EFFECT OF POST-EXERCISE ISCHEMIC CONDITIONING ON MULTISTAGE 20-KM CYCLING TIME TRIAL PERFORMANCE IN COMPETITIVE CYCLISTS

by

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ABSTRACT

Introduction: During multistage competitions (e.g., Tour de France), professional cyclists have very short recovery periods between races (<24 h). Evidence suggests that 3-4 brief cycles (5-min) of skeletal muscle ischemia and reperfusion (ischemic conditioning [IC]) applied at rest before exercise improves performance. However, few studies have assessed whether IC applied immediately post-exercise (PEIC) facilitates recovery for optimal multistage performance. Earlier research demonstrated that PEIC applied to the thighs of amateur cyclists immediately after completing a maximal incremental cycle exercise test (MICT) prevented declines in MICT performance 24 h later, suggesting that PEIC may facilitate recovery and optimize multistage exercise performance. However, MICT does not mimic the time trial nature of a multistage race; and no study has examined whether the effect of PEIC on multistage performance persists beyond 2 stages and is similarly observed in professional cyclists.

Objective: To examine the role of PEIC as a potential recovery tool by assessing its impact on multistage laboratory-based 20-km cycling time trial (20kmTT) performance among competitive cyclists.

Hypothesis: Compared to a control intervention (SHAM), the PEIC intervention would mitigate the decline in 20kmTT performance over 5 consecutive days.

Methods: Eight competitive cyclists (2F:6M) were randomized to PEIC (n=3) or SHAM (n=5). After participant characterization (*Visit 1*) and familiarization procedures (*Visit 2*), cyclists completed a baseline 20kmTT followed immediately thereafter by PEIC or SHAM (*Visit 3*). PEIC consisted of 4 x 5-min cycles of circulatory occlusion (50 mmHg above systolic blood pressure) / reperfusion (0 mmHg) applied unilaterally on alternating thighs. SHAM and PEIC protocols were

the same, but occlusion pressure during SHAM was standardized at 20 mmHg. *Visits 4-7* consisted of a 20kmTT followed by PEIC or SHAM at *Visits 4, 5 and 6*. All 20kmTTs were separated by 24±2 h. Before 20kmTTs: lower limb muscle soreness was assessed via pain pressure threshold testing; and perception of recovery scale and performance readiness ratings were collected. During 20kmTTs, performance (duration, power output, speed) and perceptual parameters were collected.

Results: On average, participants in the PEIC and SHAM intervention groups experienced a similar effect of their respective interventions on both their subsequent 20kmTT performance and their recovery rate across Visits 3-7. Mean scores of perceived recovery status were high and similar in both intervention groups. There was no significant difference in pain pressure thresholds recorded for the 3 muscles (vastus lateralis, rectus femoris, gastrocnemius) both between and within groups across *Visits 3-7*. The duration of the 20kmTT decreased (performance increased) by 0.16% in the PEIC intervention group from *Visit 3 to 7*, whereas 20kmTT duration increased (performance decreased) by 0.58% in the SHAM intervention group from *Visit 3 to 7*. It should also be noted that the typical within-subject variation in time to complete such a 20kmTT performed on subsequent days is 0.34% due to the effects of exercise on your body. Mean power output and cycling speed over the 20kmTT remained relatively constant across *Visits 3-7* in both groups, with higher values being observed at each visit in the PEIC compared to SHAM group. Mean intensity ratings of perceived exertion and leg discomfort throughout the 20kmTT remained relatively consistent both between and within groups across *Visits 3-7*. Perceived leg discomfort during the application of the intervention, consisting of a cuff inflation pressure of 50 mmHg above systolic blood pressure for the PEIC group and 20 mmHg for the SHAM group, was higher in the PEIC group throughout *Visits 3 to 6* compared with their counterparts. In the SHAM group, mild to moderate discomfort was reported despite a lower cuff pressure.

Conclusion: The preliminary results of this randomized controlled study do not provide evidence to support a potentially beneficial effect of PEIC on multistage 20kmTT performance or indices of recovery and/or performance readiness.

RÉSUMÉ

Introduction: Pendant les compétitions à plusieurs étapes (par exemple, le Tour de France), les cyclistes professionnels ont des périodes de récupération très courtes entre les courses (<24 h). Des preuves suggèrent que 3-4 brefs cycles (5 min) d'ischémie et de reperfusion des muscles squelettiques (conditionnement ischémique [CI]) appliqués au repos avant l'exercice améliorent les performances. Cependant, peu d'études ont évalué si le CI appliqué immédiatement après l'exercice (PEIC) facilite la récupération pour une performance optimale en plusieurs étapes. Des recherches antérieures ont démontré que le PEIC appliqué sur les cuisses de cyclistes amateurs immédiatement après la réalisation d'un test d'exercice maximal incrémental (MICT) prévenait le déclin de la performance MICT 24 heures plus tard, ce qui suggère que le PEIC peut faciliter la récupération et optimiser la performance des exercices à plusieurs étapes. Cependant, le MICT n'imite pas la nature du contre-la-montre d'une course à plusieurs étapes ; et aucune étude n'a examiné si l'effet de la PEIC sur la performance à plusieurs étapes persiste au-delà de deux étapes et est observé de la même manière chez les cyclistes professionnels.

