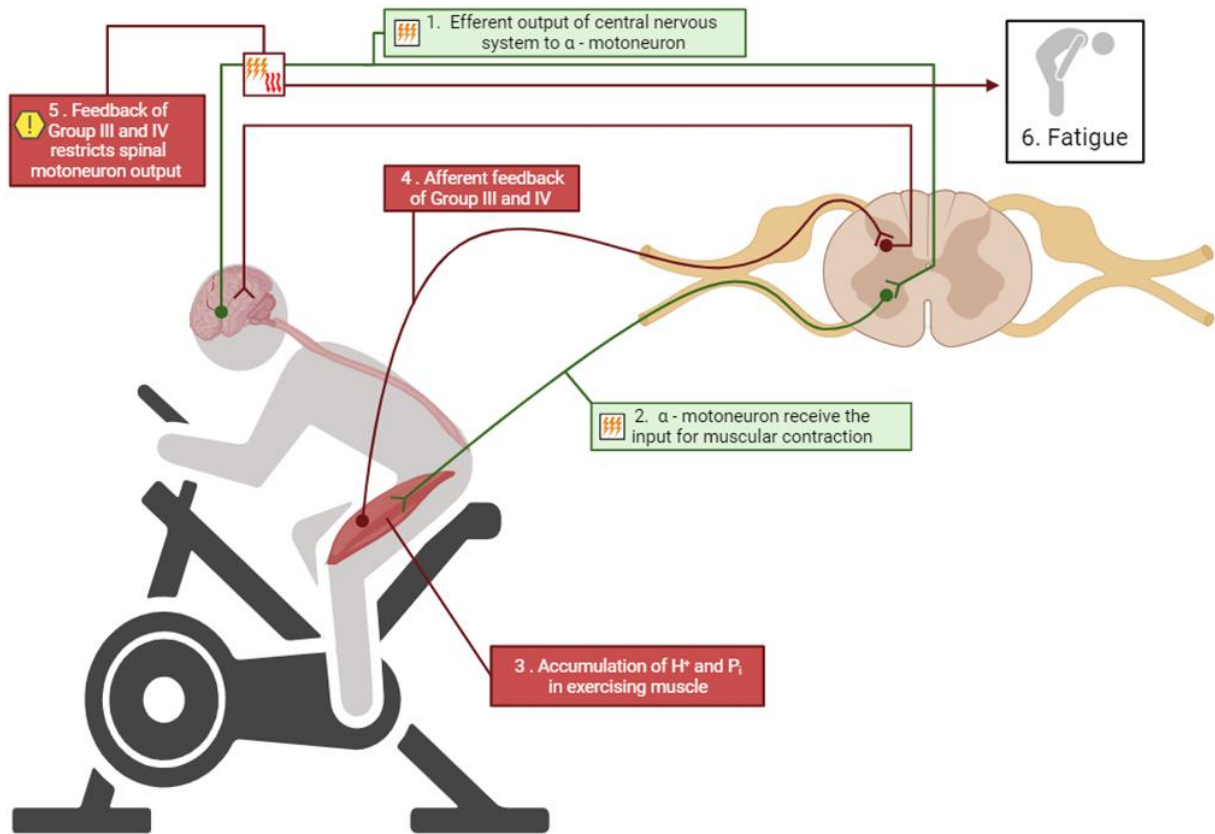


Figure 3. Consequence of group III/IV feedback for development of exercise fatigue.

In addition to that, the pattern of muscle activation individually, seems also plays a crucial role in eliciting stimuli for muscular contractions and sustaining repetitive muscular contraction (FARIA; PARKER; FARIA, 2005a; TAKAISHI et al., 1996). For example, Ducrocq & Blain (2022) demonstrated that electrical muscular activity was more pronounced in exercises of higher intensity compared to those of moderate or lower intensity, highlighting that the higher the load, the greater the tendency for neuromuscular activation to sustain the effort. Additionally, our group showed that electromyographic (EMG) signals during a CWR cycling exercise above critical intensity had a significant correlation with maximal aerobic power ($r^2 > 0.65$), raw critical intensity (i.e., respiratory compensation point intensity; $r^2 > 0.83$) and maximal oxygen uptake ($r^2 > 0.61$), supporting the idea that neuromuscular function plays an important role in exercise tolerance above critical intensity (DUTRA et al., 2022).

1.2. Severe-intensity domain continuous exercise tolerance

Considering the importance of glycolytic pathway and neuromuscular apparatus, cited previously, it could be expected that improvements in physical fitness would enhance ATP resynthesis (mainly from non-oxidative glycolysis) and neuromuscular function, consequently leading to a higher exercise tolerance at CRW exercise above critical intensity. However, some studies have reported that endurance-trained amateur athletes (i.e., systematically involved in supervised endurance training; training volume of ~ 200 minutes $\cdot$ week $^{-1}$ for 37.1 ± 11.6 km; maximal oxygen uptake of 55.9 ± 5.5 mL $\cdot$ kg $\cdot$ min $^{-1}$) presented a shorter time-to-task failure (TTF) during CWR above critical intensity exercises ($\sim -18\%$), when compared with non-trained (i.e., physically active; maximal oxygen uptake of 48.8 ± 3.3 mL $\cdot$ kg $\cdot$ min $^{-1}$) and sedentary subjects (maximal oxygen uptake of 42.8 ± 3.3 mL $\cdot$ kg $\cdot$ min $^{-1}$), at the same physiologically matched workload (ZAGATTO et al., 2017, 2023a).

Intriguingly, other studies also demonstrate that tolerance to CWR exercise performed at severe-intensity domain (expressed by TTF) was not altered in situations of modulation of glycogen concentrations (high vs low content) (HARGREAVES et al., 1997; VANDENBERGHE et al., 1995). This observation offers the opportunity to draw parallels between individuals with different levels of training, thereby corroborating with previously cited findings. Moreover, preceding studies assessing tolerance to CWR effort performed in severe-intensity domain also show that there was no change in TTF, even following exposure to a high-intensity training regimen, such as sprint interval training (SIT) (HARMER et al., 2000; NEVILL et al., 1989).

The lack of improvement in TTF observed in these studies, maybe also connected with the relation between W' (work available from non-oxidative energy sources) and critical power (CP; slope of the regression of work on time; representing an intensity) concepts (JONES et al., 2010). Therefore, considering the possible training status improvement in W' and CP (or RCP; for a detailed description see section “1.1.1 Intensity definitions”) where fatigue occurs more quickly, it is expected that the “range” of relation between W' and CP remains the same, and, thus not improve the exercise tolerance when performed at the same physiological intensity (i.e., adjusted post-training fitness status) (JONES et al., 2010). For instance, Jenkins and Quigly (1993) had observed an improvement in W' and CP intensity after 8 weeks of SIT, however they not verified any alteration in TTF in a severe-intensity domain CWR exercise ($\sim 160\%$ of RCP).

Together, the previously cited studies, implies that training status could not be influence the exercise tolerance on CRW severe-intensity domain. However, any of cited studies explain the physiological underpinnings underlying these results.

1.3. Sprint Interval Training (SIT) as a training model that could modulate glycolytic responses and underpin the maintenance of exercise tolerance at severe-intensity effort

While SIT (often comprising 4-6 Wingate bouts with 4 min intervals between each bout), has emerged in the last decade, as a time-efficient training method, due their ability to induce metabolic adaptations (mainly on oxidative pathway) within a comparable range to high-volume moderate-intensity training (BURGOMASTER et al., 2008; GIBALA et al., 2006; MACINNIS; GIBALA, 2017), the substantial demand placed on the glycolytic pathway during this training (MALTA et al., 2020; PANISSA et al., 2018), along with the elevated peripheral neuromuscular demand (FERNANDEZ-DEL-OLMO et al., 2013), may potentially modulate the glycolytic pathway and the neuromuscular apparatus, respectively (BUCHHEIT; LAURSEN, 2013a, 2013b).

With regard to the modulations of SIT in the glycolytic pathway, studies have shown the effectiveness of this training model to improve muscle glycogen content (BURGOMASTER; HEIGENHAUSER; GIBALA, 2006; GIBALA et al., 2006; MACDOUGALL et al., 1998), and increasing the activity of key enzymes responsible for ATP turnover in the glycolytic pathway, such as PFK (MACDOUGALL et al., 1998; MIYAMOTO-MIKAMI et al., 2018). This improvement may occur mainly because this training model heavily rely on the glycolytic pathway (MALTA et al., 2020), thereby enhancing the gene expression of serum/glucocorticoid regulated kinase 1 (SGK1), protein phosphatase 1, regulatory subunit 3C (PPP1R3C) and carnosine synthase 1 (CARNS1), which may also contribute in improving the functioning of the glycolytic pathways (MIYAMOTO-MIKAMI et al., 2018).

However, to the best of our knowledge, there during CWR severe-intensity domain. Harmer et al. (2000) had investigated the effects of 7 weeks of SIT on the TTF during a CWR above critical intensity (130% of peak power output) and in some muscle metabolic perturbations. They observed no significant difference in TTF at 130% of peak power output. Additionally, the authors noted a reduction in non-oxidative ATP resynthesis, indicated by lower muscle glycogen degradation and muscle lactate accumulation after the effort ($p < 0.01$).

Similarly, Nevill et al. (1989) investigated the influence of SIT (8 weeks) on muscle metabolism during a 2-minute exercise at 110% intensity associated with maximum oxygen uptake. They did not find any significant differences in glycogen degradation or accumulation of glucose-1-phosphate, glucose-6-phosphate, and muscle lactate accumulation.

In this context, considering the potential of SIT to improve activity of some enzymes of glycolytic pathway [e.g., PFK; (MACDOUGALL et al., 1998; MIYAMOTO-MIKAMI et al., 2018)] and muscle glycogen substrate (BURGOMASTER; HEIGENHAUSER; GIBALA, 2006; GIBALA et al., 2006; MACDOUGALL et al., 1998), it is noteworthy that there is no concurrent increase in ATP resynthesis within the glycolytic pathway during exercise (HARMER et al., 2000; NEVILL et al., 1989) or TTF during CWR severe-intensity domain exercise (HARMER et al., 2000). Therefore, it seems reasonable to hypothesize that a potentially higher enzymatic activity within this pathway could deplete muscular glycogen more rapidly, reaching a “critical threshold”. Consequently, this may not necessarily lead to an improvement in the utilization of the glycolytic pathway or an extension of TTF, as verified by Harmer et al (2000).

Therefore, to clarify this, further investigations are necessary to evaluate the impact of SIT on the glycolytic pathway and how it may or may not modulate tolerance during an effort performed above critical intensity.

1.4. Sprint Interval Training (SIT) as a training model that could modulate neuromuscular function responses and underpin the maintenance of exercise tolerance at severe-intensity effort

Furthermore, in addition to SIT capacity to modulate the glycolytic pathway (as outlined in section 1.3), this training model is also highly conducive to the improvement of neuromuscular function (BUCHHEIT; LAURSEN, 2013b), once this training program exercise can induce a critical reduction in central and peripheral indicators of neuromuscular function (i.e., voluntary activation and peak of twitch force at rest, respectively), providing a remarkable perturbation of the homeostasis of the neuromuscular system (FIORENZA et al., 2019).

However, few studies had measured the neural adaptations of SIT during a CWR exercise (BERTSCHINGER; GIBOIN; GRUBER, 2020; LEWIS et al., 2017). These studies, found no changes in any neuromuscular function parameters or in the fatigability after a 2-week SIT intervention (BERTSCHINGER; GIBOIN; GRUBER, 2020; LEWIS et al., 2017),

as well in TTF. These outcomes (neuromuscular function) may be ascribed to the choice of a CWR at low to moderate intensity for investigating neuromuscular function, as well as the relatively short duration of the training period (i.e., 2 weeks).

Despite the fact that moderate intensities necessitate a considerable neuromuscular contribution, both the neuromuscular activation during exercise and the patterns of neuromuscular fatigability significantly differ from those induced by SIT (BLACK et al., 2017; COLLINS et al., 2018; IANNETTA et al., 2022). In this context, when we contemplate exercise intensity exceeding the critical threshold, a distinct perspective appears to unfold. Despite ongoing debates regarding the factors contributing to task failure during exercises above the critical intensity (AMANN; SECHER, 2010; MARCORA, 2010), it becomes apparent that surpassing 'critical' concentrations of certain metabolites, stemming from heightened non-oxidative metabolism (e.g., inorganic phosphate, hydrogen ions), can predominantly diminish muscle contractile capacity at the peripheral level (i.e., peripheral fatigue) (BLACK et al., 2017; HUREAU et al., 2022). This occurs without decreasing the central drive burst input for muscle activation (i.e., central fatigue) (HUREAU et al., 2022), despite of the potential impact of these environmental disruptions on motoneurons III and IV afferences (AMANN et al., 2011; HUREAU et al., 2022). Consequently, this results in an impairment of muscle contraction performance (i.e., fatigability), akin to the effects observed in exercises performed during SIT sessions (FERNANDEZ-DEL-OLMO et al., 2013; FIORENZA et al., 2019).

In this way, further investigations are necessary to evaluate the impact of SIT on neuromuscular responses. In addition, considering the importance of neuromuscular function during severe-intensity domain exercise, it seems reasonable investigate if the neuromuscular function had a relationship with TTF in severe-intensity domain exercise.

2. Aims and program of research

The primary objective of this thesis was to investigate the relationship between the glycolytic pathway, specifically resting glycogen content, tissue buffering capacity, enzyme content, and activity, and exercise tolerance during activities above critical intensity, following a 5-week SIT program. Additionally, we explored whether a 5-week SIT regimen could enhance neuromuscular function responses, both at rest and during exercise, and fatigue mitigation and investigate if these potential neuromuscular changes could explain the possible lack of change on TTF during severe-intensity domain exercise.

To address these questions, the present thesis was divided into two independent studies. The first study (Chapter 2) examined the previously mentioned biomolecular responses to exercise above critical intensity following SIT. Meanwhile, the second study (Chapter 3) investigated the neuromuscular response to SIT, as well as its response to exercise above critical intensity.

CHAPTER 2

Glycolytic Pathway and Exercise Tolerance responses to Sprint Interval Training

(Submission cover prof in Appendix)

Manuscript 1. Five weeks of Sprint Interval Training improve muscle glycolytic content and activity but not time to task failure in severe intensity exercise

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1. Abstract

Purpose: Previous evidence suggests that, despite training induces improvements in glycolytic pathway function, exercise tolerance during constant work rate (CWR) exercise within the severe domain remains unaltered. This study examined the impact of a 5-week sprint interval training (SIT) intervention on time to task failure (TTF) during severe intensity CWR, as well as in glycolytic enzymatic content and activity, and glycogen content. *Methods:* Fourteen untrained males were randomized into SIT group (SIT_G; n=8) composed of 15 SIT-sessions over 5 weeks and in a control group (n=6). At baseline, participants performed: i) ramp incremental test to measure the cardiorespiratory function; ii) CWR cycling TTF at 150% of the power output (PO) at the respiratory compensation point (RCP-PO) with muscle biopsies at rest and immediately following task failure. After the 5-weeks, the same evaluations were repeated, and an additional cycling CWR matched to baseline RCP-PO were performed. *Results:* The content and enzymatic activities of glycogen phosphorylase (GPhos), hexokinase (HK), phosphofructokinase (PFK), and lactate dehydrogenase (LDH), as well as the glycogen content, were analyzed. RCP-PO and CWR TTF increased only for SIT_G post training, with no differences observed for TTF at intensity adjusted to post-training fitness status. The GPhos, HK, PFK, and LDH content and activity, and glycogen content also improved post-training only in the SIT_G. However, no difference in glycogen utilization was observed between groups or time. *Conclusion:* Thus, SIT improved glycolytic pathway parameters, however the use of glycogen was not altered during CWR severe intensity exercise, and TTF remained similar.

Key Words: High intensity exercise; Exercise tolerance; Muscle Glycogen; Muscle Biopsy; Enzymatic Activity

2. Introduction

Although exercise tolerance during constant work rate (CWR) exercise within the severe-intensity domain is related to multifactorial processes (BLACK et al., 2017; HUREAU et al., 2022; ZAGATTO et al., 2022), one of the primary physiological limits to severe-intensity human performance is likely associated with limitations within the glycolytic non-oxidative pathway to support the imposed metabolic demand (ALLEN; LAMB; WESTERBLAD, 2008; VIGH-LARSEN et al., 2021). Essential components for proper functioning of the glycolytic pathway, which is crucial to support the high ATP turnover during severe-intensity exercise, include: adequate glycolytic substrate availability (muscle glycogen stores), content and activity of key enzymes within the glycolytic pathway [e.g., glycogen phosphorylase (GPhos; responsible for muscle glycogen breakdown), hexokinase (HK; facilitating glucose uptake), phosphofructokinase (PFK; the rate-limiting enzyme activated allosterically), lactate dehydrogenase (LDH; interconversion of pyruvate and lactate)] (HARGREAVES; SPRIET, 2020; HEARRIS et al., 2018; SPRIET; HOWLETT; HEIGENHAUSER, 2000; VIGH-LARSEN et al., 2021), and metabolites acting as regulators for H^+ and their transporters (e.g., monocarboxylate transporter isoform 4; MCT4) (BISHOP et al., 2008, 2009).

Considering the importance of the glycolytic pathway for exercises performed within the severe-intensity domain, it could be expected that improvements in physical fitness would enhance ATP resynthesis by improving the previously mentioned metabolic parameters and, as a corollary, lengthen the time-to-task failure during severe-intensity CWR exercise (i.e., exercise tolerance). However, some studies have reported that endurance-trained amateur athletes (i.e., with greater cardiorespiratory function as assessed by maximal oxygen uptake) presented a shorter time-to-task failure during severe-intensity CWR exercises (~ -18%), when compared with non-trained and sedentary subjects, at the same physiologically matched workload (ZAGATTO et al., 2017, 2023a). These results are also consistent with studies that have modulated muscle glycogen concentrations (i.e., high vs. low content) (HARGREAVES et al., 1997; VANDENBERGHE et al., 1995), or even after training models that impose a high reliance on the glycolytic pathway, such as sprint interval training (SIT) (HARMER et al., 2000; NEVILL et al., 1989).

In relation to what stated above, Harmer et al. (2000) reported a lower muscle glycogen degradation and muscle lactate accumulation following a SIT training program compared to pre training moment. Furthermore, Nevill et al. (1989) found no alterations on the glycolytic pathway after SIT, as indicated by no differences in glucose-1-phosphate, glucose-6-phosphate,

and muscle lactate levels, following severe-intensity CWR exercise performed at the same physiological load as pre-training (e.g., 110% of peak power output) , thus indicating an unchanged activity of the glycolytic pathway.

These outcomes imply a connection between time to task failure and changes within the glycolytic pathway. For instance, studies have shown that an increased workload due to higher physical fitness after a training program, significantly enhanced the rate of glycogen degradation (HERMANSEN, 1981), and led to greater content and activity of GPhos (BENEKE; BÖNING, 2008; ESTEVE-LANAO et al., 2007; HERMANSEN, 1981). Additionally, other studies have shown that, in order to meet the energy demand of exercise, the activity of key enzymes in the glycolytic energetic pathway such as HK, PFK, and LDH are also upregulated after high intensity training (EGAN; ZIERATH, 2013; ROBERTS; BILLETER; HOWALD, 1982). Nevertheless, the enhanced activity of such enzymes may lead to a situation in which the substrate sources (i.e., primarily muscle glycogen) can be depleted faster because of the greater ability to use and resynthesize ATP relying on the glycolytic pathway (BENEKE; BÖNING, 2008; ESTEVE-LANAO et al., 2007; HERMANSEN, 1981). Additionally, none of the above-mentioned studies have investigated whether muscle buffering capacity and MCT4 content changed after training. These factors could play an important role in the attenuation of possible metabolites-induced alterations of the glycolytic pathway activity (UI, 1966), and could help explain the lack of improvement in exercise tolerance during severe-intensity CWR exercises after training. Therefore, further investigation on how changes in training status after SIT could modulate these factors is warranted. Thus, the main purpose of the present study was to investigate the relationship between the glycolytic pathway (i.e., resting glycogen content, tissue buffering capacity, enzyme content and activity) and exercise tolerance within the severe-intensity domain, following a 5-weeks of SIT cycling exercise training program.

3. Methods

3.1 Participants

A sample size calculation was performed based on an expected significant improvement in muscle glycogen content after 2 weeks of SIT (Cohen's *d* effect size: 1.6) (GIBALA et al., 2006). Considering a statistical power of 90% and an alpha value of 0.05 (COHEN, 1988), a minimum sample size of 6 participants for each group (i.e., control and SIT) was calculated.

This study recruited 15 young healthy active males (i.e., exercising at least 3 times per week without engaging in a regular training routine), with no recent history of muscular injuries or chronic metabolic disorders (e.g., diabetes mellitus) (Figure 1, Flow diagram). Females were not recruited for this study because differences in glycolytic response parameters between sexes during training and intense physical exertion have been reported (ZOUHAL et al., 2008). Given that this would potentially increase the heterogeneity of the data, and that this project could include a limited number of participants, we decided against including both females and males. A total of 14 healthy volunteers participated in study (mean $\pm$ SD; age: 30 ± 5 years; stature: 177 ± 4 cm; body mass: 76.7 ± 11.8 kg).

Throughout the study period, participants were asked to abstain from the use of any ergogenic substances and from engaging in strenuous exercise for at least 24 h before each experimental session. Additionally, participants were required to perform at least 95% of the planned-routine training sessions. Participants who engaged in fewer than 95% of the planned training sessions were excluded from the study.

The participants received verbal and written details of the experimental procedures and possible risks and benefits, and they signed an informed written consent document before undergoing any evaluation. All procedures were approved by the local Research Committee (Protocol #2.650.216/2018) and conducted in accordance with the Declaration of Helsinki. In addition to regular informed written consent document, each participant filled out a specific anamnesis related to muscle biopsy procedure concerning anesthesia, drugs allergy reaction, bleeding coagulation, and healing process amongst others.

