
**PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DO
MOVIMENTO - INTERUNIDADES**

TESE DE DOUTORADO

YAGO MEDEIROS DUTRA

**RECOVERY AFTER A RUNNING TRAINING SESSION:
INFLUENCE OF EXERCISE CHARACTERISTICS, HOT-
WATER IMMERSION, AND COLD-WATER IMMERSION**

Bauru – SP
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Prof. Dr. CARLOS MARCELO PASTRE

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“Be yourself is all that you can do” (Chris Cornell)

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ABSTRACT

Running is one of the most widely used efforts in conditioning routines, however, the characteristics and time-course of fatigue after running at different intensities and/or types of execution (i.e., continuous or interval) have not yet been described. Moreover, fatigue is known to be task-dependent, whereby its characteristics and causal mechanisms will be influenced by aspects of exercise. In this way, due to discrepancies in basic mechanisms of action between cold-water immersion (CWI) and hot-water immersion (HWI), it is possible that their effectiveness in optimizing recovery after running may be influenced by aspects of the exercise performed and the characteristics of the fatigue established. Accordingly, the overall aim of this thesis was to investigate the influence of aspects of exercise, CWI and HWI on the fatigue-recovery continuum after running. To achieve this aim, a methodological study and three experimental studies were carried out. From the data gathered, we observed that: i) neuromuscular function assessment protocol involving maximal voluntary isometric contractions of the knee extensors, peripheral stimulation and monitoring of electromyography activity provides outcomes with a coefficient of variation <10% and repeatability <25% within day and between days; ii) even with equalized volume, peripheral fatigue and perceived fatigability are more pronounced after high-intensity interval exercise performed 50% above the velocity associated with the respiratory compensation point (vRCP; HIIT_{150%RCP}) compared to exercise performed continuously at the velocity associated with vRCP (CONT_{100%RCP}); however, central fatigue was greater after CONT_{100%RCP} compared to HIIT_{150%RCP}; iii) regardless of the characteristics of the effort, running 8-km causes impairments on neuromuscular function, cardiac parasympathetic activity, mood, cognitive function and greater physiological/psychological stress in successive brief effort for at least 6 hours after exercise; however, HIIT_{150%RCP} caused greater impairment in dynamic neuromuscular function and greater perceived fatigability during successive brief efforts compared to CONT_{100%RCP}, while high-intensity interval exercise performed 30% above vRCP (HIIT_{130%RCP}) caused greater reduction in resting cardiac parasympathetic activity compared to CONT_{100%RCP}; iv) both CWI and HWI were able to mitigate central fatigue after running compared to placebo and passive rest, but CWI only had this ability after HIIT_{150%RCP}, while HWI only had this ability after CONT_{100%RCP}; neither method mitigate the impairments in maximal voluntary isometric force production or tissue contractility of knee extensors; however, after both HIIT_{150%RCP} and CONT_{100%RCP}, CWI was able to increase resting cardiac parasympathetic activity and HWI was able to decrease cardiorespiratory demand during successive brief efforts compared to placebo and passive rest. In view of the data obtained, it was concluded that the intensity/type of running has an impact on the characteristics of fatigue and that the CWI and HWI for optimize recovery should be used taking into account the characteristics of the running performed and the recovery metric to be improved.

Keywords: muscle fatigue; post-exercise recovery; exercise performance.

RESUMO

A corrida é um dos esforços mais utilizados em rotinas de condicionamento, contudo, as características e curso temporal da fadiga após corrida de diferentes intensidades e tipo de execução (i.e., contínua ou intervalada) ainda não foram descritos. Além disso, a fadiga é tarefa-dependente, ou seja, suas características e mecanismos causais são influenciados por aspectos do exercício. Desse modo, devido a discrepâncias em mecanismos de ação básicos entre a imersão em água fria (CWI) e a imersão em água quente (HWI), é possível que as suas eficácias em otimizar a recuperação após corrida possam ser influenciadas pelas características do exercício realizado e da fadiga instaurada. Diante desse contexto, o objetivo geral da presente tese foi investigar a influência de aspectos do exercício, da CWI e da HWI sobre o *continuum* fadiga-recuperação após corrida. Para alcançar tal objetivo, um estudo de caráter metodológico e três estudos experimentais foram realizados. A partir dos dados reunidos, observamos que: i) desfechos sobre a função neuromuscular obtidos por meio de teste envolvendo contrações voluntárias isométricas máximas dos extensores do joelho, estimulação periférica e monitoramento da atividade de eletromiografia apresentam coeficiente de variação <10% e repetibilidade <25% dentro do dia e entre dias; ii) mesmo com volume equalizado, a fadiga periférica e a fatigabilidade percebida são mais pronunciadas após exercício intervalado de alta intensidade realizado 50% acima da velocidade associada ao ponto de compensação respiratório (vRCP; HIIT_{150%RCP}) em comparação à exercício realizado de forma contínua na vRCP (CONT_{100%RCP}); no entanto, a fadiga central foi maior após CONT_{100%RCP} em comparação ao HIIT_{150%RCP}; iii) correr 8-km, independentemente das características do exercício, acarreta prejuízos à função neuromuscular, atividade parassimpática cardíaca em repouso, alterações no humor e em aspectos cognitivos, e maior estresse fisiológico/psicológico durante esforços breve subsequentes por pelo menos 6-horas após o fim do exercício; no entanto, o HIIT_{150%RCP} acarretou maior prejuízo à função neuromuscular e maior fatigabilidade percebida durante esforços breves subsequentes em comparação ao CONT_{100%RCP}, enquanto o exercício intervalado de alta intensidade realizado 30% acima da vRCP (HIIT_{130%RCP}) acarretou maior redução da atividade parassimpática cardíaca em repouso em comparação ao CONT_{100%RCP}; iv) ambas CWI e HWI foram eficazes em atenuar a fadiga central após corrida em comparação à intervenção placebo e descanso passivo, contudo, a CWI só acarretou tal efeito benéfico após o HIIT_{150%RCP} enquanto a HWI somente após o CONT_{100%RCP}; nenhum dos métodos atenuou os prejuízos à produção de força voluntária isométrica máxima ou à contratilidade tecidual; no entanto, após ambos HIIT_{150%RCP} e CONT_{100%RCP}, a CWI foi capaz de aumentar a atividade parassimpática cardíaca em repouso e a HWI foi capaz de reduzir a demanda cardiorrespiratória durante esforços subsequentes em comparação à intervenção placebo e descanso passivo. Diante dos dados obtidos, concluiu-se que a intensidade/tipo de execução da corrida impactam as características da fadiga após exercício e que a CWI e da HWI devem ser usadas para otimizar a recuperação após a corrida considerando as características do exercício que foi realizado e as métricas de recuperação que se deseja otimizar.

Palavras-chave: Fadiga muscular; Recuperação pós-exercício; Desempenho físico.

ABSTRACT FOR LAY PEOPLE

Different types of running are used in training and physiotherapy routines to improve physical conditioning. To avoid injuries and to increase the quality of these routines, it's important to understand what fatigue from running looks like and how long it lasts. Identifying whether a person is still tired after a running workout will help them to know whether they need more time to recover until the next training/physiotherapy session or whether they need any additional treatment, such as getting into a bath with ice-cold water or a bath with hot water, to speed up their recovery. This research observed that running for 40 minutes at moderate pace causes fatigue mainly in the nervous system, making the body send less stimuli to the thigh muscles to contract. On the other hand, running 1-3 minute sprints at high paces causes fatigue mainly in the thigh muscles, making it difficult for them to contract, even though there are stimuli to do so. Furthermore, running doing sprints at high paces also changes our mood, makes our heart more fatigued, makes us feel more achy in the legs and more unwell after exercise. All this fatigue lasts an average of 24 hours and to speed up recovery after exercise, hot baths and cold baths can help. These two treatments improve the body's ability to send stimuli to the thigh muscles to contract, which can help when doing other exercises after running. However, for this positive effect to take place, the hot bath should be used after running for 40 minutes at moderate pace and the cold bath after running doing sprints for 1-3 minutes at high pace. Furthermore, both after continuous running at a moderate pace and after running with high-speed sprints, the cold bath also helps to recover the fatigue that exercise generates in the heart, and the hot bath makes the muscle a little more efficient, so that the body needs less oxygen to perform light activity after the running exercise.

RESUMO PARA LEIGOS

Diferentes formas de corrida são utilizadas nas rotinas de treino e de fisioterapia para melhorar o condicionamento físico. Para evitar lesões e para aumentar a qualidade dessas rotinas, é importante entender como é o cansaço gerado pela corrida e por quanto tempo ele dura. Identificar se a pessoa ainda está cansada após um exercício de corrida ajudará a saber se ela precisa de mais tempo para se recuperar até a próxima sessão de treino/fisioterapia ou se ela precisa de algum tratamento adicional, como por exemplo entrar em uma banheira com água gelada ou em uma banheira com água quente, para acelerar a recuperação dela. Esta pesquisa observou que correr por 40 minutos em velocidade moderada gera cansaço principalmente no sistema nervoso, fazendo com que o corpo envie menos estímulos para os músculos da coxa se contraírem. Já correr realizando tiros de 1-3 minutos em altas velocidades gera cansaço principalmente nos músculos da coxa, fazendo com que eles tenham dificuldades para se contrair, mesmo havendo estímulos para isso. Além disso, correr realizando os tiros em alta velocidade também deixa nosso humor mais alterado, faz com que o nosso coração fique mais cansado, que a gente se sinta com mais dor nas pernas e mais indisposto após o exercício. Todo esse cansaço dura em média 24 horas e para acelerar a recuperação após o exercício, a banheira com água quente e a banheira com água gelada podem ajudar. Esses dois tratamentos melhoram a capacidade do corpo de enviar estímulos para os músculos da coxa se contraírem, o que pode ajudar na hora de fazer outros exercícios após a corrida. No entanto, para que tal efeito positivo aconteça, a banheira com água quente deve ser usada após correr por 40 minutos em velocidade moderada e a banheira com água gelada após realizar tiros de corrida de 1-3 minutos em altas velocidades. Além disso, tanto após a corrida contínua em velocidade moderada como após a corrida com tiros em alta velocidade, a banheira com água gelada também ajuda a recuperar o cansaço que o exercício gera sobre o coração, e a banheira de água quente deixa o músculo um pouco mais eficiente, fazendo com que o corpo precise de menos oxigênio para fazer uma atividade leve após o exercício de corrida.

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LIST OF ACRONYMS

ANOVA	Analysis of variance
CMJ	Countermovement jump
CNS	Central nervous system
CONT _{100%RCP}	Continuous run performed at 100% of vRCP
CV%	Coefficient of variation
CWI	Cold-water immersion
CWI _{11°}	CWI performed with water at 11°C
Db10 _{Force} /Db100 _{Force}	Ratio between the Db10 _{Force} and Db100 _{Force}
EMG	Surface electromyography activity
GXT	Graded exercise test
HIIT	High-intensity intermittent effort
HIIT _{130%RCP}	HIIT performed at 130% of vRCP
HIIT _{150%RCP}	HIIT performed at 150% of vRCP
HR	Heart rate
HRV	Heart rate variability
HWI	Hot-water immersion
HWI _{41°}	HWI performed with water at 41°C
ICC	Intraclass correlation coefficients
iMVC	Isometric MVC
iMVC _{Force}	Peak force recorded during iMVC of knee-extensors
LnRMSSd	Log-transformed RMS of successive R-R interval
MMSS	Maximal metabolic steady state
MVC	Maximal voluntary contraction
M-wave _{amp}	Peak-to-peak M-wave amplitude
PNS	Peripheral nerve stimulation
POMS	Profile of mood state
Qtw _{pot,}	Force evoked by a potentiated single stimulus
RCP	Respiratory compensation point
RER	Respiratory exchange ratio
RMS	Root mean square

$\text{RMS}_{\text{VL}}/\text{M-wave}_{\text{amp}}$	Ratio between the RMS and M-wave _{amp}
RPE	Rate of perceived effort
SD2	Poincaré plot standard deviation along the line of identity
SDNN	Standard deviation of all normal R-R
TE%	Typical error in percentage of mean
TMD	Total mood disturbance
VA	Voluntary activation
$\dot{V}\text{CO}_2$	Rate of carbon dioxide production
$\dot{V}_E$	Ventilation
$\dot{V}_E/\dot{V}\text{CO}_2$	Ventilatory equivalent of carbon dioxid
$\dot{V}_E/\dot{V}\text{O}_2$	Ventilatory equivalent of oxygen
$\dot{V}\text{O}_2$	Rate of oxygen consumption
$\dot{V}\text{O}_{2\text{max}}$	Maximal rate of oxygen consumption
vRCP	Velocity associated with RCP
$v\dot{V}\text{O}_{2\text{max}}$	Velocity associated with $\dot{V}\text{O}_{2\text{max}}$

CHAPTER 1.

INTRODUCTION

1.1. Context and rationale

Running, in all its variations (i.e. intensity, volume and type of execution), is one of the most used exercises during rehabilitation and training routines (BUCHHEIT; LAURSEN, 2013; STARES et al., 2018). Running involves repetitive, submaximal contractions which result in changes in the functioning of different body systems (i.e. cardiorespiratory, nervous, muscular) and culminate in fatigue (CARROLL; TAYLOR; GANDEVIA, 2017). Fatigue is usually defined as the temporary reduction in the ability to produce force by exercised muscles (performance fatigability) in association with the appearance of perceptions such as “tiredness” and “weakness” (perceived fatigability), followed by physiological/psychological disturbances (e.g. imbalance in the autonomic nervous system, increased core temperature, mood disorder) (ENOKA; DUCHATEAU, 2016). From a more mechanistic perspective, performance fatigability is further subdivided into central fatigue, defined as a reduction in the capacity of the central nervous system (CNS) to activate the exercised muscles, and peripheral fatigue, described as a reduction in the contractile capacity of these muscles (GANDEVIA, 2001). Due to its negative impact on performance (ENOKA; DUCHATEAU, 2016) and on health aspects (i.e., occurrence of musculoskeletal injuries, non-functional overreaching and overtraining (KELLMANN, 2010; VERSCHUEREN et al., 2020)), a better understanding of fatigue, the methods for monitoring this phenomenon and the strategies for counteracting it has been constantly pursued in the exercise sciences (DI GIULIO; DANIELE; TIPTON, 2006; GANDEVIA et al., 1995; HALSON, 2014b).

Fatigue has a task-dependent nature, whereby aspects of the exercise will influence its characteristics (BROWNSTEIN; MILLET; THOMAS, 2021). For example, in locomotion efforts, exercises above the maximum metabolic steady state (MMSS, severe and extreme domains) are usually associated with greater impairment of the tissue's contractile capacity (i.e., peripheral fatigue) and greater perceptions of pain in the lower limbs compared to exercises performed below these intensities (i.e., heavy and moderate domains) (IANNETTA et al., 2022; THOMAS et al., 2015, 2016). On the other hand, exercises performed in the heavy and moderate domains (i.e., below the MMSS) are usually associated with a greater reduction in the CNS's ability to activate the exercised muscle group (i.e., central fatigue) and a greater overall perception of fatigue (IANNETTA et al., 2022; THOMAS et al., 2015, 2016). However, despite the knowledge accumulated so far about the task-dependent nature of fatigue in locomotion

efforts, most of it comes from studies carried out on cycling (BROWNSTEIN; MILLET; THOMAS, 2021; HUNTER, 2018).

Even with similarities, several metabolic and neuromuscular differences between cycling and running prevent the direct transfer of fatigue knowledge from one mode of exercise to the other (MILLET; LEPERS, 2004). For example, for the same intensity of exercise, the metabolic demand on the quadriceps is considered greater in cycling compared to running (BIJKER; DE GROOT; HOLLANDER, 2002), which seems to induce greater impairment to contractile function of this muscle group in the former compared to the latter (BROWNSTEIN et al., 2022; TOMAZIN; MORIN; MILLET, 2017). Moreover, due to the stretch-shortening cycle present in running, muscle damage induced by eccentric contractions (NICOL; KOMI; MARCONNET, 1991; SCHWELLNUS et al., 2011) and the reduced facilitation of motoneurons by group Ia afferents (AVELA; KYRÖLÄINEN; KOMI, 1999a), seem to lead to greater impairment of knee extensors activation capacity by the CNS (i.e., central fatigue) after running compared to cycling (BROWNSTEIN et al., 2022; TOMAZIN; MORIN; MILLET, 2017). Therefore, in order to provide accurate advances on the task-dependent nature of fatigue in running, it seems most appropriate to investigate this phenomenon specifically in this mode of exercise.

Insights into the task-dependent nature of fatigue in running can be obtained from the studies conducted by Skof et al, (2006a, 2006b). From independent investigations, these authors observed that running 6-km at the velocity of the MMSS (SKOF; STROJNIK, 2006a) reduced the maximum voluntary force and contractile capacity of the knee extensors for 1 hour after exercise, without altering the capacity of the CNS to activate these muscles (assessed using the twitch interpolation technique). Conversely, after 5 sprints of 300-m (1-min interval) (SKOF; STROJNIK, 2006b), although the contractile capacity of the knee extensors remained reduced for around 20-minutes, no changes were observed either in maximal force-generating capacity or in the ability of the CNS to activate this muscle group after exercise. Taken together, the results of Skof et al. (2006a, 2006b) suggest that the dynamics of central and peripheral fatigue during running may be influenced by exercise intensity and/or type of training. However, a limited point was that these studies were carried out in different participants, using self-paced efforts, and performing exercises of different volumes and without defined metabolic stress. Such potential confounding factors in this scenario may preclude proper conclusions about the task dependency of fatigue in running. Furthermore, the acute perceived fatigability responses (e.g., pain at exercised muscles, fatigue perception, mood disturbance) after distinct running

exercises have not yet been investigated. This is an important knowledge gap in this area as perceived fatigability may acutely impair performance and chronically increase the risk of health problems such as non-functional overreaching and overtraining (KELLMANN, 2010).

Furthermore, in order to reduce the possible negative impacts of fatigue on performance and health, in addition to the basic principles of recovery such as nutrition, hydration, and sleep, additional post-exercise recovery strategies are widely investigated and used in recreational, amateur and professional sport (DUPUY et al., 2018). Among these strategies, cold-water immersion (CWI) and hot-water immersion (HWI) are some of the most popular (WILCOCK; CRONIN; HING, 2006). From a mechanistic point of view, CWI is able to reduce muscle temperature and perfusion (MAWHINNEY et al., 2013), which in combination with other secondary effects (e.g. reduction of core temperature (STEPHENS et al., 2018) and limiting proliferation of secondary damage following injury (MERRICK et al., 1999)), seems to be able to mitigate muscle soreness, improve the overall perception of “recovery” and favor the maintenance of force/power production and endurance performance for hours to days after exercise (CHOO et al., 2022; MOORE et al., 2022, 2023). On the other hand, HWI has been shown to be effective in increasing muscle temperature (RODRIGUES et al., 2020a) and blood flow (HEINONEN et al., 2011), which associated with secondary heat effects such as increase in the clearance of tissue metabolites (COTÉ et al., 1988), muscle glycogen resynthesis, sarcoplasmic reticulum Ca^{++} release, myofibrillar Ca^{++} sensitivity (CHENG et al., 2017), and nerve conduction velocity (RACINAIS; OKSA, 2010), seems to be able to attenuate the muscle soreness (PETROFSKY et al., 2015), mitigating the impairments on force/power production (SAUTILLET et al., 2024) and maintain endurance performance for hours to days after exercise (CHAILLOU et al., 2022; CHENG et al., 2017).

However, even though such reported benefits, there is a large data variability in the efficacy of CWI and HWI on improve post-exercise recovery metrics (e.g., maximal-force generating capacity, jump performance, endurance performance) (CHAILLOU et al., 2022; MOORE et al., 2023) which, among other reasons (e.g., biological individuality, preference of use) (KELLMANN et al., 2018), may be dictated by task-dependent nature of fatigue (KELLMANN et al., 2018; THORPE, 2021b). For example, after exercises performed above the MMSS, it is suggested that one of the mechanisms involved in reducing neuromuscular function is excessive and prolonged heat accumulation (KENNY et al., 2006; LEI et al., 2023; RACINAIS; OKSA, 2010). In these scenarios, given the physiological effects of CWI (i.e. reduction in muscle and

core temperature (STEPHENS et al., 2018)), it is possible that this method may be more beneficial to improve post-exercise recovery than HWI (THORPE, 2021b). In contrast, due to the ability of HWI to increase the release of calcium ions by the sarcoplasmic reticulum (CHENG et al., 2017) and nerve conduction velocity (RACINAIS; OKSA, 2010), after exercises in which large increases in muscle/core temperature are not expected (e.g., performed below the MMSS and/or at low temperatures) (LEI et al., 2023), the use of HWI may present greater benefits to post-exercise recovery compared to CWI (SAUTILLET et al., 2024). However, to the best of our knowledge, no previous study has explored the effects of CWI and HWI on recovery metrics after running considering the task-dependent nature of fatigue.

1.2. Research aims and program

In this context, the overall aim of this thesis was to investigate the influence of aspects of exercise, CWI and HWI on the fatigue-recovery continuum after running. To achieve this aim, four studies were carried out (**Figure 1.1**). The first study investigated the reliability of performance fatigability parameters obtained through an assessment protocol involving maximal voluntary isometric contractions of the knee extensors, peripheral electrical stimulation (twitch interpolation technique), and monitoring of the electromyography activity of the recruited muscles. The study is described in full in Chapter 3 entitled “*Within- and between-day reliability and repeatability of neuromuscular function assessment in females and males*” and it was published in the *Journal of Applied Physiology* (November 2023).

The overall aim of the second study was to characterize central and peripheral fatigue as well as perceived fatigability responses after (i) continuous running for 40 minutes at the velocity associated to respiratory compensation point (vRCP, CONT_{100%RCP}), (ii) interval running at intensity 30% above vRCP (HIIT_{130%RCP}) and (iii) interval running at intensity 50% above vRCP (HIIT_{150%RCP}). The overall aim of the third study was to characterize maximal isometric force-generating capacity of knee-extensors, countermovement jump performance, cardiac parasympathetic activity, mood, cognitive function, and the physiological/psychological responses during subsequent brief efforts after CONT_{100%RCP}, HIIT_{130%RCP}, and HIIT_{150%RCP}. In both studies, the exercise volume was matched between conditions and the outcomes were monitored for up to 6 hours after exercise. Studies 2 and 3 are described in full in Chapters 4 and 5, respectively.

After characterizing aspects of fatigue and some of the physiological/psychological disturbances induced by different running efforts, the fourth and main study aimed to investigate the effectiveness of CWI and HWI in attenuating these responses. The study was conducted by engaging different volunteers to $\text{CONT}_{100\% \text{RCP}}$ and $\text{HIIT}_{150\% \text{RCP}}$. The volunteers performed each of the exercises on four occasions and then underwent CWI, HWI, control intervention (passive rest) or placebo intervention (immersion in thermoneutral colored water). Outcomes of interest were monitored for up to 4 hours and then 24 hours after exercise. The study is described in full in Chapter 6.

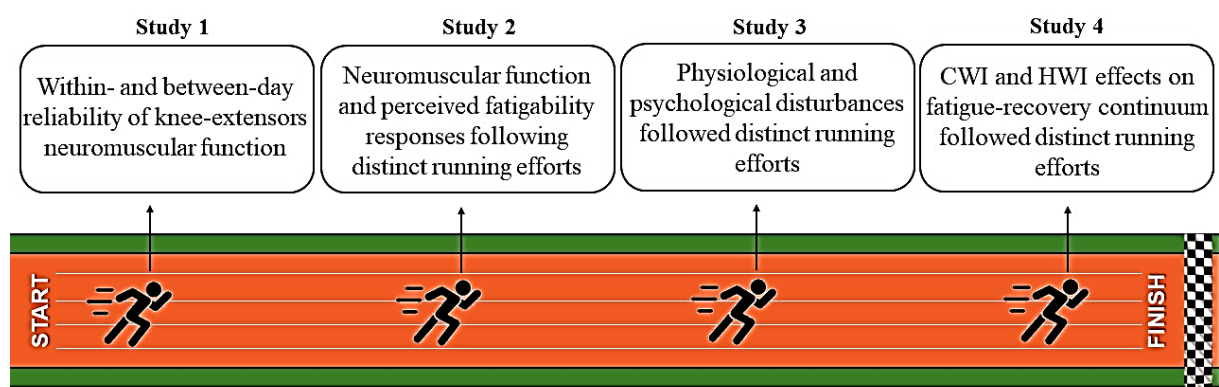


Figure 1.1. Overview of the research program and studies carried out in the present thesis.

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CHAPTER 2.

LITERATURE REVIEW

2.1. Muscle fatigue: definitions and mechanisms

The current scientific community defines fatigue as a condition in which decreased performance in a specific task is observed (performance fatigability) in association with the emergence of perceptions such as “tiredness” and “muscular soreness” (perceived fatigability) (ENOKA; DUCHATEAU, 2016) and disturbances in the body’s homeostasis (**Figure 2.1**). Performance fatigability is related to the contractile capacity of muscle tissue (peripheral fatigue), as well as the ability of the central nervous system to activate the muscles involved in the task (central fatigue) (ENOKA; STUART, 1992). Conversely, perceived fatigability arises from interactions between the individual's initial state (e.g. motivation, mood and previous experience) and the rate of change in sensations such as “perceived exertion”, “tiredness” and “muscular discomfort”, regulated by conscious and unconscious feedbacks coming from the body systems used in exercise (e.g. muscular, respiratory, cardiovascular) (ENOKA; DUCHATEAU, 2016).

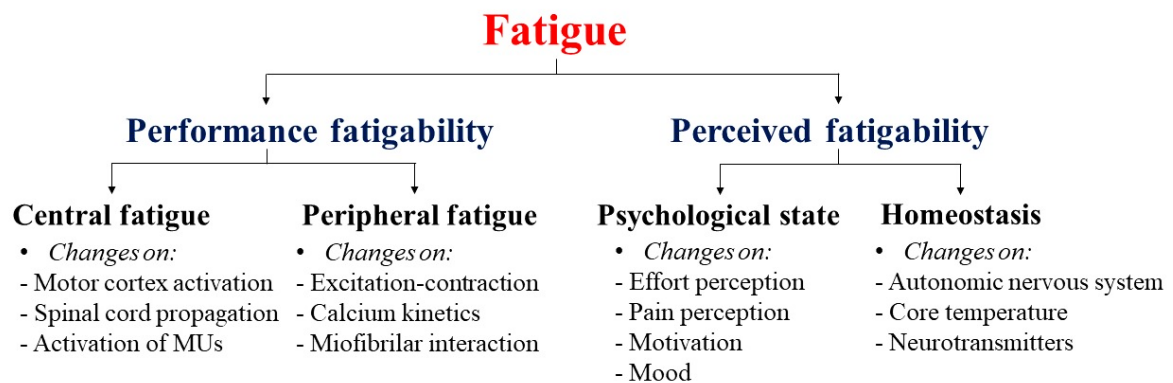


Figure 2.1. Schematic representation of fatigue as a self-reported disabling symptom, derived from two interdependent attributes: performance fatigability and perceived fatigability. Adapted from Enoka and Duchateau (2016).

Impairments to the contractile capacity of muscle tissue are generally associated with the depletion of energy substrates (e.g. creatine phosphate and glycogen), as well as the accumulation of metabolites in the tissue (e.g. lactate, inorganic phosphate, H^+ ions and K^+ ions) (BLACK et al., 2017; JONES et al., 2008)). Such changes impair different events involved in the contractile process (e.g., excitation-contraction and myofibrillar interaction (ALLEN; LAMB; WESTERBLAD, 2008; FITTS, 1994)), limiting force/power production even in the presence of “adequate” motor drive. Functional/structural changes in the muscle fiber due to exercise-induced tissue damage, caused mainly by eccentric contractions, also play a role in reduce the contractile capacity of the tissue (BEHRENS; MAU-MOELLER; BRUHN, 2012).

On the other hand, the reduction in muscle activation capacity by the CNS (central fatigue) is usually considered a reflex/protective response triggered by afferent stimuli from nerve groups III and IV, present in the skeletal muscles (KAUFMAN; RYBICKI, 1987). These nerve groups are sensitive to various mechanical (e.g., tissue damage) and metabolic (e.g., accumulation of metabolites, increase in temperature) alterations in the respiratory muscles, agonists and synergists of movement (GANDEVIA, 2001), and play an inhibitory role at different sites in the central nervous system (e.g. premotor areas, motor cortex, spinal cord) (GANDEVIA, 2001; HUREAU; ROMER; AMANN, 2018). In the presence of the stretch-shortening cycle in exercises such as running, the stimulation of groups III and IV by inflammatory mediators associated with tissue damage induced by eccentric contractions (NICOL; KOMI; MARCONNET, 1991; SCHWELLNUS et al., 2011) and the reduced facilitation of motoneurons by group Ia afferents (AVELA; KYRÖLÄINEN; KOMI, 1999b; BROWNSTEIN et al., 2022) also seem to be involved in the reduction of muscle activation capacity by the CNS. In addition, other physiological changes such as a reduction in blood glucose concentration (DAVIS; BAILEY, 1997), changes in brain concentrations of neurotransmitters such as serotonin and dopamine (MEEUSEN; WATSON; DVORAK, 2006) and increases in core temperature (NYBO; NIELSEN, 2001) are also suggested as causal mechanisms for the reduction in muscle activation capacity by the CNS.

Although less clear, it is suggested that several of the factors involved in performance fatigability are also responsible for perceived fatigability. For example, the development of perceptions such as “pain” and “muscle soreness” seem to be associated with the accumulation of metabolites such as H^+ ions and lactate in muscle tissue (GREGORY; WHITLEY; SLUKA, 2015; POLLAK et al., 2014), while reductions in blood glucose concentration (NYBO, 2003) and increases in core temperature (NYBO; NIELSEN, 2001), are associated with the appearance of a sensation of “tiredness” and increased “perception of effort” during exercise. In addition, exercise-induced changes in specific patterns of cortical activity (e.g., arousal)(KLASS et al., 2012) and in the functioning of the hypothalamic-pituitary-adrenal axis (DUCLOS; TABARIN, 2016), also seem to be involved in the development of aspects of perceived fatigability, such as reduced motivation/alertness and changes in mood.

Fatigue is considered to be multifactorial, so the interaction between the different physiological and psychological changes triggered by exercise results in the performance and perceived fatigability (ENOKA; DUCHATEAU, 2016). However, the way these interactions occur seems

to be influenced by aspects of the exercise performed, which characterizes fatigue as task-dependent (ENOKA; STUART, 1992). In locomotion efforts, aspects of exercise such as intensity (i.e., whether performed below or above the MMSS)(BROWNSTEIN; MILLET; THOMAS, 2021) and type of execution (i.e., whether performed continuously or intermittently) (BUCHHEIT; LAURSEN, 2013; MACDOUGALL et al., 2024; MACINNIS; GIBALA, 2017) are some of the most investigated, probably due to their relevant roles in conditioning routines (MACINNIS; GIBALA, 2017).

In this context, the literature has shown that exercises performed above the MMSS (i.e., severe and extreme domains) seem to induce greater impairment to the contractile capacity of muscle tissue (peripheral fatigue) and more pronounced pain perception in the exercised muscles compared to exercises performed below the MMSS (i.e., moderate and heavy domains) (BROWNSTEIN; MILLET; THOMAS, 2021). This impact of intensity on peripheral fatigue seems to occur even with small changes in work rate around the MMSS (i.e. 10% up versus 10% down) (AZEVEDO et al., 2021) and irrespective of whether the exercise is performed continuously or intermittently (MACDOUGALL et al., 2024). The metabolic response pattern observed in the severe and extreme domains (i.e., a higher rate of depletion of substrates such as phosphocreatine and glycogen and accumulation of metabolites such as H^+ ions and inorganic phosphate)(BLACK et al., 2017; JONES et al., 2008) is commonly used to explain the influence of intensity on the level of peripheral fatigue and the development of pain perception in the exercised muscles (IANNETTA et al., 2022).

Conversely, due to “controlled” systemic and metabolic stress (BLACK et al., 2017; BURNLEY; JONES, 2007; JONES et al., 2008), less impairment of tissue contractile capacity and lower pain perception in exercised muscles are observed after exercise in the moderate and heavy domains (i.e., below the MMSS) (AZEVEDO et al., 2021; IANNETTA et al., 2022; THOMAS et al., 2016). In these scenarios, the reduction in motor drive (central fatigue) due to systemic changes (e.g., reduced blood glucose and brain neurotransmitter concentrations) seems to have a greater contribution to performance fatigability (BROWNSTEIN; MILLET; THOMAS, 2021; MILLET; MARTIN; TEMESI, 2018), and the sensations of “fatigue” and “tiredness” seems to be the most relevant aspect of perceived fatigability (IANNETTA et al., 2022).

In relation to the type of execution, even if the efforts are performed at physiologically paired intensities (e.g., at MMSS), intermittent efforts seem to have a greater metabolic and

neuromuscular demand compared to continuous efforts (e.g. maintenance of higher values of oxygen consumption and blood lactate concentration during exercise)(BILLAT et al., 2000; DITTRICH et al., 2014; GROSSL et al., 2012), which results in greater fatigability of performance after the former compared to the latter (DITTRICH et al., 2014; KESISOGLOU et al., 2021). Although these responses can be influenced by the work:rest ratio (BUCHHEIT; LAURSEN, 2013), it is suggested that the greater physiological demand and consequent exacerbation of performance fatigability after interval exercise is related to the increased contribution of non-oxidative pathways to adenosine triphosphate resynthesis during this type of activity (CHIDNOK et al., 2013b; FIORENZA et al., 2019), leading to higher rates of substrate depletion and accumulation of fatigue-associated metabolites in this type of effort compared to continuous exercise (CHIDNOK et al., 2013b; FIORENZA et al., 2019).

When performed at a matched volume but at different intensities (i.e. interval efforts above MMSS and continuous efforts below MMSS), greater metabolic demands (e.g. maintaining higher blood lactate values during exercise) (NICOLÒ et al., 2014), greater performance fatigability, especially peripheral fatigue, and greater perceived fatigability (more exacerbated perceptions of effort, dyspnea and pain in the exercised muscles) are observed after interval exercises compared to continuous exercises. (MACDOUGALL et al., 2024). These responses are probably associated with the aforementioned differences in the rate of depletion of substrates and accumulation of metabolites induced by the intensity of the effort (i.e. greater above the MMSS compared to below it)(JONES et al., 2008). In contrast, also with paired volume, in efforts involving the stretch-shortening cycle such as running, continuous activities seem to exacerbate the reduction in facilitation of motoneurons by Ia afferent fibers, which may causes greater impairment of the CNS's capacity to activate muscles (central fatigue) after this type of exercise compared to intermittent exercises (BESSON et al., 2020; BROWNSTEIN et al., 2022).

The **Figure 2.2** summarizes the fatigue mechanisms discussed in this chapter and how aspects of exercise such as intensity and type of execution influence them.

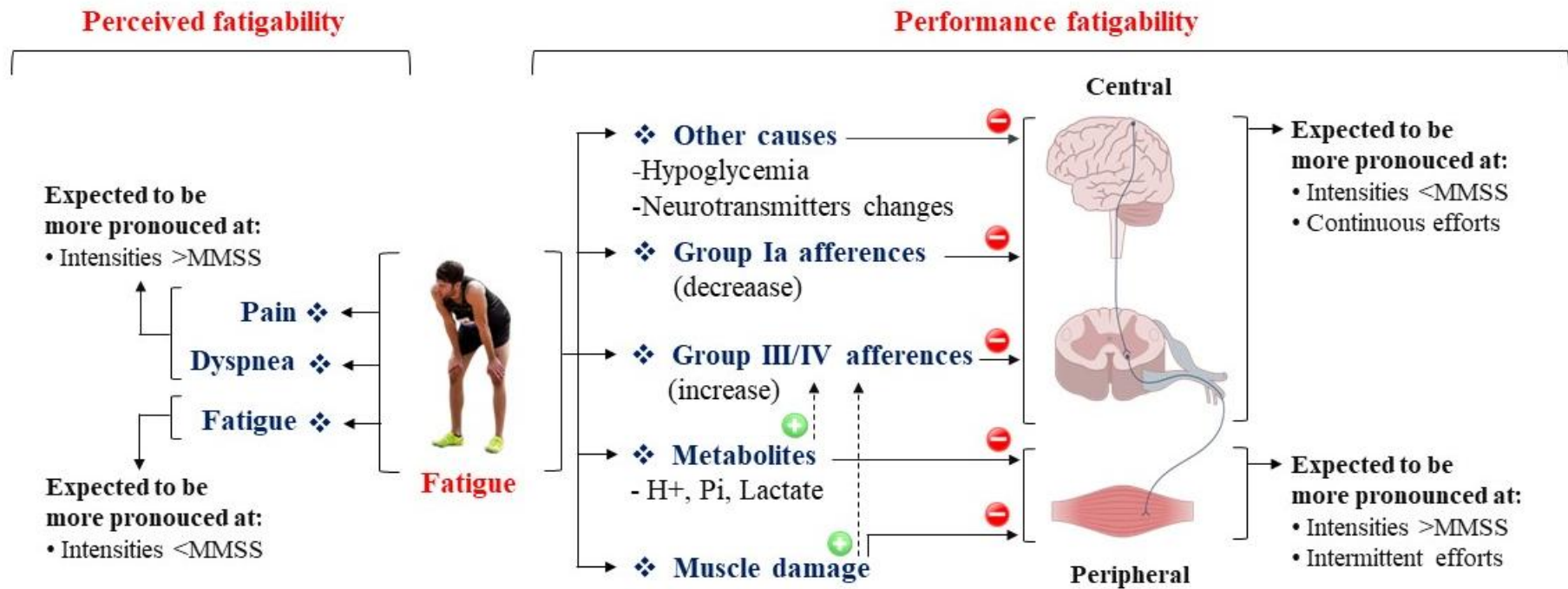


Figure 2.2. Summary of the causes of fatigue discussed in this chapter.

2.2. Methods for assessing muscle fatigue

In view of the definition of fatigue (i.e., reduced performance accompanied by psychophysiological sensations), the most ecological method for assessing it is probably by monitoring physical performance throughout a specific task, in association with recording changes in perceptions such as “level of effort”, “tiredness” and “soreness in the exercised muscles” (ENOKA; DUCHATEAU, 2016; KELLMANN, 2010). Performance fatigability will be identified by the reduction in metrics related to sustained work rate during exercise (MILLET et al., 2023), while perceived fatigability will be identified through perceptual changes estimated using psychometric instruments such as numerical/analogical scales (ENOKA; DUCHATEAU, 2016; KELLMANN, 2010).

However, despite the ecological advantage of this evaluation approach, confounding factors such as “technical aspects” and “recruitment of accessory muscles” can limit accurate conclusions about the impairment generated by exercise on neuromuscular function (ENOKA; DUCHATEAU, 2008). Furthermore, more accurate identification of the causes of reduced performance (i.e., whether due to central or peripheral fatigue) and more comprehensive monitoring of perceived fatigability (i.e., through psychometric tests and/or questionnaires) also become difficult to perform in this evaluation model (ENOKA; DUCHATEAU, 2016; MILLET et al., 2023). Thus, an alternative strategy widely used by the scientific community is to interrupt the exercise being investigated to carry out brief tests involving isolated force/power production and a more comprehensive assessment of psychophysiological aspects (**Figure 2.3**).

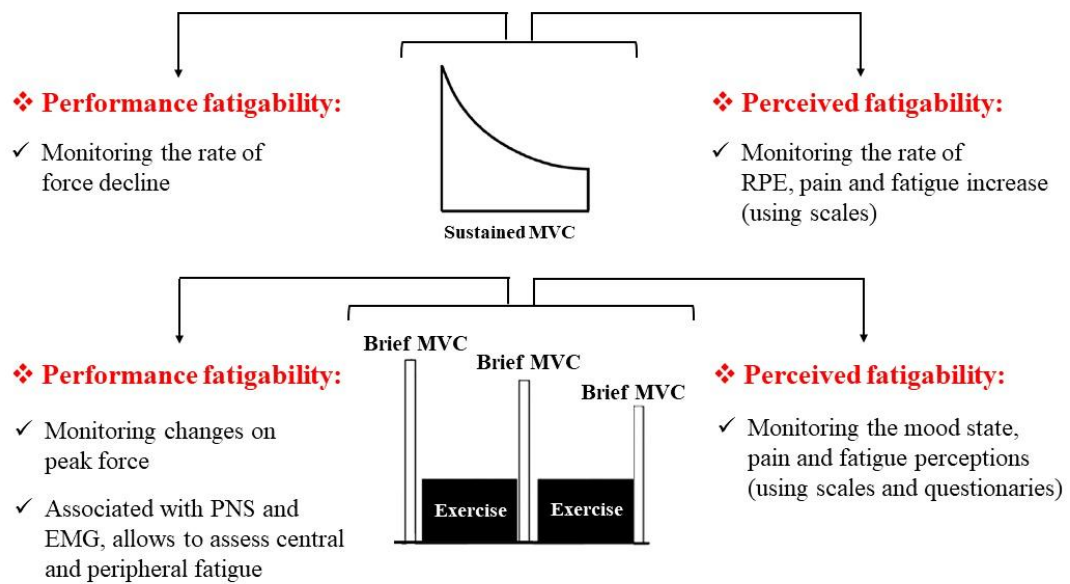


Figure 2.3. Two distinct and common protocols used to assess fatigue. MVC, maximal voluntary contractions; RPE, rating of perceived effort; PNS, peripheral stimulation; EMG, electromyography activity. Adapted from Millet et al., (2023).

To assess performance fatigability, brief tests involving maximal voluntary contractions (MVCs) of the exercised muscles are usually carried out at rest and at other times of interest (e.g., halfway through and/or immediately after the end of exercise) (MILLET et al., 2023). Reductions in the peak force produced during these tests will indicate the establishment of disorders in the neuromuscular system (performance fatigability) (ENOKA; DUCHATEAU, 2008). To obtain more details about these disorders, these tests can be performed accompanied by peripheral electrical stimulation during MVCs and with the relaxed muscle (twitch interpolation technique) and by the monitoring the electromyography activity (EMG) of the recruited muscles. With these outcomes, inferences can be made about the functioning level of the central (i.e. above the neuromuscular junction) and peripheral components of the motor pathway (i.e. below the neuromuscular junction). (MILLET et al., 2011). In this kind of evaluation protocol, reductions in the force and amplitude of the EMG responses evoked by electrical stimulation with the relaxed muscle (e.g., potentiated twitch and M-wave, respectively) indicate specific impairments to the function of the peripheral components of the neuromuscular system, since they do not involve relevant activation of the central nervous system (Millet et al., 2011). In relation to the central components of the motor pathway, the increase in force produced during MVC using doublet high-frequency stimuli (100 Hz) and the force evoked at rest are used to calculate the percentage of voluntary activation (%VA) of a

specific muscle group ($\%VA = [1 - \text{increased force}/\text{force evoked at rest}] \times 100$). (Merton, 1954). After exercise, a decrease in %VA is recognized as an indicator that the function of the central components of the motor pathway is reduced (central fatigue) (Vollestad, 1997). The force and EMG signals recorded in this protocol and the neuromuscular function outcomes obtained by them are described in **Figure 2.4**.

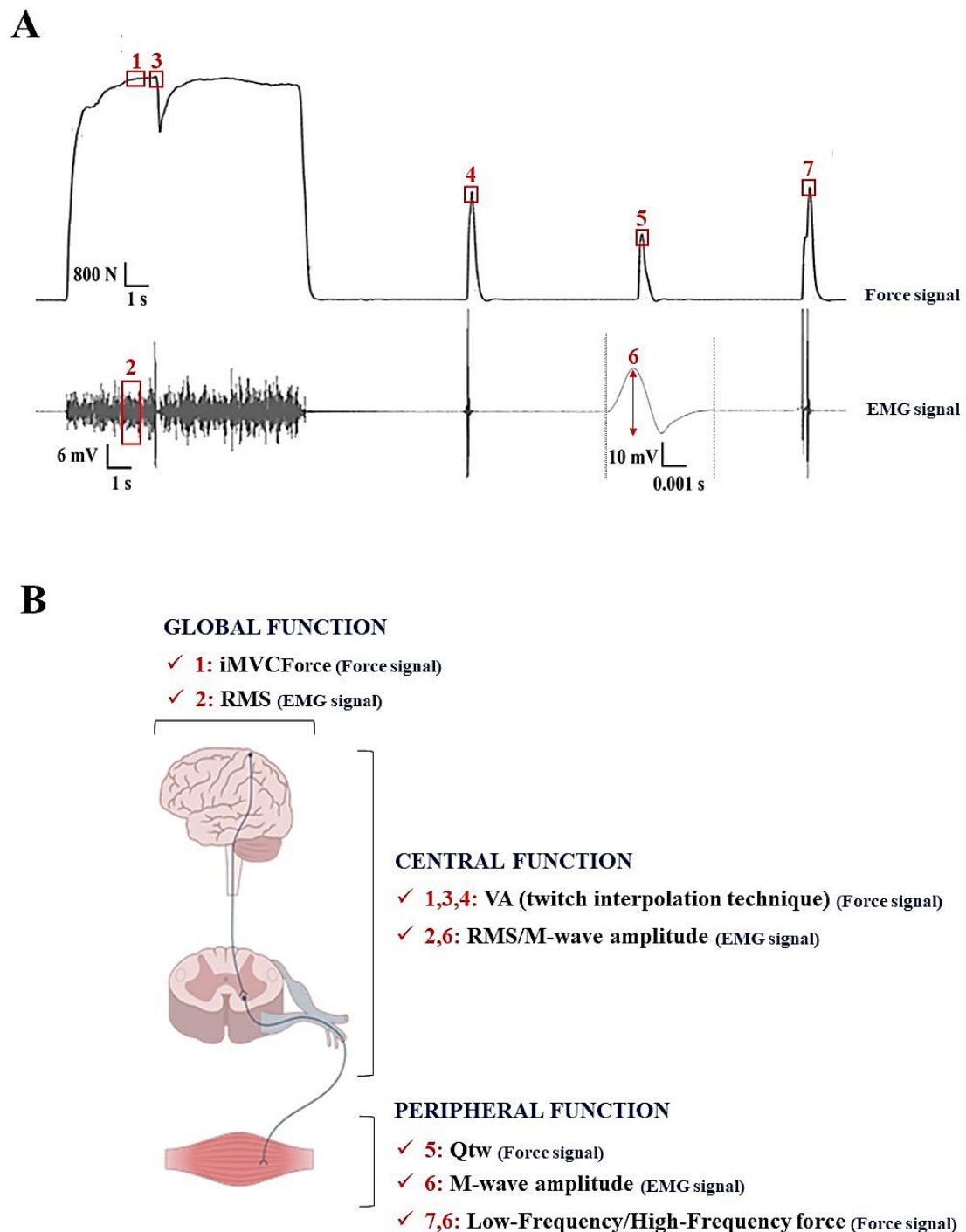


Figure 2.4. A: schematic representation of the force signal and electromyographic (EMG) activity signal recorded during a neuromuscular assessment protocol involving maximum voluntary contractions and peripheral electrical stimulation; B: neuromuscular function outcomes obtained through this assessment protocol. iMVC_{Force}: maximum voluntary force; RMS: root

In addition to protocols involving MVCs, another tool commonly used to measure performance fatigability is countermovement vertical jumps (CMJ) (Claudino et al., 2017). Besides covering the assessment of relevant functional aspects of the neuromuscular system, which are not covered by tests involving MVCs (e.g., stretch-shortening cycle) (Taylor et al., 2012),

assessment protocols using CMJs seem to be a more accessible tool for the “practical scenario” of sport (Oliver et al., 2008; Mooney et al., 2013; Freitas et al., 2014). Reduction in the height of CMJs after acute and/or blocks of exercise (days, week) is considered a sensitive indicator of disturbance in the neuromuscular system's function (Claudino et al., 2017). In addition, other findings from the kinetic and kinematic analysis of CMJs such as peak force, rate of force development, peak power and peak velocity can also be used to characterize exercise-induced changes in the function of the neuromuscular system (Cormack et al., 2008; McLean et al., 2010).

In order to assess perceived fatigability, in addition to numerical/visual scales intended to evaluate aspects such as “perception of effort” (BORG, 1982), “perception of pain in exercised muscles” (BORG et al., 2010), and “general perception of fatigue” (MICKLEWRIGHT et al., 2017)), the interruption of the investigated effort allows the use of psychometric instruments capable of providing more comprehensive information on the individual's mental/affective state (KELLMANN, 2010). Among these instruments, the Profile of Mood State Questionnaire (POMS) stands out as one of the most sensitive and widely used (LEUNES; BURGER, 2000). In POMS, using a numerical scale ranging from 0 to 4 (“0” represents “nothing” and 4 “extremely”), the individual is asked to rate how they feel in relation to 65 different “perceptions/feelings” such as “friendly”, “tense”, and “exhausted” (PRAPAVESSIS, 2000). From these results, the magnitude of 6 subcomponents of mood (“tension”, “depression”, “anger”, “vigor”, “fatigue” and “confusion”) is estimated (LEUNES; BURGER, 2000).