Objectif: Examiner le rôle de la PEIC en tant qu'outil de récupération potentiel en évaluant son impact sur la performance de la course cycliste contre la montre de 20 km (20kmTT) en laboratoire chez les cyclistes de compétition.

Hypothèse: Comparée à une intervention témoin (SHAM), l'intervention PEIC atténuerait le déclin de la performance du 20kmTT sur 5 jours consécutifs.

Méthodes: Huit cyclistes de compétition (2F:6M) ont été répartis au hasard entre PEIC (n=3) et SHAM (n=5). Après la caractérisation des participants (*visite 1*) et les procédures de familiarisation (*visite 2*), les cyclistes ont effectué un test de base de 20 km, suivi immédiatement

par le PEIC ou le SHAM (*visite 3*). Le PEIC consistait en 4 cycles de 5 minutes d'occlusion circulatoire (50 mmHg au-dessus de la pression sanguine systolique) / reperfusion (0 mmHg) appliqués unilatéralement sur des cuisses alternées. Les protocoles SHAM et PEIC étaient les mêmes, mais la pression d'occlusion pendant le SHAM était normalisée à 20 mmHg. Les *visites 4 à 7* consistaient en un TTT de 20 km suivi d'un PEIC ou d'un SHAM aux *visites 4, 5* et *6*. Tous les 20kmTT étaient séparés par 24±2 h. Avant les 20kmTT : la douleur musculaire des membres inférieurs était évaluée par un test de seuil de pression de la douleur ; et les évaluations de l'échelle de perception de la récupération et de la préparation à la performance étaient recueillies. Pendant les 20kmTT, les paramètres de performance (durée, puissance de sortie, vitesse) et de perception ont été recueillis.

Résultats: En moyenne, les participants des groupes d'intervention PEIC et SHAM ont ressenti un effet similaire de leurs interventions respectives à la fois sur leur performance ultérieure au 20kmTT et sur leur taux de récupération au cours des visites 3-7. Les scores moyens de l'état de récupération perçu étaient élevés et similaires dans les deux groupes d'intervention. Les scores de préparation à la performance étaient légèrement plus élevés dans le groupe d'intervention PEIC que dans le groupe SHAM pour la plupart des visites. Il n'y avait pas de différence significative dans les seuils de pression de la douleur enregistrés pour les 3 muscles (vastus lateralis, rectus femoris, gastrocnemius) à la fois entre et au sein des groupes à travers les visites 3-7. La durée du 20kmTT a diminué (performance accrue) de 0,16 % dans le groupe d'intervention PEIC de la visite 3 à la visite 7, tandis que la durée du 20kmTT a augmenté (performance réduite) de 0,58 % dans le groupe d'intervention SHAM de la visite 3 à la visite 7. Il convient également de noter que la variation typique au sein du sujet du temps nécessaire pour terminer un tel 20kmTT effectué les jours suivants est de 0,34 % en raison des effets de l'exercice sur votre corps. La puissance

moyenne produite et la vitesse du vélo sur les 20kmTT sont restées relativement constantes au cours des visites 3 à 7 dans les deux groupes, des valeurs plus élevées étant observées à chaque visite dans le groupe PEIC par rapport au groupe SHAM. Les évaluations moyennes de l'intensité de l'effort perçu et de l'inconfort des jambes tout au long du 20kmTT sont restées relativement constantes entre les deux groupes et au sein de chaque groupe au cours des visites 3-7. L'inconfort perçu au niveau des jambes pendant l'application de l'intervention, consistant en une pression de gonflage du brassard de 50 mmHg au-dessus de la pression artérielle systolique pour le groupe PEIC et de 20 mmHg pour le groupe SHAM, était plus élevé dans le groupe PEIC tout au long des visites 3 à 6 par rapport à leurs homologues. Dans le groupe SHAM, un inconfort léger à modéré a été signalé malgré une pression de brassard plus faible.

Conclusion: Les résultats préliminaires de cette étude randomisée et contrôlée ne fournissent pas de preuves pour soutenir un effet potentiellement bénéfique de la PEIC sur la performance du 20kmTT en plusieurs étapes ou sur les indices de récupération et/ou de préparation à la performance.

PREFACE AND CONTRIUTIONS OF AUTHORS

George, O was the primary author and played the principal role in the collection, analysis, and interpretation of the data as well as in the preparation of this thesis and the accompanying manuscript.

Van Noord, N; McManus, B; Azeem, O; Xie, T and Kim, J also played a principal support role in the collection and extraction of the data. Markus, I; Mujaddid, A; Aucoin, R; Triandafilou, J; Faria de Oliveira, M and Russell, E contributed to the collection of the data.

Jensen, D and Gepner, Y contributed to the conception, design, and financial support of this study, as well as to the analysis and interpretation of data and the preparation of this thesis and accompanying manuscript.