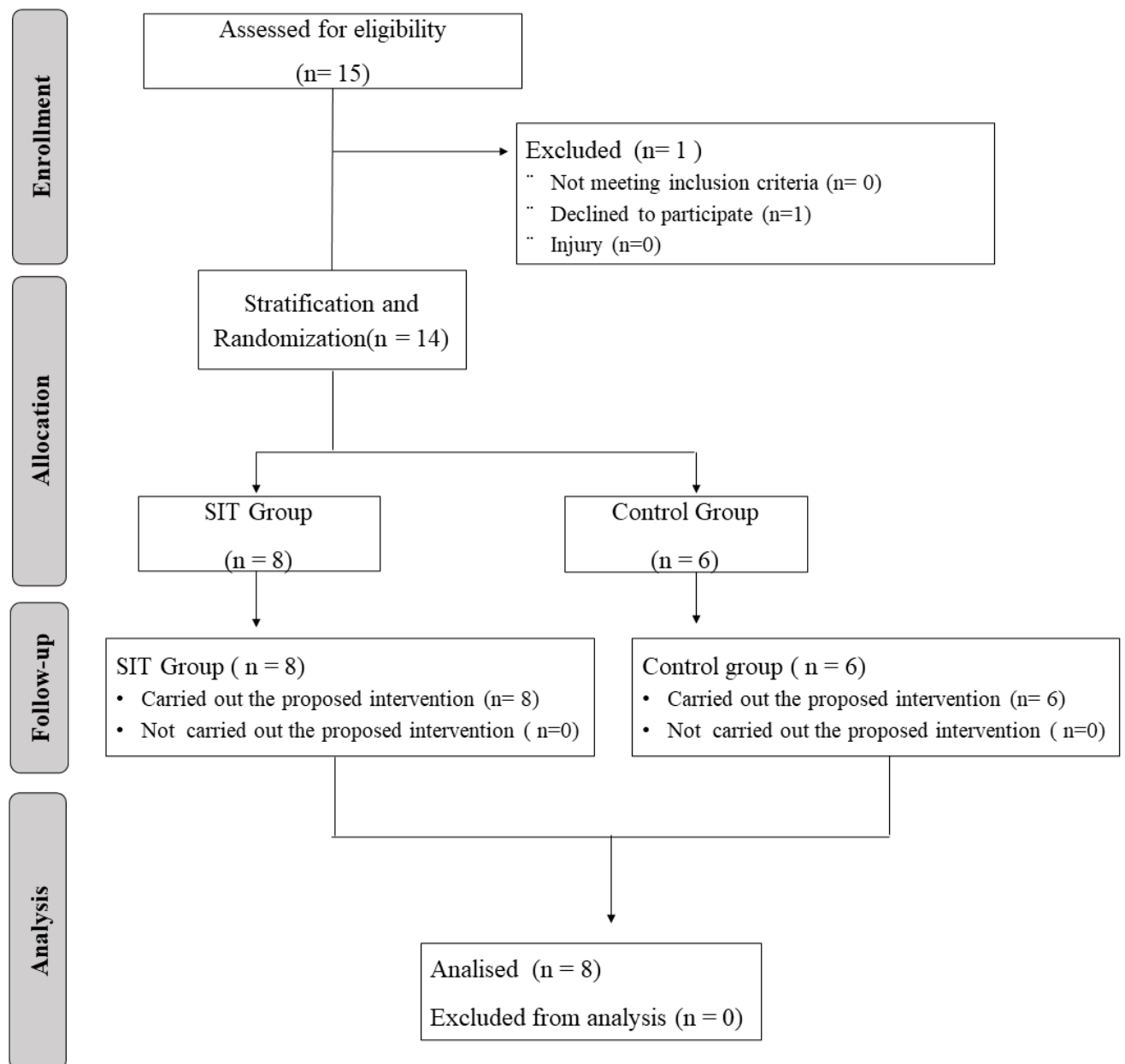


Figure 1. Study flow diagram.

3.2 Experimental Design

The study was conducted using a parallel pre-post training design model in which measurements of physical performance, physiological responses, and muscle markers were taken from participants before and after 5 weeks of SIT. During the baseline assessments (BL), participants visited the laboratory three times in the same week. The first visit involved familiarization with CWR exercise within the severe-intensity domain to ensure execution of maximal effort during the subsequent evaluations. The second visit consisted of a ramp incremental test to determine maximum oxygen consumption ($\dot{V}O_{2max}$), peak power output (PPO), the respiratory compensation point (RCP) and the power output at the RCP (RCP-PO). The third visit involved a CWR exercise until task failure performed at 150% RCP-PO, with vastus lateralis muscle biopsies taken at rest and immediately after exercise.

Following the baseline measurements, participants were matched for $\dot{V}O_{2max}$, RCP-PO, and PPO, and then randomly assigned to either the SIT group (SIT_G; n = 8) or the control group (CG; n = 6). The SIT_G underwent 5 weeks of SIT composed of 3-6 repeated 30 s all-out Wingate tests (GIBALA et al., 2006), whereas the control group maintained their usual physical activities within the same study period, without engaging in any regular training programs. After 5 weeks (Post 5-weeks), the same baseline measurements were repeated. Additionally, the CWR exercise performed during the baseline measurements was repeated using the same PO to record the time-to-task failure (Post_{BL-RCP}). A schematic of the experimental design is depicted in Figure 2. All testing and training sessions were separated by a minimum interval of 48 h and a maximum of 72 h. Participants were verbally encouraged to achieve maximum performance in all testing and training sessions, and all procedures were conducted at a similar time of the day (± 2 h). Additionally, all cycling exercises were performed on a cycle ergometer (Lode-Excalibur Sport; Lode BV, Netherlands) with a self-selected cadence selected in range between 80 and 100 rpm (± 5 rpm of the chosen cadence during all testing efforts).

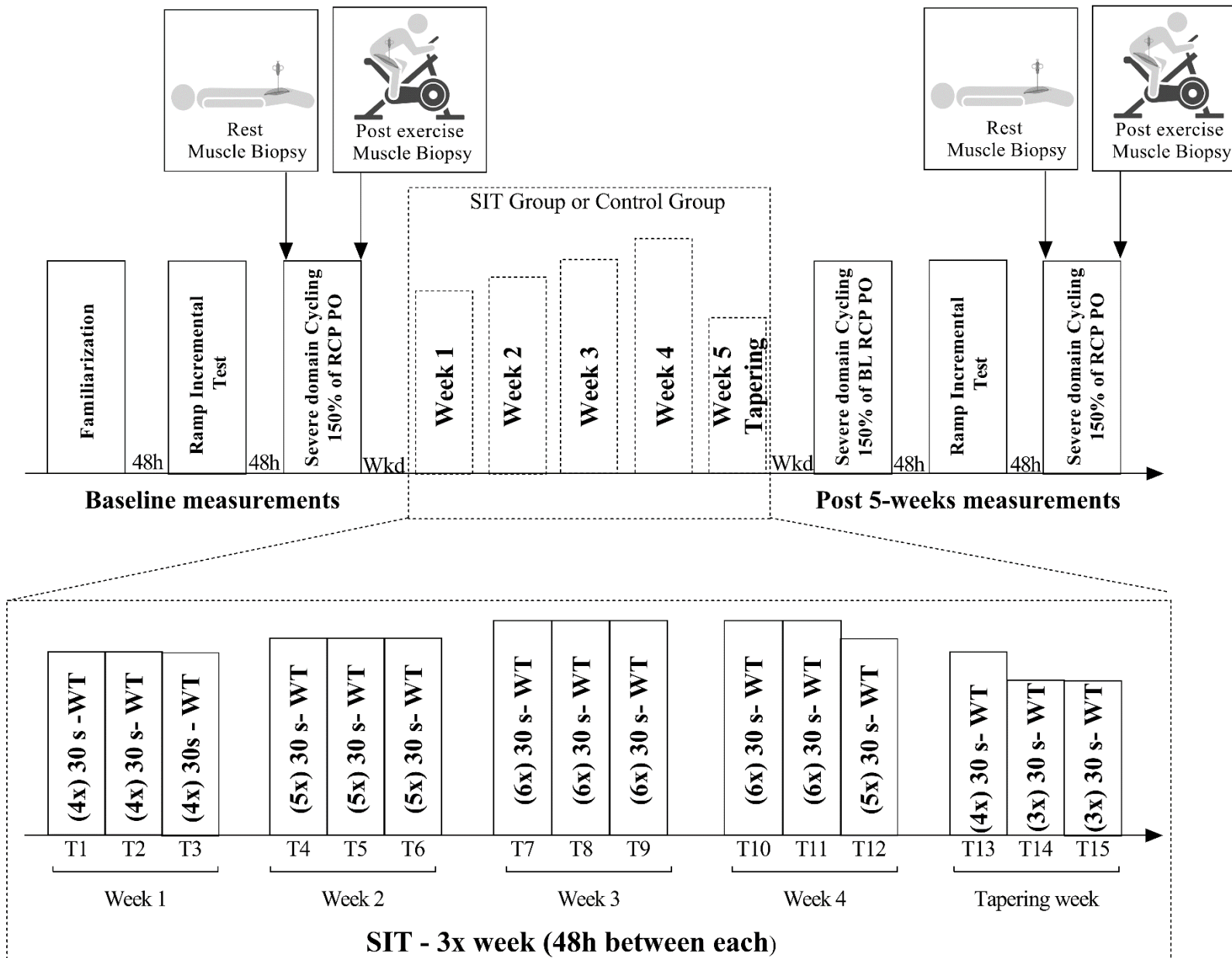


Figure 2 Study Experimental Design and training parameters. RCP-PO = respiratory compensation point power output; BL RCP-PO = Respiratory compensation point power output measured at baseline; Wkd = weekend; T1–T15 = number of training sessions

3.3 Exercise protocols

3.3.1 Ramp incremental test

The ramp incremental test was performed until task failure to measure $\dot{V}O_{2\max}$, PPO and the RCP (IANNETTA et al., 2020). The test began with a 5-minute warm-up at 75 W, followed by an increase in workload of 1 W every 2 s until the predetermined cadence could no longer be maintained or the participants stopped cycling, despite strong verbal encouragement (IANNETTA et al., 2020).

3.3.2 CWR severe-intensity domain

The CWR within the severe-intensity domain was performed at 150% of RCP-PO [i.e., corresponding to approximately 115% of PPO; (ZAGATTO et al., 2017, 2023a)]. Participants began pedalling until they achieved a self-selected cadence. Then, the workload corresponding to 150% of the RCP-PO was applied, and the time-to-task failure recorded. Task failure was determined when participants were unable to maintain a pedal cadence of -5 rpm from their self-selected cadence for more than 5 s or when volitional exhaustion occurred.

3.3.3 Sprint interval training

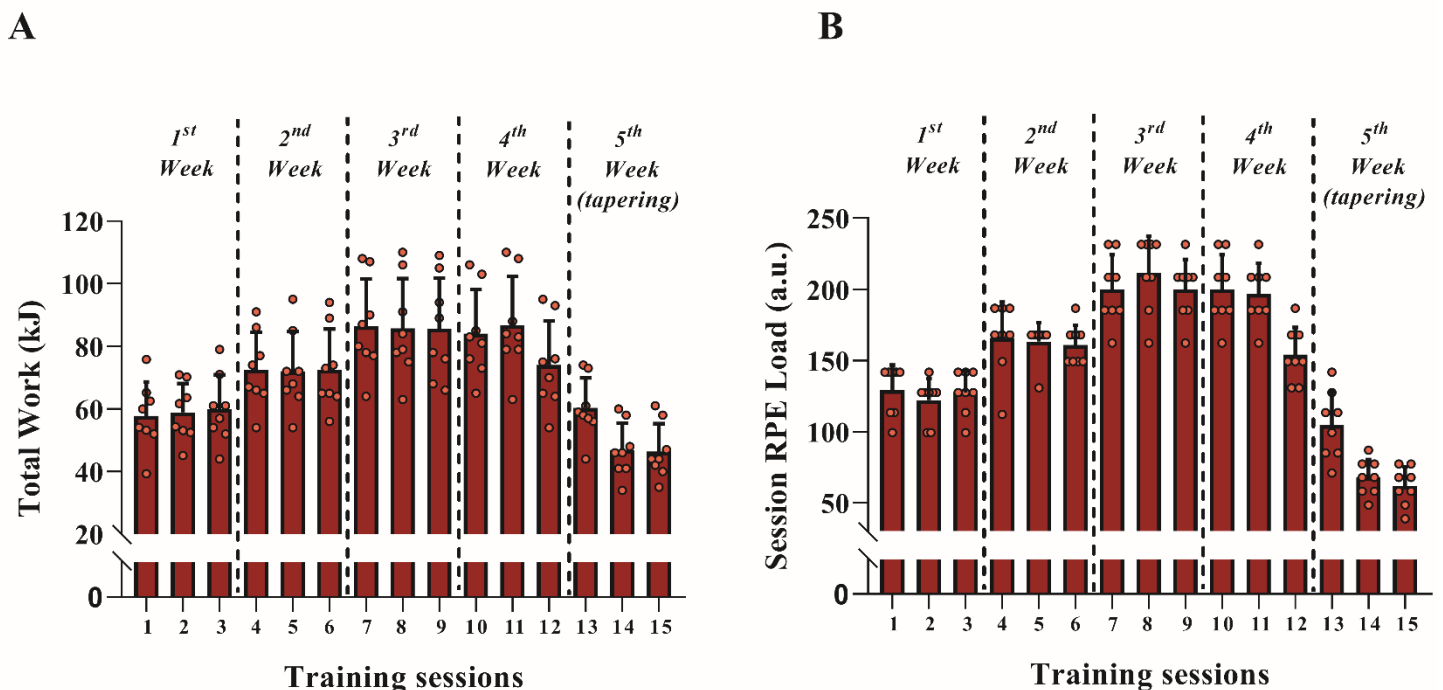
The SIT consisted of 3-6 repeated all-out bouts of the 30 s as in the Wingate test (workload: $0.70 \text{ Nm} \cdot \text{kg}^{-1}$; rest: 4 min between each sprint) (GIBALA et al., 2006). The SIT was performed 3 times per week, for 5 weeks, for a total of 15 SIT sessions. The sessions of the first training week were composed of $4 \times 30 \text{ s}$ all-out bouts. The number of bouts per session was increased by one bout each week until the third week (i.e., week 1: 4 bouts; week 2: 5 bouts; week 3: 6 bouts) (GIBALA et al., 2006). For the following training sessions, the participants underwent 6 bouts, until the last 4 training sessions (tapering component) in which 5, 4, 3, and 3 bouts of the 30 s all-out exercise were performed, respectively to reduce the training volume by approximately 16 to 50% (BOSQUET et al., 2007). All the SIT_G participants adhered to the training program with 100% compliance.

The cadence and power output were recorded during all training sessions using data acquisition at a frequency of 5 Hz with Wingate test software (Lode BV, Netherlands). The

highest power output value achieved during each sprint was recorded as peak power output (PP), while the average power output reached during each sprint was recorded as the mean power (MP). Additionally, 30 minutes after the completion of each training session, the participants rated their perceived exertion on a scale of 1-10 to quantify the internal training load of the session as described by Foster (i.e., training load = rate perceived exertion $\times$ training session duration in minutes) (FOSTER, 1998).

To characterize the training session, the energetic contribution was determined by the accumulated $\dot{V}O_2$ (oxidative pathway); the accumulated blood lactated concentration (glycolytic pathway) and by excess post oxygen consumption (DI PRAMPERO; FERRETTI, 1999; MARGARIA; EDWARDS; DILL, 1933). The energetic contribution during trainings corresponded to 82.0% oxidative metabolism and 18.0% of non-oxidative metabolisms. Of the non-oxidative sources, the glycolytic and phosphagen energetic pathways contributed 86% and 14%, respectively. These calculations consider the whole training session, including both the work and the recovery components.

The total work and session RPE load for each training session are shown in Supplemental Digital Content 1.



3.4 Data collection

3.4.1 Cardiorespiratory data

Gas exchange and ventilatory responses were measured breath-by-breath using a stationary metabolic cart (Quark PFT; COSMED, Italy) during the ramp incremental test and the CWR exercises (only 150% RCP-PO). Additionally, heart rate was measured using a wireless chest belt (HR 138 Monitor; COSMED) synchronized with metabolic cart. For all measurements, raw gas exchange was cleaned to remove outliers' data points. Then, a local mean of 5 s bins were calculated and data that exceeded more than 3 standard deviations from the local mean were removed. The data were then interpolated to 1 s intervals using OriginPro 8.0 Software (OriginLab, USA) (KEIR et al., 2014).

3.4.2 Muscle biopsies

Muscle biopsies of the *vastus lateralis* were performed at rest and immediately after the CWR exercise at task failure. First, subcutaneous local anaesthesia was administered using 3-5 mL of 2% lidocaine without vasoconstrictor, followed by execution of an incision of ~0.5 cm (skin, fascia and muscle). Muscle samples (148 ± 6 mg) were obtained using a one-hand-adapted Bergstrom needle with suction. During the exercise, a superficial dressing was applied to the incision site to prevent bleeding or potential contamination. For the biopsy sample taken after exercise, the Bergstrom needle was inserted in the same surgical incision, with the participant still seated in the cycle ergometer. The muscle samples were immediately (~12 s) frozen in liquid nitrogen, dissected to remove any blood, fat, and connective tissues, and stored at -80°C until analyses.

3.5 Data analyses

3.5.1 Ramp incremental test cardiorespiratory data

The RCP was determined by plotting the end-tidal partial pressure of CO₂ (PCO₂), ventilation ($\dot{V}_E$), and ventilation/carbon dioxide production curve ($\dot{V}_E/\dot{V}_{CO_2}$) values against oxygen consumption data ($\dot{V}_{O_2}$) (IANNETTA et al., 2020). The RCP was estimated based on

the consensus of two experienced researchers and defined as the point at which the end-tidal PCO₂ began to fall, in agreement with the second and first curve breakpoints of $\dot{V}_E$ and $\dot{V}_E/\dot{V}_{CO_2}$, respectively (IANNETTA et al., 2020). After identifying $\dot{V}_{O_2}$ at RCP, the relationship between $\dot{V}_{O_2}$ and PO (x-axis) was plotted for the determination of RCP-PO. Given that the mean response time and the slow component of the $\dot{V}_{O_2}$ response were not taken into consideration when retrieving the PO associated to the $\dot{V}_{O_2}$ identified at the RCP, it was accepted that the RCP-PO already represented an intensity in the severe domain (IANNETTA et al., 2019, 2020). The $\dot{V}_{O_{2max}}$ was determined as the highest oxygen consumption value from a 20 s rolling average, and the PPO was assumed to be the highest intensity reached during the ramp incremental test (IANNETTA; MURIAS; KEIR, 2019).

3.5.2 CWR cardiorespiratory data

Each individual's breath-by-breath $\dot{V}_{O_2}$ data were cleaned to remove the data points lying ± 3 SD from the local mean (LAMARRA et al., 1987). Thereafter, the $\dot{V}_{O_2}$ data were time aligned, 1 s interpolated and averaged into 5 s bins (KEIR et al., 2014). The first 22 s were excluded from the analysis to account for the cardio-dynamic phase (phase I) of the $\dot{V}_{O_2}$ response (MURIAS et al., 2011). Considering that the $\dot{V}_{O_2}$ kinetics within the severe and extreme intensity domains resemble a mono-exponential pattern (ÖZYENER et al., 2001; WHIPP; ROSSITER; WARD, 2002), the following equation (Eq. 1) was used to fit the data:

$$\dot{V}_{O_2}(t) = A_0 + A \cdot (1 - e^{-TD/\tau}) \quad [1]$$

Where A_0 is the baseline $\dot{V}_{O_2}$ value and A are the asymptotic amplitude for the cardiodynamic and primary exponential components (phases 1 and 2, respectively), τ is the time constant, and TD is the time delay. Additionally, a 20 s moving average was also performed on the interpolated data, and the higher value was defined as the peak of oxygen uptake achieved during the severe-intensity CWR exercise ($\dot{V}_{O_{2peak}}$).

3.5.3 Skeletal muscle tissue analysis

3.5.3.1 Enzymatic activity measurements

The enzymatic activities of the glycolytic pathway enzymes HK, PFK, and LDH were analysed using a colorimetric method with an EPOCH 2 spectrophotometer (BioTek

Instruments, USA). Commercial enzymatic activity assay kits were used for HK (MAK091; Sigma-Aldrich/Merck), PFK (ab155898; Abcam), and LDH (MAK066; Sigma-Aldrich/Merck). Wet muscle tissue (approximately 10-20 mg) was homogenized in a specific buffer and incubated in a dark environment at 37°C for 5 min. Subsequently, the homogenized sample reaction was measured every 5 min at 450 nm until the most active sample value exceeded the highest value on the standard curve. Enzymatic activity values were determined using Equation 2 and normalized to the total protein content of each sample.

$$EA = \frac{\text{Amount of NADH generated between the first and last measurement} \times \text{dilution factor}}{\text{Time of reaction} \times \text{sample volume}} \quad [2]$$

3.5.3.2 Western Blot

Western blot technique was used to quantify the protein contents of GPhos, HK, PFK, LDH, and monocarboxylate transporter 4 (MCT-4). Approximately 50 mg of wet muscle tissue was homogenized in an extraction buffer containing Triton X-100 (%), 100 mM Tris (pH 7.4), 100 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 10 mM sodium vanadate, 2 mM PMSF, and 0.1 mg/ml aprotinin at ice-cold temperature. The extract was centrifuged at 11,000 rpm for 40 min at 4°C to remove insoluble material. The supernatant was used for protein quantification using the Bradford method (25). Following this, the sample was denatured by dry boiling at 70°C for 4 min in Laemmli 4x samples (25%), and subsequently loaded onto a 12% SDS-PAGE gel and transferred to a 0.45 μ m nitrocellulose membrane using a semi-dry technique (Trans-Blot Turbo System, Bio-Rad, USA). Transfer efficiency was confirmed by briefly staining the blots with Ponceau red stain. Thereafter, the membrane was blocked with Tris-buffered saline (TBS) containing 5% BSA and 0.1% Tween-20 for 1 h at 4°C.

The specific primary antibodies used were GPhos (ab63158; Abcam), HK (ab104836; Abcam), PFK (#8164S; Cell Signaling), LDH (#3582; Cell Signaling), and MCT-4 (ab74109; Abcam) at 1:1000 dilution. Protein content was normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH; sc-47724; Santa Cruz Biotechnology). All primary proteins were incubated for 16 h at 4°C. Specific secondary antibodies (1:5000 dilution) were used for each protein with a 1 h incubation. Specific immunoreactive bands were detected using chemiluminescence with the G:Box Chemi-XRQ system (Syngene, UK), and the images were acquired and quantified using GeneSys software (Syngene, UK).

3.5.3.3 Muscle glycogen measurements

Muscle glycogen content was determined using a colorimetric method (DUBOIS et al., 1956) adapted for microsamples. Approximately 10 mg of wet muscle tissue was digested in 2 mL 30% KOH at 100°C for 1 h. Subsequently, 200 μ L Na₂SO₄ was added, and glycogen was precipitated through two passages of 6 mL ethanol in a water bath (approximately 15 s), followed by centrifugation at 2000 rpm for 5 min at 22°C in 2 mL deionized water. Colorimetric determination was performed by adding 15 μ L phenol and 2 mL H₂SO₄, followed by boiling for 15 min. The absorbance was measured in duplicate at 490 nm (coefficient of variation = 3.1%) using a spectrophotometer (EPOCH 2, BioTek Instruments, USA), and the value of absorbance for each sample was assumed to be the mean between duplicates. In addition, standard curve calibration was performed in duplicate using known glucose samples ranging from 0 (blank) to 100 μ g (coefficient of variation = 1.1%; $r^2 = 0.99$).

The muscle glycogen value was assumed to be the product of the angular coefficient of the standard curve, sample absorbance value, and dilution factor (DUBOIS et al., 1956). Subsequently, the values were converted to mmol·kg⁻¹DM according to Areta and Hopkins (ARETA; HOPKINS, 2018).

3.5.3.4 Buffering capacity in vitro measurement

Buffering capacity was determined using a titration method (BISHOP et al., 2008). Buffering capacity was determined using a titration method (27). Freeze-dried muscle samples (between 1.8 and 2.5 mg) were powdered and homogenized with sodium fluoride solution (10 mM NaF; 33 μ L NaF·g⁻¹·dm⁻¹) through five 20 s intervals on ice. The homogenate was incubated at 37°C (Thermomixer, Eppendorf, Germany) and the initial pH was measured. If necessary, the pH was adjusted to 7.2 by adding NaOH (0.02 mM). Subsequently, the homogenate was titrated by sequential addition of 2 μ L of HCl (10 mM) until a pH of 6.2 was reached. All pH measurements were performed in triplicate using a glass microelectrode (Microelectrodes; Mettler Toledo, Switzerland) connected to a pH meter (FiveEasy Plus; Mettler Toledo). The amount of HCl required to change the pH from 7.2 to 6.5 was determined by considering the quantity of dry muscle aliquoted in the sample, enabling the calculation of the buffer capacity in vitro.