Furthermore, through interactions between these subcomponents, aspects such as “total mood disturbance” (calculated by subtracting the “vigor” scores from the sum of the “tension” + “depression” + “anger” + “fatigue” + “confusion” scores) and “energy index” (calculated by subtracting the “fatigue” score from the “vigor” score) can also be estimated. (BEEDIE; TERRY; LANE, 2000). In general, increases in the “total mood disturbance” score and decreases in the “energy index” score are used to suggest the onset of fatigue (BERGLUND; SÄFSTRÖM, 1994; O’CONNOR; MORGAN; RAGLIN, 1991; RIETJENS et al., 2005; UMEDA et al., 2008).

2.3. Post-exercise recovery: definitions and monitoring

In view of the transient impairment induced by exercise on different body systems (e.g., neuromuscular, cardiovascular)(SKORSKI et al., 2019) and the negative impact of these impairments on physical and mental aspects (i.e., instauration of performance and perceived fatigability)(ENOKA; DUCHATEAU, 2016), post-exercise recovery is understood as a multifactorial process over time in which the restoration of physiological/psychological balance is observed, allowing the individual to reach and/or exceed previous performance metrics (KELLMANN et al., 2018). Aspects such as intensity, type of contraction and volume influence the stress triggered by exercise on the body's systems and, consequently, the recovery process (KELLMANN et al., 2018). Thus, it is suggested that recovery should be assessed/interpreted considering the characteristics of the exercise performed (SKORSKI et al., 2019).

For example, in exercises of short duration (e.g., <30-s), carried out at high-intensity (i.e., extreme domain), phosphocreatine stores in muscle tissue rapidly reduce, impairing the ability to maintain desired levels of force/power (i.e., instauration of performance fatigability) (CHIDNOK et al., 2013b). However, given the rate at which phosphocreatine is resynthesized (FORBES et al., 2009; MCCANN; MOLÉ; CATON, 1995), around 40-60 seconds is enough to provide satisfactory recovery of these stores and allow performance to be maintained in subsequent efforts (SKORSKI et al., 2019). However, with an increase in the duration of the effort (e.g. >30-s) or the number of efforts (e.g. >2), the contribution of the glycolytic pathway to resynthesize adenosine triphosphate increases, resulting in an higher rate of muscle glycogen depletion and higher accumulation of metabolites linked to reduction in the tissue's contractile function (e.g., Pi and H⁺) (FIORENZA et al., 2019). In this context, although the removal of metabolites from the tissue occurs within minutes (HUG et al., 2006), the restoration of glycogen stores can take hours (MACDOUGALL; WARD; SUTTON, 1977; VAN HALL; SHIRREFFS; CALBET, 2000), requiring a longer recovery time to maintain performance in subsequent efforts.

Furthermore, performing repetitive submaximal contractions (i.e., endurance efforts) not only impairs neuromuscular function, but also causes transient impairment in the function of other systems such as the cardiovascular system and the CNS (SKORSKI et al., 2019). In relation to cardiovascular system, exercise-induced changes such as increase in systemic adrenaline concentration, reductions in plasma volume (due to sweating/dehydration) and accumulation of metabolites at muscles and blood, modify, via reflex responses, how the heart and blood vessels

work to maintain blood pressure and cardiac output (MICHAEL; GRAHAM; GLEN M DAVIS, 2017). These changes involve alterations in the activity of the autonomic nervous system on heart, whereby reduction in the activity of the parasympathetic component and increase in the activity of the sympathetic component are observed (STANLEY; PEAKE; BUCHHEIT, 2013). Depending on aspects such as the concentration of metabolites accumulated in the tissue/blood and reductions in plasma volume (influenced by aspects of exercise such as intensity and volume), the return to the pre-exercise state of autonomic nervous system activity on heart can take from minutes to days (STANLEY; PEAKE; BUCHHEIT, 2013).

In relation to decreases in CNS function, these can occur for different reasons (e.g., inhibitory afferent reflex responses, reduced blood glucose, changes in brain concentrations of neurotransmitters) (MILLET; MARTIN; TEMESI, 2018) and, beyond other consequences, will cause a limitation in the ability of this system to activate a muscle group, reducing the capacity to generate force/power voluntarily (i.e., central fatigue). (ENOKA; DUCHATEAU, 2016). Depending on the causal mechanisms (e.g., whether due to glycogen depletion, increase core temperature or muscle damage), adequate recovery of this functioning aspect of CNS can last from minutes to days (CARROLL; TAYLOR; GANDEVIA, 2017). In addition, changes at the CNS level are also involved in other important aspects of recovery and performance maintenance such as concentration, motivation, decision-making, mood and the individual's perception of readiness/recovery (KELLMANN et al., 2018). Depending on physiological factors (e.g., imbalance in the autonomic nervous system (MOUROT et al., 2004), ongoing inflammatory process (SMITH, 2000)), and behavioral factors (e.g., study routine, work, competitions) (RUSSELL et al., 2019), these CNS function-related aspects can take from minutes to weeks to return to their pre-exercise values.

In order to mitigate acute/chronic decline in performance and health risks (e.g., injury, non-functional overreaching, overtraining), proper monitoring of the post-exercise recovery process is deemed mandatory (KELLMANN, 2010). In this context, evaluating performance *per se* in an ecological test, close to the physical and mental demands of the sport (i.e. intensity, volume, technical actions), is undoubtedly the most accurate method for distinguishing between the “unrecovered” and “recovered” states (KELLMANN et al., 2018). However, carrying out this type of assessment causes additional stress on the body's systems, which can delay recovery or even exacerbate the impairments already established (HALSON, 2014b). Thus, other

“surrogate markers” of recovery process are commonly used in the practical and scientific scenarios (NÉDÉLEC et al., 2012).

For monitoring the recovery of neuromuscular function (i.e. ability to produce force/power), as described in Chapter 2.2 (*Muscle fatigue assessment methods*) tests involving isolated efforts such as CVMs and CMJs are widely used (CLAUDINO et al., 2017; ENOKA; DUCHATEAU, 2008). Furthermore, in order to obtain greater detail in this scenario (e.g. muscle activation capacity by the CNS, contractile capacity of the tissue), additional evaluations involving the monitoring of EMG activity and peripheral stimulations can be carried out (MILLET et al., 2011). By reaching or exceeding performance recorded before exercise in this type of assessment, it is assumed that the individual is “recovered”, at least in terms of neuromuscular function.

In order to verify the recovery of other physiological systems beyond the neuromuscular, the analysis of heart rate variability (HRV) at rest is widely suggested (MICHAEL; GRAHAM; GLEN M DAVIS, 2017). Although it is specifically linked to the functioning of the cardiovascular system and does not correlate with the recovery from other imbalances caused by exercise (e.g. metabolic, structural), the return of HRV metrics to “baseline” values or higher is usually assumed to be an indicator of “global recovery of the individual” (STANLEY; PEAKE; BUCHHEIT, 2013). Among the possible HRV-derived metrics, those associated with activity of the parasympathetic system (e.g., RMSSd, SDNN) are often used and suggested to monitor the individual's state of recovery (BUCHHEIT, 2014).

When monitoring the recovery of psychophysiological aspects, psychometric instruments such as scales and questionnaires are usually applied (KELLMANN et al., 2018). In addition to the instruments already mentioned in Chapter 2.2 (i.e., numerical scales to assess the general perception of “fatigue”(MICKLEWRIGHT et al., 2017) and the POMS questionnaire to characterize the individual's mood state (LEUNES; BURGER, 2000)), other instruments more associated with the perception of recovery such as the Perception of Recovery Scale (KENTTÄ; HASSMÉN, 1998), the Acute Recovery and Stress Scale (NÄSSI et al., 2017), the Short Recovery and Stress Scale (NÄSSI et al., 2017) and the Recovery Stress Questionnaire (KÖLLING et al., 2020) can also be used. Moreover, specific tests to assess the recovery of other psychophysiological aspects related to cognitive function such as inhibitory control (e.g., Stroop color test) (DALLAWAY; LUCAS; RING, 2022) and decision-making ability (e.g., go/no-go test) (GUO et al., 2018) may also be employed.

Finally, monitoring physiological/psychological responses during brief efforts, similar to those of the sport/exercise, can also be an alternative for monitoring the individual's state of recovery (KELLMANN et al., 2018). This approach reduces the risk of additional impairment to body systems (as may occurs in tests closer to “real” physical/mental demands) and provides more ecological information on the individual's state of recovery compared to “surrogate markers” obtained by analyzing MVCs, CMJs, HRV, scales and questionnaires (SKORSKI et al., 2019). With the body's systems still partially impaired by the exercise (i.e. performance and perceived fatigability still present), the physiological responses during effort will tend to be different. For example, for the same work rate (e.g., power, velocity), oxygen consumption and heart rate (GLACE; MCHUGH; GLEIM, 1998; NICOL; KOMI; MARCONNET, 1991), and perceptual responses (e.g., RPE) (HAUSSWIRTH et al., 2000; SABATER-PASTOR et al., 2023) during efforts in a “fatigued state” are greater compared to the “rested state”. These changes indicate a greater physiological/psychological demand to perform a same work rate, suggesting less efficiency in the body's systems as a consequence of incomplete recovery (SKORSKI et al., 2019).

The **Figure 2.5** summarize the methods for assessing the fatigue-recovery continuum described in this chapter.

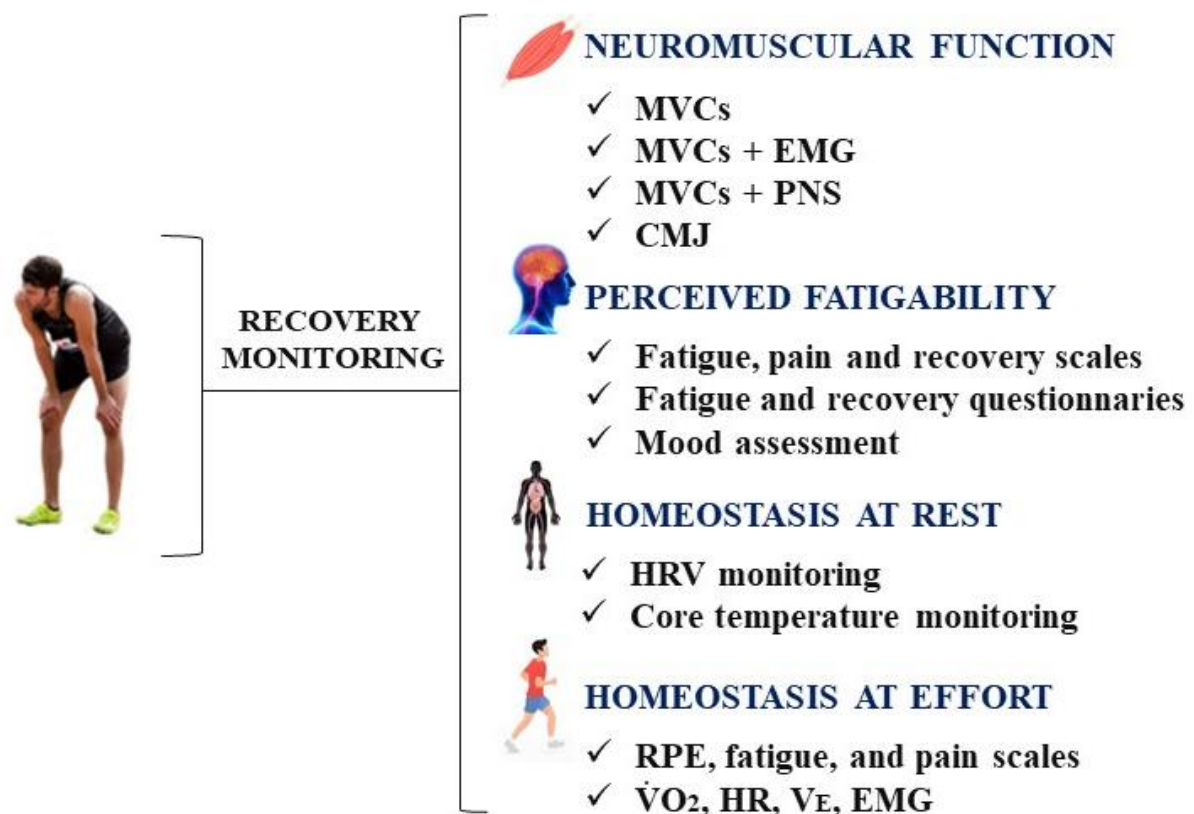


Figure 2.5. Schematic representation of the methods for assessing the fatigue-recovery continuum described in this chapter. MVC, maximal voluntary contraction; EMG, monitoring of electromyography activity of the exercised muscles; PNS, peripheral stimulation; CMJ, countermovement jumps; HRV, heart rate variability; RPE, rating of perceived effort; $\dot{V}O_2$, oxygen consumption; HR, heart rate; V_E , ventilation.

2.4. Strategies to improve post-exercise recovery

Post-exercise recovery is understood as a multifactorial process over time, in which the restoration of physiological and mental capacities is observed (KELLMANN et al., 2018). In order to ensure the proper course of this process, the scientific literature emphasizes that “primary conducts” need to be followed and that “secondary conducts” can be implemented (NÉDÉLEC et al., 2012). The “primary conducts” are those related to sleep, nutrition, and hydration. Briefly, in relation to sleep, it is recommended to implement aspects such as >7 hours of quantity, 10-20 minutes of latency (i.e. time to fall asleep) and absence of interruptions be implemented (HALSON, 2014a). For nutrition and hydration, consumption of minimal amounts of carbohydrates (1-1.2g/kg of body weight/hour) and fluids (1.25-1.5 L/kg of body weight lost during exercise) is recommended (MCCARTNEY; DESBROW; IRWIN, 2018). “Secondary conducts” are all those additional strategies that can be used to supplement the “primary conducts” with the aim of optimizing recovery (NÉDÉLEC et al., 2012). Among the wide range of possibilities for “secondary conducts” (e.g., active recovery, supplementation,

mental relaxation techniques, massage) (DUPUY et al., 2018; MIHAJLOVIC et al., 2023), some of the most widely used and researched are those related to body immersion in cold-water (ALLAN et al., 2022b; VERSEY; HALSON; DAWSON, 2013) or hot-water (CHAILLOU et al., 2022; HYLDAHL; PEAKE, 2020; MCGORM et al., 2018).

2.4.1. Cold-water immersion

Cryotherapy techniques have been used to treat illnesses and promote general well-being for centuries (TIPTON et al., 2017). Cryotherapy is an umbrella term that covers interventions aimed at removing heat from body structures (ALLAN et al., 2022a). Among the cryotherapy modalities used in the sports field to improve post-exercise recovery, cold-water immersion (CWI) is the most popular (ALLAN et al., 2022a). The literature has shown that CWI, through its basic mechanisms of action (e.g. hydrostatic pressure, reduction in temperature and local blood flow), triggers responses in different body systems that may be beneficial to the recovery process following exercise (IHSAN; WATSON; ABBISS, 2016).

One of the most well-established effects of CWI is its ability to reduce exercise-induced muscle soreness (KNOWLTON et al., 2013). It has been demonstrated that the reduction in skin temperature induced by CWI activates specific cutaneous receptors (transient receptor potential cation channel M8, TRPM8) located in the A delta and C fibers, which through inhibitory afferent inputs on interneurons in the spinal cord result in analgesia (KNOWLTON et al., 2013). Reducing the perception of pain in exercised muscles, in addition to mitigate this relevant aspect of perceived fatigability (IANNETTA et al., 2022), also aids in the recovery of performance fatigability, given the involvement of this sensation with impairments to neuromuscular function (e.g. reduced motor drive to the muscles)(ABOODARDA et al., 2020; GRAVEN-NIELSEN et al., 2002).

In addition to analgesia mediated by the nervous system, it has been suggested that CWI can mitigate the perception of pain by attenuating exercise-induced muscle damage (KWIECIEN; MCHUGH, 2021). Due to metabolic and/or mechanical stress, exercise causes tissue micro-injuries with the consequent generation of an inflammatory process and the appearance of classic signs and symptoms such as pain and oedema (PEAKE et al., 2017). The initial stages of inflammation in this scenario involve, among other events, the proliferation of the already established injury, a process known as secondary tissue damage (MERRICK, 2002). During this stage, it is suggested that CWI, through mechanisms such as reducing local tissue

temperature and blood flow, attenuates events involved in the proliferation of the inflammatory process (e.g. bleeding, migration and activation of immune system cells)(MERRICK et al., 1999). By reducing the magnitude of these events, CWI seems to reduce the magnitude of secondary tissue damage and, consequently, its repercussions (e.g., pain and loss of function) (KWIECIEN; MCHUGH, 2021; MERRICK et al., 1999). Furthermore, due to oedema, less oxygen is delivered to the muscles as a result of mechanical compression of the capillaries and an increase in the distance between them and the tissue, resulting in secondary hypoxic injury (MERRICK, 2002). By reducing blood flow to the tissue, CWI reduces the magnitude of oedema and, consequently, secondary hypoxic injury and its repercussions (YANAGISAWA et al., 2004). Importantly, it is suggested that these beneficial effects of CWI on secondary damage will be observed mainly when it is used between immediately-after and 12 hours post-exercise (KWIECIEN; MCHUGH, 2021).

Due to its ability to induce vasoconstriction and due to the effects of hydrostatic pressure, CWI also increases central blood volume and cardiac pre-load, consequently enhancing arterial pressure, stroke volume and cardiac output (MOURROT et al., 2008). These changes further activate cardiopulmonary baroreflexes linked to the inhibition of sympathetic activity and increasing of parasympathetic activity on heart (JDIDI et al., 2024). Thus, CWI has been suggested as an effective strategy to optimize the recovery process by increasing the cardiac parasympathetic activity after exercise (IHSAN; WATSON; ABBISS, 2016), a commonly used marker of “global recovery” (STANLEY; PEAKE; BUCHHEIT, 2013).

In view of the effectiveness of CWI in reducing core/body temperature, it is suggested that it may also be beneficial to the recovery process, attenuating the deleterious effects of hyperthermia on different body systems, especially the CNS (IHSAN; WATSON; ABBISS, 2016). In this context, different studies have reported the efficacy of CWI in increasing the capacity of the CNS to activate muscles after high-intensity efforts (MINETT et al., 2014; POINTON et al., 2012)). Although there is no clear understanding, it has been speculated that the improvement in CNS function after reducing body temperature by CWI occurs due to changes in cortical electrical activity such as the increase in $\alpha:\beta$ ratio (DE PAUW et al., 2013) and changes in the concentrations of neurotransmitters such as dopamine and serotonin (HIRVONEN et al., 2002; IHSAN; WATSON; ABBISS, 2016). Furthermore, from these changes in cortical electrical activity and neurotransmitters concentration, and through the release of endorphins (HIRVONEN et al., 2002), CWI seems to be able to have a positive

impact on recovery by reducing the global “perception of fatigue” (DUPUY et al., 2018), the perception of effort in subsequent tasks (DE PAULA et al., 2018; VAILE et al., 2008a), and by improving mood (YANKOUSKAYA et al., 2023) and promoting a feeling of ‘well-being’ (HUTTUNEN; KOKKO; YLIJUKURI, 2004).

In addition, it is important to highlight that the individual's beliefs and expectations about the effectiveness of the CWI can contribute to promoting and/or potentiating its effects in the recovery process (ALLAN et al., 2022b). This beneficial contribution stimulated strictly by the individual's belief is known as the placebo effect and appears to be inherent in many of the interventions that purport to benefit human health/performance (HURST et al., 2020). Despite the many cited and well-established physiological effects of CWI, it is likely that the placebo effect also contributes to the effectiveness of this method in enhancing the recovery process (BROATCH; PETERSEN; BISHOP, 2014; NASSER et al., 2023).

Despite the wide heterogeneity of protocols and the influence of individual aspects (e.g., body fat and body mass) on the effectiveness of CWI (STEPHENS et al., 2017), it is recommended that this intervention should be performed with the body submerged at least up to the height of the xiphoid process, in water with a temperature between 10-15°C, for 10-15 minutes (MACHADO et al., 2016; MOORE et al., 2022). Using these protocols and based on the mechanism mentioned above, systematic reviews of the literature have demonstrated the effectiveness of CWI in improving distinct recovery metrics, including: (i) maintaining performance during subsequent endurance efforts (60-90 minute intervals); (ii) increasing the capacity to produce maximal force/power; and (iii) reducing perceptions of muscle soreness and global fatigue between hours to days after different exercises (CHAILLOU et al., 2022; CHOO et al., 2022; MACHADO et al., 2016; MOORE et al., 2022).

The **Figure 2.6** summarize the mechanisms that support CWI as an effective method to improve recovery post-exercise.

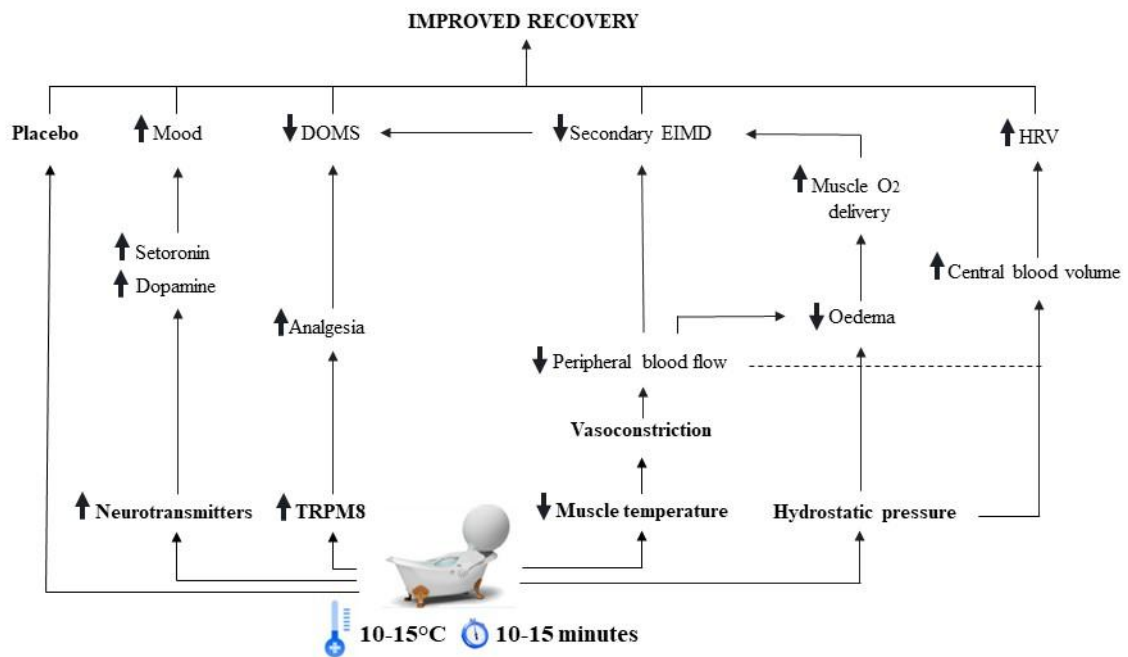


Figure 2.6. Schematic representation of the mechanisms of CWI described in this chapter, which support its use to improve the post-exercise recovery process. DOMS, delayed onset muscle soreness; EIMD, exercise-induced muscle damage; TRPM8, Transient Receptor Potential Melastatin 8. Adapted from Ihsan et al., (2016).

2.4.2. Hot-water immersion

Like cold, heat has been used to treat various illnesses and promote general well-being for centuries (PAPAIOANNOU et al., 2016). Among the therapeutic possibilities of using heat for optimize post-exercise recovery is hot-water immersion (HWI) (MCGORM et al., 2018). In addition to the effects induced by hydrostatic pressure per se (i.e., increased central blood volume) (WILCOCK; CRONIN; HING, 2006), the increase in muscle temperature (RODRIGUES et al., 2020a) and blood flow (HEINONEN et al., 2011) induced by HWI seem to be the primary physiological mechanisms that suggest its use as a beneficial method for optimize the post-exercise recovery process (VERSEY; HALSON; DAWSON, 2013).

Similarly to CWI, HWI has been shown to be beneficial for the recovery process by reducing muscle pain/soreness (PETROFSKY et al., 2017, 2015). This analgesic effect seems to occur mainly through the inhibition of purine receptors such as transient receptor potential vanilloid 1 and 4 (TRPV1 and TRPV4, respectively) (BREDEPERSON; KYM; SZALLASI, 2013), involved in creating the perception of pain (BURNSTOCK, 2013). Furthermore, due to the ability of heat to change the viscoelastic properties of tissues (increased extensibility (NADLER; WEINGAND; KRUSE, 2004)), it is suggested that HWI also promotes analgesia by interrupting the pain-spasm-pain cycle (PETROFSKY et al., 2017). As mentioned above for CWI, HWI via its analgesic effect may also contributes to alleviating impairments on force-

generating capacity (ABOODARDA et al., 2020; GRAVEN-NIELSEN et al., 2002). In addition, due to the ability of HWI in increase tissue extensibility (NADLER; WEINGAND; KRUSE, 2004), it can also favor the recovery of performance fatigability by maintaining/increasing joint range of motion, an aspect that is impaired by exercise and that contributes to force/power production, especially in dynamic contraction/tasks (THORPE, 2021b; WILCOCK; CRONIN; HING, 2006).

Another physiological response triggered by HWI that may be relevant to optimizing the recovery process is the increase in the velocity of enzymatic processes (MCGORM et al., 2018). It is suggested that for every 1°C increase in tissue temperature, the velocity of enzymatic processes increases by 10-15% (NADLER; WEINGAND; KRUSE, 2004). In the recovery process, this effect of HWI seems to be beneficial, among other reasons, because it seems to increase the rate of resynthesis of muscle glycogen stores (CHENG et al., 2017), a key substrate source for tissue contraction. Besides, HWI seems also contributes to increasing the rate of glycogen resynthesis by increasing the delivery of glucose to muscle tissue as a result of increased local blood flow and by stimulating glucose consumption by the tissue (CHENG et al., 2017). Both effects, increased muscle blood flow and glucose consumption, are mediated by the activation of heat shock proteins that stimulate, respectively, vasodilation via the nitric oxide pathway (BRUNT et al., 2016) and the translocation of glucose transporter 4 (GLUT-4) to the sarcolemma (WU; LUO; CHANG, 2015).

Furthermore, it is suggested that HWI can also impact other processes along the motor pathway that favor the recovery of the capacity to produce force/power (WILCOCK; CRONIN; HING, 2006). For example, due to the increase in local blood flow, it is suggested that HWI increases the clearance of metabolites harmful to the contractile function of the tissue (COTÉ et al., 1988), which may favor the recovery of the capacity to produce force/power. From a neural perspective, HWI seems to be able to increase nerve conduction velocity (RACINAIS; OKSA, 2010) as well as the activity of Ia fibers (MENSE, 1978; NEWTON; LEHMKUHL, 1965; SATO, 1983), involved in the facilitation of motoneurons and whose functioning is reduced after efforts involving the stretching-shortening cycle (AVELA et al., 1999; AVELA; KYRÖLÄINEN; KOMI, 1999a). By increasing the nerve conduction velocity and the activity of Ia fibers, it is suggested that HWI may favor the recovery of CNS muscle activation capacity and consequently the force/power production (MINETT; DUFFIELD, 2014; SAUTILLET et al., 2024). Besides, HWI seems also stimulates a greater release of calcium by the sarcoplasmic

reticulum and increases the sensitivity of myofibrils to this ion, which favors tissue contractility (CHENG et al., 2017; RODRIGUES et al., 2022). In addition, due to the increase in local temperature, HWI can also facilitate the unloading of oxygen from hemoglobin in the muscle capillaries (GOMEZ-CAMBRONERO, 2001), which may help to optimize repair processes in the muscle fiber and, consequently, enhance the recovery of its force-generating capacity (NADLER; WEINGAND; KRUSE, 2004).

As argued for CWI, a wide variety of HWI protocols are described in the literature. Although there is no consensus or “dose-response” studies involving HWI, in general, studies that observed most of the physiological effects mentioned above performed this intervention by submerging the body minimally up to the height of the xiphoid process in water between 38-42°C for 30-90 minutes. (BRUNT et al., 2016; COERTJENS et al., 2023; RODRIGUES et al., 2020a, 2023; SAUTILLET et al., 2024). With a less consistent literature compared to CWI (CHAILLOU et al., 2022), using such protocols and based on the mechanisms described above, HWI has been shown to be effective in accelerating the recovery of maximum force-generating capacity (KULIGOWSKI et al., 1998; SAUTILLET et al., 2024; VAILE et al., 2008b) and fatigue resistance (CHENG et al., 2017), as well as reducing perceptions of soreness in the exercised muscles (PETROFSKY et al., 2015; WANG et al., 2021), between hours to days after distinct exercises (CHAILLOU et al., 2022). As described for CWI, individuals' expectations about the effects of HWI may also contribute to its effectiveness (i.e., placebo effect) (HURST et al., 2020).

The **Figure 2.7** summarize the mechanisms that support HWI as an effective method to improve recovery post-exercise.

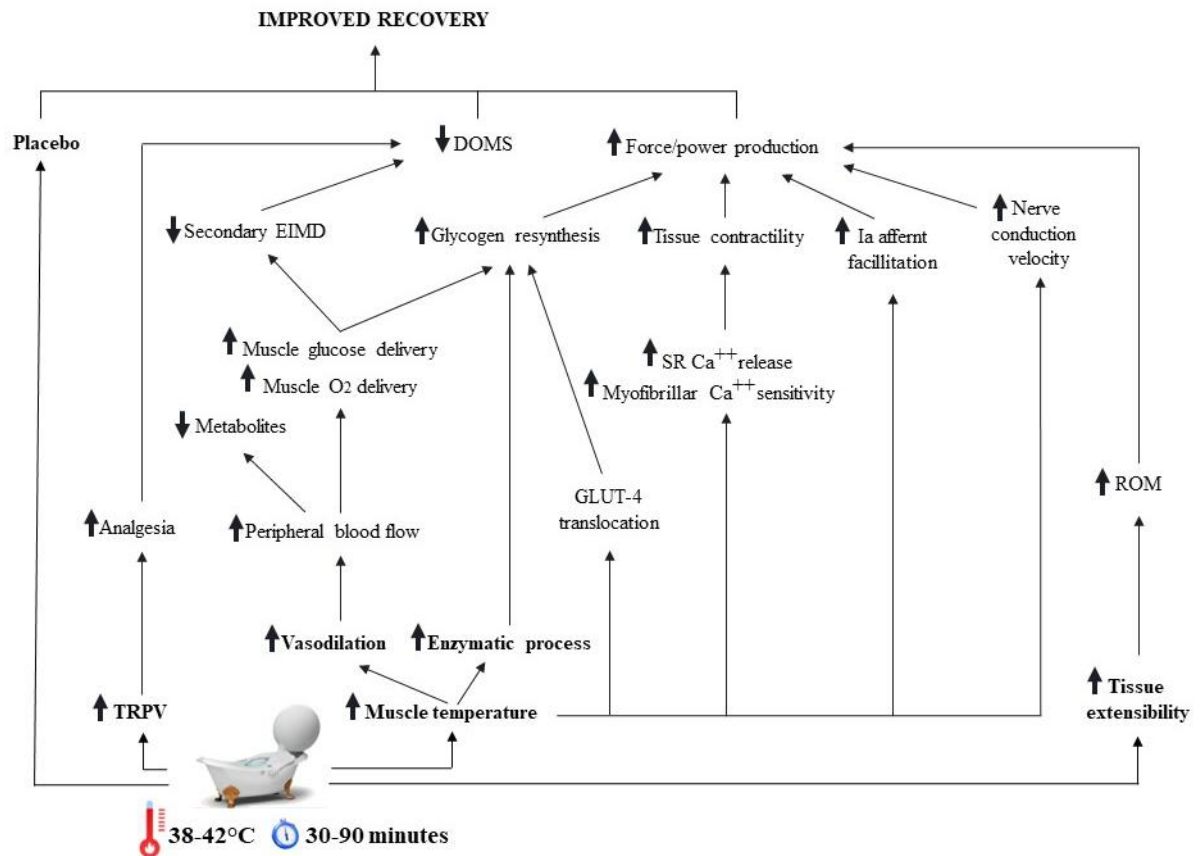


Figure 2.7. Schematic representation of the mechanisms of HWI described in this chapter, which support its use to improve the post-exercise recovery process. DOMS, delayed onset muscle soreness; EIMD, exercise-induced muscle damage; TRPV, Transient Receptor Potential Vanilloid; ROM, range of motion; SR, sarcoplasmic reticulum. Adapted from Ihsan et al., (2016).

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CHAPTER 3.
RELIABILITY OF NEUROMUSCULAR FUNCTION
ASSESSMENT IN FEMALES AND MALES

3. STUDY 1. Within- and between-day reliability and repeatability of neuromuscular function assessment in females and males

Dutra YM, Lopes JPF, Murias JM, Zagatto AM. Within- and between-day reliability and repeatability of neuromuscular function assessment in females and males. *J Appl Physiol* (1985). 2023 Dec 1;135(6):1372-1383. doi: 10.1152/jappphysiol.00539.2023.

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3.1. Abstract

The study evaluated the reliability and repeatability of the force and surface electromyography activity (EMG) outcomes obtained through voluntary and electrically evoked contractions of knee extensors in females (n=18) and males (n=20), and compared these data between sexes. Maximal isometric voluntary contractions (iMVCs) of knee extensors associated with electrical stimulation of the femoral nerve were performed over 4 days (48h-interval), with the first day involving familiarization procedures, the second involving three trials (1 h interval), and the third and fourth involving just one trial. The intraclass correlation coefficient (ICC), coefficient of variation (CV), and repeatability of outcomes from within- and between-day trials were determined for each sex. Females presented lower maximal voluntary force during iMVC ($iMVC_{Force}$) and associated *vastus lateralis* EMG activity (root mean square, RMS_{VL}), force evoked by potentiated doublet high-frequency ($Db_{100Force}$) and single stimuli (Q_{tw}), and M-wave amplitude than males ($p \leq 0.01$, partial eta squared ≥ 0.94). Voluntary activation (VA) and RMS_{VL} /M-wave amplitude did not differ between sexes. $iMVC_{Force}$, VA, $Db_{100Force}$, Q_{tw} and M-wave amplitude were the most reliable outcomes in within-day trials, with similar results between sexes (ICC > 0.62; CV < 6.4%; repeatability: 12.2 to 22.6%). When investigating between-days trials, the $iMVC_{Force}$, VA, $Db_{100Force}$ and Q_{tw} were the most reliable (ICC > 0.66; CV < 7.5%; repeatability: 13.2 to 33.45%) with similar results between sexes. In conclusion, females presented lower $iMVC_{Force}$ and evoked response than males. Although reliability and repeatability statistics vary between trials, data (e.g., from EMG or force signal) and sexes, most of outcomes obtained through this technique are reliable in females and males.

Keywords: twitch interpolation; peripheral nerve stimulation; electromyography; M-wave; muscle fatigue.

3.2. Introduction

Voluntary force-generating capacity requires the integrated action of distinct components which include the central nervous system, motor neurons, sensory neurons, and skeletal muscle fibers (1). Maximal isometric voluntary contractions (iMVC) in combination with electrical stimuli to the motor nerve (peripheral nerve stimulation; PNS) during iMVC as well as in the relaxed muscle allow to differentiate the functioning level of these components as follow: i) peripheral components (i.e., located below the neuromuscular junction); ii) central components (i.e., above the neuromuscular junction) (2, 3). More specifically, force evoked responses to PNS during iMVCs assess the capacity of the central nervous system to voluntarily activate a muscle group (4–6), while the involuntary force and electromyography activity (EMG) evoked by PNS in the relaxed muscle (e.g., potentiated twitch and M-wave, respectively), offer a way to assess the function of the neuromuscular system at the peripheral level (2).

The assessment of knee extensors iMVC and PNS are frequently used to investigate acute changes on neuromuscular function in a variety of conditions such as nutritional interventions (e.g., caffeine (7), beta-alanine (8) and creatine (9) supplementation), efforts intending to evoke post-activation potentiation (e.g., drop jumps (10)), and exercises leading to muscle performance fatigability (11–13) or damage (14, 15). In this way, assessments are generally performed several times in the same day and/or across days to track and characterize changes in neuromuscular function induced by a given intervention (14–16).

However, despite the extensive and repeated use of this technique within- and between days, data on the reliability and expected trial-to-trial noise of this neuromuscular evaluation protocol is sparse. This is surprising as, for example, performing multiple trials on the same day could produce changes in the participant's attention (17), neuromuscular strategy for performing the MVCs (18), and even fear of PNS-associated discomfort (19) may influence the force and EMG outcomes. When performing evaluations between days, in addition to some of the previously mentioned factors, slight changes in EMG electrodes placement (20), and muscle tissue properties (21) may also play a role in data noise and reliability.

Behm et al. (22) reported within-day intraclass correlation coefficient (ICC) values for some outcomes such as voluntary peak force during an iMVC (ICC=0.99) and force evoked by single and high-frequency doublet stimuli (ICC=0.83 and ICC=0.90, respectively). Other studies have provided a more comprehensive reliability analysis, reporting ICC, coefficient of variation (CV), and typical error values for some force (i.e., peak force during iMVC, voluntary

activation, force evoked by single and doublet stimuli) and EMG outcomes (i.e., root mean square, M-wave amplitude) (23–25). However, these previous studies have used small and mixed samples of females and males (23, 24), and have not systematically evaluated in the same participants the reliability of the force and EMG outcomes most used to measure neuromuscular function in sports and clinical evaluations (22–25).

Another factor to consider when examining the reliability of neuromuscular function assessments is the potential role of sex-related differences. For example, females generally have greater percentage of type I muscle fibers and have lower cross-sectional area of all fiber types than males (26). Furthermore, females have been indicated to have a distinct actin-myosin Ca^{+2} sensitivity (27, 28) and produce force via different neuromuscular recruitment strategies compared to males (29). These differences might influence some outcomes obtained during the neuromuscular function assessments (i.e., iMVC + PNS) such as the evoked force in the relaxed muscle and the EMG responses during iMVCs, which are higher (28) and show lesser amplitude variability (30, 31), respectively, in males than females. Therefore, it is possible that the reliability and measurement error of some neuromuscular evaluation outcomes might vary between sexes. Understanding whether this is the case is important as there has been an increased number of investigations addressing potential sex-related differences on neuromuscular function, muscle fatigue, and recovery (32, 33). Then, identifying the reliability of neuromuscular function assessment by iMVC + PNS protocol in each sex may be helpful when interpreting test-to-test changes in response to interventions such as nutritional treatments, conditioning activities, and exercise/training responses in each sex, or even when comparing different studies performing similar evaluations. Thus, this study aimed to identify the within-day and between days reliability and repeatability of force and EMG outcomes obtained through voluntary and electrically evoked contractions of knee extensors in females and males, and to compare the outcomes between sexes.

3.3. Materials and methods

Participants

Eighteen females (mean $\pm$ SD; age: 25 ± 4 years, height: 163.0 ± 5.5 cm, body mass: 69.4 ± 11.8 kg) and twenty males (mean $\pm$ SD; age: 24 ± 5 years, height: 178.5 ± 7.5 cm, body mass: 79.4 ± 14.7 kg) recreationally active volunteered for this study (see results section for more detailed participants' characteristics). Because this was a reliability and repeatability study, a formal power calculation for the sample size was not carried out. After being made aware of the experimental procedures, all participants provided their written informed consent. Participants were included if they were not under medical or nutritional treatments in the six months prior to the study, and if they had no known neurological, cardiovascular, or musculoskeletal disorders that could affect physiological responses during neuromuscular evaluation. There were no dropouts and all participants completed the procedures of the study in full. The study was approved by the São Paulo State University Research Committee (code number approval: #4.950.446) and all procedures were conducted in accordance with the Declaration of Helsinki.

Experimental design

Participants visited the laboratory on four different occasions with a 48-hour interval between them. On the first visit, they initially underwent anthropometric measurements and were asked to complete the International Physical Activity Questionnaire-short form (IPAQ-SF). This questionnaire contained seven questions that measure the frequency (days/week) and duration (minutes/day) of vigorous and moderate-intensity activities, and walking in bouts of at least 10 min in the last week (34). Physical activity outcomes from the IPAQ-SF for this study were as follow: weekly minutes spent in vigorous-intensity physical activity, moderate-intensity physical activity, walking and sedentary time (i.e., sitting or lying down). After these procedures were completed, participants were familiarized with the experimental procedures of the study by performing multiples submaximal and maximal isometric contractions of dominant knee-extensors (self-reported), associated with peripheral nerve stimulations and visual feedback of the force signal. On the second visit, participants performed three trials of neuromuscular function assessments interspaced by 1 hour of passive rest. On the third and fourth visits, the same neuromuscular function assessment procedures were performed but only once per visit to investigate the reliability between days (**Figure 3.1**). Prior to each visit, all participants

were instructed to avoid consumption of caffeinated food and beverages for at least 8-hours, and to abstain from vigorous physical activity for 24-hour.

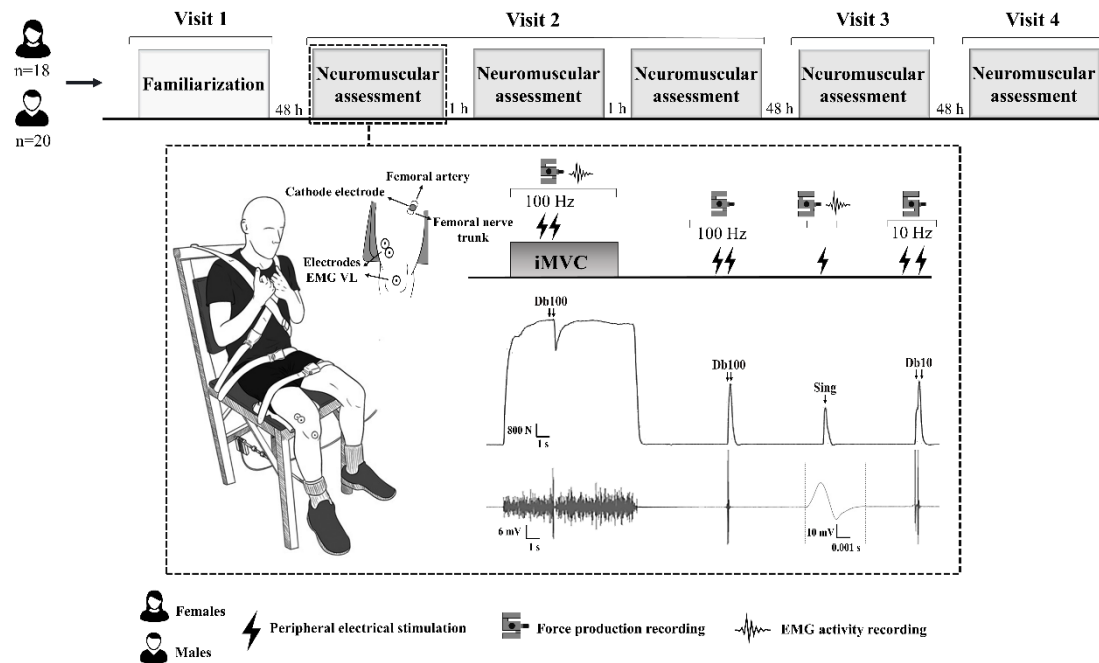


Figure 3.1. Schematic representation of the study design. iMVC: maximal isometric voluntary contraction of knee-extensors; EMG: electromyography activity; Db100: doublet supramaximal high-frequency stimuli (100Hz); Sing: single supramaximal stimulus; Db10: doublet supramaximal low-frequency stimuli (10 Hz).

Neuromuscular assessments

Maximal isometric voluntary contraction (iMVC)

In each trial, participants underwent a 5-second iMVC effort with the dominant knee-extensors (self-reported). Before each trial, participants performed a standardized warm up that consisted of 6-minutes treadmill jogging (velocity at 8 km/h for males and 6.5 km/h for females), three iMVCs with dominant knee-extensor muscles interspersed by 30 seconds, followed by 2 minutes of passive rest. For the iMVCs, volunteers were positioned on a custom-made chair designed to keep the hips and knees flexed at 90°. Volunteers were firmly secured with straps fastening legs, hips and chest. Force signal was acquired using a load cell with a 0.03 % rated output accuracy and a 0.98 N resolution (MK controle, Brazil). The load cell was attached to the ankle by a metal rod immediately above the region of the medial malleolus using straps with heavy duty Velcro. An experienced examiner performed this procedure in all trials. The rod cell was maintained aligned parallel to the ground during all trials. The load cell signal was amplified and acquired at a sampling frequency of 1000 Hz using a PowerLab system (16/35; AD Instruments, Australia). The surface EMG signal was acquired using an amplifier (FE136;

AD Instruments, Australia) and a PowerLab system (16/35; AD Instruments, Australia). The EMG signal was recorded through a pair of self-adhesive Ag/AgCl surface electrode (10 mm of diameter, Kendall MediTrace, MA). Before electrodes placement, the skin was shaved, gently abraded with a water sandpaper (1200 grit), and cleaned with alcohol. For positioning the electrodes, participants were seated in the custom-made chair and were required to perform a voluntary contraction of the knee-extensors. The electrodes were positioned 20 mm apart (center to center), in the direction of the muscle fibers and immediately above the region of the myotendinous junction detected visually after an isometric voluntary contraction. The reference electrode was placed on the patella. The position of the electrodes was marked with indelible ink. The electrodes were maintained on skin throughout the within-day trials and replaced using the indelible ink marks as reference in between-days trials. Before analysis, the EMG signal was band-pass (20-500 Hz) and notch (60 Hz) filtered. Both muscular force and EMG signals were recorded and analyzed using the LabChart 8 software (AD Instruments, Australia).

Peripheral Nerve Stimulation (PNS)

Stimulations of the femoral nerve of the dominant knee-extensors (pulse duration: 1 ms; square-wave) were delivered by a high-voltage electrical stimulator (Bioestimulador V2 400 V peak-to-peak; Insight, São Paulo Brazil) through Ag/AgCl electrodes (10 mm of diameter, Kendall MediTrace, MA) on the femoral triangle (cathode) and through carbon-rubber electrodes (32.5 mm of diameter, CARCI, Brazil) at the femoral greater trochanter level (anode). Doublet high-frequency stimulations (100 Hz) were delivered at the visual peak force of the iMVC effort (~3 after MVC started), as well as at 3-seconds post iMVC with the musculature relaxed. As previously described, volunteers' knowledge about the PNS procedure may unintentionally reduce the peak force values obtained during iMVCs + PNS (19, 25). Thus, the visually determined peak force achieved during the warm-up iMVCs was used to ensure that the volunteers reached their peak force during iMVC. Single and doublet low-frequency (10 Hz) stimulations were also delivered to the relaxed muscle at 7- and 10-seconds after iMVC, respectively. The magnitude of the electrical stimuli was determined individually in each visit by the application of single consecutive incremental stimuli on the relaxed muscle starting at 50 mA followed by 30-mA increments until reaching the involuntary force plateau. Females reached the force plateau at 156 ± 24 mA, 167 ± 30 mA, and 168 ± 30 mA in visits 1, 2, and 3, respectively ($p > 0.05$), while males reached the force plateau at 210 ± 65 mA, 223 ± 64 mA, and 219 ± 59 mA, respectively ($p > 0.05$). Thereafter, the electrical stimuli used for the PNS were further

increased by 30% (35). On Visit 1, the intensity was determined before the first trial and was used for the remaining trials of the day.

iMVC and PNS data analysis

The highest voluntary force recorded within a 100-ms period during the force plateau of the iMVC was used as the peak voluntary force ($iMVC_{Force}$). The $iMVC_{Force}$ was expressed in raw values (i.e., N) and relative to body mass (i.e., N/kg of body mass). The root mean square and median frequency of the *vastus lateralis* EMG signal (RMS_{VL} and MdF_{VL} , respectively), were recorded 0.5 seconds around the $iMVC_{Force}$. The superimposed force signal amplitude by the doublet high-frequency stimuli recorded during iMVC and the force evoked by the doublet high-frequency stimuli at rest after iMVC ($Db_{100Force}$) were used to measure the voluntary neuromuscular activation (VA) using the following equation: $VA (\%) = (1 - (\text{superimposed force} \times ((\text{force level at stimulation} / iMVC_{Force}) / Db_{100Force})) \times 100$ (5). The potentiated peak force evoked by the single stimulus (Q_{tw}) was recorded and the ratio between the force evoked by the doublet low-frequency stimuli ($Db_{10Force}$) and $Db_{100Force}$ ($Db_{10Force} / Db_{100Force}$ ratio) was calculated. The amplitude and duration of EMG response evoked by the single stimulus was also recorded (M-wave amplitude and duration, respectively). The ratio between RMS_{VL} and M-wave amplitude ($RMS / M\text{-wave}_{amp}$) was also calculated. All procedures (i.e., force and EMG signals) were performed using the LabChart 8 software (AD Instruments, Australia).

