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# Sprint interval training : time course of the physiological adaptations and recovery : Implications for physical conditioning in a sedentary population

Jaume Lloria-Varella

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**Jaume Lloria-Varella**

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**Sprint interval training: time course of the  
physiological adaptations and recovery.  
Implications for physical conditioning in a  
sedentary population**

---

Devant le jury composé de :

Klass Malgorzata	Université Libre de Bruxelles	Rapporteuse
Candau Robin	Université de Montpellier	Rapporteur
Hupin David	Université Jean Monnet	Examineur
Rahmani Abdel	Le Mans Université	Examineur
Busso Thierry	Université Jean Monnet	Directeur de thèse
Murias Juan M	Hamad Bin Khalifa University	Co-directeur de thèse

## *Abstract*

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The objectives of this thesis were to study the chronology of physiological adaptations and recovery in response to sprint interval training (SIT) in a sedentary population. Firstly, we investigated the neuromuscular and cardiac autonomic recovery after a single all-out intensity session. SIT led to significant declines in neuromuscular function in the minutes following the exercise bout. However, despite the high intensity of SIT, our results showed that both isometric and dynamic neuromuscular assessments had returned to baseline values 24 hours after the session. The heart rate variability did not exhibit significant differences between the night before and the two nights following the session. This initial study demonstrates that recovery occurs within the first 24 hours after a SIT session. In the second study, we examined the kinetics of physiological adaptations over a six-week SIT program in sedentary individuals. Significant improvements in cardiorespiratory and neuromuscular functions were observed after the first two weeks of training, but no further improvement was observed afterwards. It is important to note that the percentage of maximum heart rate achieved during training sessions was significantly reduced after the first two weeks of training. This could explain the plateau in cardiorespiratory adaptations, as it has been demonstrated that time spent at high percentages of heart rate is essential to trigger these adaptations. Differences were observed between isometric and dynamic neuromuscular assessments, indicating that these two types of measures are not interchangeable. These results confirm the effectiveness of SIT in sedentary individuals for improving maximal oxygen consumption and force production capacity, but these adaptations plateau after two weeks of training.

## *Resumé*

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Les objectifs de cette thèse étaient d'étudier la chronologie des adaptations physiologiques et de la récupération en réponse à le sprint interval training (SIT) chez une population sédentaire. Tout d'abord, nous avons étudié la récupération neuromusculaire et autonome cardiaque après une seule séance d'intensité « all-out ». Le SIT a entraîné des déclinés importants dans la fonction neuromusculaire dans les minutes suivant la séance. Cependant, malgré l'intensité élevée du SIT, nos résultats ont montré que les évaluations neuromusculaires isométriques et dynamiques étaient revenues aux valeurs de départ 24 heures après la séance. La variabilité de la fréquence cardiaque ne montrait pas de différences significatives entre la nuit précédente et les deux nuits suivant la séance. Cette première étude montre donc que la récupération intervient dans les premières 24 heures après une séance de SIT. Dans la deuxième étude, nous avons étudié la cinétique des adaptations physiologiques au cours d'un programme de SIT de six semaines chez des personnes sédentaires. Une amélioration significative des fonctions cardiorespiratoires et neuromusculaires a été observée après les deux premières semaines d'entraînement mais aucune amélioration supplémentaire n'est observée ensuite. Il est important de noter que le pourcentage de la fréquence cardiaque maximale atteint lors des séances d'entraînement a été significativement réduit après deux semaines. Cela pourrait expliquer la stagnation des adaptations cardiorespiratoires, car il a été démontré que le temps passé à des pourcentages élevés de la fréquence cardiaque est essentiel pour déclencher ces adaptations. Des différences étaient observées entre les évaluations neuromusculaires isométriques et dynamiques, montrant que ces deux types de mesures ne sont pas interchangeables. Ces résultats confirment l'efficacité du SIT chez des personnes sédentaires pour améliorer la consommation maximale d'oxygène et la capacité de production de force mais ces adaptations atteignent un plateau à partir de deux semaines d'entraînement.

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Esta tesi va per tots vosaltres, us estime molt!

## *Thesis structure*

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This thesis work comprises six main chapters. **Chapter 1** introduces the topic, terminology, and background, and outlines the aims. **Chapter 2** is constituted by a critical review of the scientific literature on the physiological responses and adaptations to sprint interval training, as well as the effects of reduced-volume sprint interval training programs on the maximal oxygen uptake. In **Chapters 3** and **4**, the two main experimental studies are presented. **Chapter 5** involves some additional results from data collected during the training period of the second study. **Chapter 6** comprises a general contextualized discussion of the body of work which includes critical appraisal and brief reminders of the main results of each publication. Moreover, **Chapter 6** also provides my personal reflections and directions for future research together with the conclusion of the thesis.



## *List of publications and conference proceedings*

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### *Publications and conference proceedings related to this thesis*

**ARTICLE | Lloria-Varella, J.,** Koral, J., Ravel, A., Féasson L., Murias JM, Busso, T. (2023). Neuromuscular and autonomic function is fully recovered within 24 h following a sprint interval training session. *European Journal of Applied Physiology*. DOI: <https://doi.org/10.1007/s00421-023-05249-6>

**ARTICLE | Lloria-Varella, J.,** Koral, J., Ravel, A., Murias JM, Féasson L., Busso, T. (2023). Cardiorespiratory and neuromuscular improvements plateau after two weeks of sprint interval training in sedentary individuals. Submitted in the *Medicine & Science in Sports & Exercise*.

**ORAL COMMUNICATION | Lloria-Varella J.,** Ravel A., Koral J., Busso T. Neuromuscular and autonomic recovery after a single sprint interval session (2022). *27<sup>th</sup> congress of the European College of Sport Science, Sevilla (Spain)*.

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### *Side works in parallel to this thesis*

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**ARTICLE | Koral J., Lloria-Varella J.,** Lazaro Romero F., and Foschia C. (2021) Effects of Three Preseason Training Programs on Speed, Change-of-Direction, and Endurance in Recreationally Trained Soccer Players. *Front. Physiol.* 12:719580. DOI: <https://doi.org/10.3389/fphys.2021.719580>

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**ARTICLE | Lloria-Varella J.,** Rossi, J. Morin, JB., Foschia C., Rousson M. Busso, T. Koral, J. (2023). Changes in power-force-velocity profile induced by 2 weeks of sprint interval training *The Journal of Sports Medicine and Physical Fitness*. 63:797-804. DOI: <https://doi.org/10.23736/s0022-4707.23.14771-2>

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**ORAL COMMUNICATION | Lloria-Varella J.,** Rossi J., Morin JB., Foschia C., Busso T., Koral J. (2021). Two weeks of running sprint interval training improve sprint mechanics, power and jump performance in young male basketball players. *19<sup>ème</sup> congrès de l'Association des Chercheurs en Activités Physiques et Sportives, Montpellier, France*.

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## *List of abbreviations*

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- AMPK: 5'-AMP-activated protein kinase
- AMP: adenosine monophosphate
- ATP: adenosine triphosphate
- p38 MAPK: ap38 mitogen activated protein kinase transcriptional coactivator
- HCO<sub>3</sub><sup>-</sup>: bicarbonate
- H<sub>2</sub>CO<sub>3</sub>: carbonic acid
- CS: citrate synthase
- CPK: creatine phosphokinase
- CT: continuous training
- COX: cytochrome c oxidase
- PHOS: glycogen phosphorylase
- HR: heart rate
- HIEE: high-intensity intermittent exercise
- HIIT: high-intensity interval training
- LDH: lactate dehydrogenase
- $\dot{V}O_2$ max: maximal oxygen uptake
- MK: myokinase
- O<sub>2</sub>: Oxygen
- $\dot{V}O_2$ : oxygen uptake
- PGC 1- $\alpha$ : peroxisome proliferator-activated receptor 1- $\alpha$
- PCr: phosphocreatine
- PFK: phosphofructokinase
- PO: power output
- PDH: pyruvate dehydrogenase
- RST: repeated sprint training
- SIT: sprint interval training
- VEGF: vascular endothelial growth factor
- WHO: World Health Organization
- W:R: work-to-rest ratio

## ***1. Chapter one – Introduction, research background and general aims***

### ***1.1.Introduction***

Exercise is an essential aspect of healthy living from the first to the last stages of life. It plays a significant role in maintaining and improving overall health since it is considered a key non-pharmacological strategy in the primary prevention and management of chronic diseases such as diabetes mellitus, cancer, obesity, hypertension, coronary heart disease and cardiovascular disease (Assi et al., 2020; Booth et al., 2012; Colberg et al., 2010). From a psychological standpoint exercise can also reduce the feelings of depression and stress, enhance mood and emotional well-being, and improve sleep (National Institute of Health, 2023; Sharma et al., 2006). Although the benefits of exercise are well known and publicized, almost a third of the population does not meet public health guidelines for recommended levels for physical activity, making sedentarism a striking global problem (Hallal et al., 2012). The 2022 WHO Guidelines on physical activity and sedentary behavior for adults aged between 18 and 64 years, recommend at least performing 150 to 300 minutes of moderate-intensity physical activity per week to achieve substantial health benefits (World Health Organization, 2023). Thus, the physiological effects of continuous training (CT) have been widely studied. However, the WHO also recognizes the added benefit of exercising at greater intensities (Ross et al., 2015), and therefore, strategies like performing 75 to 150 minutes of vigorous intensity per week are also considered in the guidelines. This is important because a greater exercise intensity can substantially reduce the total exercising time and still deliver benefits (Phillips et al., 2017; Williams et al., 2019). This is important as lack of time is one of the main reasons that people use to justify the lack of engagement in physical activity and/or exercise programs.

In the last decades, exercise scientists and coaches have been working together in order to propose effective and enjoyable training methods for promoting physical activity and, ultimately, enhance health across the general population. In this line, a growing body of research has focused on the effects of high-intensity intermittent exercise (HIIE) as a strategy to rapidly increase and maintain the overall physical fitness level and to maintain adherence to training. Physical fitness has been previously defined as “one’s ability to execute daily activities with optimal performance, endurance, and strength with the management of disease, fatigue, and stress and reduced sedentary behavior” (Campbell et al., 2013). It involves five main body-



systems: morphological, muscular, motor, cardiorespiratory and metabolic. A greater physical fitness, independently of physical activity levels, has been shown to be a strong predictor of numerous health outcomes such as cardiovascular events (Kodama et al., 2009), cancer mortality (Schmid & Leitzmann, 2015), metabolic syndrome (LaMonte et al., 2005) and even depressive symptoms (Sui et al., 2009). Thus, an increased capacity to deliver oxygen to the muscles or gains in muscular force represents an improvement in physical fitness that will, ultimately, enhance health. HIIE, that alternates short bouts of vigorous exercise with less intense or recovery periods (Atakan et al., 2021), has been related with cardiorespiratory, metabolic and muscular improvements (Brown et al., 2018; Taylor et al., 2021), and thus, it has gained popularity in the last decades.

Several high-quality reviews have been published regarding the impact of HIIE on exercise capacity and overall health in both healthy and clinical populations (Astorino & Schubert, 2018; Cassidy et al., 2017; Gibala et al., 2019). These reviews delve not only into the underlying mechanisms responsible for the positive effects of HIIE, but also recognize it as an alternative and more efficient protocol than CT, which is the approach generally recommended by the American College of Sports Medicine guidelines and the WHO. Furthermore, HIIE programs have been associated with lower dropout rates than traditional exercise programs in previously sedentary individuals, showing that HIIE is tolerable and well-accepted (Reljic et al., 2019). For the current thesis work, the acronym HIIE will be used for referring to all the non-continuous exercise forms that elicit exacerbated cardiorespiratory and metabolic responses that occur in the severe or extreme intensity domain (Buchheit & Laursen, 2013). However, depending on the manipulation of up to nine variables, different acute physiological responses and/or adaptations, could be induced. Therefore, as a first step, it is important to distinguish between the different types of HIIE that can be found in the scientific literature.

## ***1.2. Terminology and types of HIIE***

Figure 1, is an overview of the variables that can be manipulated to prescribe a HIIE session (Buchheit, 2008). Depending on these parameters and its interaction, different terminology and classifications have been used for HIIE in the scientific literature. In the current thesis, for simplicity, the different forms of HIIE will be classified as a function exercise intensity and bout duration. Exercise intensity is known to be a key factor for modulating other variables

such as bout duration, number of repetitions, number of series and the recovery time between both repetitions and series and therefore is considered a more comprehensive approach to classify the different forms of HIIE. Accordingly, the three main forms of HIIE are: i) high-intensity interval training (HIIT); ii) sprint interval training (SIT) and iii) repeated sprint training (RST) (Buchheit & Laursen, 2013).

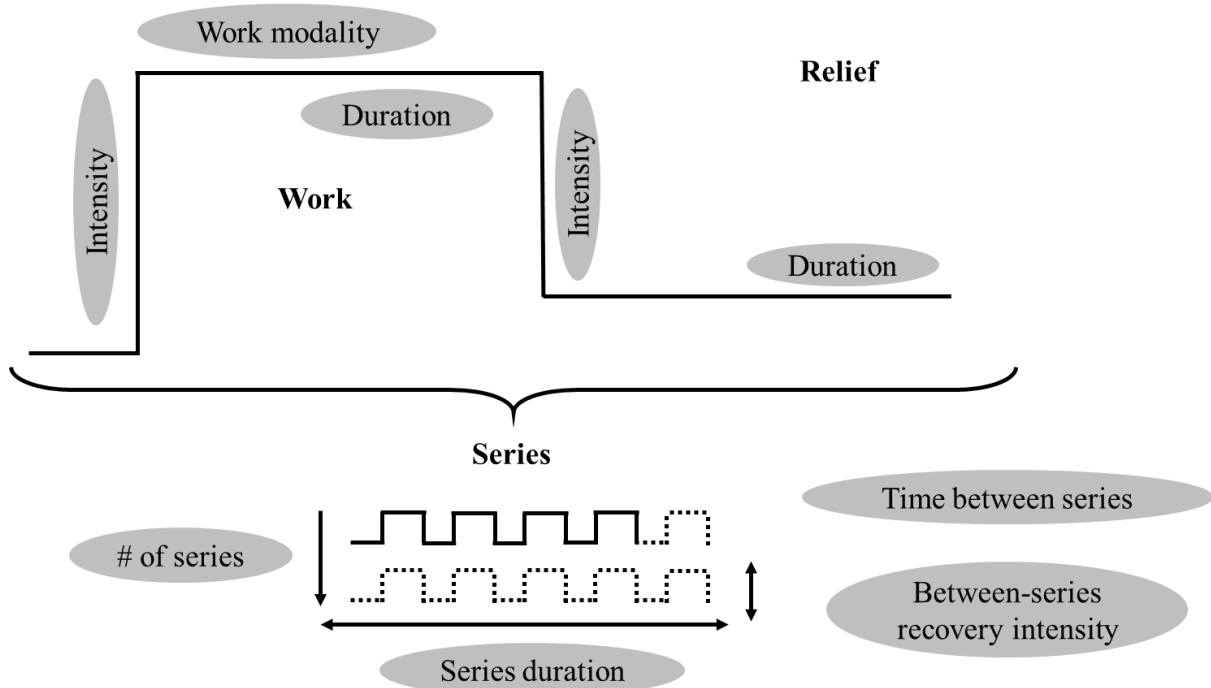


Figure 1. Schematic illustration of the nine variables characterizing a HIIE session. Reproduced and adapted from Buchheit et al. (2008).

Even though they all respond to the definition of HIIE given above, there are some aspects that need to be clarified. First, HIIT uses different work interval protocols including either short (<45 s) or long-interval (2–4 min) of rather high- but not maximal-intensity. The recovery during HIIT usually involves a few minutes of either rest, either pedaling at a substantially lower power or running speed than during the work intervals. SIT is commonly defined as brief and repeated bouts of 10 to 30 s “all-out” exercise. The recovery between sprints is established as a function of the sprint duration, usually lasting between 2 and 4.5 minutes. In this case, the recovery is mostly passive or involves some very light pedaling. Finally, RST involves very short (<10 s) repeated sprint sequences performed at an all-out intensity. The recovery during RST tends to be of a similar ratio to the one adopted during SIT but, as the work intervals last only a few seconds, it ends up being relatively short (i.e. from 30 s to 1 minute approximatively).

HIIT is usually prescribed as a percentage of a maximal anchor such as the maximal heart rate (HR), the peak power output or the maximal oxygen uptake ( $\dot{V}O_{2\max}$ ). Conversely, during SIT and RST, participants are asked to perform a maximal effort during each bout and so the intensity is not established as a function of any maximal anchor. Figure 2 reproduced from (MacInnis & Gibala, 2017) displays an overview of the classical structure of a HIIT and SIT sessions compared to a moderate-intensity CT session. In the figure, exercise intensity is depicted as a percentage of the peak power output obtained during a  $\dot{V}O_{2\max}$  test.

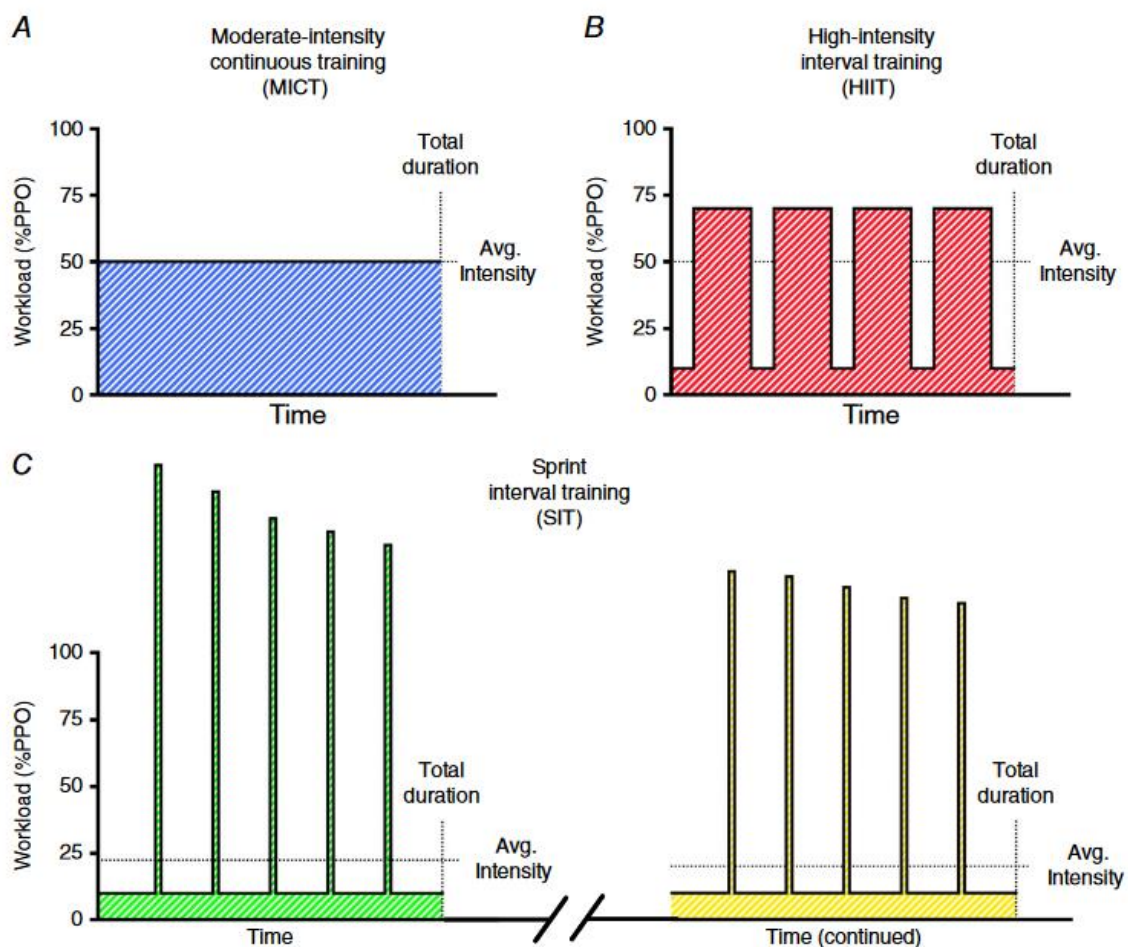


Figure 2. Classical structure of a HIIT (B), SIT (C) session compared to moderate intensity CT (A). Reproduced from MacInnis & Gibala (2017).

Based on the combinations of duration, intensity, and recovery, Buchheit & Laursen (2013) proposed a decision process for selecting a HIIE format based on the expected acute physiological response or strain of the session. Although all forms of HIIE might elicit similar physiological responses and fall within the severe and extreme intensity domain, each type of HIIE targets a different metabolic pathway according to work and recovery modalities which

will generate specific adaptations with time. The six different types of acute responses to HIIE have been categorized as: (1) metabolic, but eliciting essentially large requirements from the O<sub>2</sub> transport and utilization systems, i.e. cardiopulmonary system and oxidative muscle fibers; (2) metabolic as for (1) but with a certain degree of neuromuscular strain; (3) metabolic as for (1) but with a large anaerobic glycolytic energy contribution; (4) metabolic as for (3) plus a certain degree of neuromuscular load; (5) metabolic with essentially an important anaerobic glycolytic energy contribution and a large neuromuscular load; and (6) eliciting a high predominant neuromuscular strain (Buchheit & Laursen, 2013). Therefore, if the specificity of the discipline or the desired goal involves the use of a specific metabolic pathway or system, this approach offers a good guideline to select the training modality that adjusts most closely to the needs.

### ***1.3. Research background***

While all HIIE modalities described above have been shown to be effective for improving cardiorespiratory fitness and exercise capacity (de Oliveira-Nunes et al., 2021), the current thesis work focused on SIT. Since the seminal findings of Burgomaster et al. (2005) that observed an increased skeletal muscle oxidative potential and exercise capacity only with 16 min of all-out exercise spread over 2 weeks, a substantial amount research using SIT protocols has been conducted.

On the one hand, SIT has been identified as a very effective and time-efficient training method as various reviews and meta-analyses have reported significant gains in both aerobic and anaerobic outcomes such as the  $\dot{V}O_2$ max, repeated sprint ability, exercise capacity at a given work rate, or maximal power production during a Wingate test (Gist et al., 2014; Weston et al., 2014). The fact that such parameters can be rapidly enhanced within only a few weeks of training involving a very low time commitment has been proposed as an advantage compared to other exercise modalities such as CT (Burgomaster et al., 2008; Gibala et al., 2006). From this point, and once acknowledged that SIT is an effective approach, research has focused on optimizing SIT protocols to better adapt to the necessities of the general population. Reducing the sprint time or the number of repetitions has been shown to be effective approaches (Hazell et al., 2010; Vollaard & Metcalfe, 2017). However, little is known about the effects of the program length on the adaptations. Studying the time course of the training-induced changes

requires the inclusion of several time points to assess improvements. In this regard, two SIT-specific studies have reported a plateau in the cardiorespiratory adaptations after the first three weeks of training despite the training regimen was maintained for three or six additional weeks (Burgomaster et al., 2008; Yamagishi & Babraj, 2017). This indicates an abnormal dose-response relationship to SIT because more training was not correlated with greater adaptations. Then, two other studies reported a biphasic response to SIT showing a significant increase after a few weeks and a further increase in the cardiorespiratory adaptations with more training (Bostad et al., 2021; Gillen et al., 2016). In these cases, the training programs lasted 12 instead of 6 or 9 weeks, meaning that the actual length of the SIT program could play a significant role on the magnitude of the adaptations. From these results, further investigation seems necessary to understand how long SIT regimens continue to provide a significant stimulus for triggering physiological adaptations and to determine the optimal duration SIT programs for sedentary populations. Additionally, as many SIT models have been tested on the sedentary population, the focus has been set on cardiorespiratory and more health-related markers such as the  $\dot{V}O_2\text{max}$  or the insulin sensitivity. However, SIT has been shown to improve the mean and maximal power and the repeated sprint ability, which could be linked to adaptations occurring in the musculoskeletal system. Indeed, the resistive load used during SIT programs is usually high and the potential role of SIT on developing neuromuscular adaptations has been somewhat overlooked in the literature. Overall, a gain in force or a higher capacity to maintain a given power output for longer, could also represent an important outcome for both trained and untrained populations.

On the other hand, SIT has been sometimes identified as inappropriate for sedentary individuals. Those opposing the idea of using SIT in inactive individuals argue that the strenuous nature of SIT and, in general, of all-out exercise is likely to be a deterrent to participation because individuals tend to avoid exercise if they find it aversive (Hardcastle et al., 2014). Furthermore, it has been pointed out that SIT is a relatively complex and structured exercise regimen that can be difficult to bring out of the laboratory-settings and may not be applicable in real-life situations. The authors also highlight that, if we were to follow the original SIT protocols that involve 30-second sprints followed by 4 minutes of recovery, it would require a minimum of 20 minutes to complete a SIT session. Considering the warm-up, this time commitment could still be a significant obstacle for individuals to incorporate regular exercise into their routines. Finally, and even though the Wingate test has been previously used as an exercise model to study neuromuscular fatigue in acute terms, little is known about the

long-term fatiguing effects of an entire SIT session and/or program. Intense exercise sessions have been shown to impair recovery up to 48 hours after the exercise (Stanley et al., 2013). Thus, in order to improve SIT prescription, it would be important to determine whether all-out exercise leads to an excessive fatigue status that might impair the subsequent physiological adaptations.

In response to these critics, a growing body of research has moved away from the classic 4-6 × 30 s Wingate protocols, and focused on defining the most adapted program for promoting the greatest health benefits with the lower time commitment (Hazell et al., 2010; Metcalfe et al., 2012; Vollaard & Metcalfe, 2017; Yamagishi & Babraj, 2017). For example, after a series of studies for testing the effects of different combinations of HIIE in laboratory settings, the research team of Richard Metcalfe and Niels Vollaard analyzed the effects of their novel approach in a real-life situation. The study was conducted in an unsupervised workplace setting and involved twenty-five sedentary office-workers (Metcalfe et al., 2020). Importantly, apart from showing that their reduced-volume SIT program was very effective for improving  $\dot{V}O_2\text{max}$ , the results revealed high rates of acceptability and enjoyability towards the training program. This improved the confidence of the participants on continuing the protocol after the intervention. These results, together with others that evidence the viability of reduced-volume SIT protocols, are of great importance for the promotion of regular exercise and health enhancement. Additionally, they show that all-out exercise protocols can be used in real-life settings and be applied not only to athletes but also to sedentary individuals. Thus, reduced-volume SIT programs could even be proposed as a public health strategy since programs involving as little as two repetitions ranging from 10 to 20 s sprints remove many of the proposed barriers towards engaging a regular physical exercise routine. From these results, SIT remains as an intriguing approach for quickly enhancing overall physical fitness. Although more and more investigations are being done in relation to SIT, there are a few aspects that still need to be considered and could be crucial for the use of SIT in sedentary populations.

## ***1.4. General aims***

The main aims addressed throughout this PhD were:

- to determine the immediate and long-term fatiguing effects of SIT;
- to study the time course of the cardiorespiratory adaptations developed during a SIT program;
- to determine whether or not SIT induces improvements in neuromuscular function and its time course.

In order to address these three main points, two main experimental studies were conducted during this PhD project. In the first study (Chapter 3), we analyzed the neuromuscular and the cardiac autonomic recovery immediately after and the subsequent days after a single all-out exercise bout. A better understanding of the recovery kinetics could have important implications for the configuration of SIT programs and their prescription. In the second study (Chapter 4), we assessed the changes in cardiorespiratory adaptations at different time points to determine for how long a SIT program is effective for increasing  $\dot{V}O_2\text{max}$ . In this second study, we also analyzed whether a SIT program leads to gains in isometric or dynamic force, speed and power production in a sedentary population and the time course of these adaptations. Finally, some additional data collected during the second study are presented in Chapter 5. These data show the heart rate variability measures and the mathematical modeling of the fitness and fatigue components during the training program to assess whether long term fatigue could have interfered with the adaptations to SIT program.

## ***2. Chapter two: Literature Review***

In recent decades, there has been a growing interest in studying the effects of SIT on various populations. This chapter aims to provide a summary of the main physiological responses and adaptations observed in sedentary individuals undergoing SIT. As a matter of fact, the word “sedentary” will be used as a descriptor of the population. We are aware of the differences between sedentary and inactive as all the subjects recruited for the studies responded to both definitions, we decided to reduce it to one and ensure consistency along the current document and the published works. By examining these sections, readers will gain insight into the mechanisms underlying the improvements in health-related markers such as  $\dot{V}O_2\text{max}$  and exercise capacity. Additionally, the metabolic and neuromuscular adaptations to SIT will be reviewed. Further, this chapter will explore the recovery process following SIT, shedding light on the potential detrimental or fatiguing effects of this training modality and providing insights into the time required to recover after all-out exercise. Lastly, the effects of reduced-volume SIT models on  $\dot{V}O_2\text{max}$  will be reviewed highlighting the key findings from recent research studies.

### ***2.1. Acute responses to a single SIT session***

SIT is characterized by repeated bouts of all-out intensity exercise (Weston et al., 2014). Since a session comprises several sprints, the 10 to 30 s all-out exercise bouts need to be interspersed by rest periods that tend to be six or eight times longer than the efforts. Therefore, the recovery periods range from 2 to 4.5 minutes, depending on the effort time (Gillen & Gibala, 2014). The all-out component of SIT has been shown to be key in the development of the subsequent physiological adaptations (MacInnis & Gibala, 2017) despite the little total work time cumulated during the sessions. Therefore, maintaining the intensity as high as possible for several repetitions seems crucial for the efficacy of SIT as a training method. Although in most cases SIT is performed on a cycle ergometer and in laboratory settings, SIT can be applied to other endurance disciplines such as trail running (Koral et al., 2018), cycling (Almquist et al., 2020) or cross-country skiing (Vandbakk et al., 2017). While on the cycle ergometer, SIT is usually performed against a fixed resistance equivalent to 7.0 to 7.5 Newtons of resistive force per kg of body mass. In the other outdoor disciplines, no fixed resistance can be set.