3.6 Statistical Analyses

The data are presented as mean $\pm$ standard deviation (SD) and 95% confidence intervals (CI 95%). Shapiro-Wilk and Levene tests were used to assess the normality of the distribution and homoscedasticity of the data. A general linear model (ANOVA) was conducted to examine potential differences in biomolecular and respiratory variables [e.g., time (BL $\times$ Post 5-weeks) $\times$ group (SIT_G $\times$ CG). For the enzymatic activity, muscle glycogen content, and buffering capacity, the linear model was applied as follow: exercise time (pre $\times$ post) $\times$ period (BL $\times$ Post 5-weeks) $\times$ group (SIT_G $\times$ CG). Mauchly's sphericity test was applied, and sphericity was considered violated if the F-test was significant. In cases where sphericity was violated, the Greenhouse-Geisser epsilon correction was applied. Post hoc tests were performed using the "Bonferroni" method. Additionally, when necessary, an independent samples t-test was used to compare deltas. A Pearson product-moment correlation coefficient (r) was used to determine the level of associations between percentual of change of biomolecular results and time-to-task failure. The significance level for all analyses was set at 5% ($p < 0.05$).

4. Results

4.1 Ramp incremental test

The respiratory responses and PPO variables measured during the incremental ramp test are listed in Table 1. After the 5 weeks, just the SIT_G improved the $\dot{V}O_{2max}$, PPO, $\dot{V}O_2$ at RCP and RCP-PO (group $\times$ time interaction, $p < 0.01$; $F > 9.49$)

Table 1. Variables of the ramp incremental test measured at baseline and post-5weeks in SIT group and control group.

	SIT Group (SIT _G)			Control Group (CG)			ANOVA results		
	BL	Post 5-weeks	Δ%	BL	Post 5-weeks	Δ%	Interaction <i>p</i> (F)	Group <i>p</i> (F)	Time <i>p</i> (F)
PPO (watts)	269 ± 39 (236 to 302)	295 ± 38 (264 to 327)* [†]	+10.0 [‡]	249 ± 29 (219 to 279)	253 ± 40 (210 to 295) [†]	+0.8	<0.01 (9.52)	0.14 (2.53)	<0.01 (15.46)
VO _{2max} (L)	2.88 ± 0.37 (2.57 to 3.20)	3.06 ± 0.36 (2.76 to 3.36)* [†]	+6.6 [‡]	2.70 ± 0.44 (2.24 to 3.16)	2.67 ± 0.41 (2.23 to 3.10) [†]	-0.9	<0.01 (9.49)	0.19 (1.88)	0.04 (4.86)
RCP- PO (watts)	211 ± 31 (185 to 236)	233 ± 28 (209 to 256)* [†]	+10.8 [‡]	200 ± 23 (176 to 224)	197 ± 29 (167 to 227) [†]	-1.9	<0.01 (37.50)	0.15 (2.36)	<0.01 (21.59)
VO ₂ at RCP (L)	2.35 ± 0.30 (2.09 to 2.60)	2.52 ± 0.27 (2.29 to 2.75)*	+7.6 [‡]	2.28 ± 0.31 (1.95 to 2.61)	2.18 ± 0.35 (1.80 to 2.55)	-4.8	<0.01 (10.36)	0.23 (1.60)	0.46 (0.58)

Values are expressed as the mean ±SD (CI95%). Δ% = percentage of change between BL and Post 5-weeks for the same group; PPO = Peak power output; RCP = Respiratory compensation point; VO_{2max} = maximum oxygen consumption. *Significant difference for period × group interaction (BL < Post 5-weeks in the group). [†] Main time effect (*p* < 0.05; BL < Post 5-weeks). [‡] Significant independent t-test difference (SIT_G > CG).

4.2 CWR at severe-intensity domain

A significant time $\times$ group interaction was verified for the CWRs bouts ($p < 0.01$; $F = 7.46$; $\eta^2 = 0.38$; Power = 0.91), indicating a higher time to task failure for SIT_G during Post_{BL-RCP} when comparing different periods within the group (*post hoc* $p < 0.01$). Furthermore, the Post_{BL-RCP} values of SIT_G were also greater than those of CG for the same period (*post hoc* $p < 0.01$) (Figure 3)

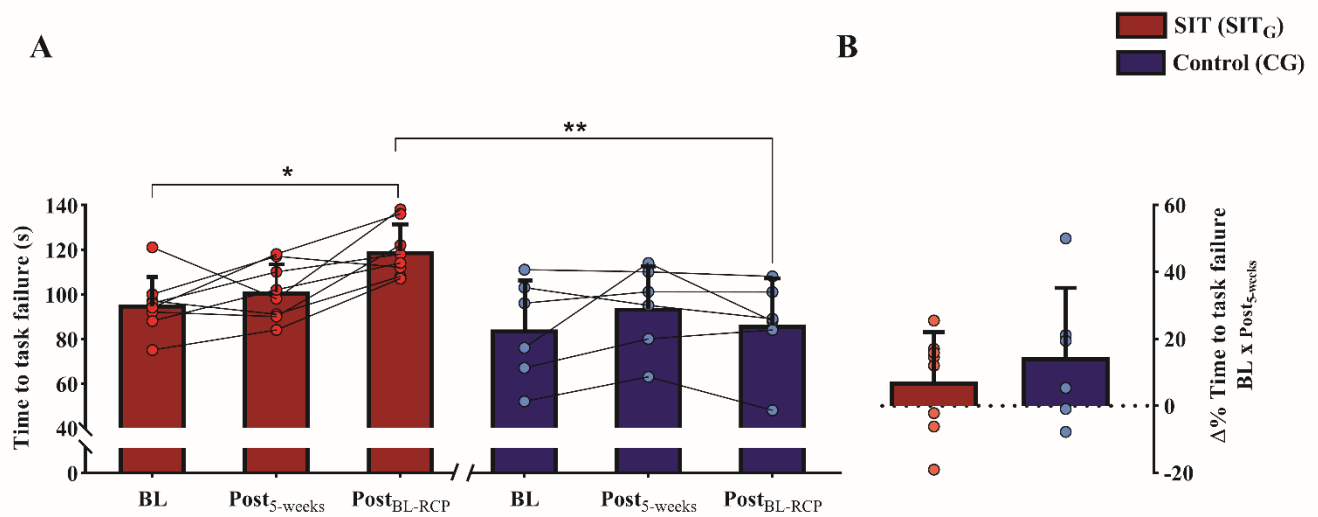


Figure 3. Comparison between time to task failure during the severe intensity domain continuous test measured at the pre-training period (BL), post-training period (Post 5-weeks), and post-training period performed at a power output corresponding to 150% of the respiratory compensation point measured at BL (Post_{BL-RCP}). * Significantly different from other periods of SIT_G group ($p > 0.05$). ** Significantly different from Post_{BL-RCP} of CG.

4.3 Cardiorespiratory responses at CWR cycling in the severe intensity domain with adjusted intensity

The $\dot{V}O_2$ kinetics profiles in the BL and Post 5-weeks periods are shown in Table 2. A significant time $\times$ group interaction was observed for τ ($p = 0.02$; $F = 6.94$), where only SIT_G present a significant lower value of τ in Post 5-weeks when compared with BL (*post hoc* $p = 0.02$). In addition, the $\Delta\%$ in τ between BL and Post 5-weeks showed a significantly smaller value for SIT_G compared to CG ($p = 0.02$). No period $\times$ group interactions were observed for A₀, A, TD, and $\dot{V}O_{2Peak}$ during severe domain exercises ($p > 0.18$; $F < 2.028$). These results remain similar when the responses where expressed as $\Delta\%$ ($p > 0.13$).

Table 2. Oxygen consumption kinetic profile during severe domain effort.

	SIT Group (SIT _G)			Control Group (CG)			ANOVA results		
	BL	Post 5-weeks	Δ%	BL	Post 5-weeks	Δ%	<i>Interaction</i> <i>p</i> (F)	<i>Group</i> <i>p</i> (F)	<i>Time</i> <i>p</i> (F)
A0 (L·min ⁻¹)	0.83 ± 0.17 (0.69 to 0.97)	0.86 ± 0.15 (0.74 to 0.99)	+4.9	0.73 ± 0.21 (0.51 to 0.96)	0.76 ± 0.21 (0.54 to 0.99)	+4.6	0.96 (0.01)	0.33 (1.04)	0.20 (1.85)
A (L·min ⁻¹)	2.39 ± 0.43 (2.03 to 2.75)	2.45 ± 0.49 (2.04 to 2.87)	+6.2	2.17 ± 0.32 (1.84 to 2.51)	2.10 ± 0.38 (1.70 to 2.50)	-3.8	0.66 (0.20)	0.11 (2.95)	0.96 (0.01)
τ (s)	29.1 ± 16.9 (14.9 to 43.3)	21.0 ± 5.5 (16.4 to 25.6)*	-13.0 [‡]	15.4 ± 2.4 (12.8 to 17.9)	23.0 ± 9.6 (12.9 to 33.0)	+46.6	0.02 (6.47)	0.25 (1.45)	0.93 (0.01)
TD (s)	2.0 ± 12.0 (-8.0 to 12.0)	5.6 ± 3.5 (2.6 to 8.5)	+41.9	8.5 ± 5.7 (2.4 to 14.5)	7.4 ± 5.6 (1.5 to 13.3)	-23.2	0.36 (0.89)	0.24 (1.53)	0.62 (0.26)
VO _{2Peak} (L)	2.92 ± 0.29 (2.68 to 3.17)	3.13 ± 0.32 (2.86 to 3.40)	+7.2	2.77 ± 0.40 (2.35 to 3.20)	2.84 ± 0.56 (2.24 to 3.42)	+1.3	0.18 (2.03)	0.30 (0.21)	0.54 (0.39)

Values are expressed as the mean ±SD (CI95%). Δ% = percentage of change between BL and Post 5-weeks for the same group; A0 = is the baseline $\dot{V}O_2$ value; A = asymptotic amplitude for the primary exponential component; τ = time constant for the primary exponential; TD = time delay; $\dot{V}O_{2peak}$ = peak oxygen consumption achieved in severe domain exercise. *Significant difference for period × group interaction (BL > Post 5-weeks in the group). [‡] Significant independent t-test difference (SIT_G < CG).

4.4 Muscle tissue analysis

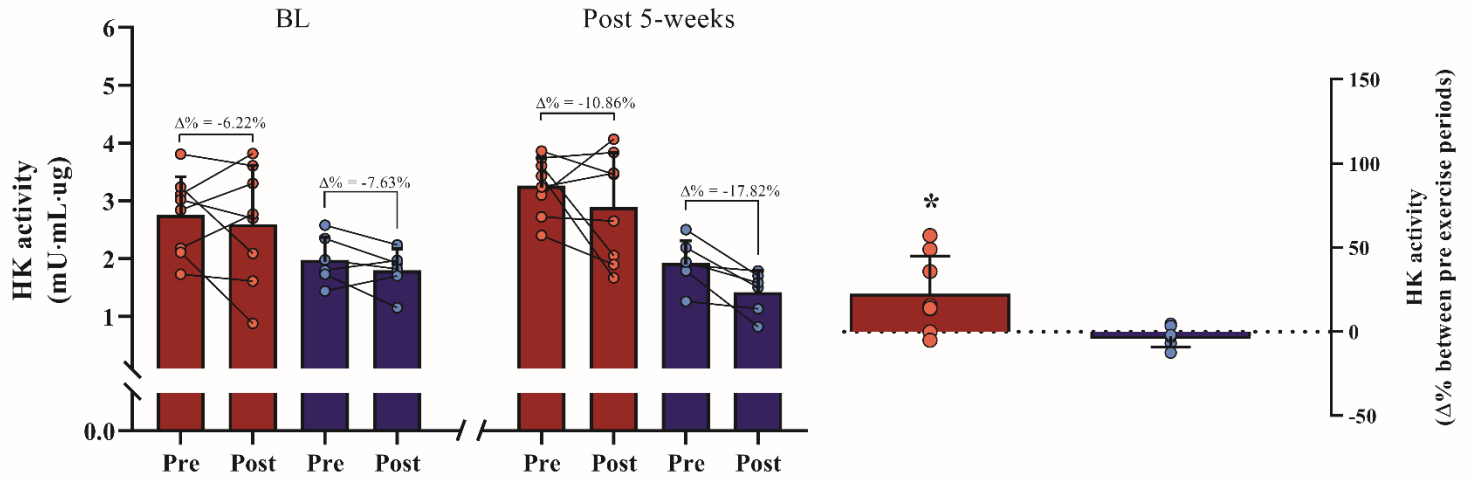
4.4.1 Enzymatic activity

The enzymatic activities of HK, PFK, and LDH are shown in Figure 4A–4C, respectively. No significant period $\times$ group $\times$ pre-or post-exercise interactions were observed for any of the enzymes ($p \geq 0.06$; $F \leq 3.55$). Despite the tendency for a decrease in enzymatic activity at post-exercise when expressed as a $\Delta\%$, no time effect was observed for any of the analyzed enzymes ($p \geq 0.11$; $F \leq 2.96$).

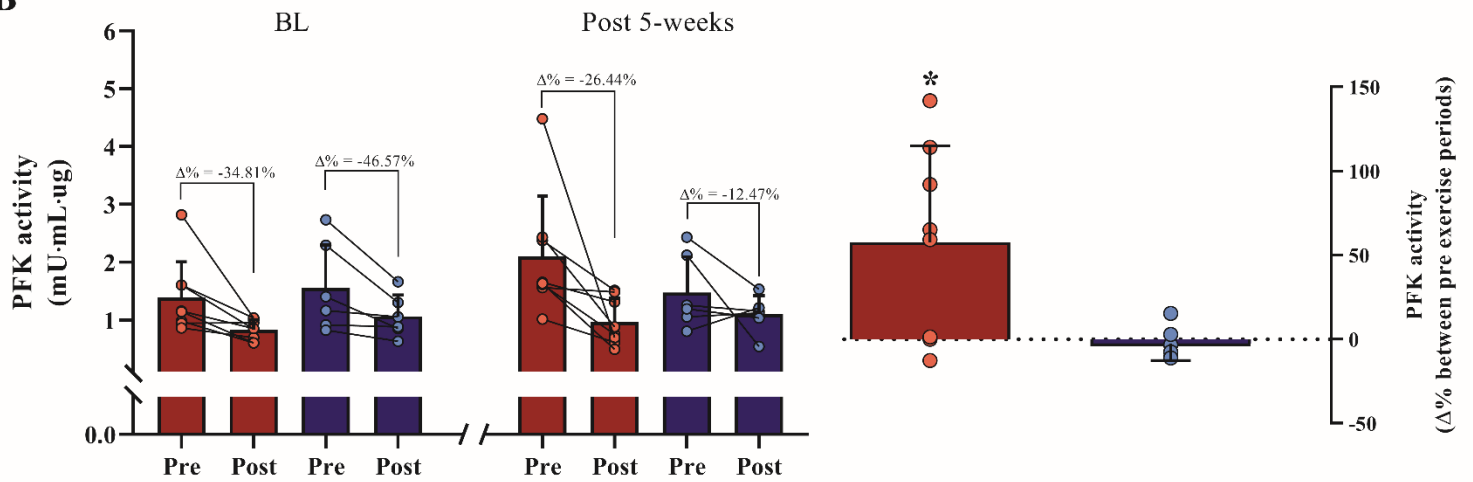
However, when comparing the $\Delta\%$ between pre-exercise BL and Post-5 weeks at rest, the SIT_G group demonstrated significant increases in HK activity ($p = 0.02$), PFK activity ($p = 0.03$), and LDH activity ($p = 0.04$) compared to the CG group.

SIT (SIT_G)
Control (CG)

A



B



C

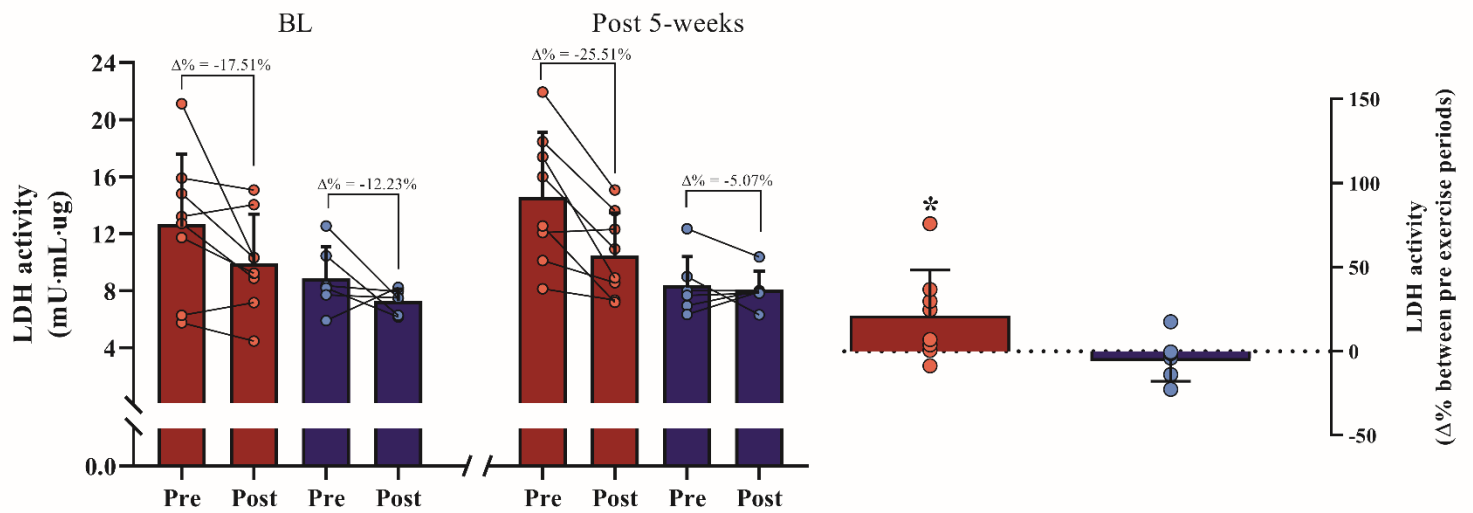


Figure 4. Comparison between hexokinase (HK; Panel A), phosphofructokinase (PFK; Panel B) and lactate dehydrogenase (LDH; Panel C) activity at pre- and post-exercise periods, measured in Post 5-weeks and BL. * Significant difference ($p < 0.05$; TG>CG).

4.4.2 Western Blot

The glycolytic enzymatic (GPhos, HK, PFK, LDH) content and the transporter protein MCT-4 are shown in Figure 5. A significant period $\times$ group interaction was observed for GPhos ($p = 0.01$; $F = 8.58$; $\eta^2 = 0.42$; Power = 0.77), HK ($p = 0.049$; $F = 4.81$; $\eta^2 = 0.29$; Power = 0.53), and PFK ($p = 0.04$; $F = 5.40$; $\eta^2 = 0.31$; Power = 0.57), indicating that only SIT_G increased the content of these proteins at Post-5weeks compared with BL (*post hoc* $p \leq 0.049$). Furthermore, when comparing the $\Delta\%$ between BL and Post-5weeks, SIT_G exhibited a greater value for GPhos and PFK ($p \leq 0.04$).

No significant period $\times$ group interaction differences were observed for LDH and MCT-4 levels ($p \geq 0.07$; $F \leq 3.79$; $\eta^2 = 0.24$; Power < 0.43). However, when expressed as $\Delta\%$, greater values were observed for the SIT_G than for the CG ($p \leq 0.047$).