Statistical analysis

Data are shown as mean $\pm$ SD and 95% confidence interval (95% CI). The normality of the data was confirmed using the Shapiro-Wilk test. An independent t test was used to compare characteristics and physical activity level between females and males. Effect sizes of the t test are reported using Cohen's d values (trivial: 0.20; small: 0.50; large: 0.80; very large: >1.3). A two-way mixed analysis of covariance (ANCOVA) was used to determine if significant difference occurred on the outcomes obtained from females and males across trials (i.e., time effect) and between sexes (i.e., group effect). Anthropometric characteristics (i.e., height, body mass and body mass index) were included as potential confounding factors. Effect sizes of the ANCOVA are reported using the partial eta square (η^2_p ; small: 0.02; medium: 0.13; large: 0.26). Relative and absolute reliability, and repeatability coefficient for all outcomes obtained from males and females were assessed for within-day trials (i.e., visit 1) and between-days trials (i.e., first trial on

visit 1, visits 2 and 3). Relative reliability (i.e., the degree to which individuals maintain their position in a sample with repeated measurements) was assessed as the intraclass correlation coefficient (ICC) using a two-way random effects model with a single measure (2,1) (36). We have not provided threshold values for ICC, as no consensus exists on this topic; as previously suggested (37) the readers has to determine the “practical significance” of the obtained reliability. Absolute reliability (i.e., the degree to which repeated measurements vary for individuals) was assessed as the coefficient of variation (CV, %) determined as follows: $CV = SD/mean \times 100$ (36). The CV values for *very good*, *good*, *fair* and *poor* reliability are 10%, 10-20%, 20-30%, and >30%, respectively (38). The repeatability of the outcomes was calculated using the following equation: $repeatability (\%) = 1.96 \times \text{standard error of measurement} \times \sqrt{2}$ (39). The standard error of measurement was calculated as the square root of the mean square error in a one-way repeated measures analysis of variance (ANOVA) and was expressed as a percentage of the mean (36). The ICC calculations and outcomes mean comparisons were performed using the software package SPSS, version 20.0 (IBM Corp., Armonk, USA). The statistical significance was set at 5% level. The CV and repeatability were calculated using the Microsoft Excel (Microsoft Corporation, Redmond, USA).

3.4. Results

Participants' characteristics

The age, anthropometric characteristics, and physical activity outcomes from IPAQ-SF of females and males are shown in **Table 3.1**. Females were significantly shorter than males ($p=0.01$, Cohen's $d > 1.2$) and had lower body mass ($p=0.03$, Cohen's $d=0.76$), but did not differ in age ($p=0.52$, Cohen's $d=0.22$) or body mass index ($p=0.38$, Cohen's $d=0.29$). According to IPAQ-SF, females and males did not differ in the weekly time spent in vigorous-intensity physical activity ($p=0.88$, Cohen's $d=0.08$), moderate-intensity physical activity ($p=0.75$, Cohen's $d=0.17$), walking ($p=0.86$, Cohen's $d=0.09$) and sedentary activities ($p=0.51$, Cohen's $d=0.33$).

Table 3.1. Participants' characteristics

	Females	Males	p value	Cohen's d
<i>Age (years)</i>	25 ± 4 (23 to 27)	24 ± 5 (22 to 27)	0.52	0.22
<i>Height (cm)</i>	163.0 ± 5.5 (160.3 to 165.7)	178.5 ± 7.5 (175.0 to 182.1)	0.01*	>1.2
<i>Body mass (kg)</i>	69.4 ± 11.8 (63.5 to 69.4)	79.5 ± 14.7 (72.6 to 86.4)	0.03*	0.76
<i>Body mass index</i>	26.2 ± 4.6 (23.8 to 28.5)	24.9 ± 4.1 (23.0 to 26.8)	0.38	0.29
IPAQ-SF				
<i>Vigorous-intensity PA (min/week)</i>	116.8 ± 111.8 (23.4 to 210.3)	108.8 ± 108.9 (30.9 to 186.9)	0.88	0.08
<i>Moderate-intensity PA (min/week)</i>	237.5 ± 131.2 (127.8 to 347.2)	208.0 ± 225.3 (46.8 to 369.2)	0.75	0.17
<i>Walking (min/week)</i>	216.3 ± 291.4 (0.0 to 459.8)	198.5 ± 113.2 (117.5 to 279.5)	0.86	0.09
<i>Sedentary time (min/week)</i>	4575.0 ± 1207.6 (3565.4 to 5584.6)	4953.0 ± 1147.9 (4131.8 to 5774.2)	0.51	0.33

Mean ± standard deviation (95% confidence interval). *p<0.05. Cohen's d was calculated as the difference between the means divided by the mean standard deviation of the data. IPAQ-SF: International Physical Activity Questionnaire-short form; PA: physical activity. Sedentary time encompasses time spent sitting or lying down. N=18 and males N=20.

Within-day and between-days differences in neuromuscular assessments in females and males

There were no statistical differences on force and EMG outcomes across trials (i.e., time effect) for females or males (p values ranging from 0.13 to 0.84; η^2_p values ranging from 0.01 to 0.08). Sex differences in global indicators of neuromuscular function across trials are shown in **Figure 3.2**. Females presented lower values (i.e., group effect) for absolute iMVC_{Force} (p<0.01, η^2_p =0.42), iMVC_{Force} relative to body mass (p<0.01, η^2_p =0.45), and RMS_{VL} (p=0.01; η^2_p =0.17) in comparison to males. There was no group effect for MdF_{VL} (p=0.36; η^2_p =0.02). There were no interaction effects for any of these outcomes.

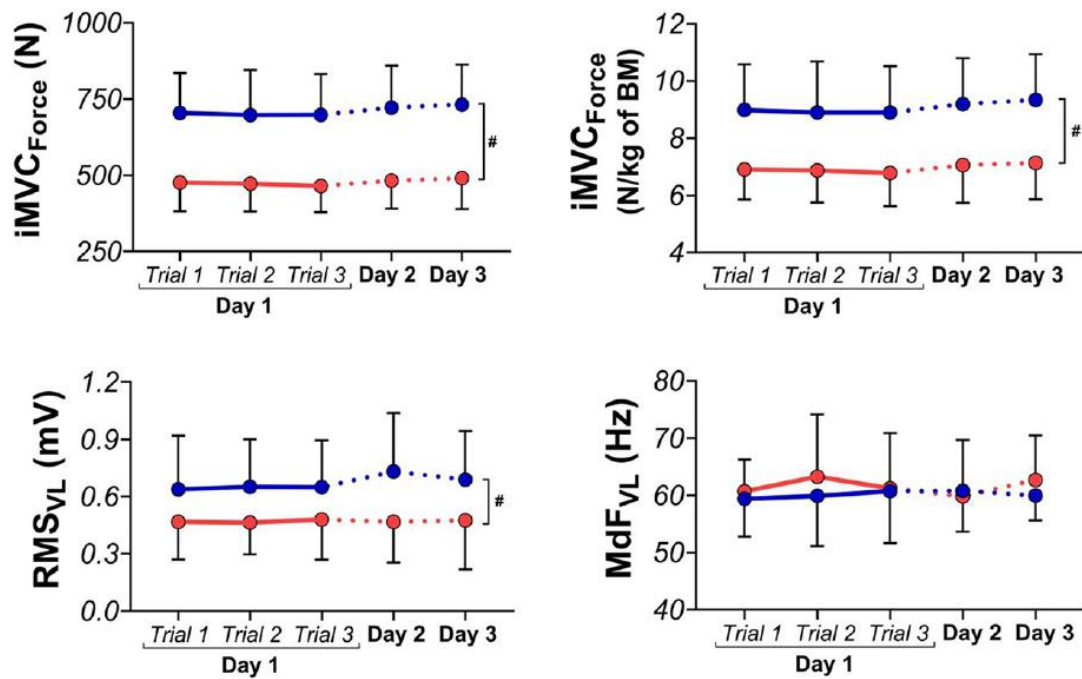


Figure 3.2. Global indicators of neuromuscular function in females and males obtained through voluntary contractions of knee extensors. #: main effect of sex ($p < 0.05$) in a two-way mixed analysis of covariance (ANCOVA) with anthropometric characteristics (i.e., height, body mass and body mass index) included as potential confounding factors. iMVC_{Force}: highest voluntary force recorded during maximal isometric voluntary contraction (iMVC); BM: body mass; RMS_{vL}: root mean square of electromyography signal from vastus lateralis during iMVC (0.5-s around iMVC_{Force}); MdF_{vL}: median frequency of electromyography signal from vastus lateralis during iMVC (0.5-s around iMVC_{Force}). Females N=18; Males N=20.

Figure 3.3 shows the central indicators of neuromuscular function across trials. There was no group effect for VA ($p = 0.62$; $\eta^2_p = 0.01$) or RMS_{vL}/M-wave amplitude ratio ($p = 0.45$; $\eta^2_p = 0.02$). There were no interaction effects in these outcomes.

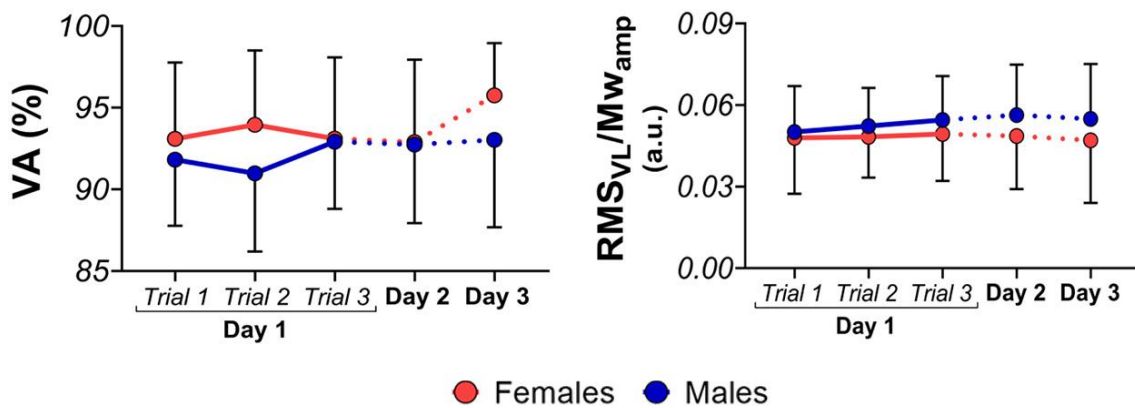


Figure 3.3. Central indicators of neuromuscular function in females and males obtained through voluntary and electrically evoked contractions of knee extensors. VA: voluntary activation measured through twitch interpolation technique; RMS_{vL}/Mw_{amp}: root mean square of surface electrography activity of vastus lateralis during iMVC_{Force} and peak-to-peak M-wave maximal amplitude ratio. Females N=18; Males N=20.

Peripheral indicators of neuromuscular function in females and males across trials are shown in **Figure 3.4**. Females presented lower values (i.e., group effect) for absolute Db_{100Force} ($p < 0.01$; $\eta^2_p = 0.47$), Db_{100Force} relative to body mass ($p < 0.01$; $\eta^2_p = 0.48$), absolute

Q_{tw} ($p<0.01$; $\eta^2_p=0.45$), and Q_{tw} relative to body mass ($p<0.01$; $\eta^2_p=0.48$). There was no group effect for $Db_{10Force}/Db_{100Force}$ ratio ($p=0.93$; $\eta^2_p<0.01$). There were no interaction effects for any of these outcomes.

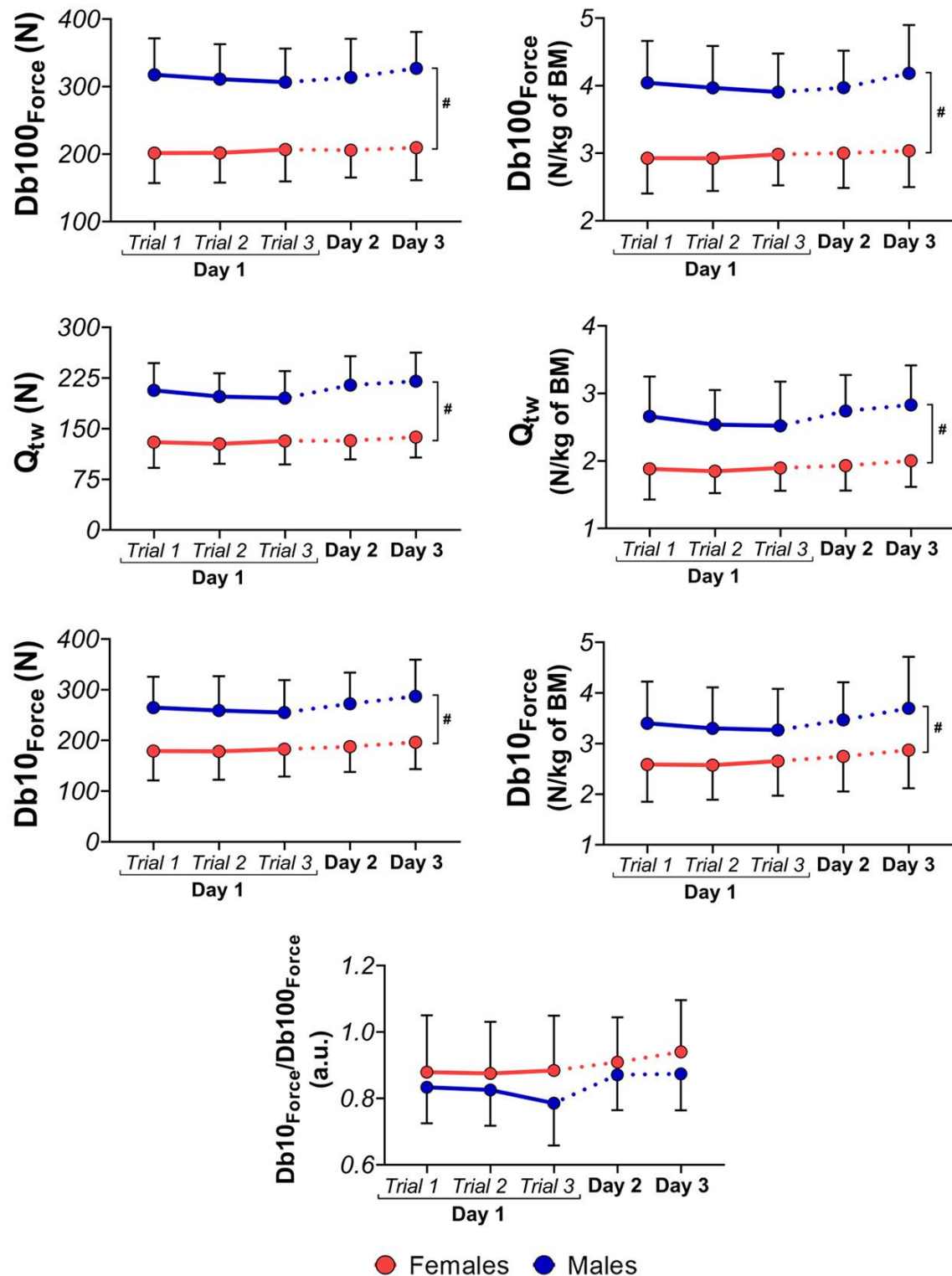


Figure 3.4. Peripheral indicators of neuromuscular function in females and males obtained through electrically evoked contractions of knee extensors. #: main effect of sex ($p<0.05$) in a two-way mixed analysis of covariance (ANCOVA) with anthropometric characteristics (i.e., height, body mass and body mass index) included as potential confounding factors. $Db_{100Force}$: force evoked by a high-frequency (100 Hz) doublet stimulus; Q_{tw} : force evoked by a single stimulus; $Db_{10Force}$: force evoked by a supramaximal low-frequency (10 Hz) doublet stimulus; BM: body mass. Females $N=18$; Males $N=20$.

Vastus lateralis M-wave amplitude and duration across trials for both sexes are presented in **Figure 3.5**. Females presented lower M-wave amplitude in comparison to males (i.e., group effect; $p<0.01$; $\eta^2_p=0.18$). There was no group effect for M-wave duration ($p=0.16$; $\eta^2_p=0.05$). There were no interaction effects in neither of these outcomes.

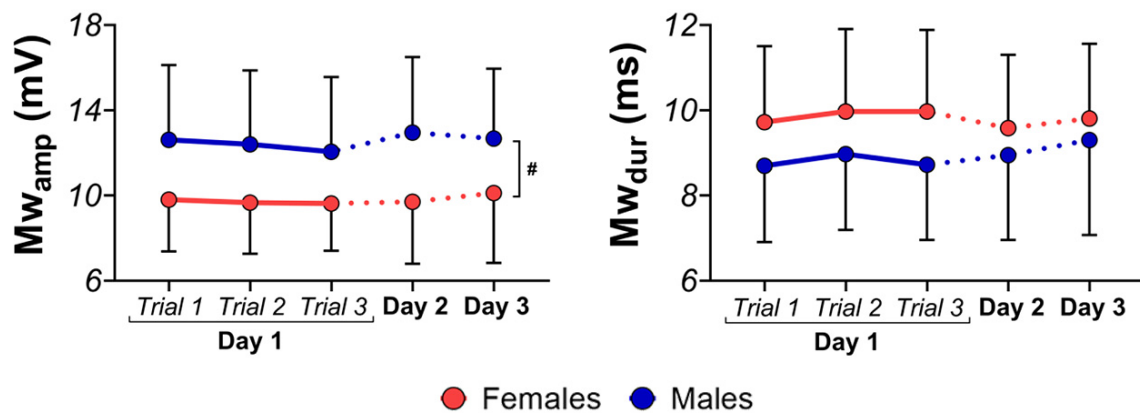


Figure 3.5. Peak-to-peak M-wave maximal amplitude (Mw_{amp}) and duration (Mw_{dur}) of electromyography signal from vastus lateralis obtained through electrically evoked contractions of knee extensors at rest. #: main effect of sex ($p<0.05$) in a two-way mixed analysis of covariance (ANCOVA) with anthropometric characteristics (i.e., height, body mass and body mass index) included as potential confounding factors. Females N=18; Males N=20.

Reliability and repeatability of neuromuscular function outcomes within-day

The ICC, CV, and repeatability values of force and EMG outcomes obtained in the within-day and between-days trial in both sexes are shown in **Table 3.2**.

iMVC_{Force}, Db_{100Force}, Q_{tw} , M-wave amplitude and duration were the most reliable outcomes obtained in within-day trials (ICC values ranging from 0.82 to 0.97) and *very good* CV values (ranging from 3.5% to 6.4%) in both sexes. ICC for these outcomes were similar for females and males, except by Q_{tw} and M-wave duration (ICC=0.91 for females in both outcomes and 0.82 and 0.84, respectively, for males). The CV values for iMVC_{Force}, Db_{100Force}, Q_{tw} , M-wave amplitude and M-wave duration were alike between sexes. The repeatability of iMVC_{Force}, Db_{100Force}, Q_{tw} , M-wave amplitude and M-wave duration varied from 12.2% to 22.6%, being alike between sexes, with exception to M-wave duration (repeatability of 15.6% and 22.4% for females and males, respectively). The VA presented ICC=0.76 and ICC=0.62 in females and males, respectively, and had *very good* CV (2.1% and 2.6% in females and males, respectively). The VA presented the lowest repeatability values among all outcomes obtained in within-day trials (6.86% and 9.04% in females and males, respectively).

The Db_{10Force}/Db_{100Force} ratio showed ICC=0.86 in females and ICC=0.56 in males. The CV values of this outcome was *very good* and alike between sexes (CV values of 5.7%

and 6.8% in females and males, respectively), but the repeatability values had little variation between sexes (19.9% and 26.3% in females and males, respectively). In both sexes, the MdF_{VL} presented ICC of 0.61 and 0.51 in females and males, respectively, *very good* CV (8.0% and 8.4%, in females and males, respectively), but large repeatability values (25.0% and 26.7% in females and males, respectively). Similar ICC values between sexes were observed in RMS_{VL} (0.86 and 0.82 in females and males, respectively) and $RMS_{VL}/M\text{-wave}_{amp}$ ratio (0.77 and 0.82 in females and males, respectively). Both sexes presented *good* CV in both RMS_{VL} (14.1% and 12.6% in females and males, respectively) and in $RMS_{VL}/M\text{-wave}_{amp}$ ratio (CV of 14.0% and 13.6% in females and males, respectively). The repeatability of these outcomes was the highest among all outcomes obtained, being alike between sexes for RMS_{VL} (49.3% and 48.9% in females and males, respectively) but showing little variation for $RMS_{VL}/M\text{-wave}_{amp}$ ratio (49.2% and 42.2% in females and males, respectively).

Reliability and repeatability of neuromuscular outcomes between-days

The $iMVC_{Force}$, $Db_{100Force}$ and Q_{tw} were the most reliable outcomes in between-days trials, with ICC values ranging from 0.80 to 0.93 and *very good* CV in both sexes (values ranging from 4.3% to 7.5%). Both ICC and CV values were similar for females and males. The repeatability was alike between sexes for $iMVC_{Force}$ (14.4% and 13.3% in females and males, respectively) and $Db_{100Force}$ (19.3% and 17.0% in females and males, respectively), but presented a little variation for Q_{tw} (33.5% and 23.1% in females and males, respectively). VA presented ICC values of 0.60 and 0.66 in females and males, respectively, *very good* CV values (2.6% in both females and males, respectively), and the lowest repeatability values among all outcomes in both sexes (repeatability values of 7.4% and 9.7% in females and males, respectively). In both sexes, MdF_{VL} presented *very good* CV (7.9% and 6.9% in females and males), ICC values of 0.19 and 0.51 in females and males, respectively, and high repeatability (26.8% and 22.3% in females and males, respectively).

The $Db_{10Force}/Db_{100Force}$ ratio presented ICC values of 0.72 and 0.56 in females and males, respectively, *very good* CV (6.4% and 7.1% in females and males, respectively), and high repeatability in both sexes (28.7% and 22.8% in females and males, respectively). In both sexes, RMS_{VL} , M-wave amplitude and duration, and $RMS_{VL}/M\text{-wave}_{amp}$ ratio presented ICC values ranging from 0.64 to 0.85 except by M-wave duration in females that presented ICC=0.48. Most of these outcomes presented *good* CV (values ranging from 9.1% to 19.4%), and high repeatability (values ranging from 29.3% to 53.4%). The ICC,

CV and repeatability of these outcomes were alike in females and males except by repeatability of M-wave duration which was 42.9% in females and 33.5% in males.

Table 3.2. Within- and between-days reliability of indicators of neuromuscular function obtained through voluntary and electrically evoked contractions of knee extensors in females and males.

<i>Indicators of global function</i>		Within-day reliability			Between-days reliability		
		ICC	CV (%)	Repeatability (%)	ICC	CV (%)	Repeatability (%)
iMVC _{Force} (N)	Females	0.94 (0.88 to 0.98)	3.5 ± 3.0 (2.1 to 5.0)	12.7	0.93 (0.85 to 0.97)	4.3 ± 3.0 (2.8 to 5.8)	14.4
	Males	0.95 (0.90 to 0.98)	3.9 ± 2.0 (3.0 to 4.9)	12.2	0.93 (0.85 to 0.97)	4.6 ± 1.8 (3.7 to 5.4)	13.3
RMS _{VL} (mV)	Females	0.86 (0.73 to 0.94)	14.1 ± 6.8 (10.7 to 17.5)	49.3	0.84 (0.69 to 0.93)	19.0 ± 11.7 (13.2 to 24.8)	53.1
	Males	0.82 (0.67 to 0.92)	12.6 ± 7.9 (8.9 to 16.3)	48.9	0.81 (0.65 to 0.91)	15.8 ± 10.3 (11.0 to 20.6)	47.8
MdF _{VL} (Hz)	Females	0.61 (0.36 to 0.82)	8.0 ± 3.9 (6.1 to 9.9)	25.0	0.19 (-0.08 to 0.52)	7.9 ± 5.7 (5.1 to 10.7)	26.8
	Males	0.51 (0.24 to 0.75)	8.4 ± 4.3 (6.4 to 10.4)	26.7	0.51 (0.24 to 0.74)	6.9 ± 3.8 (5.1 to 8.7)	22.3
<i>Indicators of the function of central components</i>							
VA (%)	Females	0.76 (0.57 to 0.89)	2.1 ± 1.5 (1.3 to 2.8)	6.9	0.60 (0.31 to 0.81)	2.6 ± 1.9 (1.6 to 3.6)	7.5
	Males	0.62 (0.38 to 0.81)	2.6 ± 1.6 (1.8 to 3.3)	9.0	0.66 (0.43 to 0.83)	2.6 ± 1.6 (1.9 to 3.4)	9.8
RMS _{VL} / M-wave _{amp} (arbitrary unit)	Females	0.77 (0.58 to 0.90)	14.0 ± 6.7 (10.7 to 17.4)	42.2	0.82 (0.65 to 0.92)	19.4 ± 12.0 (13.5 to 25.4)	53.4
	Males	0.74 (0.54 to 0.88)	13.6 ± 7.00 (10.3 to 16.9)	49.2	0.73 (0.53 to 0.87)	13.1 ± 10.7 (8.1 to 18.1)	48.3
<i>Indicators of the function of peripheral components</i>							
Db _{100Force} (N)	Females	0.96 (0.91 to 0.98)	4.1 ± 2.1 (3.0 to 5.1)	12.5	0.89 (0.79 to 0.96)	5.8 ± 1.9 (4.8 to 6.7)	19.3
	Males	0.92 (0.83 to 0.96)	4.4 ± 2.4 (3.3 to 5.5)	12.8	0.87 (0.74 to 0.94)	5.6 ± 2.9 (4.3 to 6.9)	17.0
Q _{tw} (N)	Females	0.91 (0.81 to 0.96)	6.2 ± 3.7 (4.4 to 8.1)	22.6	0.83 (0.68 to 0.93)	7.5 ± 3.5 (5.8 to 9.2)	33.5
	Males	0.82 (0.66 to 0.92)	6.4 ± 5.2 (4.0 to 8.9)	22.0	0.80 (0.64 to 0.91)	7.5 ± 3.8 (5.7 to 9.2)	23.1
Db _{10Force} (N)	Females	0.96 (0.92 to 0.99)	6.0 ± 4.7 (3.7 to 8.3)	6.0	0.87 (0.74 to 0.95)	8.7 ± 6.0 (5.7 to 11.7)	11.5
	Males	0.89 (0.78 to 0.95)	7.0 ± 4.6 (4.8 to 9.1)	8.3	0.85 (0.70 to 0.93)	7.8 ± 4.9 (5.52 to 11.7)	8.6
Db _{10Force} /Db _{100Force} (arbitrary unit)	Females	0.86 (0.73 to 0.94)	5.7 ± 4.9 (3.2 to 8.9)	19.9	0.72 (0.51 to 0.87)	6.4 ± 3.2 (4.8 to 8.0)	28.7
	Males	0.56 (0.31 to 0.77)	6.8 ± 6.6 (3.7 to 9.9)	26.3	0.56 (0.31 to 0.77)	7.1 ± 3.4 (5.5 to 8.7)	22.8
M-wave _{amp} (mV)	Females	0.95 (0.89 to 0.98)	4.4 ± 4.1 (2.4 to 6.4)	15.5	0.83 (0.68 to 0.93)	9.4 ± 5.5 (6.7 to 12.2)	33.5
	Males	0.97 (0.93 to 0.99)	4.4 ± 2.2 (3.4 to 5.5)	12.5	0.85 (0.72 to 0.93)	9.5 ± 4.4 (7.5 to 11.6)	29.3

M-wave _{dur} (ms)	Females	0.91 (0.82 to 0.96)	3.6 ± 2.9 (2.1 to 5.0)	15.6	0.48 (0.19 to 0.74)	10.4 ± 8.5 (6.1 to 14.6)	36.8
	Males	0.84 (0.69 to 0.93)	5.5 ± 4.8 (3.2 to 7.7)	22.4	0.64 (0.41 to 0.82)	9.1 ± 7.8 (5.4 to 12.7)	42.9

iMVC: highest voluntary force recorded during maximal isometric voluntary contraction (iMVC); RMS_{VL}: root mean square of electromyography signal from *vastus lateralis* during iMVC (0.5-s around iMVC_{Force}); MdF_{VL}: median frequency of electromyography signal from *vastus lateralis* during iMVC (0.5-s around iMVC_{Force}); VA: voluntary activation measured through twitch interpolation technique; Db_{100Force}: force evoked by a high-frequency (100 Hz) doublet stimulus; Q_{tw} : force evoked by a supramaximal single stimulus; Db_{10Force}: force evoked by a low-frequency (10 Hz) doublet stimulus; M-wave_{amp}: peak-to-peak M-wave amplitude; M-wave_{dur}: peak-to-peak M-wave duration; ICC: intraclass correlation coefficient; CV: mean coefficient of variation; Females: n=18; Males: n=20.

3.5. Discussion

The purpose of this study was to compare the force and EMG outcomes obtained through iMVC and PNS of knee extensors between females and males and to identify the within- and between-day reliability of these data in each sex. The main findings were that 1) females presented lower iMVC_{Force} and RMS_{VL} values than males, which seems to occur due to differences in the functioning level of the peripheral components of the neuromuscular system between sexes, as suggested by the lower Db_{100Force}, Q_{tw} , and M-wave amplitude in females compared with males and by the absence of sex differences on VA and RMS_{VL}/M-wave_{amp} ratio; 2) despite these observed (and expected) sex related differences, most force and EMG outcomes in within- and between-day trials presented ICC > 0.75, very good CV (<10%), and repeatability <25% in both sexes. This study comprehensively reports the force and EMG outcomes provided by this widespread neuromuscular evaluation technique, as well as the reliability and repeatability of the data, in a larger group of females and males compared with previous investigations (22–25). The information presented herein is important when characterizing the neuromuscular function of knee extensors in both sexes, when interpreting test-to-test changes due to acute interventions such as nutritional treatments, conditioning activities, exercise, and physical training, or even when comparing different studies performing similar evaluations. These data also expand previous reports on the reliability of assessing other muscle groups such as elbow flexors (40) and plantar flexors (22) using similar protocols, which contributes to a more robust exploration of human neuromuscular function.

Reliability and repeatability of indicators related to global neuromuscular function

iMVC_{Force} is frequently used as a “gold standard” indicator of global neuromuscular function (41). In addition, the EMG signal during iMVC generally changes due to the force production level of the muscle, providing an additional indicator of global muscle function (20). The present study demonstrates that both RMS_{VL} and MdF_{VL} presented poorer reliability and repeatability in comparison with iMVC_{Force}, regardless of sex and

interval between trials. The poorer reliability of RMS_{VL} and MdF_{VL} in comparison with $iMVC_{Force}$ probably occurs due to the random nature of motor unit action potentials (42), which lead to greater variability in the EMG signal than that observed in the force signal (43, 44). The present study offers novel information to previous investigations (23, 25), as it reports the reliability and repeatability of force and EMG indicators of global muscle function in multiple within- and between-day trials in females and males.

Reliability and repeatability of indicators related to central neuromuscular function

In addition to the global muscle function, VA evaluations offer a way to specifically assess the contribution of the central nervous system during voluntarily force production (4). VA showed ICC of 0.62–0.76, presenting the lowest CV and repeatability values ($\sim 2\%$ and $\sim 8\%$, respectively) among all outcomes, regardless of sex and interval between trials. The low ICC values, together with very good CV and low repeatability values, for VA, seem to occur due to the small variability among participants' scores, which makes it difficult to discriminate one volunteer from another (45). This reliability pattern is called ceiling and occurs when scores cluster toward the high end (or best possible score) of the measure/instrument (45). This effect was reported in previous investigations with small sample sizes (25, 46). Despite some of the methodological issues related to VA assessments (e.g., overestimation of true VA, impossibility to extrapolate results to dynamic contractions or other joint angles at isometric contractions) (47, 48), this outcome seems to be a reliable indicator of central nervous system function during voluntary force production in both sexes, both for intra- and interday evaluations. In addition to VA, the $RMS_{VL}/M\text{-}wave_{amp}$ ratio is also being used as an indicator of the function of the central components of the neuromuscular system (41). In the current study, the RMS/M-waveamp ratio showed an ICC of 0.73–0.82 and a good CV ($\sim 13\%$ – 19%), but large values for repeatability ($>42\%$). These large repeatability outcomes probably occur due to the random nature of motor unit action potentials (42) as cited earlier.

Reliability and repeatability of indicators related to peripheral neuromuscular function

The mechanical indicators of peripheral neuromuscular function (i.e., Q_{tw} , $Db100_{Force}$, and $Db10_{Force}/Db100_{Force}$ ratio) showed an ICC of 0.56–0.96 and very good CV ($\sim 4\%$ – 8%) in both females and males, regardless of the interval between trials. However, most of the repeatability results for these outcomes increased during between-day trials

(~16%–34%) compared with within-day trials (~12%–23%), for both females and males. Although it is challenging to explain the reasons for such findings, they might be related to inherent day-to-day changes on muscle tissue properties (21) and motor unit recruitment pattern (49). Independently of the reasons that might explain these findings, what they highlight is that changes in these outcomes may need to be interpreted with caution, taking into account the interval between trials in both females and males. It is also important to highlight that within-day trial repeatability for $\text{Db10}_{\text{Force}}/\text{Db100}_{\text{Force}}$ ratio presented a small variation between sexes (19.9% and 26.4% in females and males, respectively). Small sex-related differences were also observed for the between day trial repeatability for Q_{tw} (33.5% and 23.1% in females and males, respectively). It could be argued that anatomical and physiological differences between the sexes [e.g., distinct actin-myosin Ca^{+2} sensitivity (27, 28) and distinct neuromuscular recruitment strategies (29)] might contribute to explain these findings; however, the current data do not offer definite conclusions. The present study offers novel information to previous investigations (23, 25), as it reports the reliability and repeatability of Q_{tw} , $\text{Db100}_{\text{Force}}$, and $\text{Db10}_{\text{Force}}/\text{Db100}_{\text{Force}}$ ratio within and between days in both sexes.

In addition, the EMG indicators of peripheral function of the neuromuscular system (i.e., M-wave amplitude and duration) showed an ICC of ~0.95–0.97, very good CV (~3%–6%), and low repeatability (~12%–23%) in within-day trials in both sexes. For the between-day trials, both outcomes presented a poorer reliability in comparison with within-day trials in both females and males (ICC of ~0.4–0.85, CV of ~9%–10%, and repeatability ~29%–42%). This greater variability of M-wave properties between days could be due to inherent day-to-day variations of muscle tissue properties (21) and electrode placement (25). Then, changes in the EMG outcomes related to the peripheral components of the neuromuscular function might need to be interpreted with caution independently of sex, taking into account whether they were obtained from within- or between-day trials.

Sex-related differences in neuromuscular function

Although the reliability and repeatability responses were very similar in females and males, some of the outcomes indicated sex-related differences in the magnitude of the responses. This was to be expected based on previous reports (50–52). This study cannot discern the mechanisms explaining those differences, but some speculations could be made based on current data. Females presented lower $\text{iMVC}_{\text{Force}}$ (absolute and relative to

body mass) and RMS_{VL} than males. This has been usually reported by previous investigations (50–52). Voluntary force is produced through a complex chain of events that involves the capacity of the nervous system to activate a muscle group (i.e., voluntary activation) and the contractile capacity of this muscle group (i.e., action potential propagation, excitation-contraction coupling, and cross-bridge cycling) (13, 53–55). The $\text{RMS}_{\text{VL}}/\text{M-wave}_{\text{amp}}$ ratio as well as the VA did not differ between sexes in the present study. Previous investigations have already reported no differences in VA between sexes (33, 51, 52). Thus, if the neural drive to the muscles seems to be similar between sexes, it could be argued that the lower $\text{iMVC}_{\text{Force}}$ and RMS_{VL} values in females observed in the present study occur due to differences on the functioning level of the peripheral components of the neuromuscular system (i.e., located below the neuromuscular junction) between sexes (56).

Indeed, M-wave amplitude and force outcomes related to the peripheral neuromuscular function (i.e., $\text{Db100}_{\text{Force}}$ and Q_{tw} in both absolute and relative to body mass values) were lower in females compared with males. These data are supported by previous reports (33, 52, 57) describing similar sex related differences in peripheral neuromuscular function. As demonstrated in the literature, females seem to have a lower mean cross-sectional area of all fiber types in knee extensors (26), mostly type II fibers (51), as well as a lower proportion of type II fibers (26, 51). Due to well-established positive correlation between fiber cross-sectional area and force production (58, 59), such difference in fiber size and proportion between sexes may explain the lower evoked force in females in the present study. Furthermore, previous investigations have suggested that larger muscle fibers have a greater number of sodium and potassium ion channels (60), improving action potential propagation and thereby M-wave amplitude (56,61). Thus, the lower M-wave amplitude in females observed in the present study may also be explained by the sex differences in fiber size and proportion. Unfortunately, measurements of muscle properties (i.e., cross-sectional area of muscle fibers) were beyond the scope of the present study. In this way, the physiological rationale underlying the present results is based on the previous reports indicating sex differences in muscle tissue properties that apply to our sample (i.e., young adult, healthy participants, and recreationally active participants).

Limitations and practical applications

The current study has some limitations. Due to regular engagement in physical exercises, it is possible that trained volunteers of both sexes might have a more stable recruitment

of knee extensor muscles during both within- and between-day trials (62, 63). This could lead to better reliability and repeatability statistics of force and EMG outcomes than those reported in the current study. However, most investigations involving multiple trials of this kind of neuromuscular assessment are commonly performed on healthy, untrained volunteers. Thus, to increase the applicability of the findings, we chose to conduct the current study on healthy, untrained volunteers. We decided that it was best to report the repeatability values instead of SE values of the data (also known as “typical error”). Although the SE has been used in previous studies (22–25), the repeatability encompasses the SE [i.e., repeatability (%) = $1.96 \times \text{standard error of measurement} \times \sqrt{2}$ (39)] and seems to have more practical applications as it considers the measurement error of the test and the within-subject variability (64, 65). We believe that reporting both SE and repeatability data would result in unnecessary redundancies throughout the text.

Furthermore, we did not control for lower limb lever length during torque measurements or subcutaneous thickness of thigh during EMG data collection. Although reliability and repeatability results were likely not impacted, this may have some effects on raw outcomes (66, 67), which warrants caution in interpreting the herein reported sex differences, even though they corroborate previous investigations (50–52). Furthermore, we did not control for menstrual cycle phases when testing female volunteers between days. Current data indicate that neuromuscular function appears to be minimally altered by the fluctuations in ovarian sex hormones that occur during the menstrual cycle (68, 69). Thus, it seems that the reliability and repeatability statistics of force and EMG outcomes across days from the female volunteers might be trivially impacted by the menstrual cycle phases.

Finally, it is important to highlight that extrapolations from our data for future research or practical applications need to be made with caution, considering some of the specific conditions of the experimental setup, such as 1) the assessments were performed on knee extensors, which precludes extrapolating the findings for the assessment of other muscle groups such as elbow flexors (70), plantar flexors (22), or other muscle groups; 2) prior familiarization procedures were performed before the iMVCs and PNS, and previous data have shown that lack of familiarization would affect the outcomes (19); 3) specific instructions, setup, and protocol characteristics were used to acquire force and EMG signals (e.g., order of application of single and low-frequency doublet stimuli at relaxed muscle, but we would expect that similar responses would be seen if the order was altered

(71)); and 4) female and male participants in the present study were matched for age, body mass index, and physical activity level (according to IPAQSF). Thus, the results from this study need to be interpreted with caution and in the context of the experimental conditions and population that was investigated.

With this in mind, and from a practical standpoint, our results provide a helpful basis for distinguishing between measurement error and “true changes” on neuromuscular function following a variety of interventions that could affect neuromuscular assessment within hours or days such as nutritional interventions [e.g., caffeine (72) supplementation], conditioning activities (10, 73), exercises leading to muscle fatigue (74) or damage (75), and injuries (76).

3.6. Conclusions

In conclusion, although females presented lower knee extensor iMVC_{Force} and RMS_{VL} than males, which seems to be modulated by sex-related differences in peripheral but not central components of the neuromuscular system, differences in neuromuscular function outcomes induced by repetitive iMVCs and evoked contractions inherent within- and between day evaluation procedures seem to provide just small discrepancies on data reliability and repeatability in both females and males. Thus, although reliability and repeatability statistics vary according to the type of neuromuscular function outcome (e.g., from force or EMG responses), the trial interval (i.e., hours or days), and the sex of the participant, most of the force and EMG outcomes obtained through this neuromuscular evaluation technique provided reliable measures of the neuromuscular function regardless of the interval between assessments and the sex of the participant (ICC > 0.75, CV <10%, and repeatability <25%).

3.7. References

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CHAPTER 4.
NEUROMUSCULAR FUNCTION IMPAIRMENT AND
PERCEIVED FATIGABILITY IN RESPONSE TO
DISTINCT RUNNING EXERCISES

4. STUDY 2. Similar impairments of force-generating capacity, but distinct central, peripheral, and perceived fatigability after different running exercises

4.1. Abstract

Purpose: Despite the widespread practice, task dependency of fatigue during running exercise remains an under-researched topic. This study aimed to assess the extent of performance and perceived fatigability responses after distinct running exercises. **Methods:** Twelve recreationally trained runners ($\dot{V}O_{2\max}$: 52.2 ± 6.9 mL/kg/min) ran 8.1 ± 0.6 km (i) continuously for 40-min at the velocity associated to the respiratory compensation point (vRCP, CONT_{100%RCP}); and intermittently (ii) 30% above vRCP (HIIT_{130%RCP}, 10×3-min), and (iii) 50% above of vRCP (HIIT_{150%RCP}). Before and for 6-hours after exercise, voluntary and evoked contractions of the knee-extensors were assessed, along with perceived fatigue, leg pain, and mood state. **Results:** Across trials, knee-extensor peak force and voluntary activation remained reduced for at least 6-hours after exercise while contractile function was reduced for up to 2-hours (time effects, $p < 0.05$). Greater impairments in the capacity of the central nervous system (CNS) to propagate electrical signals for the *vastus lateralis* (i.e., reductions in RMS_{VL}/M-wave_{amp}) were observed after CONT_{100%RCP} compared to both HIIT_{130%RCP} and HIIT_{150%RCP} (−20, −3, and −7%, respectively), whereas greater impairments of contractile function (i.e., reductions in Q_{tw_{pot}}) were observed after HIIT_{150%RCP} compared to CONT_{100%RCP} (−8 and −2%, respectively) (trial effect, $p < 0.05$). Leg pain, perceived fatigue, and mood disturbance were greater after HIIT_{150%RCP} compared to CONT_{100%RCP} (trial effect, $p < 0.05$). **Conclusion:** Our findings highlight the task dependency of fatigue in running by showing that over a 6-hour time frame post-exercise, continuous efforts elicit greater impairments on CNS muscle activation capacity, while intermittent efforts cause greater impairments in contractile function and perceived fatigability.

Keywords: muscle fatigue, central fatigue, peripheral fatigue, post-exercise recovery, mood state

4.2. Introduction

Endurance exercises is known to induce fatigue, a transient reduction in maximal muscle force-generating capacity (performance fatigability), accompanied by sensations such as pain in exercised muscles, “tiredness” and mood disturbance (perceived fatigability) (ENOKA; DUCHATEAU, 2016). Using peripheral stimulation techniques by assessment of voluntary strength, evoked twitch contractile properties and motor unit activation of knee extensors, reductions in force-generating capacity can be explored at a more mechanistic level, being attributed to reductions in the central nervous system’s (CNS) capacity to activate the muscles (central fatigue) and/or impairments in tissue contractile function (peripheral fatigue) (HUNTER, 2018).

Fatigue is known to be task-dependent, whereby exercise characteristics determining the dynamics of central and peripheral fatigue as well as perceived fatigability responses (HUNTER, 2018; IANNETTA et al., 2022). For locomotor efforts, fatigue processes are associated to (HUNTER, 2018) the exercise intensity (i.e., whether exercise is performed below or above of the maximum metabolic steady state [MMSS]), and type of training (i.e., whether exercise is performed continuously or intermittently) have been extensively investigated (BROWNSTEIN; MILLET; THOMAS, 2021; MACINNIS; GIBALA, 2017), perhaps due to the key role of these aspects in conditioning and rehabilitation routines (MACINNIS; GIBALA, 2017).

Peripheral fatigue, pain in the exercised muscles and mood disturbances are reported to be higher after exercise in high intensity, i.e. above of the MMSS (i.e., severe and extreme domains), regardless of whether it is continuously (IANNETTA et al., 2022; MONETTE; WEBB, 2020; THOMAS et al., 2015, 2016) or intermittently (MACDOUGALL et al., 2024). Those findings are explained by specific metabolic and system-level perturbations observed in severe and extreme domains such as continuous increases in metabolic by-products (e.g., $[Pi]$, $[H^+]$, $[K^+]$, $[La^-]$) and greater stimulation of the hypothalamic-pituitary-adrenal (HPA) axis (DUCLOS; TABARIN, 2016), which are involved in impairments of contractile function (BLACK et al., 2017; JONES et al., 2008), pain sensation (GREGORY; WHITLEY; SLUKA, 2015; POLLAK et al., 2014), and mood disturbance (BAO; SWAAB, 2019), respectively.

Due to the attainment of a metabolic steady state in exercise performed below of MMSS (BLACK et al., 2017; DUCLOS; TABARIN, 2016; JONES et al., 2008), contractile function, pain in the exercised muscles and mood disturbances are lesser pronounced in

the moderate and heavy-domains regardless of the type of training (DUCLOS; TABARIN, 2016; IANNETTA et al., 2022; MACDOUGALL et al., 2024). Conversely, due to distinct mechanisms (e.g., inhibitory feedback from muscle type III/IV afferents (AMANN et al., 2008, 2011); decreased cerebral catecholamine concentrations and altered brain neurotransmitters (MILLET; MARTIN; TEMESI, 2018; TAYLOR et al., 2016), “fatigue perception” and central fatigue, usually measured by twitch interpolation technique (MILLET et al., 2011), become more pronounced at moderate and heavy-intensity domains (IANNETTA et al., 2022; THOMAS et al., 2015, 2016).

Nevertheless, despite the literature accumulated so far about the task dependency of fatigue during locomotor efforts, most of the knowledge gathered on this scenario comes from cycling while the literature report scanty evidences about the task dependency of fatigue in running (BROWNSTEIN; MILLET; THOMAS, 2021; HUNTER, 2018). There a number of differences in metabolic and neuromuscular demands between running and cycling (MILLET; LEPERS, 2004), which affect the characteristics of fatigue induced by these exercises (BROWNSTEIN et al., 2022; TOMAZIN; MORIN; MILLET, 2017) and may prevent the direct transfer of knowledge about the task dependency of fatigue from one exercise to the other. For example, for a given whole-body work rate, the delta efficiency in the quadriceps (i.e., ratio of external mechanical power output to the metabolic power required to produce it) are likely to be lower for cycling than running (25% and 42%, respectively (BIJKER; DE GROOT; HOLLANDER, 2002), which seems to induced greater peripheral fatigue in the former compared to the latter (BROWNSTEIN et al., 2022; TOMAZIN; MORIN; MILLET, 2017). Furthermore, the stretch-shortening cycles (SSCs) associated to running may cause greater muscle damage due to eccentric contractions (NICOL; KOMI; MARCONNET, 1991; SCHWELLNUS et al., 2011) and reduce Ia afferent-mediated facilitation of alpha-motoneurons (AVELA; KYRÖLÄINEN; KOMI, 1999a), resulting in a greater impairment of CNS muscle activation capacity after running compared to cycling (BROWNSTEIN et al., 2022; TOMAZIN; MORIN; MILLET, 2017).