CHAPTER 1: Review of Literature

1.1. Parameters of Endurance Exercise Performance. A commonly used outcome variable to assess for endurance exercise performance is time to completion, which can be understood as the time required to complete a given task (Coyle, 1999; Schabort et al., 1998). In the context of sport, it often describes an athlete's performance in an endurance or aerobic exercise such as cycling, running, and/or swimming. In general, athletes are able to spontaneously generate very high-power outputs for short periods of time (**Fig. 1.1**) (Morton & Hodgson, 1996). When an athlete increases their power output over a fixed distance, such as a 20-km time trial, the time to completion decreases and performance increases. To determine how to achieve this, one must first understand power output, which is calculated as work divided by time, where work is the product of force and distance. In a given sporting event with a constant distance such as a 20-km cycling time trial, an athlete's performance can either be improved by maintaining a higher muscular force output (with attendant reductions time to completion) through external factors such as riding economy, including decreased bicycle weight. Intrinsically, an athlete's ability to generate force and, by extension, power is affected by internal perceptual elements such as mood and pain, which are largely psychological, as well as a multitude of physiological factors, primarily muscle fiber size and length, fiber attachment and type, and the number of cross bridges in a muscle and their respective ability to generate force (Fitts et al., 1991; Menaspa et al., 2010). Alternatively, the force generated throughout the duration of an exercise like a 20-km time trial can be modified by supplemental physiological factors, facilitated by external influences such as recovery interventions, in the hopes of improving endurance exercise performance. In the case of elite cycling, endurance exercise performance is a frequent topic of study because its improvement also means the improvement of one's ranking in major races and competitions. Paton and Hopkins (2005; 2006), who used time to completion as the metric to define exercise performance, reported

that an improvement in experimental (laboratory-based) cycling time trials, even by a seemingly negligible amount of +0.5%, correlates with better placement in real-world cycling competitions (Paton & Hopkins, 2005; Paton & Hopkins, 2006). To see this, one need only look at the time differential between podium finishers in both the men's and women's road time trial at the 2020 Tokyo Olympics (Olympics, 2021). In the men's category, the difference between gold and silver, silver and bronze, and bronze and 4th place were 1.02 sec (1.86%), 0.42 sec (0.07%), and 0.00067 sec (0.01%), respectively. In the women's category, the time separation between these same positions were 0.941 sec (3.11%), 0.086 sec (0.28%), and 0.12 sec (0.38%), respectively.

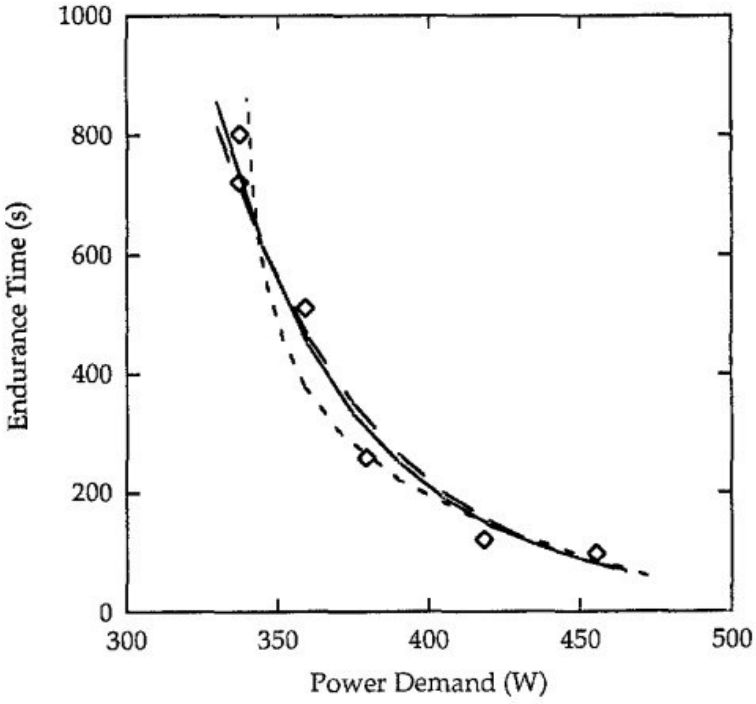

Figure 1.1. Power duration curve (reproduced from Morton & Hodgson, 1996).

1.2. Practical Scenarios of Endurance Exercise Performance. Elite cyclists, due to the nature of their sport, continually strive to improve their endurance exercise performance. This has been shown in research by Santalla et al. (2012) who demonstrated that performance parameters such

as the maximal rate of O_2 consumption (VO_2,max) and power output are essential to cyclists. At the elite level, a VO_2,max of 80 ml/kg/min seems to be the minimum threshold required to win a major competition (Santalla et al., 2012)Equally, a maximum power output of 450 watts or greater is generally observed in cyclists who excel in time trial events (Santalla et al., 2012). Notably, a study by Bell et al. (2017) on four-time Tour de France winner Chris Froome reported VO_2,max and maximum cycling power output values of 84 ml/kg/min and 525 watts, respectively (Bell et al., 2017). These results, among the highest in elite athletes, allowed the authors to posit the importance of these endurance exercise parameters, as targets for performance improvement (Bell et al., 2017).