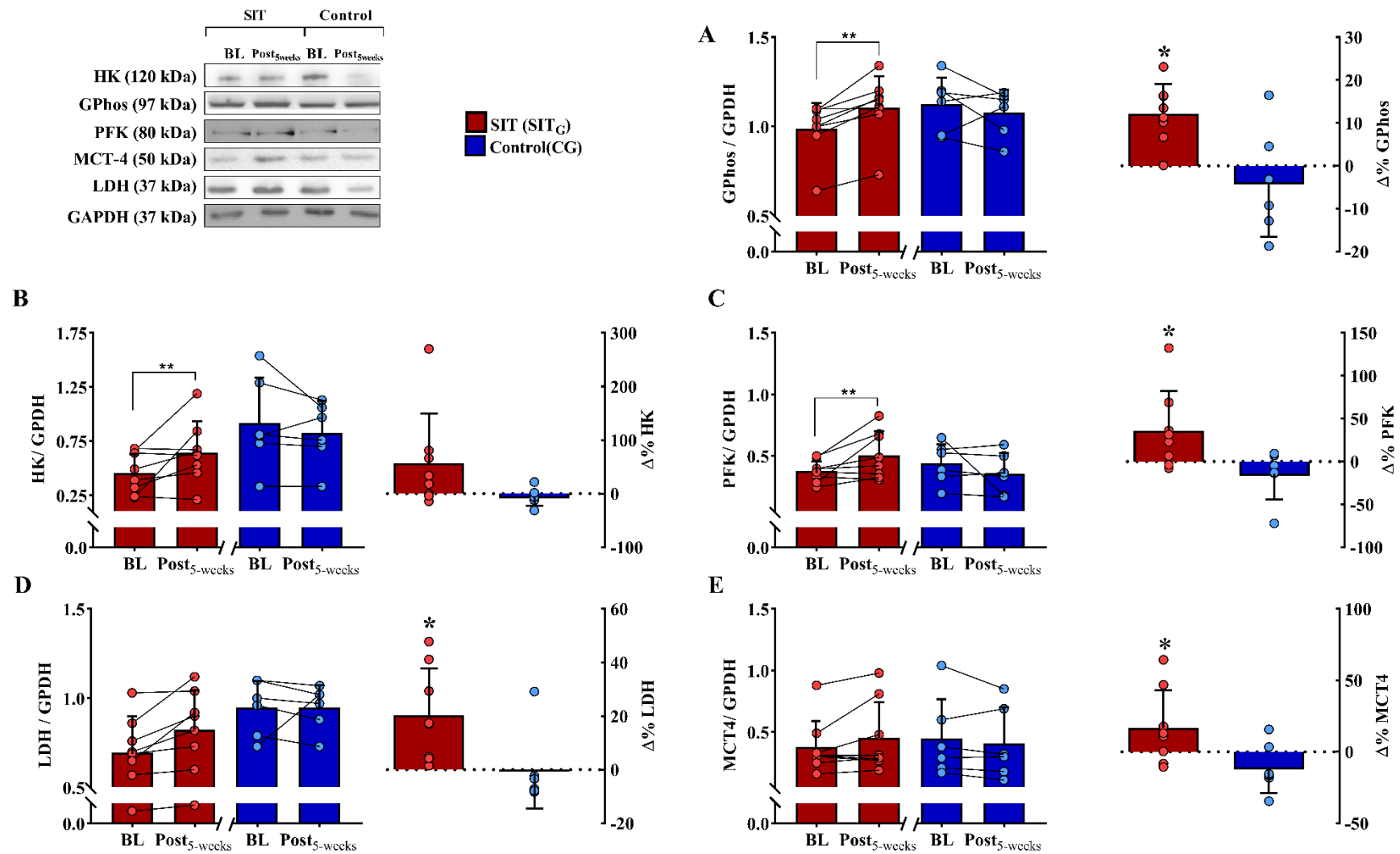


Figure 5. Muscle enzymatic content of GPhos (Panel A), HK (Panel B), PFK (Panel C), and LDH (Panel D) measured by Western Blotting technique and the percentual change between Post 5-weeks and BL for TG and CG. Panel E displays the muscle protein content of MCT-4 measured by

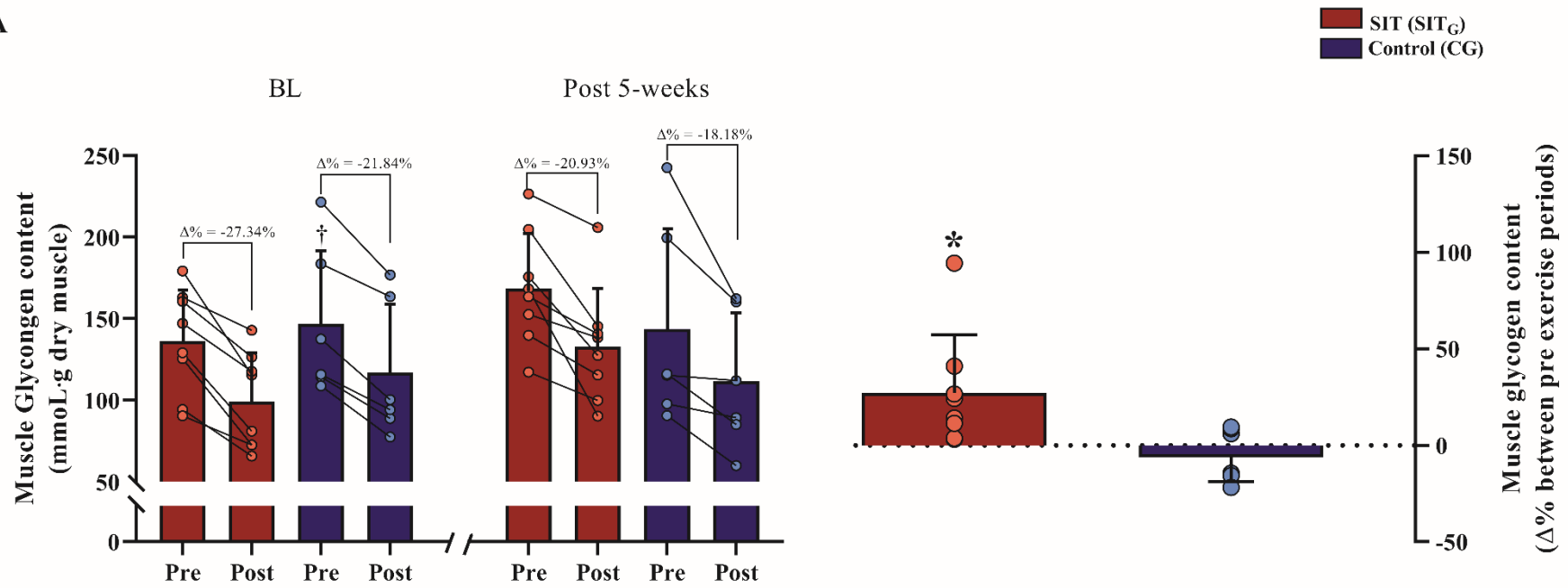
Western Blotting technic and the percentual change of difference between A1 and A2 for TG and CG. * Significant difference between groups ($p < 0.05$). ** Significant difference between Post 5-weeks and BL within the group ($p < 0.05$).

4.4.3 Muscle glycogen and buffering capacity

Muscle glycogen and buffering capacity *in vitro* are shown in Figures 6A and 6B, respectively. Muscle glycogen showed a significant period $\times$ group interaction ($p = 0.02$; $F = 6.77$; $\eta^2 = 0.36$; Power = 0.66), indicating that only SIT_G significantly increased the muscle glycogen content at rest when comparing BL and Post-5weeks (*post hoc* $p < 0.01$). In line with this, the comparison of $\Delta\%$ between BL and Post-5weeks showed a significant increase in SIT_G when compared with CG ($p = 0.03$). In addition, a time effect was verified ($p < 0.01$; $F = 69.40$; $\eta^2 = 0.85$; Power = 1.00), showing a decrease of muscle glycogen after exercise at BL and Post-5weeks periods ($p < 0.01$). However, no significative difference was observed in the percentage of glycogen depleted ($p = 0.56$; $F = 0.36$; $\eta^2 = 0.03$; Power = 0.09).

The buffering capacity *in vitro* also showed a significant period $\times$ group interaction ($p = 0.048$; $F = 4.86$; $\eta^2 = 0.29$; Power = 0.53); however, *post-hoc* analyses showed no significant difference ($p \geq 0.24$). Despite this, the $\Delta\%$ between BL and Post-5weeks showed a significant increase in buffering capacity in the SIT_G when compared with the CG at rest ($p = 0.02$). In addition, a significant time effect was observed ($p < 0.01$; $F = 38.02$; $\eta^2 = 0.76$; Power = 1.00), indicating a decrease in muscle capacity after exercise at BL and Post-5weeks periods ($p < 0.01$).

A



B

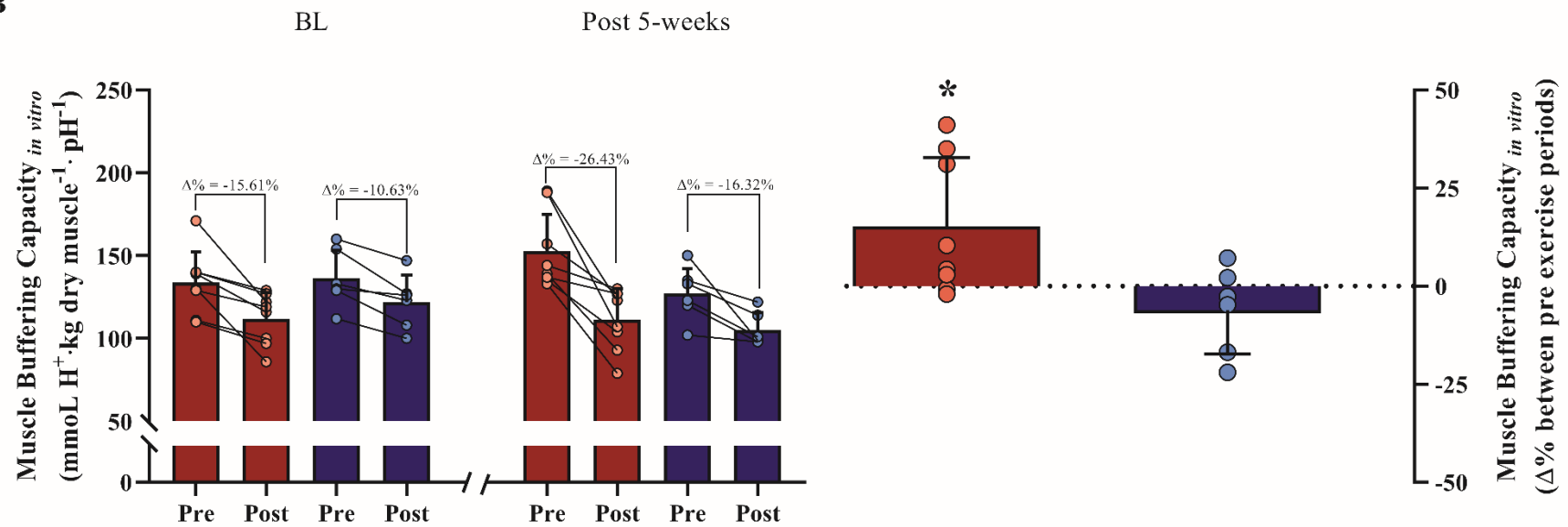


Figure 6. Muscle glycogen content (Panel A), and muscle buffer capacity (Panel B) measured at pre and post exercise at BL and Post 5-weeks, as well the percentage of buffer capacity pre-exercise in BL and Post 5-weeks (training effect). * TG > CG ($p < 0.05$); † Significant periods $\times$ group interaction, where Post 5-weeks > BL within the group ($p < 0.05$).

4.5 Correlations between $\Delta\%$ (tissue analysis vs time-to-task failure)

The correlations between $\Delta\%$ (percentual of change) of all biomolecular analyses (e.g., HK, PFK, and LDH content and activity; GPhos content; BL muscle glycogen; used muscle glycogen; and BL buffering capacity) and time-to-task failure are presented in Figure 6 for both groups.

For CG, a significative positive correlation with $\Delta\%$ for time-to-task failure was observed for $\Delta\%$ LDH content ($r = 0.87$; $p = 0.02$), $\Delta\%$ HK activity ($r = 0.87$; $p = 0.02$) and $\Delta\%$ PFK activity ($r = 0.97$; $p > 0.01$) (Figure 7). SIT_G only showed a significative negative correlation with $\Delta\%$ of time-to-task failure for $\Delta\%$ LDH content ($r = -0.74$; $p = 0.04$).

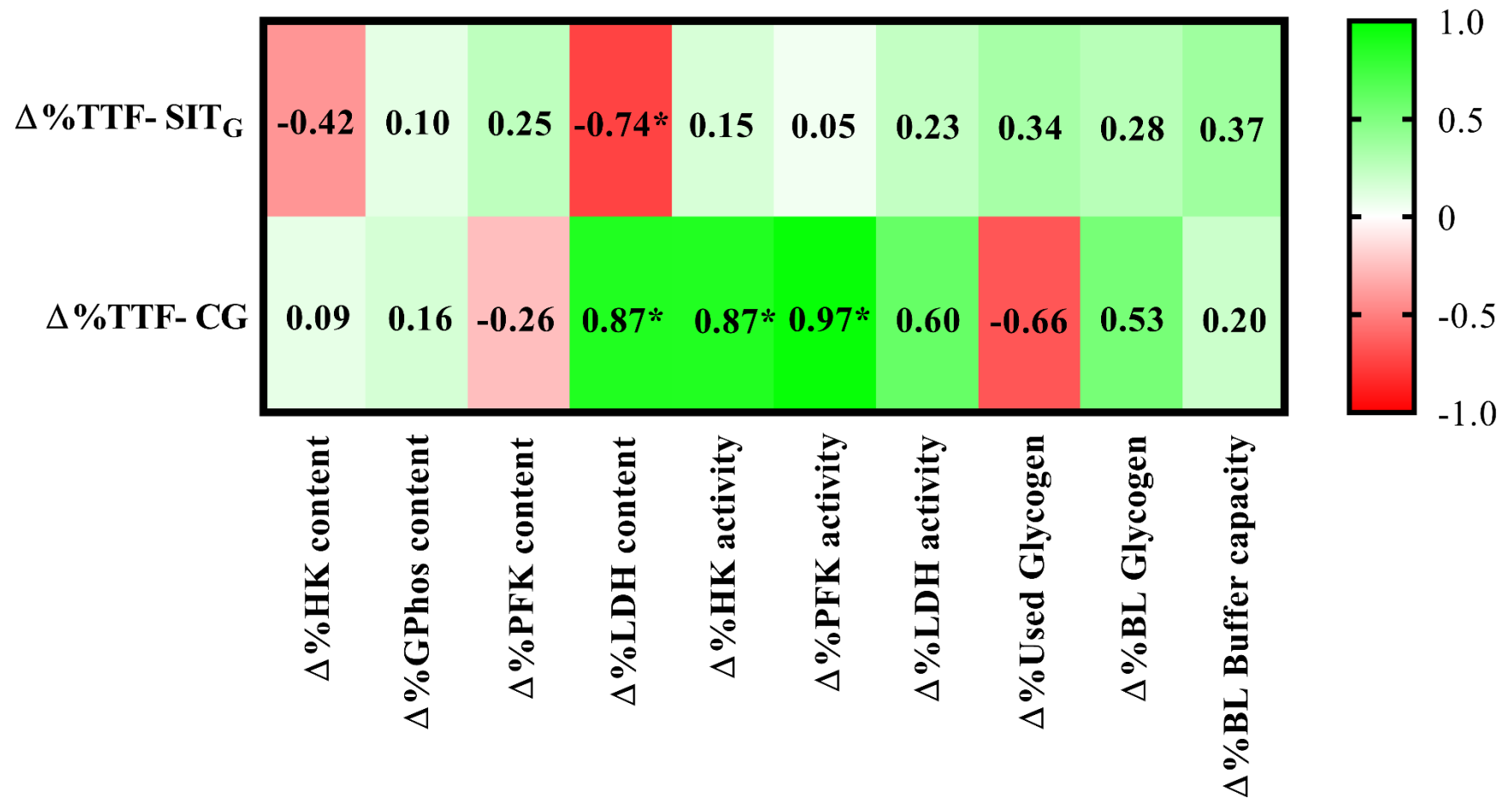


Figure 7. Heat map correlations between $\Delta\%$ (percentual alterations) of all biomolecular analysis (e.g., HK content and activity; PFK content and activity; LDH content and activity; GPhos content; BL muscle glycogen; used muscle glycogen; and BL buffering capacity) and time-to-task failure for both groups. * Significant correlation ($p < 0.05$).

5. Discussion

This study investigated the relationship between potential changes within the glycolytic pathway and exercise tolerance within the severe-intensity domain, following a 5-week SIT cycling exercise training program. The main findings were that: i) following the intervention, SIT_G increased both the content and activity of the glycolytic pathway enzymes, displayed a longer time to task failure in Post_{BL-RCP}, and showed a smaller $\tau \dot{V}O_2$; ii) the used muscle glycogen during exercise and the time-to-task failure within the severe-intensity domain, were not different between BL and Post-5weeks; and iii) the CG demonstrated a positive relationship between changes in $\Delta\%$ of LDH content, $\Delta\%$ HK activity and $\Delta\%$ PFK activity with $\Delta\%$ time-to-task failure (those who exhibited higher values had a greater exercise tolerance), whereas SIT_G displayed a negative correlation between $\Delta\%$ of LDH content and $\Delta\%$ time-to-task failure (those who showed the largest increase in %LDH had a lower exercise tolerance).

The present study demonstrated that a SIT program induces increases in glycolytic enzymes (GPhos, HK, PFK and LDH) content and activity, as well as in skeletal muscle buffering capacity when compared with CG. To the best of our knowledge, this is the first study that showed a significant increase in GPhos using a SIT model, similar to that originally proposed previously by Burgomaster et al. (2008). However, previous studies had demonstrated increases in glycolytic activity in connection to high-intensity exercise after high intensity training (BURGOMASTER; HEIGENHAUSER; GIBALA, 2006; HELLSTEN-WESTING et al., 1993; JACOBS et al., 1987; LINOSSIER et al., 1997; MACDOUGALL et al., 1998). For example, Linossier et al. (1997) found an increase in GPhos activity (faster glycogen breakdown) following 9 weeks of short-SIT sessions (i.e., 5 s sprints). These findings are in line with the results of the present study. In addition, the increases in HK, PFK and LDH and in buffering capacity observed in the present study are also in accordance with other researches reporting an increased content and activity of the same proteins following other models of SIT (i.e., different times of exercise, time of recovery and exercise volume) (HELLSTEN-WESTING et al., 1993; JACOBS et al., 1987; MACDOUGALL et al., 1998).

Such improved capacity within the glycolytic pathways can be explained by the ability of SIT to stimulate these pathways for ATP resynthesis, as evidenced by the high levels of muscle glycogen depletion found following 4 Wingate bouts (i.e., ~ -46% of muscle glycogen) (FAIRCHILD et al., 2003). This increased activity of non-oxidative glycolytic metabolism has the potential to activate the hypoxia-inducible factor-1 α (HIF-1 α) signaling pathway, which

plays a key role in the regulation of the glycolytic pathways (i.e., increasing the expression of genes related to glycolysis, glycogenesis and lactate transporters) (ABE et al., 2015). Furthermore, Miyamoto-Mikami *et al.* (2018) showed that 6-weeks of SIT can significantly increase the genes expression of serum/glucocorticoid regulated kinase 1 (SGK1), protein phosphatase 1, regulatory subunit 3C (PPP1R3C) and carnosine synthase 1 (CARNS1), which may also contribute in improving the functioning of the glycolytic pathways and to catalyze the formation of carnosine from l-histidine and β -alanine in the skeletal muscle (i.e., improving buffering capacity). However, it is important to emphasize that this explanation is speculative, since we did not measure any of the mentioned variables.

Further, it is well known that very high intensity and short exercise duration training protocols such as SIT can lead to an increased substrate storage within the skeletal muscles (i.e., muscle glycogen content) (BURGOMASTER et al., 2008; BURGOMASTER; HEIGENHAUSER; GIBALA, 2006; GIBALA et al., 2006), as well as to positive adaptations in oxidative metabolism (e.g., improvement in total cytochrome c oxidase activity and in their subunits II and IV (GIBALA et al., 2006); citrate synthase and 3-hydroxyacyl CoA dehydrogenase activity (BURGOMASTER et al., 2008)). In line with these previous findings, our results showed an increased muscle glycogen content at rest in Post 5-weeks ($\sim +27.32\%$), as well as an increase in $\dot{V}O_{2\max}$ and in the $\dot{V}O_2$ at the RCP for the SIT_G group, when compared to BL period.

Despite all the metabolic adaptations found in the present study, SIT failed to increase the time to task failure during severe-intensity exercises at a workload adjusted to post-training fitness status (i.e, 150% of RCP-PO). However, it is important to mention that while the time-to-task failure did not increase, there was a greater amount of work produced during the task (i.e., same time for a higher load). This is connected to the increased RCP-PO for SIT_G (+10.78%) following the SIT program, from which the severe-intensity CWR exercise was prescribed (i.e., 150% of RCP-PO). Additionally, it should be noted that the time to task failure was significantly increased when the evaluation was performed at the pre-training PO. These findings suggest that SIT is an effective training program that favors the enhancement of performance for exercises within the severe-intensity domain. However, the main question is yet to be answered: “Why does the time to task failure remain unchanged despite an improvement in the glycolytic pathways and buffering capacity during severe-intensity exercise?”.

The lack of improvement in time to failure during exercise performed at the same physiologically matched workload after SIT may also be explained by the concept of W' (work

available from non-oxidative energy sources) in relation to critical power (CP). For instance, the time to failure in this concept is estimated by the ratio between W' and the hyperbolic function minus CP (JONES et al., 2010). In our study, we demonstrated that muscle glycogen content improved after SIT (before exercise), indicating a potential improvement in non-oxidative energy stores (i.e., W'). Furthermore, absolute values of RCP also improved after SIT, thus enhancing CP. Therefore, considering the improvement in both W' (as exemplified by the increase in glycogen content) and CP (i.e., RCP), the time to task failure would remain unchanged after SIT. This result aligns with the findings of Jenkins and Quigly (JENKINS; QUIGLEY, 1993), who observed an improvement in ratio W' - time to task failure after 8 weeks of SIT, implying improved non-oxidative energy sources and the "maintenance" of time to task failure. However, this does not explain the physiological underpinnings underlying these results, following SIT. Muscle glycogen utilization was similar following the severe-intensity exercises performed at BL and at Post-5weeks, for both groups. Despite the increased resting glycogen content in the SIT_G, there was no significant difference in glycogen utilization within the muscle. This finding is consistent with the results of Harmer et al. (2000), that reported a non-significant difference in non-oxidative anaerobic ATP production rates during CWR at a workload adjusted to post-training fitness status (130% of $\dot{V}O_{2max}$) after SIT. Additionally, Nevill et al. (1989) also showed that the non-oxidative metabolic responses remained unchanged following a severe-intensity running exercise adjusted to post-training fitness status, after 8-weeks of SIT. However, they used an iso-time strategy (2-min exercise at 110% of $\dot{V}O_{2max}$), in which individuals finished the exercise before attaining their physiological limit of tolerance, thus possibly limiting the metabolic changes that the training program could have generated. Furthermore, Burgomaster et al. (2006) also demonstrated that the net depletion of muscle glycogen content was similar in matched-workload exercise.

Considering the lack of improvements in muscle glycogen utilization in the above cited studies, the enhancement in performance (i.e., improve in Post_{BL}-RCP) should be solely attributed to the oxidative metabolism (BURGOMASTER; HEIGENHAUSER; GIBALA, 2006; HARMER et al., 2000). Similarly, the most plausible explanation for the improvement in performance in the present study (total work during cycling within the severe-intensity domain), may be attributed to oxidative adaptations, that is a greater $\dot{V}O_{2max}$ and a faster $\dot{V}O_2$ kinetics following the SIT program.

Furthermore, the non-significant improvements in time-to-task failure seem to be dissociated from the changes in the glycolytic pathway profiles (including the buffering capacity). Our finding is in line with Hureau et al. (HUREAU et al., 2022), who indicated that

the accumulation of H^+ (due to the high activity of the glycolytic pathway during high-intensity exercise), was not associated with changes in fatigability profiles. Furthermore, the correlations observed in the present study indicated that the greater increases in LDH content had an inverse relationship with exercise tolerance during severe-intensity CWR exercise.

A potential explanation for the non-significant improvements in time-to-task failure during severe-intensity exercises and the inverse relationship between improvements in LDH content, may be related to a possible alteration arising from the sympathetic nervous system. It is already known that high-intensity exhaustive exercises stimulates the production of catecholamine (i.e., norepinephrine and epinephrine) that connects to β -adrenergic receptor on skeletal muscle and initiate the glycogenolysis process (HEARRIS et al., 2018). However, evidence has shown that the chronic and regular stimulus of catecholamine secretion by high-intensity exercise, could lead to an over-stimulation of sympathetic nervous system, with consequently reduction of catecholamine sensitivity (BRODDE; DAUL; O'HARA, 1984; TOHMEH; CRYER, 1980), which could lead to a decrease in glycogenolysis activity (SEILER; KJERLAND, 2006).

In connection to this, Harmer et al. (2000) measured the plasma level of norepinephrine and epinephrine before and after severe-intensity exercises in a group of individuals that underwent 7 weeks of SIT, showing a significantly greater concentration of these catecholamines post exercise after the SIT program, whereas no differences in muscle lactate concentration and in non-oxidative anaerobic ATP production rate at CWR severe-intensity exercise were observed following the training program. Taken together, these results may support the hypothesis of a decrease in glycogenolysis caused by a reduction in catecholamine sensitivity (while a higher LDH content could be stimulated). However, it is worth noting that the concentrations in catecholamines and the sensitivity of beta receptors within the skeletal muscle were not measured in this study, which prevents us from corroborating this hypothesis. Thus, further studies are needed to elucidate the underpinnings of the unchanged time-to-task failure following a SIT program.

In conclusion, 5 weeks of SIT increased glycolytic pathways activity, muscle buffering capacity, $\dot{V}O_{2max}$, cycling performance (Post_{TBL-RCP}) and sped the $\dot{V}O_2$ kinetics response. However, there was no improvement in time to task failure (exercise tolerance) during severe-intensity exercise with intensity adjusted to post-training status. In addition, this study suggests that the lack of improvement in time to task failure seem to be dissociated from changes in glycolytic pathway and buffering capacity, as indicated by the inverse relationship between improvements in LDH and time to task failure in SIT_G.