Insights into the task dependency of fatigue in running can be gained by independent investigations performed by Skof et al., (2006a, 2006b). These authors observed that a 6-km continuous run at MMSS (SKOF; STROJNIK, 2006a) reduced peak force during maximal voluntary contractions (MVCs) of knee-extensors and the contractile function of this muscle group for up to 60-min after exercise cessation, without impairing CNS muscle activation capacity (i.e., voluntary activation [VA] measured by the twitch

interpolation technique). On the other hand, after a 5×300-m sprint (SKOF; STROJNIK, 2006b), these authors did not demonstrated neither a reduction in peak force during MVC or VA of knee-extensors, and the contractile function of this muscle group remained impaired for up to 20-min after exercise cessation. Interestingly, in both investigations, the authors noticed an increase in evoked force for 10-min after both exercises, suggesting the coexistence of fatigue and post-activation potentiation after running (BOULLOSA et al., 2011). Taken together, the results of Skof et al., (2006a, 2006b) suggest that the dynamics of central and peripheral fatigue during running may be influenced by exercise intensity and/or type of training. However, a limited point was that these studies were carried out in different participants, using self-paced efforts, and performing exercises of different volumes and without defined metabolic stress. Such potential confounding factors in this scenario may preclude proper conclusions about the task dependency of fatigue in running. Furthermore, the acute perceived fatigability responses (e.g., pain at exercised muscles, fatigue perception, mood disturbance) after distinct running exercises have not yet been investigated. This is an important knowledge gap in this area as perceived fatigability may acutely impair performance and chronically increase the risk of health problems such as non-functional overreaching and overtraining (KELLMANN, 2010).

Therefore, the aim of this study was to provide a comprehensive evaluation of knee-extensors neuromuscular function and perceived fatigability responses following distinct running exercises at well-defined levels of metabolic stress. Exercises were performed continuously at the intensity associated to MMSS, and intermittently 30% and 50% above the MMSS intensity, ending with the volume per exercise matched. We hypothesized that the dynamics of peripheral and central fatigue as well as perceived fatigability responses would be dependent on the running exercise performed, being more pronounced as exercise-intensity increases.

4.3. Materials and methods

Participants

The findings reported by Azevedo et al., (2022) about the reductions in contractile function of knee-extensors (i.e., $Q_{tw_{pot}}$, partial eta squared = 0.25) after cycling until task failure at different intensities were used to estimate the minimum sample size in the current study. A minimum of 10 participants was estimated to achieve a power of 0.90 in f tests, setting an alpha of 0.05. Because of the sex-related differences on performance

fatigability responses (ANSDELL et al., 2020), we judged proper recruit only males to take part in the study aiming to reduce data heterogeneity. Thus, 15 healthy, recreational runners (training volume of 15 to 40 km/week) were recruited to participate of the study. Participants were included if they were not under medical or nutritional treatments in the 6 months before the study, and if they had no known neurological, cardiovascular, or musculoskeletal disorders that could potentially affect the evaluated outcomes of the investigation. After being made aware of the experimental procedures, all participants provided their written informed consent. Due to successive absences in the trials ($n=3$), the final sample of study was 12 participants (means $\pm$ SD: age 24 ± 5 years old; height 175.7 ± 6.6 cm; body mass 71.7 ± 8.9 kg; 23.3 ± 6.5 km covered/week). The study was approved by the Sao Paulo State University Research Committee (Number 50075421.9.0000.5398), and all procedures were conducted in accordance with the Declaration of Helsinki.

Experimental Design

The experimental design of the study is presented in **Figure 4.1** Participants visited the laboratory 4 times with a 48-72-h interval between them. On the visit 1, participants underwent height and body mass measurements, performed a graded exercise test (GXT), and were familiarized with the experimental procedures of the study (i.e., questionnaires, scales, maximal knee-extensor contractions, peripheral electrical stimulation, countermovement jumps). On the visits 2, 3 and 4, participants performed volume-matched runs in three different types of training, in a randomized order: continuous run at velocity associated to respiratory compensation point (vRCP, CONT_{100%RCP}); high-intensity interval run 30% above vRCP (HIIT_{130%RCP}); and high-intensity interval run 50% above vRCP (HIIT_{150%RCP}). The vRCP was used to non-invasively determine an exercise intensity threshold for training prescription in the heavy-domain ($\leq$ vRCP) and severe-domain ($>$ vRCP) (KEIR et al., 2022). Despite differences in exercise intensities and type of training (i.e., continuous vs interval), each participant performed the same volume load among training types (8.1 ± 0.6 km).

Throughout CONT_{100%RCP}, HIIT_{130%RCP}, and HIIT_{150%RCP}, rate of pulmonary oxygen consumption ($\dot{V}O_2$) and surface electromyography activity (EMG) from *vastus lateralis* (dominant lower limb) were recorded. Every 20% of exercise-completion, blood samples were taken from the earlobe to measure blood lactate concentrations ($[La^-]$). In these same moments, the rating of perceived exertion (RPE), pain in lower limbs (“Pain”) and global fatigue (“Fatigue”) were also collected. At rest, immediately after exercises and 1, 2, 4

and 6-hours post exercises, neuromuscular assessments consisting of maximal isometric contractions of knee-extensors (iMVC) associated with peripheral electrical stimulation (PNS) were performed. Rating of “Pain” and “Fatigue” were also collected at the same times. The mood state was assessed at rest, 1, 2, 4 and 6-hours after exercises.

All running efforts (i.e., GXT, CONT_{100%RCP}, HIIT_{130%RCP}, and HIIT_{150%RCP}) were performed on a motorized treadmill (ATL, Inbramed, Brazil) with the treadmill gradient fixed at 1% (JONES; DOUST, 1996).

Prior to each visit, all participants were instructed to avoid consumption of food and caffeinated beverages for at least 2 and 8 h, respectively, and to abstain from vigorous physical activity for 24-h. Before the visit 2 (i.e., first exercise trial), the participants were instructed to carry out a dietary recall and to repeat the same eating pattern prior to visits 3 and 4. Before visits 2, 3 and 4, participants were required to rate their overall perception of sleep quality according to Snyder et al., (2018) (visual scale 0-10; 0-terrible to 10-excellent). If sleep quality was rated below 6 (fair), the participants were released, and the visit was rescheduled (it was the case on 3 occasions). On visits 2, 3 and 4, while waiting for the post-exercise assessments, participants consumed cereal bars (Nutry[®], Paraná, Brazil) and processed juices (Maguary[®], São Paulo, Brazil) aiming to ingest 1.1 g/kg of body mass/hour of carbohydrate, which has been recommended to maximize glycogen repletion (MCCARTNEY; DESBROW; IRWIN, 2018). Water was consumed *ad libitum*. The amount cereal bars, juice and water ingested post-exercise during visit 2 was recorded and repeated in visits 3 and 4. All participants performed visits 2, 3 and 4 in the same period of the day and all tests were performed in an environmentally controlled room (temperature: 20–22°C; humidity 40%–60%).

EXPERIMENTAL DESIGN

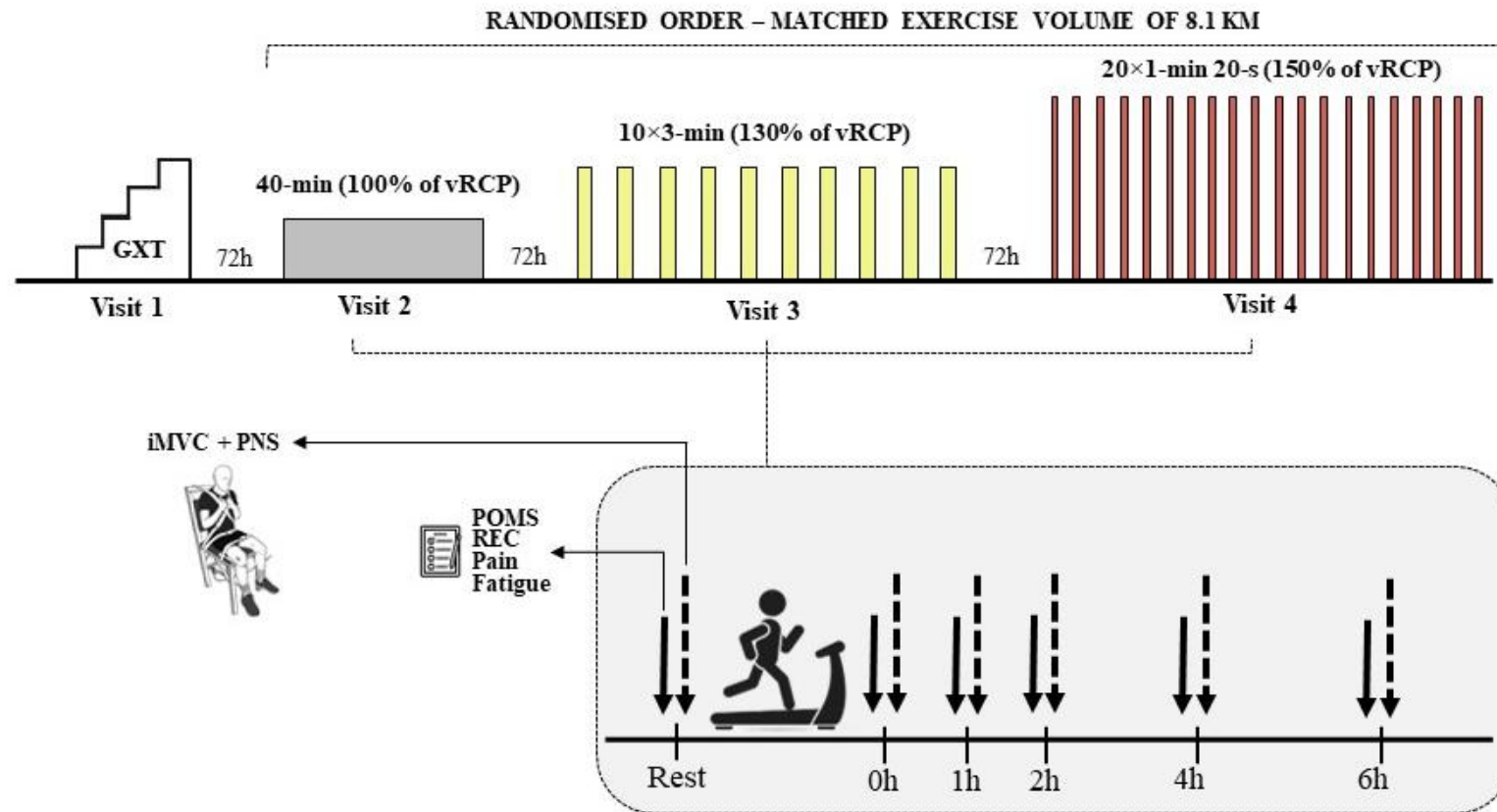


Figure 4.1. Schematic representation of the investigation design. GXT, graded exercise test to assess cardiorespiratory function; iMVC, maximal voluntary isometric contraction of knee-extensors (dominant lower-limb, self-reported); PNS, peripheral electrical stimulation; CMJ, countermovement jump performance analysis; $\dot{V}O_2$, rate of oxygen consumption; HR, heart rate; RER, respiratory exchange ratio; RPE, rate of perceived effort; “Pain”, perception of pain in lower-limbs; “Fatigue”, perception of a global fatigue; vRCP, velocity associated with the respiratory compensation point; HRV, heart rate variability assessment at res; POMS, profile of mood state questionnaire; REC, perception of recovery. N=12.

Graded exercise test

The GXT was performed to determine the maximal oxygen consumption ($\dot{V}O_{2\max}$), RCP and the velocities associated to them (i.e., $v\dot{V}O_{2\max}$ and $vRCP$, respectively). The $vRCP$ was used to set the intensities of running exercises on visits 2, 3 and 4 (i.e., $CONT_{100\%vRCP}$, $HIIT_{130\%vRCP}$, and $HIIT_{150\%vRCP}$). The GXT started at 8 km/h and the velocity was increased by 1.5 km/h each 2-min stage until task failure (BENTLEY; NEWELL; BISHOP, 2007). Five minutes after the end of the GXT, the participants performed a constant work rate exercise until task failure at a velocity 5% greater than the highest velocity achieved during GXT (SCHAUN, 2017). In both cases, task failure was determined when participants disengaged from the effort despite the use of strong verbal encouragement.

Different types of running training: $CONT_{100\%RCP}$, $HIIT_{130\%RCP}$, and $HIIT_{150\%RCP}$.

The $CONT_{100\%RCP}$ consisted of 40-min of continuous running at $vRCP$, while the $HIIT_{130\%RCP}$ consisted of intermittent exercise composed by 10×3 -min running bouts 30% above $vRCP$ (exercise to rest ratio of 1:1.5) and the $HIIT_{150\%RCP}$ also an intermittent exercise consisted of 20×1 -min and 20-s running bouts 50% above $vRCP$ (exercise to passive rest ratio of 1:2). The duration of the bouts in $HIIT_{130\%RCP}$ and $HIIT_{150\%RCP}$ were chosen aiming to match the estimated distance covered by the participants between $CONT_{100\%RCP}$, $HIIT_{130\%RCP}$, and $HIIT_{150\%RCP}$ (i.e., distance in km = velocity in km/h $\times$ exercise time in hours). For example, with a $vRCP$ of 10km/h, after 40-min (the usual duration of the this type of training session (MACINNIS; GIBALA, 2017)), the participant will cover ~ 6.7 -km. To cover this same distance at 13km/h (i.e., 130% of $vRCP$) and 15 km/h (i.e., 150% of $vRCP$), the participant will take ~ 31 -min (i.e., $\sim 10 \times 3$ -min) and ~ 26 min (i.e., $\sim 20 \times 1$ -min and 20-s). Thirty minutes following exercises, participants were asked to rate their perceived exertion with the RPE-CR-10 scale, which was multiplied by the duration of the exercise in minutes to quantify RPE session (RPE_{session}) (FOSTER et al., 2001). All participants were able to complete the proposed running exercise on all the three visits.

Data collection and analysis

Respiratory data

Breath-by-breath pulmonary gas exchange and ventilation were continuously measured, breath-by-breath, using a metabolic cart (Quark, CPET, COSMED, Rome, Italy) during GXT,, $CONT_{100\%RCP}$, $HIIT_{130\%RCP}$, and $HIIT_{150\%RCP}$. The metabolic cart was calibrated

before each test according to the recommendations from the manufacturer. All the breath-by-breath data were examined to exclude aberrant breaths and those values varying more than ± 3 SD from the local mean were removed (ROSSITER et al., 2002). The breath-by-breath data were subsequently interpolated every 1-s (ÖZYENER et al., 2001) using the software OriginPro 8.0 (Origin Lab Corporation, Massachusetts, United States). In the GXT, the highest $\dot{V}O_2$ average from the last 20-s of each stage was assumed as $\dot{V}O_{2max}$ when it was similar (± 2.1 mL/kg/min) to $\dot{V}O_2$ average from the last 10-s recorded in the CWR exercise carried out 5 minutes after GXT (SCHAUN, 2017). All participants reached the $\dot{V}O_{2max}$ according to this criterion. The minimal exercise velocity at which $\dot{V}O_{2max}$ was reached was recorded as $v\dot{V}O_{2max}$ (BILLAT; BLONDEL; BERTHOIN, 1999). For the estimation of RCP, raw profiles of pulmonary ventilation ($\dot{V}_E$), carbon dioxide output ($\dot{V}CO_2$), their ratio (i.e., $\dot{V}_E/\dot{V}CO_2$), RER, and end-tidal pressure of CO_2 (Pet CO_2) were plotted against time. RCP was identified as the point at which Pet CO_2 began a precipitous fall after a period of isocapnia concomitantly with a second and first breakpoint in the $\dot{V}_E$ and $\dot{V}_E/\dot{V}CO_2$ relationships, respectively (KEIR et al., 2022). The $vRCP$ was identified by the projection of RCP (time at which it occurs) at GXT stages. When necessary, $v\dot{V}O_{2max}$ and $vRCP$ were adjusted according to Kuipers et al., (1985). During $CONT_{100\%RCP}$, $HIIT_{130\%RCP}$, and $HIIT_{150\%RCP}$, the $\dot{V}O_2$ average of the last 20-s of each time-window corresponding to 20% of exercise completion calculated (i.e., 20, 40, 60, 80 and 100% of exercise completion) (DUTRA et al., 2020). During the brief running efforts, the $\dot{V}O_2$, HR and RER average of the last 20-s of effort was calculated (DUTRA et al., 2020).

Neuromuscular function

EMG activity during $CONT_{100\%RCP}$, $HIIT_{130\%RCP}$, and $HIIT_{150\%RCP}$

The EMG activity from the *vastus lateralis* of the dominant lower limb (self-reported) was recorded. The EMG signal was acquired through a Wave Wireless EMG device (Cometa System, Milan, Italy) via self-adhesive Ag-AgCl surface electrodes (Meditrace 100, Covidien, USA) placed over the 2/3 of muscle in a bipolar configuration and with a 20-mm interelectrode distance, as recommended by SENIAM (HERMENS et al., 2000). Before electrode placement, the skin sites were shaved, lightly abraded using a sandpaper, and cleaned with rubbing alcohol. Signals were acquired at a sampling rate of 2,000 Hz by PowerLab system (16/35, ADInstruments, Dunedin, New Zealand). The EMG activity was posteriorly band-pass filtered (20–500 Hz) and analyzed offline using the LabChart

8 software (ADInstruments, Dunedin, New Zealand). The root mean square (RMS) of the signal in the last 20-s of each time-window corresponding to 20% of exercise completion calculated (i.e., 20, 40, 60, 80 and 100% of exercise completion) (DUTRA et al., 2020).

Maximal isometric voluntary contraction and peripheral nerve stimulation

iMVCs with dominant knee-extensors (self-reported) associated to PNS during force production and at rest were performed by the participants prior to, immediately-post, 1, 2, 4 and 6 hours after CONT_{100%RCP}, HIIT_{130%RCP}, HIIT_{150%RCP}. For the iMVCs, participants were positioned on a custom-made chair designed to keep the hips and knees flexed at 90°. Participants were firmly secured with straps fastening legs, hips, and chest. Force signal was acquired using a load cell with a 0.98-N resolution (MK Controle, Brazil). The load cell was attached to the ankle by a metal rod immediately above the region of the medial malleolus using straps with heavy duty Velcro. The EMG signal from *vastus lateralis* was also acquired by a pair of self-adhesive Ag/AgCl surface electrode (Meditrace 100, Covidien, USA) placed on the 1/3 distal of muscle using an amplifier FE136 (ADInstruments, Dunedin, New Zealand). Both muscular force and EMG signals were recorded using the LabChart 8 software (DUTRA et al., 2023).

Electrical stimulations of the femoral nerve of the dominant knee extensors (pulse duration: 1 ms; square wave) were delivered by a high-voltage electrical stimulator (Bioestimulador V2 400 V peak-to-peak; Insight, São Paulo, Brazil) through Ag/AgCl electrodes (10 mm of diameter, Kendall Medi-Trace, Massachusetts) on the femoral triangle (cathode) and through carbon rubber electrodes (32.5 mm of diameter, CARCI, Brazil) at the femoral greater trochanter level (anode). Doublet high-frequency stimulations (i.e., 100 Hz) were delivered at the visual peak force of the iMVC effort (~2-3-s after iMVC started) as well as at 3-s after iMVC with the musculature relaxed. Single and doublet low-frequency (10 Hz) stimulations were also delivered to the relaxed muscle at 7 and 10 s after iMVC, respectively. The magnitude of the electrical stimuli was determined individually in each visit by the application of single consecutive incremental stimuli on the relaxed muscle starting at 30 mA followed by 30-mA increments until reaching the involuntary force plateau. The force plateau was 280±58 mA across the trials, The electrical stimuli intensity used for the PNS was further increased by 30% (DUTRA et al., 2023)

For the trials carried out immediately after exercise, it took 57±9-s, to maneuver the participants from the treadmill to the chair used for the neuromuscular assessments and

to perform the methodological procedures needed to start the test (e.g., electrodes and wires placement, ankle attachment, signal stabilization). Except in the assessments carried out immediately after exercise, three iMVCs interspersed by 30-s and followed by 2-min rest were performed before all trials as a warm-up procedure. The peak force achieved during these warm-up iMVCs was used to ensure that participants reached their peak force during iMVCs associated to PNS trials, since described that participants' knowledge about the PNS may unintentionally reduce the voluntary peak force during this kind of test (BUTTON; BEHM, 2008).

The highest voluntary force recorded within a 100-ms period during the force plateau of the iMVC was used as the peak voluntary force ($iMVC_{Force}$). The $iMVC_{Force}$ was expressed in raw values (i.e., N). The superimposed force signal amplitude by the doublet high frequency stimuli recorded during iMVC and the force evoked by the doublet high-frequency stimuli at rest after iMVC ($Db100_{Force}$) were used to measure the voluntary neuromuscular activation (VA) using the following equation: $VA (\%) = [1 - (\text{superimposed force}) \times (\text{force level at stimulation}/iMVC_{Force}) / (Db100_{Force})] \times 100$ (STROJNIK; KOMI, 1998). The potentiated peak force evoked by the single stimulus ($Q_{tw_{pot}}$) was recorded and the ratio between the force evoked by the doublet low-frequency stimuli ($Db10_{Force}$) and $Db100_{Force}$ (i.e., $Db10_{Force}/Db100_{Force}$ ratio) was calculated. The EMG signal was initially band-pass (20–500 Hz) filtered and then the RMS from the signal 0.5 s around the $iMVC_{Force}$ was calculated ($MVC_{RMS_{VL}}$). The amplitude of EMG response evoked by the single stimulus were also recorded (M-wave amplitude). The ratio between $MVC_{RMS_{VL}}$ and M-wave amplitude ($RMS/M\text{-wave}_{amp}$) was also calculated. All analysis procedures were performed using the LabChart 8 software. All data is presented in percentage change from the rest (*raw* values are presented in supplementary file).

Perceptual responses

Thirty minutes following $CONT_{100\%RCP}$, $HIIT_{130\%RCP}$, and $HIIT_{150\%RCP}$, participants were asked to rate their RPE through a RPE-CR-10 scale, which was multiplied by the duration of the exercise in minutes in order to quantify the internal load of the exercises (FOSTER et al., 2001). The RPE, rating of “Pain” in the lower limbs and global “Fatigue” were recorded each 20% of exercise-completion. RPE was assessed by 6-20 top-to-bottom Borg's scale (BORG, 1982); exercising legs “Pain” was assessed by 0-10 top-to-bottom Borg's scale (BORG et al., 2010); and global “Fatigue” was assessed by 10-0 top-to-bottom Micklewright's scale (MICKLEWRIGHT et al., 2017).

The mood state was assessed using the profile of mood state questionnaire (POMS) (BEEDIE; TERRY; LANE, 2000). The Total Mood Disturbance (TMD) score, which is a summary of positive mood from adding subscales and subtracting vigor was calculated. Higher scores for the TMD indicate a greater mood disturbance (BEEDIE; TERRY; LANE, 2000).

Blood lactate concentration during exercise

Blood samples from the earlobe were collected each 20% of exercise completion during CONT_{100%RCP}, HIIT_{130%RCP}, and HIIT_{150%RCP} to measure [La⁻]. Samples (25 μ L) were collected in heparinized micro-hematocrit tubes, immediately deposited into polyproline microtubes containing 50 μ L of sodium fluoride at 1%, stored at – 20° Celsius and then analyzed in a biochemical analyzer YSI 2900 (Yellow Spring Instruments, Ohio, USA). The equipment error is $\pm$ 2%, according to the manufacturer's information.

Statistical analysis

The SPSS the software package version 20.0 (IBM Corp., Armonk, NY) was used for all statistical analysis. All data are presented as mean $\pm$ SD. Statistical significance was set at α of 0.05. Normality of the data was verified using the Shapiro-Wilk test. A two-way repeated measures ANOVA was used to assess the effect of training type (CONT_{100%RCP}, HIIT_{130%RCP}, and HIIT_{150%RCP}) and time (rest until 100% of exercise completion; rest until 6-h post-exercise) on the outcomes. Assumptions of sphericity were explored and controlled for all variables with the Greenhouse–Geisser adjustment, where necessary. If significant main effects or interactions (training type $\times$ time) were observed, a Bonferroni post hoc pairwise comparison was performed. Partial eta squared (η_p^2) was calculated to estimate effect sizes, with values of 0.01, 0.06, and 0.14 representing small, moderate, and large effects, respectively.

4.4. Results

Maximal and submaximal responses recorded during GXT are displayed in **Table 4.1**. The CONT_{100%RCP} was performed at 12.1 ± 0.9 km/h while HIIT_{130%RCP} and HIIT_{150%RCP} were performed at 15.7 ± 1.2 km/h and 18.3 ± 1.4 km/h, respectively ($F_{2,22} = 1914.47$, $p < 0.01$, $\eta_p^2 = 0.99$). These intensities corresponded to $75 \pm 5\%$, $98 \pm 7\%$ and $113 \pm 8\%$ of $\dot{V}O_{2max}$, respectively ($F_{2,22} = 1946.01$, $p < 0.01$, $\eta_p^2 = 0.99$). There was an exercise effect for session-RPE ($F_{2,22} = 44.66$, $p = 0.01$, $\eta_p^2 = 0.80$), whereby it was lower in CONT_{100%RCP} (210 ± 71 a.u.) compared to both _{130%RCP} (571 ± 188 a.u., *post hoc* $p < 0.01$) and _{150%RCP}

(618 ± 128 a.u. *post-hoc* $p < 0.01$), with no difference between the latter. Each participant covered the same distance of 8.1 ± 0.6 km during exercises.

Table 4.1. Maximal and submaximal physiological parameters measured during GXT.

$\dot{V}O_{2\max}$ (L/min)	3.70 ± 0.39 (3.45 to 3.95)
$\dot{V}O_{2\max}$ (mL/kg/min)	52.2 ± 6.9 (47.8 to 56.5)
$v\dot{V}O_{2\max}$ (km/h)	16.1 ± 1.5 (15.1 to 16.9)
RCP	
$\dot{V}O_2$ (L/min)	3.08 ± 0.35 (2.85 to 3.29)
$\% \dot{V}O_{2\max}$	83 ± 8 (78 to 88)
Velocity (km·h ⁻¹)	12.1 ± 0.9 (11.5 to 12.7)
$\% v\dot{V}O_{2\max}$	75 ± 5 (72 to 78)

Data are presented as mean $\pm$ standard deviation (95% of confidence interval). $\dot{V}O_{2\max}$: maximal oxygen consumption; $v\dot{V}O_{2\max}$: velocity associated to $\dot{V}O_{2\max}$; RCP: respiratory compensation point; $\dot{V}O_2$: oxygen consumption.

Metabolic, respiratory, muscle activation and perceptual responses during CONT_{100%RCP}, HIIT_{130%RCP}, and HIIT_{150%RCP}

Physiological and perceptual responses measured during running exercises are displayed in **Figure 4.2**. During all training types, the $\dot{V}O_2$, $[La^-]$, and RMS_{VL} increased from warm-up until 20-60% of exercise completion (time effect, $p < 0.01$) with no further increase across the exercise, whereas the RPE, “Pain” and “Fatigue” progressively increased throughout effort reaching its greatest scores at 80%-100% of exercise completion (*post-hoc* $p \leq 0.01$). Over the entire exercise (i.e., , $\dot{V}O_2$, RMS_{VL} , $[La^-]$, RPE and fatigue perceptions were lower in CONT_{100%RCP} compared to both HIIT_{130%RCP} and HIIT_{150%RCP} (main effect of training type, $p \leq 0.01$), with no difference between the latter ($p > 0.05$),

except by $[La^-]$ which was also lower in HIIT_{130%RCP} compared to HIIT_{150%RCP} (main effect of training type, $p<0.01$).

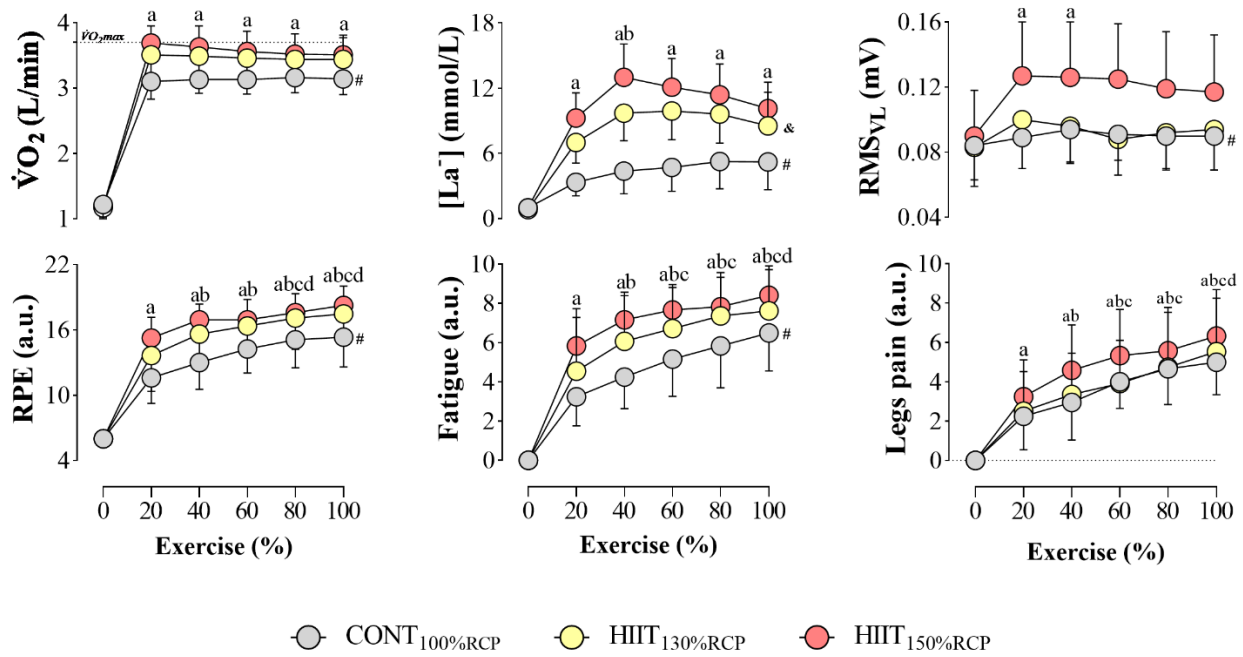


Figure 4.2. Physiological and perceived responses during exercise. $\dot{V}O_2$, rate of oxygen consumption; $[La^-]$, blood lactate concentration; RMS_{VL} (root mean square of vastus lateralis electromyography activity signal); RPE, rating of perceived exertion; “Fatigue”, perception of overall fatigue; “Pain”, perception of pain in lower-limbs. CONT_{100%RCP} (40-min continuous running at velocity associated to respiratory compensation point [vRCP]), HIIT_{130%RCP} (10×3-min running bouts 30% above vRCP, 1:1.5 work:rest ratio), and HIIT_{150%RCP} (20×1-min and 20-s running bouts 50% above vRCP, 1:2 work:rest ratio). Statistical reports come from a two-way (time and training type) repeated measures analysis of variance (ANOVA). a, different from the beginning of the effort ($p<0.05$, main effect of time); b, different from 20% of exercise duration ($p<0.05$, main effect of time); c, different from 40% of exercise duration ($p<0.05$, main effect of time); d, different from 60% of exercise duration ($p<0.05$, main effect of time); #, different from HIIT_{130%RCP} and HIIT_{150%RCP} ($p<0.05$, main effect of training type); &, different from CONT_{100%RCP} and HIIT_{150%RCP} ($p<0.05$, main effect of training type). N=12.

Neuromuscular function after CONT_{100%RCP}, HIIT_{130%RCP}, and HIIT_{150%RCP}

The changes in neuromuscular function after exercises are displayed in **Figure 4.3**. There was a main effect of time for $iMVC_{Force}$ ($F_{5,55}=13.71$, $p<0.01$, $n_p^2=0.55$), VA ($F_{5,55}=6.12$, $p<0.01$, $n_p^2=0.36$), $Db10_{force}/Db100_{force}$ ($F_{5,55}=6.20$, $p<0.01$, $n_p^2=0.36$), Qtw_{pot} ($F_{5,55}=0.24$, $p<0.01$, $n_p^2=0.24$), and $RMS_{VL}/M-wave_{amp}$ ($F_{5,55}=0.24$, $p=0.03$, $n_p^2=0.24$), evidencing that independently of type of training, the neuromuscular function was impaired over time. Immediately after exercise, the $iMVC_{Force}$, VA and the $Db10_{force}/Db100_{force}$ reduced (rest vs 0-h: *post-hoc* $p\leq0.01$). $Db10_{force}/Db100_{force}$ remained reduced for 1-h post-exercise (rest vs 2-h, 4-h and 6-h: *post-hoc* $p>0.05$), while $iMVC_{Force}$ and VA remained reduced for at least 6-hours after exercise (rest vs 0-h, 1-h, 2-h, 4-h, and 6-h: *post-hoc* $p\leq0.01$). Besides, $iMVC_{Force}$ presenting its lowest scores 4-hours after exercises (0-h vs 4-h: *post-hoc* $p=0.01$). The Qtw_{pot} reduced in comparison to rest only 2-hours after exercise (rest vs 2-h: *post-hoc* $p=0.01$), while the $RMS_{VL}/M-wave_{amp}$ reduced only 2- and

4-hours after exercise (rest vs 2-h, 4-h: *post-hoc* $p < 0.01$). Throughout the 6-hours post-exercise monitoring time, there was a main effect of training type for $Q_{tw_{pot}}$ ($F_{2,22} = 5.06$, $p = 0.02$, $\eta_p^2 = 0.31$) and $RMS_{VL}/M\text{-wave}_{amp}$ ($F_{2,22} = 9.45$, $p < 0.01$, $\eta_p^2 = 0.46$) with greater reductions in $Q_{tw_{pot}}$ being evidenced after $HIIT_{150\%RCP}$ compared to $CONT_{100\%RCP}$ (*post-hoc* $p = 0.02$), and greater reductions in the $RMS_{VL}/M\text{-wave}_{amp}$ being observed after $CONT_{100\%RCP}$ compared to both $HIIT_{130\%RCP}$ and $HIIT_{150\%RCP}$ (*post-hoc* $p \leq 0.02$). There were no time or training type effects on $M\text{-wave}_{amp}$.

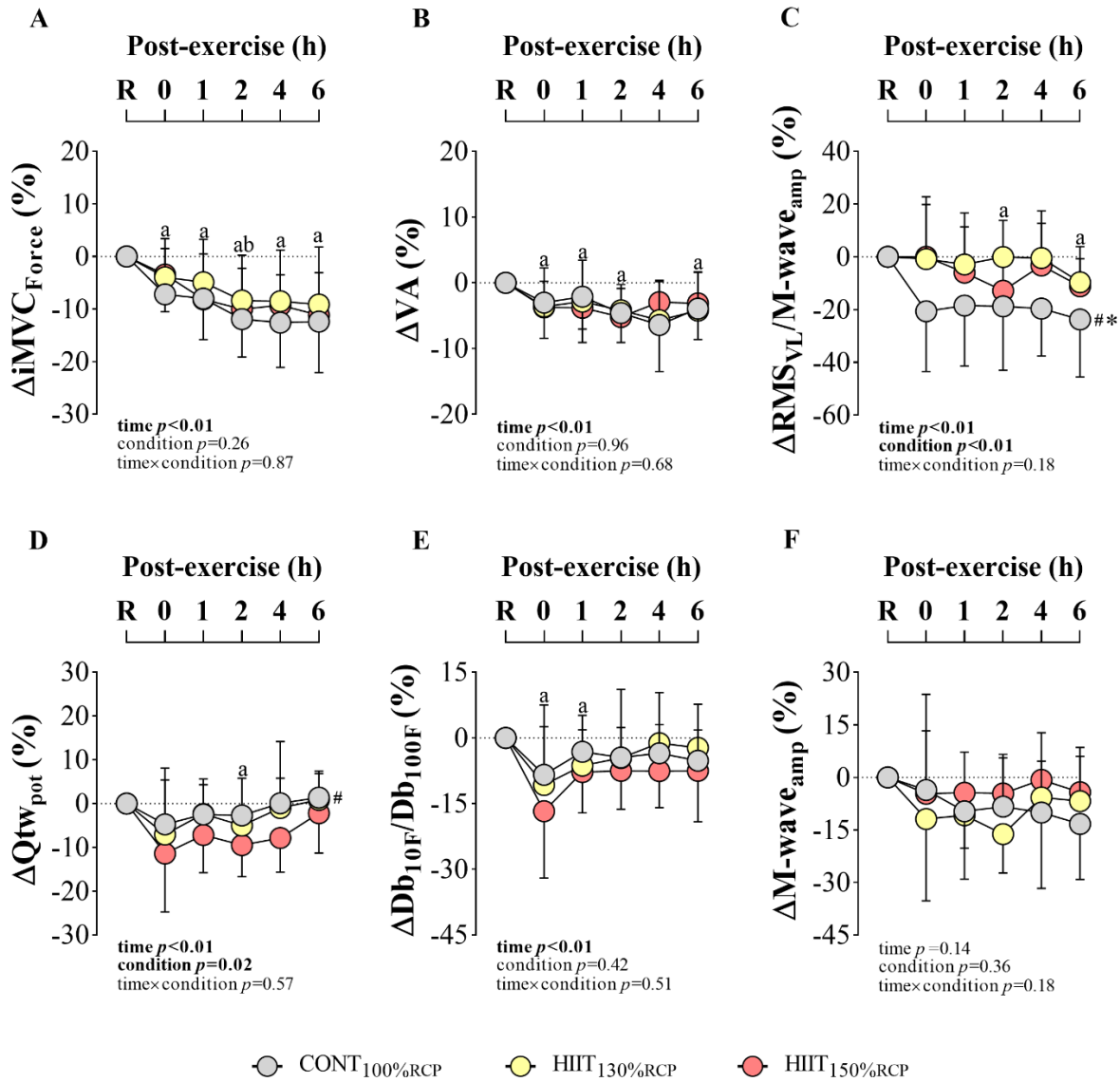


Figure 4.3. Percentage change from rest on knee-extensors function outcomes and countermovement jump performance indicators after exercise. CONT_{100%RCP} (40-min continuous running at velocity associated to respiratory compensation point [vRCP]), HIIT_{130%RCP} (10 $\times$ 3-min running bouts 30% above vRCP, 1:1.5 work:rest ratio), and HIIT_{150%RCP} (20 $\times$ 1-min and 20-s running bouts 50% above vRCP, 1:2 work:rest ratio). R, rest assessments; $iMVC_{Force}$, highest voluntary force recorded during maximal voluntary isometric contractions of knee-extensors; VA, voluntary activation measured through twitch interpolation technique; $RMS_{VL}/M\text{-wave}_{amp}$, ratio between the root mean square of electromyography signal from vastus lateralis during $iMVC$ (0.5-s around $iMVC_{Force}$) and peak-to-peak M-wave amplitude ($M\text{-wave}_{amp}$); $Q_{tw_{pot}}$, force evoked by a potentiated single stimulus at relaxed knee-extensors; Db_{10F}/Db_{100F} , ratio between the force evoked by a supramaximal high-frequency (100Hz) and low-frequency (10 Hz) doublet stimulus at

relaxed knee-extensors. Statistical reports come from a two-way (time and training type) repeated measures analysis of variance (ANOVA). a, different from the rest ($p<0.05$, main effect of time); b, different from 0-h ($p<0.05$, main effect of time); #, different from HIIT_{150%RCP} ($p<0.05$, main effect of training type). N=12. All raw data is presented in supplementary file.

Perceived fatigability responses after CONT_{100%RCP}, HIIT_{130%RCP}, and HIIT_{150%RCP}

The changes on perceived fatigability responses are presented in **Figure 4.4**. There was a main time effect for legs pain ($F_{5,55}=20.73$, $p<0.01$, $\eta_p^2=0.67$) and fatigue perception ($F_{5,55}=62.63$, $p<0.01$, $\eta_p^2=0.85$), whereby they both remained decreased for at least 6-hours after exercise (rest vs 0-h, 1-h, 2-h, 4-h, and 6-h: post hoc $p\leq0.01$). Over the 6-hours after exercise, there was a main effect of training type for legs pain ($F_{2,22}=8.70$, $p<0.01$, $\eta_p^2=0.44$) and fatigue perception ($F_{2,22}=4.91$, $p=0.02$, $\eta_p^2=0.33$), whereby legs pain were lower in CONT_{100%RCP} compared to both HIIT_{130%RCP} (*post-hoc* $p=0.03$) and HIIT_{150%RCP} (*post-hoc* $p=0.04$), and perception of fatigue was lower in CONT_{100%RCP} compared to HIIT_{150%RCP} (*post-hoc* $p<0.01$). There was no main effect of time on POMS_{TMD} scores, but the HIIT_{150%RCP} elicited greater “mood disturbance” compared to CONT_{100%RCP} throughout the 6-hours monitoring time (main effect of training type, $F_{2,22}=3.70$, $p=0.04$, $\eta_p^2=0.27$).

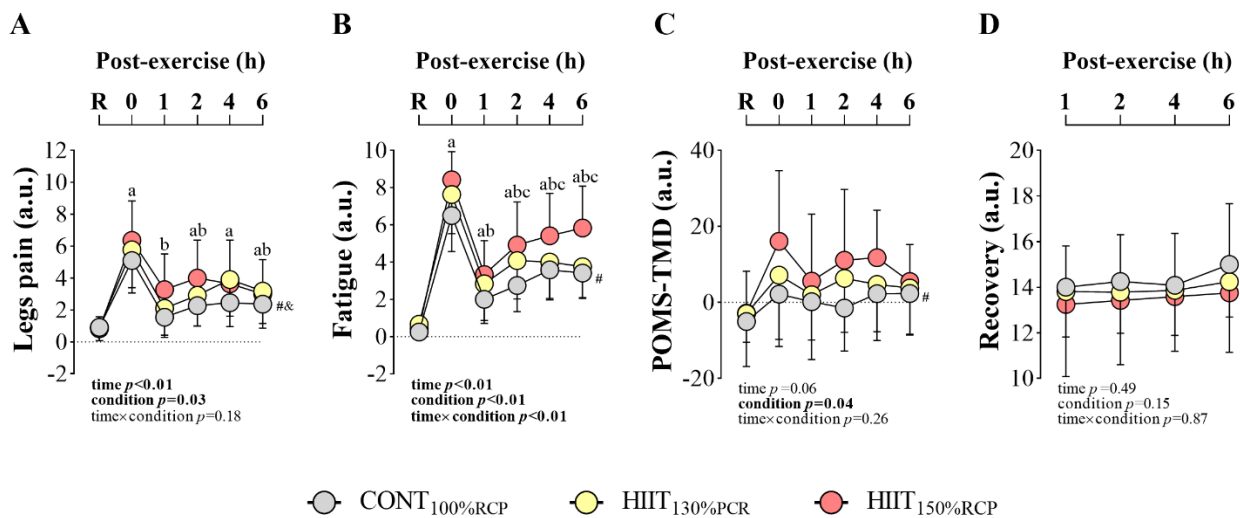


Figure 4.4. Perceived fatigability responses after exercise. CONT_{100%RCP} (40-min continuous running at velocity associated to respiratory compensation point [vRCP]), HIIT_{130%RCP} (10 $\times$ 3-min running bouts 30% above vRCP, 1:1.5 work:rest ratio), and HIIT_{150%RCP} (20 $\times$ 1-min and 20-s running bouts 50% above vRCP, 1:2 work:rest ratio). R, rest assessments; POMS_{TMD}, total mood disturbance calculated through the profile of mood state questionnaire. Statistical reports come from a two-way (time and training type) repeated measures analysis of variance (ANOVA). a, different from the rest ($p<0.05$, main effect of time); b, different from 0-h ($p<0.05$, main effect of time); c, different from 1-h ($p<0.05$, main effect of time); #, different from HIIT_{150%RCP} ($p<0.05$, main effect of training type); &, different from HIIT_{130%RCP} ($p<0.05$, main effect of training type). N=12. Raw data is presented in supplementary file.

4.5. Discussion

To the best of our knowledge, this is the first study to characterize the knee-extensors function and perceived fatigability responses after distinct running exercises (i.e., CONT_{100%RCP}, HIIT_{130%RCP}, and HIIT_{150%RCP}). The main findings are that despite similar reductions on iMVC_{Force}, over 6- hours after exercise, CONT_{100%RCP} induced greater reduction in RMS_{VL}/M-wave_{amp} compared to both HIIT_{130%RCP} and HIIT_{150%RCP} (i.e., main effect of training type). Conversely, over 6-hours after exercise, greater reductions in Qtw_{pot} were observed followed HIIT_{150%RCP} compared to CONT_{100%RCP} (i.e., main effect of training type). In terms of perceived fatigability responses, there was a similar perception of recovery after exercise, but leg pain was greater after HIIT_{130%RCP} and HIIT_{150%RCP} compared to CONT_{100%RCP}, and “fatigue perception” and mood disturbance were greater after HIIT_{150%RCP} compared to CONT_{100%RCP}. Overall, these findings highlight the importance of task dependency of fatigue in running, demonstrating that greater central fatigue can be expected after exercises such as CONT_{100%RCP}, whereas greater peripheral fatigue and perceived fatigability are more likely to be observed after exercise such as HIIT_{150%RCP}.

Neuromuscular function impairments after CONT_{100%RCP}, HIIT_{130%RCP}, and HIIT_{150%RCP}: maximal force-generating capacity of the knee-extensors

The current study extends previous investigations (ROSS et al., 2010; SKOF; STROJNIK, 2006a, 2006b) by reporting the magnitude and time course of reductions in iMVC_{Force} after running at CWR, performed at well-defined metabolic stress level (i.e., at, 30% or 50% above vRCP), and with matched volume between training type (i.e., 8.1 ± 0.6 km). Such methodological precautions helps to understand the dynamics of iMVC_{Force} reduction without possible confounding factors in this scenario, such as variations in running velocity and pacing strategy (as observed in self-paced time-trial efforts)(ROSS et al., 2010; SKOF; STROJNIK, 2006a, 2006b) and differences in exercise volume (as observed in time-to-task failure tests at different intensities) (IANNETTA et al., 2022). It was observed that iMVC_{Force} showed a similar decrease for CONT_{100%RCP}, HIIT_{130%RCP}, and HIIT_{150%RCP} (~ 10.1% at 2-h post-exercise, the lowest values recorded). This degree of knee-extensor force loss is higher than that reported by Skof et al., after a 5×300-m sprint (~ 4%) (2006b) and after a 6-km track run at velocity associated to lactate threshold (~ 6%) (2006a). However, it is lower than those reported by Brownstein et al., (2022) after 17-km treadmill running at velocity associated to lactate threshold (~ 14%) and Ross et al., (2010) after a 20-km self-paced running (~ 17%). Taken together, the

findings of the current study and those reported by Skof et al., (2006a, 2006b), Brownstein et al., (2022), and Ross et al., (2010), suggest that in endurance running exercises up to 2-h duration, the increase in exercise volume seem to increase the degree of knee-extensor force-loss (GIANDOLINI et al., 2016).

Furthermore, the similar reduction in $iMVC_{Force}$ between $CONT_{100\%RCP}$, $HIIT_{130\%RCP}$, and $HIIT_{150\%RCP}$ in the current study suggests that, once the same distance has been covered, aspects of exercises such as intensity or type of training do not seem to affect the magnitude of impairment of the maximal force-generating capacity of the knee-extensors. Similar responses were reported by Besson et al., (2020), who did not found differences in knee-extensors $iMVC_{Force}$ reductions after an ultramarathon run (169 km) performed in single versus multi-stages (~ 31% and 25%, respectively). However, in cycling, MacDougall et al., (2024) demonstrated that even matching effort total work, greater reductions on knee-extensors $iMVC_{Force}$ were observed after 10×2-min effort at 80% of peak power output (~14%) compared to 38-min continuous effort at 54% of peak power output (-25%). Although it is difficult to explain such a discrepancy between running and cycling, it may be due to differences in neuromuscular and metabolic demands between the exercises (BIJKER; DE GROOT; HOLLANDER, 2002; BROWNSTEIN et al., 2022; MILLET; LEPERS, 2004). Nevertheless, it reinforces that what is known about exercise-induced impairments of neuromuscular function is not entirely interchangeable between these exercises.

In addition, we also observed that the lowest values of $iMVC_{Force}$ were recorded 2-hours after exercise. As evidenced in previous studies (SKOF; STROJNIK, 2006a, 2006b), this “delay” in the reduction of $iMVC_{Force}$ suggests that potential confounding factors such as post-activation potentiation (BOULLOSA et al., 2011; RASSIER; MACINTOSH, 2000) may prevent proper conclusions about the real degree of exercise-induced force-loss when assessments are performed shortly after the end of running. However, this assumption needs to be further investigated.