Some of the most notable competitions in the sport of cycling are three races known as the Grand Tours, the most famous of which is the Tour de France. The Tour de France is a stage race, which means that the total distance athletes must cycle during the event is divided into multiple smaller stages to be ridden over several days across multiple countries and a variety of terrains and distances. The Tour de France is composed of 21 stages that are spread over a period of 23-days. Of these 23 days, two are full rest days and one day is a so-called "transfer day" that allows athletes and their teams to travel to the starting point of the next stage (Tour de France, 2022). In 2022, this transfer day saw athletes and their teams travel from Sønderborg, Denmark, where the third stage of the race took place, to Dunkirk, France, where the athletes began the fourth stage. On the 2022 Tour de France course, the average distance covered was 159.7 km, with the first stage being the shortest time trial at 13.2 km and the sixth stage the longest at 220 km (Tour de France, 2022). Given the exceptionally demanding nature of these distances on an athlete's body, copious amounts of rest would be required to allow the subsequent stages of the race to be completed with maximum endurance performance (i.e., shortest times to completion of each stage distance).

In other words, these athletes are expected to cycle an average distance of 159.7 km per day, for 6 consecutive days at times, before receiving a full 24-hour rest period throughout the competition. As noted by Faria et al. (2005), one of the dangers of these rigorous competitions is a phenomenon called “over-reaching”, which reflects an imbalance in the amount of effort exerted through intense cycling and the amount of rest and recovery time afforded to the body. This phenomenon, which is associated with a significant level of accumulated fatigue, can decrease cycling performance and, in some cases, result in the athlete's withdrawal from competition (Faria et al., 2005). Due to the current structure of cycling competitions and athletes’ desire to gain an advantage over their competitors, it is imperative to identify (through research) safe and effective methods for athletes to recover in the limited time available in the fast-paced competition schedule.

1.3. Physiological Impacts on Cyclists. During particularly intense aerobic exercise such as competitive cycling, the body undergoes several physiological changes at the cellular level. Under such strenuous conditions, the mitochondria perform a process called oxidative phosphorylation to provide the cells with the energy, Adenosine Triphosphate (ATP), needed to contract the muscles and generate force and power. As crucial as this process may be for the body's adaptation after exercise, research has stipulated that this process also initiates some undesirable side reactions that produce excess reactive oxygen-containing chemical species (ROS). In competitive situations where rest is minimal, elite cyclists undergo prolonged periods of mitochondrial oxidative phosphorylation rendering them highly vulnerable to a condition called oxidative stress, where the ROS production exceeds the body's ability to remove oxidative by-products, resulting in oxidative damage within a cell (Andreadou et al., 2020; Córdova Martínez et al., 2015). These substances result in the modulation of the body’s signaling pathways, mitochondrial dysfunction,

contractile anomalies and the necrosis of cells (Andreadou et al., 2020). The accumulation of ROS also triggers a pro-inflammatory response in the cell initially necessary for muscle repair and potential adaptation (Andreadou et al., 2020). Generally, within 1-2 hours post-exercise, these desirable effects become detrimental if the inflammatory response persists in excess, with an elevated baseline level of pro-inflammatory mediators at the onset of each new stage of a multistage race (MacIntyre et al., 1995; Piper et al., 2003). When present for 7 or more days post-exercise, these inflammatory mediators can inhibit a muscle's ability return to baseline function (**Fig. 1.2**) (Córdova et al., 2015; MacIntyre et al., 1995). Ultimately, such damages within the muscle create a phenomenon known as exercise-induced muscle damage (EIMD), which can lead to a decreased ability to generate high muscular forces resulting in a diminished power output capacity and time to completion, i.e., performance (Markus et al., 2021).