CHAPTER 3

Neuromuscular Function and Exercise Tolerance responses to Sprint Interval Training

Manuscript 2. Five weeks of Sprint Interval Training improves training workload but not neuromuscular function and fatigability performances

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1. Abstract

Purpose: The study aimed to investigate whether 5-weeks of sprint interval training (SIT) improve the neuromuscular function [NMF; isometric maximal voluntary contraction (iMVC) + peripheral nerve stimulations (PNS) with evoked force and electromyography signal assessment (EMG)] and the time to task failure (TTF) in cycling at constant work rate (CWR) above of critical-intensity domain. *Methods:* Twenty-one males were randomly allocated into SIT group (SIT_G; n=11) that underwent 5-weeks of SIT (i.e., 4-6 30-s Wingate bouts) and control group (CON; n=10) that remained out of any regular training programs., in a pre-post training, parallel study. Pre- and post-training (i.e., 5-weeks), the participants underwent a cycling ramp test and, a CWR above of critical-intensity domain [i.e., at 150% of respiratory compensation point power output (RCP-PO) cycling until task failure (TTF), with the NMF assessed pre- and immediately post-effort. The assessments were repeated in the Post-training moment. *Results:* No differences on the TTF matched to current 150% of RCP-PO (-3.46%; $p=0.79$) were verified. Still, the SIT_G showed a significative improvement over time in Wingate trials outcomes [peak power (+12.22%; $p < 0.01$) and mean power (+6.65%; $p < 0.01$)] Notably, the SIT did not induce alterations in NMF measured by iMVC ($p \geq 0.11$) as well as fatigability ($p \geq 0.07$) and EMG signal during CWR ($p > 0.20$). *Conclusion:* These findings indicate that 5-weeks of SIT can lead to dynamic neuromuscular adaptation, however, this neuromuscular adaptation was not transferred to isometric neuromuscular responses assessments, as well as to TTF.

Key-Words: High intensity exercise; Exercise tolerance; Twitch Interpolation; Fatiguability.

2. Introduction

During cycling efforts, the neuromuscular system assumes a pivotal role in eliciting stimuli for muscular contractions and sustaining repetitive movements of the lower limbs (FARIA; PARKER; FARIA, 2005a; TAKAISHI et al., 1996). Consequently, in the pursuit of enhanced the neuromuscular system responses (i.e., muscular neural activity), cyclists turn to training regimens that consistently impose heightened demands on the neuromuscular apparatus, a practice which may culminate in substantial improvements in neuromuscular function (NMF) (FARIA; PARKER; FARIA, 2005b), mainly assuming that gains are stimuli-dependent (RICH; CAFARELLI, 2000).

In recent decades, the sprint interval training (SIT) composed by short-term repeated all-out efforts (i.e., often comprising 4-6 x 30-s Wingate efforts), beyond demonstrated efficient in improves the oxidative mitochondrial and respiratory capacities, (BURGOMASTER et al., 2008; GIBALA et al., 2006; MACINNIS; GIBALA, 2017), has been also suggested to induce beneficial gains in neuromuscular parameters due to high-peripheral demand (BUCHHEIT; LAURSEN, 2013a, 2013b). This can be evidenced by improvements observed after 4 weeks of SIT in muscle activation during 30-second Wingate efforts, as estimated by root mean square (RMS), and in power output (PO) achieved during this effort (i.e., peak and mean power) (CREER et al., 2004).

In addition, the all-out efforts and training (i.e., SIT) lead to significant perturbation in the homeostasis of the neuromuscular system reducing the NMF (i.e., fatigability) mainly in peripheral situ (FERNANDEZ-DEL-OLMO et al., 2013; FIORENZA et al., 2019). Therefore, it is expected that SIT could improve the NMF and mitigate the neuromuscular fatigability (post-exercise) after a constant workload rate (CWR) exercise (BERTSCHINGER; GIBOIN; GRUBER, 2020; LEWIS et al., 2017). However, only two studies had investigated the effects of SIT on NMF and in exercise NMF fatigability after CWR performed at low/moderate intensity (i.e., NMF and fatigability measured by twitch interpolation technique), reporting no improvements in any NMF parameters or in NMF fatigability after 2 weeks of SIT (BERTSCHINGER; GIBOIN; GRUBER, 2020; LEWIS et al., 2017). These results can be attributed to the selection of a CWR at low/moderate intensity for the investigation of NMF and also due to short-training time (i.e., 2-weeks). Despite moderate intensities requiring substantial neuromuscular contribution, both neuromuscular activation during exercise and neuromuscular fatigability patterns are significantly different from those that SIT could induce (BLACK et al., 2017; COLLINS et al., 2018; IANNETTA et al., 2022).

In this way, when we consider exercise intensity above of the critical intensity, a different scenery seems to emerge. For instance, although the causes of task failure during exercises above critical intensity are still under debate (AMANN; SECHER, 2010; FITTS, 2016; MARCORA, 2010; WESTERBLAD, 2016; ZAGATTO et al., 2022), it seems that the exceeding of ‘critical’ concentrations of some metabolites provided by the high utilization of non-oxidative metabolism (e.g., inorganic phosphate, hydrogen ions) can primarily reduce muscle contractile capacity at the peripheral level (i.e., peripheral fatigue) (BLACK et al., 2017; DE POLI et al., 2020; HUREAU et al., 2022; THOMAS et al., 2016), without interfering with the central drive burst input for muscle activation (i.e., central fatigue) (HUREAU et al., 2022), despite the potential influence of these *milieu* perturbations in motoneurons III and IV afferences (AMANN et al., 2011; HUREAU et al., 2022). This ultimately establishes an impairment of muscle contraction performance (i.e., fatigability) similar to exercise performed in SIT sessions (FERNANDEZ-DEL-OLMO et al., 2013; FIORENZA et al., 2019). In this way, it is possible to hypothesize that SIT lead to remarkable adaptations on NMF fatigability, as well in muscle activity during exercise, contributing to enhance exercise tolerance (i.e., time to task failure; TTF) during CWR at severe domain. In addition, these gains could be observed with improvement of the 30-s Wingate performances over the training weeks.

Therefore, the aim of our study was to investigate whether a 5-week SIT regimen can improve the NMF responses during exercise and mitigate fatigability, enhancing the TTF during CWR exercise above critical intensity. Additionally, we also investigated the impact of SIT on the dynamic neuromuscular performance, assumed as 30-s Wingate performance outcomes (peak power and mean power) over time.

3. Methods

3.1 Participants

A minimum sample size of 10 participants for each experimental group was calculated, for statistical power of 95% and an alpha value of 0.05 according Cohen guidelines for sample size calculation (COHEN, 2013). The sample size was calculated based on significantly attenuation of peripheral fatigue (i.e., force level at high frequency peripheral nervus electrical stimulation after a 10km time trial exercise; ~20 min of moderate intensity exercise) after 4-weeks of SIT (Cohen’s d effect size: 1.33) (LEWIS et al., 2017). The Cohen’s d was estimated by mean differences at G*Power 3.1 software (Kiel, Germany) (FAUL et al., 2009)].

As an inclusion criterion, the participants were males, healthy, not currently engaged in a periodized training routine (i.e., exercising at least 3 times per week), and should have no recent history of muscle or neuromuscular injuries. To minimize data heterogeneity, females were not included, as previous research had demonstrated sex differences in neuromuscular responses (STERN et al., 2012). Assuming a pre- and post-training study design, with parallel experimental and control groups, a total of 21 volunteers participated in study (mean $\pm$ SD; age: 30 ± 4 years; height: 176 ± 5 ; body mass: 76.4 ± 11.0 kg). Flow diagram of the study is shown in Figure 1.

During the study period, participants received specific instructions, including the abstention from use of performance ergogenic aid substances, and from engaging in any other training/exercise routines. Furthermore, participants were required to perform at least 95% of the scheduled training sessions to remain eligible for the study (exclusion criteria). All participants were informed about possible risks and benefits of the study, and they signed an informed consent document. All procedures were approved by local Research Committee (Protocol #2.650.216/2018), which was conducted according to the Declaration of Helsinki.

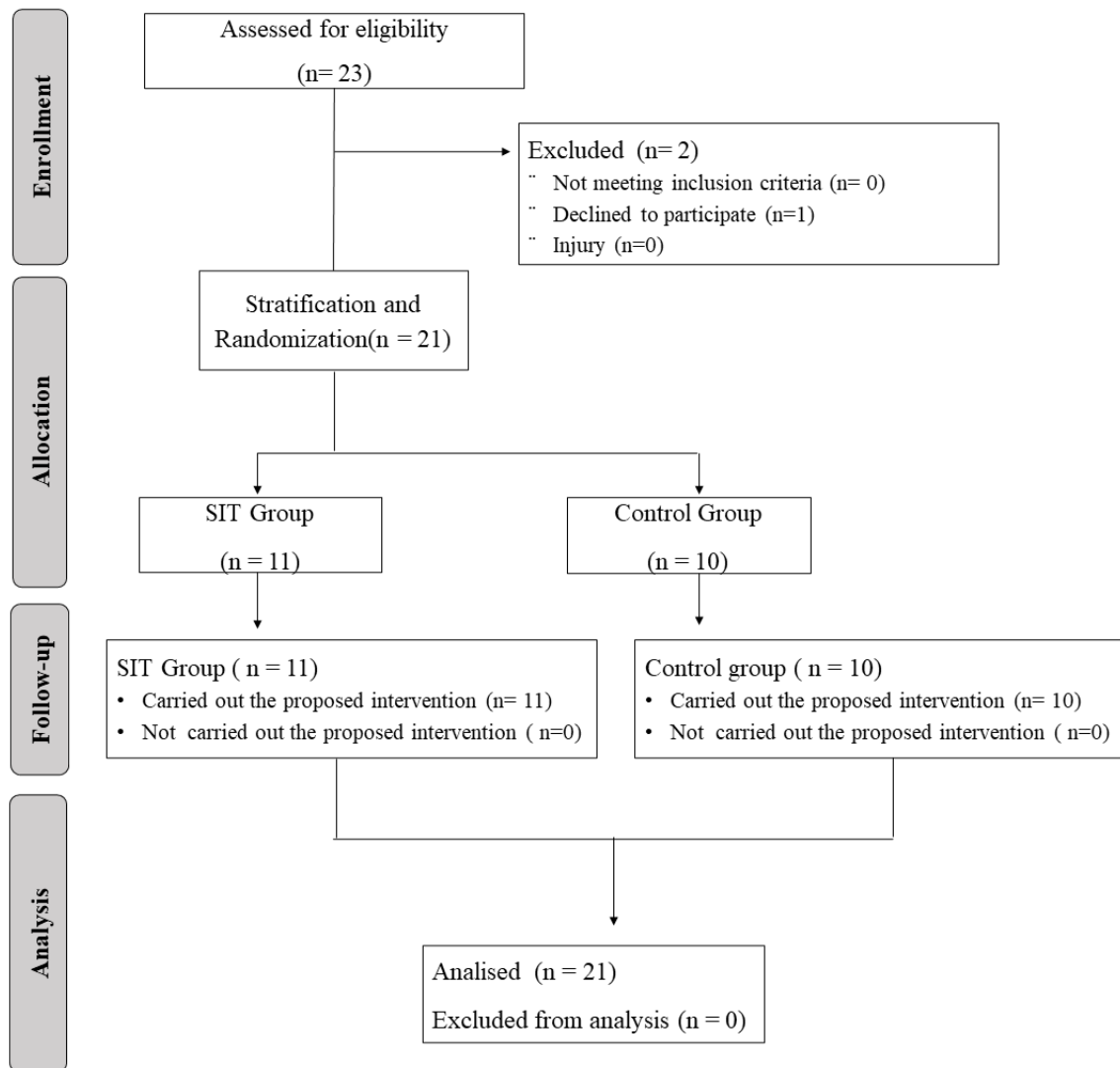


Figure 1. Flow diagram.

3.2 Experimental Design

The study was conducted using a parallel pre- and post-training design model, with cycling and neuromuscular performances measured before and after 5 weeks. The participants were allocated in two groups, a SIT group (SIT_G), and a control group (CON; non-engaged in training). During the baseline (i.e., pre-training) assessments, participants visited the laboratory three times in the same week: i) the 1st visit involved a familiarization with CRW exercise and with NMF assessments to ensure the maximal execution during subsequent evaluations; ii) The 2nd visit consisted of a ramp incremental test realized until the task failure to determine the

maximal oxygen consumption ($\dot{V}O_{2\max}$), peak power output (PPO), respiratory compensation point (RCP) and the RCP power output (RCP-PO); iii) the 3rd visit involved a CWR exercise at 150% of RCP-PO performed until failure to record the TTF, with the surface electromyography (EMG) analysis during exercise NMF assessment by peripheral nerve stimulation (PNS) performed before and immediately to the exercise failure (delay of 25 ± 7 s).

Following the pre-training measurements, participants were matched for $\dot{V}O_{2\max}$, RCP-PO, and PPO, and then randomly assigned to either the SIT_G (n = 11) or the CON (n = 10). The SIT_G underwent 5 weeks of SIT composed of 4-6 x 30-s Wingate efforts interspaced by 4-min (GIBALA et al., 2006), while the CON did not perform any exercise training program. After 5-weeks of SIT (post-training), the same procedures applied at pre training measurements were repeated. A schematic of the experimental design is shown in Figure 2.

All exercise procedures were performed in an electromagnetic braked cycle ergometer (Excalibur Sport, Lode, Holland) in self-selected cadence between 80 and 100 rpm (± 5 rpm of the chosen cadence during all efforts), while the NMF was assessed by an isometric maximal voluntary contraction (iMVC) torque of the knee extension. In addition, all testing and training sessions were separated by a minimum interval of 48h and a maximum of 72h, and were realized in same period of the day (± 2 h) to avoid any circadian influences. The participants were verbally encouraged to obtain the maximum performance in all tests and training sessions.

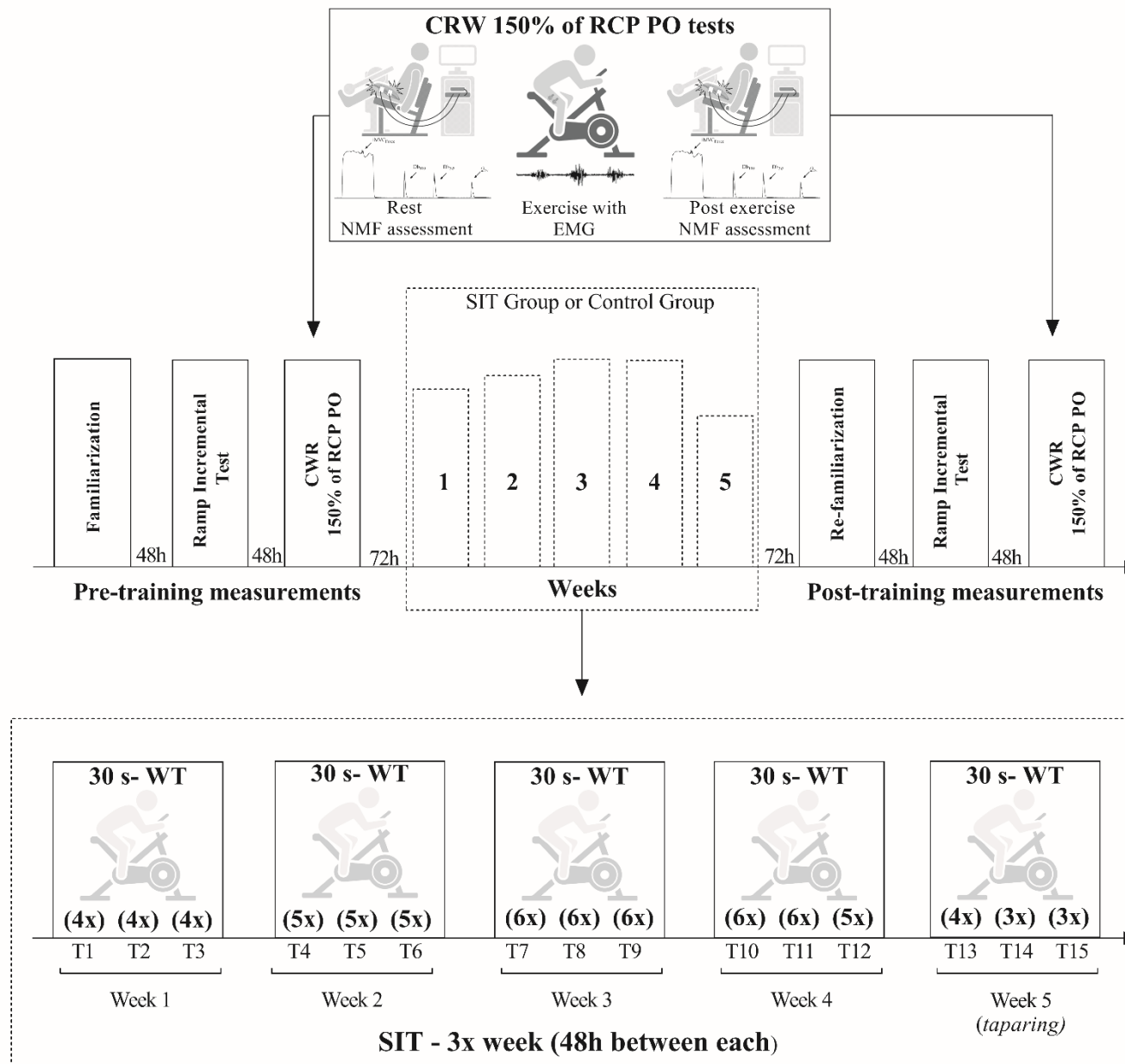


Figure 2. Study Experimental Design and training parameters. CWR = constant work rate cycling; RCP PO = respiratory compensation point power output; Wkd = weekend; T1–T15 = number of training sessions

3.3 Experimental procedures and analysis

3.3.1 Ramp incremental test

The test was conducted using a ramp protocol, performed until task failure to measure $\dot{V}O_{2\max}$, PPO and RCP oxygen consumption ($\dot{V}O_2$) (IANNETTA et al., 2020). The test began with a 5-minute warm-up at 75W followed by an workload increase of 1W every 2 seconds until the predetermined cadence could no longer be maintained (GIBALA et al., 2006). During the ramp test, cardiorespiratory responses were measured breath-by-breath using a previously calibrated stationary gas analyzer (Quark CPET; COSMED, Italy). All cardiorespiratory raw data were cleaned to remove the data points lying ± 3 SD from the local mean (LAMARRA et al., 1987). Then data were interpolated to 1 second intervals using OriginPro 8.0 Software (OriginLab, USA) (IANNETTA; MURIAS; KEIR, 2019).

The end-tidal partial pressure of CO₂ (PCO₂), ventilation ($\dot{V}_E$), and ventilation/carbon dioxide production curve ($\dot{V}_E/\dot{V}CO_2$) values were plotted against $\dot{V}O_2$ data for RCP determination (IANNETTA et al., 2020). The RCP was estimated through the consensus of two experienced researchers and defined as the point where end-tidal PCO₂ started to decrease, at aligning with the second and first curve breakpoints of $\dot{V}_E$ and $\dot{V}_E/\dot{V}CO_2$, respectively (IANNETTA et al., 2020). Subsequently of $\dot{V}O_2$ at RCP be identify, the RCP-PO was determined based on $\dot{V}O_2$ and PO (x-axis) relationship (IANNETTA; MURIAS; KEIR, 2019). Finally, the $\dot{V}O_{2\max}$ was assumed as the highest $\dot{V}O_2$ value from a 20-s rolling average, while the PPO was assumed as the highest intensity reached during the ramp incremental test (IANNETTA; MURIAS; KEIR, 2019).

3.3.2 CWR at severe-intensity domain

The CWR was performed at 150% of RCP-PO [i.e., corresponding to approximately 115% of PPO; (ZAGATTO et al., 2017, 2023a)]. The workload was applied after the participants achieve their self-selected cadence, then the TTF was recorded. Task failure was determined by a volitional exhaustion or by the inability to maintain a pedal cadence of -5 rpm from their self-selected cadence for more than 5 seconds. The EMG signal was recorded during the whole effort.

3.3.3 Surface Electromyography during the exercise

The EMG signal from muscles *vastus lateralis* (VL), *rectus femoris* (RF), *biceps femoris* (BF) and *gluteus maximus* (GM) of propulsive leg were recorded using a wireless device (Wave Plus wireless Cometa System, Italy). Before the electrodes placement, the skin surface was gently shaved and sanding to remove dead skin cells and cleaned and degreasing by isopropyl alcohol to reduce the impedance ($<10\text{ k}\Omega$) (HERMENS et al., 2000). After that, silver / silver chloride (Ag/AgCl) electrodes (recording area, 78.5 mm^2 ; Medi-trace, Dublin, Ireland) were placed lengthwise in the belly of each muscle according surface EMG for a non-invasive assessment of muscles (SENIAM) recommendations (HERMENS et al., 2000).

EMG signals data acquisition was performed at 2,000 Hz associated with PowerLab signal acquisition hardware (Adinstruments, New Zealand) and subsequently filtered with a band pass filter of 20–500 Hz and notch (60 Hz) (DUTRA et al., 2023). The evaluation of muscular activity was estimated by RMS data, which was calculated and normalized by the first 5 seconds of exercise (RMS%), and then expressed in 4 quarter time window according to TTF (i.e., 0-25%, 25-50%, 50-75% and 75-100%) (DE POLI et al., 2020; DUTRA et al., 2022). In addition, the ratio between raw RMS (for each time window) and CRW PO (PO/RMS) was calculated to estimate the neuromuscular efficiency during the exercise pre- and post-training.

3.3.4 Maximal voluntary contractions

3.3.4.1 Force data acquisition

Two 5 seconds iMVC of the knee extensors of dominant leg were performed, being one performed before and one as soon as possible after the CWR ($25\pm 7\text{ s}$). The iMVCs were performed with knee and hip flexion at 90° and with trunk and thighs fixed by a belt (DE POLI et al., 2020; MILIONI et al., 2019). An inextensible bar was attached above the region of the medial malleolus (e.g., $\sim 1\text{--}2\text{ cm}$ above; ankle region) using heavy-duty straps, connected to a load cell (strain gauge sensibility: $2.0 \pm 0.02\text{ mV}\cdot\text{V}$; maximum capacity: 100 kgf; 0.03 % rated output accuracy; 0.98 N resolution; MKControle, Brazil) (DUTRA et al., 2023). The force data was acquired by a digital-to-analog converter PowerLab (Adinstruments, New Zealand), with a sampling frequency of 2,000 Hz. The force data was filtered by a fourth-order Butterworth filter in LabChart 11 (Adinstruments, New Zealand). The peak force peak measured during iMVC ($\text{iMVC}_{\text{Force}}$) was assumed as the higher value of plateau of force around mean of 100 ms and assumed as global indicator (BEHM; ST-PIERRE; PEREZ, 1996).