Neuromuscular function impairments after $CONT_{100\%RCP}$, $HIIT_{130\%RCP}$, and $HIIT_{150\%RCP}$: central fatigue

In the current study, central fatigue was measured through VA and $RMS_{VL}/M-wave_{amp}$. From the VA it was observed that the CNS activation capacity of the knee extensors during $iMVCs$ remained similarly reduced for at least 6-hours after $CONT_{100\%RCP}$, $HIIT_{130\%RCP}$, and $HIIT_{150\%RCP}$. Although it is possible to expect some effect of exercise

intensity on the degree of VA reduction (i.e., greater reductions after exercises performed below MMSS compared to above it (MILLET; MARTIN; TEMESI, 2018; THOMAS et al., 2015)), it is also arguable that the extent of central fatigue is independent from exercise intensity (IANNETTA et al., 2022), especially in exercises performed in heavy and severe-domains (AZEVEDO et al., 2021; IANNETTA et al., 2022). VA was reduced $\sim 3.9\%$ in the current study, which is higher than that reported by Skof et al., (2006b) after 5 $\times$ 300-m sprint and Skof et al., (2006a) after 6-km track run at vLT (both $\sim 1\%$). However, the reductions demonstrated here are lower than those reported by Brownstein et al., (2022) after 17-km treadmill run at vLT ($\sim 10.3\%$), and by Ross et al., (2010) after a self-paced 20-km treadmill run ($\sim 12\%$). As suggested for knee-extensors iMVC_{Force} reductions, when the above findings are combined with those reported here, it could be argued that the previously observed impact of distance on central fatigue after prolonged running (≥ 2 -h) (GIANDOLINI et al., 2016) also appears to occur at shorter running (≤ 2 -h).

Despite the similar reductions in VA between exercises in the current study, looking to the greater reduction in RMS_{VL}/M-wave_{amp} after CONT_{100%RCP} compared to HIIT_{130%RCP} and HIIT_{150%RCP} (main effect of training type, $p < 0.05$), suggests that the mechanisms underpinning the impairments in CNS activation capacity of knee-extensors after these exercises are likely to be different. Previous studies have shown that, in response to repeated SSC, the activity of Ia afferent decreases, further reducing the excitatory input from Ia afferents to alpha motoneurons (NICOL; AVELA; KOMI, 2006). This reduction in reflex sensitivity has been linked to reductions in peak voluntary force and CNS muscle activation capacity (AVELA; KYRÖLÄINEN; KOMI, 1999a, 1999b), and has been argued to play a role in the decreased CNS muscle activation capacity after running (BESSON et al., 2020; BROWNSTEIN et al., 2022; TOMAZIN; MORIN; MILLET, 2017). Another generally used explanation for the reduction in CNS muscle activation capacity after running is the increase in group III/IV afferent stimuli, which are known to provide inhibitory feedback to various CNS sites due to increases in intramuscular metabolite concentration (e.g., Pi, H⁺), tissue damage, increases on body temperature, among other physiological changes (HUREAU; ROMER; AMANN, 2018; TAYLOR et al., 2016).

As CONT_{100%RCP} was performed at vRCP, it is likely that there was less metabolic disturbance in this exercise compared to HIIT_{130%RCP} and HIIT_{150%RCP} (JONES et al., 2008). Indeed, the difference in [La⁻] between exercises (i.e., CONT_{100%RCP} < HIIT_{130%RCP}

<HIIT_{150%RCP}) supports that there was a distinct degree of metabolic disturbance between them. In this way, although other possible mechanisms cannot be ruled out, it may be suggested that the reduction in reflex facilitation of Ia afferent fibers due to repetition of SSC played a key role in reducing CNS muscle activation capacity specifically after CONT_{100%RCP}. Due to the rest between the bouts in HIIT_{130%RCP} and HIIT_{150%RCP} (~4 and ~2 minutes, respectively), it is possible that there was enough time for the Ia afferent fibers partially recover from the exercise-induced impairments, as there is evidence that 90-s intervals allow satisfactory recovery of this reflex (BUDINI et al., 2018, 2020). Thus, we speculate that a “less intense” disfacilitation of Ia occurred after HIIT_{130%RCP} and HIIT_{150%RCP} compared to CONT_{100%RCP} (BESSON et al., 2020). Conversely, the greater metabolic disturbance in HIIT_{130%RCP} and HIIT_{150%RCP}, may have elicited a greater degree of group III/IV afferent stimulation (TAYLOR et al., 2016). In this way, although other mechanisms may also be involved, it is possible to suggest that the reflex inhibition induced by group III/IV may have played a key role in impairing the CNS activation capacity of the knee-extensors specifically after HIIT_{130%RCP} and HIIT_{150%RCP} compared to CONT_{100%RCP}.

Furthermore, as exercise performed above MMSS seems to result in large heat storage (LEI et al., 2023) and prolonged increases in muscle/body temperatures (KENNY et al., 2006), this mechanism may also be involved in the impairment of CNS function after HIIT_{130%RCP} and HIIT_{150%RCP} via the inhibitory feedback from group III/IV (MORRISON; SLEIVERT; CHEUNG, 2004). Finally, it was observed that legs pain was greater in HIIT_{130%RCP} and HIIT_{150%RCP} compared to CONT_{100%RCP} throughout 6-hours after exercise (main effect of training type, $p < 0.05$), suggesting a possible higher degree of tissue damage and inflammatory process after the formers compared to latter (PEAKE et al., 2017). This possible higher degree of tissue damage and inflammatory process after HIIT_{130%RCP} and HIIT_{150%RCP} compared to CONT_{100%RCP} might also have triggered greater afferent stimuli from group III/IV neurons after the formers compared to latter (DAVIS; BAILEY, 1997; DOUSSET et al., 2007).

As direct assessments of body temperature and muscle damage/inflammation were beyond the scope of this study, future research should include these measurements in combination with the assessment of central fatigue in order to better understating of the possible causes of central fatigue following exercises such as CONT_{100%RCP}, HIIT_{130%RCP}, and HIIT_{150%RCP}.

Neuromuscular function impairments after CONT_{100%RCP}, HIIT_{130%RCP}, and HIIT_{150%RCP}: peripheral fatigue

We demonstrated that the $Q_{tw_{pot}}$ reduction in the current study was $\sim 5.7\%$ and that it was only observed 2-hours after exercises (main time effect, $p < 0.05$). This level of significant reduction has only been reported after prolonged running (≥ 2 -h) (GIANDOLINI et al., 2016). Previous studies performing 17-km (BROWNSTEIN et al., 2022) and 20-km (ROSS et al., 2010) treadmill runs or shorter efforts on the track (SKOF; STROJNIK, 2006a, 2006b) did not observe significant reductions on contractile function indices of knee-extensors after exercise. As shown in previous investigations (BOULLOSA et al., 2011; SKOF; STROJNIK, 2006a, 2006b), post-activation potentiation may coexist with fatigue after running, which may prevent the evaluation of the true extent of peripheral fatigue in assessments performed shortly after exercise cessation (BOULLOSA et al., 2011). By performing assessments throughout 6-hours after exercises, the current study expands previous investigation by capturing the extent of impaired contractile function after running without confounding factors such as post-activation potentiation.

We observed that HIIT_{150%RCP} induced greater impairments on knee-extensor contractile function compared to CONT_{100%RCP} (i.e., greater reductions in $Q_{tw_{pot}}$, main effect of training type, $p < 0.05$). This finding is in line with the previously reported impact of intensity on the degree of peripheral fatigue (BROWNSTEIN; MILLET; THOMAS, 2021) and seems to occur irrespective whether exercise is performed continuously or intermittently (MACDOUGALL et al., 2024). Indeed, a greater accumulation of metabolites correlated with impairments in contractile function (e.g., P_i , H^+ , K^+) is observed during exercise above MMSS compared to lower intensities, leading to higher indices of peripheral fatigue after the formers compared to latter (BLACK et al., 2017; CHIDNOK et al., 2013a; JONES et al., 2008). Indeed, just slight changes on intensity around MMSS (i.e., 10% above or below it) seems to be capable of impact the degree of peripheral fatigue (AZEVEDO et al., 2021). The current study expands previous investigations by showing, for the first time, the impact of intensity/type of training on the degree of peripheral fatigue in running.

In addition, it is well known that muscle damage can occur after repeated SSC cycles and eccentric muscle contractions in efforts like high-intensity running (PETERSEN et al., 2007; SCHWELLNUS et al., 2011). These events are associated with disruption of excitation-contraction coupling, calcium release from the sarcoplasmic reticulum and disruption at the level of sarcomeres (PROSKE; MORGAN, 2001), which impairs the

contractile capacity of the tissue (BEHRENS; MAU-MOELLER; BRUHN, 2012). As we already argued, looking at the findings on legs pain after exercise (i.e., HIIT_{150%RCP} > CONT_{100%RCP}), it is possible to suggest that a difference in the degree of muscle damage/inflammation between HIIT_{150%RCP} and CONT_{100%RCP} might also play a role in the distinct degree of peripheral fatigue demonstrated between these exercises (BEHRENS; MAU-MOELLER; BRUHN, 2012). Nevertheless, further studies are warranted to better understand the relationships between distinct running exercises, muscle damage/inflammatory processes and the impairment of contractile function.

Perceived fatigability responses after CONT_{100%RCP}, HIIT_{130%RCP}, and HIIT_{150%RCP}

To the best of our knowledge, this is the first study to comprehensively assess perceived fatigability responses after distinct running exercises at well-defined levels of metabolic stress. This is an important knowledge gap to fill in this area since perceived fatigability may acutely impair performance and chronically increase the risk of health issues such as non-functional overreaching and overtraining (KELLMANN, 2010). It was observed that after ~ 8.1-km of treadmill run, legs pain and fatigue perception remained increased for at least 6-hours, regardless of exercise intensity or type, although there was no time effect on mood state. (KELLMANN, 2010; KELLMANN et al., 2018). However, participants rated legs pain, fatigue perception and mood disturbance higher after HIIT_{150%RCP} than CONT_{100%RCP} (main effect of training type, $p < 0.05$). As argued above, the possible muscle damage/inflammatory process induced by the repeated SSC and eccentric muscle contractions during running may have involved in the legs pain sensation after exercise (PETERSEN et al., 2007; SCHWELLNUS et al., 2011). As we speculate that such events may have been greater after HIIT_{150%RCP} compared to CONT_{100%RCP}, this may lead to a higher legs pain score in the former compared to latter. On the other hand, the mechanism behind fatigue perception after exercise is less clear and may be related to the HPA axis (DUCLOS; TABARIN, 2016) and other neurobiological aspects such as decreased cerebral catecholamine concentrations and altered brain neurotransmitters (MILLET; MARTIN; TEMESI, 2018; TAYLOR et al., 2016). As argued for legs pain, it is expected that such system-level perturbations would be higher in HIIT_{150%RCP} compared to CONT_{150%RCP}, resulting in higher fatigue perception and mood disturbance in the former compared to the latter. Clearly, the current study is unable to provide a more mechanistic point of view about perceived fatigability responses underpinning running. Nonetheless, our findings highlight the need for exercise professionals to consider perceived fatigability after running as it coexists with impairments in neuromuscular

function and can have acute and chronic effects on performance and health (KELLMANN, 2010). Furthermore, our findings highlight that in addition to the impact on central and peripheral fatigue, running characteristics also appears impact the perceived fatigability responses. Even so, despite the insights derived from the current study, further research is encouraged to provide a better understanding of the impact running on perceived fatigability responses.

Further considerations

Despite the advances in knowledge provided by the current study, we are aware of some of its methodological limitations. There was a delay of 57 ± 9 seconds between the start of the neuromuscular assessments and the end of the exercise session. Although there is a setup to assess neuromuscular function immediately after exercise in cycling (KRÜGER et al., 2019), this methodological apparatus is difficult to implement for treadmill running and appears to be an inherent methodological flaw when investigating locomotor exercises closer to real-world scenarios (CARROLL; TAYLOR; GANDEVIA, 2017). Nevertheless, recovery of neuromuscular outcomes has been suggested to be up to 2% (not statistically significant) in the first minute after locomotor exercises (KRÜGER et al., 2019). Therefore, it is likely that this delay had a negligible influence on the findings and conclusions of the current study. Furthermore, it is important to note that only males were included in the current study. As comparisons between the sexes were not within the scope of the study and due to the sex-induced differences in fatigability (ANSDELL et al., 2020; AZEVEDO et al., 2022), we deemed it appropriate to recruit only males in order to reduce the heterogeneity of the data and increase the statistical power of the analysis. Therefore, future studies should assess the interactions of sex on dynamics of performance and perceived fatigability responses after running.

Nevertheless, despite these limitations, the current study is the first to address the dynamics of central and peripheral fatigue as well as perceived fatigability responses after distinct running efforts, controlling potential confounding factors in this scenario, such as fluctuations in exercise intensity (as observed in self-paced efforts)(SKOF; STROJNIK, 2006a, 2006b), exercise volume (as observed in time to exhaustion task at different intensities)(IANNETTA et al., 2022; THOMAS et al., 2016). Besides, we also controlled for water and food ingestion post-exercise, which may be important confounding factors in this kind of investigation (BEELLEN et al., 2010). Therefore, we felt that the findings reported here provide new and valuable insights for the exercise professionals and

scientific community, helping to improve real-world scenarios (i.e., rehabilitation, training, and competition routines) and to continuously develop this area of knowledge.

4.6. Conclusion

In conclusion, this study showed that a running exercise of ~8 km performed as CONT_{100%RCP}, HIIT_{130%RCP} and HIIT_{150%RCP} similarly impaired the maximal force-generating capacity and voluntary activation of the knee extensors, including the recovery time-course for at least 6 h. However, a greater impairment in the capacity of the CNS to propagate electrical signals to knee extensor muscles during iMVCs (i.e., a greater reduction in RMS_{VL}/M-wave_{amp}) was observed after CONT_{100%RCP} compared to HIIT_{130%RCP} and HIIT_{150%RCP}, while a greater impairments in knee extensor contractile function (i.e., greater reduction in Qtw_{pot}) as well as greater perceived fatigability (e.g., leg pain, fatigue perception, and mood disturbance), were observed after HIIT_{150%RCP} compared to CONT_{100%RCP}. Taken together, these findings describe the task dependency nature of fatigue in running, which may be very useful in promoting performance and reducing health risks, especially in a busy rehabilitation, training, and competition agenda.

4.7. References

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4.8. Supplementary files

The supplementary files of the Study 4 (*Neuromuscular function impairment and perceived fatigability in response to distinct running exercises*) are available in the following link:

<https://docs.google.com/document/d/1LXQon-S10KHI792SJBjUffqCg50zvdCw/edit?usp=sharing&ouid=115558097214527656085&rtipof=true&sd=true>

CHAPTER 5.
**MARKERS FOR MONITORING THE FATIGUE-
RECOVERY RELATIONSHIP IN ENDURANCE RUNNING**

5. STUDY 3. Markers for monitoring the fatigue-recovery relationship in endurance running.

5.1. Abstract

Proper monitoring of running-induced fatigue provides useful information to favor performance and reduce health risks in successive tasks. This study described changes in different markers for the routine assessment of running-induced fatigue. Twelve recreationally trained runners ($\dot{V}O_{2\max}$: 52.2 ± 6.9 mL/kg/min, training volume: 23.3 ± 6.5 km/week) ran 8.1 ± 0.6 km (i) continuously at velocity associated to respiratory compensation point (vRCP, CONT_{100%RCP}); and intermittently (ii) 30% above and (iii) 50% above of vRCP (HIIT_{130%RCP} and HIIT_{150%RCP}, respectively). Before and followed 6-h after exercise, maximal isometric voluntary contractions (iMVCs) of knee-extensors, countermovement jumps (CMJs), analysis of heart-rate variability (HRV), and evaluation of mood state (by POMS questionnaire), and cognitive function (by Stroop test) were performed at rest, while assessments of physiological (e.g., $\dot{V}O_2$, heart rate, respiratory exchange ratio) and psychological responses (e.g., perception of effort, fatigue and legs pain) were performed during successive brief efforts (3-min run at 80% of vRCP). For at least 6-h, reduced peak force during iMVC, reduced cardiac parasympathetic activity, and disrupted mood and cognitive function were observed, accompanied by lower respiratory exchange ratio and higher $\dot{V}O_2$, heart rate, perceived effort, fatigue, and legs pain during successive brief efforts (time effects, $p < 0.05$). Main condition effects ($p < 0.05$) suggest that compared to CONT_{100%RCP}, HIIT_{130%RCP} induced greater reduction in cardiac parasympathetic activity and HIIT_{150%RCP} induced greater psychological responses in successive brief efforts. The findings demonstrate that running-induced fatigue involves changes in a wide range of body systems/capacities for at least 6-h and suggest that a multivariate approach should be used to monitor it properly.

Keywords: training, performance, wellness, recovery, injury.

5.2. Introduction

Running is a widely used exercise in rehabilitation, training, and competition routines [20,62]. Due to the metabolic, mechanical, and system-level stress caused by running, it induce a state of temporary imbalance called fatigue [58]. Fatigue is defined as a transient reduction in maximal muscle force-generating capacity accompanied by sensations such as “tiredness” and “weakness” and changes in body homeostasis (e.g., core temperature, cardiac parasympathetic activity, mood state) [27]. Recovery is a multifaceted restorative process relative to time that compensates for fatigue [35]. Recovered individuals have reached their pre-exercise homeostasis status and will be able to meet or exceed previous performance levels [58]. Because fatigue is linked to acute impairments in performance and chronic health problems (e.g., musculoskeletal injury, non-functional overreaching, and overtraining) [34,66], the ability to monitor the fatigue-recovery relationship is an invaluable aspect of recreational, amateur, and professional sport [34].

A multitude of non-invasive markers have been suggested to monitor the fatigue-recovery relationship [29]. For example, to track exercise-induced changes in neuromuscular function, tests that assess force production in brief maximal voluntary contractions (MVCs) [47] and the analysis of countermovement jump (CMJ) performance [22] are commonly used. To carry out monitoring of other systems disturbed by exercise, assessing the level of parasympathetic activity at rest by means of heart rate variability (HRV) analysis [61], and psychometric instruments such as scales (e.g., to measure perception of legs pain, overall fatigue and overall recovery) and tests/questionnaires (e.g., Stroop test, profile of mood state questionnaire) are also employed [29,34]. When possible to carry out, analyzing performance and physiological/psychological responses in a specific task close to “real-world” scenarios is suggested as the most relevant marker for differentiating between fatigued and recovered state [6,37].

Nevertheless, fatigue is known to be task-dependent, with aspects of exercise such as intensity (i.e., whether performed below or above the maximum metabolic steady state [MMSS]) and type of execution (i.e., whether performed continuously or intermittently) influencing physiological and psychological imbalances induced by exercise [9]. In this way, depending on the characteristics of the exercise performed, and consequently, the characteristics of exercise-induced body imbalance, some markers may be more appropriate than others for monitoring the fatigue-recovery relationship. [35]. For example, after running in which neuromuscular function is expected to be highly compromised (e.g., due to the great metabolite accumulation and/or tissue damage;

exercises far above MMSS)[55], neuromuscular function indices obtained through MVCs and CMJs tests may be more relevant to track the fatigue-recovery relationship [23]. On the other hand, after running with high cardiorespiratory demand (i.e., due to maintaining high oxygen consumption and/or heart rate during exercise) [20], HRV metrics may be more useful in track the fatigue-recovery relationship [61].

In this way, the aim of this study was to provide a comprehensive assessment of the fatigue-recovery relationship after distinct running exercises, measuring day-long fluctuations in markers related to knee extensor force production, CMJ performance, cardiac parasympathetic activity, mood state and cognitive function, as well as physiological (e.g. oxygen consumption, heart rate) and psychological responses (e.g. perceived effort, fatigue and leg pain) during successive brief running efforts. The exercises were performed continuously at the velocity associated with respiratory compensation point (vRCP, non-invasive surrogate marker of maximum metabolic steady state [33]), and intermittently 30% and 50% above vRCP, ending with the volume per exercise matched. Our hypothesis was that the running exercises would lead to relevant changes in the markers assessed, with more pronounced changes after running performed at 30% and 50% above the vRCP compared to running performed at vRCP.

5.3. Materials and methods

Participants

Based on the findings reported by Brownstein et al., [18] about the reductions in peak force after 1.5-h running at moderate domain (partial eta squared = 0.72), a minimum sample size of 9 participants was estimated to reach a power of 0.90 in *f* tests setting an alpha of 0.05. Because of the sex-related differences on performance fatigability responses [3], we judged proper recruit only males to take part in the study aiming to reduce data heterogeneity. Thus, 15 healthy, recreationally runners (training volume of 15 to 40 km/week) were recruited to participate of the study. Participants were included if they were not under medical or nutritional treatments in the 6 months before the study, and if they had no known neurological, cardiovascular, or musculoskeletal disorders that could potentially affect the evaluated outcomes of the investigation. After being made aware of the experimental procedures, all participants provided their written informed consent. Due to successive absences in the trials (*n*=3), the final sample of study was 12 participants (means $\pm$ SD: age 24 $\pm$ 5 years old; height 175.7 $\pm$ 6.6 cm; body mass 71.7 $\pm$ 8.9 kg; 23.3 $\pm$ 6.5 km covered/week). These same volunteers and part of this data set were

included in a previous study (Study 2 of this thesis) that addressed a different research question, therefore, data from the participants' characteristics and running' characteristics are repeated.

Experimental Design

The experimental design of the study is presented in **Figure 5.1**. Participants visited the laboratory on four occasions with a 48-72-h interval between them. On the visit 1, participants underwent height and body mass measurements, performed a graded exercise test (GXT), and were familiarized with the experimental procedures of the study (i.e., questionnaires, scales, maximal knee-extensor contractions, peripheral electrical stimulation, countermovement jumps). On the visits 2, 3 and 4, participants performed in a randomized order three different exercises: continuous run at velocity associated to respiratory compensation point (vRCP, CONT_{100%RCP}); high-intensity interval run 30% above vRCP (HIIT_{130%RCP}); and high-intensity interval run 50% above vRCP (HIIT_{150%RCP}). Despite differences in exercise intensities and type (i.e., continuous vs interval), the volume load was equal among exercises. At rest, and 1, 2, 4 and 6 hours post exercises, the following measurements were performed: (i) assessment of mood state using the profile of mood state questionnaire (POMS); (ii) assessment of cognitive aspects using the Stroop color and word test; (iii); analysis of cardiac parasympathetic activity from HRV-derived metrics; and (iv) neuromuscular assessments consisting of maximal isometric contractions of knee-extensors (iMVC) and countermovement jump (CMJ) performance analysis. In addition, in these same time points, brief running efforts associated with the monitoring of $\dot{V}O_2$, heart rate (HR), respiratory exchange ratio (RER), and perceived responses (i.e., effort, fatigue, and legs pain) were performed. All running protocols (i.e., GXT, CONT_{100%RCP}, HIIT_{130%RCP}, HIIT_{150%RCP}, and brief running efforts) were performed on a motorized treadmill (ATL, Inbramed, Brazil) with the treadmill gradient fixed at 1% [32].

Prior to each visit, all participants were instructed to avoid consumption of food and caffeinated beverages for at least 2 and 8 h, respectively, and to abstain from vigorous physical activity for 24-h. Before the visit 2 (i.e., first exercise trial), the participants were instructed to carry out a dietary recall and to repeat the same eating pattern prior to visits 3 and 4. Before visits 2, 3 and 4, participants were required to rate their overall perception of sleep quality according to Snyder et al., [60] (visual scale 0-10; 0-terrible to 10-excellent). If sleep quality was rated below 6 (fair), the participants were released, and the visit was rescheduled (it was the case in 3 occasions). On visits 2, 3 and 4, while

waiting for the post-exercise assessments, participants consumed cereal bars (Nutry[®], Paraná, Brazil) and processed juices (Maguary[®], São Paulo, Brazil) aiming to ingest 1.1 g/kg of body mass/hour of carbohydrate, which has been recommended to maximize glycogen repletion [43]. Water was consumed *ad libitum*. The amount cereal bars, juice and water ingested post-exercise during visit 2 was recorded and repeated in visits 3 and 4. All participants performed the visits 2, 3 and 4 in the same period of the day and all tests were performed in an environmentally controlled room (temperature: 20–22°C; humidity 40%–60%).

EXPERIMENTAL DESIGN

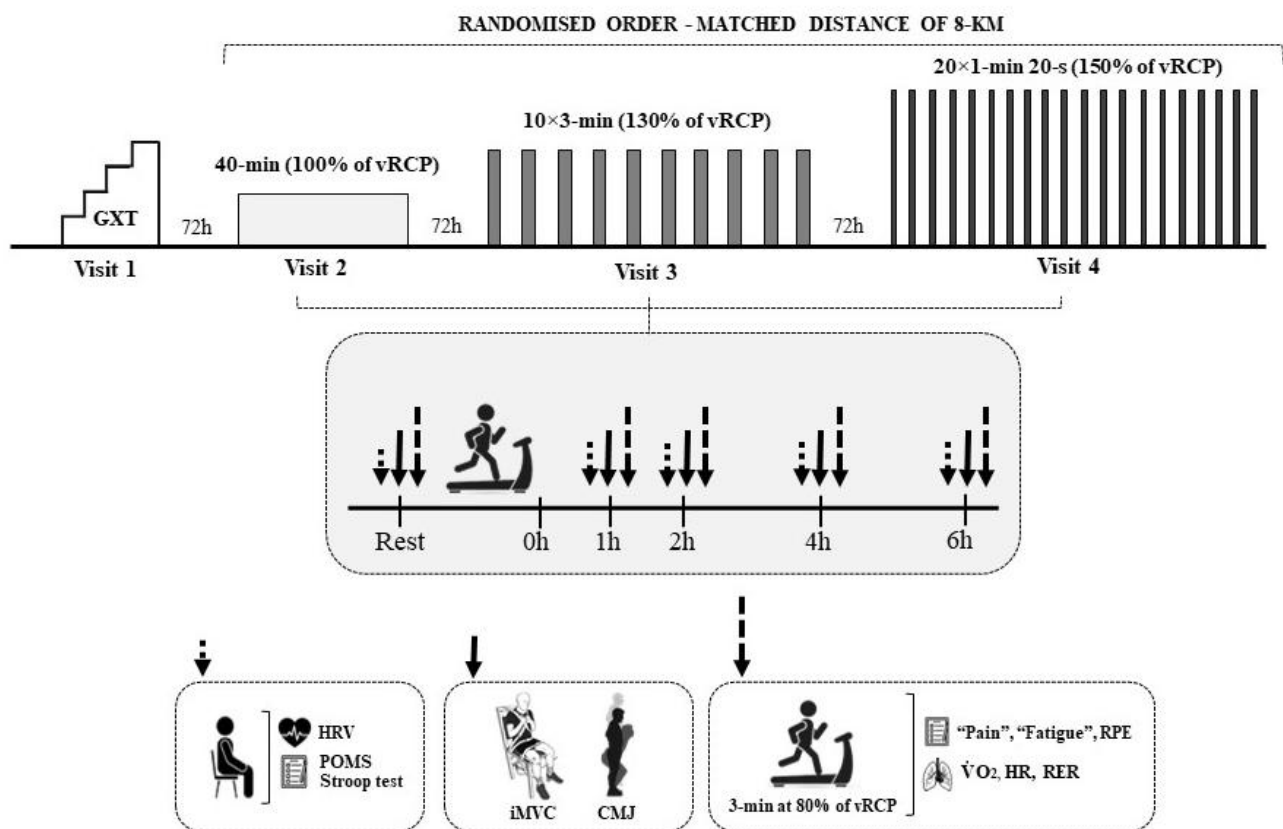


Figure 5.1. Schematic representation of the experimental design. GXT: graded exercise test to assess cardiorespiratory function; vRCP: velocity associated to respiratory compensation point; iMVC, maximal voluntary isometric contraction of knee-extensors (dominant lower-limb, self-reported); CMJ: countermovement jump performance analysis; HRV: heart rate variability assessment at rest; POMS: profile of mood state questionnaire; $\dot{V}O_2$: rate of oxygen consumption; HR: heart rate; RER: respiratory exchange ratio; RPE: rate of perceived effort; “Pain”: perception of legs pain; “Fatigue”: perception of global fatigue. N=12.

Exercise protocols

Graded exercise test

The GXT was performed to determine the maximal oxygen consumption ($\dot{V}O_{2max}$), RCP and the velocities associated to them (i.e., $v\dot{V}O_{2max}$ and vRCP, respectively). The vRCP

was used to set the intensities of running exercises on visits 2, 3 and 4 (i.e., $CONT_{100\%vRCP}$, $HIIT_{130\%vRCP}$, and $HIIT_{150\%vRCP}$). The GXT started at 8 km/h and the velocity was increased by 1.5 km/h each 2-min stage until task failure [10]. Five minutes after the end of the GXT, the participants performed a constant work rate exercise also until task failure at a velocity 5% greater than the highest velocity achieved during GXT (SCHAUN, 2017). In both cases, task failure was determined when participants disengaged from the effort despite the use of strong verbal encouragement.

$CONT_{100\%RCP}$, $HIIT_{130\%RCP}$, and $HIIT_{150\%RCP}$.

The $CONT_{100\%RCP}$ consisted of 40-min of continuous running at vRCP, while the $HIIT_{130\%RCP}$ consisted of 10×3 -min running bouts 30% above vRCP (exercise to rest ratio of 1:1.5) and the $HIIT_{150\%RCP}$ consisted of 20×1 -min and 20-s running bouts 50% above vRCP (exercise to passive rest ratio of 1:2). The duration of the bouts in $HIIT_{130\%RCP}$ and $HIIT_{150\%RCP}$ were chosen aiming to match the estimated distance covered by the participants between $CONT_{100\%RCP}$, $HIIT_{130\%RCP}$, and $HIIT_{150\%RCP}$ (i.e., distance in km = velocity in km/h $\times$ exercise time in hours). For example, with a vRCP of 10km/h, after 40-min (the usual duration of the this type of training session [42]), the participant will cover ~ 6.7 -km. To cover this same distance at 13km/h (i.e., 130% of vRCP) and 15 km/h (i.e., 150% of vRCP), the participant will take ~ 31 -min (i.e., $\sim 10 \times 3$ -min) and ~ 26 min (i.e., $\sim 20 \times 1$ -min and 20-s). Throughout $CONT_{100\%RCP}$, $HIIT_{130\%RCP}$, and $HIIT_{150\%RCP}$, HR during exercise was monitored and 3 and 5 minutes after exercise, blood samples were taken from the earlobe to measure peak of blood lactate concentrations ($[La^-]$). All participants were able to complete the proposed running exercise in all the three visits.

Brief running efforts

To provide a more ecological evaluation of the fatigue-recovery relationship [35], an assessment of the physiological and psychological responses during brief running efforts was carried out. The effort was performed at 80% of vRCP and lasted 3 minutes. This low intensity and short duration was chosen to avoid causing additional physiological/psychological disturbance in the participants [29]. All participants were able to complete the proposed efforts.

Data collection and analysis

Respiratory data

Breath-by-breath pulmonary gas exchange and ventilation were continuously measured during GXT, brief running efforts, CONT_{100%RCP}, HIIT_{130%RCP}, and HIIT_{150%RCP} using a metabolic cart (Quark, CPET, COSMED, Rome, Italy), which was calibrated before each test according to the recommendations from the manufacturer. All the breath-by-breath data were examined to exclude errant breaths and those values lying more than ± 3 SD from the local mean were removed [52]. The breath-by-breath data were subsequently interpolated every 1-s [49] using the software OriginPro 8.0 (Origin Lab Corporation, Massachusetts, United States). In the GXT, the highest $\dot{V}O_2$ average from the last 20-s of each stage was determined as $\dot{V}O_{2max}$ when it was similar (± 2.1 mL/kg/min) to $\dot{V}O_2$ average from the last 10-s recorded in the CWR exercise carried out 5 minutes after GXT [54]. All participants reached the $\dot{V}O_{2max}$ according to this criterion. The minimal exercise velocity at which $\dot{V}O_{2max}$ was reached was recorded as $v\dot{V}O_{2max}$ [11]. For the estimation of RCP, raw profiles of ventilation ($\dot{V}_E$), carbon dioxide output ($\dot{V}CO_2$), their combination (i.e., $\dot{V}_E/\dot{V}CO_2$), RER, and end-tidal pressure of CO₂ (PetCO₂) were plotted against time. RCP was identified as the point at which PetCO₂ began a precipitous fall after a period of isocapnia concomitantly with a second and first breakpoint in the $\dot{V}_E$ and $\dot{V}_E/\dot{V}CO_2$ relationships, respectively [33]. The vRCP was identified by the projection of RCP (time at which it occurs) at GXT stages. When necessary, $v\dot{V}O_{2max}$ and vRCP were adjusted according to Kuipers et al., [39]. During the brief running efforts, the $\dot{V}O_2$, HR and RER average of the last 20-s of effort was calculated [25].

Neuromuscular function

Maximal isometric voluntary contraction

iMVCs with dominant knee-extensors (self-reported) were performed associated with the monitoring of EMG activity of *vastus lateralis*. The participants were positioned on a custom-made chair designed to keep the hips and knees flexed at 90°. Participants were firmly secured with straps fastening legs, hips, and chest. Force signal was acquired using a load cell with a 0.03% rated output accuracy and a 0.98-N resolution (MK Controle, Brazil). The load cell was attached to the ankle by a metal rod immediately above the region of the medial malleolus using straps with heavy duty Velcro. The EMG signal from *vastus lateralis* was also acquired by a pair of self-adhesive Ag/AgCl surface electrode (Meditrace 100, Covidien, USA) placed on the 1/3 distal of muscle using an amplifier FE136 (ADInstruments, Dunedin, Nova Zelandia). Both muscular force and EMG signals were recorded using the LabChart 8 software [26].

The highest voluntary force recorded within a 100-ms period during the force plateau of the iMVC was used as the peak voluntary force ($iMVC_{Force}$). The EMG signal was initially band-pass (20–500 Hz) filtered and then the RMS from the signal 0.5 s around the $iMVC_{Force}$ was calculated (RMS_{VL}). The ratio between RMS_{VL} and $iMVC_{Force}$ (i.e., $RMS_{VL}/iMVC_{Force}$) was also calculated. All analysis procedures were performed using the LabChart 8 software. All data is presented in percentage change from the rest (*raw* values are presented in supplementary file).

Countermovement jump performance analysis

The participants performed 5 CMJs interspaced by 20-s rest on a force plate (Cefise, Nova Odessa, Brazil). Participants were instructed to keep both hands akimbo and to quickly flex the knees to 90° and then jump as high as possible. Force plate signal (1000 Hz) was acquired using the PowerLab system (16/35, ADInstruments, Dunedin, Nova Zelandia) and the LabChart 8 software (AD Instruments, Dunedin, Nova Zelandia). The data was initially filtered with a fourth-order Butterworth filter (low-pass cutoff 25 Hz) and using the equations described by Linthorne et al., [41], the CMJ height (CMJ_{Height}), concentric peak force ($CMJ_{Force_{con}}$), and eccentric peak force ($CMJ_{Force_{ecc}}$) were analyzed by a custom-made Matlab algorithm (MathWorks, Natick, USA). The average obtained from the five CMJ trials was calculated [22] and has been presented in percentage change from the rest (*raw* values are presented in supplementary file).

Perceptual responses

The mood state and cognitive function were assessed individually in a silent room using the POMS questionnaire [8] and the Stroop test [53], respectively. The POMS is a 65-item questionnaire in which responses are rated on a Likert scale of 1 (not at all) to 4 (extremely). The POMS provides a measure of six mood states (Tension, Depression, Anger, Vigor, Fatigue, Confusion). The *raw* values of each POMS subscales were described. To the Stroop test, participants received a paper sheet with 112 color naming, presented in a randomly order, and all the color's names were printed with a color distinct from the color naming. The test was composed of two tasks: 1) Reading: the participants were required to sound reading the 112 words; and 2) Naming: the participants were required to identify the ink color. The participants were required to complete both tasks as fast as possible. If they were unable to complete the test in 120 seconds, the task was finished. For the final score (arbitrary unit; a.u.) of the test, the time to complete the task and the time value corresponding to the errors and unread words in each task were used

(Final score = score + unread words $\times$ time = (total of words/time to complete the task)) [53]. Both tasks required inhibitory control, as the paper sheet did not present congruence. The scores of the first task (Naming) reflect verbal fluency skills, while the second task (Reading) and the Naming-Reading (also called interference) characterizes inhibitory control and cognitive flexibility skills [53]. Finally, immediately after the brief running efforts, participants were asked to rate their perception of effort (RPE) by a 6-20 top-to-bottom Borg's scale [16]; exercising legs pain by 0-10 top-to-bottom Borg's scale [14]; and global fatigue perception by a 10-0 top-to-bottom Micklewright's scale [45]. Data are presented in *raw* values (arbitrary units).

Heart rate variability

Measurements of HRV occurred at rest, with the participants remaining seated alone in a dim lighting, silent room for 6-min. The HRV signal was acquired through a Bio-amplifier FE136 (ADInstruments, Dunedin, Nova Zelandia) and a PowerLab system (16/35, ADInstruments, Dunedin, Nova Zelandia) via self-adhesive Ag-AgCl surface electrodes (Meditrace 100, Covidien, USA) in a modified lead-II arrangement [28]. Before electrode placement, each site was cleaned by rubbing alcohol. The LabChart 8 software (AD Instruments, Dunedin, Nova Zelandia) was used to offline calculation of metrics related to parasympathetic activity in the final 5-min of signal recording. The root mean square of successive R-R interval differences (RMSSd, time domain analysis), the standard deviation of all normal R-R (SDNN, time domain analysis), and the poincaré plot standard deviation along the line of identity (SD2, frequency domain analysis) were calculated. All these outcomes were further transformed in based-ten logarithm (i.e., LnRMSSd, LnSDNN, and LnSD2) and are herein presented in percentage of change from the rest [19]. *Raw* values are presented in supplementary file.

Blood lactate concentration

Blood samples from the earlobe (25 μ L) were collected in heparinized micro-hematocrit tubes, immediately deposited into polyproline microtubes containing 50 μ L of sodium fluoride at 1%, stored at -20° Celsius and then analyzed in a biochemical analyzer YSI 2900 (Yellow Spring Instruments, Ohio, USA). The equipment error is $\pm 2\%$, according to the manufacturer's information.

Statistical analysis

All data are presented as mean $\pm$ SD. Statistical significance was set at α of 0.05. Normality of the data was verified using the Shapiro-Wilk test. A one-way repeated

measures ANOVA was used to compare outcomes between CONT_{100%RCP}, HIIT_{130%RCP}, and HIIT_{150%RCP}. A two-way repeated measures ANOVA was used to assess the effect of exercise (CONT_{100%RCP}, HIIT_{130%RCP}, and HIIT_{150%RCP}) and time (rest until 6-h post-exercise) on the further outcomes. Assumptions of sphericity were explored and controlled for all variables with the Greenhouse–Geisser adjustment, where necessary. If significant main effects or interactions (exercise $\times$ time) were observed, a Bonferroni post hoc pairwise comparison was performed. Partial eta squared (η_p^2) was calculated to estimate effect sizes, with values of 0.01, 0.06, and 0.14 representing small, moderate, and large effects, respectively. The SPSS the software package version 20.0 (IBM Corp., Armonk, NY) was used for all statistical analysis.

5.4. Results

Exercise characterization

The CONT_{100%RCP} was performed at 12.1 ± 0.9 km/h, while HIIT_{130%RCP} and HIIT_{150%RCP} were performed at 15.7 ± 1.2 km/h and 18.3 ± 1.4 km/h, respectively ($F_{2,22} = 1914.47$, $p < 0.01$, $\eta_p^2 = 0.99$; HIIT_{150%RCP} > HIIT_{130%RCP} > CONT_{100%RCP}). The estimated distance covered by the participants during exercises was 8.1 ± 0.6 km. The mean HR during CONT_{100%RCP}, HIIT_{130%RCP} and HIIT_{150%RCP} was 176 ± 9 bpm, 185 ± 8 bpm, and 182 ± 10 bpm, respectively ($F_{2,22} = 13.79$, $p < 0.01$, $\eta_p^2 = 0.55$, HIIT_{130%RCP} > HIIT_{150%RCP} > CONT_{100%RCP}). The peak of $[La]^-$ after CONT_{100%RCP}, HIIT_{130%RCP} and HIIT_{150%RCP} was 4.5 ± 2.5 mmol/L, 8.7 ± 3.1 mmol/L, and 10.2 ± 3.3 mmol/L, respectively ($F_{2,22} = 62.13$, $p < 0.01$, $\eta_p^2 = 0.85$; HIIT_{150%RCP} > HIIT_{130%RCP} > CONT_{100%RCP}).

Neuromuscular function after CONT_{100%RCP}, HIIT_{130%RCP}, and HIIT_{150%RCP}

The changes in the neuromuscular function of knee extensors, assessed using MVCs, are shown in **Figure 5.2 (A, B, and C)**. There was a main effect of time for iMVC_{Force} ($F_{4,44} = 23.16$, $p < 0.01$, $\eta_p^2 = 0.68$) and RMS_{VL} ($F_{4,44} = 18.78$, $p < 0.01$, $\eta_p^2 = 0.63$), whereby both remained reduced for at least 6 hours after exercise. There were no exercise or interaction effects on iMVC_{Force} or RMS_{VL} ($p > 0.05$). There were no time, exercise, or interaction effects on RMS_{VL}/iMVC_{Force}. The changes in CMJ performance are shown in **Figure 5.2 (D, E, and F)**. There were no time, exercise, or interaction effects for CMJ_{Height} or CMJ_{Fconc} ($p > 0.05$). However, CMJ_{Fecc} reduced 1 hour after exercise (main effect of time, $F_{4,44} = 4.49$, $p < 0.01$, $\eta_p^2 = 0.29$) and, throughout the 6 hours after exercise, it was lower after

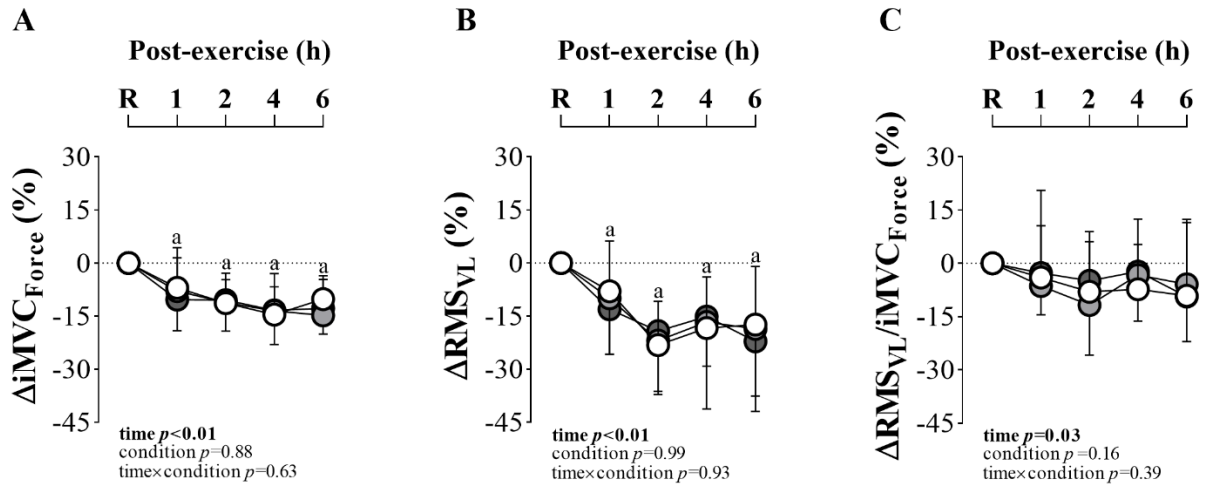
HIIT_{150%RCP} compared to CONT_{150%RCP} (main effect of exercise, $F_{2,22}=4.26$, $p<0.01$, $\eta_p^2=0.28$).

Cardiac parasympathetic activity, profile of mood state and cognitive function after CONT_{100%RCP}, HIIT_{130%RCP}, and HIIT_{150%RCP}

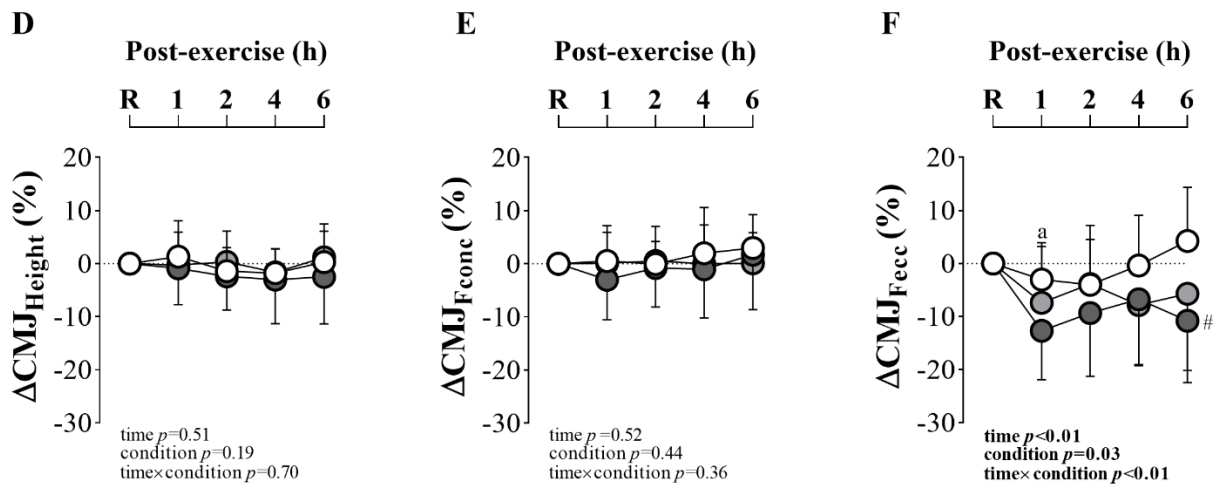
The HRV metrics related to parasympathetic activity are shown in **Figure 5.2 (G, H, and I)**. There was a main effect of time for LnRMSSd ($F_{4,44}=17.95$, $p<0.01$, $\eta_p^2=0.62$), LnSDNN ($F_{4,44}=18.68$, $p<0.01$, $\eta_p^2=0.63$), and LnSD2 ($F_{4,44}=18.19$, $p<0.01$, $\eta_p^2=0.62$), whereby they both remained reduced for at least 6 hours after exercise. Besides, throughout this time, LnRMSSd presented greater reduction after HIIT_{130%RCP} compared to CONT_{100%RCP} (main effect of exercise, $F_{2,22}=5.93$, $p<0.01$, $\eta_p^2=0.35$). In addition, specifically 1 hour after exercise, LnRMSSd was lower after both HIIT_{130%RCP} and HIIT_{150%RCP} compared to CONT_{100%RCP} (time×exercise effect, $F_{8,88}=4.35$, $p<0.01$, $\eta_p^2=0.28$).

The POMS subscales scores are shown in **Figure 5.3 (A, B, C, D, E, and F)**. Main effects of time were observed only for “Tension” ($F_{4,44}=4.21$, $p<0.01$, $\eta_p^2=0.27$), “Fatigue” ($F_{4,44}=31.85$, $p<0.01$, $\eta_p^2=0.74$), and “Vigor” ($F_{4,44}=7.34$, $p<0.01$, $\eta_p^2=0.42$). “Tension” scores decreased 1, 2, and 6 hours after exercise; “Fatigue” scores increased from 1 to 6 hours after exercise; and “Vigor” scores decreased 1, 4 and 6 hours after exercise. There were no exercise or interaction effects for any of the POMS subscales ($p>0.05$). Performance in the Stroop Test is shown in **Figure 5.3 (G, H, and I)**. There were main effects of time only for “Reading” ($F_{4,44}=13.71$, $p<0.01$, $\eta_p^2=0.37$) and “Reading-Naming” ($F_{8,88}=5.33$, $p<0.01$, $\eta_p^2=0.33$). The “Reading” decreased 1, 2, and 6 hours after the exercise, while the “Reading-Naming” increased 2, 4, and 6 hours after the exercise. There were no exercise or interaction effects for these outcomes.