The intensity domains theory suggests that there are two physiological landmarks, the first and the second lactate threshold, that divide the oxygen uptake ( $\dot{V}O_2$ ) responses within the whole intensity spectrum into three exercise intensity domains: moderate, heavy and severe/extreme. SIT and, in general, the short bouts of all-out exercise, generate a sufficiently high metabolic disturbance for falling into the extreme domain. As a consequence, the blood lactate, muscle phosphocreatine (PCr), inorganic phosphate,  $H^+$  and muscle pH fail to stabilize impairing the myosin-actin turnover and calcium ( $Ca^{2+}$ ) release, reducing the force output and/or shortening velocity (Hultman et al., 1986). During longer and intense exercises, a  $\dot{V}O_2$  slow component would progressively drive the  $\dot{V}O_2$  towards  $\dot{V}O_{2max}$ . However, the high metabolic stress and the short duration of the all-out bouts do not allow the  $\dot{V}O_2$  response to be developed and therefore, participants' records fail to show a clear  $\dot{V}O_2$  slow component. Thus, this implies that the  $\dot{V}O_{2max}$  is not usually reached during SIT because the task finishes before. From a metabolic point of view, the all-out intensity that is asked to the participants during the SIT sessions implies a high energy turnover in a very short time. Although the bioenergetics of sprint exercises differs markedly depending on the sprint duration and recovery intervals (Ross & Leveritt, 2001), the three main energetic systems are known to be involved even in sprint exercises lasting between 10 to 30 s. During the initial seconds of the sprint, the energy turnover is primarily derived from the breakdown of stored muscle phosphagens such as the muscle adenosine triphosphate (ATP) and the PCr. As the energy turnover rate of the phosphagens energy system is high, the power output (PO) increases and reaches the peak after the first 2-3 seconds. Once the stores of PCr fall dramatically (within the first 10 s from the onset of the exercise), the ATP resynthesis relies more on glycolytic processes that are known to resynthesize ATP at slower rates compared to the phosphagens energy system. This explains a reduction of the power that is being maintained during this phase of the sprint. The increased reliance on the anaerobic glycolysis, which accounts for most of the energy produced during all-out sprinting (Smith & Hill, 1991), results in disproportioned accumulation of lactate and inorganic phosphate, with a subsequent increase in  $H^+$  and a lowering of the muscle pH. Then, as the sprint time gets prolonged (>15 – 20 s), the energy demands are progressively relying on the aerobic energy system. Bogdanis et al. (1998) measured the relative contributions of each energetic system during 10 and 20 s sprints and observed that, while in the first 10 s the aerobic system accounted for a 13% of the energy production, this relative contribution got doubled (i.e. 27%) during the second half of the sprints. Then, Smith & Hill (1991) reported that the aerobic energy system provided approximately 35% of the energy during the last 5 s of a 30 s sprint, suggesting that the energetic contribution of the aerobic metabolism is not negligible

despite the short exercise time. From a muscular point of view, the very high power-production required during all-out sprinting implies a large muscle fiber recruitment, in particular type II fibers. Indeed, the stress placed on type II fibers is believed to be key for future muscle-related adaptations (Gibala et al., 2006). Finally, Freese et al., 2013 studied the cardiorespiratory responses during a  $4 \times 30$  s SIT session. The authors reported that ventilation,  $\dot{V}O_2$  and HR reached around the 80% of the estimated maximal values on average during the session. The intermittent nature of SIT generated sharp rises in the three variables that peaked at the end of a few seconds the completion of the exercise bout. Then, during the recovery periods, a progressive decline of the  $\dot{V}O_2$ , ventilation and HR occurred until the next bout of exercise started.

## ***2.2. Effects of SIT on $\dot{V}O_{2max}$***

The mechanisms behind the increases in  $\dot{V}O_{2max}$  after SIT or HIIE, which has been shown to result in similar or greater improvements compared to CT, are not well-known yet. The  $\dot{V}O_{2max}$ , by definition, involves central and peripheral parameters (Adolf Eugen Fick, 1870). On the one hand, there is the cardiac output, which is defined as the amount of blood pumped by the heart in a given period of time (typically in a minute). Then, on the other hand, there is the arteriovenous oxygen difference, which is a measure of the amount of oxygen taken up from the blood by the tissues. Therefore, any detectable change in  $\dot{V}O_{2max}$  could come from either one or the other. CT has been shown to improve both central and peripheral parameters. First, the enlargement and hypertrophy of the left ventricle that increases cardiac output contributes to an increase in  $\dot{V}O_{2max}$  (Levine, 2008). Secondly, increases in capillary density and growth, that results in greater oxygen extraction from the blood (Andersen & Henriksson, 1977; Denis et al., 1986), plus the enhanced enzymatic activity that leads to an increased oxidative capacity (Bassett, 2000) all contribute to increases in  $\dot{V}O_{2max}$ . However, although increases in capillary growth and enzymatic activity are known to occur earlier, the cardiac remodeling through exercise is likely to be a longer-term adaptation (Levine, 2008). Thus, considering that most of the SIT training programs last only for a few weeks, this kind of adaptation is not usually expected. Interestingly, Jensen et al. (2004) reported an increased capillary growth within the first four weeks of HIIT. Furthermore some studies have reported increases in capillary density (Bonafiglia et al., 2017; Raleigh et al., 2016; Scribbans et al., 2017) and enzymatic activity after SIT (Burgomaster et al., 2005, 2008) while others have failed to detect

changes in central parameters after short HIIE/SIT interventions (Jacobs et al., 2013; Jalaludeen et al., 2020; Laursen et al., 2005; Macpherson et al., 2011; Raleigh et al., 2018). This might be the reason why an important body of the literature has mainly focused on the muscle-related metabolic or peripheral adaptations, suggesting that the changes behind  $\dot{V}O_{2\max}$  after SIT could be principally explained by peripheral rather than central parameters, which had been somewhat overlooked in the literature (Mandić et al., 2022). However, a recent series of acute and chronic mechanistic studies have reported that, indeed, a combination of both could be responsible for the changes in  $\dot{V}O_{2\max}$ . The following section provides a review of the main peripheral and central adaptations to SIT that lead to changes in  $\dot{V}O_{2\max}$ . The discussion of muscle-enzymatic adaptations to SIT, which is also considered peripheral, will be addressed in a subsequent section of the manuscript.

### ***2.2.1. Peripheral adaptations to SIT***

The mechanical and metabolic stress produced during exercise can promote the growth of new capillaries (Hudlicka et al., 1992). When exercising, an increased number of capillaries enables the active muscles to extract more oxygen from the blood, resulting in enhanced performance and exercise capacity (Saltin et al., 1977). This phenomenon, referred to as angiogenesis, is primarily driven by vascular endothelial growth factor (VEGF) in skeletal muscle, known for its potent pro-angiogenic properties (Hoier & Hellsten, 2014). In general, HIIE has been shown to produce increases in both the mRNA expression of VEGF and the VEGF content (Hoier & Hellsten, 2014; C. W. Taylor et al., 2016). Specific to SIT, Cocks et al. (2013) were the first to show that muscle capillarization of the vastus lateralis occurred after 8 weeks of either SIT or CT. Then, Scribbans et al. (2014) also observed a similar increase in capillary density after 6 weeks of a SIT protocol involving  $8 \times 20$  s at 170% of  $\dot{V}O_{2\max}$ . Interestingly, the authors did not observe a significant difference compared to the CT group, confirming that both training modalities led to very similar adaptations. These results were important because SIT involved a marked reduction in the total exercise time per session and have been lately replicated by other studies. Specifically, Bonafiglia et al., 2017; Raleigh et al., 2016; Scribbans et al., 2017 observed increases in capillary density using a 20 s sprints protocol, confirming that SIT is an effective method to increase capillarization and therefore,  $\dot{V}O_{2\max}$ . In addition to improving the ability to deliver oxygen into skeletal muscle, it is important to consider the impact of SIT on the well-known indicators of mitochondrial biogenesis. Although this specific subject will

be addressed in a later section, increases in mitochondrial biogenesis are known to result in an enhanced capacity to utilize oxygen for the ATP turnover. Thus, SIT might presumably have a positive impact in two of the major peripheral determinants of aerobic exercise capacity despite an important difference in the total exercising time.

### ***2.2.2. Central adaptations to SIT***

One of the most important central adaptations to exercise is the enhancement of the maximal cardiac output (Hellsten & Nyberg, 2015) which is, the product between the heart rate and the stroke volume. After a period of regular endurance training, the enlargement of the left ventricle and its improved contractility allow for a greater ejection fraction, which increases volume of blood expelled with each beat, ultimately increasing the stroke volume and, therefore, the cardiac output. Furthermore, increases in blood volume, which occur much earlier after the training onset (Fellmann, 1992; Gore et al., 1992), are also known to induce significant changes in  $\dot{V}O_{2\max}$  due to an increased cardiac output. These modifications allow for a better delivery and transport of the oxygen because more blood is ejected towards the circulatory system, resulting in a concomitant increase in  $\dot{V}O_{2\max}$  (Montero et al., 2015). Despite all these adaptations have been traditionally linked to endurance training, recent research has observed a close relationship between the increases in  $\dot{V}O_{2\max}$  and the increase in blood volume after SIT (Mandić et al., 2022).

A temporary decrease in plasma volume that triggers mechanisms that increase albumin production and content have been shown to occur after traditional endurance training (Montero & Lundby, 2018). The increase in albumin content leads to an expansion in plasma volume, that accounts for nearly all the increase in total blood volume during the first 2-3 weeks after the onset of exercise (Fellmann, 1992; Gore et al., 1992). With time, this is followed by a later increase in erythrocyte volume, until reaching the equilibrium (Sawka et al., 2000). As a result of the overall increased blood volume there is a greater cardiac diastolic filling, stroke volume and cardiac output that ultimately increases  $\dot{V}O_{2\max}$ . Interestingly, a recent study has shown that a single 3x30-s SIT session also causes a significant reduction (~16%) in plasma volume, indicating that a similar mechanism may be responsible for the later increase in  $\dot{V}O_{2\max}$  observed after SIT (Mandić et al., 2021). These findings are of interest because they could help explaining why SIT is such an efficient training method for improving  $\dot{V}O_{2\max}$ . As a proof of

concept, the same research team investigated the effects of a six-week  $3 \times 30$ -s SIT protocol on central hemodynamic factors (Mandić et al., 2022). The main findings were that SIT led to significant increases  $\dot{V}O_{2\max}$  that were accompanied by significant increases in plasma and total blood volume, total hemoglobin mass and cardiac output. The rise in blood volume was likely caused by an increase in plasma volume, as there was a decrease in hemoglobin concentration and hematocrit. This hemodilution reflected the typical time-course of exercise-induced hypervolemia, where plasma volume increases quickly and then erythrocyte volume increases until a balance is achieved and hematocrit returns to normal levels. Importantly, the authors concluded that, even though central hemodynamic components seemed to be key after SIT, the overall change in  $\dot{V}O_{2\max}$  was greater than the changes in the central parameters by themselves, indicating that peripheral adaptations could likely explain part of the change in  $\dot{V}O_{2\max}$ . Nevertheless, a recent study from the same research team, showed that when 276 mL of blood are withdrawn using phlebotomy after the SIT intervention,  $\dot{V}O_{2\max}$  and cardiac output got restored to pre-intervention values whereas the parameters related to the extraction of the  $O_2$  remain unaffected (Mandić et al., 2023). These studies, help to understand the relationship between the changes in blood volume and cardiac output and the improvements in  $\dot{V}O_{2\max}$  after SIT. Indeed, the authors argued that despite the peripheral adaptations might play a role on explaining the changes in  $\dot{V}O_{2\max}$ , the central adaptations are key for allowing for a greater amount of oxygen to reach the tissues to be extracted. In other words, without a substantial increase in  $O_2$  delivery, the peripheral adaptations cannot fully explain the increased  $\dot{V}O_{2\max}$  after SIT. These findings are consistent with a previous interval training intervention that compared CT with high volume interval training. After eight weeks of exercise training, cardiac output increased in parallel with the improvements in  $\dot{V}O_{2\max}$  (Daussin et al., 2007) leading to think that the contribution of central and peripheral factors to the improvement in  $\dot{V}O_{2\max}$ , is similar between SIT and other interval training modalities like HIIT. Previous to these studies, other research teams had reported increases in peak cardiac output (Bostad et al., 2021) and blood volume (Satiroglu et al., 2021) after all-out SIT. Although peripheral adaptations draw most of the attention due the short length of the SIT programs, more and more research work seem to agree on that the central adaptations primarily regulate changes in  $\dot{V}O_{2\max}$  after a SIT intervention and, therefore, should also be considered.

### ***2.3. Metabolic adaptations to SIT***

The skeletal muscle is a dynamic tissue that adapts in many different ways to an exercise stimulus. The metabolic adaptations in this tissue are associated to the ability of the muscle to produce energy. As the mitochondria are in charge of the energy production in cells, one can understand how increasing its density or its function can help to increase the ATP turnover to fuel several cellular activities (Granata et al., 2018). According to Ross & Leveritt (2001), three main mechanisms can help increasing the muscle ability to increase the energy turnover. First, an increase in the activity of key regulatory enzymes can result in a faster rate of energy production across the three main energetic systems. Second, increasing the amount energetic substrate stored in the muscle or the rate at which the energetic substrate is used would allow for an increase in the total energy production. Third, through exercise, the muscle can enhance its capacity to resist the build-up of specific metabolites that contribute to fatigue and therefore, maintain a higher energy production.

#### ***2.3.1. Mitochondrial function and density***

Comparative studies have shown a correlation between the  $\dot{V}O_2\text{max}$  and the whole-body mitochondrial content (Schwerzmann et al., 1989), suggesting that the total quantity of mitochondrial respiratory enzymes in cells is a key determinant of the oxidative capacity of the muscles. Indeed, increased mitochondrial function or density have been linked to better endurance performance and overall health (Jacobs & Lundby, 2013). Moreover, the skeletal muscle mitochondrial density is known to regulate substrate metabolism during submaximal exercise, promoting a greater reliance on fat oxidation and a proportional decrease in carbohydrate oxidation (Holloszy & Coyle, 1984). Consequently, exercise training could lessen glycogen utilization and lactate production at a given intensity by increasing the lactate threshold. These changes, together with the enhanced enzymatic activities discussed in the following point, could potentially enable individuals to exercise for longer periods at greater intensities and percentages of their  $\dot{V}O_2\text{max}$ , increasing their exercise capacity (Joyner & Coyle, 2008). Therefore, optimizing mitochondrial function and/or content through physical training has become a focus for improving both performance and health outcomes. Although the mechanisms behind the improvements in mitochondrial content and function are not yet fully understood, the effects of volume and intensity of the exercise have been studied

separately to determine their relative impact on these parameters. On the one hand, exercise volume has been shown to have significant effects on mitochondrial content. For example, a study comparing five different training volumes reported that participants training four or five times per week increased mitochondrial density to a greater extent than those training only once or twice a week (Montero & Lundby, 2017). Additionally, they reported that as little as six exercise sessions were sufficient to increase the mitochondrial content. However, previous research suggested that training volume is not a primary determinant of the changes in mitochondrial function (i.e. mitochondrial respiration) (Bishop et al., 2014). On the other hand, a single SIT session activated signaling pathways associated with mitochondrial biogenesis, (Gibala et al., 2009; Little et al., 2011; Metcalfe et al., 2015) highlighting the effects of exercise intensity on mitochondrial density. Moreover, SIT has been found to be a more efficient form of exercise to improve mitochondrial respiration when compared to disciplines involving greater training volumes (Granata et al., 2016, 2018). As the total work time is very little during SIT, the all-out component of the effort is critically important to generate homeostatic perturbations and activate several key protein kinases involved in signal transduction or cell signaling. At the muscle cellular level, the kinases act as sensors for regulating other cell functions. Two of the main kinases are the 5'-AMP-activated protein kinase (AMPK) and the p38 mitogen activated protein kinase transcriptional coactivator (p38 MAPK). The AMPK plays an important role during contraction-induced metabolic disturbances, serving primarily to restore cellular energy status. This protein kinase gets activated in response to an imbalance between the adenosine monophosphate (AMP) and the ATP or in situations with low muscle glycogen availability (Hardie, 2011). A greater AMP:ATP ratio together with a decrease in the muscle glycogen reserves are two scenarios that often occur during SIT or HIIE. Furthermore, the exercise-induced activation of AMPK is known to occur in an intensity-dependent manner as cycling at 80% of  $\dot{V}O_2\text{max}$  generated an increase in the response compared to cycling at 40% of  $\dot{V}O_2\text{max}$  (Egan et al., 2010). On the other hand, the p38 MAPK has emerged as an important signaling protein implicated in regulating contraction-induced peroxisome proliferator-activated receptor 1- $\alpha$  (PGC 1- $\alpha$ ) and adaptations of an oxidative phenotype in response to exercise-induced mechanical stress (Bartlett et al., 2012; Little et al., 2010, 2011). When phosphorylated, and thus activated, both converge towards the cell nucleus to directly stimulate potential targets. One of these targets is the PGC 1- $\alpha$ , which is commonly referred to as the “master regulator” of mitochondrial biogenesis (Jäger et al., 2007) and has also been found to play an important role in the capillary growth (Arany et al., 2008; Geng et al., 2010). Specific to SIT, Gibala et al., (2009) observed that four 30 s bouts of all-out cycling increased

phosphorylation of AMPK and p38 MAPK immediately after the exercise. In addition, the mRNA expression of PGC-1 $\alpha$  was also greater after 3 h of recovery. However, the PGC-1 $\alpha$  protein content measured at the muscle level was unchanged after SIT. The authors suggested that it is possible that more than one dose of intense intermittent exercise might be necessary to increase the PGC-1 $\alpha$  protein content in human muscle. These findings agree with those observed by Little et al. (2011) after the same SIT session. They observed a ~100% increase in cytosolic ACC phosphorylation, a recognized marker of AMPK activation, as well as signs of p38 MAPK activation. Moreover, when they measured the mitochondrial protein content 24 h after the exercise a significant increase compared to baseline and the 3 h time-point was also observed.

In sum, these investigations demonstrate that a single session of SIT can increase mitochondrial function and biogenesis in human skeletal muscle and provides important mechanistic insights into this adaptive process. Additionally, given the potential benefits of increased skeletal muscle PGC-1 $\alpha$  for improving insulin sensitivity (Benton et al., 2008) and preventing age-related sarcopenia and metabolic decline (Kang & Ji, 2013) SIT may represent an effective strategy for improving metabolic health in a variety of populations.

### ***2.3.2. Enzymatic adaptations***

PCr stores have been shown to experience a large depletion after all-out exercise (Spriet, 2006) due to the high energetic demand. The creatine phosphokinase (CPK), that catalyses the breakdown of PCr, and the myokinase (MK), that resynthesizes ATP from ADP and PCr, are consequently key enzymes that regulate the energy production at the beginning of the exercise. The activity of CPK and MK have been shown to significantly increase after both shorter (i.e. 5 s) (Thorstensson et al., 1975) and longer (i.e. 30 s) (Parra et al., 2000) maximal sprints. That, in sum leads to a faster depletion of PCr due to an enhanced ability to deplete the substrate but also a faster resynthesis of ATP using PCr. Although these findings seem promising, other studies failed to detect any change in CPK after sprint training in sedentary individuals (Cadefau et al., 1990; Jacobs et al., 1987). Cadefau et al. (1990) argued that considering that the CPK is already largely activated in the sedentary muscle, the stress induced by a sprint training may not be enough to stimulate a significant increase. The effects of all-out exercise in the regulatory enzymes of the glycolytic anaerobic system have been extensively examined. For example,



MacDougall et al. (1998), after 7 weeks of 30 s SIT, observed a ~49% significant increase on the phosphofructokinase (PFK) activity, an enzyme responsible for catalyzing the phosphorylation of fructose 6-phosphate to fructose 1,6-biphosphate and therefore key for the glycolytic regulation. Additionally, a number of studies have found the PFK activity to be increased after short (Linossier et al., 1993) or long sprints (Cadefau et al., 1990; Parra et al., 2000). Similarly, the activities of other enzymes such as the lactate dehydrogenase (LDH), that catalyzes the conversion of pyruvate to lactate, and glycogen phosphorylase (PHOS), that enhances the glycogen mobilization into the glycolytic pathway, have been found to increase after all-out exercise (Ross & Leveritt, 2001). All these changes are important because they enhance the capacity of the muscle to break down glycogen and therefore improve the overall energy availability during exercise.

The contribution of the aerobic energy system during a single sprint is low compared to the phosphagens and the anaerobic glycolysis. However, when multiple all-out bouts are repeated within the same session and/or when the session involves longer (i.e. ~30 s) sprints, the anaerobic energy production decreases and a greater amount of the total energy produced is derived from the aerobic metabolism. Therefore, an enhanced activity of the oxidative enzymes can be of importance during SIT. For example, Burgomaster et al., (2006) examined whether a 2-week 30 s SIT program could alter the pyruvate dehydrogenase (PDH) function during aerobic exercise. This enzyme converts pyruvate into acetyl-coA, increasing the influx of acetyl-coA from glycolysis towards the Krebs cycle. During a work-matched 250 kJ cycling time trial, the authors observed a net muscle decrease of the glycogenolysis and an increased capacity for pyruvate oxidation through PDH. These findings are of importance because they establish a clear relationship between SIT training and an improved exercise capacity during aerobic exercise. Moreover, the same research team also found an 11% increase in the maximal activity of the citrate synthase (CS), that catalyzes the condensation of oxaloacetate and acetyl coenzyme A to form citrate in the Krebs cycle (Siu et al., 2003) after only six SIT sessions (Burgomaster et al., 2005, 2006). These results were lately confirmed by De Smet et al. (2016) that observed a +35-50% increase in the CS maximal activity after a 5-week SIT training program involving 4 to 6 repetitions of 30 s. Finally, Gibala et al. (2006) also observed increases in the maximal activity of cytochrome c oxidase (COX), that catalyzes the final step in mitochondrial electron transfer chain, after only two weeks of SIT. Overall, all these changes at the enzymatic level, reflect an enhanced capacity of substrate degradation across all the energetic pathways.

### ***2.3.3. Muscle substrate storage***

An increased availability of metabolic substrates before exercise can enhance or maintain energy production at a high rate for longer (Hargreaves & Spriet, 2020). As mentioned before, during HIIE and specially SIT or all-out exercise, the metabolic substrates stored in the muscle such as ATP, PCr or glycogen allow for a quick energy turnover. Although the lipid degradation plays a non-negligible role during SIT, it is known that the ATP regeneration through the aerobic metabolism is slower than the phosphagens system or the anaerobic glycolysis (Spriet, 2006) and, therefore, the other systems are used to meet the high energy demands. Some studies have shown no change or even a decrease in resting muscle levels of ATP and/or PCr after short sprint training (Sharp et al., 1986; Thorstensson et al., 1975). Importantly, in studies such as Thorstensson et al. (1975), in which the sprint training increased leg muscle volume, an increased ATP storage was found, but SIT did not directly increase the ATP and PCr reserves. Although the effects of all-out exercise on glycogen storage highly depend on the carbohydrate intake and there is equivocal data on the topic (Ross & Leveritt, 2001), some studies have been able to better isolate the effects of nutrition and have found significant increases in resting glycogen content after SIT (Harmer et al., 2000; Parra et al., 2000). Indeed, Gibala et al. (2006) observed that SIT resulted in a 28% increase in the muscle glycogen content compared to 17% increases during CT. Although the difference between training groups was not significant, SIT involved a ~90% less training volume since they only performed six sessions of 4 to 6 bouts of 30 s of all-out exercise spread over two weeks. These findings coincide with Burgomaster et al. (2005), who observed a 26% increase in resting glycogen muscle after two weeks of SIT involving the exact same protocol. Finally, apart from the total amount of substrate available, the rate at which this substrate is used can also help to maintain energy production for longer. In this line, some studies observed net decreases in the glycogenolysis, which is the breakdown of glycogen to produce energy, after a SIT training regimen (Burgomaster et al., 2006).

### ***2.3.4. Build-up of metabolites***

Finally, an important adaptation usually observed in the skeletal muscle is an increased capacity to resist the effects of the build-up of metabolic by-products that occur due to the use of the phosphagens and anaerobic glycolysis during HIIE. During the first seconds of SIT, where the energy demands are mostly met by the phosphagens and the anaerobic glycolysis energy

systems, the breakdown of PCr and the hydrolysis of ATP, combined with the dissociation of lactic acid into lactate and  $H^+$ , result in a sudden increase of both inorganic phosphate and  $H^+$ . The accumulation of  $H^+$  combined with an acidification of the muscle are known to impair muscle contraction and, therefore, force and power production, through several mechanisms. These are principally the decrease in  $Ca^{2+}$  release and uptake from the sarcoplasmic reticulum, the decrease of sensitivity of troponin C to  $Ca^{2+}$ , which finally interferes directly with cross-bridge cycling (Allen & Westerblad, 2001; Westerblad et al., 2002). Although the accumulation of inorganic phosphate is directly dependent on the breakdown of PCr, the ability to buffer lactate can delay the onset of fatigue during HIIE (Joyner & Coyle, 2008). The main buffer for lactate is the bicarbonate ( $HCO_3^-$ ). The  $HCO_3^-$  ion combine with the  $H^+$  in the blood to forming carbonic acid ( $H_2CO_3$ ). This combination reduces the amount of free  $H^+$  in the blood, which results in a raise of the blood pH. Gibala et al. (2006) measured muscle buffering capacity and found an increase of 7.6 and 4.2% for the SIT and endurance training group respectively, with no significant difference between groups. Burgomaster et al., (2006) observed a decrease in net glycogenolysis and lactate accumulation during exercise after 2 weeks of 30 s SIT. The lactate accumulation during the same bout of aerobic exercise measured at the muscle level was significantly lower after the training compared to baseline, indicating that training enhanced the capacity to buffer this metabolic by-product and also delayed the onset of fatigue.

In sum, these main mechanisms of adaptation described in the three previous points, together with the increases in mitochondrial function and density, have been linked to performance increments during endurance tasks such as cycling time trials or constant work rate trials (Burgomaster et al., 2005; Yamagishi & Babraj, 2017)

## ***2.4. Neuromuscular adaptations to SIT***

Skeletal muscle composition may influence sprint performance. It is known that muscle contraction speed and strength are partly determined by the muscle fiber type and the size or the muscle cross-sectional area. Based on the size principle, which states that small motor units and type I fibers are recruited before the larger ones (i.e. type II), different intensities of exercise might elicit different responses and adaptations in the long term (Skelly et al., 2021). For example, during low intensity endurance training a larger proportion of fibers type I is recruited and in the long-term, this type of training is known to induce a shift towards type I fiber type (IIX  $\rightarrow$  IIa  $\rightarrow$  I). During HIIE and specifically, during SIT, a greater proportion type II fibers is recruited due to the high energy demands (Skelly et al., 2021). These fibers are known to count with an enhanced glycolytic capacity which is an important determinant of performance during short but intense bouts of exercise. Within the type II fibers, two main types are usually differentiated, the type IIa and the type IIX. While the IIa have a high oxidative and glycolytic capacity, the type IIX are principally glycolytic. Furthermore, the IIa tend to be more resistant to fatigue than the type IIX. Consequently, if a fiber type is repeatedly recruited and exposed to larger metabolic disturbance than the others during SIT, changes in that specific type may also happen in a larger proportion.

### ***2.4.1. Muscle fiber type and size***

Sprint runners or track cyclists are known to have a larger percentage of type II fibers than other athletes (Costill et al., 1976). Furthermore, performance during sprint events has been strongly correlated with the proportion of type II fibers (Esbjörnsson et al., 1993). Thus, as for the endurance training, a specific adaptation towards the fiber that is used more often could be expected. Some studies have shown that this seems to be the case after different cycling sprint training programs. For example, Jacobs et al. (1987), tested the effects of a six-week 15 and 30 s cycle all-out program and observed a significant decrease in type I and type IIX fibers and an increase in type IIa, obtaining a shift towards the IIa (I  $\rightarrow$  IIa  $\leftarrow$  IIX). Similarly, Allemeier et al., (1994) performed a similar training program involving 3  $\times$  30 s efforts and observed the same pattern of shifting towards type IIa muscle fibers. These results were lately confirmed by De Smet et al. (2016), who performed a 5-week 30 s sprints program and obtained a significant increase in the number of type IIa fibers. Since the IIa fiber type is known to have an enhanced

fatigue resistance and higher glycolytic capacity, this might be the reason why the capacity to maintain a greater mean and maximal power outputs during a SIT session, is usually improved after training. It is interesting to note that, following 7 weeks of 5 s all-out sprints with 55 s recovery periods, Linossier et al. (1993) observed an 8.5 % decrease in type IIX combined with an 8% increase in type I. Also, Simoneau et al., (1985) who performed longer efforts 15 to 90 s across a range of intensities, also detected a shift towards type I. In these cases, the shift was similar to the one observed after traditional endurance training (IIX → I) and highlights that the shift towards type Iia seems to be highly dependent on sprint and recovery length. On the one hand, prolonged sprints require a considerable amount of aerobic energy production and, on the other hand, short recovery periods can limit the ability of the muscle to resynthesize PCr leading to a decreased ability to rapidly produce high power outputs (Ross & Leveritt, 2001). Additionally, it has been argued that contraction frequency is known to be an important factor when determining the fiber-type synthesis (Pette & Vrbová, 1985). In this regard, Scribbans et al. (2014) obtained an increase in type I fibers accompanied by a decrease in Iia fibers after 6 weeks of SIT. The authors argue that although their protocol could have been considered similar to SIT (8 × 20 s at 170% of  $\dot{V}O_{2max}$ ), the cadence during the exercise bouts was set to 80 to rotations per minute controlled in order to be comparable to a CT group. Then, from an intensity point of view their protocol could have been comparable. However, as SIT intervals reached much higher cadences, the corresponding specific adaptations of the Iia fiber types could have been expected.

Muscle growth during hypertrophy occurs through a net increase in protein synthesis. The activation of ribosomal RNAs and muscle-specific genes such as mTORC1 play a key role in the regulation of protein synthesis and ribosomal biogenesis (Schiaffino et al., 2021). An increase in the muscle fiber size correlated with increases in maximal muscle strength or power (Douglas et al., 2021). Although further research on this topic seems warranted, some studies examined the effects of SIT on muscle fiber cross sectional area. For example, Cadefau et al. (1990) obtained a 8 to 16% significant increase on muscle cross sectional area after an 8-month SIT based program. Conversely, Allemeier et al. (1994), observed a non-significant increase of 6 to 12 % in fiber cross sectional area after performing 3 × 30 s SIT two times a week for six weeks. Similarly, De Smet et al. (2016) did not observe any significant change in muscle fiber hypertrophy after five weeks of SIT training. These results suggest that the extent of hypertrophy could be dependent on the length of the training program, although other factors like sex could also play a role (Ross & Leveritt, 2001). In this regard, Esbjörnsson Liljedahl

et al., (1996) reported that females increased the type IIX fiber cross sectional area more than males after 4 weeks of SIT. This phenomenon was linked to the fact that females were found to be less trained than males, and therefore, they benefited more from the same training regimen. In contrast, Scalzo et al. (2014) observed that males showed 150% greater rates of muscle protein synthesis than females 48 hours after 3 weeks of SIT, suggesting that in this case, males would benefit from SIT more than females. Finally, Bagley et al. (2021) observed a 3.25 cm<sup>2</sup> increase in the quadriceps cross sectional area without sex differences after 12 weeks of 4 × 20 s SIT. Although these results shed some light on the muscle-related adaptations occurring after cycle sprint training, further mechanistic research specifically on SIT is needed to understand how these changes translate to improved sprint performance or enhanced exercise capacity.