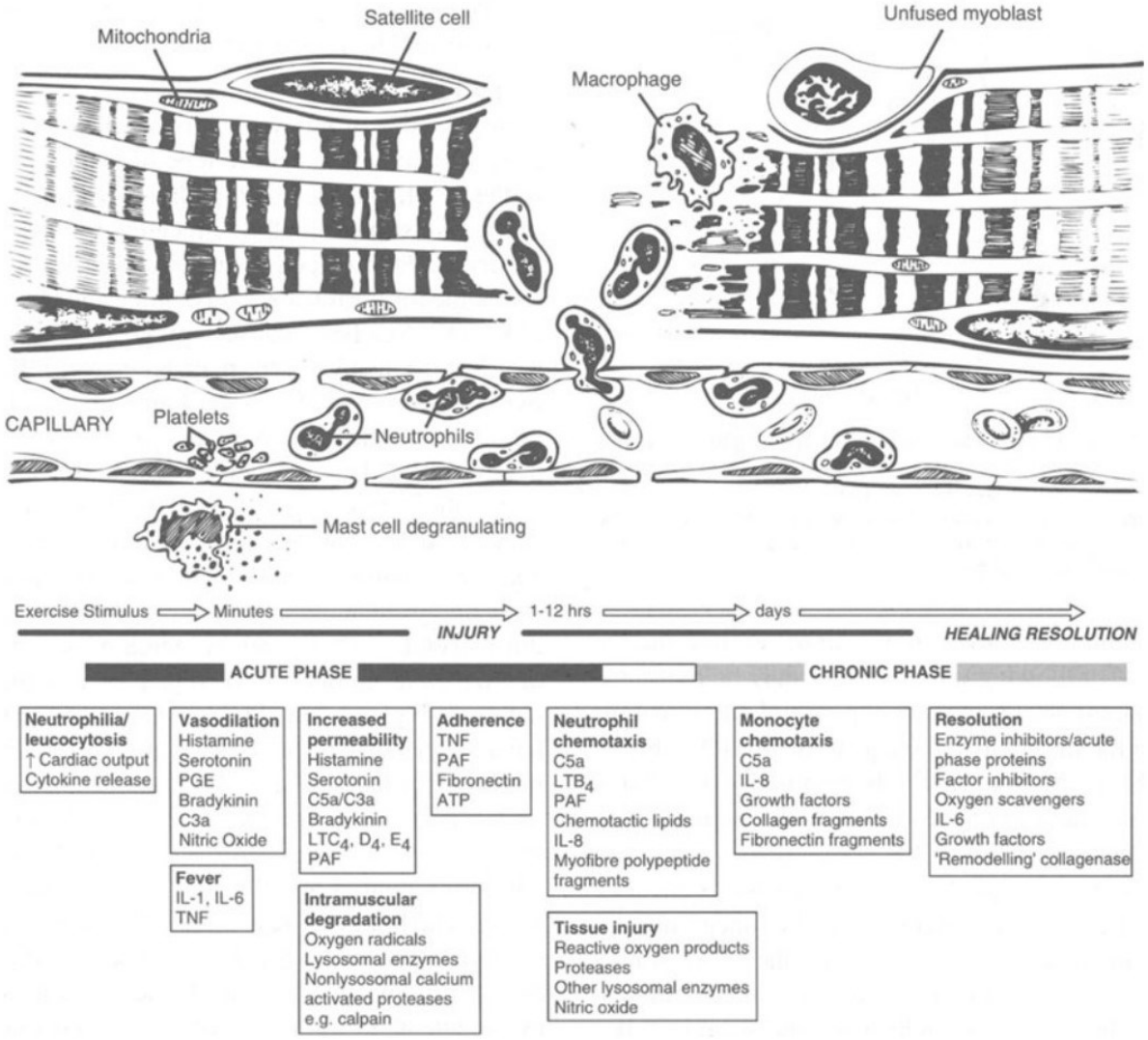

Figure 1.2 Proposed timeline of inflammation and muscle damage following exercise Abbreviations: ATP = adenosine triphosphate; IL = interleukin; LT = leukotriene; PAF = platelet activating factor; PGE = prostaglandin E; TNF = tumour necrosis factor (reproduced from MacIntyre et al., 1995).

The research summary by Markus et al. (2021) elaborates by stating that EIMD is composed of two stages, primary EIMD which is the result of the mechanical stress encountered during an exercise stimulus, and secondary EIMD which is the result of higher levels of metabolic stress thought to begin with the increased production of ROS that triggers an inflammatory

response (Markus et al., 2021). Damage to an athlete's skeletal muscle has several substantial repercussions on their performance during endurance exercise, whether through increased soreness and/or pain, or a physiological decrease in muscle force production capacity (Markus et al., 2021). Specifically, EIMD causes a redox imbalance in the sarcoplasmic reticulum (SR) of skeletal muscle leading to the opening of the calcium-sensitive ryanodine receptor (RyR) resulting in excess release of calcium. This overabundant influx of calcium causes myofibrillar hyper-contracture and the closing of mitochondrial permeability transition pore (MPTP) pumps, further damaging the SR (Hausenloy & Yellon, 2013). EIMD can be measured by magnetic resonance imaging, biopsies, subjective pain scales, or blood biomarkers such as those used in research conducted by Cordova et al. (2014), which indicated that splitting cycling exercise into multiple stages, as is done in most major competitions, exacerbates the effects of muscle damage (Córdova et al., 2015). This study, involving a three-stage cycling competition totaling 460 km over 4 consecutive days revealed higher levels of creatine kinase (CK), a blood biomarker of skeletal muscle damage, in athletes at stage 4 compared to levels recorded after completion of stage 1. Furthermore, a study done by Burt & Twist (2011) on 17 healthy university students featured a 15-minute cycling time-trial followed by a plyometric exercise protocol, a maximal vertical countermovement jump followed by 10 sets of 10 maximal vertical jumps with 1 minute of rest between sets, designed to induce EIMD and a second time-trial 48 hours post-plyometric exercise. This study indicated that, EIMD resulted in increased perceived muscle soreness, a decrease in peak knee extensor isokinetic torque measured 48 hours post-exercise, and a decrease in distance covered during a 15 minute time-trial (from 4754.05m at baseline to 4480.28m) following the initiation of EIMD all measured after a period of 48 hours post-exercise (Burt & Twist, 2011).