Maximal isometric voluntary contraction of knee-extensors



Countermovement jump performance



Cardiac parasympathetic activity

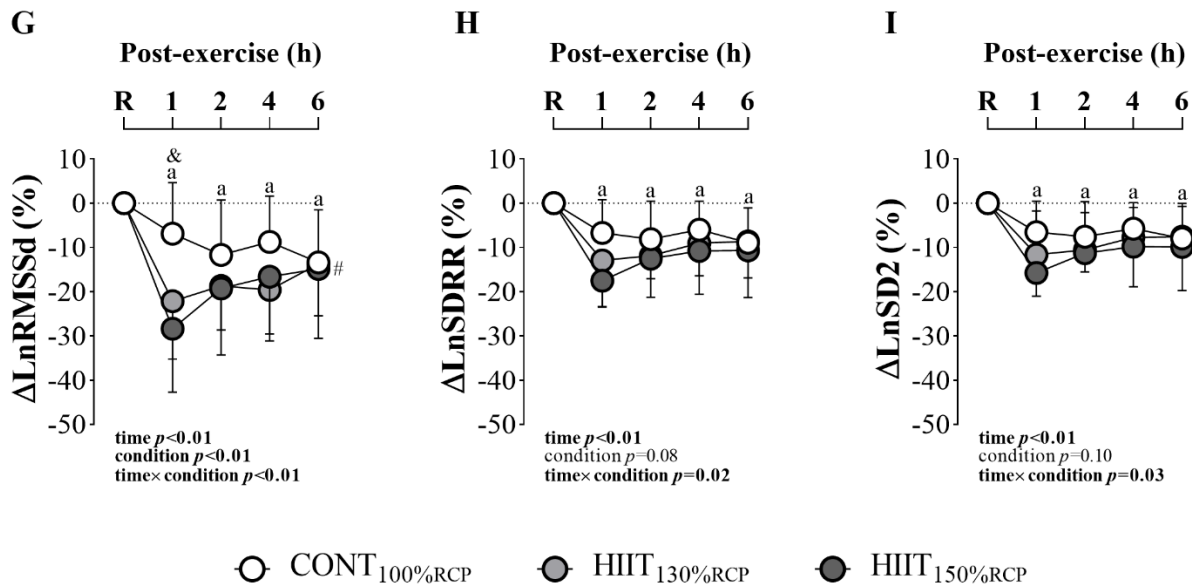
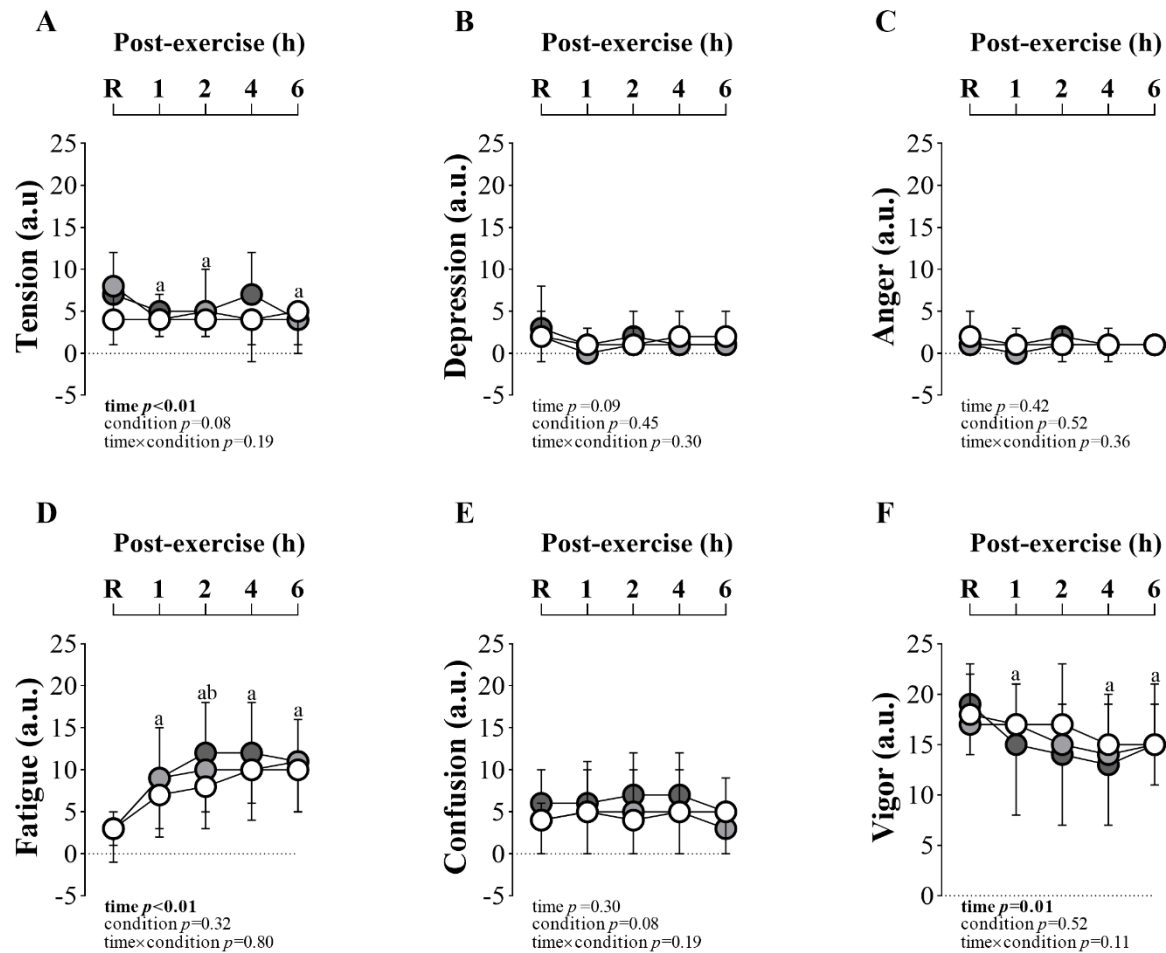


Figure 5.2. Percentage change from rest on knee-extensors function outcomes during maximal isometric voluntary contractions (iMVCs), countermovement jump (CMJ) performance indicators, and cardiac

parasympathetic activity metrics derived from heart-rate variability analysis. R: rest assessments; iMVC_{Force}: highest voluntary force recorded during iMVCs; RMS_{VL}: root mean square of electromyography signal from *vastus lateralis* during iMVC (0.5-s around iMVC_{Force}); CMJ_{Height}, CMJ_{Fconc}, and CMJ_{Fecc}: average of the height, concentric force, and eccentric force recorded in 5 CMJs; LnRMSSd, LnSDNN, and LnSD2: based-ten logarithm of the root mean square of successive R-R interval differences (RMSSd), standard deviation of all normal R-R (SDNN), and poincaré plot standard deviation along the line of identity (SD2), respectively. Statistical reports come from a two-way (time and condition) repeated measures analysis of variance (ANOVA). a: different from the rest ($p < 0.05$, main time effect); #: different from CONT_{100%RCP} ($p < 0.05$, main condition effect); &: different from HIIT_{130%RCP} and HIIT_{150%RCP} 1-h after exercise cessation ($p < 0.05$, time $\times$ condition effect). CONT_{100%RCP}: 40-min continuous running at velocity associated to respiratory compensation point [vRCP]); HIIT_{130%RCP}: 10 $\times$ 3-min running bouts 30% above vRCP, 1:1.5 work:rest ratio), and HIIT_{150%RCP}: 20 $\times$ 1-min and 20-s running bouts 50% above vRCP, 1:2 work:rest ratio. N=12.

Profile of mood state



Stroop test performance

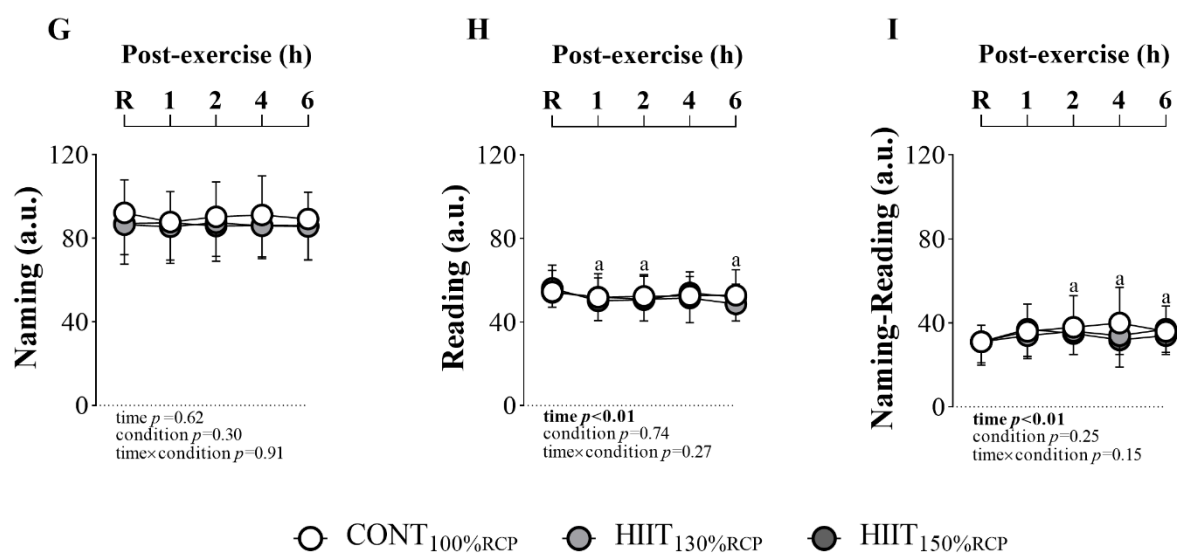


Figure 5.3. Changes from rest in the scores of Profile of Mood State questionnaire (POMS) and Stroop test. For the Stroop test, in “Reading”, the participants were required to sound reading the 112 words and in “Naming”, the participants were required to identify the ink color. For the final score (arbitrary unit; a.u.) of the test, the time to complete the task and the time value corresponding to the errors and unread words in each task were used (Final score = score + unread words $\times$ time = (total of words/time to complete the

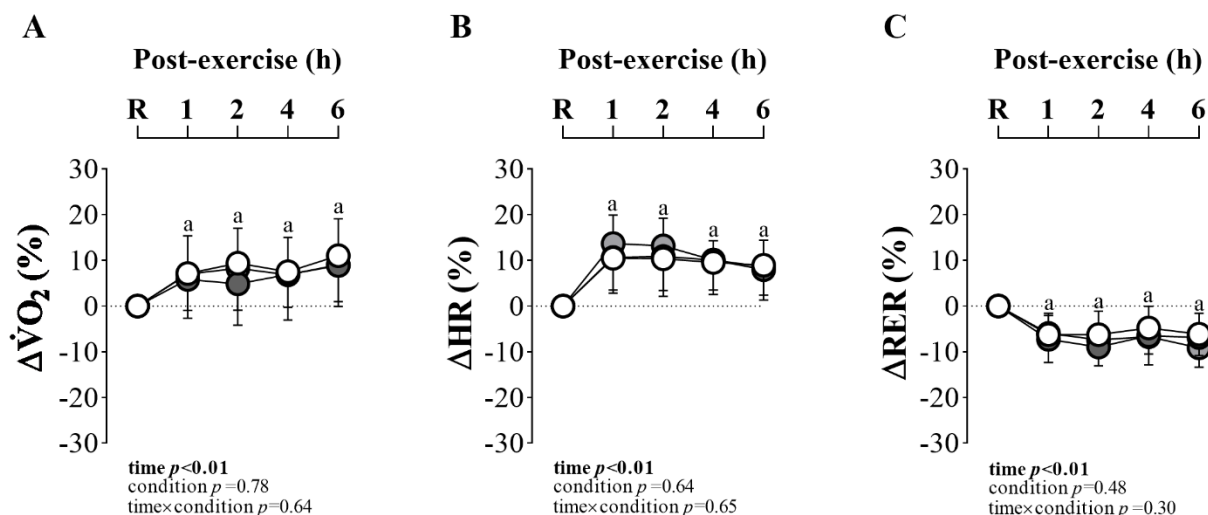
task)). The scores of the first task (Naming) reflect verbal fluency skills, while the second task (Reading) and the Naming-Reading (also called interference) characterizes inhibitory control and cognitive flexibility skills. R: rest assessments. Statistical reports come from a two-way (time and condition) repeated measures analysis of variance (ANOVA). a: different from the rest ($p<0.05$, main time effect); b: different from the 1-h ($p<0.05$, main time effect). CONT_{100%RCP}: 40-min continuous running at velocity associated to respiratory compensation point [vRCP]; HIIT_{130%RCP}: 10×3-min running bouts 30% above vRCP, 1:1.5 work:rest ratio), and HIIT_{150%RCP}: 20×1-min and 20-s running bouts 50% above vRCP, 1:2 work:rest ratio. N=12.

Physiological and psychological responses during brief running efforts after CONT_{100%RCP}, HIIT_{130%RCP}, and HIIT_{150%RCP}.

The physiological responses during brief-running efforts performed after CONT_{100%RCP}, HIIT_{130%RCP}, and HIIT_{150%RCP} are shown in **Figure 4 (A, B, C, D, E, and F)**. There were main effects of time for $\dot{V}O_2$ ($F_{4,44}=19.70$, $p<0.01$, $n_p^2=0.63$), HR ($F_{4,44}=33.67$, $p<0.01$, $n_p^2=0.75$), and RER ($F_{4,44}=32.80$, $p<0.01$, $n_p^2=0.75$). Over 6 hours after exercise, $\dot{V}O_2$ and HR were higher compared to resting values, while RER was lower during the successive brief running efforts. There were no exercise or interaction effects in these outcomes.

The psychological responses during brief running efforts performed after CONT_{100%RCP}, HIIT_{130%RCP}, and HIIT_{150%RCP} are displayed in **Figure 4 (G, H, and I)**. There were main effects of time for RPE ($F_{4,44}=38.06$, $p<0.01$, $n_p^2=0.77$), fatigue perception ($F_{4,44}=39.99$, $p<0.01$, $n_p^2=0.78$), and legs pain perception ($F_{4,44}=28.41$, $p<0.01$, $n_p^2=0.72$). All these outcomes remained increased in the brief running efforts performed 1, 2, 4, and 6 hours after exercise, compared to resting values. Besides, there were also main effect of exercise for RPE ($F_{2,22}=4.60$, $p=0.02$, $n_p^2=0.29$), fatigue perception ($F_{2,22}=3.98$, $p=0.03$, $n_p^2=0.26$), and legs pain perception ($F_{2,22}=5.43$, $p=0.01$, $n_p^2=0.35$), whereby they were higher in the brief running efforts performed after HIIT_{150%RCP} compared to CONT_{100%RCP}.

Physiological responses during brief-running



Psychological responses during brief-running

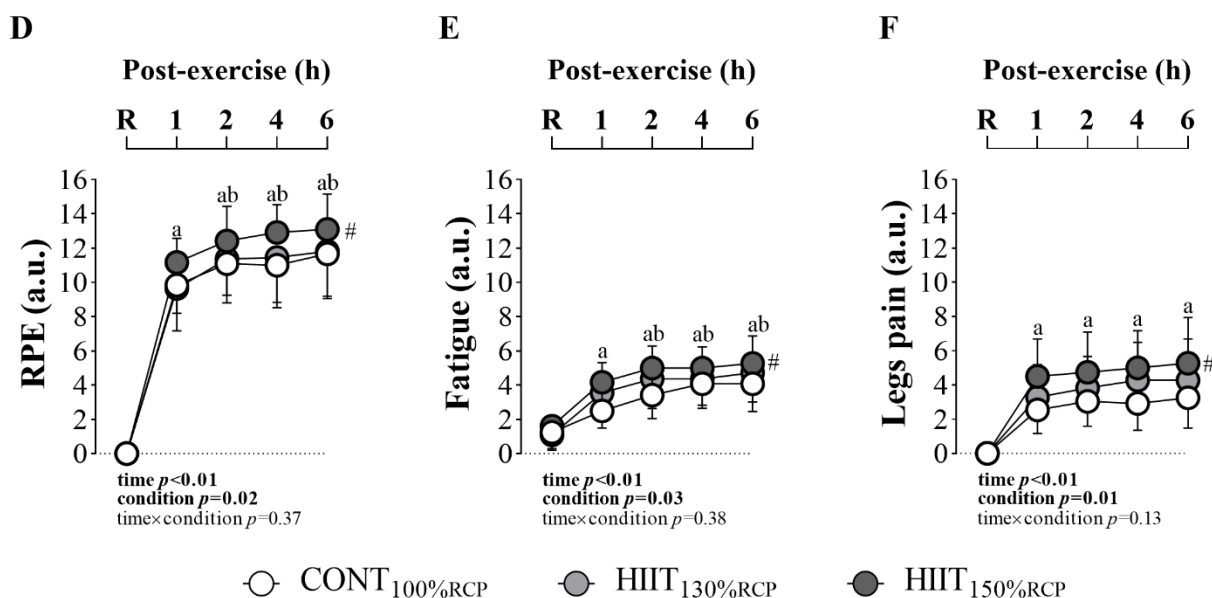


Figure 5.4. Changes from rest on physiological and psychological responses during brief running efforts (3-min at 80% of velocity associated to respiratory compensation point [vRCP]). $\dot{V}O_2$: rate of oxygen consumption; HR: heart rate; RER: respiratory exchange ratio; RPE: rate of perceived effort; “Pain”: perception of legs pain; “Fatigue”: perception of global fatigue. Statistical reports come from a two-way (time and condition) repeated measures analysis of variance (ANOVA). a: different from the rest ($p < 0.05$, main time effect); b: different from 1-h ($p < 0.05$, main time effect); #: different from CONT_{100%RCP} ($p < 0.05$, main condition effect). CONT_{100%RCP}: 40-min continuous running at velocity associated to vRCP; HIIT_{130%RCP}: 10 $\times$ 3-min running bouts 30% above vRCP, 1:1.5 work:rest ratio, and HIIT_{150%RCP}: 20 $\times$ 1-min and 20-s running bouts 50% above vRCP, 1:2 work:rest ratio. N=12.

5.5. Discussion

Proper monitoring of the fatigue-recovery relationship after exercise provides information on the physiological/psychological state of individuals, useful for favor performance and reduce health risks in subsequent tasks [29,34]. Although running-based efforts are one of the most used in rehabilitation and training routines [20,62], as far as

we know, this is the first study to describe changes in a wide range of markers for the routine assessment of the fatigue-recovery relationship after distinct running exercises. The main findings are that run 8-km induced impairments in neuromuscular function (i.e., decreased $iMVC_{Force}$ and RMS_{VL}), reductions in cardiac parasympathetic activity (i.e., $LnRMSSd$, $LnSDNN$, and $LnSD2$), changes in mood state (i.e., decrease “Vigor” and increase “Fatigue”), and in cognitive aspects (i.e., performance in Stroop test) for at least 6 hours after exercise, regardless of effort characteristics. However, compared to $CONT_{100\%RCP}$, it seems that $HIIT_{130\%RCP}$ induced greater decrease in cardiac parasympathetic activity and $HIIT_{150\%RCP}$ induced greater decrease in CMJ_{Fec} (main effect of exercise). During successive brief running efforts performed over 6 hours, RER were lower compared to rest state, while $\dot{V}O_2$, HR, RPE, fatigue and legs pain perceptions were higher. Compared to $CONT_{100\%RCP}$, $HIIT_{150\%RCP}$ induced greater increase in RPE, fatigue perception and legs pain over these subsequent efforts (main effect of exercise). Overall, these findings highlight that running induce changes in a wide range of body systems/capacities for at least 6 hours and suggest that in order to properly monitoring the fatigue-recovery relationship after this exercise, a multivariate approach combining the assessment of different physiological, psychological and performance markers should be employed.

Markers for monitoring the fatigue-recovery relationship: neuromuscular function

Despite no differences between efforts in the reduction of derived $iMVC$ outcomes (i.e., $iMVC_{Force}$ and RMS_{VL}), CMJ_{Fec} presented greater reduction after $HIIT_{150\%RCP}$ compared to $CONT_{100\%RCP}$ over 6 hours after exercise (main effects of exercise). Because $HIIT_{150\%RCP}$ was performed in a higher velocity compared to $CONT_{100\%RCP}$ (18.3 ± 1.4 km/h *versus* 12.1 ± 0.9 km/h), it may be argued that events associated with the impairments on force-generating capacity (i.e., accumulation of metabolites such as H^+ , Pi , K^+ ; muscle damage; inhibitory inputs into CNS) were more pronounced after the former compared to the latter [13,21,50,55]. Indeed, the greater peak of $[La]^-$ after $HIIT_{150\%RCP}$ compared to $CONT_{100\%RCP}$ reinforces this assumption (i.e., 4.5 ± 2.5 mmol/L *versus* 10.2 ± 3.3 mmol/L). As consistently evidenced, reductions in $iMVC_{Force}$ tend to be similar after exercises with distinct system-level and metabolic disturbances [5,31,64], suggesting that additional measurements seem to be needed to identify further differences in neuromuscular impairments between exercises (e.g., use of twitch interpolation technique to assess central and peripheral fatigue) [5,31,64]. The findings of the current study suggest that metrics related to CMJ performance as CMJ_{Fec} may be useful in this

scenario, helping to identify distinct level of neuromuscular function impairment induced by distinct exercise. Interestingly, the CMJ_{Height} did not change after running, suggesting that this derived CMJ outcome may not be sensitive to identify acute neuromuscular impairments induced by running. Although CMJ_{Height} is deemed to be appropriate to track the fatigue-recovery relationship after blocks of exercise routines (i.e., days, weeks) [22], confounding factors such as post-activation potentiation may preclude its use to identify neuromuscular impairments minutes to hours after exercise [17]. In this way, caution is advisable when use CMJ_{Height} to infer about the fatigue-recovery relationship within hours after exercise cessation.

Markers for monitoring the fatigue-recovery relationship: cardiac parasympathetic activity

Exercise-induced changes such as increase in systemic adrenaline concentration, reductions in plasma volume (due to sweating/dehydration) and accumulation of metabolites at muscles and blood, modify, via reflex responses, how the heart and blood vessels work to maintain blood pressure and cardiac output [44]. These changes involve alterations in the activity of the autonomic nervous system toward reduction in the activity of the parasympathetic component and increase in the activity of the sympathetic component [61]. The current study evidenced that distinct HRV-derived metrics related to cardiac parasympathetic activity (i.e., LnRMSSd, LnSDNN, and LnSD2) can identify such exercise-induced changes. Besides, over the 6 hours post-exercise, it was also observed that HIIT_{130%RCP} induced greater reduction in LnRMSSd compared to CONT_{100%RCP}. It may be argued that this difference occurred due to the higher cardiovascular demand in HIIT_{130%RCP} (mean HR of 185 ± 8 bpm) compared to CONT_{100%RCP} (mean HR of 176 ± 9 bpm, $p < 0.05$) [44], highlighting the ability of HRV-derived to identify differences in the level of disturbance on the cardiac parasympathetic activity induced by different exercises. Although HRV metrics are specifically associated with the functioning of the cardiovascular system and is not associated with recovery from other imbalances caused by exercise (e.g., neuromuscular, metabolic), the return of cardiac parasympathetic activity assessed by HRV-derived metrics to “baseline” values or higher is usually assumed to be an indicator of “global recovery of the individual” [61].

Markers for monitoring the fatigue-recovery relationship: mood state and cognitive function

Chronic changes in mood state during exercise routine are strictly related to health problems such as non-functional overreaching and overtraining, which further leads to performance impairment [36]. The POMS is deemed as a low-cost, simple and valid tool in detecting mood fluctuations in this context [34,35]. The current study observed that “Tension” and “Vigor” decreases after exercise, while “Fatigue” increases, regardless of the characteristics of the effort. While reduce in “Tension” can be explained by acute exercise-induced increase on endogenous opioids (e.g., β -endorphins) linked to “well-being” sensation and relaxed state, the decrease on “Vigor” and increase on “Fatigue” seems to occur due to exercise-induced changes on brain temperature, fluid balance, and neurotransmitters (e.g., serotonin and dopamine) (BASSO; SUZUKI, 2017). Mood disturbance such as decreased “Vigor” and increased “Fatigue” have been observed a few days after starting to participate in strenuous exercise scenarios, such as block of intensified training routines [51,59]. The current study expands these investigations by showing that an 8-km run may already be capable of induce such changes on mood state, regardless of it was performed as CONT_{100%RCP}, HIIT_{130%RCP}, or HIIT_{150%RCP}.

In addition, we observed that scores on Stroop test changed after exercise, suggesting the acute capacity of running in impact executive function, a “higher-level” cognition aspect embracing working memory, planning, inhibition, among other cognitive functions [1]. The Stroop test is strictly related to inhibition, the ability to sustain mental processes while inhibiting automatic evoked responses [53]. In the current study, the “Reading” in Stroop test (i.e., correct read the words inhibiting the color-naming evoked response), decreased after exercise, suggesting a more-efficient inhibitory process [63]. Such positive effect corroborate previous reports on acute bouts of exercises (i.e., 20 minutes, 50-60% of maximal HR) in improve executive function and seems to be explained by the exercise-induced acute increase in brain blood flow and oxygenation [2,56]. However, it was also evidenced in the current study that “Naming-Reading” in Stroop test (i.e., correct name the color of the words inhibiting the word-reading evoked response *minus* to correct read the words inhibiting the color-naming evoked response) increased after exercise, suggesting a worsen inhibitory control [63]. Although we cannot fully explain this mismatch in the results, it seems that the inhibition of color-naming response in the “Reading” task requires a less demanding inhibitory process in comparison to inhibition of word-reading response in the “Naming” and consequently “Naming-Reading” task [63]. In this way, it might be suggested that although exercise facilitate inhibitory process in less demanding tasks, it seems to impair inhibitory process in more demanding tasks

[65]. Because inefficient inhibition could result in inefficient selective attention, longer processing time and impaired recognition of relevant information in the environment [38], the monitoring of this cognitive aspect is advisable.

Markers for monitoring the fatigue-recovery relationship: responses during successive brief efforts

In addition to resting assessments, we also measured physiological and psychological responses during successive brief running efforts (3 minutes at 80% of vRCP). It is argued that this kind of assessment, involving efforts with physical/motor demands closer to “real world scenario”, provide more accurate information to differentiate between “fatigue” and “recovery” states [35]. Over the efforts performed 6 hours followed CONT_{100%RCP}, HIIT_{130%RCP} and HIIT_{150%RCP}, participants had a greater cardiorespiratory demand (i.e., higher $\dot{V}O_2$ and HR) compared to rest state. Due to the evidenced neuromuscular function impairment (i.e., reduced iMVC_{Force}) and incomplete recovery of cardiovascular system (i.e., reduced cardiac parasympathetic activity), it may be argued that this greater cardiorespiratory demand occurred due to decreased muscle [46] and cardiovascular efficiency [61], demanding more work from the cardiorespiratory system to perform the task [58]. Interestingly, it was also evidenced that RER was lower over the efforts performed after CONT_{100%RCP}, HIIT_{130%RCP} and HIIT_{150%RCP} compared to the rest state. It suggests that carbohydrates were used less to provide energy in these successive efforts [57], likely due to insufficient repletion of muscle glycogen stores, even ensuring adequate carbohydrate consumption by the participants.

In line with the changes on physiological responses, RPE, perceived fatigue and legs pain were also higher during the successive brief efforts compared to the rest state. While the increases in RPE and perceived fatigue are probably linked to the cited increase in physiological responses during effort such as higher HR [14,15], the increased legs pain perception may be occurred due to exercise-induced damage and inflammatory process elicited by the eccentric contractions involved in the stretch-shortening cycle observed in running [48,55]. Besides, RPE, legs pain and perceived fatigue during the brief efforts were greater after HIIT_{150%RCP} compared to CONT_{100%RCP}. Although we cannot fully explain such differences, it may be argued that intensity-dependent alterations in aspects such as exercise-induced muscle damage [48,55], increase in body temperature [40], stimulation of the hypothalamic-pituitary-adrenal axis [24], and brain neurotransmitter alterations [7], might be involved in such differences evidenced between HIIT_{150%RCP} compared to CONT_{100%RCP}. Indeed, the greater peak of $[La]^-$ and greater mean HR during

HIIT_{150%RCP} (4.5 ± 2.5 mmol/L and 176 ± 9 bpm, respectively) compared to CONT_{100%RCP} (10.2 ± 3.3 mmol/L and 182 ± 10 bpm, respectively) suggest a distinct level of metabolic and cardiovascular stress between exercises. Interestingly, just the psychological metrics assessed during the successive brief efforts were able to identify this probably distinct level of *stress* between HIIT_{150%RCP} and CONT_{100%RCP}, reinforcing the relevance of these markers when monitoring the fatigue-recovery relationship.

Collectively, the findings obtained in the brief running efforts indicate a greater physiological/psychological disturbance during tasks following CONT_{100%RCP}, HIIT_{130%RCP} and HIIT_{150%RCP}, suggesting a decrease in the efficiency of the body systems involved in the task and highlighting a state of incomplete recovery [58]. In this way, it may be suggested that a decline in performance and a greater internal load in subsequent tasks can be expected for at least 6 hours after running (~8 km), even if these activities involve submaximal muscle contractions and even if adequate consumption of carbohydrates is ensured during this recovery time [29]. Besides, looking for psychometric measures, it is possible that the increase in internal load during subsequent tasks will be greater after HIIT_{150%RCP} compared to CONT_{100%RCP} [29].

Further considerations

Despite the novel findings of the current study, we are aware of some of its limitations. It was not within the scope of this study to analyze more mechanistic outcomes related to running-induced fatigue such as blood concentration of markers related to muscle damage (e.g., creatine kinase activity) and inflammation (e.g., interleukins), and central and peripheral neuromuscular function (i.e., by means of twitch interpolation technique) [47]. Although such outcomes could undoubtedly provide more detailed information on the running-induced imbalances in body systems, the current study was designed to evaluate a well-founded selection of practical tests that can be easily applied for routine assessment of fatigue in daily “real-world” scenarios (i.e., iMVCs, CMJs, HRV measures, POMS, Stroop test), also performing an ecological assessment of the fatigue-recovery relationship (i.e., by measuring the physiological and psychological responses in successive brief efforts after running). Besides, due to the reluctance of subjects to deviate from their normal training routine [12], we were not able to monitor the fatigue-recovery relationship for days after exercise cessation (e.g., 24, 48 hours after), which is an important knowledge gap that needs to be covered by future studies. In addition, it is important to note that only males were included in the current study. As comparisons between the sexes were not within the scope of the study and due to the sex-induced

differences in fatigue and recovery [4,30], we deemed it appropriate to recruit only males in order to reduce the heterogeneity of the data and increase the statistical power of the analysis. Therefore, future studies should assess the interactions of sex on the markers of fatigue-recovery relationship herein addressed. Nevertheless, the current study is the first to describe the fatigue-recovery relationship after running with distinct metabolic stress, using non-invasive assessment markers that are less expensive and commonly used in practical scenarios, also measuring the physiological/psychological disturbances in an ecological task closer to the “real” physical/motor demands of the modality. Thus, we feel that, even with the aforementioned limitations, the results of the current study can be very useful to (i) help coaches and exercise professionals make informed decisions about the markers to be used to properly monitor the fatigue-recovery relationship after different running exercises; (ii) to plan appropriate recovery schedules during routines of rehabilitation, training and competition; and (iii) to properly judge the need to use additional recovery strategies to favor performance and/or decrease the internal load and health risks in subsequent tasks [35].

5.6. Conclusion

The current study showed that an 8-km run as CONT_{100%RCP}, HIIT_{130%RCP}, and HIIT_{150%RCP} reduces knee-extensor maximal force-generating capacity (i.e., decreased iMVC_{Force}) and resting cardiac parasympathetic activity, change mood aspects (measured by POMS subscales), impair cognitive function (measured by Stroop test performance), and exacerbate physiological (e.g., $\dot{V}O_2$ and HR) and psychological responses (e.g., RPE, perceived fatigue and leg pain) in successive brief running efforts for at least 6 hours. According to the running characteristics, greater decrease in cardiac parasympathetic activity (i.e., HIIT_{130%RCP}>CONT_{100%RCP}) and greater neuromuscular impairment and perceived responses in the successive brief efforts was also evidenced (i.e., HIIT_{150%RCP}>CONT_{100%RCP} for both). Thus, it can be suggested that running induces changes in a wide range of body systems/capacities for at least 6 hours and that a multivariate approach combining the assessment of different physiological, psychological and performance markers should be used to adequately monitor the fatigue-recovery relationship after this exercise.

5.7. References

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CHAPTER 6.
HOT AND COLD-WATER IMMERSION EFFICACY IN
MITIGATE FATIGABILITY FOLLOWING RUNNING

6. STUDY 4. Hot and cold-water immersion efficacy in mitigate fatigability following running depends on exercise characteristics and parameters evaluated.

6.1. Abstract

Cold- (CWI) and hot- (HWI) water immersion are commonly applied to improve post-exercise recovery. However, due to the wide range of task-dependent fatigability responses and mechanisms linked to CWI and HWI, their effectiveness in improve post-exercise recovery may be influenced by the exercise performed and parameters evaluated. Accordingly, we carried out two independent studies to investigate the impact of water immersion in 41°C (HWI_{41°}, 45-min) and 11°C (CWI_{11°}, 15-min) in mitigate fatigability responses after a continuous running at velocity associated to respiratory compensation point (vRCP, CONT_{100%RCP}, n=12) and after a high-intensity intermittent running at velocity 50% above vRCP (HIIT_{150%RCP}, n=10). The effects of HWI_{41°} and CWI_{11°} were compared to a thermoneutral colored water (placebo, 10-min) and passive rest (control). HWI_{41°} increased the *vastus lateralis* activation during maximal isometric voluntary contractions (i.e., increase RMS/M-wave_{amp}) after CONT_{100%RCP}, while CWI_{11°} elicited such beneficial effect after HIIT_{150%RCP} (condition×time effects, $p<0.05$). Neither method improved peak voluntary force or contractile function of knee-extensors, nor countermovement jump performance after exercise (condition or condition×time effects, $p>0.05$). However, the CWI_{11°} increased resting cardiac parasympathetic activity (i.e., RMSSd_{Log}), while HWI_{41°} decreased the rate of oxygen consumption in successive brief running efforts (condition or condition×time effects, $p<0.05$) after both CONT_{100%RCP} and HIIT_{150%RCP}. These findings suggest that HWI_{41°} and CWI_{11°} improve muscle activation after running efforts like CONT_{100%RCP} and HIIT_{150%RCP}, respectively, with no impact on peak force on contractile function. However, CWI_{11°} increases resting cardiac parasympathetic activity and HWI_{41°} reduces metabolic demand during successive brief efforts, regardless of running characteristics.

Keywords: post-exercise recovery; muscle fatigue; recovery modalities; water immersion; cryotherapy; heat therapy.

6.2. Introduction

Endurance exercises result in disturbances on several physiological (e.g., neuromuscular, cardiovascular) and psychological process (e.g., mood, alertness) which ultimately leads to fatigue. Fatigue can be defined as a reduction in force-generating capacity (performance fatigability), accompanied by sensations as tiredness and weakness (perceived fatigability) that may last for minutes to days after task cessation (Enoka & Duchateau, 2016; Brownstein *et al.*, 2021). Acutely, fatigue can impair performance, increase injury risk, and chronically, it may lead to deleterious conditions as nonfunction overreaching and overtraining syndrome (Kellmann, 2010; Halson, 2014). Therefore, cold-water immersion (CWI) and hot-water immersion (HWI) have been widely used and investigated (Wilcock *et al.*, 2006; Chaillou *et al.*, 2022) to mitigate the fatigue process in addition to the basic principles of recovery such as nutrition, hydration, sleep and other (Kellmann *et al.*, 2018).

CWI decreases muscle temperature and blood flow (Mawhinney *et al.*, 2013), which in combination with secondary cold-induced events (e.g., reduction of core temperature (Stephens *et al.*, 2018), limiting proliferation of secondary damage following injury (Merrick *et al.*, 1999), and increase of central blood volume (Mourots *et al.*, 2008)), are argued to be capable to reduce cardiovascular strain (Jdidi *et al.*, 2024) and to improve muscle soreness, force production, and perceived feeling of recovery within hours to days after endurance exercises (Machado *et al.*, 2016; Choo *et al.*, 2022; Moore *et al.*, 2022). On the other hand, HWI increases muscle temperature (Rodrigues *et al.*, 2020a) and blood flow (Heinonen *et al.*, 2011), which associated with secondary effects such as increase in the clearance of tissue metabolites (Coté *et al.*, 1988), muscle glycogen resynthesis, sarcoplasmic reticulum Ca^{++} release and myofibrillar Ca^{++} sensitivity (Cheng *et al.*, 2017), are argued to be capable of decrease muscle soreness (Petrofsky *et al.*, 2015), enhance force production (Sautillet *et al.*, 2024) and maintaining submaximal performance in subsequent tasks (Cheng *et al.*, 2017) within hours after endurance exercise. Besides, in addition to physiological effects, subject's belief or expectation in the effectiveness of CWI and HWI can also influence their effects as post-exercise recovery strategies (Hurst *et al.*, 2020).

Even though such reported benefits, there is a large variability in the efficacy of CWI and HWI on improve post-exercise recovery metrics (e.g., maximal-force generating capacity, jump performance, endurance performance) (Chaillou *et al.*, 2022) which, among other reasons, may be dictated by the task-dependent characteristic of the fatigue (Thorpe,

2021). It is well established that characteristics of the exercise such as intensity (e.g., whether performed below or above maximum metabolic steady state [MMSS]) and type (e.g., heavy-intensity continuous vs high-intensity intermittent effort) may impact in different ways the fatigability responses (MacInnis & Gibala, 2017). For example, greater impairments on components of motor pathway located below neuromuscular junction (Thomas *et al.*, 2016; Azevedo *et al.*, 2021), greater amount of heat storage in the body (Lei *et al.*, 2023), and greater “muscle soreness” and lower “fatigue” perception (Iannetta *et al.*, 2022) are observed after exercise performed above MMSS compared to below it. Besides, it is suggested that greater cardiorespiratory stress (Michael *et al.*, 2017) and less mood disturbance (Thum *et al.*, 2017; Olney *et al.*, 2018) occur after high-intensity intermittent efforts compared to heavy-intensity continuous effort. In this way, due to specific mechanisms associated to CWI and HWI, it may be hypothesized that their effectiveness in improve metrics of recovery (e.g., maximal-force generating capacity, jump performance, endurance performance) may be impacted by the characteristics of the exercise-induced disturbances. For example, CWI may be more helpful after endurance exercises in which a great heat storage and muscle damage are observed, whereas HWI may be less beneficial in this scenario (Thorpe, 2021).

Furthermore, due to the wide range of fatigability responses (Halsen, 2014), modulated in some cases by distinct physiological and psychological process (Enoka & Duchateau, 2016), it may be also hypothesized that CWI and HWI may have different efficacies according to the outcome that it is intended to improve. For example, CWI has been shown to be effective in improve cardiac parasympathetic activity at rest (Jdidi *et al.*, 2024), a physiological metric commonly used to monitor the exercise-induced disturbance in the cardiovascular system (Stanley *et al.*, 2013). This CWI effect is usually explained, among other reasons, by the increase on central blood flow mediated by peripheral vasoconstriction (Jdidi *et al.*, 2024). Because of the opposite effects of HWI in this scenario (i.e., increase peripheral blood flow) (Heinonen *et al.*, 2011), such effect on cardiac parasympathetic activity seems do not occur after HWI (Parker *et al.*, 2018). On the other hand, due to heat capacity on enhance sarcoplasmic reticulum Ca^{++} release (Cheng *et al.*, 2017) and nerve conduction velocity (Rutkove, 2001), it seems to be more effective in mitigate the decline in rate of force development compared to CWI (Sautillet *et al.*, 2024), which may be even detrimental in this scenario (Choo *et al.*, 2022) (by decreasing nerve conduction velocity (Rutkove, 2001)).

Therefore, beyond the task-dependent characteristic of exercise, the outcomes that are intended to improve may also be influenced by CWI and HWI efficacy in mitigating fatigability responses after endurance exercise. Although previous studies have already compared CWI and HWI efficacy as post-exercise recovery methods (Sautillet *et al.*, 2024; Menzies *et al.*, 2024), the impact of task-dependent characteristic of exercise on their effects as recovery strategies has yet to be established. In addition, despite the current knowledge about the mechanisms of CWI and HWI, few studies have investigated their impact on more insightful responses such as voluntary activation level and muscle tissue contractility (Bouchiba *et al.*, 2022), as well as on more ecological tasks closer to “real-world” scenarios as by monitoring physiological and psychological responses during brief-running efforts (Menzies *et al.*, 2024). Such knowledge may provide a clearer understanding about CWI and HWI use as recovery methods, helping individuals to make informed decisions about which exercise scenarios and which fatigability responses are more prone to be benefited using these strategies.

Thus, this study aimed to investigate the effects of CWI and HWI as post-exercise recovery strategies after two distinct commonly used endurance exercises: (i) continuous running at heavy-domain and (ii) intermittent running at severe-domain. A comprehensive assessment of fatigability responses was performed addressing outcomes such as global, central, and peripheral neuromuscular function, countermovement jump performance, heart rate variability (HRV) analysis at rest, as well as physiological and psychological responses during brief running efforts. Besides, a placebo control was also used trying to compensate for potential bias on the outcomes and conclusions elicited by placebo effect (Hurst *et al.*, 2020). We hypothesized that CWI will be more helpful for mitigating fatigability responses after intermittent running at severe-intensity and that it will have a greater beneficial impact on cardiac parasympathetic activity. On the other hand, the effectiveness of HWI will be greater after continuous running at heavy-domain and on outcomes associated with neuromuscular function.

6.3. Materials and methods

The current investigation was divided into 2 independent crossover studies. The study 1 was carried out to investigate the effects of CWI and HWI on fatigability responses after a continuous running performed at velocity associated with RCP (vRCP; CONT_{100%RCP}), while the study 2 was carried out to investigate the effects of CWI and HWI on the same outcomes, but after an intermittent running performed 50% above vRCP (HIIT_{150%RCP}).

Participants

The findings reported by Dantas et al., (2020) on the CWI-induced improvement in knee-extensors force 24-h after a 10-km run (effect size = 0.33) were used to estimate the number of participants. A minimum of 8 participants was estimated to achieve a power of 0.90 in *f* tests, setting an alpha of 0.05. Because of the sex-related differences on performance fatigability responses (Ansdell *et al.*, 2020), we judged proper recruit only males to take part in the studies aiming to reduce data variability. Thus, 15 healthy, recreationally active males were recruited to participate of each study. Participants were included if they were not under medical or nutritional treatments in the 6 months before the studies, and if they had no known neurological, cardiovascular, or musculoskeletal disorders that could potentially affect the evaluated outcomes of the investigation. After being made aware of the experimental procedures, all participants provided their written informed consent. Due to successive absences in the trials ($n=2$ in study 1 and $n=3$ in study 2) and due to injuries not related do the research ($n=1$ in study 1 and $n=2$ in study 2), the final sample of study 1 was 12 participants (mean $\pm$ SD; age:26 $\pm$ 7 years; stature: 177 $\pm$ 7 cm ; body mass: 74.2 $\pm$ 6.3 kg), while 10 participants completed the study 2 (mean $\pm$ SD; age:24 $\pm$ 5 years ; stature: 175 $\pm$ 4 cm ; body mass: 74.6 $\pm$ 6.5 kg). The studies were approved by the Sao Paulo State University Research Committee (Number 50075421.9.0000.5398), and all procedures were conducted in accordance with the Declaration of Helsinki.

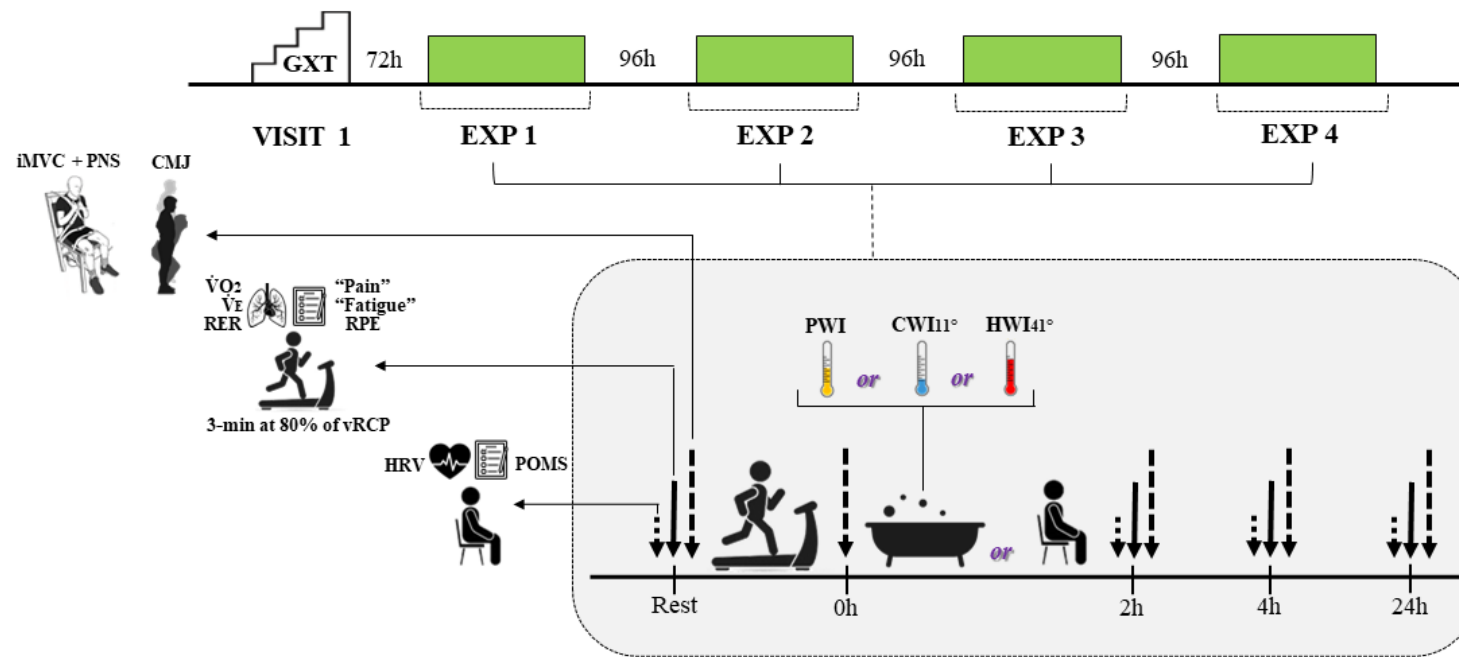
Experimental Design

The experimental design of the current investigation is presented in **Figure 6.1**. The studies were very similar to each other, differing only in the type of running performed by the participants, whether CONT_{100%RCP} (study 1) or HIIT_{150%RCP} (study 2). Both studies consisted of 9 visits to the laboratory performed within 5-6 weeks. The first visits were used to evaluate anthropometric characteristics, to assess cardiorespiratory fitness level through a graded exercise test (GXT) and to familiarize the participants with the experimental procedures of the investigation. On the visits 2, 4, 6 and 8, participants performed CONT_{100%RCP} or HIIT_{150%RCP}, according to the study in which they took part, and following a randomized order, they underwent HWI, CWI, placebo intervention or passive rest (control condition) after exercise. On the visits 3, 5, 7 and 9, participants visited the laboratory for follow-up evaluations 24-h after exercise. Neuromuscular

assessments were performed at rest, immediately after exercise, 2, 4 and 24-h post exercise. The neuromuscular assessments comprised maximal isometric contractions of knee-extensors (iMVC) associated with peripheral nerve electrical stimulation (PNS) and countermovement jump (CMJ) performance analysis. Heart rate variability (HRV) and the mood state were assessed at rest, 2, 4 and 24-h post-exercise, while rating of perceived recovery (“Recovery”) was assessed 2-, 4-, and 24-h post-exercise. In addition, brief running efforts with the monitoring of physiological and psychological responses were also performed at rest, 2, 4 and 24-h post-exercise aiming to perform a more ecological assessment of the fatigue-recovery relationship as previously suggested (Kellmann *et al.*, 2018). All running efforts (i.e., GXT, 3-min runs at 80% vRCP, CONT_{100%RCP} and HIIT_{150%RCP}) were performed on a motorized treadmill (ATL, Inbramed, Brazil) with the treadmill gradient fixed at 1% (Jones & Doust, 1996).

Prior to all visits, participants were instructed to avoid consumption of food and caffeinated beverages for at least 2 and 8-h, respectively, and to abstain from vigorous physical activity for 24-h. Before visits 1, 2, 4, 6 and 8 (i.e., prior to GXT, and when CONT_{100%RCP} or HIIT_{100%RCP} were performed), participants were required to rate their overall perception of sleep quality according to Snyder *et al.*, (2018) (visual scale 0-10; 0-terrible to 10-excellent). If sleep quality was rated below 6 (fair), the participants were released, and the visit was rescheduled (it was the case in 2 occasions throughout the investigation). Before the visit 2 (i.e., first trial of CONT_{100%RCP} or HIIT_{150%RCP}), the participants were instructed to carry out a dietary recall and to repeat the same eating pattern prior to visits 4, 6 and 8. All participants performed the visits 2, 4, 6 and 8 (and consequently visits 3, 5, 7 and 9) in the same period of the day ($\pm$ 1-h) and all tests were performed in an environmentally controlled room (temperature: 20–22°C; humidity 40%–60%).