3.3.4.2 *Peripheral nerve stimulation*

During (i.e., after 3-s of the iMVC) and after the iMVCs, peripheral nerve electrical stimuli were delivered on femoral nerve (cathode: femoral triangle; anode: femoral greater trochanter) by a high voltage electrical stimulator Biostimulator V3 (400V peak to peak; Insight, Brazil). The electrical stimuli was assumed at 120% of stimuli in which the force plateau was reached (NEYROUD et al., 2014), which corresponded to 263 ± 34 mA. The force plateau verification was determined individually before each NMF assessment by application of consecutive incremental single stimulus.

Doublet high-frequency stimulus (i.e., square wave, 100Hz, pulse duration of 1 ms) was delivered during iMVC (Db_{100} -superimposed). After iMVC, in relaxed muscle another doublet high-frequency stimulus was delivered, following by doublet low-frequency stimulus (10 Hz) and single (Q_{tw}), respectively each 5 seconds after the iMVC ends. The force amplitude for each electrical stimulus (onset torque) were measured for determination of force level at doublets high-frequency stimulus (Db_{100}) and low-frequency stimulus (Db_{10}) (peripheral parameters) and voluntary activation level (VA; central parameter) by equation 1 (NEYROUD et al., 2014). In addition, low-frequency muscle function (Db_{10} / Db_{100}) was calculated as ratio between low and high frequency stimuli.

$$VA = 1 - (Db_{100}\text{-superimposed} \times (\frac{\text{Force level at stimulus moment}}{iMVC\text{Force}})) / Db_{100} \times 100 \quad [1]$$

The pre-exercise data at both moments were used to compare possible effects of training on NMF. The comparison between pre and post-exercise data was used to estimate possible effects of SIT on fatigability patterns (i.e., peripheral and central parameters).

3.3.4.3 *Surface Electromyography during the iMVCs*

During all iMVCs, the surface EMG signal was monitored in VL distal part (MIURA; WATANABE, 2016). Before the pre-exercise iMVC, the placement of electrode was performed as described in *Surface Electromyography during the exercise* section. The EMG signal was

acquired by BioAmp (ADInstruments, New Zealand) and was converted from analog to digital at a sampling rate of 2,000 Hz by PowerLab system (ADInstruments, New Zealand) band-pass filter (20-500 Hz). The EMG data were analyzed in LabChart 11 software (ADInstruments, New Zealand) offline, being filtered with a band pass filter of 20–500 Hz and notch (60 Hz). Subsequently the RMS from 500-ms iMVC_{Force} (RMS_{iMVC}) was measured. In addition, without the band pass filter the maximal amplitude and duration of M-Wave evoked by the single stimuli was measured. In addition, the maximal amplitude of M-Wave was also used to normalize RMS values (RMS_{iMVC}/M-Wave) (MILLET et al., 2011).

All parameters measured in iMVC with peripheral neuromuscular stimulation (including force and EMG responses) presented have a good reliability and repeatability as recently demonstrated by our study group (DUTRA et al., 2023).

3.3.5 Training

3.3.5.1 Sprint interval training

The SIT comprised 4-6 repeated bouts of the 30 seconds Wingate efforts (workload:0.70 Nm·kg⁻¹; rest:4 min between each bout) (GIBALA et al., 2006). Training sessions were performed 3 times per week, for 5 weeks, completing a total of 15 SIT sessions, always respecting a 48h of interval between sessions (e.g., Monday-Wednesday-Friday; Tuesday-Thursday-Saturday). The first week of training was composed by 4×30 seconds Wingate bouts, then successively session bouts were increased in one bout each training week until the third week (i.e., week 1: 4 bouts; week 2: 5 bouts; week 3: 6 bouts) (GIBALA et al., 2006). In the week 4, during the first two training sessions the participants underwent 6 Wingate bouts, followed of a reducing in the number of Wingate bouts in the last 4 training sessions around 16% to 50% in the total training volume (BOSQUET et al., 2007) to induce a tapering response. Thus, these subsequently sessions were composed by 5, 4, 3, and 3 bouts of 30-seconds Wingate all-out efforts, respectively (further details in the Figure 1).

During all training sessions, the cadence, velocity, and power output were recorded using the Wingate test software (Lode BV, Netherlands) in a data acquisition frequency of 5 Hz. The highest power data value achieved during each bout was recorded as peak power (PP), while the average power reached during each sprint was recorded as the mean power (MP). To assess the dynamic neuromuscular performance during the training sessions, the mean of PP

and MP of the first 3 30-second Wingate bouts in each training session were calculated. Subsequently, a weekly average was determined (e.g., the mean of the first three 30-second Wingate bouts on training days 1, 2, and 3 = the mean of week 1).

In addition, in a descriptive manner, the energy demand of the training sessions was calculated [see details (DI PRAMPERO; FERRETTI, 1999; MARGARIA; EDWARDS; DILL, 1933; MIYAGI et al., 2017)]. Energetic contribution corresponded to 82.0% oxidative metabolism and 18.0% of non-oxidative metabolisms (i.e., glycolytic and phosphagen energetic pathways; 86% and 14% respectively), when assumed the whole session including recovery interval.

3.4 Statistical Analyses

The data are presented as mean $\pm$ standard deviation (SD) and 95% confidence intervals (CI 95%). Shapiro-Wilk and Levene tests were used to assess the normality distribution and homoscedasticity of the data. A general linear model (ANOVA) was conducted to examine potential differences in NMF parameters [e.g., exercise time (pre $\times$ post) $\times$ procedure evaluation time (Pre-training $\times$ Post-training) $\times$ group (SIT_G $\times$ CON)], TTF [procedure evaluation time (Pre-training $\times$ Post-training) $\times$ group (SIT_G $\times$ CON)]. In addition, one way repeated-measure ANOVA was used to compare training parameters (PP and MP) of SIT_G. Mauchly's sphericity test was applied, and sphericity was considered violated if the F-test was significant. In cases where sphericity was violated, the Greenhouse-Geisser epsilon correction was applied. Post hoc tests were performed using the "Bonferroni" method. Additionally, when necessary, an independent samples t-test was used to compare deltas ($\Delta\%$; percentage of change between BL and Post_{5-weeks}). A Pearson product-moment correlation coefficient (r) was used to determine the level of associations between percentual of change of neuromuscular function results and time-to-task failure. The significance level for all analyses was set at 5% ($p < 0.05$).

4. Results

4.1 Ramp incremental test

The cardiorespiratory values and performance parameters measured in the ramp test are shown in the Table 1. Only SIT_G showed a significant training time $\times$ group interaction (p

≤ 0.02 ; $F \geq 6.10$), improving PPO (*post hoc* $p < 0.01$), $\dot{V}O_{2\max}$ (*post hoc* $p < 0.01$), RCP-PO (*post hoc* $p < 0.01$) and $\dot{V}O_2$ at RCP (*post hoc* $p < 0.01$). Corroborating with that, the percentage of change ($\Delta\%$) had also showed a significative improvement for these parameters only for SIT_G ($p < 0.01$).

Table 1. Parameters of the ramp incremental test measured at pre and post intervention in SIT group and control group.

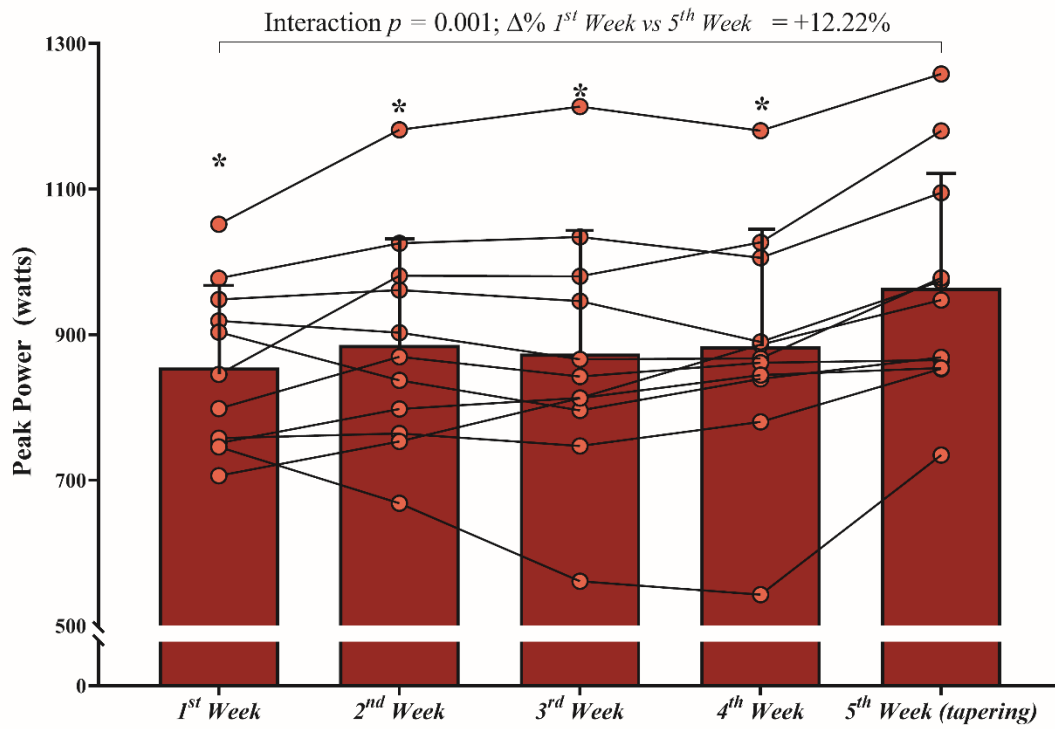
	SIT Group (SIT _G)			Control Group (CON)			ANOVA results		
	Pre-training	Post-training	Δ%	Pre-training	Post-training	Δ%	Interaction <i>p</i> (F)	Time <i>p</i> (F)	Group <i>p</i> (F)
PPO (watts)	267 ± 37 (242 to 292)	290 ± 34 (267 to 314)* [†]	+9.38 [‡]	267 ± 43 (237 to 298)	269 ± 46 (237 to 302)	+0.60	<0.01 (15.30)	<0.01 (21.43)	0.56 (0.36)
VO _{2max} (L)	2.83 ± 0.39 (2.57 to 3.10)	3.03 ± 0.37 (2.78 to 3.27)* [†]	+7.15 [‡]	2.76 ± 0.44 (2.45 to 3.07)	2.78 ± 0.42 (2.48 to 3.09)	+0.99	0.01 (7.25)	<0.01 (11.46)	0.38 (0.82)
RCP- PO (watts)	210 ± 29 (190 to 229)	232 ± 26 (214 to 250)* [†]	+8.79 [‡]	210 ± 27 (190 to 230)	209 ± 32 (186 to 231)	-1.01	<0.01 (62.22)	<0.01 (51.94)	0.37 (0.85)
VO ₂ at RCP (L)	2.35 ± 0.35 (2.12 to 2.59)	2.55 ± 0.35 (2.32 to 2.78)*	+4.30 [‡]	2.33 ± 0.35 (2.08 to 2.58)	2.31 ± 0.39 (2.02 to 2.59)	+0.05	0.02 (6.10)	0.07 (3.60)	0.39 (0.79)

Values are expressed as the mean ±SD (CI95%). Δ% = percentage of change between BL and Post 5-weeks for the same group; PPO = Peak power output; VO_{2max}= maximum oxygen consumption; RCP = Respiratory compensation point; RCP-PO = Power output at respiratory compensation point. *Significant difference for period × group interaction (Pre-training < Post-training in the group). [†] Main time effect (*p* < 0.05; Pre-training < Post-training). [‡] Significant independent t-test difference (SIT_G > CON).

4.2. Dynamic Neuromuscular performance during SIT

The mean of PP and MP for the first 3 sprints of the weekly sessions (i.e., considering only the first 3 bouts of the 30-Wingate effort) was showed in Figure 3A and 3B, respectively. The PP, had improved ($p = 0.009$; $F = 6.61$), where only the tapering week (5th week; last training sessions) presented greater PP values than 1st (*post hoc* $p = 0.04$), 2nd (*post hoc* $p = 0.01$), 3rd (*post hoc* $p > 0.01$) and 4th week (*post hoc* $p < 0.01$). Similarly, the MP showed also a significant improvement ($p < 0.01$; $F = 6.61$), where only the last week presented greater MP values than 1st (*post hoc* $p = 0.01$), 2nd (*post hoc* $p = 0.04$), 3rd (*post hoc* $p = 0.01$) and 4th weeks (*post hoc* $p = 0.04$).

A



B

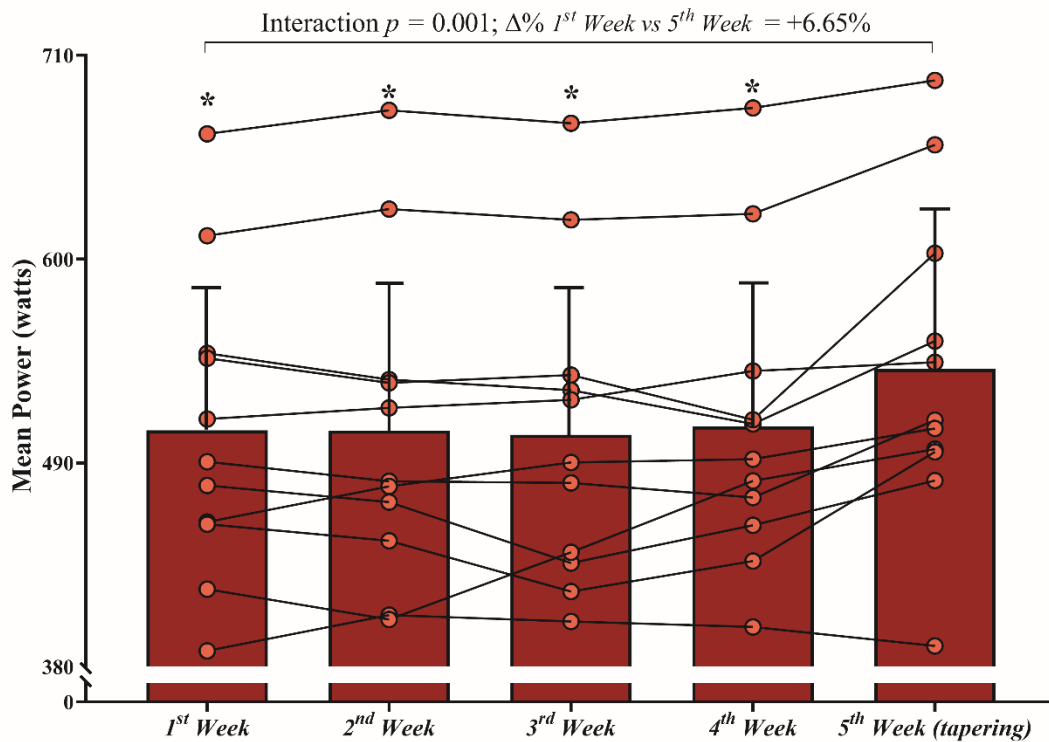


Figure 3. A. Mean of peak power measured during first 3 sprints of 1st to 5th weeks trainings sessions. B. Mean of mean power measured during first 3 sprints of 1st to 5th weeks trainings sessions. * Significant interaction ($p < 0.05$; < 5th).

4.3 Time to task failure recorded in CWR

The intensity of 150% of RCP-PO in pre-training was 319 ± 48 W (CI95%: 287 to 350 W) for SIT_G and 315 ± 41 W (CI95%: 285 to 344 W) for CON. While, 150% of RCP-PO in and Post 5 weeks was 348 ± 39 W (CI95%: 322 to 374 W) for SIT_G and 313 ± 47 W (CI95%: 280 to 347W) for CON. The workload at CRW present a significant training time $\times$ group interaction ($p < 0.01$; $F = 33.63$), where only SIT_G present showed higher values of CWR workload in post-training than pre-training (*post hoc* $p < 0.01$). In addition, a significative time effect was verified ($p < 0.01$, $F = 27.80$), showing that pos-training values were higher than pre-training (*post hoc* $p < 0.01$). However, no group effect was verified ($p = 0.32$; $F = 1.02$)

For TTF, no significant training time $\times$ group interaction ($p = 0.23$; $F = 1.51$), time ($p = 0.13$; $F = 0.13$) or group effect ($p = 0.85$; $F = 0.04$) were verified (Figure 4). In addition, corroborating with that, when it considering only the $\Delta\%$ between pre-training and post-training CRW TTF, no difference was observed ($p = 0.16$).

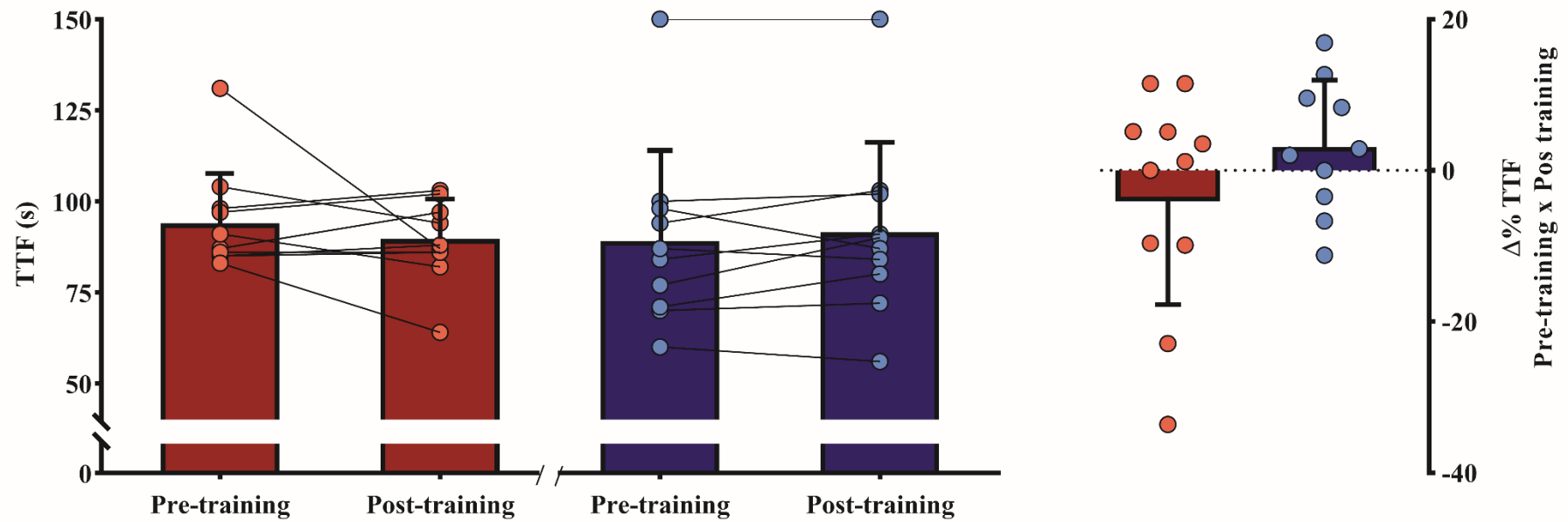


Figure 4. Comparison between time to task failure during the severe intensity domain continuous test measured at the pre-training period (BL), post-training period (Post 5-weeks), performed at a power output corresponding to 150% of the respiratory compensation point. * Significantly different from other period of SIT_G group ($p > 0.05$)

4.4 NMF - Maximal voluntary contractions and Peripheral neuromuscular stimulus

Parameters of NMF measured before and post exercise, at pre- and post-training are reported in Table 2 (raw data) and fatigability indicators are showed in Figure 5A and 5B.

The NMF data indicate no significative exercise time $\times$ training time $\times$ group interaction for any of measured parameter ($p \geq 0.07$; $F \leq 3.79$). Despite that, $iMVC_{Force}$, Db_{100} , Db_{10} , RMS_{iMVC} , $RMS_{iMVC}/M\text{-Wave}$ showed an exercise time effect ($p \leq 0.04$; $F \geq 4.61$), demonstrating a significative decrease of values measured immediately post-exercise, indicating a peripheral fatiguability state instauration after CWR.

Further, no significative training time $\times$ group interaction was found for any fatigue indicator parameters expressed in pre and post exercise percentual difference ($p \geq 0.07$; $F \leq 3.72$).

Table 2. Raw data of neuromuscular system function indicators pre and post constant work load severe domain cycling measured in Pre and Post intervention.

		Pre-training		Post-training		ANOVA		
		Pre exercise	Post exercise	Pre exercise	Post exercise	Interaction <i>p</i> (F)	Time <i>p</i> (F)	Group <i>p</i> (F)
iMVC _{Force} (N)	SIT _G	611.24 ± 120.15	457.04 ± 162.38 [†]	613.94 ± 131.67	475.59 ± 113.24 [†]	0.19	< 0.01	0.93
	CON	599.83 ± 89.44	525.21 ± 92.76 [†]	567.89 ± 86.73	450.79 ± 56.40 [†]	(1.88)	(81.03)	(0.07)
Db ₁₀₀ (N)	SIT _G	263.81 ± 81.18	193.65 ± 92.55 [†]	257.38 ± 76.49	185.19 ± 74.94 [†]	0.35	0.03	0.75
	CON	268.34 ± 39.51	197.80 ± 53.53 [†]	226.29 ± 42.59	173.49 ± 45.33 [†]	(0.92)	(5.21)	(0.10)
Db ₁₀ (N)	SIT _G	230.61 ± 84.64	122.12 ± 71.71 [†]	220.44 ± 85.25	108.49 ± 59.12 [†]	0.14	0.04	0.70
	CON	226.04 ± 59.17	120.21 ± 51.85 [†]	186.42 ± 42.65	109.16 ± 33.36 [†]	(2.39)	(4.61)	(0.16)
Db ₁₀₀ / Db ₁₀ (a.u.)	SIT _G	0.87 ± 0.11	0.60 ± 0.19	0.84 ± 0.16	0.57 ± 0.17	0.46	0.79	0.95
	CON	0.84 ± 0.17	0.59 ± 0.15	0.83 ± 0.14	0.63 ± 0.12	(0.57)	(0.07)	(0.04)
VA (%)	SIT _G	90.72 ± 5.05	81.92 ± 24.58	91.83 ± 4.26	85.34 ± 9.82	0.44	0.81	0.29
	CON	90.84 ± 7.76	93.69 ± 4.91	89.61 ± 8.36	88.38 ± 8.46	(0.61)	(0.06)	(1.21)
RMS _{iMVC} (μV)	SIT _G	645.40 ± 305.59	545.88 ± 270.94 [†]	903.62 ± 406.87	693.38 ± 393.75 [†]	0.07	< 0.01	0.22
	CON	560.28 ± 523.84	443.24 ± 485.57 [†]	455.88 ± 459.42	448.12 ± 460.77 [†]	(3.66)	(8.63)	(1.63)
M-Wave amp. (mV)	SIT _G	14.09 ± 3.04	13.99 ± 2.83	15.67 ± 4.97	14.30 ± 3.65	0.19	0.75	0.98
	CON	14.47 ± 3.18	14.86 ± 3.99	14.12 ± 5.28	14.48 ± 5.29	(1.81)	(0.10)	(0.01)
M-Wave dur. (ms)	SIT _G	0.009 ± 0.001	0.010 ± 0.002	0.010 ± 0.003	0.011 ± 0.003	0.14	0.15	0.65
	CON	0.009 ± 0.001	0.010 ± 0.002	0.011 ± 0.002	0.011 ± 0.002	(2.43)	(2.20)	(0.21)
RMS _{iMVC} /M-Wave (a.u.)	SIT _G	0.05 ± 0.03	0.04 ± 0.02 [†]	0.07 ± 0.04	0.05 ± 0.02 [†]	0.06	0.26	0.24
	CON	0.04 ± 0.04	0.03 ± 0.04 [†]	0.04 ± 0.03	0.03 ± 0.03 [†]	(4.17)	(1.34)	(1.47)

Values are expressed as the mean ±SD. iMVC_{Force} = peak force peak measured during isometric maximal voluntary contraction; Db₁₀₀ = force level at doublet high frequency stimulation; Db₁₀ = force level at doublet low frequency stimulation; Db₁₀ / Db₁₀₀ = ratio between Db₁₀ and Db₁₀₀; VA = voluntary activation; RMS_{iMVC} = root mean square from 500-ms iMVC peak force; M-Wave amp. = maximal M-Wave amplitude; M-Wave dur. = duration of M-Wave; RMS_{iMVC}/M-Wave = RMS_{iMVC} normalized by maximal M-Wave amplitude. [†] Main time effect (*p* < 0.05; Pre exercise > Post exercise).