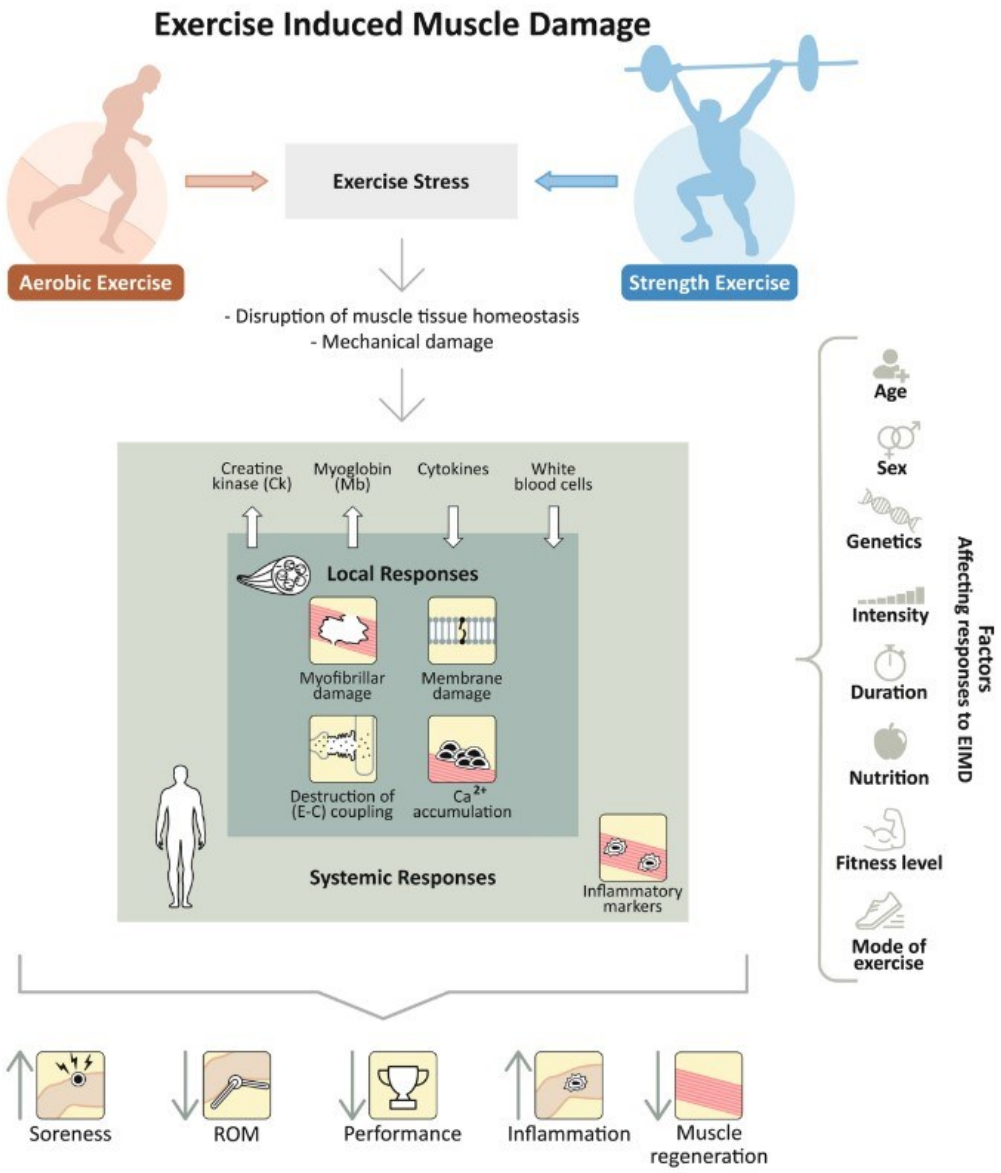

Figure. 1.3. Schematic representation of exercise-induced muscle damage processes and effects (reproduced from Markus et al., 2021).

For an athlete attempting to perform endurance activities as strenuous as Grand Tour cycling, an accumulation of muscle damage will effectively lead to structural damage that will cause a decrease in the capacity of the muscle to generate force and power. All of these elements lead to a direct negative effect on performance, which could affect an athlete's potential ranking in a given competition (**Fig 1.3**). With the goal of optimizing performance by maintaining the

capacity for muscle force and power development, cyclists and their coaches, performance directors, and sports medicine doctors are constantly seeking to identify novel strategies to accelerate the rate of recovery between and during multistage races.

1.4 Recovery and exercise performance. Recovery techniques play an exceptionally important role in maintaining an athletes' peak exercise/sport performance. In the current literature, scientists have studied the effects of countless interventions designed to optimize recovery, ranging from fluid replenishment and nutritional modifications to the use of cold-water immersion and compression garments (Montgomery et al., 2008). Lane & Wenger (2004) stated that optimizing and maintaining high level exercise performance is highly dependent on the active application of recovery techniques. This is particularly relevant in situations involving multiple repeated bouts of exercise over short periods of time wherein the ability to recover quickly is essential to mitigate performance losses (Lane & Wenger, 2004). Furthermore, Filho et al. (2015) indicated that recovery and stress factors have an incredibly strong impact on an athlete's performance. The authors asserted that athletes are more likely to experience decreased performance when unable to maintain a favorable balance between recovery demands and stress states, i.e., when they are subjected to periods of high stress and low recovery (Filho et al., 2015). The results of this study indicated that improving athletes' recovery allows them to avoid impaired cognitive functions and better respond to sport-related stressors, such as lack of energy or physical discomfort. The Tour de France, a grueling multistage competition, is a perfect example of participants trying to both perform at their highest capacity and maintain a balance between recovery and stress while being simultaneously exposed to a multitude of external challenges such as variable terrain and weather

(Filho et al., 2015). As recovery is known to play an important role in attenuating declines in athletic performance, further research into recuperative techniques is needed.