#### ***2.4.2. Force and power production after SIT***

As a significant body of the SIT-related research has mainly focused on health-related markers to understand the benefits of SIT in untrained populations. However, less attention has been paid to the neuromuscular changes following SIT. Although the experiments cited in the previous section refer to changes in fiber type and size, no clear links have been established with changes in the capacity to produce force or power after SIT. Additionally, factors such as sex have been shown to potentially influence the results. For example, Esbjörnsson Liljedahl et al. (1996) showed that females increased their power output more than males after 4 weeks of SIT. On the contrary, Astorino et al. (2011) reported that adaptations to six sessions of several Wingate tests were independent of sex. In this study, both males and females progressed to the same extent in terms of mean and peak power output (~10%) during a Wingate test. The increments in mean and peak power output have been widely reported by other studies involving different sprint length SIT models even though, in most cases, no mechanistic analyses of the fiber type or size were performed (Barnett et al., 2004; Bayati et al., 2011; Burgomaster et al., 2006, 2008; Whyte et al., 2010). Essentially, these studies show that SIT was effective for improving mean and peak power production during a single (Bayati et al., 2011; Hazell et al., 2010; Whyte et al., 2010) but also during repeated Wingate tests (Burgomaster et al., 2005; Yamagishi & Babraj, 2017). These results, apart from showing an enhanced capacity to produce higher powers per se, suggest that participants might also benefit from an increased repeated sprint ability and fatigue resistance (Yamagishi & Babraj, 2017). An increased fatigue resistance together with a greater power output during the sprints, could

lead to increases in the total work per session, making SIT program even more efficient. Additionally, previous meta-analyses also reported a significant main effect for improvements in Wingate sprint mean and peak power after SIT (Gist et al., 2014; Sloth et al., 2013). Although the Wingate test was initially proposed as a reliable measure of the “anaerobic capacities”, more recent research has shown that shorter sprints might better reveal the maximal power production capacities (Driss & Vandewalle, 2013). This was linked to the fact that the 30 s sprints are known to rely more on aerobic energy pathways for ATP resynthesis compared to shorter sprints that rely almost exclusively on anaerobic sources. Furthermore, although this is not usually discussed within these studies, participants that are not accustomed to SIT might change their strategy to perform an effort as a function of its length. Therefore, and even though the Wingate test has been used as the goal standard for anaerobic capacity assessments, a shorter sprint might better represent the capacity to reach high power outputs. Thus, although SIT has been shown to be effective for enhancing the repeated sprint ability and the mean and peak power production during Wingate tests, whether or not the maximal expression of power output in short sprint events can be also improved, remains unknown. Furthermore, the actual force and velocity production capacities on a cycle ergometer could be also analyzed to better understand the effects of SIT.

On the other hand, only two studies have directly analyzed the effects of SIT on changes in the isometric force. Astorino et al. (2011) and Bagley et al. (2021) performed a 3 and a 12-week SIT training program consisting of 20 and 30 s sprints respectively, and did not observe changes in the knee extensors maximal isometric force. The authors argued that that the mode of exercise training (cycling) might not have trained the neural control needed for isolated knee extensors to increase their force production. Although SIT has been also tested in other exercise modes like running, Sökmen et al. (2018) did not report changes in isometric strength at two different contraction velocities after a 10-week SIT period. However, isometric and dynamic neuromuscular assessments have been shown to give different information about the individual’s capabilities under fatigue conditions (Koral et al., 2021; Krüger et al., 2019) which suggests that these measurements might not be interchangeable and caution should be taken when interpreting the results. Indeed, and although the isometric measurements are known to reflect the individuals’ capabilities to produce force, a more in-depth analysis of how the force and speed converge dynamically to produce power on the cycle ergometer, could be of importance for truly determining whether or not SIT produces specific neuromuscular adaptations. Previous studies have shown how the use of the calculations for maximal power

described in Morin & Belli (2004), the so-called power-force-velocity profile (PFVP), can be assessed (Cross et al., 2017; Rudsits et al., 2018). However, these types of analyses have not been performed yet after a SIT regimen and, therefore, further research is warranted.



## ***2.5.Recovery after SIT***

From an exercise prescription point of view, recovery constitutes a key aspect. Each training session imposes a certain load (or stress) to the organism (Passfield et al., 2022). When the training load is well managed, this load should represent the trigger for many physiological processes that lead to adaptations. However, when the load is too high for the individual's capabilities or the load is imposed very often, the time course of recovery can get prolonged (Stanley et al., 2013). Inadequate or poor recovery is known to negatively impact the functioning of several systems such as the cardiovascular, hormonal or musculoskeletal (Current Sports Medicine Reports, ACSM, 2019). This, could impair beneficial adaptations to exercise training and reduce performance (Skorski et al., 2019), but also negatively impact general health and/or increase the risk of injury (Horgan et al., 2021). For example, Parra et al. (2000) observed that the enzymatic activity a group of participants performing a SIT session every day for two weeks, improved significantly less than the group that took two days of rest between sessions. This supports the idea that adequate rest periods should be given between SIT sessions to allow the organism to recover and to be in optimal conditions to be ready for the next training stimuli. Research in exercise science has focused on the development of protocols or tools to evaluate recovery after exercise. The analysis of psychological and/or physiological indicators such as questionnaires, resting heart rate and its variability, blood markers like creatine kinase, urea acid, and hormonal markers like cortisol reflect the effects of physical exercise on the different components of system. In addition, other factors like age, sex, fitness level, external conditions or even the nature of the exercise performed can also modulate recovery (Baird et al., 2012). For example, trained individuals are known to restore quicker their HR (Bunn et al., 2018) and lactate (Gmada et al., 2005) after exercise compared to untrained individuals. Moreover, light isometric contractions induce less muscle damage than maximal eccentric exercise for the same number of series and repetitions (Nosaka & Newton, 2002). These results show that recovery is a multifactorial phenomenon, which should be kept in mind when analyzing recovery after a given exercise session.

In addition to the mentioned markers, the scientific literature has traditionally linked the recovery process to the concept of fatigue. Physical fatigue has been previously defined by Bigland-Ritchie & Woods (1984) as “any exercise-induced reduction in force or power regardless the task can be sustained or not”. This logic assumes that if an individual is not able

to execute a given task at the same level than before performing a bout of exercise, this is because a certain degree of fatigue interferes with that individual's capabilities. The assessment of neuromuscular fatigue usually involves tasks in which a specific muscle or muscle group is evaluated in isolated like during a knee extension or biceps curl exercise (Aboodarda et al., 2017). By these means, the position of the participant is standardized and fatigue can be objectively measured as the loss in isometric force compared to the baseline measure. Furthermore, these standardized tasks allow for the use of muscle function assessment techniques like the electromyography (EMG) or the electrical stimulation to better characterize the origin of fatigue. For example, a classic electrical stimulation-based neuromuscular assessment involves the use of double-pulse stimulations during contraction and at rest (Millet et al., 2011; Place et al., 2007). Then, from the amplitude of the evoked forces, different parameters like the percentage of voluntary activation (VA) (a marker of central fatigue) and the muscle contractile function and membrane excitability (markers of peripheral fatigue) can be obtained.

Although the isometric evaluation of the neuromuscular fatigue through electrical stimulation has been shown to be valid, other measurements such as jumps or short sprints have been also used to assess the detrimental effects of a given exercise on the neuromuscular system. These measurements are mostly applied in the field and, although they might be less accurate and repeatable than lab-based measurements, they tend to better represent the conditions or the kind of activity being previously performed. Therefore, this type of evaluations can be considered as more ecological measurements. For example, after a SIT cycling-based exercise session, it might be better to assess the capacity to generate force, speed and therefore power on the same bike, rather than to evaluate only the isometric force. In the following point, a summary of the literature studying the effects of SIT or HIIE on post-exercise neuromuscular and cardiac autonomic recovery will be presented. There will be particular emphasis on studying the duration of impairment in these two systems following all-out exercise, with the goal of determining how long they remain affected during the recovery process.

### ***2.5.1. Neuromuscular recovery***

The study of neuromuscular fatigue has been widely used for assessing recovery from minutes to days or even weeks after the exercise. A review on this topic concluded that the time course

and mechanisms of recovery after fatiguing exercises are highly dependent on the characteristics of the preceding exercise bout (Carroll et al., 2017). The Wingate test has been extensively used as an all-out exercise model for analyzing the immediate fatiguing effects of a SIT session. As described in the 2.1 Section (e.g. Acute responses to a single SIT session), the exercise capacities in the extreme intensity domain are limited by the high ATP demands and the accumulation of metabolites (Black et al., 2017). This metabolite accumulation is known to interfere with the  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum and the turnover of myosin-actin cross-bridges (Hultman et al., 1986) leading to peripheral neuromuscular fatigue. Important declines of about  $\sim 20\%$  for the isometric maximal voluntary contraction of the knee extensors (iMVC) and  $\sim 30\%$  for the evoked electrical stimulations have been found after one minute in response to a single all-out 30 s sprint (Fernandez-del-Olmo et al., 2013). Moreover, VA decreased by  $\sim 34\%$ . Interestingly, Fernandez-del-Olmo et al. (2013), included a series of neuromuscular assessments during a 35 min recovery period before completing a second Wingate test. Although the evoked forces did not completely recover at 14- and 23-min post-exercise, the iMVC and the voluntary activation were back to baseline values in a similar fashion 14 min after the test. The second Wingate test led to greater declines in all the measured variables. These results suggest that the fatigue generated by all-out exercise seems to be not only peripheral but also central in origin. The main hypothesis behind these findings was that the all-out exercise led to important declines in force due to an impaired the excitation-contraction coupling as a result of metabolite accumulation. Additionally, and although the reasons were unclear, the exercise also impaired mechanisms acting upstream to the motor cortical neurons. These results are important since short but intense exercise sessions, were not believed to result in high amount of central fatigue (Carroll et al., 2017). These results offer a good overview of the neuromuscular fatigue and the recovery after all-out exercise. Nevertheless, it could be argued that two bouts of 30 s of exercise with 35 min of recovery in between, might not fully reflect the potential detrimental effects of a longer SIT session. Other studies have analyzed post-exercise recovery after a session of SIT. For example, Lievens et al., (2020) observed significant declines in iMVC ( $\sim 25\%$ ) 10 minutes and up to five hours after the a  $3 \times 30$  s SIT session. Interestingly, the VA did not show any important decline, suggesting that the central command was not impaired after the session. Similarly, the evoked forces showed significant declines up to five hours after the fatiguing task, suggesting that peripheral fatigue was most likely explaining the drop in isometric force. Furthermore, Lievens et al. (2020) performed muscle biopsies before the fatiguing trial. Importantly, the participants with a greater proportion of fast twitch fibers displayed a longer post-exercise time to recovery than

the participants with a lower proportion. Specifically, the fast-fiber group, did not restore the iMVC up to five hours after the session whereas the slow-fiber group. These results indicate that fiber-typology might be an important factor to consider after all-out exercise. After the timeframe of five hours, conflicting results on whether the neuromuscular system is recovered after a SIT session have been observed. For example, 24 h after a  $6 \times 30$  s SIT session, Place et al. (2015), Schlittler et al. (2019) and Zanou et al. (2021) did not detect any decline in the iMVC. However, for a similar  $6 \times 30$  s session or even a session with fewer sprints in young females (i.e.  $3 \times 30$  s), Krusnauskas et al. (2020) and Venckunas et al., (2019), observed an average significant decline for the iMVC after 24 h. Moreover, when the femoral nerve was electrically stimulated at low frequencies, the results resulted to be similar. Those who did not observe declines in iMVC, did not observe either an increased low frequency fatigue (LFF), while Venckunas et al. (2019) found a significant increased LFF measured through a double-pulse stimulation at 10 Hz. The main hypothesis for the increased LFF 24 h after the SIT session is related to the reduction in free myoplasmic  $Ca^{2+}$  or reduced myofibrillar  $Ca^{2+}$  sensitivity. Indeed, it has been shown that muscle biopsies from recreationally active men taken 24 h after completing a  $3 \times 30$  s SIT exhibited a large fragmentation of the ryanodine receptor 1 protein complex (RyR1), which is the main channel of  $Ca^{2+}$  release from the sarcoplasmic reticulum (Place et al., 2015). From this point, a less effective  $Ca^{2+}$  handling would, ultimately, lead to a force depression at low stimulation frequencies due to the sigmoidal shape of the force-calcium relationship.

Altogether, these results show that i) SIT generates a significant amount of neuromuscular fatigue closely related to peripheral mechanisms; ii) the restoration of the isometric force and the contractile properties of the muscle at rest, seem to be dependent on a combination of factors which could explain the differences between studies. Further research is warranted to determine whether the neuromuscular system is recovered 24 h after SIT. Furthermore, the latest research on SIT recommends shortening of the sprint length since short sprints have been shown to lead to similar physiological adaptations than the classic 30 s all-out efforts. However, whether such shortened SIT models lead to shorter recovery times is still unknown.

### ***2.5.2. Cardiac autonomic recovery***

The heart rate variability (HRV) that is the fluctuation in the time intervals between adjacent heartbeats, has been shown to be a useful technique to assess the recovery process after physical exercise. It indexes neurocardiac function since it is generated by heart-brain interactions and non-linear autonomic nervous system processes (Shaffer & Ginsberg, 2017). In brief, the balance between the sympathetic and the parasympathetic nervous system activities is known to reflect deviations in homeostasis. Although these deviations in HRV are known to be influenced by pathological, physiological, psychological, environmental, lifestyle and genetic factors (Tiwari et al., 2021), repeated and standardized measurements within the same individual have been shown to be appropriated for measuring exercise-mediated changes.

For example, Seiler et al. (2007) demonstrated that an exercise session consisting of continuous pedaling above the second ventilatory threshold prolonged the recovery of various HRV indices to a greater extent than a session performed below the first ventilatory threshold in trained individuals. These results suggest that the greater the exercise intensity, the larger the disturbances to HRV will be. Although the relationship between exercise intensity and post-exercise cardiac autonomic recovery needs to be further studied, greater exercise intensities have been found to be correlated with longer recovery times after HIIE (Michael et al., 2017; Stanley et al., 2013). From a mechanistic point of view, the effect of intensity on HRV recovery is known to be associated with the amount of non-oxidative energy contribution and the subsequent stimulation of the muscle metaboreflex through the accumulation of metabolites such as lactate and inorganic phosphate (Buchheit et al., 2007). Based on this assumption and on the data available, at least 48 h of rest have been recommended after a HIIE session (Stanley et al., 2013). However, this explanation might be true for HIIE sessions but not for the whole high-intensity spectrum. From all the studies reviewed by Stanley et al. (2013), only one performed an actual SIT or, at least, an all-out protocol. Niewiadomski et al. (2007) conducted a Wingate-based intervention that did not result in a great HRV disturbance the day(s) after performing two consecutive 30 s sprints. This suggests that although HRV recovery after HIIE or exercise above the second ventilatory threshold might get prolonged, little is known about the HRV recovery after SIT. A recent investigation including the comparison of two SIT sessions consisting of  $2 \times 30$  s and  $4 \times 30$  s aimed to determine the effects of increased high intensity volume on cardiac autonomic recovery (Ye et al., 2022). They recruited fifteen young

recreationally active males and made them perform both SIT sessions and a non-exercise control trial. Although the  $2 \times 30$  session tended to show a faster immediate recovery than the  $4 \times 30$  s, no differences were observed between sessions during the nocturnal HRV readings. These results suggest that the cardiac autonomic recovery occurs faster after SIT since, from the first night after the exercise ( $\sim 10$  h from the exercise session), all subjects presented values similar to baseline. During the trial, the group presented stable measures from the baseline to the night after the exercise. These results seem promising because SIT would represent an efficient training method useful for delivering an intense with few detrimental effects. Given the fast recovery, another training session could potentially be placed the immediate next day after the SIT. However, further research regarding the recovery after a single SIT session is needed. The effects of SIT on females or the influence of shortened SIT models on the HRV have not been quantified yet.

## ***2.6. Effects of reduced-volume SIT programs on $\dot{V}O_{2max}$***

SIT has been shown to be a very effective method to improve overall physical function across several populations including young (Burgomaster et al., 2005) inactive (Metcalf et al., 2012), older (Adamson et al., 2020), obese (Whyte et al., 2010), type 2-diabetic (Jiménez-Maldonado et al., 2020) and cardiometabolic disease participants (Weston et al., 2014). The rapid and positive impact of SIT on  $\dot{V}O_{2max}$  can hold significant value for individuals with lower fitness levels. This is crucial as lower endurance fitness has been found to be powerful indicator of potential cardiovascular complications, mortality, and premature death (Ross et al., 2016). A growing body of research on SIT has aimed to optimize SIT protocols to maximize the increase in  $\dot{V}O_{2max}$  while minimizing the overall training volume or time required. For example, Richard's Metcalf and Niels's Volland research team have done a series of articles in which, within the same training program, a specific variable has been changed to study what was the impact on  $\dot{V}O_{2max}$  and other important outcomes. As such, the length and number of the sprints per session, the recovery periods, the resistive load, or the training frequency have been modified to find the most appropriated SIT protocol for a regular unfit population. This research has been impactful because, as long as the associated health benefits are not affected, reducing the total volume of sprint exercise could be expected to enhance the adherence to SIT (Reljic et al., 2019). In the following sections, the effects of different reduced-SIT protocols on  $\dot{V}O_{2max}$  will be reviewed. In Table 1, a general overview of the experimental design, training characteristics and main results of the SIT protocols proposed in the different studies that are discussed within this section is presented.

### ***2.6.1. Effects of reduced-sprint time and/or number of sprints on $\dot{V}O_{2max}$***

Hazell et al. (2010) was the first to examine the effects of significantly reducing the total sprint time in more detail. The study evaluated whether two modified 10 s SIT models could generate the same aerobic and anaerobic adaptations than the classic and effective 30:4 SIT over 2 weeks of training. Thus, they compared a group that performed the classic 30:4 protocol, with two others that performed 10 s of sprints followed by four and two minutes of recovery respectively (10:4 and 10:2). Importantly, the number of repetitions was kept the same for all the SIT models (it progressed from 4 to 6 repetitions within the two weeks of training). The results showed significant increases in  $\dot{V}O_{2max}$  of 9.5% and 8.5% for the for the 30:4 and the 10:4. The 10:2

group also improved significantly (4.2%). However, the increase in  $\dot{V}O_{2\max}$  was statistically smaller compared to the other two conditions. The authors suggested that the generation of a high peak power output during the first few seconds of each sprint was more likely responsible for the SIT adaptations than the total work completed and that therefore, there was no need to perform the longer intervals. These results were important since they show that the total work time can be substantially reduced without compromising training adaptations. Moreover, the same study reported that participants found that the shorter protocols were easier to perform, which could have a significant impact on training adherence when it comes to a SIT program (Hu et al., 2022). Subsequently, more research has been conducted to study the effects of different SIT protocols across a variety of populations. For example, Metcalfe et al. (2012) assessed the effects of a six-week SIT protocol termed “REHIT” (Reduced Exertion High Intensity Training) on the insulin sensitivity among other outcomes. A comparison between a classic SIT and a REHIT session is displayed in Figure 3. Participants trained three times a week and the duration of the sprints increased from 10 s in week 1, to 15 s in weeks 2 and 3, and 20 s in the final 3 weeks. The sessions included only two sprints, as the authors aimed for decreasing substantially the total training time and making the sessions less strenuous and more applicable to a largely sedentary population. The REHIT protocol led to important gains of 15% and 12% in  $\dot{V}O_{2\max}$  for males and females respectively after six weeks of training. Remarkably, the insulin sensitive only improved in males. Four years later, the REHIT protocol was applied to a larger cohort to study the differences between sedentary males and females on the insulin sensitivity. The results showed a larger inter-individual difference but no significant differences between sex. Moreover, both groups improved their  $\dot{V}O_{2\max}$  by +10.1% and 9.3% in males and females, respectively. These two studies extended the previous literature showing the beneficial effects of SIT but reinforcing the fact that the sprint length and number could be substantially reduced whilst still maintaining positive effects.

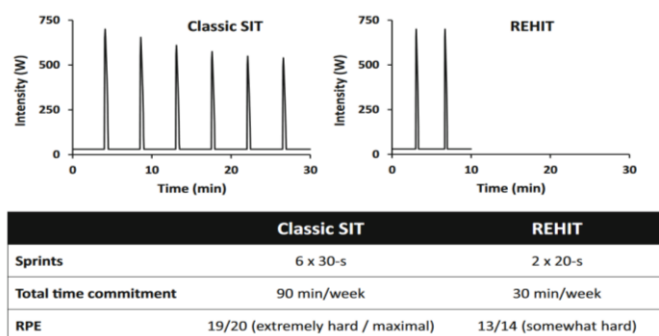


Figure 3. Comparison of the classic SIT protocol with the novel REHIT protocol. Reproduced from Metcalfe et al. (2012).



Other studies have compared the effects of traditional endurance training versus two SIT protocols: a classic 30:4 and a new 15:4.75 min of active recovery for four weeks (Zelt et al. 2014). The results showed no differences between the three protocols, evidencing again that SIT protocols with reduced sprint time could be as effective as the classic 30:4 protocols and even as effective as the regular endurance training. Yamagishi & Babraj (2017) compared the effects of the classic 30:4 with a 15:2 model involving an active recovery in several outcomes for nine weeks against a control group. The particularity of this study was that their protocol lasted for 9 weeks, which is considerably more time than usual (2 to 6 weeks). The results showed that halving the total training time did not negatively impact the magnitude of adaptations. Specifically, the  $\dot{V}O_2\text{max}$  was not different between groups (~17%). Surprisingly, although the protocol involved progressive increases in the external load during the nine weeks, the  $\dot{V}O_2\text{max}$  plateaued after the first three weeks of training. These findings suggest that SIT can be a very effective training method for rapidly boosting the general fitness in a sedentary population, but that some other form of physical training might be needed after some weeks of SIT.

A study conducted by Songsorn et al. (2016) evaluated whether a single 20 s sprint per session was enough to increase  $\dot{V}O_2\text{max}$ . The training involved three exercise sessions per week for 4 weeks, consisting of a single 20 s Wingate sprint, without any prior warm-up or cool-down. Although there was a slight average increase of 3%, the authors concluded that a single 20 s bout per session does not provide a sufficient training stimulus for developing significant adaptations in  $\dot{V}O_2\text{max}$  in moderately active males and females. These gains were low compared to the original REHIT protocol in which the mean percent increase was 14%. Finally, Nalçakan et al. (2018) involved thirty-six participants to test if a reduced REHIT protocol involving only 10 s sprints could be as effective as the 20 s program originally proposed by Metcalfe et al. (2012). Six weeks of training led to a 4% increase in  $\dot{V}O_2\text{max}$  for the group that performed the REHIT of 10 s. In contrast, the group that performed the original REHIT with 20 s sprints, improved their  $\dot{V}O_2\text{max}$  by 10%, showing that reducing the sprint length, in this case, significantly attenuated the efficacy of the program. Moreover, the authors demonstrated that reducing the sprint length from 20 to 10 s did not lead to more positive affective responses, changes in the mood state post-exercise or reduce the ratings of perceived exertion. This suggests that 20 s sprints can be an optimal protocol from the psychological and from the psychological point of view. These findings combined with those from Songsorn et al. (2016) help to understand to which extent the number and/or the length of sprints can be reduced to

determine the optimal all-out protocol for increasing  $\dot{V}O_{2\max}$  while minimizing the high level of physical exertion. Finally, the REHIT protocol was taken out of the laboratory to test its effects in an unsupervised workplace setting. The results showed that the protocol was feasible and helped participants to improve their  $\dot{V}O_{2\max}$  by 7.4%. Moreover, they assessed the task self-efficacy, enjoyment and acceptability of the intervention with very positive results for the proposed training regimen.

A meta-analysis including 34 studies to determine the effect of the number of sprints in a SIT session on the changes in  $\dot{V}O_{2\max}$  observed small negative effect of the maximum number of sprints in a training session (Vollaard et al., 2017). This suggested that more sprints were not necessarily connected with greater gains in  $\dot{V}O_{2\max}$  and had important implications for the design of practical SIT interventions. The authors proposed that the increased blood volume and increased mitochondrial density could plausibly be explained by the rapid glycogen depletion associated with supramaximal exercise. Indeed, in a previous experiment they showed that the high glycogen depletion during SIT could be closely related to mechanisms like a decrease in plasma volume and the release of AMPK that may promote changes in both total blood volume and mitochondrial density through PGC1- $\alpha$ . However, these findings were recently contrasted by another meta-analysis by Hall et al. (2023). Their work showed a positive effect for greater training volume. The authors defined training volume as an increment in the length or the number of the sprints, as both resulted on an increased total training time. They stated that more and longer sprints would result in greater disturbances of the metabolic environment, potentially increasing the response to training and therefore, the magnitude of adaptations. Importantly, the authors argued that within a SIT program, volume could be increased throughout different approaches (i.e. sprint time, number of sprints per session, training frequency, weeks of training) and this was difficult to assess and analyze between studies, and that this could potentially influence the results. Hall et al. (2023) seems to be the only reference directly supporting the idea that longer sprints would lead to greater adaptations and, therefore, further research is needed to elucidate the topic. To summarize, the presented results show that i) the number and the length of repetitions during SIT can be substantially reduced without attenuating the magnitude of physiological adaptations and ii) the “shortened-SIT” models may represent a better approach to introduce all-out exercise to a non-fit population.

### ***2.6.2. Effects of training frequency and work-to-rest ratio on $\dot{V}O_{2max}$***

As outlined in Figure 1 (section 1.2), other variables like training frequency and the work-to-rest (W:R) ratio are key factor that can very likely influence the training adaptations to HIIE or SIT. Therefore, they should be considered prior to the start of a training regimen. The changes in the intensity and duration of work and rest intervals are known to alter the relative demands of metabolic pathways (Holloszy & Coyle, 1984). Given the all-out nature of SIT, this fact is of great importance because the duration of the recovery period could highly influence the overall intensity of the session. Remarkably, for a given sprint duration, a shorter recovery time between repetitions has been shown to lead to decrements in performance in the subsequent exercise bout (Selmi et al., 2016). The most studied SIT protocol consists of 4 to 6 repetitions of 30 s separated by 4 minutes of recovery (Burgomaster et al., 2005; MacDougall et al., 1998). That leads to a W:R ratio of 1:8, which, given the metabolic and muscular demands of a 30 s all-out effort, is usually enough for allowing a complete recovery. However, as for the length of the recovery, very few studies have directly investigated the impact of different W:R ratios on the physiological adaptations and performance outcomes. Moreover, some of those studies did an RST rather than a SIT protocol in which, inherently, the recovery times are shorter due to a reduced sprint time.

For example, Hazell et al. (2010) compared the effects of the classic 30 s protocol against two reduced-SIT protocols consisting of 10 s repetitions with 2 and 4 minutes of recovery respectively. That lead to a WR ratio of 1:8 for the 30 s protocol and to 1:12 and 1:24 for the other to SIT protocols. The results showed significant increases in  $\dot{V}O_{2max}$  and other more performance-related outcomes for the 1:8 and the 1:24 groups, and smaller but still significant increase for the 1:12 group. The authors did not specifically address the question of the W:R ratio, but they argued that adaptations occurred in all groups because the mean power during the 1:12 and 1:24 was significantly higher than during the 1:8 and therefore, the differences in sprint time were offset. Lloyd Jones et al., (2017), observed that both short (6 s) and long (30 s) sprints, when matched for a 1:8 work-to-rest ratio and a total sprint duration of 2 minutes, yielded similar effects on endurance and sprint performance adaptations after a 2-week period. Surprisingly, although the authors reasoning behind the performance adaptations relies on positive changes in the enzymatic activity, the  $\dot{V}O_{2max}$  did not change for any of the groups. In a study conducted by Olek et al. (2018), the effects of different W:R ratios on aerobic and

anaerobic measures were compared. A group followed a 10 s sprint with 1 min of recovery while the other did 10 s with 4 minutes of recovery. Unlike the findings of Lloyd Jones et al. (2017), who did not observe changes in  $\dot{V}O_2\text{max}$ , Olek et al. (2018) discovered significant improvements in  $\dot{V}O_2\text{max}$  after only two weeks of SIT, with a total of six sessions spread over two weeks. The increase in  $\dot{V}O_2\text{max}$  was slightly greater in the 1:6 W:R group (13.6%) compared to the 1:24 W:R group (11.9%), but the difference between the groups was not statistically significant. The comparable changes in  $\dot{V}O_2\text{max}$  in both groups were attributed to increases in the CS activity, which remained unaffected by the recovery duration. Additionally, the study authors also noted a significantly enhanced power output at the end of a 30 s Wingate test when the recovery time between bouts was reduced (1:6 W:R vs. 1:24 W:R), indicating greater fatigue resistance during maximal efforts. Finally, La Monica et al. (2019) that tested the effects of upper-body SIT with the same groups than Hazell et al. (2010) and therefore, with the same W:R ratios, observed significant gains in  $\dot{V}O_2\text{max}$  in the 1:8 group while the 1:12 and 1:24 group did not significantly improve. The authors argued that given the differences between the upper and lower body musculature, the upper body may benefit from longer sprint times that provide more work to be accomplished during the training. Although there are more studies investigating the effects of modified W:R ratios on aerobic and anaerobic outcomes after sprint interval exercise, most of them are based on RST rather than SIT protocols. The reader is directed towards the works of Glaister (2005); Lloyd Jones et al. (2019); Monks et al. (2017); Ohya et al. (2013) and Shi et al. (2018) for further read.