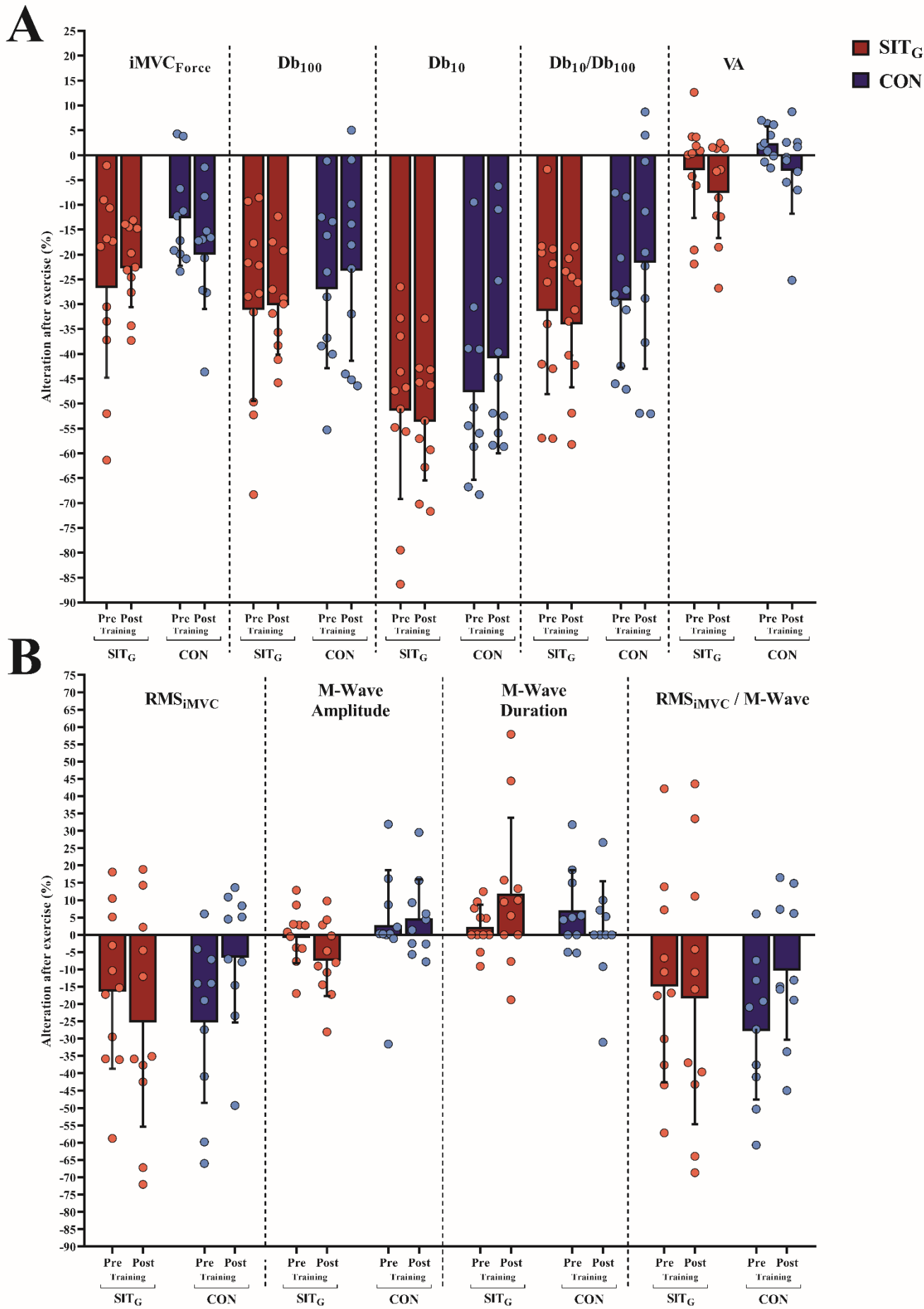


Figure 5. Comparison between fatigue indicator (alterations caused by exercise in neuromuscular function parameters) in Pre and Post 5 weeks periods.

4.5 EMG signal responses during the CWR

No significant within CWR-exercise window $\times$ training time $\times$ group interaction was observed for RMS ($p \geq 0.30$; $F \leq 1.33$) (Figure 6A to 6G) for any muscle. However, it was found main effect of time window for all analyzed muscles RMS ($p \leq 0.02$; $F \geq 3.06$), with significant increase since the effort begun until the ends for *recto femoris*, *vastus lateralis* and *gluteus maximus* (*post hoc* $p < 0.01$). For *biceps femoris* only 75 and 100% time-window values showed significant higher values when compared with others time windows (*post hoc* $p < 0.01$). Further, there was no significant time window $\times$ differences for RMS pre- and post-training change ($p \geq 0.07$; $F \leq 2.52$).

The Supplemental Digital Content 1 show no significant within CWR-exercise window $\times$ training time $\times$ group interaction was observed for PO/RMS ($p \geq 0.11$; $F \leq 1.94$) for any measured muscle. Despite that, a significant main effect of time window was showed for *recto femoris*, *vastus lateralis* and *gluteus maximus* ($p < 0.01$; $F > 27.24$), demonstrating a continuous decrease of muscle activation efficiency during exercise. In addition, no significant within CWR-exercise window $\times$ differences were verified for PO/RMS pre- and post-training change ($p \geq 0.12$; $F \leq 1.92$).

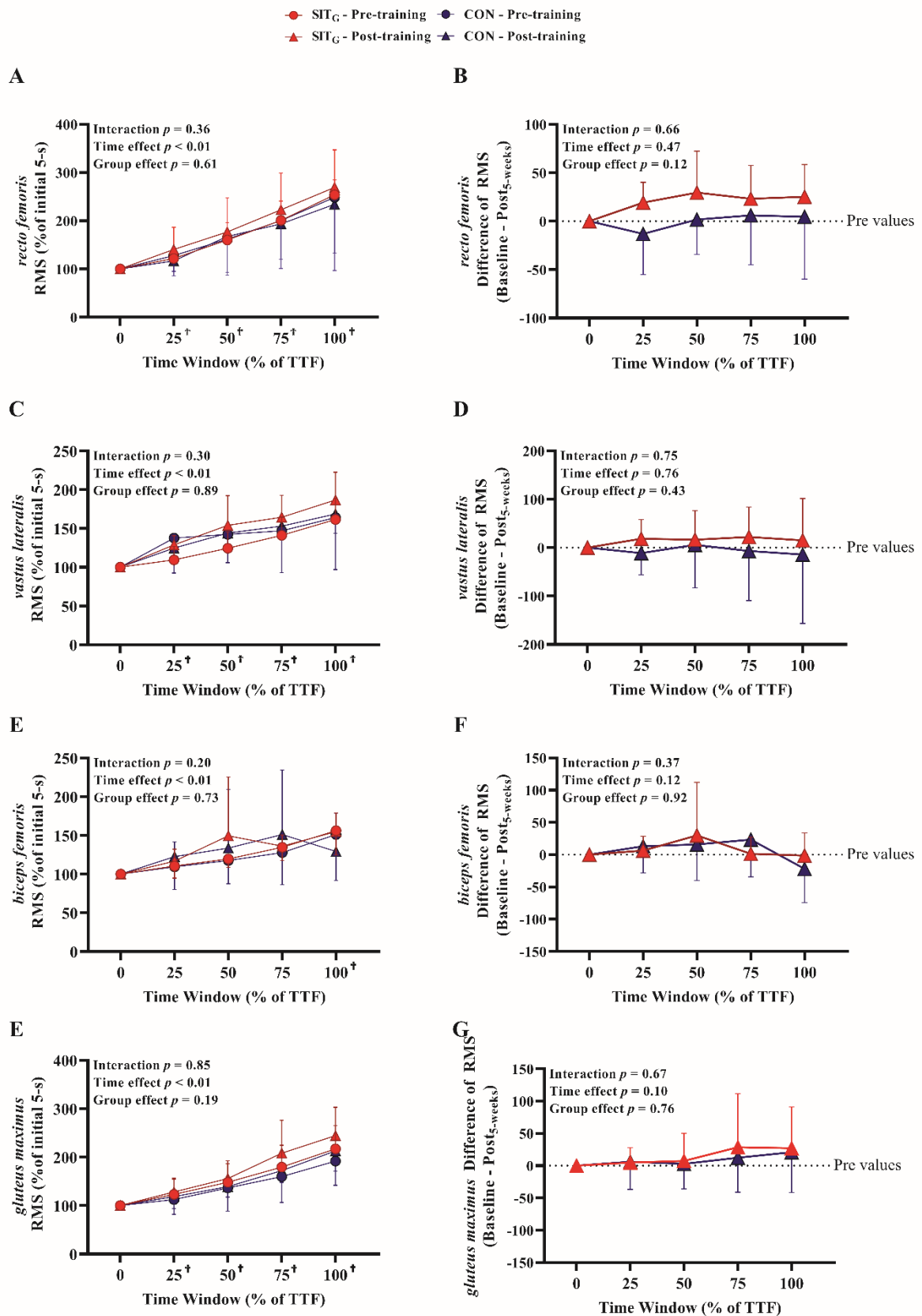
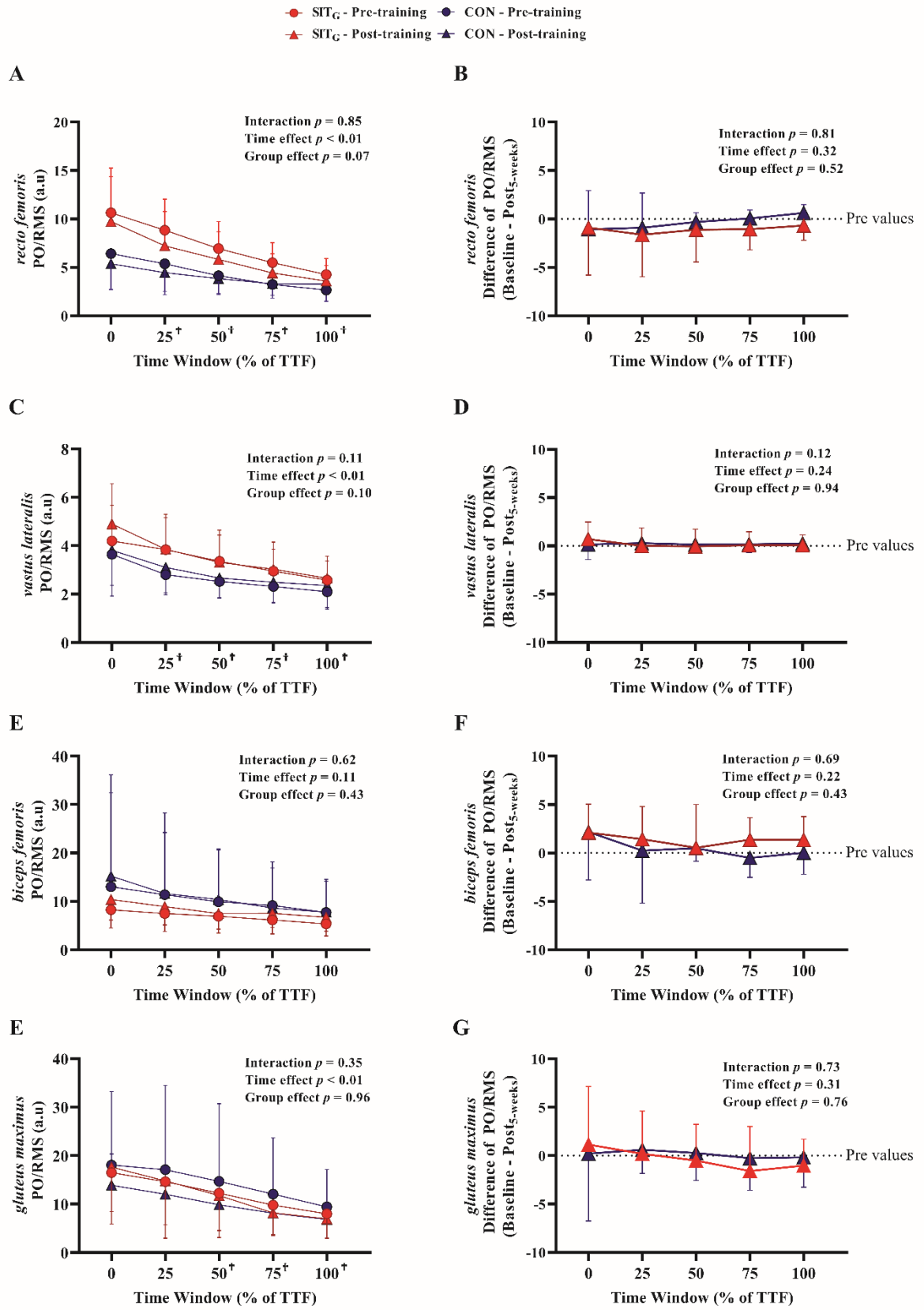


Figure 6. The comparison between RMS measured during exercise (i.e., fragmented every 25% of the time until exhaustion) in pre and post 5 weeks periods of recto femoris (Panel A), vastus lateralis (Panel C), biceps femoris (Panel E) and gluteus maximum (Panel G). The comparison

of differences between pre and post 5 weeks values of recto femoris (Panel B), vastus lateralis (Panel D), biceps femoris (Panel F) and gluteus maximum (Panel H). † Main time effect ($p < 0.05$; $\neq$ of all percentage moments).



Supplementary Digital Content 1. The comparison between PO/RMS measured during exercise (i.e., fragmented every 25% of the time until exhaustion) in pre and post 5 weeks periods of recto femoris (Panel A), vastus lateralis (Panel C), biceps femoris (Panel E) and gluteus maximum (Panel G). The comparison of differences between pre and post 5 -weeks values of recto femoris (Panel B), vastus lateralis (Panel D), biceps femoris (Panel F) and gluteus maximum (Panel H). † Main time effect ($p < 0.05$; $\neq$ of all percentage moments).

4.6 Correlations between $\Delta\%$ (NMF analysis vs time-to-task failure)

The correlations between $\Delta\%$ (percentual of change) of NMF analysis and time-to-task failure are presented in Figure 7 for both groups. No significant correlation was observed ($p > 0.21$) for any group or dataset.

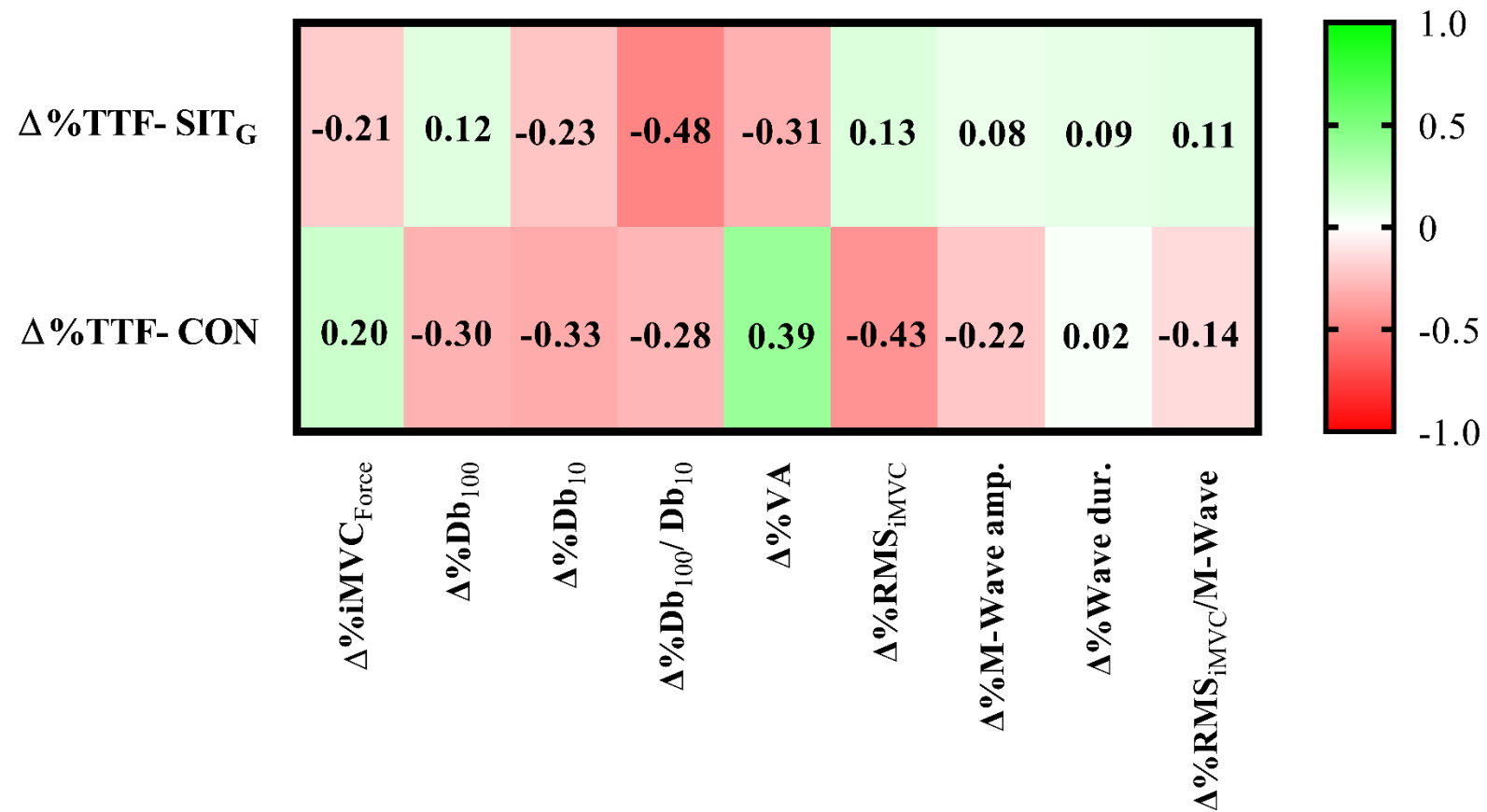


Figure 7. Heat map correlations between $\Delta\%$ (percentual alterations) of pre exercises maximal voluntary contraction and peripheral nerve stimulation data and time-to-task failure for both groups.

5. Discussion

Despite that SIT provides a high neuromuscular demand (FERNANDEZ-DEL-OLMO et al., 2013; FIORENZA et al., 2019), it has been strongly recommended for enhancing the NMF (given that neuromuscular gains are stimulus-dependent) and until to date no study has conducted a comprehensive evaluation of NMF (considering potentials alterations in force generation, neuromuscular activity during exercise, fatigability and exercise tolerance) during CRW performed above critical intensity.

Our findings demonstrated that: i) The SIT_G increased cardiorespiratory capacity (e.g., $\dot{V}O_{2Peak}$, RCP and RCP-PO) after intervention. ii) SIT was not effective to improve the exercise tolerance (TTF). iii) No alterations were verified in muscular electrical activity during CWR above critical intensity, NMF parameters measured pre-exercise (e.g., iMVC_{Force}, Db100, Db10, percentage of VA, RMS_{iMVC}, M-wave) or in establishment of fatigue etiology after exercise for SIT_G. iv) However, SIT_G showed improvements in dynamic neuromuscular performance assumed as PP (12.22%) and MP (6.65%) of the first 3 bouts of 30-s Wingate all-out efforts.

Cardiorespiratory function

The improvements by SIT in cardiorespiratory capacity ($\dot{V}O_{2Peak}$, RCP and RCP-PO) reported herein is not a novelty, aligning with other studies that have demonstrated the impact of this training mode on oxidative parameters (BURGOMASTER et al., 2008; GIBALA et al., 2006; MACINNIS; GIBALA, 2017). These effects may be attributed to the ability of the training program to induce the expression of peroxisome proliferator-activated receptor γ coactivator-1 α (PGC-1 α) through adenosine monophosphate (AMP) accumulation (PGC-1 α) (LAURSEN, 2010), with consequently coordination of mitochondrial biogenesis and improvement of oxidative biomolecular parameters (i.e., improvement of citrate synthase and cytochrome c oxidase activity and content) (BURGOMASTER; HEIGENHAUSER; GIBALA, 2006; GIBALA et al., 2006). In addition, the literature has also shown the ability of SIT to improve skeletal muscle capacity (GIBALA et al., 2006), which may be related to improvements in RCP and RCP-PO in the present study.

Time to Task Failure and EMG activity during CWR

No improvements were observed in TTF in post-training period (i.e., 150% of RCP PO). These results corroborate with other finds that demonstrated no alterations in TTF in CWR

above critical intensity after 7 (HARMER et al., 2000) and 8 weeks of SIT (NEVILL et al., 1989). This result may be related with the lack of NMF and EMG changes in post-training.

The EMG during 150% of RCP CWR measured in pre- and in post-training, showed no difference between groups, showing that regardless of the training, the development of fatigue (in muscle recruitment) was the same. In addition, when we normalized the RMS values by the exercise PO, we also did not observe any changes, supporting the fact that there was no alteration in the muscle recruitment pattern during the CWR. To the best of our knowledge, there are no studies investigating muscle recruitment during CWR above critical intensity after SIT. Despite this, Ducrocq and Blain (2022) had compared RMS of *vastus lateralis* and *recto femoris* in different time trials (e.g.; pairwise; 3-min at ~120% and 6-min ~110% of critical intensity) showing no difference RMS between these efforts. Therefore, considering that SIT_G RCO-PO in the present study improved ~8%, seems that results are in agreement with previously cited findings. However, Dutra et al. (2022), had showed that recreative endurance cyclists (i.e., training volume > 40 km· week⁻¹) had higher *rectus femoris* activation during CWR above critical intensity (i.e., 60% Of the difference between maximal aerobic power and RCP intensity) when compared with untrained group. In addition, the authors reported a strong correlation between neuromuscular activation during CWR and RCP-PO ($r = 0.83$), suggesting that higher activation may express a higher TTF. In this way, it seems that SIT cannot transfer its possible neuromuscular demand to neuromuscular recruitment adaptation in another type of exercise (e.g., CWR) (SCHLITTLER et al., 2019).

Moreover, despite the unchanged in RMS data measured during CWR, the RMS behavior for the measured muscles during exercise are in according with previously studies (i.e., continuous improvement in RMS during exercise, demonstrating the augmentation in the recruited motor units within the assessed muscles as an endeavor to extend the duration of the exercise.) (CAMATA et al., 2011; DE POLI et al., 2020; DUTRA et al., 2020, 2022; ZAGATTO et al., 2023b).