A



B

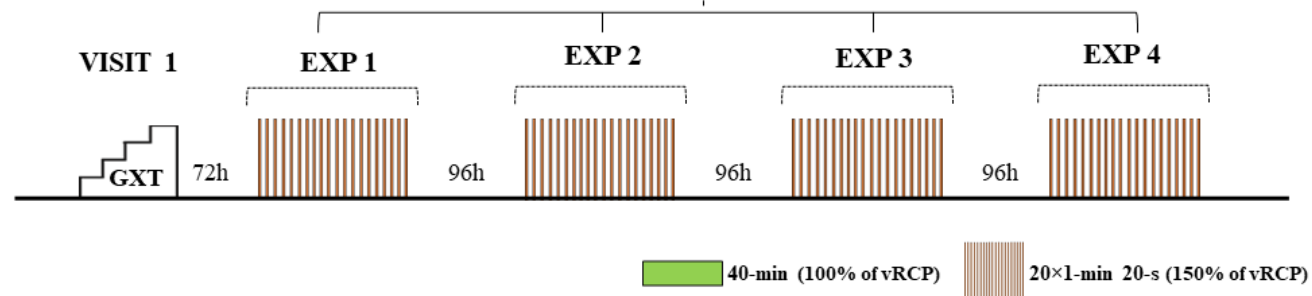


Figure 6.1. Schematic representation of the investigation design. GXT, graded exercise test to assess cardiorespiratory function; EXP, experimental trials consisted of two visits to the laboratory, spaced 24-h apart; iMVC, maximal voluntary isometric contraction of knee-extensors (dominant lower-limb, self-reported); PNS, peripheral electrical stimulation; CMJ, countermovement jump; $\dot{V}O_2$, rate of oxygen consumption; $\dot{V}_E$, ventilation; RER, respiratory exchange ratio; “Pain”, perception of pain in lower-limbs; “Fatigue”, perception

of o global fatigue; RPE, rate of perceived effort; vRCP, velocity associated with the respiratory compensation point; HRV, heart rate variability; POMS, profile of mood state questionnaire; PWI, placebo water immersion (colored thermoneutral water for 10-min); CWI_{11°}, cold-water immersion (11°C for 15-min); HWI_{41°}, hot-water immersion (41°C for 45-min).

Experimental protocols

Graded exercise test and familiarizations

During the first visits in study 1 and 2, participants performed a graded exercise test (GXT) to determine the maximal rate of oxygen consumption ($\dot{V}O_{2\max}$), respiratory compensation point (RCP) and the velocities associated to them (i.e., $v\dot{V}O_{2\max}$ and $vRCP$, respectively). The GXT started at 8 km/h the velocity was increased by 1.5 km/h each 2-min stage until task failure (Bentley *et al.*, 2007). Five minutes after the end of the GXT, the participants performed a constant-load exercise also until task failure at a velocity 5% greater than the highest velocity achieved during GXT (Schaun, 2017). In both cases, task failure was determined when participants disengaged from the effort despite the use of strong verbal encouragement. After testing, participants were familiarized with the experimental methods of the study by (i) performing multiple submaximal and maximal isometric contractions of dominant knee-extensors (self-reported), associated with PNS and visual feedback of the force signal; (ii) performing CMJs associated with visual feedback of the force signal; (iii) rating their rate of perceived exertion (RPE), “Fatigue”, and “Pain” at lower-limbs in relation to the final stage of the GXT; (iv) rating their mood; and (v) rating their “Recovery” perception in relation to the end of the GXT.

CONT_{100%RCP} and HIIT_{150%RCP}

The CONT_{100%RCP} consisted in 40-min continuous run effort at $vRCP$ while the HIIT_{150%RCP} consisted of 20×1 -min and 20-s running bouts 50% above $vRCP$ (exercise to passive rest ratio of 1:2). The duration of the bouts in HIIT_{150%RCP} were chosen aiming to match the estimated distance covered by the participants between CONT_{100%RCP} and HIIT_{150%RCP} (i.e., distance in km = velocity in km/h $\times$ exercise time in hours). Blood samples from the earlobe (25 μ L) were collected 3- and 5-min after the end of all CONT_{100%RCP} and HIIT_{150%RCP} for further evaluating the peak of blood lactate concentration after exercise ($[La^-]_{peak}$) through a biochemical analyzer YSI 2900 (Yellow Spring Instruments, Ohio, USA). Thirty minutes following all CONT_{100%RCP} and HIIT_{150%RCP}, participants were asked to rate their RPE through a RPE-CR-10 scale, which was multiplied by the duration of the exercise in minutes in order to quantify the internal load of the exercises (Foster *et al.*, 2001).

Brief running efforts

The brief running efforts consisted in 3-min run efforts at 80% of vRCP. This low-intensity (i.e., moderate domain) and short-duration were chosen aiming to reduce the risks of additional disorders to the body's system already impaired by CONT_{100%RCP} and HIIT_{150%RCP}. During efforts, the monitoring of $\dot{V}O_2$, ventilation ($\dot{V}_E$), respiratory exchange ratio (RER), as well as rate of perceived exertion (RPE), legs "Pain", and overall "Fatigue" was performed. The analysis of these responses during effort was performed aiming to carry out a more ecological assessment of the fatigue-recovery relationship after running (Kellmann *et al.*, 2018)

Recovery interventions

Immediately after the post-exercise assessments (nearly 20-min after exercise cessation), participants performed the assigned interventions for visits 2, 4, 6 and 8 in each study (order determined by simple randomization with sealed opaque envelopes). The control condition (CON) comprises a passive rest recovery seated in a comfortable desk during 10-min in an environmentally controlled room (temperature: 20–22°C; humidity 40%–60%). In the HWI, CWI and placebo recovery interventions, participants seated with legs fully extended in an inflatable bath (120cm×55cm×50cm, VG plus, Brazil) with the water set to the level of xyphoid process. In HWI intervention (HWI_{41°}), participants were submerged for 45-min in water at 41.2±0.6°C (Rodrigues *et al.*, 2020b). In CWI intervention (CWI_{11°}), participants were submerged for 15-min in water at 11.4±0.5°C (Machado *et al.*, 2016). The water temperature was maintained in HWI_{41°} by removing 5-L of water from the bath and adding 5-L of pre-warmed (45°C) water in it when necessary. The water was warmed in separated tanks using electric boilers. In CWI_{11°}, the water temperature was maintained with the remotion of 2-L of water from bath and the addition of 20-g ice cubes in it when necessary. Assessments of water temperature were performed until 5-min using a digital thermometer. In the placebo intervention (PWI), participants were submerged for 10-min in a colored water at neutral temperature of 26.1±0.8°C. An unidentified pH-balanced water coloring agent (Pinta água, Color Brinque, Brazil) was added to the water in PWI immediately before immersion. The participants were told that the substance had been provided for testing in the laboratory and that the manufacturers claimed that "it was able to accelerate recovery after exercise". They were also told that it was not possible to provide details about the substance's mechanisms of action or the manufacturer until the results of the study were published. (Broatch *et al.*, 2014). Given the popularity of water recovery interventions (most notably CWI), it is possible that participants had previous knowledge and positive expectations toward these

interventions, which may lead to potential bias on the outcomes due to the placebo effect (Hurst *et al.*, 2020). High-resolution thermal images (FLIR C3-X, 128×96 pixels infrared resolution, thermal sensitivity <70 mK; Flir systems, USA) were taken from 1.5 m distance immediately before and after the recovery interventions. After processing it using commercially available software (Flirt tools+, Flir systems, USA), it was observed that the skin temperature on the anterior area of the thigh (1/3 medial, dominant lower-limb) increased from 30.3±1.95°C to 32.6±1.4°C after CON; decreased from 30.3±1.1°C to 27.1±3.7°C after PWI; decreased from 29.8±2.5°C to 17.8±2.6°C after CWI_{11°}; and increased from 29.9±1.0 to 35.5±1.5°C after HWI_{41°} (all $p < 0.05$ in paired t tests).

Throughout the recovery interventions, while waiting for the 2-h and 4-h post-exercise assessments, participants consumed water *ad libitum* and 1.1 g/kg of body mass/hour of carbohydrate in the form of cereal bars and artificial juices (McCartney *et al.*, 2018). The amount of water and carbohydrate ingested post-exercise during visits 2 (i.e., the first trial of CONT_{100%RCP} or HIIT_{150%RCP}) was recorded and repeated in visits 4, 6 and 8. Before the visit 3 (i.e., 24-h after the first trial of CONT_{100%RCP} or HIIT_{150%RCP}), the participants were instructed to carry out a dietary recall and to repeat the same eating pattern prior to visits 5, 7 and 9. Aiming to qualitatively assess the feeling/beliefs of the participants toward PWI, HWI_{41°} and CWI_{11°}, after completing all visits, the participants were asked about: (i) “which method was the most pleasant to receive”; (ii) “which method was the least pleasant to receive”; (iii) “which methods to you think was helpful for improve post-exercise recovery”; and (iv) “which methods to you think was not helpful for improve post-exercise recovery”.

Data collection and analysis

Respiratory data collection and analysis

Breath-by-breath pulmonary gas exchange and $\dot{V}_E$ were continuously measured during GXT and brief running efforts performed after CONT_{100%RCP} and HIIT_{150%RCP} using a metabolic cart (Quark, CPET, COSMED, Rome, Italy), which was calibrated before each test according to the recommendations from the manufacturer. Initially, all the breath-by-breath data were examined to exclude errant breaths and those values lying more than ±3 SD from the local mean were removed (Rossiter *et al.*, 2002). The breath-by-breath data were subsequently interpolated every 1-s (Özyener *et al.*, 2001) using the software OriginPro 8.0 (Origin Lab Corporation, Massachusetts, United States). In the GXT, the highest $\dot{V}O_2$ average from the last 20-s of each stage was determined as $\dot{V}O_{2max}$ when it was similar (±2.1 mL/kg/min) to $\dot{V}O_2$ recorded in the constant-load exercise carried out

5-min after GXT (Schaun, 2017). All participants reached the $\dot{V}O_{2\max}$ according to this criterion. The minimal exercise velocity at which $\dot{V}O_{2\max}$ was reached was recorded as $v\dot{V}O_{2\max}$ (Billat *et al.*, 1999). For the estimation of RCP, raw profiles of $\dot{V}_E$, carbon dioxide output ($\dot{V}CO_2$), their combination (i.e., $\dot{V}_E / \dot{V}CO_2$), RER, and end-tidal pressure of CO_2 (Pet CO_2) were plotted against time. RCP was identified as the point at which Pet CO_2 began a precipitous fall after a period of isocapnia concomitantly with a second and first breakpoint in the $\dot{V}_E$ and $\dot{V}_E / \dot{V}CO_2$ relationships, respectively (Keir *et al.*, 2022). The vRCP was identified by the projection of RCP (time at which it occurs) at GXT stages. When necessary, $v\dot{V}O_{2\max}$ and vRCP were adjusted according to Kuipers *et al.*, (1985). During the brief running efforts, the $\dot{V}O_2$, $\dot{V}_E$ and RER average of the last 20-s of effort was calculated (Dutra *et al.*, 2020) being further presented in percentage change from the rest (*raw* values are presented in supplementary file).

Neuromuscular function assessments and analysis

Knee-extensors function outcomes

iMVCs with dominant knee-extensors (self-reported) associated to PNS during force production and at rest were performed by the participants prior to, immediately-post, 2-, 4-, and 24-h after all CONT_{100%RCP} and HIIT_{130%RCP}. For the iMVCs, participants were positioned on a custom-made chair designed to keep the hips and knees flexed at 90°. Participants were firmly secured with straps fastening legs, hips, and chest. Force signal was acquired using a load cell with a 0.03% rated output accuracy and a 0.98-N resolution (MK Controle, Brazil). The load cell was attached to the ankle by a metal rod immediately above the region of the medial malleolus using straps with heavy duty Velcro. The load cell signal was acquired at a sampling frequency of 1,000 Hz using a PowerLab system (16/35, ADInstruments, Dunedin, Nova Zelandia). The surface electromyography activity (EMG) signal from *vastus laterallis* was acquired using an amplifier FE136 (ADInstruments, Dunedin, New Zealand) and the PowerLab system. The EMG was recorded through a pair of self-adhesive Ag/AgCl surface electrode (Meditrace 100, Covidien, USA) placed on the 1/3 distal of muscle. The reference electrode was placed on the patella. Before electrode placement, the skin was shaved, lightly abraded, and cleaned with rubbing alcohol. Both muscular force and EMG signals were recorded using the LabChart 8 software (Dutra *et al.*, 2023).

Stimulations of the femoral nerve of the dominant knee extensors (pulse duration: 1 ms; square wave) were delivered by a high-voltage electrical stimulator (Bioestimulador V2

400 V peak-to-peak; Insight, São Paulo, Brazil) through Ag/ AgCl electrodes (10 mm of diameter, Kendall Medi-Trace, Massachusetts) on the femoral triangle (cathode) and through carbon rubber electrodes (32.5 mm of diameter, CARCI, Brazil) at the femoral greater trochanter level (anode). Doublet high-frequency stimulations (100 Hz) were delivered at the visual peak force of the iMVC effort (~2-3-s after iMVC started) as well as at 3-s after iMVC with the musculature relaxed. Single and doublet low-frequency (10 Hz) stimulations were also delivered to the relaxed muscle at 7 and 10 s after iMVC, respectively. The magnitude of the electrical stimuli was determined individually in each visit by the application of single consecutive incremental stimuli on the relaxed muscle starting at 30 mA followed by 30-mA increments until reaching the involuntary force plateau. The force plateau was 267 ± 57 mA across study 1 and 281 ± 74 mA across study 2. Thereafter, the electrical stimuli intensity used for the PNS were further increased by 30%.

For the trials carried out immediately after exercise, it took 66 ± 5 -s to maneuver the participants from the treadmill to the chair used for the neuromuscular assessments and to perform the methodological procedures needed to start the test (e.g., electrodes and wires placement, ankle attachment, signal stabilization). Except in the assessments carried out immediately after exercise, three iMVCs interspersed by 30-s and followed by 2-min rest were performed before all trials as a warm-up procedure. The peak force achieved during these warm-up iMVCs was used to ensure that participants reached their peak force during iMVCs associated to PNS trials, since described that participants' knowledge about the PNS may unintentionally reduce the voluntary peak force during this kind of test (Button & Behm, 2008).

The highest voluntary force recorded within a 100-ms period during the force plateau of the iMVC was used as the peak voluntary force ($iMVC_{Force}$). The $iMVC_{Force}$ was expressed in raw values (i.e., N). The EMG signal was initially band-pass (20–500 Hz) filtered and then the root mean square of the *vastus lateralis* EMG signal (RMS_{VL}) was recorded 0.5 s around the $iMVC_{Force}$. The superimposed force signal amplitude by the doublet high-frequency stimuli recorded during iMVC and the force evoked by the doublet high-frequency stimuli at rest after iMVC ($Db100_{Force}$) were used to measure the voluntary neuromuscular activation (VA) using the following equation: $VA (\%) = [1 - (\text{superimposed force}) \times (\text{force level at stimulation}/iMVC_{Force}) / (Db100_{Force})] \times 100$ (Strojnik & Komi, 1998). The potentiated peak force evoked by the single stimulus ($Q_{tw_{pot}}$) was recorded and the ratio between the force evoked by the doublet low-

frequency stimuli ($\text{Db10}_{\text{Force}}$) and $\text{Db100}_{\text{Force}}$ (i.e., $\text{Db10}_{\text{Force}}/\text{Db100}_{\text{Force}}$ ratio) was calculated. The amplitude of EMG response evoked by the single stimulus were also recorded (M-wave amplitude). The ratio between RMS_{VL} and M-wave amplitude ($\text{RMS}/\text{M-wave}_{\text{amp}}$) was also calculated. All analysis procedures were performed using the LabChart 8 software. All data is presented in percentage change from the rest (*raw* values are presented in supplementary file).

Countermovement jump performance indicators

Before, after (~ 2-min), 2-, 4- and 24-h after $\text{CONT}_{100\%\text{RCP}}$ and $\text{HIIT}_{130\%\text{RCP}}$, participants performed 5 CMJs interspaced by 20-s rest on a force plate (Cefise, Nova Odessa, Brazil). Participants were instructed to keep both hands akimbo and to quickly flex the knees to 90° and then jump as high as possible. Force plate signal (1000 Hz) was acquired using the PowerLab system (16/35, ADInstruments, Dunedin, New Zealand) and the LabChart 8 software (AD Instruments, Dunedin, New Zealand). The data was initially filtered with a fourth-order Butterworth filter (low-pass cutoff 25 Hz) and using the equations described by Linthorne et al., (2001), the CMJ height ($\text{CMJ}_{\text{Height}}$), concentric peak force ($\text{CMJ}_{\text{Force}}$), and power ($\text{CMJ}_{\text{Power}}$) were analyzed by a custom-made Matlab algorithm (MathWorks, Natick, USA). The average obtained from the five CMJ trials was calculated (Claudino *et al.*, 2017) and has been presented in percentage change from the rest (*raw* values are presented in supplementary file).

Heart rate variability measurement

Before, 2-, 4- and 24-h after $\text{CONT}_{100\%\text{RCP}}$ and $\text{HIIT}_{150\%\text{RCP}}$, measurements of HRV occurred at rest, with the participants remaining seated alone in a dim lighting, silent room for 6-min. The HRV signal was acquired through a Bio-amplifier FE136 (ADInstruments, Dunedin, New Zealand) and a PowerLab system (16/35, ADInstruments, Dunedin, New Zealand) via self-adhesive Ag-AgCl surface electrodes (Meditrace 100, Covidien, USA) in a modified lead-II arrangement (Esco *et al.*, 2017). Before electrode placement, each site was cleaned by rubbing alcohol. The LabChart 8 software (AD Instruments, Dunedin, New Zealand) was used to offline calculation of root mean square of successive R-R interval differences (RMSSd) from the final 5-min of signal recording. The RMSSd was further transformed in based-ten logarithm ($\text{RMSSd}_{\text{Log}}$) and is herein presented in percentage of change from the rest (Buchheit, 2014). *Raw* values are presented in supplementary file.

Perceptual responses assessments

Participants' perception of "Recovery" was assessed using the Total Quality Recovery scale proposed by Kentta and Hassmen (6-20 top-to-bottom scale, "very, very poor recovery" to "very, very good recovery") (Kenttä & Hassmén, 1998). Participants were inquired to rate their current recovery status in relation of the end of the exercise and, before answering, they were asked to direct their attention to signs as sleepiness, mood states, soreness or heaviness legs sensation. Before, 2-, 4- and 24-h after CONT_{100%RCP} and HIIT_{150%RCP}, the mood of the participants was assessed using the profile of mood state questionnaire (POMS) (Beedie *et al.*, 2000). The Total Mood Disturbance (TMD) score, which is a summary of positive mood from adding subscales and subtracting vigor was calculated and presented in percentage change from the rest (*raw* values are presented in supplementary file). Higher scores for the TMD indicate a greater mood disturbance (Beedie *et al.*, 2000). During the brief running efforts performed at rest, 2-, 4- and 24-h after CONT_{100%RCP} and HIIT_{150%RCP}, RPE, rating of "Pain" in the lower-limbs and global "Fatigue" were recorded immediately after effort cessation. RPE was assessed by 6-20 top-to-bottom Borg's scale (Borg, 1982); exercising legs "Pain" was assessed by 0-10 top-to-bottom Borg's scale (Borg *et al.*, 2010); and global "Fatigue" was assessed by 10-0 top-to-bottom Micklewright's scale (Micklewright *et al.*, 2017).

Statistical analysis

The SPSS the software package version 20.0 (IBM Corp., Armonk, NY) was used for all statistical analysis. All data are presented as mean $\pm$ SD. Statistical significance was set at α of 0.05. Normality of the data was verified using the Shapiro-Wilk test. A one-way repeated measures ANOVA (recovery intervention) was used to compare post-exercise responses between CONT, PWI, CWI_{11°}, HWI_{41°}. A two-way repeated measures ANOVA (time and recovery intervention) was used to assess the effects of time (rest, immediately-post, 2-, 4-, and 24-h post-exercise) and recovery interventions (CONT, PWI, CWI_{11°}, HWI_{41°}). Assumptions of sphericity were explored and controlled for all variables with the Greenhouse–Geisser adjustment, where necessary. If significant main effects or interactions (time $\times$ recovery intervention) were observed, a Bonferroni post hoc pairwise comparison was performed. Partial eta squared (η_p^2) was calculated to estimate effect sizes, with values of 0.01, 0.06, and 0.14 representing small, moderate, and large effects, respectively.

6.4. Results

Study 1: Effects of CWI_{11°} and HWI_{41°} on fatigability responses after CONT_{100%RCP}

The participants anthropometric characteristics and results in GXT are displayed in **Table 6.1**. The CONT_{100%RCP} was performed at 11.4 ± 1.2 km/h ($73.5 \pm 5.0\%$ of $v\dot{V}O_{2max}$) and had a volume of 7.5 ± 0.8 km. Participants reached the same ($p > 0.05$) RPE-SESSION and $[La]_{Peak}^-$ after exercise in CONT (252.0 ± 101.3 a.u. and 2.8 ± 1.4 mmol/L, respectively), PWI (248.9 ± 114.5 a.u. and 2.8 ± 2.1 mmol/L, respectively), CWI_{11°} (244.0 ± 95.1 a.u. and 2.9 ± 2.3 mmol/L, respectively), and HWI_{41°} (248.9 ± 116.2 a.u. and 2.9 ± 2.1 mmol/L, respectively).

Table 6.1. Anthropometric characteristics and cardiorespiratory responses recorded during GXT.

CONT _{100%RCP} (n=12)	
Age (years)	26 ± 7
Height (cm)	177.1 ± 7.5
Body mass (kg)	74.1 ± 6.2
$\dot{V}O_{2max}$ (L/min)	3.6 ± 0.3
$\dot{V}O_{2max}$ (mL/kg/min)	48.5 ± 3.4
$v\dot{V}O_{2max}$ (km/h)	15.46 ± 1.44
RCP	
$\dot{V}O_2$ (L/min)	2.9 ± 0.4
$\dot{V}O_{2max}$ (%)	82.3 ± 5.8
km/h	11.4 ± 1.2
$v\dot{V}O_{2max}$ (%)	73.4 ± 4.9

Mean $\pm$ standard deviation. $\dot{V}O_{2max}$: maximal rate of oxygen consumption; $v\dot{V}O_{2max}$: velocity associated to $\dot{V}O_{2max}$; RCP: respiratory compensation point; $\dot{V}O_2$: rate of oxygen consumption; $[La]_{Peak}^-$: peak of blood lactate concentration after exercise.

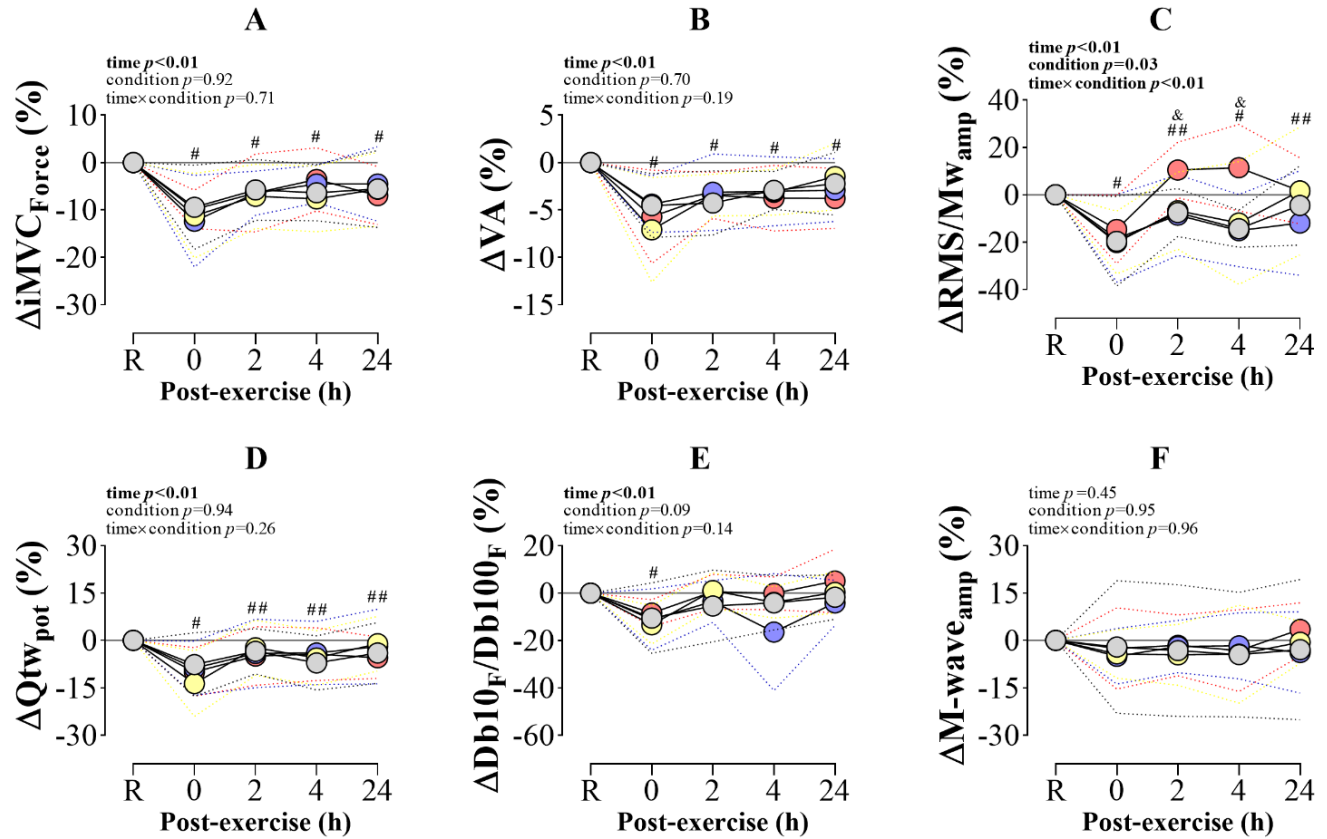
The performance fatigability indicators after CONT_{100%RCP} are presented in **Figure 6.2**. Compared to rest, $iMVC_{Force}$ and VA decreased immediately after exercise and remained reduced for at least 24-h (main effect of time: $p \leq 0.01$; $\eta_p^2 \geq 0.49$; post hoc: $p < 0.05$). $RMS_{VL}/M-wave_{amp}$, $Q_{tw_{pot}}$, and $Db_{10Force}/Db_{100Force}$ presented lower values compared to rest only immediately post-exercise (main effect of time: $p \leq 0.02$; $\eta_p^2 \geq 0.37$; post hoc: $p < 0.05$). The $RMS/M-wave_{amp}$ was higher 2 and 4-h after HWI_{41°} compared to CWI_{11°}, PWI and CON (time $\times$ recovery intervention effect: $p > 0.01$; $\eta_p^2 = 0.18$; post-hoc: $p > 0.01$). There were no other time, recovery intervention or interaction effects on the knee-extensors function outcomes evaluated. CMJ_{Power} increased immediately post-exercise (main effect of time: $p < 0.01$; $\eta_p^2 = 0.59$; post hoc: $p < 0.05$) with no other time, recovery intervention, or interaction effects on the CMJ performance indicators evaluated.

The physiological and psychological responses evaluated at rest and during brief-running efforts after CONT_{100%RCP} are presented in **Figure 6.3**. Although $RMSSd_{Log}$ decreased only immediately post-exercise (main effect of time: $p < 0.01$; $\eta_p^2 = 0.75$; post hoc: $p < 0.05$), it was higher 2-h after CWI_{11°} compared to CON, PWI and HWI_{41°} (time $\times$ recovery

intervention effect: $p<0.01$; $\eta_p^2=0.34$; post-hoc: $p=0.01$). POMS_{TMD} remained decreased for 4-h after CONT_{100%RCP} (main effect of time: $p<0.01$; $\eta_p^2=0.38$; post hoc: $p<0.05$), suggesting a “mood” impairment after this type of exercise. There were no recovery intervention or interaction effects on POMS_{TMD}. The “Recovery” perception increased just 24-h after exercise (main effect of time: $p<0.01$; $\eta_p^2=0.64$; post hoc: $p<0.05$) being higher throughout the assessments after CWI_{11°} compared to PWI and CON (recovery intervention effect: $p<0.01$; $\eta_p^2=0.27$; post-hoc: $p=0.01$).

Throughout the brief running efforts performed after CONT_{100%RCP}, the $\dot{V}_E$ and $\dot{V}O_2$ were higher for at least 4-h compared to rest values while RER remained lower for at least 24-h after exercise (main effect of time: $p<0.01$; $\eta_p^2\geq 0.58$; post-hoc: $p=0.01$). The $\dot{V}O_2$ was lower after HWI_{41°} compared to CWI_{11°}, PWI and CON (recovery intervention effect: $p=0.02$; $\eta_p^2=0.26$; post-hoc: $p<0.01$). There were no other recovery intervention or interaction effects on the cardiorespiratory responses evaluated. During these same brief running efforts, “Pain” at lower limbs and “Fatigue” perception were higher for at least 24-h after CONT_{100%RCP} while RPE was higher for just 4-h after this exercise (main effect of time: $p<0.01$; $\eta_p^2\geq 0.53$; post-hoc: $p=0.01$). The “Fatigue” perception was lower after CWI_{11°} compared to PWI and CON (recovery intervention effect: $p<0.01$; $\eta_p^2=0.28$; post-hoc: $p=0.01$). There were no other recovery intervention or interaction effects of HWI_{41°} or CWI_{11°} on these fatigability responses. Finally, a quality assessment about the participant’s feeling and beliefs towards the recovery methods used demonstrated that most of the participants considered PWI the “most pleasant” method used (67% of the answers), the CWI_{11°} as “helpful” method to improve recovery (75% of the answers), and the HWI_{41°} as the “least pleasant” (75% of the answers) and “not helpful” method (67% of the answers) (**Figure 6.3**).

Knee-exetensors function outcomes



CMJ performance outcomes

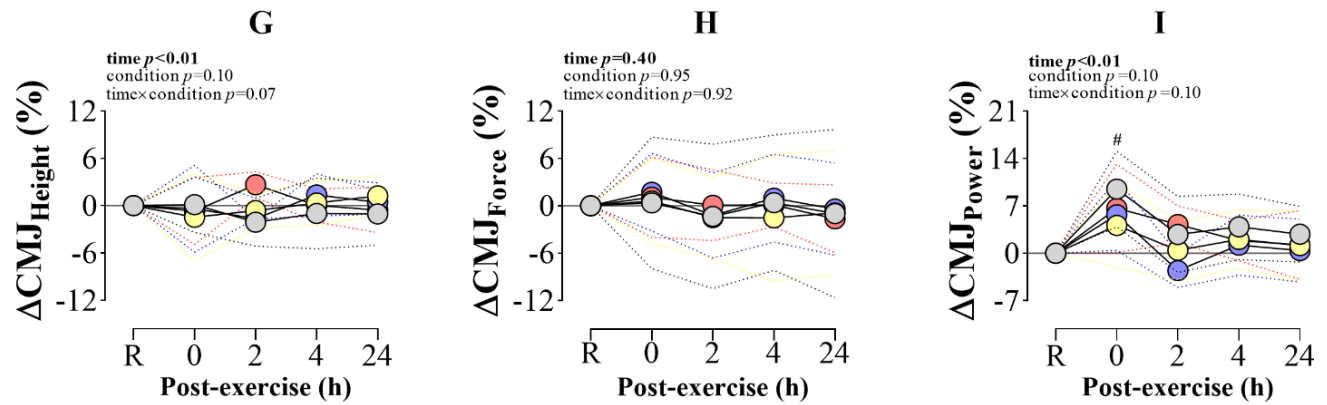
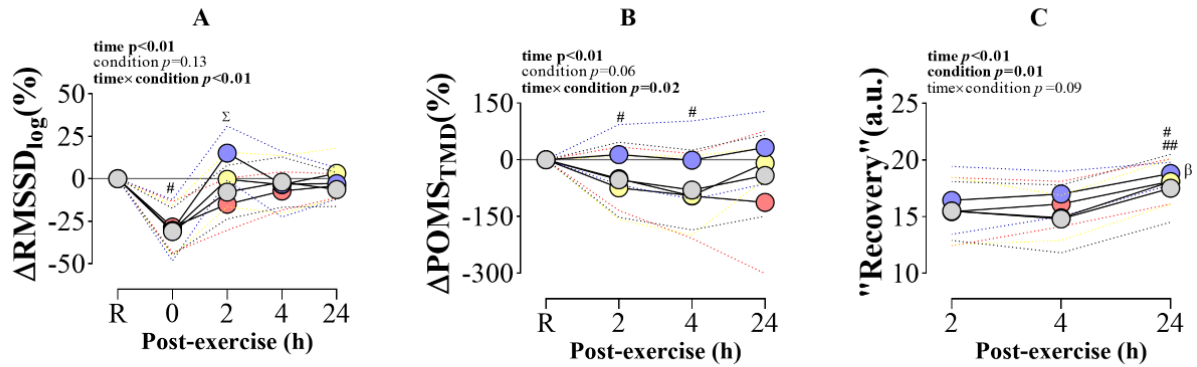
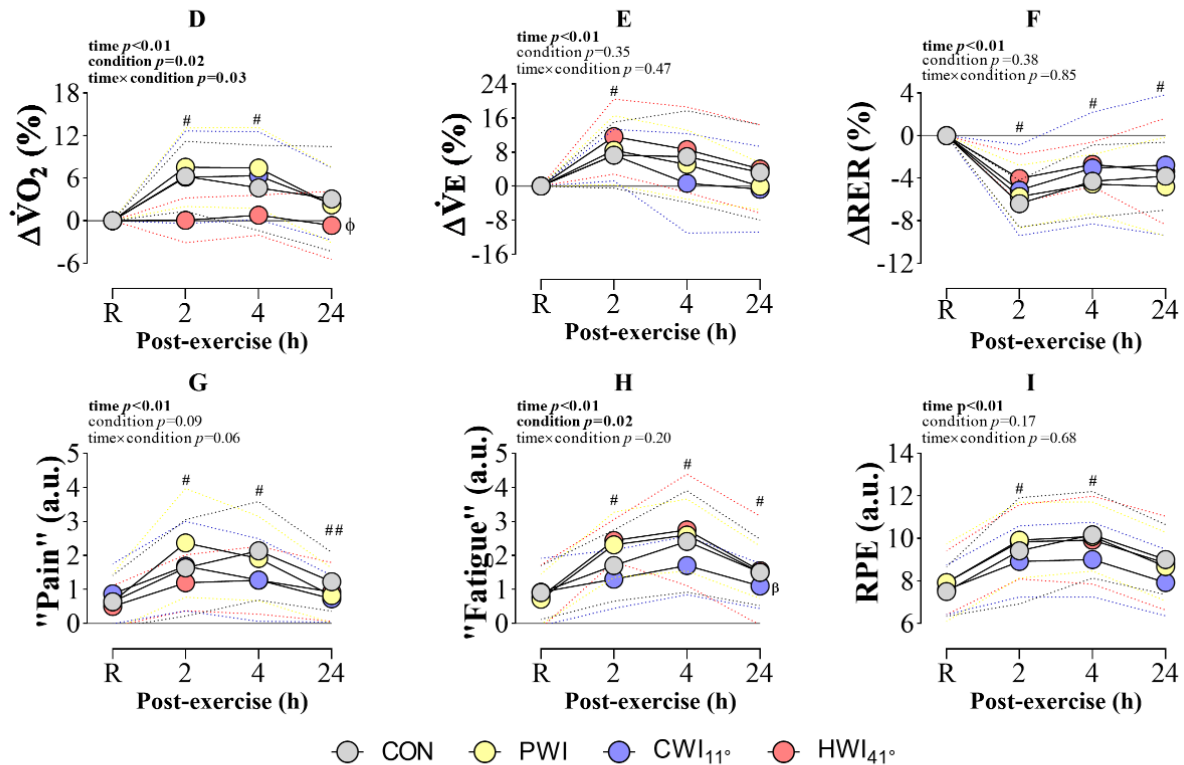


Figure 6.2. Percentage change from rest on knee-extensors function outcomes and countermovement jump performance indicators after a 40-min continuous running at velocity associated to respiratory compensation point (CONT_{100%RCP}) followed by different recovery strategies. CWI_{11°}, cold-water immersion at 10°C for 15-min; HWI_{41°}, hot-water immersion at 42° for 45-min; PWI, immersion on thermoneutral colored water for 10-min (placebo condition); CON, passive rest (control condition). Bold type indicates statistical main effects in repeated measures analysis of variance (ANOVA), $p < 0.05$. #, different from rest (main effect of time); ##, different from 0-h (main effect of time); &, HWI_{42°} different from PWI and CON (time $\times$ recovery intervention effect). R, rest assessments; $iMVC_{Force}$, highest voluntary force recorded during maximal voluntary isometric contractions of knee-extensors; VA, voluntary activation measured through twitch interpolation technique; $RMS/M_{wave_{amp}}$, ratio between the root mean square of electromyography signal from vastus lateralis during $iMVC$ (0.5-s around $iMVC_{Force}$) and peak-to-peak M-wave amplitude ($M_{wave_{amp}}$); Qtw_{pot} , force evoked by a potentiated single stimulus at relaxed knee-extensors; $Db10_{Force}/Db100_{Force}$, ratio between the force evoked by a supramaximal high-frequency (100Hz) and low-frequency (10 Hz) doublet stimulus at relaxed knee-extensors; CMJ_{Height} , CMJ_{Force} , and CMJ_{Power} , average of the height, force and power recorded in 5 CMJs. All raw data is presented in supplementary file. N=12

Physiological and psychological responses at rest



Physiological and psychological responses at brief running



Participants' feelings and beliefs about the recovery methods used

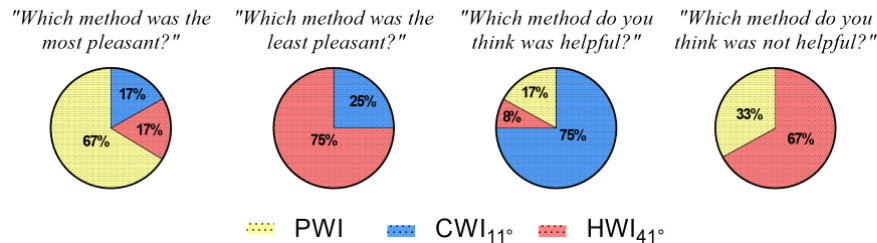


Figure 6.3. Physiological and psychological responses at rest and during brief running efforts (3-min, 80% of vRCP) performed after a 40-min continuous running (CONT_{100%RCP}) followed by different recovery strategies. CWI_{11°}, cold-water immersion at 10°C for 15-min; HWI_{41°}, hot-water immersion at 42°C for 45-min; PWI, immersion on thermoneutral colored water for 10-min (placebo condition); CON, passive rest (control condition). Bold type indicates statistical main effects in repeated measures analysis of variance (ANOVA). R, rest assessments; #, different from rest (main effect of time); ##, different from 0-h (main effect of time); Σ , CWI_{11°} different from HWI_{41°}, PWI, and CON (time $\times$ recovery intervention effect); β , CWI_{11°} different from PWI and CON (main effect of recovery intervention); Φ , HWI_{41°} different from CWI_{11°}, PWI, and CON (main effect of recovery intervention). $\dot{V}\text{O}_2$, $\dot{V}\text{E}$, and

RER, rate of oxygen consumption, ventilation, and respiratory exchange ratio, respectively, in a brief running effort; Pain”, “Fatigue” and RPE, pain perception, global fatigue perception, and rate of perceived effort, respectively, in a brief running effort; RMSSd_{log}, based-ten logarithm of root mean square of successive R-R interval differences; POMS_{TMD}, total mood disturbance calculated through the profile of mood state questionnaire; “Recovery”, perception of global recovery. All raw data is presented in supplementary file. N=12.

Study 2: Effects of CWI_{11°} and HWI_{41°} on fatigability responses after HIIT_{150%RCP}

The participants anthropometric characteristics and results in GXT are displayed in **Table 6.2**. The HIIT_{150%RCP} was performed at 16.7 ± 1.3 km/h ($105.5 \pm 5.7\%$ of $\dot{V}O_{2\max}$) and had a volume of 7.4 ± 0.6 km. Participants reached the same ($p > 0.05$) RPE-SESSION and $[La]_{\text{Peak}}$ after exercise in CONT (592.0 ± 142.1 a.u. and 5.2 ± 0.9 mmol/L, respectively), PWI (608.0 ± 160.9 a.u. and 5.6 ± 1.8 mmol/L, respectively), CWI_{11°} (613.3 ± 141.1 a.u. and 5.4 ± 1.4 mmol/L, respectively), and HWI_{41°} (666.7 ± 69.3 a.u. and 5.1 ± 0.9 mmol/L, respectively).

Table 6.2. Anthropometric characteristics and cardiorespiratory responses recorded during GXT.

HIIT _{150%RCP} (n=10)	
Age (years)	24 ± 5
Height (cm)	175.1 ± 4.2
Body mass (kg)	74.3 ± 6.5
$\dot{V}O_{2\max}$ (L/min)	3.7 ± 0.4
$\dot{V}O_{2\max}$ (mL/kg/min)	49.5 ± 4.5
$v\dot{V}O_{2\max}$ (km/h)	15.8 ± 1.4
RCP	
$\dot{V}O_2$ (L/min)	2.9 ± 0.4
$\dot{V}O_{2\max}$ (%)	79.5 ± 4.2
km/h	11.1 ± 0.8
$v\dot{V}O_{2\max}$ (%)	70.2 ± 3.7

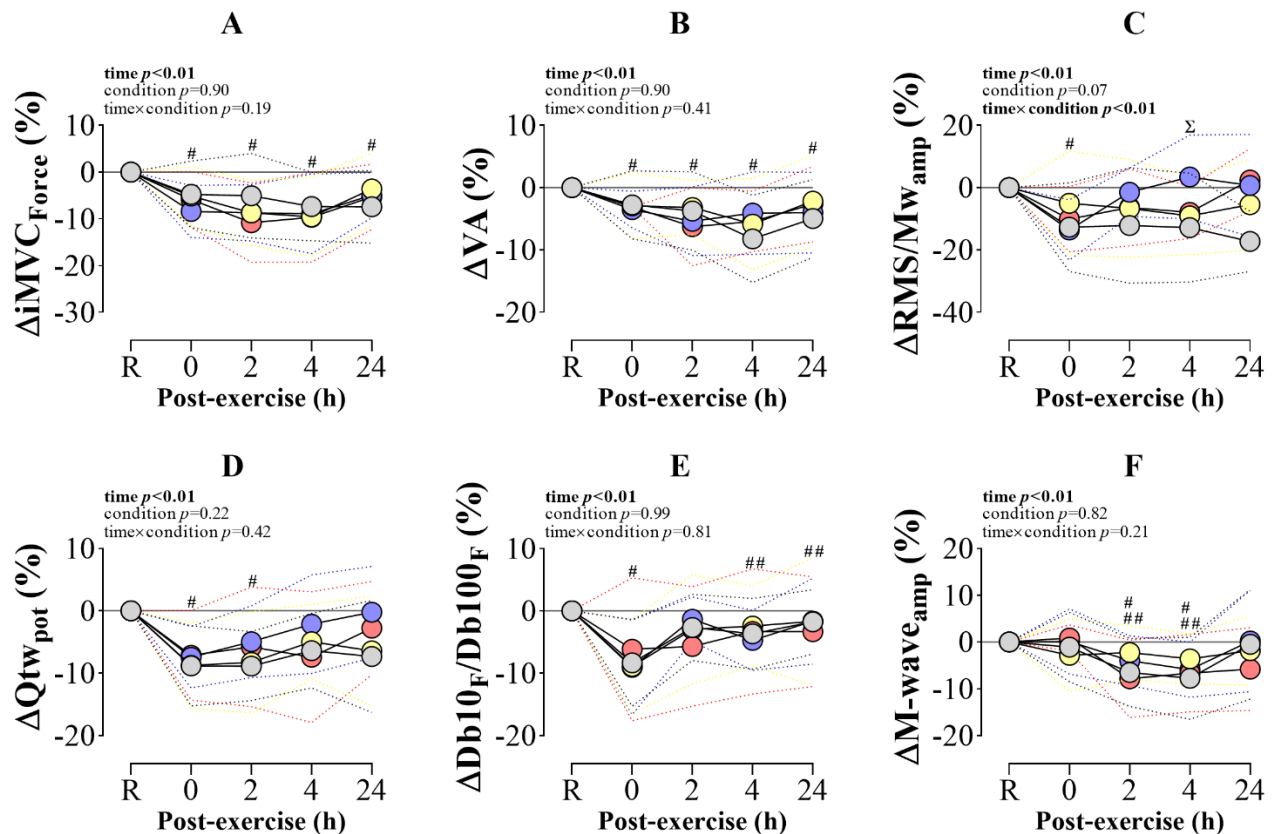
Mean $\pm$ standard deviation. iMVC_{Force}: highest voluntary force recorded during maximal isometric voluntary contraction; $\dot{V}O_{2\max}$: maximal rate of oxygen consumption; $v\dot{V}O_{2\max}$: velocity associated to $\dot{V}O_{2\max}$; RCP: respiratory compensation point; $\dot{V}O_2$: rate of oxygen consumption; $[La]_{\text{Peak}}$: peak of blood lactate concentration after exercise.

The knee-extensors function outcomes in HIIT_{150%RCP} are presented in **Figure 6.4**. Compared to rest, iMVC_{Force} and VA reduced immediately after exercise and remained decreased for at least 24-h (main effect of time: $p \leq 0.01$; $\eta_p^2 \geq 0.51$; post hoc: $p < 0.05$). RMS_{VL}/M-wave_{amp} and Db_{10Force}/Db_{100Force} presented lower values compared to rest only immediately post-exercise (main effect of time: $p \leq 0.02$; $\eta_p^2 \geq 0.28$; post hoc: $p < 0.05$). The Qtw_{pot} reduced immediately after exercise compared to rest and remained decreased for 2 hours (main effect of time: $p < 0.01$; $\eta_p^2 = 0.46$; post hoc: $p < 0.05$). The M-wave_{amp} decreased specifically 2 and 4-h post-exercise compared to rest values (main effect of time: $p < 0.01$; post hoc: $p < 0.05$). The RMS_{VL}/M-wave_{amp} was higher 4-h after exercise in CWI_{11°} compared to HWI_{41°}, PWI and CON (time $\times$ recovery intervention effect: $p < 0.01$;

$\eta_p^2=0.50$; post-hoc: $p<0.05$). There were no other recovery intervention or interaction effects of CWI_{11° or HWI_{41° on the knee-extensor outcomes evaluated. There were no time, recovery intervention, or interaction effects on CMJ_{Height} , CMJ_{Force} , or CMJ_{Power} .

The physiological and psychological responses evaluated at rest and during brief-running efforts after $HIIT_{150\%RCP}$ are presented in **Figure 6.5**. $RMSd_{Log}$ remained decreased for 4-h after $HIIT_{100\%RCP}$ (main effect of time: $p<0.01$; $\eta_p^2=0.64$; post hoc: $p<0.05$), and as observed in $CONT_{100\%RCP}$, it was higher 2-h after CWI_{11° compared to PWI and CON (time $\times$ recovery intervention effect: $p<0.01$; $\eta_p^2=0.25$; post-hoc: $p=0.01$). $POMS_{TMD}$ decreased specifically 4-h after $HIIT_{150\%RCP}$ (main effect of time: $p<0.01$; $\eta_p^2=0.39$; post hoc: $p<0.05$), suggesting a “mood” impairment after this type of exercise. There were no recovery intervention or interaction effects on it. As observed in $CONT_{100\%RCP}$, the “Recovery” perception increased just 24-h after exercise (main effect of time: $p<0.01$; $\eta_p^2=0.83$; post hoc: $p<0.05$) with no further recovery intervention or interaction effects on it. Throughout the brief running efforts performed after $HIIT_{150\%RCP}$, the $\dot{V}_E$ were higher for at least 4-h compared to rest values while $\dot{V}O_2$ and RER remained lower for at least 24-h after exercise (main effect of time: $p<0.01$; $\eta_p^2\geq 0.67$; post-hoc: $p=0.01$). As observed in $CONT_{100\%RCP}$, the $\dot{V}O_2$ was lower after HWI_{41° compared to CWI_{11° , PWI and CON throughout the brief running efforts (recovery intervention effect: $p=0.02$; $\eta_p^2=0.41$ post-hoc: $p<0.01$). There were no other recovery intervention or interaction effects on the cardiorespiratory responses evaluated. During these same brief running efforts, “Pain” at lower limbs were higher for at least 24-h after $HIIT_{150\%RCP}$ while “Fatigue” perception and RPE were higher for just 4-h after this exercise (main effect of time: $p<0.01$; $\eta_p^2\geq 0.68$; post-hoc: $p=0.01$). There were no recovery intervention or interaction effects of HWI_{41° or CWI_{11° on these responses. A quality assessment about the participant’s feeling and beliefs towards the recovery methods used demonstrated that most of the participants considered PWI as the “most pleasant” method used (90% of the answers), CWI_{11° as “helpful” method to improve recovery (70% of the answers), and HWI_{41° as the “least pleasant” (60% of the answers) and “not helpful” method (50% of the answers). These answers were very similar to obtained after $CONT_{100\%RCP}$.