When it comes to training frequency, the research team of Richard Metcalfe and Niels Volland reduced the number of training sessions per week to reduce the total volume after having reduced the sprint duration (Nalçakan et al., 2018) and the number of repetition per session (Songsorn et al., 2016). In the study of Thomas et al. (2020) it was compared whether reducing the training frequency to 2 sessions per week could attenuate the magnitude of the changes following their REHIT protocol. The results showed that reducing training frequency from 3 or 4 to 2 sessions/week did not attenuate the increases in  $\dot{V}O_2\text{max}$  after 6 weeks of training. Indeed, there were no between-group differences, but the group that did only two sessions per week, progressed slightly more (10.2%) than the group that did three (8.1%) and four (7.3%). Thus, as mentioned above, even though a greater training volume could be expected to further enhance adaptations, it appears that a low dose of all-out exercise is sufficient to stimulate the mechanisms by which SIT induces increases  $\dot{V}O_2\text{max}$ . The literature investigating the changes in training frequency specifically on SIT is scarce. However, Adamson et al. (2020)

demonstrated that one session of  $6 \times 6$  s all-out per week was sufficient to produce health benefits as shown by the increases in  $\dot{V}O_2\text{max}$  compared to the group that did a second weekly session in older adults. Also, the study of Stavrinou et al., (2019), reported that improvements in  $\dot{V}O_2\text{max}$  with twice-weekly HIIT ( $10 \times 60$  s cycling at  $\sim 83\%$  of peak power) were not significantly different from those with the same protocol performed three times/week. Remarkably, the authors assessed exercise enjoyment, and the intention to implement HIIT in the future concluding that both variables were well-maintained in both training programs. Although these two studies did not specifically assess the training adaptations to SIT, they are good examples of how training volume during HIIE exercise can be reduced without reducing the magnitude of adaptations.

### ***2.6.3. Effects of program length on $\dot{V}O_2\text{max}$***

An important question that arises when implementing a SIT program is the total length in weeks. Typically, a positive relationship between the number of weekly hours of training and the changes in  $\dot{V}O_2\text{max}$  can be established (Gilliat-Wimberly et al., 2001; Gim & Choi, 2016; Scribbans et al., 2016). Although the development of adaptations through physical training might take several months or even years for an unfit population, Burgomaster et al. (2008) provided evidence for robust and comparable metabolic adaptations when 6-weeks of SIT were compared against conventional CT, despite markedly reduced total training volume in the SIT group (66% less time, 90% less energy expenditure). These results suggest that for training methods like HIIE or SIT in which the total volume is inherently low and the intensity high, the relationship between the training volume and the magnitude of the adaptations might be somewhat different when compared to other disciplines such as endurance or resistance training. However, since most of the physiological adaptations to SIT have been shown to occur very early from the training onset (Burgomaster et al., 2005), many studies have only focused on the short-term adaptations going from two (Burgomaster et al., 2005; Hazell et al., 2010; Koral et al., 2018) to four (Raleigh et al., 2018; Willoughby et al., 2016) to six (Burgomaster et al., 2008; Gibala et al., 2006; Metcalfe et al., 2012) weeks of training. As a consequence, the literature specifically aiming to determine the time course of the physiological adaptations to SIT as a function of the program length is scarce. Indeed, only four studies aimed to do so or included a discussion on it (Bostad et al., 2021; Burgomaster et al., 2008; Gillen et al., 2016; Yamagishi & Babraj, 2017). Investigating on this concrete research question implies several testing points and,

consequently, reader's attention is drawn to the fact that the Pre-Post designs determining the effects of SIT for a given number of weeks will not be reviewed in the current section. This decision has been made to avoid the bias introduced by the methodological differences between studies that did not assess the time course of the adaptations. Therefore, a summary of the results of the studies together with meta-analyses and review papers that specifically address the effects of the program length will be given here.

The protocol from Burgomaster et al. (2008) involved a progressive overload from four to six 30 s repetitions per session during the six-week of duration of the training regimen. In other words, the external training load was increased over time. The intermediate measurement-point three weeks after the beginning of the training, revealed a percent change of  $\sim 7.3\%$  in  $\dot{V}O_{2\max}$ . Surprisingly, no significant differences were observed after this time-point, as the Post-training measurements the change in  $\dot{V}O_{2\max}$  from baseline resulted to be similar. The SIT group improved to the same extent than the endurance training group but with a much lower total time commitment. Interestingly, a similar study involving only  $3 \times 20$  s sprints per session conducted by the same research team, showed a significant change between the intermediate (six weeks) and the post-study measurements (twelve weeks) (Gillen et al., 2016). The percent changes in  $\dot{V}O_{2\max}$  were of  $\sim 12\%$  and  $\sim 19\%$  respectively. These results contrast those from the previous study since more training led to more adaptations and suggest that, even though the external training load was kept the same, a lengthened training program could induce greater adaptations. These results were confirmed by a more recent study conducted by Bostad et al. (2021). With the same protocol as Gillen et al. (2016), they reported a 8% gain after two weeks followed by a non-significant increase of 15% and 21% after six and twelve weeks. Finally, a study that also included a shortened-sprint model group (15 instead of 30 s) and a control group, reported that most of the gains in  $\dot{V}O_{2\max}$  occurred within the first three weeks of the training protocol (Yamagishi & Babraj, 2017). The training regimen included increases in training load and went up to nine weeks of training with intermediate measurements at the third and sixth week. The average percent gain for the 15 and the 30 s sprints groups was of 16.6 and 17% at three weeks respectively with no between-groups differences. Then, at the nine-weeks' time point the  $\dot{V}O_{2\max}$  went down to 12.1 and 12.8% respectively, compared to baseline. These results show that first, most of the gains occurred in the first three weeks of training and secondly, that there was a plateau in  $\dot{V}O_{2\max}$  gains with time. Importantly, although  $\dot{V}O_{2\max}$  tended to plateau, other performance-related outcomes like the time to exhaustion kept improving until the end of the nine weeks of training, highlighting that other variables still

benefited from SIT. The authors suggested that the training stimulus might need to be altered through different strategies to see continuous cardiorespiratory improvements.

Overall, the presented studies show contrasting results regarding the effects of the program length on  $\dot{V}O_2\text{max}$ . Despite regular increases in external training load, Burgomaster et al., (2008) and Yamagishi & Babraj (2017) observed a plateau three weeks after the beginning of the training. Then, Bostad et al. (2021) and Gillen et al. (2016) that maintained the exact same  $3 \times 20$  s with two minutes of recovery throughout the twelve weeks of training, showed a greater increase in  $\dot{V}O_2\text{max}$  at the end compared to the six-week measurement point of the training program. Importantly, four meta-analyses on the topic including a great number of studies, reported trivial, unclear or no changes when performing the analyses for determining the effects of the length of the SIT intervention on the magnitude of  $\dot{V}O_2\text{max}$  improvement (Gist et al., 2014; Sloth et al., 2013; Vollaard et al., 2017; Weston et al., 2014). However, and as mentioned above, most of the studies included in these meta-analyses did not specifically aim to determine the time course of the adaptations and therefore, these results should be interpreted with caution. Moreover, the studies of Bostad et al. (2021), Gillen et al. (2016) and Yamagishi & Babraj (2017) were published after these meta-analyses, suggesting that the latest literature reviewing the time course of the adaptations was not taken into account for the results. Altogether, these results seem to indicate that the length of the training program does not play an important role because most of the adaptations occur early. However, the results (Bostad et al. (2021) and Gillen et al. (2016) indicate that further research is required for elucidating whether or not the training program length plays a role in the time course of the  $\dot{V}O_2\text{max}$  within a SIT program.

## ***2.7. Summary***

This chapter focused on the significant concepts and ideas associated with the research conducted on SIT and all-out exercise. Overall, SIT has been shown to be a very efficient training method for improving key aerobic and anaerobic outcomes. The mechanisms behind these improvements are related to a series of central and peripheral adaptations like the increase in blood volume and mitochondrial biogenesis, enhanced enzymatic activity and muscle substrate storage, as well as the capacity to resist the effects of the build-up of metabolic by-products. Moreover, changes in muscle fiber type and size have been observed following all-

out sprint protocols. However, further SIT-specific research is needed to link these neuromuscular adaptations with the increases in mean and peak power output, repeated sprint ability, fatigue resistance, and isometric force. Additionally, whether or not the neuromuscular system is still impaired 24 h after a SIT session, remains unclear based on the results of previous studies in the topic. The autonomic nervous system seems to recover quickly after SIT, although additional research is needed since most of the studies have analyzed the effects of HIIT rather than SIT. Finally, the findings from the studies that investigated the different ways to reduce volume during SIT indicate that: i) the number and the length of the sprints and the training frequency could be lower than the classical 4-6 × 30 s protocol without attenuating the adaptations; ii) when respecting the same W:R ratio on a reduced-sprint protocol, similar gains are achieved; and iii) the effects of the program length on  $\dot{V}O_{2\max}$  are still unclear.



Table 1. Summary of the studies analyzing the effects of reduced-volume SIT protocols on  $\dot{V}O_2\text{max}$

Reference	Population characteristics (sample size; descriptor of fitness level; groups (if more than one), relative $\dot{V}O_2\text{max}$ )	Study goal	Intervention duration (weeks)	Frequency (sessions / week)	Number of sprints	Sprint length (s)	Recovery Duration (s)	$\dot{V}O_2\text{max}$ improvements (%)
Metcalf 2012	n=29; sedentary and healthy young males; 2 groups: control ( $38 \pm 2.7$ ) and REHIT ( $36 \pm 2.2$ )	To investigate the effects of a reduced-exertion SIT intervention on insulin sensitivity in previously sedentary subjects.	6	3	2	10 to 20 s (increased progressively)	200-220	+15% in men +12% in women
Metcalf 2016	n=30, sedentary; 2 groups: men ( $38.6 \pm 8.3$ ) and women ( $31.6 \pm 4.6$ )	To examine the effects of REHIT on $\dot{V}O_2\text{max}$ and OGTT-derived insulin sensitivity in a larger cohort of men and women.	6	3	2	10 to 20 s (increased progressively)	200-220	+10.1 in men +9.3 in women
Nalçakan 2018	n=36; sedentary/recreationally active; 2 groups: REHIT <sub>10s</sub> ( $34 \pm 9$ ) and REHIT <sub>20s</sub> ( $39 \pm 7$ )	To test the effects of a reduced sprint length on the magnitude of physiological adaptations.	6	3	2	REHIT <sub>10s</sub> : 10 s sprints REHIT <sub>20s</sub> : 20 s sprints	200-220	REHIT10: +4% REHIT 20: +10%
Thomas 2020	n=42; healthy and inactive; 3 groups: REHIT 2, REHIT 3 and REHIT 4 sessions a week	Determine the effect of training frequency on changes in $\dot{V}O_2\text{max}$ in response to a REHIT protocol in inactive individuals.	6	2, 3 or 4	2	10 to 20 s (increased progressively)	200-220	REHIT 2: +10.2% REHIT 3: +8.1% REHIT 4: +7.3%
Metcalf 2020	n=25; sedentary and healthy; 2 groups control ( $28 \pm 7$ ) and REHIT ( $28 \pm 7$ )	To investigate the acceptability and effectiveness of REHIT when applied in an unsupervised workplace setting.	6	2	2	10 to 20 s (increased progressively)	180	REHIT: +7.4 Control: -2.3%
Hazell 2010	n=48; young and recreationally active students; 4 groups: SIT <sub>30:4</sub> (~47), SIT <sub>10:4</sub> (~45), SIT <sub>10:2</sub> (~46), control (~45)	To compare the established, effective SIT protocol (30 s “all-out” efforts with 4 min recovery) versus two modified types of SIT (10 s “all-out” efforts with either 4 min or 2 min recovery) on both aerobic and anaerobic performance.	2	3	4-6	SIT <sub>30:4</sub> : 30 s SIT <sub>10:4</sub> : 10 s SIT <sub>10:2</sub> : 10 s	120 to 240	SIT <sub>30:4</sub> : +9.5% SIT <sub>10:4</sub> : +8.5% SIT <sub>10:2</sub> : +4.2% (not significantly different from Pre) Control: no change
Zelt 2014	n=36; recreationally active; 2 groups: SIT <sub>30:4.5</sub> (~48), SIT <sub>15:4.75</sub> (~43)	The purpose of this study was twofold: (1) to confirm that reductions in SIT work-interval duration do not result in reduced	4	3	4-6	SIT <sub>30:4.5</sub> : 30 s SIT <sub>15:4.75</sub> : 15s	SIT <sub>30:4.5</sub> : 270 s SIT <sub>15:4.75</sub> : 285	SIT <sub>30:4.5</sub> : +4% SIT <sub>15:4.75</sub> : +8%

		adaptations in aerobic and anaerobic capacity, and (2) to examine the effects of reduced work interval duration on submaximal determinants of exercise performance, namely lactate threshold and critical power.						
Yamagishi 2016	n=27; recreationally active; 3 groups: SIT <sub>30:4</sub> (40.6 ± 9.6), SIT <sub>15:2</sub> (42.2 ± 5.4) and control group (47.4 ± 7.9)	The purposes of the current study were twofold: to determine the effects of sprint duration (15 vs 30 seconds) on physiological and performance adaptations while matching sprint-to-rest ratio between training protocols (sprint: rest ratio of 1:8) and to determine the time course of those adaptations over 9 weeks (baseline, weeks 3, 6, and 9).	9	3	4-6	SIT <sub>30:4</sub> : 30 s SIT <sub>15:2</sub> : 15 s	SIT <sub>30:4</sub> : 240 s SIT <sub>15:2</sub> : 120 s	SIT <sub>30:4</sub> : +12.8% SIT <sub>15:2</sub> : +12.1%

### ***3. Chapter three: Neuromuscular and autonomic function is fully recovered within 24 hours following a sprint interval training session (Study 1)***

#### ***3.1. Introduction***

Sprint interval training (SIT) is an effective method to improve overall physical function in different populations including young (Burgomaster et al., 2005), inactive (Metcalf et al., 2012), old (Adamson et al., 2020), obese (Whyte et al., 2010), type 2-diabetic (Jiménez-Maldonado et al., 2020) and cardiometabolic disease participants (Weston et al., 2014). SIT is generally characterized by brief bouts of all-out exercise lasting ~10-30 s, performed against a fixed resistance, and interspersed by ~2-4.5 min of recovery (Gillen & Gibala, 2014). Although the 30 s all-out efforts interspersed with 4 min breaks have been widely used in various studies (Burgomaster et al., 2005; Gillen & Gibala, 2014; Whyte et al., 2010), recent research has shown that shorter sprints lead to more feasible and equally effective all-out exercise protocols for improving overall physical function (Vollaard et al., 2017; Vollaard & Metcalfe, 2017). In these instances, where the total work time is minimal, the all-out component of the effort plays a crucial role in initiating the physiological processes that enhance both aerobic and anaerobic energetic pathways (Hazell et al., 2010; Olek et al., 2018). For example, in response to a single SIT session, it has been shown that, despite the decrease in external power output with the succession of sprints, the all-out efforts resulted in a greater muscle O<sub>2</sub> extraction after each bout, which resulted in very high cardiorespiratory responses and blood lactate accumulations (Buchheit et al., 2012). Furthermore, a single 6 x 30 s session was sufficient to upregulate the muscle expression of genes involved in adaptations related to the endurance phenotype (Little et al., 2011). Additionally, as little as a 1 x 30 s all-out session could speed up the kinetics of O<sub>2</sub> consumption ( $\dot{V}O_2$ ), with a session of 3 x 30 s all-out upregulating the response for up to three days after the intervention (Iannetta et al., 2022). Although the beneficial effects of SIT are well-documented, the recovery time between SIT sessions, which is a key component for restoring homeostasis after physical exercise (Stanley et al., 2013) and optimizing exercise training prescription, is often not justified. Based on the idea that the recovery process might take longer depending on factors such as the accumulation of metabolites (Buchheit et al., 2011) or the dysregulation of reactive oxygen species generated during high-intensity exercise (Lamb & Westerblad, 2011), one to two rest days are typically given between SIT training sessions. This is because SIT results in a great accumulation of fatigue inducing metabolic by-products

due to a large use of the anaerobic energy-system. Thus, a delayed recovery could be expected, which could negatively impact adaptations to training (Burgomaster et al., 2005). However, a more precise assessment of the recovery time following a session of SIT needs to be carried out to optimize training programs and, ultimately, to enhance fitness and health-related outcomes in those adopting SIT.

An approach to evaluate recovery is the analysis of the neuromuscular function for determining fatigue after physical exercise. Specific to SIT, conflicting results have been observed. On the one hand, a significant drop in isometric force 24 h after the completion of a 3 x 30 s and 6 x 30 s session has been observed in young untrained males (Krusnauskas et al., 2020; Venckunas et al., 2019). Similarly, a significant increase in low frequency fatigue (LFF) was observed 24 h after performing a 3 x 30 s all out session in young females (Krusnauskas et al., 2018) and untrained males (Venckunas et al., 2019). However, since no further measurements were performed after 24 h, whether fatigue persisted for more than one day remained unknown. On the other hand Schlittler et al., 2019; Zanou et al., 2021 did not observe any significant reductions in isometric force or LFF 24 h after a 6 x 30 s SIT session. Furthermore, no studies have investigated the effects of shorter all-out sprints on neuromuscular function which could also be a determinant factor influencing recovery time. Another aspect to consider is that neuromuscular fatigue is usually measured during isometric tasks to isolate specific muscle groups. This is important as recent studies have shown that the magnitude of fatigue is affected by the characteristics of the task (i.e., dynamic vs. isometric measurements). This indicates that measurements in isometric and dynamic conditions might give different information about the individual's fatigue status (Koral et al., 2021). In this line, a 72 h recovery period was enough for both young and older participants to restore their peak power output after a 3 x 20 s all-out session (Yasar et al., 2019). However, no measurements were performed before the 72-h period, which did not allow to determine if recovery occurred before that point. Since SIT is principally performed on a cycle-ergometer, the capacity to develop force, velocity and power might provide additional useful information to practitioners to understand for how long traces of neuromuscular fatigue persists after a SIT session.

Another commonly used technique to assess the recovery process is by measuring heart rate variability (HRV). Since changes in cardiac autonomic activity are known to reflect a deviation from homeostasis, the measurement of HRV is an important and easy to access tool for evaluating the stress/recovery status. Reviews examining the cardiac parasympathetic

reactivation after exercise have reported that high-intensity exercise necessitated, in general, 48 h to allow parasympathetic activity to return to pre-exercise levels (Stanley et al., 2013). Furthermore, it has been reported that the higher the intensity of the exercise, the slower the post-exercise HRV recovery (Michael et al., 2017), highlighting the detrimental effects of the exercise intensity on recovery and subsequent performance. The extrapolation of these results to the recovery following a SIT session is not straightforward as those studies analyzed responses to high intensity interval training (HIIT), which imply much longer work and rest intervals (~ 45 s to 4 minutes) than SIT, and with the intensity being usually established as a percentage of the maximal responses to incremental testing instead of all-out efforts. In fact, a recent experiment with continuous monitoring of the time course of recovery of two different SIT sessions (i.e. 2 x 30 and 4 x 30 s) observed that the suppression of the cardiac parasympathetic activity was fully resolved within a resting period of about 10 h comprising nighttime (Ye et al., 2022). However, there is a need to confirm and further investigate whether SIT also including shorter all-out sprints generate disturbances of the autonomic nervous system lasting longer than hours after the exercise.

Previous works investigating recovery of less intense training methods such as HIIT have reported a delayed recovery up to one or two days after the exercise. Thus, the main aim of this study was to determine whether the neuromuscular and autonomic nervous systems would be impaired 24 h to 48 h after a single session of SIT. We hypothesized that SIT would imply a quicker recovery due to the much shorter total work time under which physiological and metabolic responses are exacerbated. A secondary goal of the study was to determine whether there were any neuromuscular or autonomic differences in the recovery time-course of two SIT protocols with the same work-to-rest ratio and total work time. In this case, as the total work time was the same, we hypothesized that no differences would be observed between protocols.

### ***3.2. Methods***

#### ***Participants***

Twenty-five (11 females) recreationally active participants (i.e., performing light physical activity) volunteered to take part in this study (age:  $24 \pm 5$  years; height:  $1.73 \pm 0.10$ ; body mass:  $73.4 \pm 16.7$  kg). All participants were free from neuromuscular or musculoskeletal

disorders and performed a medical check before enrolling the experiments. They were informed of the procedures and risks involved in the study and all provided written informed consent to participate. This experimental protocol was approved by the institutional medical ethics committee (IRB IORG0007394 Ref NCT05013021) and it was conducted according to the Declaration of Helsinki.

### ***Experimental procedures***

The experimental design included 4 visits and is outlined in Figure 4: i. a familiarization session to the neuromuscular evaluation, the power-force-velocity profile, and to SIT; ii. Pre SIT measurements and the SIT session; iii. the 24 h post SIT measurements (Post<sub>24h</sub>), and iv. the 48 h post SIT measurements (Post<sub>48h</sub>). A previously validated SIT protocol involving 4 x 30 s sprints and 4 minutes of recovery (SIT<sub>30:4</sub>) (1) was modified by shortening the sprint and the recovery times to 15 s and 2 minutes, respectively (SIT<sub>15:2</sub>). Then, the total repetitions were increased to 8 in order to maintain the same work-to-rest ratio. This decision was taken based on the previous findings suggesting that shorter sprints would lead to more feasible and equally effective all-out SIT programs (Vollaard et al., 2017; Vollaard & Metcalfe, 2017). However, to control for potential differences between the SIT<sub>15:2</sub> and the SIT<sub>30:4</sub>, a subset of the sample composed by eleven participants (8 males and 3 females) performed, in a randomized order, an additional SIT<sub>30:4</sub> session followed by the same experimental procedures aforementioned. Additionally, neuromuscular assessments were performed at 1, 2 and 8 minutes after the completion of the last sprint for both sessions on the eleven participants to characterize the immediate neuromuscular fatigue derived from the two SIT sessions (i.e. Post<sub>1min</sub>, Post<sub>2min</sub> and Post<sub>8min</sub>). All the sessions were performed in the same room under similar temperature and humidity conditions. Although all the sessions were aimed to be placed in the morning (9 to 12 h), 4 participants had to be tested in the afternoon (from 15 to 19 h) due to personal-, health- or work-related issues. The isometric chair (i.e. vertical displacement of the force sensor), the saddle height and the handlebar position in the cycle ergometer were adjusted for every participant and kept similar for the next sessions. The familiarization session comprised a warm up of a series of submaximal right leg knee extensors contractions in an isometric chair ergometer, 2 to 3 knee extensor maximal voluntary contractions, and 2 to 3 knee extensor maximal voluntary contraction with a complete neuromuscular assessment (see details below). Then, and after a 5-min warm-up at a fixed power (e.g., 100 W and 80 W for males and females, respectively), one 7 s sprint with a load equivalent to 0.5 N.kg<sup>-1</sup> of the participant's bodyweight

was performed and the procedures of the power-force-velocity profile were explained. Finally, 4 sprints of 15 seconds interspersed by 2 minutes of passive recovery were performed. At least one week was given between the familiarization session and the SIT sessions. In the second visit, the participants performed the same neuromuscular evaluation than in the familiarization, plus two 7 s sprints with 2 different loads and then, the corresponding SIT session. Finally, in the Post<sub>24h</sub> and Post<sub>48h</sub> visits, the same tests performed during the session two were administered to the participants. During the length of the study, participants were instructed to avoid strenuous physical activity and to keep their habitual nutritional and sleep routines.

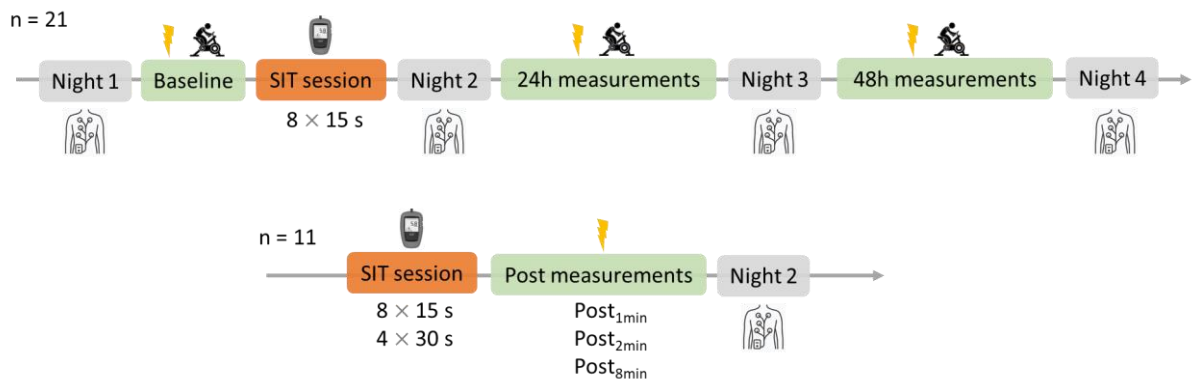


Figure 4. Experimental protocol of the study.

### ***Sprint Interval Training session***

The SIT session was performed on an instrumented Monark cycle ergometer (see below for more specifications about the device). The flywheel was mechanically braked with a resistance equivalent to 0.7 N per kg of bodyweight and participants were asked to perform an 8 x 15 s all-out session interspersed by two minutes of recovery between repetitions. Instructions to pedal as strong and as fast as possible throughout the duration of the sprint and strong verbal encouragement was given to the participants. These procedures were kept the same for the subset of the sample that performed the SIT<sub>30:4</sub> session. The power output (PO; in W) was recorded during the sprints. Then, the Maximal (PO<sub>max</sub>) and mean (PO<sub>mean</sub>) power output were obtained from every sprint and the total work (T<sub>work</sub>; in kJ) of each session was computed as follows:

$$T_{work}(kJ) = \sum Mean_{PO} \times 15 \text{ or } 30/1000 \quad (1)$$

Blood lactate concentration was measured prior to the sessions and 3 minutes after the completion of the last sprint of the session with a portable device (Lactate Scout 4; EKF Diagnostics, Cardiff, United Kingdom).

### *Assessment of the neuromuscular function*

Measures of neuromuscular function of the right knee-extensors were assessed at Pre, Post<sub>24h</sub> and Post<sub>48h</sub> with electrical stimulation of the femoral nerve during isometric maximal voluntary contractions (iMVC) and at rest. The cathode electrode (Kendall MediTrace) was placed on the inguinal triangle while the anode, a 50 × 90 mm electrode (Durastick Plus; DJO Global, Vista, CA) was placed on the gluteal fold. All stimuli were delivered through a constant current stimulator (DS7R, Digitimer, Welwyn Garden City, UK) using 2 ms square-wave stimuli. During contractions, the participants' ankles were attached with a non-compliant cuff to a load cell (Meiri F2732 200 daN; Celians, Montauban, France) fixed to a custom-built chair. Participants sat with hips and knees at 90° flexion and a non-compliant strap around the hips to avoid lower-limb movements. The neuromuscular assessment at Pre, Post<sub>24h</sub> and Post<sub>48h</sub> began with two practice iMVC's to ensure potentiation of subsequent evoked measures, followed by three 3 to 5 s iMVC's, all separated by one minute. During these 3 iMVC's, paired motor nerve stimulation at 100 Hz was delivered when peak force plateaued (super imposed doublet; SID) and 2 s after the iMVC (Db<sub>100</sub>). Then, 3 s after the contraction, a double pulse electrical stimulus at 10 Hz (Db<sub>10</sub>) was delivered. For the subset of the sample that was also evaluated right after the SIT sessions, only one 3 to 5 s iMVC and the nerve stimulation protocol was performed at each timepoint (i.e. 1, 2 and 8 minutes). All data were analyzed offline using Labchart software (v8, ADInstruments). iMVC was defined as the average of the highest 0.5-s window for each iMVC. Muscle voluntary activation (VA) was calculated using the following equation:

$$VA (\%) = \left(1 - \frac{SID}{Pt}\right) \times 100 \quad (2)$$

where SID is the amplitude of the superimposed twitch evoked by the stimulation during the force plateau and Pt is the amplitude of the resting twitch evoked by PNS. When the stimulations were given slightly before or after iMVC peak force value, a correction equation was applied according to (Strojnik & Komi, 1998). Finally, the ratio between the stimuli delivered after the iMVC (i.e. Db<sub>10</sub>/Db<sub>100</sub>) was used as a measure of low frequency fatigue.



### ***Assessment of the power-force-velocity profile***

All specifications of the used equipment are described in previous works (Arsac et al., 1996; Morin & Belli, 2004) . Briefly, a Monark cycle ergometer was equipped with a strain gauge (FN 3030 type; FGP Instrumentation, Les Clayes-sous-Bois, France) to measure the friction force and an optical encoder (100 pts/turn, Hengstler type RI 32.0; Aldingen, Germany) to quantify the flywheel displacement. Angular velocity and pedaling frequency were calculated from filtered displacement. Data were sampled at 200 Hz, recorded in LabVIEW software (NI, Austin, TX) and filtered with a fourth-order low-pass Butterworth filter at 12 Hz. The saddle height for the cycle ergometer was retrieved from the familiarization session and toe clips were well fastened to avoid foot displacement from the pedal. Participants started each 7 s trial with the right pedal at 45° from the vertical axis and were asked to remain seated for the duration of the measurement. After ensuring the departure position by visual inspection, a 3s countdown was launched and two 7-s resisted sprints interspersed by 2 minutes of recovery were performed. The load was fixed first to 0.5 N and then to 0.7 N and 0.3 N and 0.5 N of resistive force per kg of bodyweight for males and females respectively. For each sprint, participants were instructed to pedal as fast as possible and strong verbal encouragement was provided.