1.5 Ischemic Conditioning. Ischemic conditioning (IC) is a process by which a tissue of interest (e.g., heart or locomotor skeletal muscle) is subjected to a brief period of controlled ischemia *via* vascular occlusion followed by reoxygenation *via* vascular reperfusion. This cycle is performed sequentially, one or more times, to provide increased tolerance against future ischemic events such as those experienced during a myocardial infarction, surgery or during intense physical effort (Dick HJ Thijssen et al., 2016). The delivery of O_2 to the mitochondria of skeletal muscle cells is critical to their proper functioning especially during periods of increased O_2 demand, such as exercise. When O_2 supply is interrupted, especially for a prolonged period, and then restored, the reperfusion process can be very damaging because it often results in a wide range of cellular damage. Powers et al. (2008) reported that myocardial ischemia that persists beyond 20 minutes can create irreversible damage, namely cell death (Powers et al., 2008). It should be noted, however, that skeletal muscle has a higher tolerance to ischemia than cardiac tissue with damage appearing when exposed to 4 hours of ischemia (Gillani et al., 2012). The basic mechanisms of reperfusion injury following periods of ischemia lasting longer than 4 hours in skeletal muscle are quantified by several factors such as the modulation of acidic pH, elevated intracellular calcium concentrations, and the elevated formation of ROS within the affected tissue area (Rosenberg et al., 2018). Specifically, these modulations result in cell death through a direct action on a structure known as the mitochondrial permeability transition pore (MPTP), which effectuates the release of certain molecules, such as cytochrome C, from the mitochondria into the cytoplasm of a cell. These substances eventually initiate the caspase system which ultimately leads to the death of a given

cell (Rosenberg et al., 2018; Dick HJ Thijssen et al., 2016). Distinctively, the process of IC aimed at a particular target tissue, can also result in remote downstream effects, known as remote ischemic conditioning (RIC) or remote ischemic preconditioning (RIPC) when applied prior to the ischemic event.

Regarding the application of IC in clinical research, a consensus has not yet been reached regarding the most favorable (or efficacious) protocol given the large number of variables involved in its use, including the number (2-4) and duration (3-5 minutes) of RIPC cycles as well as the position of RIPC administration (the limb where it is applied) and the cuff inflation pressure used to occlude blood flow (Sharma et al., 2015). Likewise, in the field of exercise performance, Incognito et al. (2016) reported that the variance in the muscle mass of limbs undergoing ischemia-reperfusion cycles, the number (2-8) and length of these cycles (2-10 minutes) and the amount of time afforded between the application of IC and the onset of an exercise stimulus (ranging from minutes to 5-7 days prior to the stimulus) are all examples of protocol variations that have previously been used in IC studies (Maxime Caru et al., 2019; Anthony V Incognito et al., 2016). The variability in protocol described in the paragraph above is an illustration of the dose response related to various individual characteristics such as fitness level, with highly trained subjects appearing to require longer periods of ischemia over an increased number of ischemic cycles when compared to trained and untrained individuals (Cocking et al., 2018). This is the case seeing as elite athletes are closer to the higher upper limits of human physiological potential (Rhaí André Arriel et al., 2020). Ultimately, additional research is needed to determine the optimal protocol for the application of IC in both clinical settings and human exercise performance research.

1.6. Clinical applications of Ischemic Conditioning. IC, specifically ischemic preconditioning (IPC) in a preclinical setting, was first described by Murry et al. (1986), who studied the protective

effects of brief episodic coronary artery occlusions before a prolonged ischemic insult on the heart of anesthetized dogs. They found that the preconditioned dogs had significantly less severe myocardial infarction than the control group of dogs that had not been preconditioned (Murry et al., 1986). Subsequently, RIPC was first studied unintentionally by Przyklenk et al. (1993). They demonstrated the extensive protective effect of IC, similarly, observed by Murry et al. (1986) through the significantly smaller infarct size in the heart tissue of dogs in the IC intervention group at the site of occlusion. More importantly, the conditioning effect was also observed in tissue downstream of the initial occlusion site and was termed "preconditioning at a distance" (Przyklenk et al., 1993). Another pioneering study by Zhao et al. (2003) demonstrated the widespread application of IC by observing its impact when applied after an ischemic event. Like Murry et al. (1986) and Przyklenk et al. (1993), Zhao et al. (2003) examined the cardioprotective effects of IPC on areas of the canine heart and how it could mitigate the damage caused by reperfusion injury, namely the production of ROS and cell death. The results showed that IPC significantly reduced the infarct size following a period of ischemia, reduced the amount of CK in the plasma during reperfusion, and attenuated the edema resulting from an ischemic period comparable to those of post-conditioning (Zhao et al., 2003).