Knee-extensors function outcomes



CMJ performance outcomes

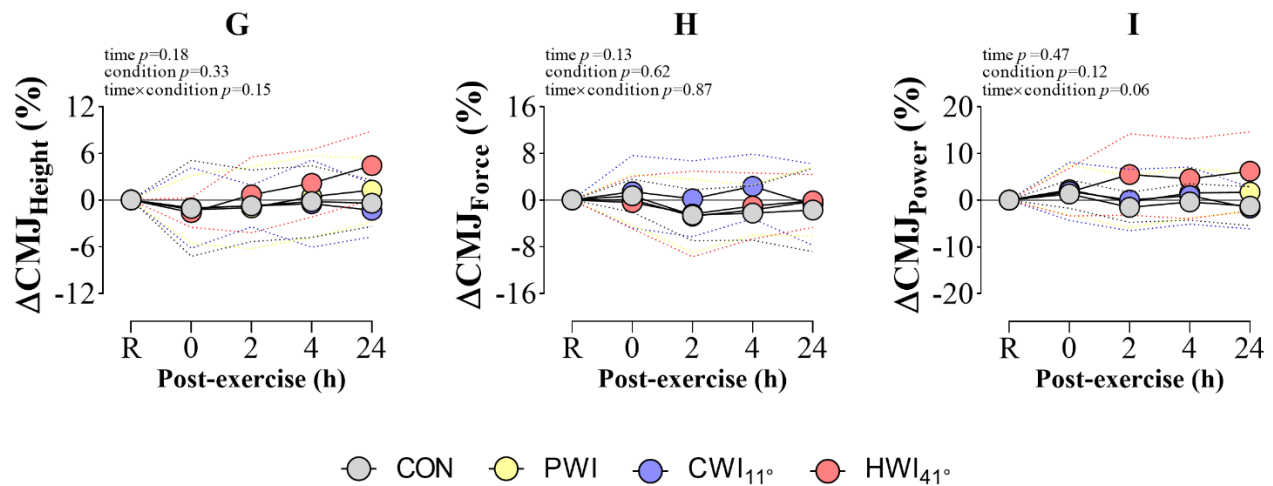
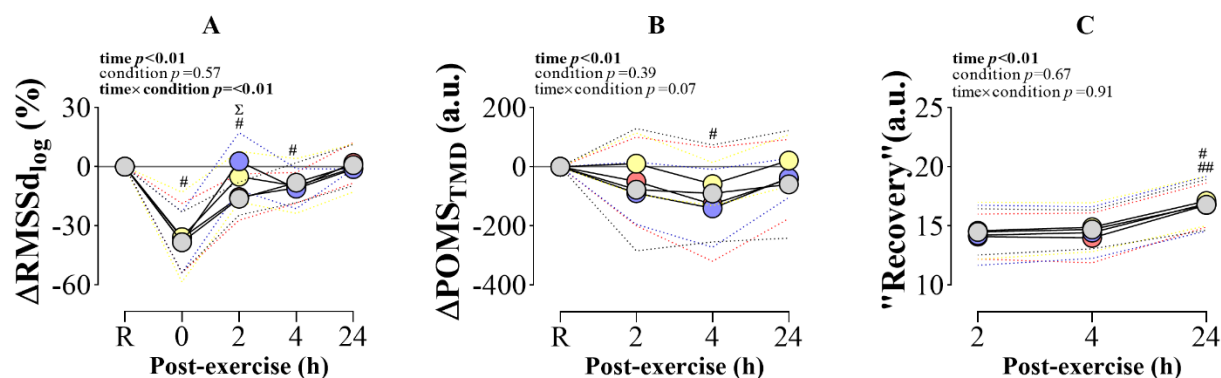
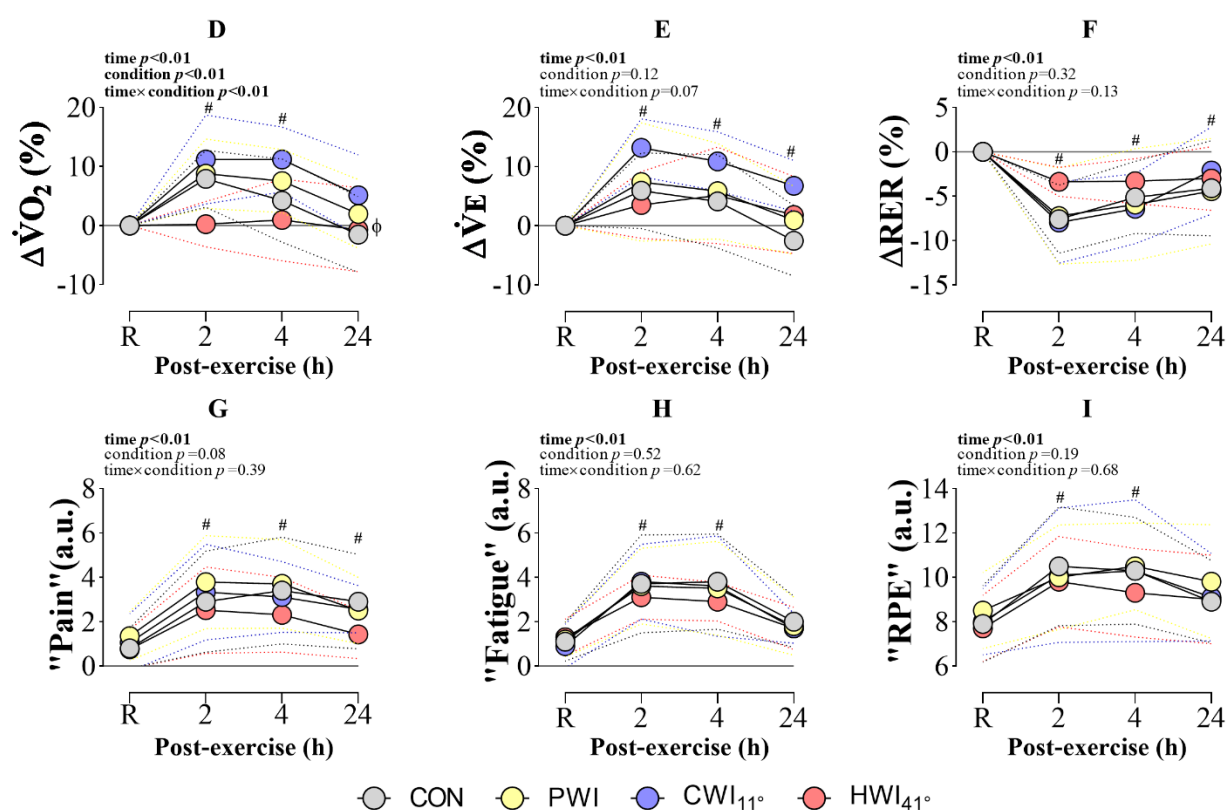


Figure 6.4. Percentage change from rest on knee-extensors function outcomes and countermovement jump performance indicators after 20 $\times$ 1-min and 20-s running bouts (HIIT_{150%RCP}) followed by different recovery strategies. CWI_{11°}, cold-water immersion at 10°C for 15-min; HWI_{41°}, hot-water immersion at 42° for 45-min; PWI, immersion on thermoneutral colored water for 10-min (placebo condition); CON, passive rest (control condition). Bold type indicates statistical main effects in repeated measures analysis of variance (ANOVA), $p < 0.05$. #, different from rest (main effect of time); ##, different from 0-h (main effect of time); Σ , CWI_{11°} different from PWI, and CON (time $\times$ recovery intervention effect). R, rest assessments; $iMVC_{Force}$, highest voluntary force recorded during maximal voluntary isometric contractions of knee-extensors; VA, voluntary activation measured through twitch interpolation technique; $RMS_{VL}/M-wave_{amp}$, ratio between the root mean square of electromyography signal from vastus lateralis during $iMVC$ (0.5-s around $iMVC_{Force}$) and peak-to-peak M-wave amplitude ($M-wave_{amp}$); Qtw_{pot} , force evoked by a potentiated single stimulus at relaxed knee-extensors; $Db10_{Force}/Db100_{Force}$, ratio between the force evoked by a supramaximal high-frequency (100Hz) and low-frequency (10 Hz) doublet stimulus at relaxed knee-extensors; CMJ_{Height} , CMJ_{Force} , and CMJ_{Power} , average of the height, force and power recorded in 5 CMJs, respectively. All raw data is presented in supplementary file. N=10.

Physiological and psychological responses at rest



Physiological and psychological responses at brief running



Participants' feelings and beliefs about the recovery methods used

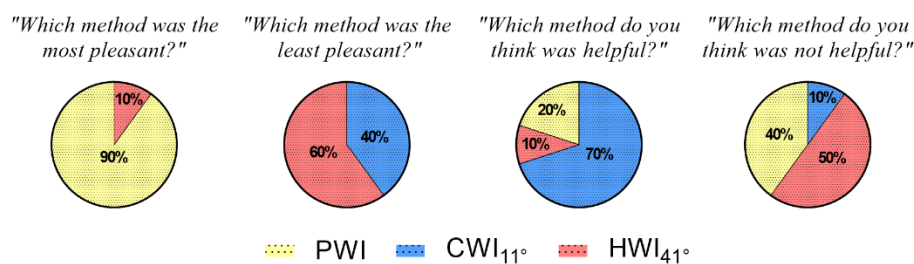


Figure 6.5. Physiological and psychological responses at rest and during brief running efforts (3-min, 80% of vRCP) performed after 20×1-min and 20-s running bouts (HIIT_{150%RCP}) followed by different recovery strategies. CWI_{11°}, cold-water immersion at 10°C for 15-min; HWI_{41°}, hot-water immersion at 42° for 45-min; PWI, immersion on thermoneutral colored water for 10-min (placebo condition); CON, passive rest (control condition). Bold type indicates statistical main

effects in repeated measures analysis of variance (ANOVA). R, rest assessments; #, different from rest (main effect of time); ##, different from 0-h (main effect of time); Σ , CWI_{11°} different from HWI_{41°}, PWI, and CON (time×recovery intervention effect); Φ , HWI_{11°} different from CWI_{11°}, PWI, and CON (main effect of recovery intervention). $\dot{V}O_2$, $\dot{V}_E$, and RER, rate of oxygen consumption, ventilation, and respiratory exchange ratio, respectively, in a brief running effort; Pain”, “Fatigue” and RPE, pain perception, global fatigue perception, and rate of perceived effort, respectively, in a brief running effort; RMSSd_{log}, based-ten logarithm of root mean square of successive R-R interval differences; POMS_{TMD}, total mood disturbance calculated through the profile of mood state questionnaire; “Recovery”, perception of global recovery. All raw data is presented in supplementary file. N=10.

6.5. Discussion

To the best of our knowledge, this is the first study to investigate the effectiveness of CWI_{11°} and HWI_{41°} in improving post-exercise recovery metrics after two distinct running efforts and using a placebo-controlled design. Due to the differences in effort characteristics between CONT_{100%RCP} and HIIT_{150%RCP} and due to specific mechanisms linked to CWI (Ihsan *et al.*, 2016) and HWI (McGorm *et al.*, 2018), we hypothesized that the effectiveness of CWI_{11°} and HWI_{41°} in improving post-exercise recovery would differ between CONT_{100%RCP} and HIIT_{150%RCP}, as well as between the parameters evaluated (e.g., neuromuscular, cardiovascular). We corroborate our hypothesis by evidencing that despite no effects on voluntary peak force (i.e., $iMVC_{Force}$), contractile function (i.e., $Q_{tw_{pot}}$) or CMJ performance (i.e., height, power), HWI_{41°} was able to increase the activation of *vastus lateralis* during knee-extensors $iMVCs$ (i.e., increase RMS/M-wave_{amp}) only after CONT_{100%RCP} while CWI_{11°} elicited this beneficial effect only after HIIT_{150%RCP}. Furthermore, regardless of the characteristics of the running (i.e., whether CONT_{100%RCP} or HIIT_{150%RCP}), CWI_{11°} was also able to increase resting cardiac parasympathetic activity (i.e., RMSSd_{log}) while HWI_{41°} was also able to decrease cardiorespiratory demand (i.e., $\dot{V}O_2$) in successive brief running efforts. Only after CONT_{100%RCP}, CWI_{11°} was also able to increase the “Recovery” perception at rest and decrease “Fatigue” at successive brief running efforts. In addition, it was also possible to observe that the participants had different feelings/beliefs as users of the CWI_{11°} and HWI_{41°}, with most of them indicating CWI_{11°} as a “helpful method” and HWI_{41°} as “not helpful” and “least pleasant” method. Taken together, these findings suggest that to enhance the potential benefits of CWI_{11°} and HWI_{41°} on mitigate fatigability after running, they may be used in the light of the exercise characteristics (e.g., CONT_{100%RCP} or HIIT_{150%RCP}) and the parameter that are intended to improve (e.g., RMSSd_{log} at rest or $\dot{V}O_2$ at running). The subject’s different feeling and beliefs about CWI_{11°} and HWI_{41°} may also be considered, as the effectiveness surrounding recovery methods can be influenced by these individual aspects (Hurst *et al.*, 2020).

HWI_{41°} and CWI_{11°} effects on CNS muscle activation capacity

Although HWI_{41°} and CWI_{11°} were not able to mitigate the reductions on voluntary peak force, contractile function or CMJ performance, indicators of performance, they increased activation of *vastus lateralis* during knee-extensors iMVCs (i.e., higher RMS/M-wave_{amp} compared to CON and PWI). These findings suggest that HWI_{41°} and CWI_{11°} likely triggered a compensatory response of CNS to underpin the desired voluntary force output (Decorte *et al.*, 2012; Froyd *et al.*, 2016). However, the pathways that led HWI_{41°} to increase CNS muscle activation capacity after CONT_{100%RCP} are probably different from those that led CWI_{11°} to induce this same response after HIIT_{150%RCP}.

After CONT_{100%RCP}, it may be suggested that HWI_{41°} increase CNS muscle activation capacity due to the heat capacity in increase activity of Ia fibers (Newton & Lehmkuhl, 1965; Mense, 1978; Sato, 1983). Previous studies have evidenced that in response to repeated stretch-shortening cycles as occurred in running, the activity of Ia afferent decreases further reducing the excitatory input from Ia afferents onto alpha motoneurons (Nicol *et al.*, 2006). This reduction in reflex sensitivity has been linked to decrements in voluntary peak force and CNS muscle activation impairments (Avela *et al.*, 1999b, 1999a) and seems to play a role in the decreased CNS muscle activation capacity observed after running (Tomazin *et al.*, 2017; Besson *et al.*, 2020; Brownstein *et al.*, 2022). Although we were not able to perform muscle temperature measurements in the current study, it was possible to observe that HWI_{41°} increased quadriceps surface temperature from 29.9±1.0 to 35.5±1.5°C ($p<0.05$), which may represent at least a 2°C increase on muscle temperature (Rodrigues *et al.*, 2020b). Thus, it may be suggested that the increase in muscle temperature induced by HWI_{41°} was able to mitigate the impairment in the reflex facilitation of Ia afferent fibers after CONT_{100%RCP}, favoring the sending of electrical signals from CNS to *vastus lateralis* during iMVCs. Indeed, HWI seems to be effective in mitigate the decline in rate of force development following exercise (Sautillet *et al.*, 2024), which, among other reasons, may be linked to increases on CNS muscle activation capacity (Maffiuletti *et al.*, 2016). Besides, an increase in CNS muscle activation capacity may also be played a role in the previous reported heat-induced improvements on *all out* endurance performance 2-h after exhaustive cycling (Minett & Duffield, 2014; Cheng *et al.*, 2017). Future studies with additional measurements such as electroencephalography (EEG) and transcranial magnetic stimulation (TMS) should be performed to provide a better understating about the possible HWI impacts on CNS muscle activation capacity after exercise.

In contrast to CONT_{100%RCP}, HIIT_{150%RCP} was performed above the MMSS (i.e., severe-domain) which may result in a great heat storage (Lei *et al.*, 2023) and long-lasting increase in muscle/body temperatures (Kenny *et al.*, 2006). Therefore, it may be argued that the inhibitory feedback of group III/IV onto CNS triggered by increases on muscle/body temperature (Morrison *et al.*, 2004) may be involved in the impairments of CNS function after HIIT_{150%RCP}. In this way, CWI_{11°} capacity on reduce muscle/body temperature (Stephens *et al.*, 2018) might be mitigated this inhibitory feedback of group III/IV, favoring the sending of electrical signals from CNS to *vastus lateralis* during iMVCs after HIIT_{150%RCP}. Interestingly, disfacilitation of Ia afferents due to repeated stretch-short cycles may have had less influence on neuromuscular function impairments after HIIT_{150%RCP} compared to CONT_{100%RCP}. Due to the work:rest ratio of 80:160-s in HIIT_{150%RCP}, it is possible that there was satisfactory time for the Ia afferent fibers to recover between running bouts since brief disruptions in this reflex seems to be almost fully recovered after 90-s (Budini *et al.*, 2018, 2020). Thus, we speculate that a “less intense” disfacilitation of Ia occurred after HIIT_{150%RCP} compared to CONT_{100%RCP} (Besson *et al.*, 2020).

Furthermore, it is well known that muscular damage and inflammation can exist after repeated stretch-shortening cycles and eccentric muscle contractions in efforts like high-intensity running (Petersen *et al.*, 2007; Schwellnus *et al.*, 2011). Beyond the impairments on contractile function (Behrens *et al.*, 2012), these events are associated to the release of substances like cytokines, which are also involved in the increased activity of group III/IV afferences and further impairment of CNS function (Davis & Bailey, 1997; Dousset *et al.*, 2007). In the current study, the “Pain” perception at lower limbs during brief running efforts remained increased for at least 24-h after HIIT_{150%RCP}, suggesting the presence of at least minor tissue damage and inflammatory process after exercise (Peake *et al.*, 2017). In this way, it may also be suggested that CWI_{11°} attenuated inhibitory feedbacks onto CNS triggered by process linked to muscle damage and inflammation (Ihsan *et al.*, 2016), consequently mitigating the negative impact of these events on CNS function and further enhancing CNS muscle activation capacity after HIIT_{150%RCP}. Such effects of CWI_{11°} on CNS function evidenced in the current study may be used to provide a better understanding about the CWI benefits on improve subsequent endurance performance (<4-h interval between exercises) in temperate (De Paula *et al.*, 2018) and hot environments (Vaile *et al.*, 2008, 2011). Nevertheless, future studies using EEG and/or

TMS should be performed to provide a better understating about the possible CWI impacts on CNS function after exercise.

HWI_{41°} effects on cardiorespiratory demand at brief running efforts after CONT_{100%RCP} and HIIT_{150%RCP}

HWI is linked to increase in local blood flow (Heinonen *et al.*, 2011). This effect of HWI seems to be mediated, among other reasons, by the expression of heat shock proteins (HSP), such as Hsp90, which acts as an agonist for nitric oxide production by endothelial nitric oxide synthase (Pritchard *et al.*, 2001). As evidenced by Hoekstra *et al.*, (2018), HWI (39°C) for 1-h was able to increase the bioavailability of nitric oxide in plasma for at least 2-h after the intervention. Given the well-established positive relationship between nitric oxide bioavailability and vasodilation (Tousoulis *et al.*, 2012), it may be suggested that HWI_{41°} in the current study was able to increase nitric oxide bioavailability and hence local blood flow after CONT_{100%RCP} and HIIT_{150%RCP}, facilitating oxygen transport to the lower limb muscles and consequently reducing cardiorespiratory demand (i.e., lowering $\dot{V}O_2$) during a steady-state brief exercise (i.e., 3-min of running at 80% of vRCP). Similar findings were observed by Koga *et al.*, (1997) while participants performed a heavy-domain cycling exercise with simultaneous lower limb passive heat therapy. The current study expands this finding by evidencing that this reduction of cardiorespiratory demand during submaximal exercise may be achieved even with heat interventions performed hours prior to the effort.

In addition, it is possible that the increases in muscle temperature due to the HWI_{41°} in the current study also facilitated the unloading of oxygen from hemoglobin in the muscle capillaries (Gomez-Cambronero, 2001), as well as increased muscle efficiency by enhancing calcium kinetics (Rodrigues *et al.*, 2022), respectively improving oxygen delivery and reducing oxygen cost of muscles during submaximal exercise (Poole & Jones, 2012). Such changes may also help to explain the lower $\dot{V}O_2$ at brief running efforts after HWI_{41°} use in the current study. Nevertheless, future investigations addressing outcomes such as muscle oxygen consumption is needed to provide a better understanding of the mechanisms of HWI as a post-exercise recovery method capable of improving cardiorespiratory function.

CWI_{11°} effects on RMSSd_{Log} at rest after CONT_{100%RCP} and HIIT_{150%RCP}

CWI results in peripheral vasoconstriction which is association with the hydrostatic pressure effects, increases central blood volume and cardiac pre-load, consequently

enhancing arterial pressure, stroke volume and cardiac output (Mourot *et al.*, 2008). These changes further activate cardiopulmonary baroreflexes linked to the inhibition of cardiac sympathetic activity and increasing of parasympathetic activation (Jdidi *et al.*, 2024). Such events were probably the responsible for the increase of RMSSD_{Log} by CWI_{11°} after CONT_{100%RCP} and HIIT_{150%RCP} in the current study. Interestingly, the CWI_{11°} effects were observed just 2-hour after the exercise (approximately 90-min after the end of treatment). It has been argued that CWI impact on parasympathetic activation last for a just a few minutes after exposure (Jdidi *et al.*, 2024). In this way, although CWI may be potentially capable of improve parasympathetic activation after exercise, such benefits are more prone to be observed just in a few hours after use it. Perhaps, this short-term influence of CWI on hemodynamic and parasympathetic activity may be some of the mechanisms involved in the enhancement of subsequent endurance performance a few hours after use it as described by a previous meta-analysis (Choo *et al.*, 2022).

CWI_{11°} effects on perceived fatigability responses after CONT_{100%RCP}

It was evidenced that CWI_{11°} increased perceived “Recovery” and “Fatigue” during brief-running efforts performed after CONT_{100%RCP}. CWI has been linked to increases in the plasma concentration of dopamine (Srámek *et al.*, 2000) and β -endorphins (Suzuki *et al.*, 2007), which are further related to the CWI-improvements on perception of “well-being” (Tipton *et al.*, 2017). It may be suggested that such events likely drive participants to feel “more recovered” and “less fatigued” after CWI_{11°} in the current study. Although it is hard to explain why CWI_{11°} elicited the cited benefits only after CONT_{100%RCP}, we speculated that this kind of exercise may be more “boring” and “less enjoyable” than HIIT_{150%RCP} (Thum *et al.*, 2017), which may have created a scenario more prone to be benefit by CWI_{11°} in the former compared to the latter. Nevertheless, it is important to highlight that the beliefs toward CWI benefits as post-exercise recovery methods (75% thought CWI_{11°} as a “helpful” method) may also be played a role in this scenario (Allan *et al.*, 2022).

Further considerations

Although both HWI_{41°} and CWI_{11°} increased the amount of electrical signal sent from the CNS to the *vastus lateralis* during iMVCs (i.e., higher RMS/M-wave_{amp}), VA (obtained through twitch interpolation technique) remained decreased for at least 4-h to 24-h after CONT_{100%RCP} and HIIT_{150%RCP}, suggesting that impairments on peripheral factors (i.e., below neuromuscular junction) such as changes on excitation-contraction coupling or tissue contractility aspects may have limiting the voluntary force production of the knee-

extensors even with increased muscle activation (Dotan *et al.*, 2021). However, as mentioned above, this increase on muscle activation capacity triggered by HWI_{41°} and CWI_{11°} in a task-dependent characteristic may be involved in the previous reported improvements of CWI (Vaile *et al.*, 2011; De Paula *et al.*, 2018) and heating (Cheng *et al.*, 2017) on subsequent endurance performance (<4-h interval between tasks). Besides, although CWI_{11°} was able to increase cardiac parasympathetic activity at rest (i.e., RMSSD_{Log}) after CONT_{100%RCP} and HIIT_{150%RCP}, it is important to highlight that HRV metrics are strictly related to the recovery of cardiovascular system (Stanley *et al.*, 2013) and does not necessarily indicated a “complete” restorative process because other physiological resources may still be impaired (e.g., metabolic, neuromuscular, mental) (Skorski *et al.*, 2019). Similarly, although HWI_{41°} was able to decrease cardiorespiratory demand (i.e., $\dot{V}O_2$) during brief submaximal efforts carried out after CONT_{100%RCP} and HIIT_{150%RCP}, its impact on performance during longer and/or higher intensity efforts has yet to be established, especially because of the possible simultaneous and antagonistic effects of increased muscle/body temperatures on cardiorespiratory system and CNS function (i.e., possibly improving the former (Hoekstra *et al.*, 2018) but limiting the latter (Morrison *et al.*, 2004)). In addition, the benefits of CWI_{11°} on perceived “Recovery” and “Fatigue” (herein evidenced only after CONT_{100%RCP}) may be interpreted in the light of other markers of recovery (e.g., force production, HRV metrics). Although a better post-exercise recovery also encompasses sensations such as “less tiredness” and better “well-being”, with a mismatch between these sensations and the recovery of the physiological systems (i.e., metabolic, cardiovascular, neuromuscular), subjects may increase the risk of injury and suboptimal performance in a subsequent task, and, chronically, be susceptible to issues such as non-functional overreaching and/or overtraining (Kellmann *et al.*, 2018).

Finally, we are aware of some limitations of the present study. Due to the lack of proper methodological support, we cannot carry out measurements of muscle temperature, blood flow or oxygen consumption. Such knowledge will provide a more accurate understanding about the mechanisms of HWI_{41°} and CWI_{11°} as post-exercise recovery methods. Besides, further measurements of CNS function as EEG and TMS were also not able to be performed. In addition, there was a delay of 66 ± 5 seconds between the start of the neuromuscular assessments and the end of the exercise session. Although approach exists to assess neuromuscular function immediately post-exercise in cycling (Krüger *et al.*, 2019), such methodological apparatus is difficult to implement for treadmill running

and seems to be an inherent methodological flaw when investigating locomotor exercises closer to real world scenarios (Carroll *et al.*, 2017). In any case, recovery of neuromuscular outcomes is up to 2% (not statistically significant) in the first minute after cessation of locomotor exercises (Krüger *et al.*, 2019). In this way, this delay is likely to have had a negligible influence on the results and conclusions of the current study. Furthermore, as comparisons between the sexes were not within the scope of the study and due to the sex-induced differences in fatigability (Ansdell *et al.*, 2020; Azevedo *et al.*, 2022), we deemed it appropriate to recruit only males in order to reduce the heterogeneity of the data and increase the statistical power of the analysis. Therefore, future studies should assess the interactions of sex on CWI and HWI efficacy in mitigate fatigability responses after running.

Nevertheless, the current study is the first to carry out a comprehensive assessment about HWI_{41° and CWI_{11° efficacy on improve fatigability responses after two types of commonly used running and using a placebo control. Therefore, despite these limitations, we felt that the current study provides new and valuable insights for the exercise professionals and scientific community, suggesting which scenarios and which fatigability responses may be most favorably influenced by CWI and HWI, as well as further research topics that will help in continuous development of this area of knowledge (e.g., CWI and HWI effects on CNS function after exercise).

6.6. Conclusion

In summary, this study showed that both HWI_{41° and CWI_{11° enhance *vastus lateralis* activation during iMVCs (i.e., enhance $\text{RMS}/\text{M-wave}_{\text{amp}}$), but CWI_{11° only had this effect after $\text{HIIT}_{150\%\text{RCP}}$, while HWI_{41° only did so after $\text{CONT}_{100\%\text{RCP}}$. Furthermore, CWI_{11° was able to improve resting cardiac parasympathetic activity (i.e., enhance $\text{RMSSD}_{\text{Log}}$) and HWI_{41° was able to attenuate cardiorespiratory demand (i.e., reduce $\dot{\text{V}}\text{O}_2$) in brief successive efforts followed both $\text{CONT}_{100\%\text{RCP}}$ and $\text{HIIT}_{150\%\text{RCP}}$. Collectively, these findings suggest that in order to increase the potential beneficial effects of HWI_{41° and CWI_{11° as recovery methods after running, they should be used according to the characteristic of the exercise and the parameter that it is intended to improve.

6.7. References

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6.8. Supplementary files

The supplementary files of the Study 4 (*Hot and cold-water immersion efficacy in mitigate fatigability following running*) are available in the following link:

<https://docs.google.com/document/d/11DZ8v2tlp9hxdxNb7wvFbcnxhxeu0Zcp/edit?usp=sharing&ouid=115558097214527656085&rtpof=true&sd=true>

CHAPTER 7.

THESIS SUMMARY

7.1. Conclusion

The overall aim of this thesis was to investigate the influence of aspects of exercise, CWI and HWI on the fatigue-recovery continuum after running. To achieve this aim, four studies were carried out. Study 1 aimed to assess the intra- and inter-day reliability and repeatability of a key evaluation protocol used to measure the neuromuscular function (i.e., iMVC+PNS). The main findings of this study were although reliability and repeatability of knee extensors vary according to the type of neuromuscular function outcome (e.g., from force or EMG responses) and the trial intervals (i.e., hours or days), most force and EMG outcomes obtained through these neuromuscular assessment protocol present ICC>0.75, very good CV (<10%), and repeatability <25% in within- and between-day.

Study 2 aimed to characterize central and peripheral fatigue and perceived fatigability responses after (i) continuous running for 40 minutes at the velocity associated to respiratory compensation point (vRCP, CONT_{100%RCP}), (ii) interval running at intensity 30% above the vRCP (HIIT_{130%RCP}) and (iii) interval running at intensity 50% above the vRCP (HIIT_{150%RCP}). Despite similar reductions on peak voluntary force of knee-extensors (i.e., iMVC_{Force}), CONT_{100%RCP} elicited greater central fatigue (i.e., greater reduction in RMS/M-wave_{amp}) compared to both HIIT_{130%RCP} and HIIT_{150%RCP}. Conversely, impairments in contractile function of this muscle group (i.e., greater reduction in Qtw_{pot}) were greater after HIIT_{150%RCP} compared to CONT_{100%RCP}. In terms of perceived fatigability responses, legs pain was greater after HIIT_{130%RCP} and HIIT_{150%RCP} compared to CONT_{100%RCP}, and “fatigue perception” and mood disturbance were greater after HIIT_{150%RCP} compared to CONT_{100%RCP}. To the best of our knowledge, this is the first study to provide a comprehensive evaluation of knee-extensors neuromuscular function and perceived fatigability responses following distinct running exercises at well-defined levels of metabolic stress.

Study 3 aimed to characterize maximal isometric force-generating capacity of knee-extensors, countermovement jump performance, cardiac parasympathetic activity, mood, cognitive function, and the physiological/psychological responses during subsequent brief efforts after CONT_{100%RCP}, HIIT_{130%RCP}, and HIIT_{150%RCP}. The main findings are that, running 8-km causes: (i) impairments in neuromuscular function (i.e., decreased iMVC_{Force} and RMS_{VL}); (ii) reductions in resting cardiac parasympathetic activity (i.e., decreased RMSSD_{Log}); (iii) changes in mood state (i.e., decrease “Tension” and increase “Fatigue”) and in cognitive aspects (i.e., performance in Stroop test); and (iv) greater

cardiorespiratory demand (i.e., higher $\dot{V}O_2$ and HR) and psychological responses (i.e., higher RPE, legs pain and fatigue perceptions) during successive brief efforts (3-min run at 80% of vRCP). These findings were observed regardless of the characteristic of the effort and over 6 hours following exercise. However, HIIT_{150%RCP} caused greater reduction in eccentric peak force of countermovement jump and greater perceived fatigability during successive brief efforts compared to CONT_{100%RCP}, while HIIT_{130%RCP} caused greater reduction in resting cardiac parasympathetic activity compared to CONT_{100%RCP}. To the best of our knowledge, this is the first study to provide a comprehensive evaluation of physiological and psychological disturbances following distinct running exercises at well-defined levels of metabolic stress.

Finally, Study 4 aimed to investigate the effectiveness of CWI and HWI in attenuating the responses identified in Studies 2 and 3. In comparison to passive rest and placebo, both CWI and HWI were able to mitigate central fatigue (i.e., increase RMS/M-wave_{amp}), despite no effects on voluntary peak force (i.e., $iMVC_{Force}$), contractile function (i.e., $Q_{tw_{pot}}$) or countermovement jump performance (i.e., height, power). However, CWI elicited this beneficial effect only after HIIT_{150%RCP}, while HWI did so only after CONT_{100%RCP}. CWI was also able to increase resting cardiac parasympathetic activity (i.e., increase RMSSD_{Log}) and HWI was able to decrease cardiorespiratory demand (i.e., decrease $\dot{V}O_2$) in successive brief running efforts after CONT_{100%RCP} and HIIT_{150%RCP}. In addition, it was also possible to observe that the participants had different feelings/beliefs as users of the CWI and HWI, with most of them indicating CWI as a “helpful method” and HWI as “not helpful” and “least pleasant” method. To the best of our knowledge, this is the first study to provide a comprehensive evaluation of CWI and HWI efficacy as post-exercise recovery methods taking into account the task dependency nature of fatigue and using a placebo condition to control for HWI effects.

7.2. Practical applications

- When using $iMVC+PNS$ to assess neuromuscular function, exercise scientists and professionals should be aware of the measurement error and expected trial-to-trial noise of this data (i.e., coefficient of variation <10% and repeatability <25%). This knowledge may be very useful for drawing correct conclusions about changes in neuromuscular function in different scenarios, such as after efforts intended to evoke postactivation potentiation and exercises leading to performance fatigability or muscle damage.

- After running 8-km, maximal force-generating capacity and voluntary activation of knee extensors will be reduced for at least 6 hours. Moreover, it can be expected that performing this volume of exercise as CONT_{100%RCP} will cause greater impairments in CNS muscle activation capacity compared to HIIT_{150%RCP}, while performing it as HIIT_{150%RCP} will cause greater impairments in contractile function and perceived fatigability compared to CONT_{100%RCP}. This knowledge may be very useful for (i) providing insights on the potential causes of performance decline in subsequent tasks; (ii) planning proper rest intervals during rehabilitation, training, and competition routines; and (iii) suggesting “appropriate targets” for recovery interventions following distinct running efforts.
- To properly monitor the fatigue-continuum recovery after running efforts, markers other than the commonly used neuromuscular function indicators should be used. These includes the assessment on resting cardiac parasympathetic activity, mood state, cognitive function, and physiological/psychological responses during brief-efforts. This knowledge can be very helpful to better understand the “causes” of performance decline in subsequent tasks, as well as to identify which body systems/capacities should be recovered followed distinct running efforts.
- To increase the potential beneficial effects of CWI and HWI as recovery methods after running, they should be used according to the characteristic of the exercise and the parameter desired to be improved. Hot-water immersion (41°C for 45-min) should be used to mitigate central fatigue after running efforts like CONT_{100%RCP}, while cold-water immersion (11°C for 15-min) should be used to mitigate central fatigue after running efforts like HIIT_{150%RCP}. Regardless of running characteristics, CWI should be used to improve resting cardiac parasympathetic activity and HWI should be used to decrease cardiorespiratory demand in successive brief running efforts.

7.3. Limitations and considerations

In conjunction with the practical applications mentioned above, it is also important to acknowledge any potential shortcomings and limitations of this research; these limitations are discussed below.

- The reliability and repeatability of the iMVC+PNS protocol described in the Study 1 were identified using untrained volunteers. Due to regular engagement in

physical exercises, it is possible that trained volunteers might have a more stable recruitment of knee extensor muscles during both within- and between-day trials (CALLEWAERT et al., 2013). This could lead to better reliability and repeatability statistics of force and EMG outcomes than those reported in the Study 1. However, most investigations involving multiple trials of this kind of neuromuscular assessment are commonly performed on healthy, untrained volunteers. Thus, to increase the applicability of the findings, we chose to conduct the study on healthy, untrained volunteers.

- Although we provided a characterization of central and peripheral fatigue, as well as perceived fatigability responses after different running efforts, it was not within the scope of this thesis to provide a more mechanistic investigation of these events (e.g., exercise-induced muscle damage, tissue metabolite accumulation, glycogen depletion, change in brain activity, among others).
- Although we provided a comprehensive characterization of physiological and psychological disturbances induced by different running efforts, we were not able to measure other relevant aspects in this scenario such as changes in core temperature, hydration status, cortical electrical/metabolic activity, reaction time, decision-making among others.
- Due to the lack of proper methodological support, we cannot carry out measurements of muscle temperature, blood flow or oxygen consumption after CWI and HWI. Such knowledge will provide a more accurate understanding about the mechanisms/efficacy of CWI and HWI as post-exercise recovery methods.
- There was a delay of 50-60 seconds between the start of the neuromuscular assessments and the end of the exercise in Studies 2 and 4. Although there is a setup to assessing neuromuscular function immediately after exercise in cycling (KRÜGER et al., 2019), this methodological apparatus is difficult to implement for treadmill running and appears to be an inherent methodological flaw when investigating locomotor exercises closer to real-world scenarios (CARROLL; TAYLOR; GANDEVIA, 2017). Even so, the recovery of neuromuscular outcomes is suggested to be to 2% (not statistically significant) in the first minute after locomotor exercises (KRÜGER et al., 2019). Therefore, it is likely that this

delay had negligible influence on the findings and conclusions drawing from these studies.

- As comparisons between sexes were not within the scope of this thesis and due to the sex-induced differences in fatigability and recovery (HUNTER et al., 2023), we deemed appropriate to recruit only males in order to reduce data heterogeneity and increase the statistical power of the analysis in Studies 2, 3, and 4. Therefore, caution is advised when transferring the knowledge reported here to females.

7.4. Future directions

This thesis has demonstrated how exercise aspects (e.g., intensity and/or type of execution), CWI and HWI impact fatigability responses, as well as physiological and psychological disturbances following running. However, the findings from the present series of studies have identified a number of considerations for future research.

- To further improve knowledge in this area, future studies could investigate the impact of the intensity/type of running, carrying out a complementary assessment of neuromuscular function (e.g., using transcranial magnetic stimulation, assessing the H-reflex, concentric peak force and rate of force development); and performing more mechanistic assessments of fatigue, such as analysis of (i) exercise-induced muscle damage; (ii) the magnitude of glycogen depletion and accumulation of metabolites in the tissue; (iii) changes in core temperature; and (iv) changes in the electrical/metabolic activity of the cortex.
- Future studies could investigate the correlations between aspects of fatigue (e.g. central and peripheral fatigue; physiological and psychological disturbances) and aspects of performance/stress (e.g. pace, total time, internal training load, mean heart rate) during subsequent workouts close to “real world” scenarios, such as time trials (e.g. 400-m, 1500-m, 5 km) and/or resistance training (e.g., repetition until failure, bar velocity). This knowledge can be very useful to better understand which “recovery metrics” are related to possible performance impairment in efforts carried out after running.
- To further improve knowledge about the effectiveness of CWI and HWI in mitigating fatigue after running, future studies should investigate the effects of these methods by also performing mechanistic assessments, such as changes in

muscle blood flow, muscle metabolism (e.g. oxygen consumption) and muscle/core temperature. Investigating correlations between the level of change in these physiological aspects and the level of change in recovery metrics (e.g. force/power production, muscle soreness) may provide a better understanding of the effectiveness of CWI and HWI in improving post-exercise recovery.

- Future studies should investigate in females how exercise aspects (e.g., intensity and/or type of execution), CWI and HWI impact fatigability responses, as well as physiological and psychological disturbances following running.

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APPENDIX

APPENDIX 1. RESEARCH ETHICS COMMITTEE APPROVAL

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



PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Time course da recuperação neuromuscular após sessão de treino de corrida: influência da intensidade, da imersão em água quente e da imersão em água fria.

Pesquisador: Alessandro Moura Zagatto

Área Temática:

Versão: 2

CAAE: 50075421.9.0000.5398

Instituição Proponente: UNIVERSIDADE ESTADUAL PAULISTA JULIO DE MESQUITA FILHO

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.950.446

Apresentação do Projeto:

Como informado pelo pesquisador responsável no Projeto de Pesquisa e Documento de Informações Básicas, gerado por meio dos dados fornecidos no ato do preenchimento da Plataforma Brasil, "o presente projeto tem como objetivos: (i) caracterizar o time course e (ii) investigar a influência da imersão em água quente e da imersão em água

fria sobre o desempenho neuromuscular após diferentes sessões de treino de corrida. Para alcançar os objetivos propostos, dois estudos independentes serão realizados (estudos 1 e 2). Doze homens saudáveis, praticantes regulares de corrida, com idade entre 18 e 35 anos serão convidados a participar do estudo 1. Esse estudo será um estudo descritivo, consistirá em 4 visitas ao laboratório e será conduzido de forma cruzada e randomizada. Na visita 1, um teste incremental máximo será realizado em esteira para determinação das intensidades associadas ao máximo consumo de oxigênio e ao ponto de compensação respiratório. Nas visitas 2,3 e 4, os voluntários realizarão, em cada dia, uma sessão aguda de treinamento: treinamento contínuo na intensidade do ponto de compensação respiratório (iPCR, 30 minutos), treinamento intervalado de alta intensidade e longa duração (HIITLongo, 2 minutos, 1:1) e treinamento intervalado de alta intensidade e curta duração (HIITcurto, 1 minuto, 1:1). Nas visitas 2,3 e 4, contrações isométricas voluntárias máximas de extensores do joelho associada a técnica de twitch interpolation e saltos verticais contramovimento serão realizados antes, imediatamente após e transcorrido 1, 2, 4 e 6

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Continuação do Parecer: 4.950.446

horas do fim das sessões de treino. Serão convidados a participar do estudo 2, trinta e seis homens saudáveis, praticantes regulares de corrida, com idade entre 18 e 35 anos. Esse estudo será um ensaio clínico randomizado e placebo-controlado conduzido com grupos independentes e consistirá em 4 visitas ao laboratório. Na visita 1, um teste incremental máximo será realizado em esteira para determinação das intensidades associadas ao máximo consumo de oxigênio e ao ponto de compensação respiratório. Em seguida, os voluntários serão divididos em três grupos: grupo que realizará as sessões de treinamento contínuo na intensidade do ponto de compensação respiratório (iPCR, 30 minutos), grupo que realizará as sessões de treinamento intervalado de alta intensidade e longa duração (HIITlongo, 2 minutos, 1:1) e grupo que realizará o treinamento intervalado de alta intensidade e curta duração (HIITcurto, 1 minuto, 1:1). Cada grupo repetirá a mesma sessão de treino em três dias distintos (visitas 2,3 e 4). Após cada sessão, os voluntários de cada grupo serão submetidos a tratamento placebo, tratamento de imersão em água fria ou tratamento de imersão em água quente. Contrações isométricas voluntárias máximas de extensores do joelho associada a técnica de twitch interpolation e saltos verticais contramovimento serão realizados antes, imediatamente após, 1, 2, 4 e 6 horas após o fim das sessões de treino".

Objetivo da Pesquisa:

Como informado pelo pesquisador responsável no Projeto de Pesquisa e Documento de Informações Básicas, gerado por meio dos dados fornecidos no ato do preenchimento da Plataforma Brasil, "Objetivo Primário: Avaliar a influência da intensidade, da imersão em água quente e da imersão em água fria sobre o desempenho neuromuscular após sessões de treinamento de corrida. Objetivo Secundário: O presente projeto terá dois objetivos secundários: I) Caracterizar o desempenho neuromuscular após três sessões distintas de treinamento de corrida: treinamento contínuo de moderada intensidade, treinamento intervalado de alta intensidade e longa duração (HIITlongo) e treinamento intervalado de alta intensidade e curta duração (HIITcurto). II) Avaliar a influência da imersão em água quente e da imersão em água fria sobre o desempenho neuromuscular após três sessões distintas de treinamento de corrida: treinamento contínuo de moderada intensidade, HIITlongo e HIITcurto".

Avaliação dos Riscos e Benefícios:

Como informado pelo pesquisador responsável no Projeto de Pesquisa e Documento de Informações Básicas, gerado por meio dos dados fornecidos no ato do preenchimento da Plataforma Brasil, "os riscos atribuídos à participação no presente projeto se relacionam aos envolvidos na prática de exercícios físicos extenuantes e aos

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procedimentos de avaliação da função neuromuscular. Apesar de raro, há possibilidade de alterações fisiológicas durante a realização de qualquer tipo de esforços máximos que podem ser respostas atípicas de pressão arterial, arritmias, desmaios e tonturas. Portanto, profissionais qualificados e experientes com esse tipo de situação estarão à disposição para tais eventualidades e caso alguma dessas alterações ocorra, o laboratório ficará responsável por lhe promover total auxílio. Em relação aos procedimentos de avaliação da função neuromuscular, estímulos elétricos serão entregues na região da sua virilha. Apesar de ter uma duração bastante curta (0,001 s) e de apenas provocar a contração dos músculos da coxa, esse procedimento gera ligeiro desconforto e ansiedade. O estímulo elétrico não acarreta influências sobre órgãos como coração, pulmão e rins, e não acarreta riscos à sua vida. É importante destacar que a participação nesta pesquisa não infringe normas legais e éticas. Todos os procedimentos adotados nesta pesquisa obedecem aos Critérios da Ética em Pesquisa com Seres Humanos conforme Resolução no. 466/2012 do Conselho Nacional de Saúde".

Quanto aos benefícios da participação dos sujeitos na pesquisa, no TCLE informa o pesquisador responsável: "Ao participar desta pesquisa o Sr. não terá nenhum benefício direto como pagamentos ou renda. O Sr. será convidado a realizar um teste para a avaliação da capacidade física, obtendo resultados tais quais, Frequência cardíaca máxima, VO2máx, iVO2máx e Limiares ventilatórios. Esses resultados proporcionam uma visão do estado geral da condição física do Sr. e podem ser usados para a periodização e prescrição de treinamentos físicos. Além de disso, o Sr. será notificado dos aspectos mais importantes do estudo após sua conclusão".

Comentários e Considerações sobre a Pesquisa:

A pesquisa se mostra relevante social e cientificamente, além de apresentada por meio do projeto de forma coerente em relação aos aspectos éticos, ou seja, em conformidade com as resoluções vigentes, a saber: 466/12 e 510/16, do CNS.

Considerações sobre os Termos de apresentação obrigatória:

Após ter se submetido a apreciação deste CEP e recebido orientações para a realização de correções e melhor acatamento das exigências relativas às Resolução 466/12 e 510/16, do Conselho Nacional de Saúde, os termos ora apresentados encontram-se adequados.

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Recomendações:

Nenhuma.

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 - Comitê de Ética em Pesquisa.

Lembramos que é dever do pesquisador responsável, ao término da pesquisa e conforme o cronograma informado à Plataforma Brasil, apresentar o relatório final da mesma.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1796372.pdf	16/08/2021 16:03:04		Aceito
Projeto Detalhado / Brochura Investigador	Projeto_YMD_AMZ_atualizado.pdf	16/08/2021 16:00:01	Yago Medeiros Dutra	Aceito
Outros	Carta_resposta.pdf	16/08/2021 15:59:38	Yago Medeiros Dutra	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_Yago.pdf	23/07/2021 16:40:38	Yago Medeiros Dutra	Aceito
Folha de Rosto	folhaDeRosto_assinada_Zagatto_Jair.pdf	23/07/2021 16:34:36	Yago Medeiros Dutra	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

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BAURU, 02 de Setembro de 2021

Assinado por:
Mário Lázaro Camargo
(Coordenador(a))

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APPENDIX 2. PERMISSION TO REUSE PUBLISHED FULL-TEXT STUDY 1

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Within- and between-day reliability and repeatability of neuromuscular function assessment in females and males
Author: Yago Medeiros Dutra, João Pedro Fialho Lopes, Juan M. Murias, et al
Publication: Journal of Applied Physiology
Publisher: The American Physiological Society
Date: Dec 1, 2023

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