The friction force measured at the strain gauge ( $F_{friction}$ ), the force derived from the inertia of the wheel ( $F_{inertia}$ ) and the velocity of the flywheel ( $V$ ) were all considered for the PO calculations in Watts as initially proposed by Morin & Belli (2004).  $F_{inert}$  was obtained by the linear relationship between force and velocity described by free decelerations of the flywheel at different loads,  $V$  was derived from the flywheel displacement measured by the optical encoder. Therefore,  $P$  was computed considering the sum of both forces ( $F$ ) and  $V$  at each instant during the sprint as follows:

$$PO = (F_{friction} + F_{inertia}) \times V \quad (3)$$

An average value for  $F$ ,  $V$ , and  $P$  for each pedal downstroke was recorded allowing to obtain the linear ( $F$ - $V$ ) and the second-order polynomial ( $P$ - $V$ ) relationships that characterizes the maximal mechanical capabilities of the lower limb's neuromuscular system. With the equation obtained from the linear  $F$ - $V$  relationship of the loads, the slope ( $S_{FV}$ ) and the two intercepts with the axes could be then calculated for obtaining the theoretical maximum force at zero

velocity ( $F_0$ ; N) and the theoretical maximum velocity until which force could be produced ( $V_0$ ;  $\text{rad}\cdot\text{s}^{-1}$ ). From  $F_0$  and  $V_0$ , the maximal power output ( $P_{\max}$ ; W) was then calculated as follows:

$$P_{\max} = \frac{(F_0 \times V_0)}{4} \quad (4)$$

Using the methods described above,  $F_0$ ,  $V_0$ ,  $P_{\max}$  and the  $S_{fv}$  were then calculated for every participant by pooling together the two trials performed at every time point (i.e., both loads) (Figure 5). As the distribution of the F and V points and the slope of the FV equation could largely influence the intercepts with the axes (i.e.,  $F_0$  and  $V_0$ ) the maximal F, V and P values recorded by the strain gauge in each sprint were also kept for further analysis. The coefficient of determination ( $r^2$ ) of the equations obtained from the distribution of the F and V points was used for determining whether or not the 7 s sprint was appropriate enough for then extrapolating to the intercepts with the axes as the main outputs of the power-force-velocity profile (PFVP). In the cases in which the  $r^2$  of the equation was not superior to 0.9, the sprint was discarded and not used for the posterior statistical analysis. Four of the participants that performed only the SIT<sub>15-2</sub> systematically failed to reach a  $r^2$  due to uneven pedal strokes between the right and the left leg and therefore, only the data from 21 participants are presented in Table 1 for  $F_0$ ,  $V_0$ ,  $P_{\max}$  and the  $S_{fv}$ .

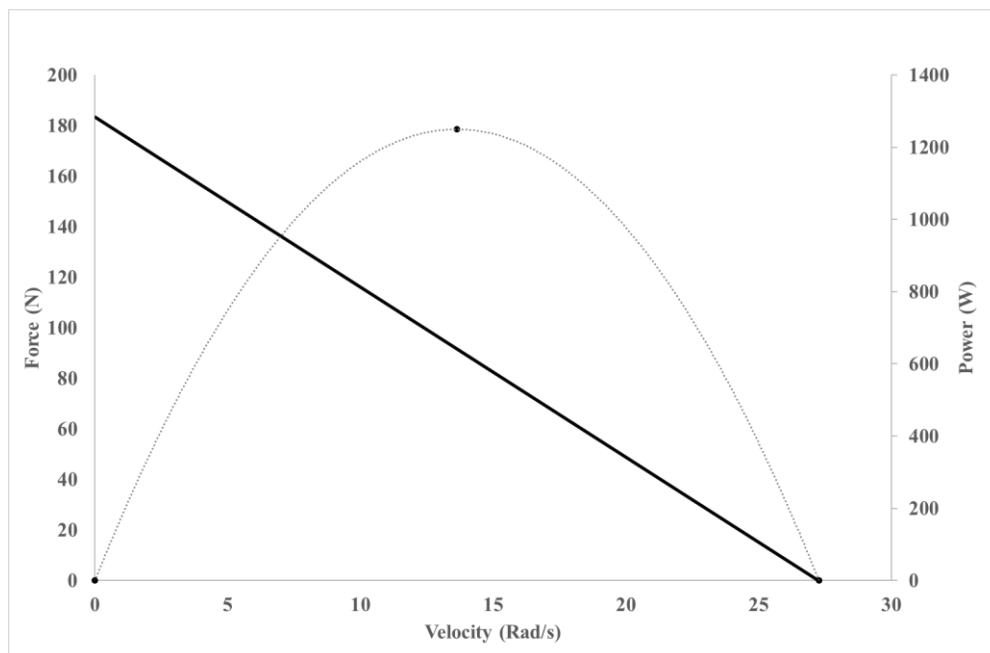


Figure 5. Representation of the Power-force-velocity profile for one participant

### *Assessment of the heart rate variability*

The heart rate variability was assessed using the NeuroCoach ECG-ambulatory system (NBATEchno, Saint-Jean Bonnefonds, France). A device was given to each participant at the end of the familiarization session. The functioning of the device and the placement of the electrodes (Kendall MediTrace) was carefully explained. The cathode electrode was placed on the left collar bone and the anode was placed on the nearest point to the 5<sup>th</sup> or 6<sup>th</sup> rib ensuring that the heart would be located between both electrodes. Participants were instructed to place the electrodes and turn on the device before going to bed, ensuring that the recording will cover several sleep cycles. The ECG recordings began on the night before the SIT session and continued for the following three nights. As for the neuromuscular and the PFVP assessments, the subset of the sample that did the additional SIT<sub>30:4</sub> session, the HRV was also measured. Participants were asked to draw a circle around the electrodes with an indelible ink marker to ensure the same night-to-night placement.

The ECG trace sampled at the frequency of 512 Hz was extracted from ECG-NeuroCoach ambulatory system and then exported to HRVAnalysis (HRVAnalysis v1.1, Saint-Étienne, France (Pichot et al., 2016). In the cases where, for technical problems, the recordings were not superior to four hours, the entire night was discarded to avoid biases generated by the influence of the sleep cycle on HRV. Then, each R peak was detected from the ECG to provide the R-R interval series. The missing beats, isolated extra-systoles, and artifacts were corrected using a spline cubic interpolation and the RR signal was then resampled at 2 Hz to obtain a regular sample base-time (Pichot et al., 2016). From this point, the mean heart rate and the mean R-R interval (average peak-to-peak R) were calculated and then the time-domain analysis was applied on the entire night-time to obtain the standard deviations of normal-to-normal (N-N) intervals (SDNN) and the root mean square of successive difference of N-N intervals (RMSSD). Additionally, the Fourier transform was used to obtain the following frequency-domain indices: low (LF: 0.04 –0.15 Hz) and high (HF: 0.15 –0.4 Hz) frequency power bands and the ratio between both (i.e. LF/HF), based on the recommendations of the Task Force (1996). The frequential analysis was applied on the mean of successive 5-min epochs for avoiding possible influences from the sleep cycle (Task Force, 1996). For each recorded night, the highest number of 5-min epochs without excluded R-R intervals or artefacts were automatically detected and processed by HRVAnalysis. Finally, SDNN was interpreted as an expression of the global cardiac autonomic activity and RMSSD and HF as an expression of parasympathetic activity.

Since both sympathetic and parasympathetic activity are known to contribute to LF, the LF/HF ratio was considered to reflect sympatho/vagal balance (Task Force, 1996).

### *Statistics*

Data are presented as mean values and standard deviations in the text and tables. Because of generally skewed distributions, all HRV variables but the LF/HF ratio were log-transformed (i.e. LnSDNN, LnRMSSD, LnLF, LnHF). Then, normal distribution of the data was checked using the Shapiro-Wilk normality test. Student paired t-tests were performed on the lactate measurements collected before and after the sessions. For all the other neuromuscular or HRV variables concerning the SIT<sub>15:2</sub>, one-way repeated measures analysis of variance was used to determine the effect of time. Then, the Tukey test was used as post-hoc when a significant effect was observed. For comparing the T<sub>work</sub>, lactate, neuromuscular and HRV data between the SIT<sub>15:2</sub> and the SIT<sub>30:4</sub>, a two-way repeated measures analysis of variance was used to determine whether there was any time, session (i.e., vs. SIT<sub>30:4</sub> SIT<sub>15:2</sub>), or time x session interaction effect. The Tukey post-hoc test was used if there was any significant main difference. All data were analyzed using the 9.4.0 version of Prism 2022 software (GraphPad Software, San Diego, CA) and the significance level for all statistics was set at  $p < 0.05$ .

### 3.3. Results

#### *Sprint interval training session*

$PO_{max}$  and  $PO_{mean}$  across the sprints for the SIT<sub>15:2</sub> are displayed in Figure 6. Significant gradual declines in both outcomes were found starting from the second ( $90.7 \pm 5.8$  and  $92.2 \pm 9\%$ ) to the last ( $69.2 \pm 22.2$  and  $75.9 \pm 9.6\%$ ) in comparison to the first sprint. No further significant differences were found for  $PO_{max}$  and  $PO_{mean}$  between the 4<sup>th</sup> and 5<sup>th</sup>, 6<sup>th</sup> and 7<sup>th</sup> and 7<sup>th</sup> and 8<sup>th</sup> sprint. The drops in PO were accompanied by a high accumulation of blood lactate which was of  $13.5 \pm 2.3$  mmol/L three minutes after the last sprint performed. The average  $T_{work}$  of the session for males and females was  $69.1 \pm 13.7$  and  $46.7 \pm 7.7$  kJ respectively.  $T_{work}$  for SIT<sub>15:2</sub> ( $65.9 \pm 15.1$  kJ) was significantly greater than for SIT<sub>30:4</sub> ( $56.8 \pm 10.9$  kJ) ( $p < 0.001$ ), but there was no significant difference for the lactate accumulation three minutes after SIT<sub>15:2</sub> and SIT<sub>30:4</sub> ( $15.0 \pm 1.7$  and  $16.0 \pm 1.5$  mmol/L, respectively).

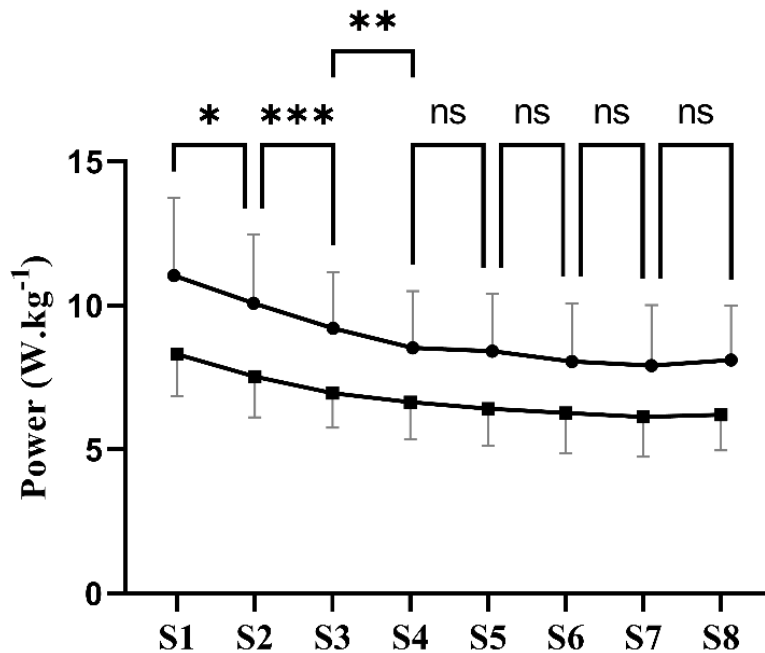


Figure 6. Maximal (circles) and mean (squares) power output  $\pm$  SD across the eight sprints of the SIT<sub>15:2</sub> session. For clarity, only differences from one sprint to the previous are displayed. Asterisks indicate a significant difference from the previous sprint for both maximal and mean power. \*, \*\* and \*\*\* stand for  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$  respectively.

## *Neuromuscular Function*

Table 2 and Figure 7 contain the isometric and the dynamic neuromuscular assessments performed during the iMVC's and the PFVP at Pre, Post<sub>24h</sub> and Post<sub>48h</sub>. No significant differences were found Post<sub>24h</sub> and Post<sub>48h</sub> for iMVC, VA, Db<sub>100</sub>, Db<sub>10</sub> or the Db<sub>10</sub>/Db<sub>100</sub> or for F<sub>0</sub>, V<sub>0</sub>, P<sub>max</sub> and S<sub>FV</sub> compared to Pre.

Table 2. Mean  $\pm$  SD for the neuromuscular assessments and power-force velocity profile at Pre, Post<sub>24h</sub>, Post<sub>48h</sub> after the SIT session.

	<i>Pre</i>	<i>Post<sub>24h</sub></i>	<i>Post<sub>48h</sub></i>
iMVC (N.kg <sup>-1</sup> )	7.96 $\pm$ 2.28	7.98 $\pm$ 2.34	8.20 $\pm$ 2.49
VA (%)	91.5 $\pm$ 6.2	90.9 $\pm$ 4.5	91.9 $\pm$ 5.3
Db100 (N.kg <sup>-1</sup> )	2.94 $\pm$ 0.68	2.95 $\pm$ 0.78	3.01 $\pm$ 0.52
Db10 (N.kg <sup>-1</sup> )	2.91 $\pm$ 0.64	2.77 $\pm$ 0.78	2.74 $\pm$ 0.77
Ratio Db <sub>10</sub> /Db <sub>100</sub>	0.94 $\pm$ 0.12	0.90 $\pm$ 0.16	0.92 $\pm$ 0.14
F <sub>0</sub> (N.kg <sup>-1</sup> )	1.88 $\pm$ 0.29	1.88 $\pm$ 0.29	1.90 $\pm$ 0.32
V <sub>0</sub> (rad.s <sup>-1</sup> )	25.1 $\pm$ 2.9	25.1 $\pm$ 2.4	24.8 $\pm$ 2.5
S <sub>FV</sub> (N.kg <sup>-1</sup> .rad.s <sup>-1</sup> )	-5.57 $\pm$ 1.37	-5.65 $\pm$ 1.54	-5.40 $\pm$ 1.40
P <sub>max</sub> (W.kg <sup>-1</sup> )	11.9 $\pm$ 2.3	11.9 $\pm$ 2.5	11.4 $\pm$ 2.7

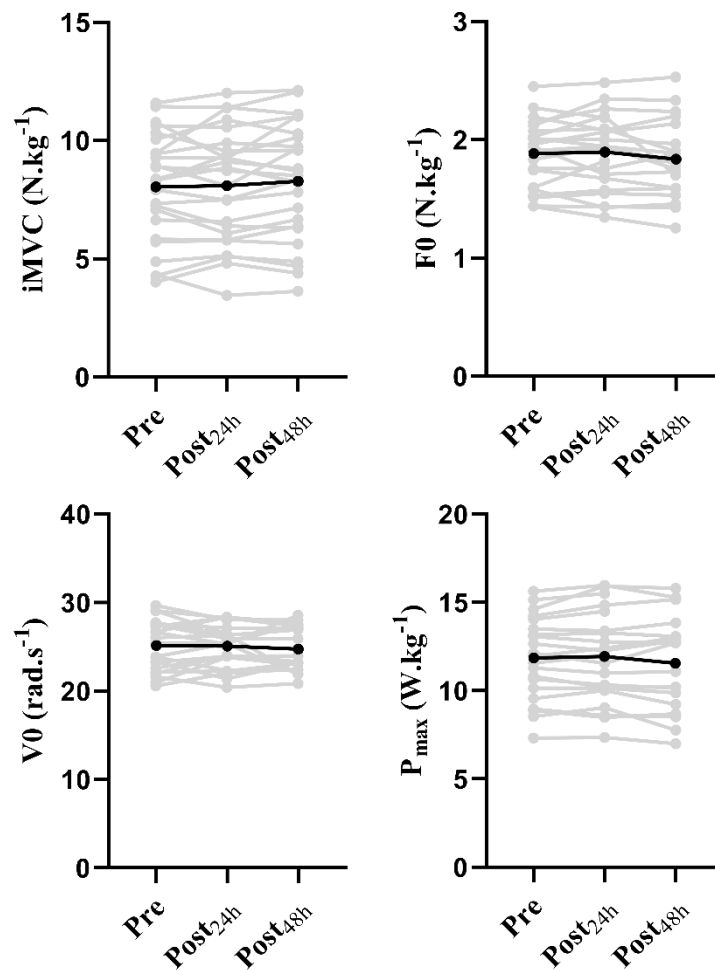


Figure 7. Mean and individual values for the isometric maximal voluntary contraction, maximal theoretical force and velocity and maximal power at Pre, Post<sub>24h</sub> and Post<sub>48h</sub>.

Figure 8 displays the original force-recordings from one of the participants during the neuromuscular assessment at Pre, Post<sub>1min</sub>, and Post<sub>8min</sub>, Post<sub>24h</sub> and Post<sub>48h</sub> for the SIT<sub>15:2</sub>. Figure 9 shows the results of neuromuscular assessments performed on the eleven participants who completed iMVC's evaluations at Pre, Post<sub>1min</sub>, Post<sub>2min</sub> and Post<sub>8min</sub>, Post<sub>24h</sub> and Post<sub>48h</sub> for both, SIT<sub>15:2</sub> and SIT<sub>30:4</sub>. No significant session or interaction effects were observed between sessions. A significant effect of time ( $p < 0.01$ ) was found at Post<sub>1min</sub>, Post<sub>2min</sub> and Post<sub>8min</sub> for both sessions for iMVC, Db<sub>100</sub>, Db<sub>10</sub> or the Db<sub>10</sub>/Db<sub>100</sub> in comparison to Pre, Post<sub>24h</sub> and Post<sub>48h</sub>. Despite the important declines observed for iMVC, Db<sub>10</sub> and Db<sub>100</sub> shortly after the sessions, none of those variables showed a time effect at Post<sub>24h</sub> and Post<sub>48h</sub> when compared to Pre. VA did not display a significant difference from Pre at any of the tested time-points.

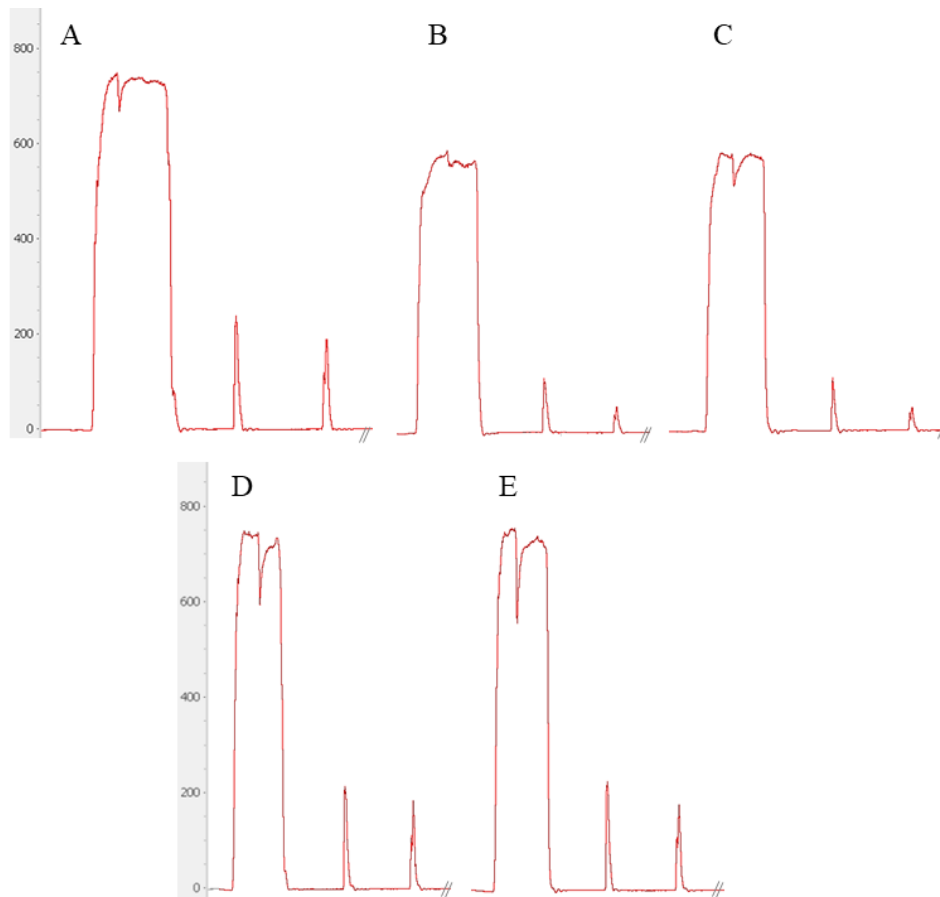


Figure 8. Original force traces from a single participant during the neuromuscular assessments of the SIT15-2 at Pre (A), Post<sub>1min</sub> (B), Post<sub>8min</sub> (C), Post<sub>24h</sub> (D) and Post<sub>48h</sub> (E).



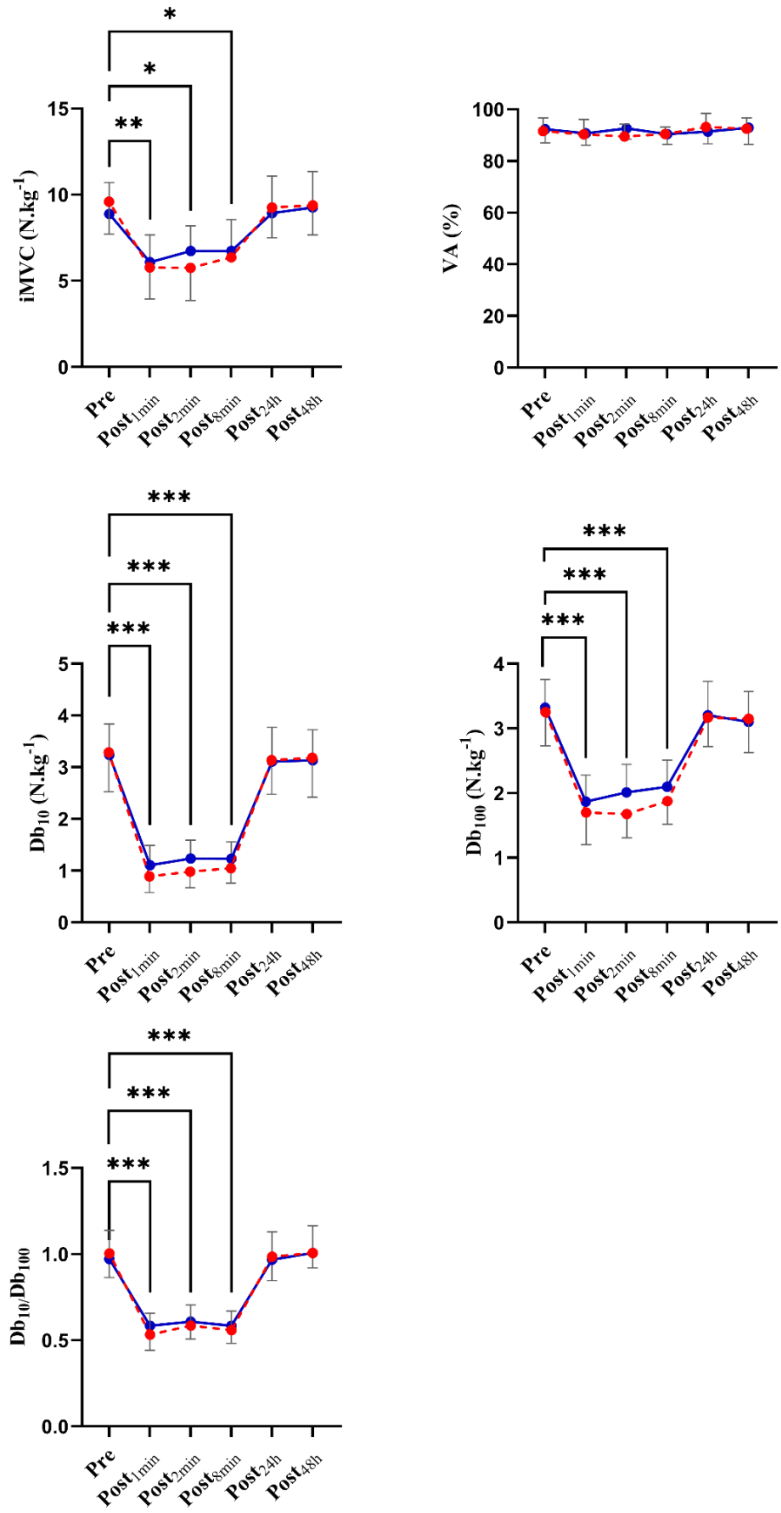


Figure 9. Mean and SD values for the isometric maximal voluntary contraction, voluntary activation, low frequency stimulation and ratio between low and high frequency stimulation at Pre, Post<sub>1min</sub>, Post<sub>2min</sub> and Post<sub>8min</sub>, Post<sub>24h</sub> and Post<sub>48h</sub> for the eleven subjects that performed immediate post-session neuromuscular assessments after SIT<sub>15:2</sub> (black lines) and SIT<sub>30:4</sub> (dashed lines). Only significant differences from Pre are shown. \*, \*\* and \*\*\* stand for  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$  respectively.

### ***Power-force-velocity profile***

Changes in the PFVP parameters for the participants that did the SIT<sub>15:2</sub> are presented in Table 1. No significant differences were found for  $F_0$ ,  $V_0$ ,  $P_{\max}$  and  $S_{FV}$  when comparing Pre to Post<sub>24h</sub> and Post<sub>48h</sub>. The comparison analysis between the SIT<sub>15:2</sub> and the SIT<sub>30:4</sub> did not reveal a significant time, session or interaction effect for  $F_0$ ,  $V_0$ ,  $P_{\max}$  or  $S_{FV}$ .

### ***Heart rate variability***

The night time, the average heart rate and the changes for the time- and frequency-domains of HRV parameters for the participants that performed the SIT<sub>15:2</sub>, are presented in Table 2 and Figure 10. The electrodes of four participants session came off during the night, noising the signal and reducing the length of the recording time available for analysis. Therefore, data from twenty-one participants are presented in the Table 3 and Figure 10. No significant differences were found at Post<sub>24h</sub> and Post<sub>48h</sub> in comparison to Pre for any of the studied variables. Additionally, no significant effect of time, session or interaction effect was found when comparing SIT<sub>15:2</sub> and the SIT<sub>30:4</sub>.

Table 3. Mean  $\pm$  SD of heart rate and the temporal and frequential HRV parameters.

	<i>Night 1</i>	<i>Night 2</i>	<i>Night 3</i>	<i>Night 4</i>
Night time (hours:minutes)	6:48 $\pm$ 1:41	6:46 $\pm$ 1:55	6:48 $\pm$ 2:05	6:47 $\pm$ 2:06
Heart Rate (beats per minute)	60 $\pm$ 9	61 $\pm$ 8	60 $\pm$ 9	63 $\pm$ 10
Ln RMMSD (ms)	4.20 $\pm$ 0.35	4.14 $\pm$ 0.45	4.21 $\pm$ 0.48	4.08 $\pm$ 0.48
Ln SDNN (ms)	4.83 $\pm$ 0.27	4.73 $\pm$ 0.33	4.79 $\pm$ 0.29	4.75 $\pm$ 0.39
Ln HF (ms <sup>2</sup> )	6.82 $\pm$ 0.67	6.71 $\pm$ 0.88	6.86 $\pm$ 0.80	6.75 $\pm$ 0.77
Ln LF (ms <sup>2</sup> )	7.57 $\pm$ 0.46	7.58 $\pm$ 0.65	7.65 $\pm$ 0.56	7.48 $\pm$ 0.50
LF/HF	2.03 $\pm$ 0.75	2.26 $\pm$ 0.95	2.27 $\pm$ 0.92	2.15 $\pm$ 0.91

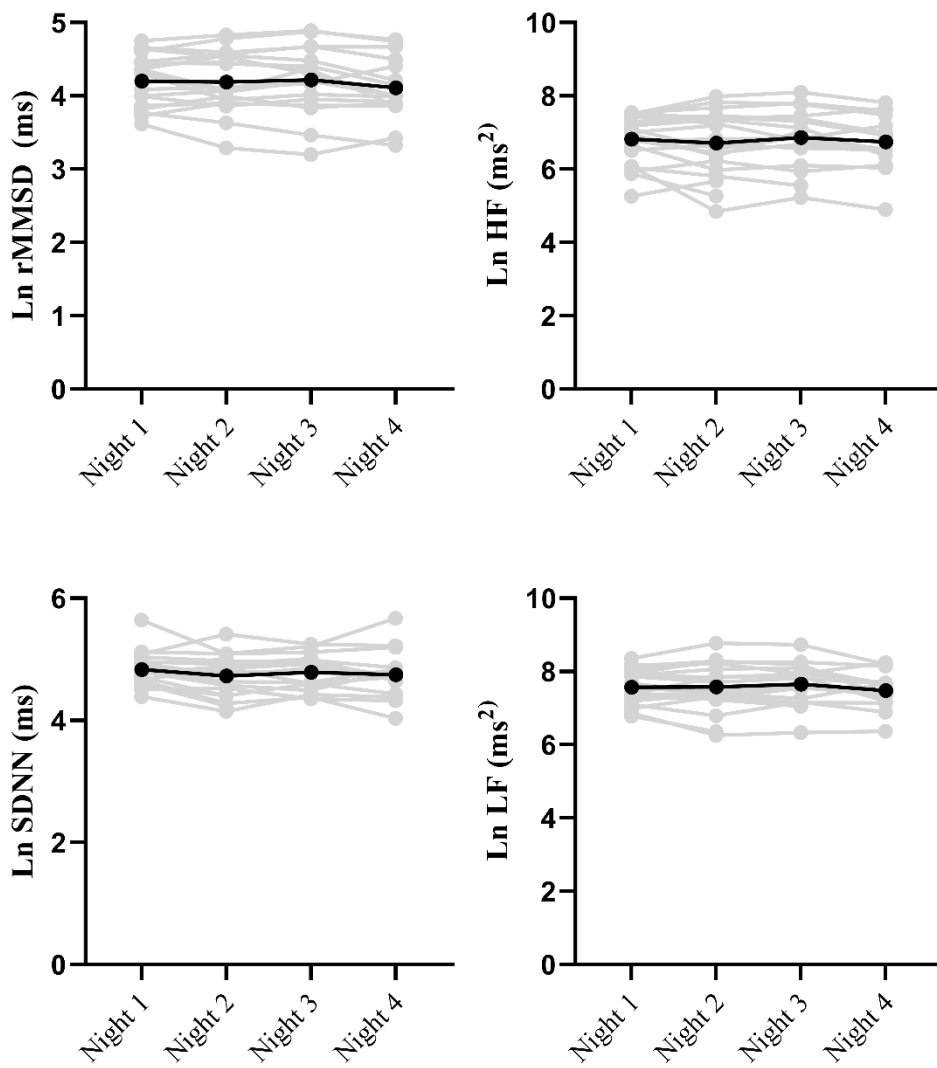


Figure 10. Mean and individual values for the temporal and frequential HRV parameters at Pre, Post<sub>24h</sub> and Post<sub>48h</sub>.

### ***3.4. Discussion***

The primary aim of this study was to evaluate the neuromuscular and parasympathetic recovery after a single SIT<sub>15:2</sub> session in a recreationally active population. The main finding was that, although a significant decline in neuromuscular function existed in the subset of participants that were evaluated immediately after the session, no significant changes were found neither for neuromuscular function nor for heart rate variability indices even 24 h after the SIT session. The present results suggest that impairments in both the neuromuscular and the autonomic nervous system, are fully resolved within the first 24 h following a single session of SIT. Furthermore, given that no session or interaction effects were found for any of the variables when comparing the SIT<sub>15:2</sub> to the SIT<sub>30:4</sub> approach, our results might be extended to all-out sessions involving longer sprints.