Fatigable response after CWR at severe-domain

Fatigability results revealed no differences between pre- and post-training periods for any group. Thus, consistently across all times and groups, CWR induced peripheral fatigue. This result also, aligns with other studies that have also demonstrated the onset of peripheral fatigue following CWR above critical intensity effort (BLACK et al., 2017; DE POLI et al., 2020; FIORENZA et al., 2019; IANNETTA et al., 2022; THOMAS et al., 2016).

Efforts above critical intensity are associated with high utilization of non-oxidative metabolism, resulting in the accumulation of intramuscular hydrogen ions and inorganic phosphate (BLACK et al., 2017; ZAGATTO et al., 2022). Additionally, during this exercise intensity, a 'critical' concentration can be surpassed. The Pi entering the sarcoplasmic reticulum leads to the precipitation of calcium (Ca^{2+}), reducing their release excitation-contraction coupling (HUREAU et al., 2022), consequently diminishing the number of high-force cross-bridges and, thereby, power production (i.e., a decrease in type I and II fibers' force output) (FITTS, 2008). These physiological alterations are associated with peripheral fatigue (BLACK et al., 2017; HUREAU et al., 2022).

Furthermore, the lack of changes in fatigue pattern post-training may be attributed to the potential stability in the 'critical threshold' of fatigue. Amann (AMANN, 2011) introduced this term, referring to the significance of afferent group III and IV fibers in responding to the accumulation of certain metabolites (e.g., Pi), which constrains the central motor drive, leading to the attenuation of circulatory and ventilatory responses, as well as skeletal muscle contraction (AMANN et al., 2011). This occurs irrespective of external modulations [e.g., a reduction in inspired oxygen fraction (AMANN et al., 2007) or prior fatigue-inducing effort (AMANN et al., 2008)] that can decrease the TTF; however, the level of peripheral fatigue remains the same.

Neuromuscular Function

Despite the absence of alterations or correlations in NMF parameters (i.e., pre-exercise measures by twitch interpolated technique), notable improvements were observed in training performance outcomes, PP and MP. These findings align with the results presented by Creer et al. (CREER et al., 2004) who reported 6% of increase in both PP and MP after a 4-weeks of SIT (also using Wingate). This reported enhancement in performance is associated with an improvement in dynamic neuromuscular function, marked by an increase in motor unit recruitment during the 30-s Wingate test, as exemplified by *vastus lateralis* RMS (CREER et al., 2004). It is worth emphasizing that these performance improvements observed in present study were noted following a taper strategy, a pattern consistent with the conclusions drawn by Flockhart et al. (FLOCKHART et al., 2021). Their research has clarified that an excessive increase in training intensity can lead to physiological impairments, ultimately resulting in a decrease or no change rather than an improvement in performance.

The disparity between the NMF outcomes and the Wingate outcomes results may be ascribed to the non-transferability of "dynamic" neuromuscular adaptations to isometric adaptations. For instance, several studies have reported no changes in isometric knee-extension after a 2-week SIT regimen (4-6 efforts per training session) in recreationally active participants (ASTORINO et al., 2012; BERTSCHINGER; GIBOIN; GRUBER, 2020; FORBES; SLADE; MEYER, 2008). Furthermore, Schlittler et al. (2019) observed that a more extended SIT program (i.e., 3 weeks) similarly had no impact on isometric knee-extension force. In addition, we must consider that Wingate present ~20% an oxidative energetic contribution (BENEKE et al., 2002), possibly a much higher value than a 5-second iMVC. Therefore, the adaptations on oxidative parameters may cause any influence on these improvements.

In this regard, the improvements in dynamic neuromuscular performance and the absence of improvement in force production after 5 weeks of SIT may be clarified by the specificity of neural adaptation to the exertion, wherein the potentially higher forces generated may not be translated to translated to iMVC.

Limitations

It is important to highlight the absence of a Wingate protocol at the pre-training and post-training periods for the control group, or the incorporation of another training group (e.g., neuromuscular resistance training). This limitation prevents us from definitively attributing these results to the observed non-transferability.

Conclusion

In summary, this study provides evidence that 5 weeks of SIT neuromuscular adaptations in training workload (i.e., PP and MP; dynamic neuromuscular performance) cannot be transferred to iMVC neuromuscular evaluations, and may be related with oxidative improvements. In addition, the fatigability during the CWR exercise seems to be intensity-dependent, being independent of possible changes caused by SIT.

CHAPTER 4

Thesis Conclusions

Conclusion: Theoretical model integrating evidence, directions for future research

Despite literature indicating that TTF during CWR in the severe-intensity domain appears to remain consistent different training levels (ZAGATTO et al., 2017, 2023a), modulation of glycogen concentrations (high vs low content) (HARGREAVES et al., 1997; VANDENBERGHE et al., 1995) or even after a SIT (HARMER et al., 2000; NEVILL et al., 1989), there is limited understanding of the physiological factors contributing to the prevention of exercise prolongation.

In this context, considering the importance of the glycolytic pathway and neural function in severe-intensity domain exercise (DUTRA et al., 2022; HARGREAVES; SPRIET, 2020), and the potential of SIT to improve both aspects (BUCHHEIT; LAURSEN, 2013b; MACDOUGALL et al., 1998; MIYAMOTO-MIKAMI et al., 2018). The present thesis investigated the relationship between the glycolytic pathway—specifically, resting glycogen content, tissue buffering capacity, enzyme content, and activity—and exercise tolerance during activities above critical intensity following a 5-week SIT program. Additionally, we explored whether a 5-week SIT regimen could enhance neuromuscular function responses, both at rest and during exercise, as well as fatigue mitigation. We also investigated if these potential neuromuscular changes could explain the possible lack of change in TTF during severe-intensity domain exercise.

Based on the findings of this thesis, although 5 weeks of SIT can induce changes in the glycolytic pathway (i.e., increased content and activity of important enzymes in the non-oxidative glycolytic flux, as well as an increase in muscle glycogen content at rest) and also cause dynamic neuromuscular adaptations, the training was not effective in causing greater tolerance in continuous exercise performed at 150% of critical intensity. This resulted in similar use of muscle glycogen between pre- and post-training moments. Furthermore, despite an improvement in dynamic neuromuscular variables observed during training, these adaptations do not seem to be transferable to different exercise models (e.g., iMVC and CWR at 150% of RCP). In addition, the correlations indicate that probably alterations on LDH after SIT seems reflect negative influence on TTF, while the NMF, showed no correlations with changes on TTF.

Taken together, these findings indicate that neither glycolytic changes nor neuromuscular function can explain the absence of improvements in exercise tolerance at the severe-intensity domain when assessed physiologically (i.e., adjusted to post-training status).

Ultimately, further studies are still necessary to underpin the maintenance of exercise tolerance during severe-intensity efforts.

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Appendix

Appendix 1. Research Ethics Committee Review

UNESP - FACULDADE DE
CIÊNCIAS CAMPUS BAURU -
JÚLIO DE MESQUITA FILHO



PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Glicogênio muscular, atividade enzimática e pH muscular em exercício supramáximo em ciclo ergômetro após 4 semanas de treinamento intervalado de alta intensidade: Uma investigação do processo de fadiga muscular.

Pesquisador: Alessandro Moura Zagatto

Área Temática:

Versão: 1

CAAE: 88448418.5.0000.5398

Instituição Proponente: UNIVERSIDADE ESTADUAL PAULISTA JULIO DE MESQUITA FILHO

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.650.216

Apresentação do Projeto:

Adequada e atendendo as normas relativas a ética em pesquisa.

Objetivo da Pesquisa:

Segundo consta no projeto, seu objetivo é: "Investigar o impacto da atividade enzimática da via glicolítica, oferta de substrato energético e pH na fadiga muscular em exercício supramáximo em ciclo ergômetro, assim como o efeito de 4 semanas de treinamento intervalado de alta intensidade sobre esses fatores".

Avaliação dos Riscos e Benefícios:

Riscos e benefícios estão suficientemente apresentados e explicados no corpo do projeto, no documento que contém as informações básicas do projeto e, principalmente, no TCLE, favorecendo a compreensão do participante da pesquisa e sua escolha.

Comentários e Considerações sobre a Pesquisa:

Adequada.

Considerações sobre os Termos de apresentação obrigatória:

Segue de forma exemplar as orientações da Resolução 466/12, do CNS.

Recomendações:

Nenhuma.

Endereço: Av. Luiz Edmundo Carrijo Coube, nº 14-01

Bairro: CENTRO

CEP: 17.033-360

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Município: BAURU

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**UNESP - FACULDADE DE
CIÊNCIAS CAMPUS BAURU -
JÚLIO DE MESQUITA FILHO**



Continuação do Parecer: 2.650.216

Conclusões ou Pendências e Lista de Inadequações:

Aprovado.

Considerações Finais a critério do CEP:

Projeto considerado aprovado por estar em conformidade com os parâmetros legais, metodológicos e éticos analisados pelo colegiado deste CEP.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1122015.pdf	25/04/2018 16:48:23		Aceito
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1122015.pdf	25/04/2018 16:40:02		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	25/04/2018 16:39:16	Fabio Milioni	Aceito
Outros	Questionario_Triagem_Biopsia.pdf	25/04/2018 16:38:57	Fabio Milioni	Aceito
Outros	Carta_anuencia_local.pdf	25/04/2018 16:38:27	Fabio Milioni	Aceito
Outros	Carta_anuencia_medico.pdf	25/04/2018 16:37:39	Fabio Milioni	Aceito
Projeto Detalhado / Brochura Investigador	Projeto.pdf	25/04/2018 16:34:06	Fabio Milioni	Aceito
Folha de Rosto	FRFabio.pdf	25/04/2018 16:23:26	Alessandro Moura Zagatto	Aceito

Situação do Parecer:

Aprovado

Necessita apreciação da CONEP:

Não

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UNESP - FACULDADE DE
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Continuação do Parecer: 2.650.216

BAURU, 11 de Maio de 2018

Assinado por:
Mário Lázaro Camargo
(Coordenador)

Endereço: Av. Luiz Edmundo Carrijo Coube, nº 14-01
Bairro: CENTRO **CEP:** 17.033-360
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Telefone: (14)3103-9400 **Fax:** (14)3103-9400 **E-mail:** cepesquisa@fc.unesp.br

Appendix 2. Term of Free and Informed Consent (TFIC) in the native language**TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE)**

(Conselho Nacional de Saúde, Resolução 466/12).

O Sr. está sendo convidado a participar da pesquisa intitulada “Glicogênio muscular, atividade enzimática e pH muscular em exercício supramáximo em ciclo ergômetro após 4 semanas de treinamento intervalado de alta intensidade: Uma investigação do processo de fadiga muscular” que tem como responsável o pesquisador Rodrigo de Araujo Bonetti de Poli, Mestre na área de Educação Física, supervisionado pelo Prof. Dr. Alessandro Moura Zagatto. O projeto tem como objetivo investigar como funciona o fornecimento de energia e a fadiga muscular durante exercícios exaustivos após as adaptações proporcionadas por treinamento intervalado de alta intensidade. Leia cuidadosamente o que se segue e pergunte ao responsável em caso de qualquer dúvida.

Os requisitos para participar do estudo é ser fisicamente ativo, não fazer uso regular de bebidas alcoólicas e/ou tabagismo, não estar fazendo uso de suplementos alimentares proteicos, especialmente creatina e não apresentar problemas de saúde como insuficiências cardiorrespiratórias ou limitações músculo-esqueléticas.

Caso o Sr. aceite participar, antes do início da pesquisa serão realizadas avaliações físicas em 4 dias diferentes. No primeiro dia o Sr. irá realizar um teste na bicicleta ergométrica no qual a cada dois minutos a resistência irá aumentar até o momento que o Sr. não conseguir mais manter o ritmo de pedalada. Após aproximadamente 48 h o Sr. será familiarizado a um exercício bastante intenso na bicicleta ergométrica, que irá durar o máximo que o Sr. conseguir sustentar, sendo que a resistência constante do teste será baseada nos resultados atingidos no teste feito anteriormente. No terceiro e quarto dias de avaliações, observando 48 h de repouso entre eles, o Sr. irá realizar o procedimento ao qual foi familiarizado, entretanto diferentes avaliações serão realizadas nesses dois dias de teste. Em um dos dias serão realizadas avaliações da capacidade muscular antes e depois da realização do exercício na bicicleta. As avaliações da capacidade muscular serão feitas através de contrações máximas do músculo da coxa, sendo que durante as contrações serão aplicados impulsos elétricos no nervo da coxa por um aparelho produzido especificamente para isso. A intensidade dos impulsos elétricos irá até o limite do seu desconforto, e o impulso elétrico será aplicado por eletrodos de gel sobre a pele. Ao mesmo tempo será monitorada a atividade elétrica (eletromiografia) produzida pelo músculo, utilizando aparelho específico e eletrodos posicionados na pele após raspagem dos pêlos e limpeza do local com material estéril, descartável e de uso único. Já no teste do quarto dia, será novamente realizado o mesmo exercício na bicicleta, mas antes e imediatamente após

o teste, serão coletados uma pequena amostra de músculo da perna por meio de biópsia muscular.

A biópsia muscular é uma técnica laboratorial e não cirúrgica, e será realizada por um médico competente (Dra. Daniela Moraes de Franco Seda, RG 30.311.157-4, CRM-SP 130526), que irá utilizar materiais estéreis. Nesse procedimento será aplicado abaixo da pele um anestésico local (lidocaína 2%) e após a anestesia será feita uma pequena incisão de aproximadamente 0,5 cm na parte lateral da perna, para que seja possível inserir uma agulha específica (agulha de Bergstrom) e a retirada de uma pequena quantidade de músculo (aproximadamente 100 miligramas). Em seguida será feito um pequeno curativo com esparadrapo estéril específico e compressão do local com bandagem durante 24h, não havendo a necessidade de ponto cirúrgico (sutura). Nesse procedimento, são raríssimos os casos de complicações como infecções ou quelóide, e há somente sensação de desconforto no momento do procedimento, sendo bastante incomum a ocorrência de dor e sangramento abundante. O local da biópsia pode ficar sensível ou ligeiramente dolorido durante 24 h e podem ocorrer hematomas e pequenos sangramentos durante esse período. Ainda, você deverá responder um questionário específico (em anexo), para que seja certificado que você não possui reação adversa ao anestésico a ser utilizado ou mesmo sobre o processo de cicatrização. Certifique-se de discutir todas as suas dúvidas com o médico.

Todos os exercícios na bicicleta serão realizados juntamente com o uso de equipamento que mede os gases da respiração (ergoespirometria), e ao final desses testes será realizada uma punção do lóbulo da orelha com uma pequena lâmina estéril e descartável para coleta de quantidade mínima de sangue (aproximadamente 1 gota). Ambos os procedimentos são rotineiros no laboratório, porém podem gerar um ligeiro desconforto para pessoas não familiarizadas.

Terminada a primeira fase de avaliações físicas, o Sr. irá iniciar período de 5 semanas de treinamento específico, individualizado e monitorado. Serão 3 sessões de treino por semana entre a primeira e a quarta semana e 2 sessões na quinta semana. Cada sessão terá duração entre 20 – 45 minutos. Os treinos serão compostos por exercícios em que o Sr. será solicitado a pedalar o mais forte e rápido que conseguir durante 30 segundos contra uma resistência referente a aproximadamente 7% do seu peso corporal. Na primeira semana essa tarefa será realizada 3 vezes por sessão, na segunda semana a tarefa será realizada 4 vezes por sessão, na terceira semana a tarefa será realizada 5 vezes por sessão e na quarta semana a tarefa será realizada 6 vezes por sessão. Período de 4 minutos de recuperação será realizado entre as tarefas em todas as sessões. Na quinta semana serão apenas duas sessões em que o Sr. será solicitado a realizar a tarefa apenas uma vez. Após o final das 5 semanas de treinamento, o Sr. irá realizar novas avaliações similares às que aconteceram antes do período de treinos. A primeira avaliação será o teste em que a

resistência vai aumentando a cada 2 minutos até o Sr. não sustentar mais o ritmo de pedalada. Em dias seguintes, com no mínimo de 48 h de descanso entre os dias de teste, serão realizados novamente 3 testes de alta intensidade com resistência constante. Em um deles a resistência será a mesma que foi usada antes do período de treinamento, e nos dois restantes, a resistência será baseada nos resultados do teste progressivo realizado depois dos períodos de treinamento. Nos testes realizados com resistência baseada nos resultados obtidos após o treino, um deles será monitorado a capacidade muscular e no outro será realizado biópsia muscular, exatamente da mesma maneira que foi feito nos testes realizados antes do período de treinamentos.

São benefícios da participação no estudo, a possibilidade de realizar um teste para verificar sua capacidade pulmonar e cardíaca, obter índices para prescrição de exercício individualizada que poderá contribuir para a melhora da sua condição cardiopulmonar e condicionamento físico. Além disso, poderá conhecer sua capacidade de exercício, treinar de forma orientada durante o período da pesquisa e conhecer as adaptações físicas após o treinamento. Você será notificado dos aspectos mais importantes do estudo após sua conclusão e poderá inteirar-se do estado geral de sua saúde e condição física.

É importante deixar claro que o Sr. tem liberdade de se recusar a participar e ainda se recusar a continuar participando em qualquer fase da pesquisa, sem qualquer prejuízo para o Sr.. Sempre que quiser poderá pedir mais informações sobre a pesquisa através do telefone do pesquisador do projeto e sobre seus direitos aos representantes do Comitê de Ética. A participação nesta pesquisa não traz complicações legais e os riscos e desconfortos são os mesmos que uma pessoa que pratica atividade física teria, tais como, leve mal-estar decorrente do esforço, arritmias cardíacas leves e desconfortos musculares durante a pesquisa e em dias seguintes (dor tardia). Entretanto, todos os procedimentos serão acompanhados por profissionais, que no caso de alguma ocorrência estarão aptos à execução de técnicas de socorros urgência e transporte imediato para unidade de socorro mais próxima.

NÃO será concedida nenhuma premiação financeira ou de qualquer outra natureza pela sua participação no estudo, entretanto o Sr. será ressarcido de qualquer despesa decorrente da participação na mesma, tais como transporte e/ou estacionamento, assim como será indenizado por eventuais danos decorrentes de sua participação.

Os procedimentos adotados nesta pesquisa obedecem aos Critérios da Ética em Pesquisa com Seres Humanos conforme Resolução no. 466/12 do Conselho Nacional de Saúde e nenhum dos procedimentos usados oferece riscos à sua dignidade. Todas as informações coletadas neste estudo são estritamente confidenciais sendo que somente o pesquisador e o supervisor terão conhecimento dos dados. Todo material biológico coletado (sangue e tecido) será utilizado exclusivamente para a presente pesquisa e então devidamente

descartado em local apropriado e inutilizado a partir de incineração por empresa especializada prestadora de serviços para UNESP/Bauru, de forma que NÃO será integrado a um banco de material biológico e NÃO será utilizado para outro fim.

Após estes esclarecimentos, convido-o a participar dessa pesquisa e solicito o seu consentimento de forma livre para participar da mesma, assinando este Termo de Consentimento Livre e Esclarecido que será elaborado em duas vias, as quais uma ficará de posse do pesquisador responsável e a outra em sua posse.

Título do Projeto: Glicogênio muscular, atividade enzimática e pH muscular em exercício supramáximo em ciclo ergômetro após 4 semanas de treinamento intervalado de alta intensidade: Uma investigação do processo de fadiga muscular.

Pesquisador Responsável: Prof. Ms. Rodrigo de Araujo Bonetti de Poli.

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Dados do participante da pesquisa:

Nome: _____

RG: _____ Data de Nascimento: ____/____/____

Endereço: _____

E-mail: _____

Telefone: (____) _____

Bauru, _____ de _____ de 20____.

Assinatura do participante

Dr. Alessandro Moura Zagatto
Supervisor

Ms. Rodrigo de Araujo Bonetti de Poli
Pesquisador Responsável

Appendix 3. Biopsy Screening in the native language

1) Você já teve alguma reação adversa ou alérgica a algum anestésico local (ex.: em procedimentos dentários)?

a) sim

b) não

2) Você tem alguma tendência a sangrar facilmente ou a contusões (ex.: com pequenos cortes ou ao barbear)?

a) sim

b) não

3) Atualmente, você está tomando alguma medicação que pode aumentar a chance de hemorragias ou hematomas (ex.: aspirinas, anti inflamatórios)?

a) sim

b) não

4) Você já desmaiou ou tem tendência a desmaiar quando assiste ou é submetido a algum procedimento médico?

a) sim

b) não

5) Você vai entrar em contato com o médico que fez a biópsia, caso você tenha alguma dúvida sobre o local do procedimento, incluindo: vermelhidão excessiva, inchaço, infecção, dor ou rigidez na perna?

a) sim

b) não

6) Você está disposto a visitar o médico que realizou a biópsia 7 a 10 dias depois do procedimento para uma avaliação?

a) sim

b) não

Appendix 3. Proof of article submission

Medicine & Science in Sports & Exercise

Five weeks of SIT improve muscle glycolytic content and activity but not time to task failure in severe intensity exercise --Manuscript Draft--

Manuscript Number:	
Full Title:	Five weeks of SIT improve muscle glycolytic content and activity but not time to task failure in severe intensity exercise
Short Title:	SIT improve muscle glycolytic but not TTF
Article Type:	Original Investigation
Keywords:	High intensity exercise; Exercise tolerance; Muscle Glycogen; Muscle Biopsy; Enzymatic Activity.
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Order of Authors Secondary Information:	
Abstract:	Purpose: Previous evidence suggests that, despite training induces improvements in glycolytic pathway function, exercise tolerance during constant work rate (CWR) exercise within the severe domain remains unaltered. This study examined the impact of a 5-week sprint interval training (SIT) intervention on time to task failure (TTF) during severe intensity CWR, as well as in glycolytic enzymatic content and activity, and glycogen content. Methods: Fourteen untrained males were randomized into SIT group (SITG; n=8) composed of 15 SIT-sessions over 5 weeks and in a control group (n=6). At baseline, participants performed: i) ramp incremental test to measure the cardiorespiratory function; ii) CWR cycling TTF at 150% of the power output (PO) at the respiratory compensation point (RCP-PO) with muscle biopsies at rest and immediately following task failure. After the 5-weeks, the same evaluations were repeated, and an additional cycling CWR matched to baseline RCP-PO were performed. Results: The content and enzymatic activities of glycogen phosphorylase (GPhos), hexokinase (HK), phosphofructokinase (PFK), and lactate dehydrogenase (LDH), as well as the glycogen content, were analyzed. RCP-PO and CWR TTF increased only for SITG post training, with no differences observed for TTF at intensity adjusted to post-training fitness status. The GPhos, HK, PFK, and LDH content and activity, and glycogen content were not significantly different between SITG and control groups.

	Conclusion: Thus, SIT improved glycolytic pathway parameters, however the use of glycogen was not altered during CWR severe intensity exercise, and TTF remained similar.	
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	Fundação de Amparo à Pesquisa do Estado de São Paulo (21/08479-0)	Mr Yago Medeiros Dutra
	Fundação de Amparo à Pesquisa do Estado de São Paulo (18/21126-6)	Dr Alessandro Moura Zagatto
	CNPq (422193/2016-0)	Dr Alessandro Moura Zagatto