#### ***Evidence of neuromuscular fatigue during and immediately after the session***

The SIT<sub>15:2</sub> session led to large average declines of ~30% and ~25% for PO<sub>max</sub> and PO<sub>mean</sub>, respectively. These declines in performance were evident after the first four sprints and are comparable to those found following the classical 30 s sprint efforts (McCartney et al., 1986). The subset of participants that performed the neuromuscular measurements at Post<sub>1min</sub>, Post<sub>2min</sub> and Post<sub>8min</sub>, presented significant drops for iMVC as well as in the amplitude of the electrically evoked muscular contractions. One minute after the SIT<sub>15:2</sub> session, the iMVC, Db<sub>100</sub> and Db<sub>10</sub> showed a decline of  $29.7 \pm 15.6\%$ ,  $42.7 \pm 14.4\%$  and  $67.9 \pm 17.5\%$ , respectively (Figure 9). Then, only a 5.4% of the force was restored at Post<sub>8min</sub>, with the decline at that time being still significantly different from Pre. Our results coincide with those previously presented by Krusnauskas et al., (2018, 2020) and Venckunas et al., (2019). Importantly, no significant differences between the magnitude of impairment for the neuromuscular indices between the SIT<sub>15:2</sub> and the SIT<sub>30:4</sub>, suggesting that both sessions generated a similar amount of fatigue. The greater decrease in Db<sub>10</sub> following SIT explains the lower Db<sub>10:100</sub> ratio and suggests that the neuromuscular fatigue was predominantly associated with an excitation–contraction coupling failure (Jones, 1996) and reduced Ca<sup>2+</sup> availability. The non-significant drop observed for VA after the SIT sessions suggests that peripheral rather than central factors are most likely to explain the drop in performance during the short-duration high intensity exercise (Carroll et al., 2017).

### *Evidence of the neuromuscular recovery*

Compared to Pre, no significant differences were observed for any of the neuromuscular indices evaluated 24 h and 48 h after the SIT<sub>15:2</sub> session. A strength of the current study was that, in addition to performing the classic neuromuscular evaluations during isometric contractions, the ability to produce force, velocity and power on cycle ergometer was also evaluated to better assess the fatigue/recovery status of the participants. The average and individual datapoints showed no declines for the iMVC,  $F_0$ ,  $P_{max}$ ,  $V_0$  or the electrically evoked contractions meaning that, from a neuromuscular point of view, our participants were back to baseline levels 24 h after the SIT<sub>15:2</sub> session. Furthermore, the comparison for the subset of the sample that performed both sessions indicated that neuromuscular recovery also occurred within the first 24 h for a session with longer sprints and recovery times. These results are in line with those presented by Schlittler et al. (2019) and Zanou et al. (2021) 24 h after a 6 x 30 s SIT session. However, our results are in contrast with some of the previous literature on the topic. Studies performed on similar populations to the one recruited for the present work, had reported significant drops in isometric force (Krusnauskas et al., 2020) and LFF (Krusnauskas et al., 2018; Venckunas et al., 2019) 24 h after a 6 x 30 s and 3 x 30 s SIT session, respectively. In the present study, neither the iMVC nor the LFF presented a significant decline a day after the SIT<sub>15:2</sub> or the SIT<sub>30:4</sub>. The main hypothesis for the increased LFF 24 h after the SIT session is related to reductions in free myoplasmic  $Ca^{2+}$  or myofibrillar  $Ca^{2+}$  sensitivity (Krusnauskas et al., 2018; Venckunas et al., 2019). Then, a less effective  $Ca^{2+}$  handling would lead to a force depression at low stimulation frequencies due to the sigmoidal shape of the force-calcium relationship. Indeed, muscle biopsies from recreationally active men taken 24 h after completing a 3 x 30 s SIT exhibited a large fragmentation of the ryanodine receptor 1 protein complex (RyR1), which is the main channel of  $Ca^{2+}$  release from the sarcoplasmic reticulum (Place et al., 2015). However, in our experiment, the LFF measured throughout the Db<sub>10</sub>/Db<sub>100</sub> was unchanged a day after the exercise. Considering that the SIT<sub>30:4</sub> could have generated a higher intracellular disruption likely connected to the greater reliance on glycolytic pathways during the 30 s all-out intervals compared to 15 s, two main ideas could help explaining the lack of difference in neuromuscular fatigue between sessions: i) the important drop in PO typically observed during the last ~10-12 s of a classic 30 s Wingate exercise implied a significantly lower  $T_{work}$  for the SIT<sub>30:4</sub> compared to the SIT<sub>15:2</sub>. Indeed, during the 15 s bouts, participants maintained a higher  $PO_{mean}$  and therefore, produce a higher  $T_{work}$ , that could also contribute to a greater fatigue despite the shortened sprint length; ii) lactate accumulation,

which is a commonly used marker of exercise intensity, was not significantly different between the SIT<sub>30:4</sub> and the SIT<sub>15:2</sub> suggesting that, from a metabolic point of view, the sessions were similar. Nevertheless, these arguments still contrast previous findings (Krusnauskas et al., 2018, 2020; Venckunas et al., 2019), leading to think that the changes in LFF and iMVC might be depending on other factors. For example, the same 6 x 30 s session resulted in a lower fragmentation of RyR1 24 h after the exercise in trained men compared to their recreationally active counterparts (Place et al., 2015). Also, three weeks of SIT training provided protection against RyR1 degradation for some but not for other participants (Schlittler et al., 2019). Then, young females did not present a significant change in LFF after a 3 x 30 s SIT session (Krusnauskas et al., 2018) and, finally, a more pronounced drop in force and LFF was observed 5 h after a 3 x 30 s session for the individuals with a higher percentage of fast-twitch fibers (Lievens et al., 2020). Therefore, a combination of factors like sex, training status or even fiber typology might also help explaining the lack of low frequency- and neuromuscular fatigue observed 24 h after SIT in our study. We attempted to account for the sex difference in our sample by performing a two-way analysis of variance (sex × time) for the 25 participants that completed the SIT<sub>15:2</sub>. Although a main sex effect was found for force, speed and power, no significant time or sex × time interaction effects were observed for any of the neuromuscular indices meaning that, irrespective of their sex, participants had recovered from the session 24 h after its completion.

### ***Evidence of the autonomic nervous system recovery***

In the current experiment, HRV was measured to determine the time course of the recovery of the parasympathetic branch of the autonomic nervous system after all-out exercise. None of the temporal or frequential indices nor the mean heart rate, displayed a significant variation related to the effects of the SIT<sub>15:2</sub> nor the SIT<sub>30:4</sub> (Table 2, Figure 10). Recovery after a bout of exercise has been previously assessed by the restoration of the vagal-mediated indicators of HRV (i.e. Ln rMMSD and Ln HF) (Stanley et al., 2013) and, even though the literature describing the effects of all-out exercise on the autonomic nervous system disturbance is scarce, our results support recent findings showing a quick autonomic recovery after either 2 x 30 or 4 x 30 s all-out sessions (Ye et al., 2022). Although we did not continuously monitor HRV for evidencing the immediate changes after the session, the cardiac parasympathetic activity was comparable to baseline values already from the first night after the exercise. Furthermore, and even though our study was not initially powered to evaluate sex-related differences, the two-way analysis of

variance did not show any sex or sex  $\times$  time interaction effect. This suggests that a similar response between males and females might be expected and expands previous results in which only males were tested (Ye et al., 2022).

These findings have to be interpreted with caution as they only apply to a population of young recreationally active individuals and some limitations need to be considered. In this study, autonomous nocturnal recordings were performed by our participants to reduce subjective and external factors influencing HRV. However, this implied that the breathing frequency and the brain activity through electroencephalography could not be controlled. Since it is known that breathing frequency could be a confounding factor for the frequential HRV parameters, only Ln rMMSD, which has been shown to be independent of breathing (Penttilä et al., 2001), was kept for stating that recovery was achieved from the first night after the SIT<sub>15-2</sub> and the SIT<sub>30-4</sub> from a temporal perspective. Moreover, the frequential HRV indices were obtained by averaging several 5 min epochs of the sleep-time recordings to avoid sleep cycle-related biases according to previous guidelines (Task Force, 1996). Considering these limitations, monitoring HRV during the nights after SIT sessions, gave us mainly information on the potential perturbation of the cardiac parasympathetic activity.

Previous literature reviews assessing the post-exercise recovery of the vagal-mediated indices of HRV have suggested that the greater the exercise intensity the slower the post-exercise parasympathetic activity recovery (Michael et al., 2017; Stanley et al., 2013). However, given the small number of SIT interventions available for analysis in those reviews it can be concluded that the post-exercise HRV recovery has not been rigorously investigated throughout the whole intensity spectrum. For example, in the studies included for analysis by Stanley et al. (2013), only Niewiadomski et al. (2007) conducted a Wingate-based intervention and the results do not show a great disturbance the day after performing two consecutive 30 s sprints. Additionally, only three out of eight studies, assessed HRV at 24 h and 48 h after the intervention. Thus, based on our findings and those from Ye et al. (2022), we suggest that 24 h are enough to restore the parasympathetic activity following one SIT session. Moreover, we did not observe any difference between SIT sessions when the work-to-rest ratio and the total sprint time was kept the same. Then, the idea that changes in HRV responses are intensity dependent might be true under some but not all conditions. We postulate that the total exercise time and, more specifically, the length of the intervals, might be a key factor to consider when analyzing the long-term post-exercise disturbance of the ANS among the different high-intensity forms

of exercise. For example, an average SIT session ranges from 120 to 210 s of total work time and a 1 to 8 work-to-rest ratio. In contrast, a regular HIIT session often consist of several intervals from 3 to 5 min of exercise with greater work-to-rest ratios. The fact that HIIT sessions imply a much greater time spent at high percentages of the maximal heart rate and, therefore, a larger solicitation of the ANS could be related to a greater and more prolonged post-exercise fatigue. Under this scenario, it could be argued that if the goal is to minimize fatigue but still deliver an intense training load, performing a SIT session might be more appropriate than HIIT.

From a practical point of view, both the neuromuscular and the autonomic nervous system would be ready to cope with the next training stimulus the very next day. However, and despite occasionally two intense sessions could be performed consecutively, rest days have been shown to be necessary for promoting adaptations and improve performance within a SIT program (Burgomaster et al., 2005; Parra et al., 2000). Moreover, current evidence has shown that sharp increments in training frequency yielded to longer recovery times after each HIIT session, evidencing the greater cumulative fatigue due to the additional high intensity training (Busso et al., 2002). Thus, despite the scheduling of consecutive intense training loads does not seem to be recommended, it might be possible to combine all-out with other type of training stimuli to promote different physiological adaptations.



#### ***4. Chapter four: Cardiorespiratory and neuromuscular improvements plateau after two weeks of sprint interval training in sedentary individuals (Study 2)***

##### ***4.1. Introduction***

Sprint Interval Training (SIT) consisting of repeated and brief all-out exercise bouts (J. Gillen & Gibala, 2014) has been shown to be a time-efficient approach to increase cardiorespiratory fitness, as shown by the demonstrated increases in maximal oxygen uptake ( $\dot{V}O_{2\max}$ ) (Sloth et al., 2013; Volllaard et al., 2017). The rapid and positive effects of SIT on  $\dot{V}O_{2\max}$  can be particularly important for less fit individuals as lower endurance fitness is a strong predictor of future cardiovascular complications, mortality, and premature death (R. Ross et al., 2016). Increases in  $\dot{V}O_{2\max}$  in response to SIT are often connected to peripheral changes such as improvements in skeletal muscle mitochondrial function and content (MacInnis & Gibala, 2017), as well as maximal enzymatic activity (Burgomaster et al., 2008). Nevertheless, recent research has also shown that central hemodynamic factors such as increases in total hemoglobin mass, blood volume and cardiac output are also connected to the greater  $\dot{V}O_{2\max}$  observed after six weeks of SIT (Mandić et al., 2022). This indicates that both peripheral and central adaptations are important contributors to changes in  $\dot{V}O_{2\max}$  after a SIT intervention.

Since the finding of (Burgomaster et al. (2005) demonstrating that as few as six sessions of repeated 30 s all-out efforts were sufficient to enhance muscle oxidative capacity, research has focused on optimizing SIT protocols to provide the greatest increase in  $\dot{V}O_{2\max}$  with the lowest total training volume or time commitment. Indeed, the manipulation of variables such as sprint and recovery duration, number of repetitions, or training frequency has resulted in a substantial number of different but still effective SIT protocols. For instance, reducing the sprint length to 20, 15 or even 10 s and, therefore, substantially reducing the total work time compared to the 30 s efforts, did not diminish the magnitude of physiological adaptations on healthy participants (Hazell et al., 2010; Naççakan et al., 2018; Yamagishi & Babraj, 2017; Zelt et al., 2014). Furthermore, no between-group differences were observed when reducing training frequency from 3 or 4 to 2 weekly sessions (Thomas et al., 2020). Also, two studies reported that  $\dot{V}O_{2\max}$  rapidly increased in the first two or three weeks, but then stagnated after three (Burgomaster et al., 2008) and six (Yamagishi & Babraj, 2017) additional weeks of training. Finally, a meta-analysis concluded that more sprints did not necessarily correlate with greater gains in  $\dot{V}O_{2\max}$

(Vollaard et al., 2017). Overall, these findings suggest that SIT programs do not need to be very long to elicit significant increases in  $\dot{V}O_{2\max}$ . Furthermore, it appears that sprint length can be shortened and training frequency and the number of sprints reduced with no detrimental consequences for the increases in  $\dot{V}O_{2\max}$ . This information is relevant as SIT could elicit adverse affective responses and lower adherence to training given its very intense nature (Hu et al., 2022). Moreover, showing that similar improvements in cardiorespiratory fitness can be obtained with fewer and/or shorter sprints is important for promoting physical exercise in the general population.

In contrast to the previous statements, a recent meta-analysis demonstrated an increased effectiveness of SIT with more and longer sprints in a variety of aerobic and anaerobic outcomes (Hall et al., 2023). It was argued that more and longer sprints would result in greater disturbances of the metabolic environments, potentially increasing the response to training and therefore, the magnitude of adaptations. In addition, a study on sedentary males showed an increase in  $\dot{V}O_{2\max}$  of ~12% after 6 weeks of SIT, and a further 7% increase after 12 weeks of training (J. B. Gillen et al., 2016). And although the statistical significance was not reached, Bostad et al. (Bostad et al., 2021) reported a 8% gains after two weeks followed by a 15% and 21% after six and twelve weeks suggesting that longer programs might be more beneficial for developing  $\dot{V}O_{2\max}$ . Of note, the percent increase in  $\dot{V}O_{2\max}$  in Gillen et al. (2016) and Bostad et al. (2021) was considerably greater than what previous meta-analyses had reported (Vollaard et al., 2017). Altogether, these results indicate that it is not clear whether  $\dot{V}O_{2\max}$  continues to increase following the first weeks of a SIT program.

In addition to the evaluation of changes in  $\dot{V}O_{2\max}$ , some studies have focused on the effects of SIT on other outcomes such as time-trials and critical power tests (Yamagishi & Babraj, 2017), fatigue resistance in endurance tasks (Bagley et al., 2021), and the repeated sprint performance (Yamagishi & Babraj, 2017). Although these improvements can be partly explained by the enhanced aerobic and/or anaerobic capacities, the high and constant pedal resistances combined with the great metabolic stress used during SIT could also result in specific neuromuscular adaptations. Bagley et al. (Bagley et al., 2021) reported significant increments in the quadriceps cross sectional area after 12 weeks of SIT, with no changes in the isometric and concentric torque. Consistent with that, no differences in isometric force or muscle excitability at rest were observed after two-weeks of SIT (Lewis et al., 2017). However, the ability to produce force and velocity, and therefore power during a dynamic task as cycling

and not only under isometric conditions might be an important factor to consider when evaluating neuromuscular adaptations to SIT. Indeed, a previous study from our laboratory observed increases in the power-force-velocity profile after two weeks of running SIT in basketball players (Lloria-Varela et al., 2023), suggesting that a better characterization of the participant's neuromuscular system capabilities could be achieved with the evaluation of dynamic tasks.

Thus, this study aimed to assess the time course of the physiological and neuromuscular adaptations during a 6-week SIT intervention in sedentary participants. The main goal was to determine whether the expected increases in  $\dot{V}O_2\text{max}$  would plateau after two and four weeks of training. The secondary goal was to evaluate the effectiveness of SIT to improve neuromuscular function associated to the ability to produce force and velocity during cycling sprints.

## 4.2. Methods

### Experimental design

The experimental design included five testing and fifteen SIT sessions (Figure 11) spread over a 6-week training period. Participants were familiarized to SIT and to the experimental procedures as they completed an initial familiarization session consisting of 4 x 15 s and a much shorter protocol to determine the acute effects of a single 8 x 15 s SIT session on different outcomes. The testing sessions took place before (Pre), and after two (Week<sub>2</sub>), four (Week<sub>4</sub>), six (Week<sub>6</sub>) and 7 weeks (Week<sub>7</sub>) from the start of the training intervention. Testing in each evaluation session included a force assessment, a power-force-velocity profile (PFVP), and a  $\dot{V}O_2$ max test. Therefore, each training block involved, one testing and five training sessions scheduled 48 h apart in which the number of sprints per session evolved as follows: 10, 12, 12, 14 and 8 (Figure 11). All the sessions were performed in the same room under similar temperature and humidity conditions. During the length of the study, participants were instructed to avoid strenuous physical activity to and keep their habitual nutritional and sleep routines. This experimental protocol was approved by the Comité de Protection des Personnes and the Agence Nationale Sanitaire et du Médicament (ANSM-France) and was performed in accordance with the Declaration of Helsinki. The study was sponsored by the University Hospital of Saint-Etienne (France) under the following ClinicalTrials.gov identifier: NCT05013021. Informed written consent was obtained from all participants before the start of the study.

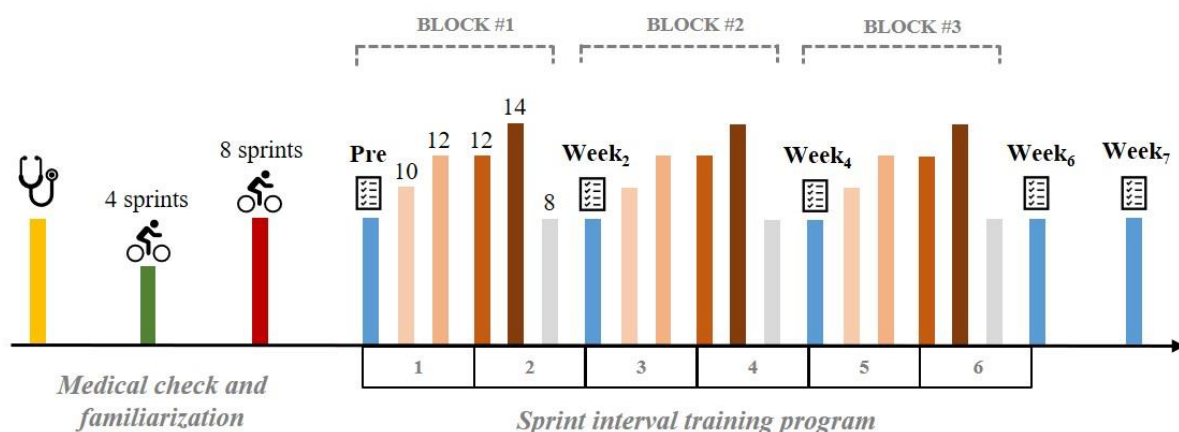


Figure 11. Overview of the experimental design of the study.

## ***Participants***

Minimal sample size was calculated using G-power 3.1 based on a test-retest error of  $2 \text{ mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  for measurements of  $\dot{V}\text{O}_2\text{max}$ . It was calculated that 13 participants were needed in order to detect a difference of  $2 \text{ mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  between two tests with a power of 90% and an  $\alpha$  error of 5%. Thus, eighteen sedentary participants were initially enrolled in this study. All reported to perform less than two hours of planned physical exercise per week and most of them reported to do not perform any by the time of the study. After the inclusion visit, a participant had to be excluded because was not vaccinated against COVID-19. Another participant stated that they could not participate in the study because of scheduling problems. Finally, from sixteen participants that initiated the training protocol, three failed to complete at least 90% of the sessions due to illness or COVID-19 isolation. Therefore, thirteen individuals (five males) that completed the entire training protocol were considered for statistical analysis (see Table 1 for participant's characteristics). All participants reported to perform less than 1 h of planned physical activity per week and to be free from neuromuscular or musculoskeletal disorders. Prior to enrollment, a medical-check including an anamnesis and a resting ECG to rule out any possible contraindications to physical exercise was performed. During the medical-check, the participants were informed of the procedures and risks involved in the study and they all provided written informed consent to participate.

Table 4. Participant's characteristics.

Weight (kg)	$71.0 \pm 19.5$
Height (cm)	$171 \pm 11$
Age (years)	$23 \pm 5$
$\dot{V}\text{O}_2\text{max}$ ( $\text{L} \cdot \text{min}^{-1}$ )	$2.66 \pm 0.88$
$\dot{V}\text{O}_2\text{max}$ ( $\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ )	$37.36 \pm 5.56$
$\text{HR}_{\text{max}}$ ( $\text{beats} \cdot \text{min}^{-1}$ )	$194 \pm 10$

## ***Sprint Interval Training program***

The SIT program was performed on an instrumented Monark cycle ergometer (see the PFVP section for more specifications about the device). The flywheel was mechanically braked with a resistance equivalent to 0.7 N per kg of bodyweight for both males and females. Then, instructions to pedal as strong and as fast as possible throughout the 15 s of the sprint and strong

verbal encouragement were given to the participants. Each sprint was interspersed by 2 minutes of recovery in which participants were asked to maintain an unloaded pedaling during the first minute and then rest until the next sprint. The number of repetitions per session was increased from 10 to 14 and then dropped to 8 in the last session of every training block (Figure 11). The power output (PO; in W) was recorded during the sprints. Maximal and mean PO were obtained from every sprint and the total work ( $T_{work}$ ; in kJ) of the session was computed as follows:

$$T_{work}(kJ) = \sum Mean\ power \times 15/1000 \quad (5)$$

A heart rate (HR) monitor Polar H10 (Polar Electro Oy, Kempele, Finland) was given to the participants and the HR was recorded continuously during the sprint and the recovery. Then, an analysis was performed on the time spent at the following arbitrary HR zones: 50-60%, 60-70%, 70-80%, 80-90% and  $\geq 90\%$  of the maximal HR obtained from the  $\dot{V}O_{2max}$  tests. Blood lactate concentrations were measured with a portable device at participant's arrival and 3 minutes after the last sprint of the 8-sprints familiarization session and the last session of every block (Lactate Scout 4; EKF Diagnostics, Cardiff, United Kingdom). As these four sessions took place before the training and at the end of each block, the abbreviations Pre, Week<sub>2</sub>, Week<sub>4</sub> and Week<sub>6</sub> will be also used for referring to the performance comparison between sessions.

## ***Procedures***

### *Assessment of the maximal oxygen uptake ( $\dot{V}O_{2max}$ )*

The incremental protocol proposed by Iannetta et al. (Iannetta et al., 2019) was used for the current study. The test started with a 2-minutes bout at 20 W followed by a 6-minutes constant load bout at 100 W for males and 60 W for females. Then, after 2 min at 20 W, the continuous increase in PO started until volitional exhaustion occurred. The ramp increments were of 20 and 30 W·min<sup>-1</sup> for males and females, respectively. During the test participants were monitored with a gas exchange and ventilatory measurement system with breath-by-breath technology and calibrated according to manufacturer's recommendations before each test (Metalyzer 3b, CORTEX Biophysik GmbH, Leipzig, Germany). Blood lactate concentration was measured before and three minutes after the test.

Breath-by-breath gas exchange and ventilatory data were subsequently exported from Metasoft software (CORTEX Biophysik GmbH, Leipzig, Germany) to the Exercise Thresholds App (Keir et al., 2022) for further analysis. Then, the portion of the data containing the incremental part of the test was selected and a 20 s rolling average was used to calculate the  $\dot{V}O_{2\max}$  in  $\text{ml}\cdot\text{min}^{-1}$ . After that, an extended analysis allowed for identification of the gas exchange threshold (GET) and the respiratory compensation point (RCP) which were identified as proposed in Keir et al. (Keir et al., 2022), following the model originally proposed by Beaver et al. (Beaver et al., 1986). GET and RCP were visually determined by two independent experts and consensus was reached for all participants. Maximal HR ( $HR_{\max}$ ) was defined as the highest HR value reached throughout the incremental test. Peak power output was defined as the last target power before cadence dropped more than 10 turns per minute compared to the average cadence during the ramp. Oxygen pulse at  $\dot{V}O_{2\max}$  was used as an estimation of the stroke volume. It was calculated and corrected for body weight as proposed by Oliveira et al. (Oliveira et al., 2011) using the equation 2. The  $\dot{V}O_2$  and the HR are in  $\text{ml}\cdot\text{min}^{-1}$  and  $\text{beats}\cdot\text{min}^{-1}$  respectively and the body weight in kg.

$$O_2 \text{ pulse} = \left( \frac{\frac{\dot{V}O_2}{HR}}{\text{Body weight}} \right) \times 100 \quad (6)$$

#### *Assessment of maximal isometric voluntary force*

The isometric maximal voluntary contraction of the right knee extensors (iMVC) was measured by attaching the participant's ankle with a non-compliant cuff to a load cell (Meiri F2732 200 daN; Celians, Montauban, France) fixed to a custom-built chair. Participants sat with hips and knees at 90° flexion and a non-compliant strap in the hips to avoid lower-limb movements. After a warm-up of submaximal contractions, a practice iMVC to ensure potentiation of subsequent contractions was performed. Then 2 iMVC were performed separated by one minute. In the cases in which a difference greater than 5% was detected between trials, a third iMVC was performed. All data were analyzed using Labchart software (v8, ADInstruments) and iMVC was defined as the average of the highest 0.5 s window for each trial. Then, the iMVC was corrected by body weight in kg.

### ***Power-force-velocity profile (PFVP)***

The saddle height for the cycle ergometer was retrieved from the familiarization session and toe clips were well fastened to avoid foot displacement from the pedal. Participants started each 7 s trial with the right pedal at 45° from the vertical axis and were asked to remain seated for the duration of the measurement. After ensuring the departure position by visual inspection, a 3 s countdown was launched and two 7 s resisted sprints interspersed by 2 minutes of recovery were performed. The load was fixed first to 0.5 and then to 0.7 N and 0.3 and 0.5 of resistive force per kg of bodyweight for males and females, respectively. For each sprint, participants were instructed to pedal as fast as possible and strong verbal encouragement was provided.

A Monark cycle ergometer was made up of a strain gauge FN 3030 type; FGP Instrumentation, Les Clayes-sous-Bois, France) to measure the friction force and an optical encoder (100 pts/turn, Hengstler type RI 32.0; Aldingen, Germany) to quantify the flywheel displacement. Data were sampled at 200 Hz, recorded in LabVIEW software (NI, Austin, TX) and were filtered with a fourth-order low-pass Butterworth filter at 12 Hz. Then, from the product between the sum of forces (friction and inertia) (F) and velocity of the wheel (V), the power output can be obtained as indicated by Morin & Belli (2004). An average value for F, V, and P for each pedal downstroke was recorded allowing to obtain the linear (F-V) and the second-order polynomial (P-V) relationships that characterize the maximal mechanical capabilities of the lower limb's neuromuscular system. With the equation obtained from the linear F-V relationship of the both loads, the slope ( $S_{FV}$ ) and the two intercepts with the axes could be then calculated for obtaining the theoretical maximum force at zero velocity ( $F_0$ ; N) and the theoretical maximum velocity until which force could be produced ( $V_0$ ;  $\text{rad}\cdot\text{s}^{-1}$ ). From  $F_0$  and  $V_0$ , the maximal power output ( $P_{\max}$ ; W) was then calculated as follows:

$$P_{\max} = \frac{(F_0 \times V_0)}{4} \quad (7)$$

Using the methods described above,  $F_0$ ,  $V_0$ ,  $P_{\max}$  and the  $S_{FV}$  were then calculated for each participant by pooling together the two trials performed at every time point (i.e. the both loads). As the distribution of the F and V points and the slope of the F-V equation could largely influence the intercepts with the axes (i.e.  $F_0$  and  $V_0$ ) the coefficient of determination ( $r^2$ ) was used for determining whether or not the sprint was appropriate enough for then extrapolating to the intercepts with the axes as the main outputs of the PFVP. In the cases in which the  $r^2$  of the



equation was not superior to 0.9, the sprint was discarded and not used for the posterior statistical analysis. Therefore, only the data from 12 participants is presented for  $F_0$ ,  $V_0$ ,  $P_{\max}$  and the  $S_{FV}$  in the results section corresponding to the PFVP.

### *Statistical analysis*

Data are presented as mean values and standard deviations in the text and tables. Normal distribution of the data was checked using the Shapiro-Wilk normality test. Student paired t-tests were performed on the lactate samples collected before and after the 8-sprints sessions and the  $\dot{V}O_{2\max}$  tests. A one-way repeated measures analysis of variance was used to determine the effect of time in  $\dot{V}O_{2\max}$ , PFVP, iMVC, HR and the  $T_{\text{work}}$  from the four sessions in which performance was measured. Then, the Tukey test was used as post-hoc when a significant effect was observed. All data were analyzed using the 9.4.0 version of Prism 2022 software (GraphPad Software, San Diego, CA) and the significance level for all statistics was set at  $p < 0.05$ .

### 4.3. Results

$T_{\text{work}}$  from the last session of each training block was significantly greater at Week<sub>2</sub>, Week<sub>4</sub> and Week<sub>6</sub> compared to Pre for a similar lactate accumulation at the end of the session (Table 5). No further differences were observed between the sessions. The  $T_{\text{work}}$  for the first sprint and the Peak PO remained constant throughout the training protocol. Compared to Pre, the time spent above the 80% of  $HR_{\text{max}}$  was lower at Week<sub>6</sub> while the time spent above the 90% of  $HR_{\text{max}}$  was reduced at Week<sub>4</sub> and Week<sub>6</sub>.

Table 5. Responses to the 8-sprints sessions before training (Pre) and at the end of the first (Week<sub>2</sub>), second (Week<sub>4</sub>) and third block of training (Week<sub>6</sub>).

	Pre	Week <sub>2</sub>	Week <sub>4</sub>	Week <sub>6</sub>
$T_{\text{work}}$ session (kJ)	51.8 ± 15.0	58.4 ± 19.6* +11.7 ± 7.4%	63.7 ± 24.7* +20.9 ±	59.9 ± 21.2* +22.0 ± 9.7%
$T_{\text{work}}$ of the 1 <sup>st</sup> sprint (kJ)	8.2 ± 2.7	8.1 ± 2.4 -0.1 ± 13.8%	8.6 ± 3.0 +4.8 ± 8.8%	8.7 ± 2.6 +7.9 ± 8.8%
Peak PO session ( $W \cdot kg^{-1}$ ) 1)	10.1 ± 1.8	10.1 ± 1.9 +1.2 ± 8.9%	10.1 ± 1.6 +0.7 ± 12.1%	10.3 ± 2.1 +3.2 ± 13.6%
Lactate <sub>Post3</sub> ( $mmol \cdot L^{-1}$ )	13.2 ± 1.9	13.4 ± 2.7 +3.2 ± 17.1%	13.6 ± 2.5 +4.0 ± 23.1%	13.5 ± 3.5 +3.3 ± 29.0%
Time ≥ 80% of $HR_{\text{max}}$ session (s)	662 ± 206	553 ± 275 -23.2 ± 40.6%	402 ± 175* -38.7 ±	370 ± 230* -46.2 ± 26.9%
Time ≥ 90% of $HR_{\text{max}}$ session (s)	107 ± 88	61 ± 87 -49.3 ± 42.8%	9 ± 11* -77.6 ±	26 ± 44* -59.1 ± 27.8%

\*denotes a significant difference from Pre ( $p < 0.05$ )

The time spent at the different percentages of HR and the  $T_{\text{work}}$  for the three training blocks is displayed in Figure 12. A significant decrease of the time spent above the 80% and 90% of  $HR_{\text{max}}$  was observed throughout the training program, being greater in the first compared to the second and third block of training (Figure 12). There was a numerical increase in the time spent below the 80% of  $HR_{\text{max}}$  between training blocks, but it did not change significantly. The  $T_{\text{work}}$  increased significantly during the second block compared to the first with no further differences between the rest of the time points.

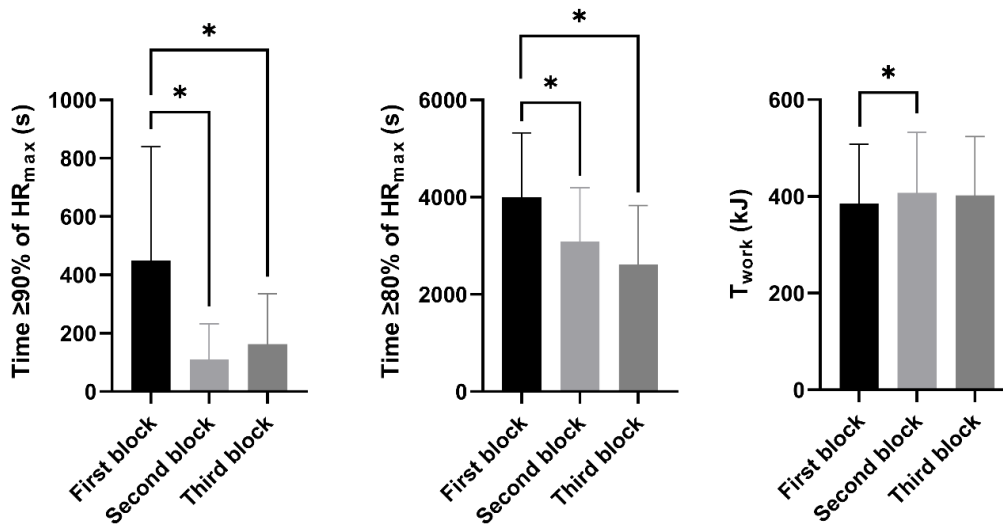


Figure 12. Time spent at the different percentages of HR and Twork for the first, second and third block of training. \*denotes a significant difference from the first block ( $p < 0.05$ ).

A significant time effect at Week<sub>2</sub>, Week<sub>4</sub>, Week<sub>6</sub> and Week<sub>7</sub> compared to Pre was observed for the absolute and relative  $\dot{V}O_{2max}$  and the  $O_2$  pulse with no further differences between time-points (Table 6). The  $\dot{V}O_2$  at GET displayed a significant increase at Week<sub>2</sub> and at Week<sub>7</sub> compared to Pre, and the  $\dot{V}O_2$  at RCP was greater at Week<sub>2</sub>, Week<sub>4</sub>, Week<sub>6</sub> and Week<sub>7</sub> compared to Pre. The peak power output showed a significant time effect from Week<sub>2</sub> and Week<sub>6</sub> compared to Pre.

Table 6. Time course of the main parameters related to the assessment of the  $\dot{V}O_{2max}$ .

	Pre	Week <sub>2</sub>	Week <sub>4</sub>	Week <sub>6</sub>	Week <sub>7</sub>
$\dot{V}O_{2max}$ (L·min <sup>-1</sup> )	2.66 ± 0.9	2.99 ± 1.0*	2.85 ± 1.0*	2.89 ± 0.9*	2.87 ± 0.9*
		+8.7 ± 4.2%	+7.1 ± 4.9%	+9.4 ± 3.9%	+8.3 ± 5.6%
$\dot{V}O_{2max}$ (ml <sup>-1</sup> ·min <sup>-1</sup> ·kg <sup>-1</sup> )	37.4 ± 5.6	41.5 ± 5.7*	39.8 ± 6.9*	40.3 ± 5.9*	39.9 ± 6.5*
		+7.8 ± 4.8%	+6.3 ± 5.0%	+7.9 ± 4.1%	+6.8 ± 6.4%
$O_2$ pulse (ml·beat <sup>-1</sup> ·kg <sup>-1</sup> )	19.2 ± 2.7	21.3 ± 2.9*	20.3 ± 3.3*	20.8 ± 2.9*	20.7 ± 3.3*
		+13.5 ± 15.3%	+9.5 ± 18.7%	+11.3 ± 15.4%	+9.6 ± 16.2%
$\dot{V}O_2$ at GET (L·min <sup>-1</sup> )	1.49 ± 0.40	1.66 ± 0.51*	1.57 ± 0.45	1.60 ± 0.38	1.60 ± 0.46*
		+9.1 ± 9.1%	+5.0 ± 7.1%	+7.9 ± 8.1%	+8.4 ± 10.6%
$\dot{V}O_2$ at RCP (L·min <sup>-1</sup> )	2.05 ± 0.59	2.19 ± 0.68*	2.15 ± 0.70*	2.20 ± 0.63*	2.21 ± 0.71*
		+6.4 ± 6.5%	+4.9 ± 5.5%	+9.3 ± 3.8%	+9.4 ± 8.7%
Peak power output (W·kg <sup>-1</sup> )	3.14 ± 0.4	3.34 ± 0.6*	3.33 ± 0.5*	3.35 ± 0.6*	3.27 ± 0.6
		+5.6 ± 7.5%	+5.6 ± 7.5%	+6.0 ± 10.9%	+3.8 ± 10.5%

\*denotes a significant difference from Pre ( $p < 0.05$ )

A significant increase in  $F_0$  was observed at Week<sub>2</sub>, Week<sub>6</sub> and Week<sub>7</sub> compared to Pre (Table 7).  $V_0$  only showed a significant difference at Week<sub>7</sub> compared to Pre. The increase in  $F_0$  led to an increased (i.e. steeper)  $S_{FV}$  at Week<sub>2</sub> and Week<sub>7</sub> compared to Pre. Furthermore,  $S_{FV}$  at Week<sub>7</sub> was significantly different from Week<sub>4</sub>. The  $P_{max}$  and the absolute and relative iMVC remained unchanged throughout the training protocol.

Table 7. Time course of the main parameters related to the assessment of the PFVP and the isometric force.

	Pre	Week <sub>2</sub>	Week <sub>4</sub>	Week <sub>6</sub>	Week <sub>7</sub>
$F_0$ (N·kg <sup>-1</sup> )	1.59 ± 0.29	1.73 ± 0.23*	1.68 ± 0.20	1.71 ± 0.22*	1.81 ± 0.22*
		+9.1 ± 9.8%	+7.4 ± 11.2%	+9.4 ± 12.6%	+15.8 ± 12.3%
$V_0$ (rad·s <sup>-1</sup> )	26.0 ± 3.4	24.7 ± 4.6	24.7 ± 2.8	24.3 ± 2.5	23.4 ± 2.7*
		-5.4 ± 10.2%	-4.5 ± 10.1%	-6.0 ± 9.5%	-9.7 ± 7.8%
$S_{FV}$	-4.3 ± 1.3	-5.1 ± 1.3*	-4.8 ± 1.0	-5.0 ± 0.9	-5.5 ± 1.1*#
		+18.3 ± 22.2%	+14.9 ± 20.5%	+19.9 ± 22.6%	+31.6 ± 22.2%
$P_{max}$ (W·kg <sup>-1</sup> )	10.2 ± 1.7	10.6 ± 1.9	10.4 ± 1.7	10.4 ± 1.8	10.6 ± 1.8
		+2.6 ± 9.2%	+2.2 ± 12.1%	+2.3 ± 10.6%	+4.1 ± 9.6%
iMVC (N)	575 ± 192	609 ± 183	594 ± 194	579 ± 168	588 ± 170
		+3.9 ± 13.2%	+6.6 ± 17.2%	+9.1 ± 16.0%	+9.9 ± 19.4%
iMVC (N·kg <sup>-1</sup> )	7.9 ± 2.1	8.3 ± 2.1	8.1 ± 2.0	8.2 ± 2.0	8.3 ± 2.1
		+3.0 ± 12.2%	+5.8 ± 17.0%	+7.6 ± 15.5%	+8.3 ± 19.0%

\*denotes a significant difference from Pre ( $p < 0.05$ ); # significant difference from Week<sub>4</sub> ( $p < 0.05$ ).

#### ***4.4. Discussion***

The aim of this study was to determine the time course of the physiological and neuromuscular adaptations during a 6-week SIT intervention. The main findings were that: i) SIT was an effective approach for rapidly increasing  $\dot{V}O_{2max}$  in a group of previously inactive participants, as reflected by the rapid increase within the first two weeks of training and the further plateau in this outcome thereafter; ii) although the isometric force remained unchanged, cycling-specific neuromuscular adaptations were observed after the SIT intervention.

##### ***Improvements in $\dot{V}O_{2max}$***

The ~ 8% average increase in  $\dot{V}O_{2max}$  observed after two weeks of SIT coincides with the range provided by previous meta-analyses (Gist et al., 2014; Vollaard et al., 2017) and confirms the efficacy of SIT for enhancing cardiorespiratory fitness and physical capacity in short periods of time. Importantly, the present study maintained the work-to-rest ratio traditionally used in 30 s all-out SIT (i.e., 1:8) but halved the length of both effort and recovery and doubled the number of repetitions. Under these training conditions, the magnitude of improvement was similar compared to the results reported by Burgomaster et al. (Burgomaster et al., 2005), who proposed a protocol involving 30 s efforts and 4 minutes of recovery with less sprints. These findings are important because shorter sprints are known to induce more positive affective responses and cause less unpleasant feelings than longer sprints in non-accustomed populations (Hu et al., 2022). In addition, the results of the comparison between the 8-sprints sessions show that the participants were able to maintain a higher mean PO over the sprints for a similar lactate accumulation, reflecting an increased fatigue resistance and exercise capacity after SIT (Forbes et al., 2008). It is noteworthy that the training program did not lead to any improvements in the maximal expression of PO during the sessions, as evidenced by the unchanged  $T_{work}$  for the first sprint and peak PO of the sessions over time.

##### ***Time course of changes in $\dot{V}O_{2max}$***

The present study demonstrates a rapid increase in  $\dot{V}O_{2max}$  during the initial two weeks of training, which subsequently reached a plateau despite the continuation of the same training regimen for an additional four weeks. These results are consistent with Burgomaster et al.

(2008) and Yamagishi & Babraj (2017), which noted a similar plateau effect after the three first weeks of SIT, with no subsequent increases after the next 3 and 6 weeks of additional training, respectively. Although the three SIT protocols employed in these studies varied in their structure and Yamagishi & Babraj (2017) and Burgomaster et al. (2008) included progressive increases in training load, it was found that more training did not necessarily result in greater improvements and, to date, there is no clear physiological explanation for this phenomenon. Prior research has indicated that a combination of central and peripheral adaptations plays a role in the rapid increases in  $\dot{V}O_{2\max}$ . Improved transport, extraction, and utilization of oxygen are known to result from an increase in plasma and total blood volume (Mandić et al., 2022, 2023) and cardiac output (Bostad et al., 2021) as well as enhanced enzymatic activity and/or mitochondrial density (Burgomaster et al., 2008; MacInnis & Gibala, 2017), leading to concomitant increases in  $\dot{V}O_{2\max}$ . In our case, the  $O_2$  pulse increased significantly from Week<sub>2</sub> suggesting that the stroke volume might have also increased along with the  $\dot{V}O_{2\max}$ . Although the present study did not explore the mechanisms underlying the changes in  $\dot{V}O_{2\max}$ , the analysis of the HR data during the training sessions revealed that our participants spent considerably less time at high percentages of their  $HR_{\max}$  during the last four weeks compared to the first two weeks of training (Figure 12). This same pattern was observed when comparing the last training session of each block where performance was evaluated based on the same number of sprints. Previous research has shown that exercising at or above the 90% of the  $HR_{\max}$  is crucial for achieving further improvements in  $\dot{V}O_{2\max}$  (Gormley et al., 2008). As a result, the observed plateau in  $\dot{V}O_{2\max}$  could be primarily attributed to the reduction in time spent at high percentages of  $HR_{\max}$  during the second and third block, which might have limited the potential for further central and peripheral adaptations. Although an untrained population could likely benefit from exercising at lower percentages than 90% of  $HR_{\max}$  (Gormley et al., 2008) of HR, it is worth noticing that the time spent between 80% and 90% of  $HR_{\max}$  was also significantly reduced during the second and third blocks compared to the first. Interestingly, the  $T_{\text{work}}$  per block increased with time, suggesting that the participants were able to maintain higher PO during the sessions despite the reductions in HR. Two factors might help explaining the reduction in time spent at greater percent of  $HR_{\max}$ : first, habituation to the training protocol might have induced sufficient physiological adaptations that resulted in a faster HR recovery during the 2-minute breaks consequently reducing the time spent at high HR percentages; second, the prolonged SIT protocol and the high number of repetitions could have resulted in the development of pacing strategies among participants affecting the all-out nature of the sprints as the SIT regimen progressed (Vollaard et al., 2017). Therefore, an alternative view is

that instead of saying that more SIT training does not result in more adaptations, it could be considered that the training stimuli changed over time (i.e., the all-out component was reduced and/or the pacing increased), leading to a plateau in the adaptations. The hypothesis of lower engagement of the participants after the first block of training could be supported by the similar time course followed by other outcomes. The  $\dot{V}O_2$  at GET and RCP, the  $F_0$  and the  $T_{work}$  during 8-sprint session rapidly increased in the first two weeks of training, with no substantial changes thereafter. This observation may have implications for the effectiveness of SIT to enhance overall cardiorespiratory fitness and physical capacity beyond the first weeks of training. Based on these data, perhaps this type of training should be used in the beginning of a program as a “booster” before other type of training is introduced. Despite these results, it is important to highlight that the studies conducted by (Bostad et al., 2021) and Gillen et al. (2016), which examined the effects of twelve weeks of SIT training, reported substantial improvements when comparing to the measurements taken after the second and the sixth week respectively. Although it is difficult to determine, other factors such as the individual response to the training and the sex and not only to the length of the training program, could also play a role in the time course of the adaptations.

### ***Neuromuscular adaptations to SIT***

The PFVP has been shown to be a reliable indicator of the maximal capacities of the neuromuscular system during multi-joint tasks (Morin & Samozino, 2018) making it an appropriate tool for evaluating the development of force, speed and power in cycling exercises. Our study showed an increase in  $F_0$  from Week<sub>2</sub>, while iMVC did not significantly change throughout the training protocol, indicating that both variables are not interchangeable, as previously reported (Koral et al., 2021). Additionally, a significant decrease in  $V_0$  and a steeper  $S_{FV}$  were observed at Week<sub>7</sub> compared to Pre, demonstrating that cycling-specific neuromuscular adaptations occur during SIT. Surprisingly,  $P_{max}$  did not change throughout the training intervention. However, the absence of changes in  $P_{max}$  could be attributed to the model used, which considers both force and speed to calculate power. Therefore, an increase in one parameter coupled with a decrease in the other could explain the absence of change. To address this limitation, the raw power during the PFVP was examined, but no significant variations in  $P_{max}$  were observed. This contrasts the results of Hazell et al. (2010) and Zelt et al. (2014) that performed a 10 s and 15 s SIT protocols, respectively, and reported significant increases in maximal PO during a 30 s Wingate test after two and four weeks of training. One possible

explanation for this discrepancy could be that the present study used much shorter 7 s all-out tests for assessing  $P_{\max}$ . Driss & Vandewalle (2013) suggested that the use of the Wingate test to assess  $P_{\max}$  may be influenced by other factors, and that therefore, shorter trials with different loads should be used instead. The changes observed in both  $F_0$  and  $V_0$  were attributed to the heavy resistance load utilized during the training program. First, repeatedly overcoming a high resistance force from a stationary position may require a high force output, resulting in an increase in  $F_0$ . Secondly, once the sprint is initiated, the heavy resistance load may hinder the participants' ability to maintain a high pedaling speed, leading to a decrease in  $V_0$ . These results are consistent with a running-SIT model applied to young male basketball players (Lloria-Varela et al., 2023) and suggest that SIT induces significant changes in the PFVP of both sedentary and athletic populations. Regardless, an important aspect to consider is that isometric evaluations of neuromuscular performance might not fully capture changes related to SIT and, thus, dynamic evaluations provide a more accurate way of evaluation.

### ***Conclusions***

The results of this study showed that SIT is an effective method to rapidly enhance cardiorespiratory capacity and the neuromuscular function in sedentary participants. However, given that SIT is usually prescribed as a maximal effort exercise without targeting a specific percentage of  $HR_{\max}$ , it is advisable to monitor the participants' heart rate during the sessions to ensure that they spend sufficient time within the HR range necessary to keep triggering adaptations in  $\dot{V}O_{2\max}$  after the first weeks of training. In practice, SIT may be useful for short-term interventions to enhance overall physical fitness. However, for further improvement of the aerobic and/or the force and power capacities, other training modalities such as endurance or resistance training may need to be incorporated with time.



## ***5. Chapter five: Use of heart rate variability and mathematical modeling for monitoring fatigue during a SIT program.***

The following chapter presents part of the data that was collected during the Study 2 but was not included in the manuscript presented in Chapter 4. As the population targeted for the Study 2 was mainly sedentary and, therefore, not familiar with SIT, we aimed to determine whether or not the accumulation of intense training sessions would lead to a continuous fatigue state that could potentially impair the subsequent physiological adaptations. This postulate assumes that, within a training program, adequate rest periods should intersperse the training sessions in order to allow the organism to trigger post-exercise adaptative reactions (Skorski et al., 2019). The HRV was assessed at Week<sub>2</sub>, Week<sub>4</sub>, Week<sub>6</sub> and Week<sub>7</sub> in the same time point as previously described for other measurements. Specifically, the procedures for the HRV recordings are described in Study 1 (Chapter 3). Briefly, participants were asked to sleep with a Neurocoach the night before the test evaluation day (i.e. the night from Sunday to Monday) to collect information regarding the training-induced changes in the cardiac sympathetic reactivation at the different time-points. Subsequently, mathematical models were used to account for the fitness and fatigue components of training taking into account the daily training dose and measures of performance.

### ***5.1. Heart rate variability***

In some previous longitudinal studies, the HRV has been used as a marker of fatigue or non-recovery status. For example, Pichot et al. (2000) reported a progressive decrease in nocturnal HRV (i.e. lower parasympathetic and higher sympathetic drives) in response to a three-week high-volume period. Interestingly, when the training load was reduced in a fourth week, an abrupt compensation occurred, restoring HRV indices to baseline levels. To our knowledge, the second study performed in this PhD is the first investigation that combines SIT and HRV assessment during a training program. The following figure shows the mean and individual traces of the 10 participants that collected enough measures for performing statistical analyses (Figure 13). Although two more participants had collected four out of five measures, they missed the first time point because of technical complications. Therefore, these two participants were excluded for analysis as a baseline measure without training could not be established.

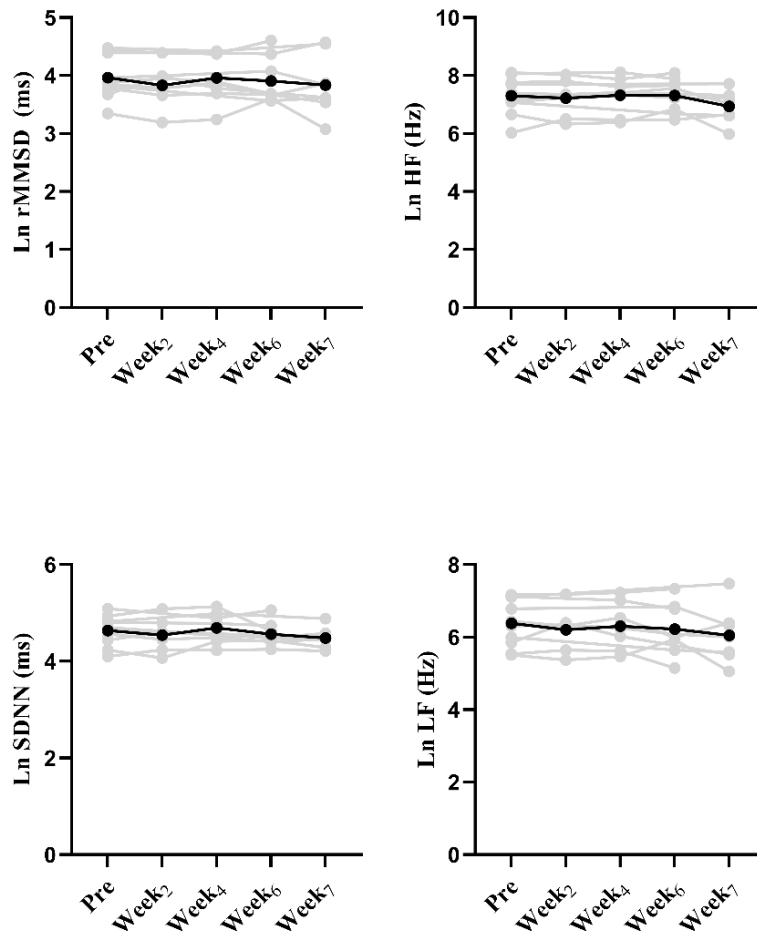


Figure 13. Mean and individual values for temporal and frequential parameters of HRV at Pre, Week<sub>2</sub>, Week<sub>4</sub>, Week<sub>6</sub> and Week<sub>7</sub>.

The analysis of variance did not reveal any significant differences between time points for any of the HRV variables presented here. These results suggest that the training regimen did not impact the HRV and that the rest periods between training sessions were long enough for the organism to recover. These results are important for the prescription of SIT since little is known about how the cardiac sympathetic system reacts to an all-out protocol. Moreover, although these measures were taken 48 h after the last training session of each block, they essentially confirmed the main result obtained in Study 1: the autonomic recovery after SIT seems to occur rapidly after the all-out exercise session. Previous literature reviews have shown impaired cardiac autonomic functions up to 48 h or more after a single session of HIIE (Stanley et al., 2013). Although the authors seem to establish a direct relationship between exercise intensity and the time course of the recovery of the cardiac autonomic function, this postulate might not apply to SIT. Moreover, rather than evaluating a single exercise session, the results presented

here show the effects of a multiple-weeks SIT program, and show that the HRV remained stable independently of the training time. Overall, the reduced total sprint time during the SIT sessions, combined with the separation of the training sessions by more than 24 h, could play a crucial role in the time course of HRV recovery.

Additionally, daily HRV measures were obtained in eleven out of sixteen subjects. The goal of these daily assessments was to increment the accuracy of the measure. Since the HRV is known to be influenced by multiple factors, (Tiwari et al., 2021) and not only respond to the fluctuations in training load, including a measure every two weeks without having the context in which the measure was taken could be highly influenced. The daily measurements were taken using the HRV4Training app, which has been previously validated (Plews et al., 2017) and used in research and training contexts (Altini & Plews, 2021), and a Polar H10 chest belt (Polar Electro Oy, Kempele, Finland). The app also includes a questionnaire that needs to be filled out after each measurement. In this questionnaire, participants can report several variables to provide additional information and context to the measure. The variables that the participants reported in this study were: muscle soreness, overall feeling of fatigue, night time, night quality, sicknesses and alcohol intake.

The following figures show the day-to-day variations in rMMSD for six out of the eleven participants that recorded the HRV in a daily basis. Annotations comprising the responses to the questionnaire related to some of the measurements were added. It is important to notice that these annotations served as “context” for understanding how the HRV could have been affected by other factors apart from the SIT program.

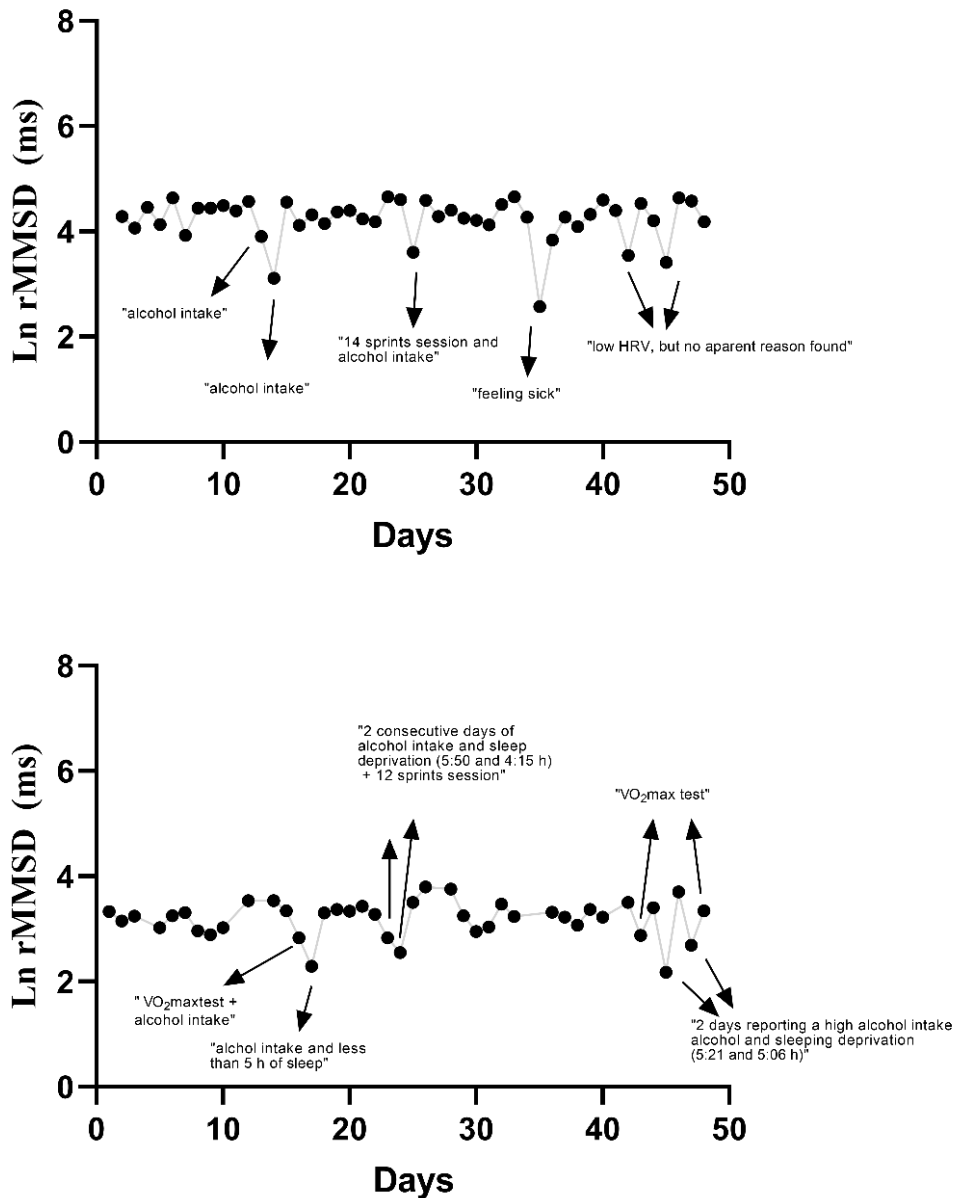


Figure 14. Daily HRV variations for the first (top) and second (bottom) participants.

As it can be seen in Figure 14, these two participants followed a similar HRV pattern. In general, there was a relatively stable HRV with some declines that, in most cases, are associated with sleep deprivation or high alcohol intake. These two participants knew each other due their studies and the talks maintained with both of them during the training sessions revealed that they attended to the same events organized by their university. This fact could very likely explain why the declines for both of them follow a similar tendency and highlights the effects of alcohol intake and sleep deprivation on HRV.

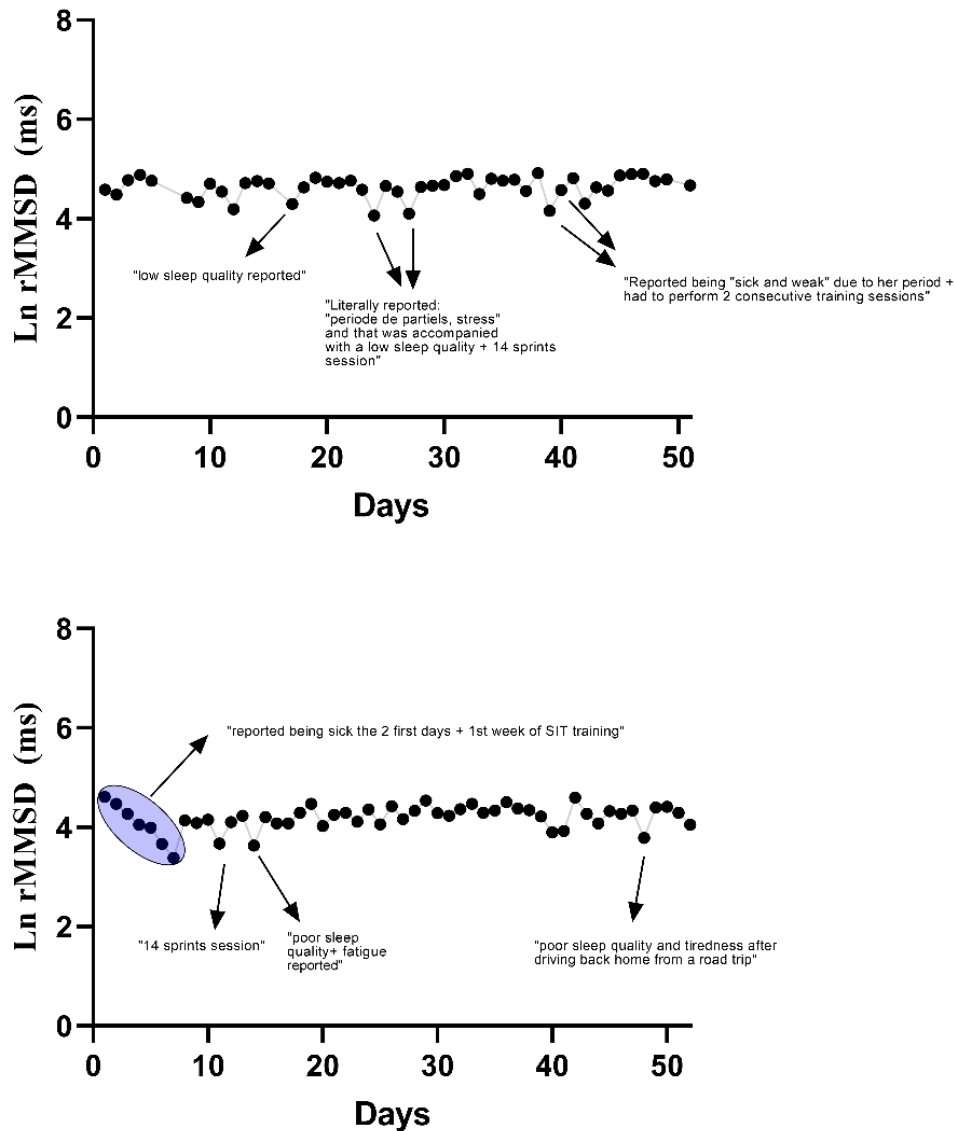


Figure 15. Daily HRV variations for the third (top) and fourth (bottom) participants.

The top panel in Figure 15 shows the case of a participant that, in general, showed a regular tendency with some declines partly caused by a low sleep quality and menstruation period in females. Contrasting results have been shown regarding the effects of the different phases of the menstrual cycle on HRV (Brar et al., 2015; Teixeira et al., 2012). In this case, this participant exhibited peaks and declines. The bottom panel displays the case of a participant started the training regimen reporting being sick but a high motivation towards the training sessions. In HRV terms, we observed a progressive decline in the first seven measurements which correspond to the first week comprising a  $\dot{V}O_2\text{max}$  test, and two training sessions of ten and twelve sprints respectively. This is the clearest and the greatest training-induced drop in HRV observed among the participants of the study. Apart from that, this participant exhibited

a regular HRV with some declines that were mainly related to a poor sleep quality or a general feeling of fatigue.

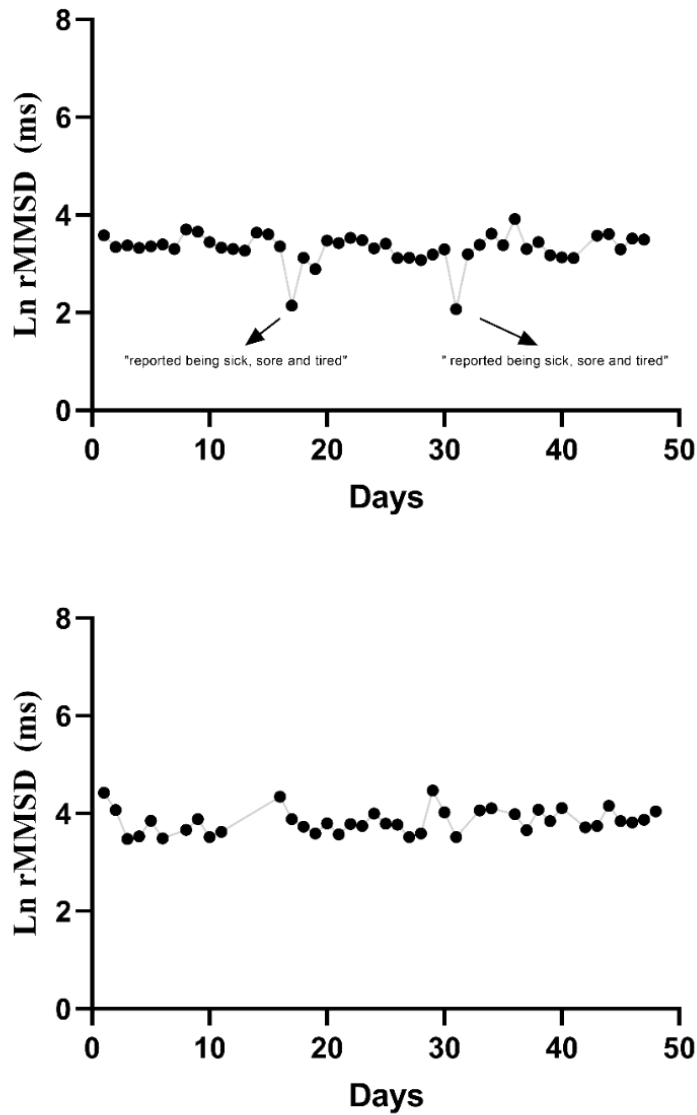


Figure 16. Daily HRV variations for the fifth (top) and sixth (bottom) participants.

Figure 16 shows the case of two participants who did not show great HRV fluctuations during the training regimen. Particularly, in the first panel, the two big declines observed can be associated with the participant reporting to be sick, sore and tired. This participant resulted to be the most compliant towards the training regimen and the measures taken during the study, showing a very stable routine in a day-to-day basis. In the second case, no particular reasons were observed that could potentially explain the changes in HRV. In general, the measurements

taken by these last five participants showed a relative stability that seems to be disrupted sporadically. Since these participants did not show a high compliance towards the daily measurements, no in-depth analysis was performed to understand how the HRV could have been influenced by other factors rather than only the training regimen.

The results presented here suggest that our SIT program did not have a big impact on HRV, confirming the results of Study 1 and the every-two-weeks HRV analyses. In most cases, the declines observed could have been, at least, partly explained by reasons other than the participation to the all-out training program. This finding confirms that the recovery periods between sessions were long enough for the autonomic nervous system to recovery after all-out exercise. Moreover, these results open a possibility towards the monitoring of the fatigue/recovery status during SIT. The HRV has been shown to reflect well how training affects practitioners, showing that HRV-based training could help on training prescription, planification and, ultimately, improve performance (Granero-Gallegos et al., 2020). Daily HRV was assessed to understand the influence of an intense training such as SIT on an unfit population. In this case, the HRV seemed to be unaffected by the training regimen, but whether or not this is extensible to other populations/conditions, remains unknown. Furthermore, as the app can be connected to a larger platform, the sleep and nutritional routines together with the feelings of the participants can be monitored during the training. Such tools could ultimately help participants understand how to self-assess their training performance and their trainers to have a more accurate control of the training.

## ***5.2. Mathematical modeling of the fitness and fatigue components***

As for the HRV, an *a posteriori* analysis was performed to determine whether the SIT program could exacerbate an excessive cumulative fatigue due to an incomplete recovery between sessions. Specifically, too much training with insufficient recovery between exercise sessions could lead to a decreased performance (Lehmann et al., 1997). By quantifying the exercise dose while accounting for both intensity and duration, these mathematical models have been able to differentiate between the influence of fatigue and adaptation on performance. For that purpose, the training dose was calculated following the method of Foster et al. (2001) from exercise duration weighted by session rate of perceived exertion using a 0 to 10 scale. For one SIT session, the exercise duration is proportional to the number of sprints and, since the sessions

were meant to be all-out, the perceived exertion was fixed as a constant value to 10 giving training dose ranged from 20 for the sessions with less sprints (*i.e.* 8) to 35 for the sessions with the higher number of sprints (*i.e.* 14). It was arbitrary fixed to 0 for the rest days and to 10 for the days of testing at the end of each block of training. Subsequently, the mean power over the first eight sprints of the training sessions was analyzed as a marker of performance. The rationale behind this choice relies on the fact that participants were asked to perform all-out (and therefore maximally) in each training session and for each repetition. Two mathematical models were used for describing the interaction between fitness (positive effects of the training) and fatigue (negative effects of the training). The first one is the seminal model proposed by Banister et al., (1975) which considered both positive and negative effects of training using two exponentials. The second one is the variable-dose response model proposed by Busso (2003) which considered that fatigue induced by a single session varies according to past sessions. Between these two last models, the one proposed by Busso (2003) is believed to be the most appropriate one, since increases in training frequency have been shown to yield to a progressive increase in the magnitude and duration of the fatigue induced by a same training bout (Busso et al., 2002) and the Banister's model would not account for this component. Both models were compared to a simple model without fatigue component, *i.e.* a single exponential. The idea was to test if a fatigue component in the model allowed a better fit of the data which could be interpreted as a significant cumulative fatigue with the accumulation of the sessions during the 6-week program. When the models accounting for the fatigue component were used, no significant improvement of the goodness of fit was obtained,  $r^2 = 0.40 \pm 0.26$  for the model with only one component for positive effect and  $r^2 = 0.41 \pm 0.27$  and  $0.42 \pm 0.27$  for the model of Banister et al (1975) and Busso (2003) respectively. In other words, the model with a fatigue component failed to show any traces of cumulated fatigue due to an uncomplete recovery between SIT sessions. These results go in line with the HRV-related data showing that 48 h are more than enough to recover after a SIT session and that therefore the SIT protocol proposed here did not generate enough fatigue for impairing subsequent adaptations. However, some limitations need to be considered. The data processed by the model was obtained from training sessions and not from specific measures of performance. The fact of analyzing performance from a several-sprints session, instead of specifically doing a performance test, could have influenced the results. Moreover, the day-to-day variability of the marker of performance used was considerably high, and therefore, the signal-to-noise ratio could be also playing a role on the lack of detection of cumulated fatigue (Hemingway et al., 2021).



## ***6. Chapter six: General discussion and perspectives***

The main aims to the current thesis work were to study the acute effects of all-out exercise on neuromuscular and cardiac autonomic functions, to investigate the neuromuscular adaptations generated by a SIT program, and to determine the time course of the cardiorespiratory and neuromuscular adaptations to SIT. The main findings were as follows: i) recovery after a single session of SIT occurred rapidly, as both the neuromuscular and the cardiac autonomic systems were found to be back to baseline levels 24 h after the session; ii) substantial improvements in cardiorespiratory and neuromuscular functions took place within the first two weeks of training and exhibited a plateau thereafter; and iii) even though the isometric force did not significantly increase after a 6-week SIT program, increases in the force and speed production on the cycle-ergometer were observed during and after the program, evidencing some level of neuromuscular adaptations.

In the discussion section that follows, the primary objective is to provide an overview of the key findings from the experimental part of this PhD project while also offering contextual information to explore the practical implications of these findings.

### ***6.1. Fatigue and recovery during SIT programs: implications for exercise prescription in the short and long term***

Although, in general, two days of recovery are usually recommended after HIIE (Michael et al., 2017; Stanley et al., 2013), in the first study of the current PhD work we demonstrated that this does not necessarily need to be the case for SIT. Both the neuromuscular and the cardiac autonomic system were found to be recovered in the first 24 h after an  $8 \times 15$  s SIT session. Additionally, we observed the same time course of the recovery for a session consisting of  $4 \times 30$  s, suggesting that when the W:R ratio and the total work time are kept the same, recovery also occurs rapidly. These results might have important implications for: i) SIT prescription; ii) the combination of SIT with other training modalities; and iii) the assessment of fatigue during SIT programs.

The fatigue-recovery balance is known to be an important for aspect of training planification (Skorski et al., 2019). Among the different ways to assess fatigue after physical exercise, those

that better represent the conditions and nature of the exercise performed should be emphasized. In Study 1 of the current work, we observed important declines in the voluntary and electrically evoked forces one, two, and eight minutes after two different SIT sessions. Our results evidence and confirm that all-out exercise induces a notable degree of neuromuscular fatigue. In our case, the neuromuscular fatigue was mainly peripheral in origin, since the VA was not significantly different from the baseline. Although we did not perform continuous HRV measures after the sessions, a recent experiment conducted by Ye et al. (2022) showed important declines in rMMSD up to 140 minutes after a 2 and 4 × 30 s compared to baseline and to a non-exercise control group. These data indicate that the autonomic nervous system activity is also strongly impaired after SIT and that, therefore, adequate recovery should be ensured before the next training stimulus is imposed. Interestingly, our findings show a quick recovery of these two systems after the session. Both the neuromuscular and the cardiac autonomic functions were found to be back to baseline levels 24 h after SIT. Moreover, as the isometric approaches for evaluating neuromuscular fatigue might not be very ecological compared to cycling-based SIT, we assessed the force, speed and power production on the same cycle-ergometer in which the sessions were performed. The results of the PVPF did not reveal an impaired capacity to perform a 7 s maximal sprint, meaning that from isometric but also from a dynamic perspective, participants were able to replicate the same maximal effort.

These results provide evidence on how fast the neuromuscular and autonomic nervous systems recover after a single bout of all-out exercise. In addition, the individual HRV data presented in Chapter 5 show that the fatigue/recovery balance is well maintained when all-out training sessions are scheduled 48 h apart during a SIT program. In practice, both the neuromuscular and the autonomic nervous system would be ready to cope with the next training stimulus the very next day after the sessions. However, despite occasionally two intense training sessions could be performed consecutively, rest days have been shown to be necessary for promoting adaptations and improve performance within SIT programs (Burgomaster et al., 2005; Parra et al., 2000). In addition to that, a previous study showed how the sharp increments in training frequency yielded to longer recovery times after each intense session, evidencing the greater cumulative fatigue due to the additional intense training (Busso et al., 2002). Thus, even though our data showed a complete recovery 24 h after a SIT session, suggesting that the system would be ready for absorbing the effects of another all-out session 24 h after the previous one, care should be taken when scheduling successive SIT sessions, as the cumulative effects of SIT on performance fatigability have not been extensively studied yet. Thus, the scheduling of

consecutive intense training loads should be done with caution and it might be recommended that a combination of all-out exercise with other type of training stimuli to promote different physiological adaptations is performed. In this regard, previous research has shown that the first ventilatory threshold acts, in the short term, as a binary threshold for HRV recovery (Seiler et al., 2007). Furthermore, Brownstein et al., (2022) have recently shown how cycling above the first ventilatory threshold led to a greater fatigability and impaired maximal exercise performance capacity. While such hypothesis is purely speculative, it is conceivable that the prompt recovery seen following SIT, coupled with the observation that low-intensity exercise below the first ventilatory threshold appears to induce minimal fatigue, could potentially allow for the combination of SIT with low-intensity training on consecutive days. This could apply not only to highly trained athletes but also to individuals who are non-active or engage in recreational physical activity.

## ***6.2. Sprint interval training programs in sedentary populations: considerations for the implementation and practical applications***

SIT has been shown to be a very effective method for improving general physical fitness across different specific populations (Adamson et al., 2020; Jiménez-Maldonado et al., 2020; Whyte et al., 2010). In the second study of this PhD project, we investigated the time course of the cardiorespiratory and neuromuscular adaptations to SIT in a sedentary and young population. The training protocol proposed here was composed of 15 s sprints, interspersed with recovery periods of 2 minutes. The number of repetitions increased progressively throughout the 2-weeks training blocks, and ranged between 8 and 14. This SIT model is very similar to the one initially proposed by Burgomaster et al., (2005). The only difference is that the sprint and the recovery time were divided by two and the number of repetitions doubled. This decision was made in accordance previous results showing that shorter-sprints SIT models could still be a sufficient stimulus to develop adaptations (Hazell et al., 2010; Metcalfe et al., 2012; Yamagishi & Babraj, 2017). Although the number of repetitions in the protocol of Metcalfe et al. (2012) was also reduced to make the training even more feasible for untrained populations, we decided to maintain the same total work time than Burgomaster et al. (2005). For that purpose, we doubled the number of repetitions and obtained a SIT model that was slightly different but, overall, very similar to one that has been widely used (Burgomaster et al., 2005; Hazell et al., 2010; Yamagishi & Babraj, 2017). Our results showed significant gains of ~8% in  $\dot{V}O_{2max}$ , one of

the most used health-related markers and predictor of all-cause mortality (Ross et al., 2016), after only two weeks of training. This shows that shortening the sprint, while maintaining the same W:R ratio and total work time, does not negatively impact the magnitude of adaptations.

Previous research has shown that an increase of one metabolic equivalent ( $3.5 \text{ ml}^{-1} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ) resulted in a 10-25% improvement in survival over an approximate 10-year follow-up (Nes et al., 2014). Remarkably, these results were adjusted for other risk factors of premature mortality such as age, health status, smoking, blood pressure or cholesterol levels. Even though our study was not powered to perform these kinds of calculations, an average  $\dot{V}O_2\text{max}$  increase of  $4.1 \text{ ml}^{-1} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  was observed. These results have been widely replicated in previous studies adopting SIT and they are within the range reported by various high-quality SIT-related meta-analyses (Gist et al., 2014; Vollaard et al., 2017). Although these results have important implications for the promotion of physical exercise, there has been strong critics towards the use of SIT in sedentary populations. It has been argued that all-out exercise could be considered too arduous and may evoke anticipated perceived incompetence, lower self-esteem and potential failure towards the training (Hardcastle et al., 2014). In addition, another main argument is that SIT is a complex and structured regime that requires high level of self-discipline and self-regulation that is unlikely to be adopted outside of the laboratory environment. Even though the training sessions were considered hard and extenuating by our participants, we obtained a high compliance to the training program. Out of 16 participants and a total of 240 training sessions, only 6 sessions were missed. Additionally, this 2.5% of unattendance was mainly due to sicknesses non-related with the training regimen. This suggests that the SIT model proposed here could be used in a young and sedentary population for improving cardiorespiratory and neuromuscular markers. All the sessions in the current study were supervised and strong verbal encouragement was provided by one or more experimenters. We acknowledge that this fact could be a limitation for the use of SIT out of the laboratory settings and that this, combined with the all-out nature of the activity, could reduce adherence to training among non-accustomed individuals. However, when other training modes such as endurance and resistance training are applied in non-accustomed populations, they might also require the presence/advise of a coach to ensure that, at least, the basic principles of physical training and a correct technique are respected. Given this fact, and considering that previous research has compared the effects of CT vs. SIT standardizing for the same amount of work performed (Gibala et al., 2006; Scribbans et al., 2014), SIT is undoubtedly an effective way to rapidly increase the overall physical fitness.

We found a quick and significant increase in several cardiorespiratory parameters after two weeks of training. It is important to mention that, despite the training regimen was maintained for an additional four additional weeks, no significant differences were observed thereafter. From a cardiorespiratory point of view, these results had been previously observed in the literature in studies specifically aiming to determine the time course of the adaptations (Burgomaster et al., 2008; Yamagishi & Babraj, 2017). Although no physiological explanation to this phenomenon was provided, we observed that the average time spent at high percentages of the maximal HR during the training sessions comprised in the last four weeks of training was significantly lower compared to first two weeks. Since the time spent at high percentages of the HR, which is an indicator of cardiac solicitation, has been found to be key in the development of the adaptations in  $\dot{V}O_2\text{max}$  (Gormley et al., 2008), we think that this could be the main reason behind the observed stagnation in cardiorespiratory improvements. Two main hypotheses might help us understanding this phenomenon. Firstly, habituation to training could have lowered the mean HR responses for the same training session during the recovery periods. Secondly, participants might have reduced their willingness to perform all-out efforts as the training progressed. Although this second reason could confirm one of the criticisms to SIT previously mentioned, the analysis on the power production during the sessions revealed that our participants managed to maintain an overall higher mean power, because the  $T_{\text{work}}$  of the second and third block of training was significantly greater or equal compared to the first. Thus, we recommend the monitoring of the HR during SIT sessions, as it is a simple parameter that might reveal enough information to determine whether or not the participant is performing the session adequately for triggering the desired adaptations and maximizing the effects of the training.

To further address the point about the participants willingness to perform maximally after the first sessions of SIT, we performed further analysis to understand what were the specific power responses to the SIT sessions. In Figure 17, the individual and group power traces for the four 8 sprints sessions comprised in the training protocol are shown as a percentage of the mean power of the first sprint. Remarkably, although the group trace shows a progressive decline in the mean power output as the session progresses, the individual traces of some participants show a different response. Such observation suggests that some participants are able to develop similar or even higher mean power outputs in the last sprints of the sessions, showing that different pacing strategies were adopted during these sessions. In other words, the exercise was probably not always conducted in an all-out manner for every repetition in every session. This occurred not only for the 8-sprint sessions but also for longer sessions during the training

program. Pacing strategies, such as maintaining a relatively high mean power throughout the session instead of peaking at the beginning and then declining, might also allow for a notable increase in the total work.

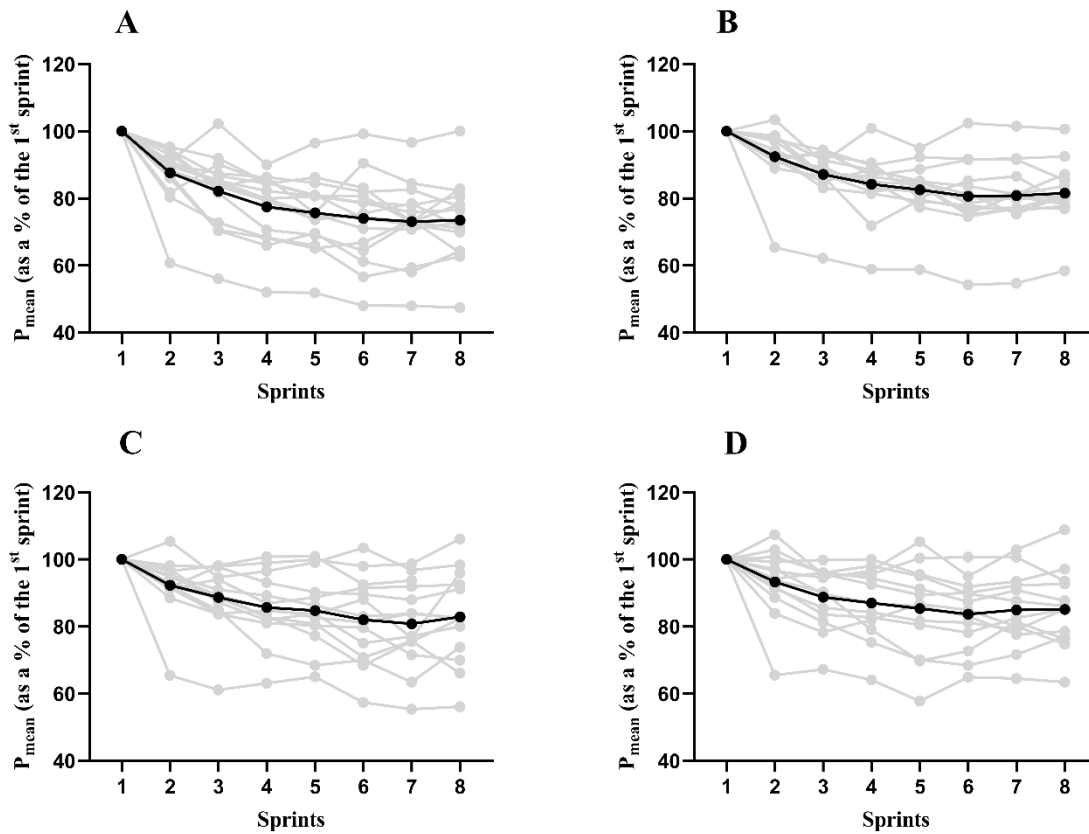


Figure 17. The individual and group power traces for the four 8 sprints sessions comprised in the training protocol as a percentage of the first sprint. A, B, C and D correspond to the familiarization, the fifth, tenth and fifteenth training sessions respectively.

Thus, instead of arguing that habituation to training might have made our participants improve less for a given training session, a combination of a decreased willingness and increased pacing might be the reason why our participants spent less time at high percentages of their maximal HR as the protocol progressed. This could ultimately explain why the cardiorespiratory adaptations plateaued even though the training was kept constant for four more additional weeks. Under this scenario, SIT could be used for two or three weeks as an exercise mode to rapidly increase physical fitness and then combined with other training modes, such as endurance and/or resistance training, to keep promoting cardiorespiratory adaptations and ensuring adherence to training.

### ***6.3. Neuromuscular adaptations to SIT***

In the current thesis work, we studied the time course of the neuromuscular adaptations to a SIT program. As the isometric and dynamic neuromuscular assessments have been shown not to be interchangeable (Koral et al., 2021; Krüger et al., 2019), we included measurements of the isometric force together with speed, force, and power generation capacities on a cycle ergometer. Our results show progressive but non-significant increases in the iMVC as the training progressed. Specifically, the iMVC relative to bodyweight was increased from 3% at Week<sub>2</sub>, 5.8% at Week<sub>4</sub>, 7.6% at Week<sub>6</sub> and 8.3% at Week<sub>7</sub>. We observed a great inter-subject variability in this measure, indicating that not every participant responded in the same way. These results agreed with the ones previously presented (Astorino et al., 2011; Bagley et al., 2021). On the other hand, the dynamic force production ( $F_0$ ) was increased of 9% at Week<sub>2</sub>, stabilized in the two subsequent time-points, and increased again by 15.8% at Week<sub>7</sub>. Additionally, the standard deviations were smaller, indicating that participants were more likely to evolve in the same direction in response to the training. These results show that the isometric and dynamic force production might not respond to the same extent to a given training regimen, providing evidence for the use of both or, at least, the most ecological assessment. The speed production during the PFVP was found to be decreased significantly by ~10% at the end of the protocol (Week<sub>7</sub>) while, in contrast to the previous literature, the  $P_{\max}$  did not improve with training. Overall, although the maximal expression of power generation did not change, these results indicate SIT induced cycling-specific neuromuscular adaptations. Previous research had reported increases in mean and peak power production during a 30 s Wingate test. However, these results might not be comparable to ours since we measured the power production during a two different-load 7 s sprints separated by a few minutes of rest.

It is worth-mentioning that both  $F_0$  and  $V_0$  were found to be increased and decreased to a greater extent at Week<sub>7</sub> compared to Pre. During this last week, as the training program had already finished, participants only performed two evaluation sessions separated from 96 h of rest. The fact of not performing any kind of training, combined with some additional recovery, might have acted as a form of tapering, allowing our participants to perform better on the measures. Additionally, the intrinsic motivation of accomplishing a 6-week training program could have also played a role in this last measurement. An idea that could help explaining these results is the high resistance load used during the training. Typically, during SIT training programs,

friction loads of about  $0.7 \text{ N} \times \text{kg}$  of bodyweight is used. The high loads combined with the instructions to accelerate, overcome the resistance, and reach the peak power as fast as possible, might have improved participants' capacities to develop high forces in a short period of time. Secondly, the high and constant resistance throughout the duration of the sprint might hinder participants' ability to maintain a high pedaling speed during the repetitions. Therefore, as the training program progressed, a shift towards the force-side involving might have happened.

Interestingly, these results go in line with a side study that took place at the beginning of this PhD project. The aim of the study was to test the efficacy of SIT on the PFVP and jump performance in young trained basketball players. As all the training sessions took place out of laboratory settings to better adapt to the availabilities of the team, the SIT program had to be modified accordingly. Thus, instead of performing the classic cycling-based SIT, we proposed a running-based SIT that consisted of 30 s sprinting and 4 minutes of recovery (Lloria-Varela et al., 2023). During the sprints, the players were asked to cover the highest possible distance performing back and forth trips of 5, 10, 20 and 25 m in the basketball court. Since all the trips were performed in the same line, the sprints involved several decelerations, sharp changes of direction and accelerations. The results showed that two weeks of running SIT induced significant changes in the PFVP measured on the field with a radar gun. The  $F_0$  was found to be increased, the  $V_0$  remained constant and the  $P_{\max}$  increased. Moreover, although sprint performance increased over the first five and ten meters of a linear 20 m sprint, the time at 20 m and the maximal velocity were unchanged. All these adaptations were accompanied by a significant increase in jump height measured through a countermovement jump test. The main hypothesis behind the changes in the PFPV was linked to the fact that the training involved several decelerations and accelerations. As the players were asked to reach a line, turn and then sprint back to another line to cover the highest distance possible, all these re-accelerations might have made them more able to orient the forces forward during the sprint. This hypothesis was confirmed by the fact that the ratio of force, a parameter used in the model to compute the runners' ability to orientate the forces forward, was significantly increased. Moreover, high correlations ( $r = -0.93$  and  $-0.82$ ;  $p < 0.001$ ) were observed between the changes in  $F_0$  and the split times at five and ten meters, meaning that the players with higher  $F_0$  were the fastest covering these two distances due to improved acceleration capacities and horizontal force orientation.



It is interesting to observe how two SIT modalities applied in totally different conditions and populations induce similar changes in the PFVP. The results from both works are in favor of the hypothesis supporting that SIT induces significant neuromuscular adaptations. Furthermore, the use of the PFVP might better capture the potential changes in neuromuscular function during a cycling- or running-based sprint than the isometric assessments. More mechanistic research is thus needed to link the changes in fiber type and size with the changes in the different parameters of the PFVP.

#### ***6.4. General conclusion***

In summary, SIT was proven to be a suitable strategy to efficiently enhance physical fitness and improve health among sedentary individuals. Both our second study and the existing literature demonstrate remarkable improvements in health-related markers such as  $\dot{V}O_2\text{max}$  with little time investment. This bears significant implications for promoting efficient and effective physical activity programs. Some individuals may benefit from using SIT as their primary training mode to achieve better physical fitness before transitioning to other exercises, while others can simply incorporate short SIT sessions into their existing exercise routines. In addition, our second study revealed that despite the high intensity of SIT, it induces little fatigue and allows for quick recovery. This suggests that it could be easily combined with other exercise modes such as endurance or strength training. Considering our results, it could be logical to speculate that if SIT can rapidly improve health-related markers with little fatigue, it may also hold potential benefits for other populations. In conclusion, although studying the effects of SIT in young and sedentary populations provides valuable insights, SIT programs should be first adapted and then tested in order to determine whether or not can be effectively implemented in elderly or pathological populations.

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