These foundational studies were extended to early clinical applications with the first clinical trial on IPC by Günaydin et al. (2000), who examined patients receiving IPC before undergoing coronary surgery and found similar cardioprotective effects to those observed in the earlier preclinical work in canines (GÜNAYDIN et al., 2000). Specifically, they observed an increase in anaerobic glycolysis in the affected tissue through elevated levels of the blood biomarker lactate dehydrogenase facilitating the re-supply of ATP to the body's cells even in the absence of O_2. Similarly, work conducted by Staat et al. (2005), was the first application of post-

ischemic conditioning in a clinical setting where the authors denoted potential protective mechanisms of ischemic conditioning when applied after coronary angioplasty, namely decreased levels of circulating serum CK and reduced infarct size in patients undergoing IC (Staat et al., 2005). These successful applications fueled further work by Cheung et al (2006), which was one of the first studies to present the use of non-invasive remote ischemic conditioning by applying a blood pressure cuff to a patient's lower extremity prior to cardiac surgery to alleviate the extent of tissue necrosis of the heart muscle caused by physician-induced occlusion or coronary blood flow during these same cardiac surgeries. The authors reported that RIPC resulted in a decreased inflammatory response through the increased levels of anti-inflammatory cytokines, specifically interleukin (IL)-10 which inhibits the overexpression of the pro-inflammatory mediator known as TNF-alpha, diminished myocardial injury expressed through significantly lower levels of the blood marker troponin I in RIPC group participants 6 hours after the application of IC (Cheung et al., 2006). Given the apparent clinical benefits of both IPC and RIPC, researchers began to postulate that IPC applied to the limbs might also benefit human exercise performance (Sharma et al., 2015).

IC can be broken down into early and late protective phases, which are respectively referred to as the first and second protective windows. Thijssen et al. (2016) proposed that early and immediate protective mechanisms of IC are the result of the rapid recruitment of signaling molecules such as nitric oxide (NO), hydrogen sulfide, opioid agonists, adenosine, and ROS, whereas late protective effects, typically occurring 12 to 24 hours after the initial ischemic event, appear to depend on the generation of protective proteins, such as cytokine-inducible nitric oxide synthase (iNOS) and cyclo-oxygenase-2 (COX-2) (Bolli et al., 2007; Dick HJ Thijssen et al., 2016). These substances are postulated to initiate a complex cascade system involving the

activation of stress-induced transcription factors, such as NF-κB, STAT 1 and STAT 3, which ultimately result in the upregulation of cardioprotective genes (**Fig 1.4**) (Bolli et al., 2007). Therefore, it has been suggested that upregulation of these proteins may contribute to the much longer-lasting protection offered by IC (Dick HJ Thijssen et al., 2016).

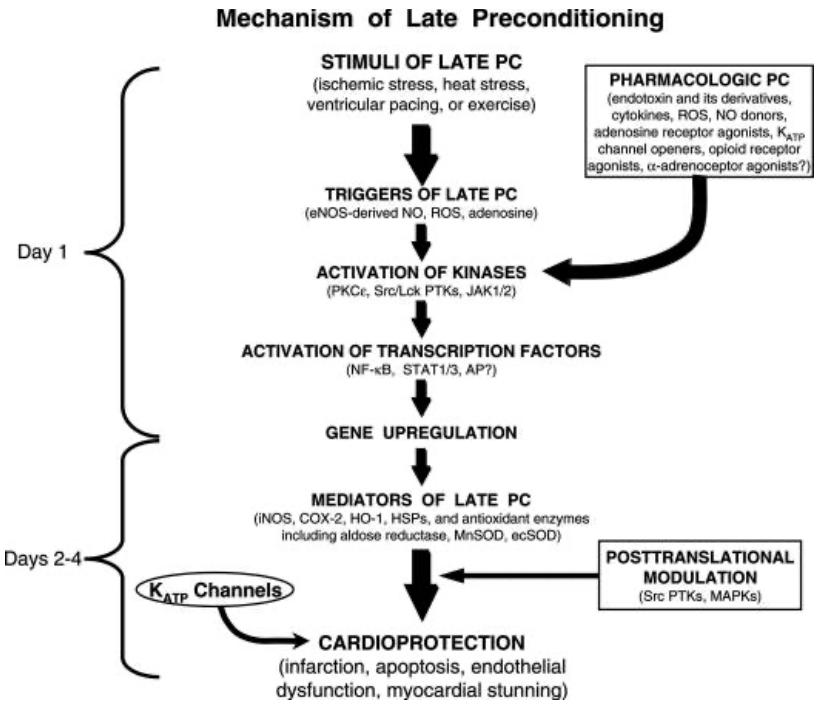

Figure 1.4: Schematic representation of the cellular mechanisms underlying late Preconditioning (reproduced from (Bolli et al., 2007).

This promising intervention is intriguing given that it is non-invasive and can be performed on the body extremities using blood pressure cuffs or tourniquets to promote local or remote beneficial effects on skeletal muscle. Such ergogenic assistance has been shown to induce cardioprotective effects, offering an exciting approach to improving human exercise performance.

1.7. Ischemic Preconditioning (IPC) and Human Exercise Performance. De Groot et al. (2010) were among the first to propose use of IPC as a non-invasive intervention to improve

exercise performance. Their study consisted of 3 cycles of 5-minute ischemia-reperfusion using a blood pressure cuff inflated to a pressure of 220 mmHg applied to both legs of 15 healthy, young (27 years), well-trained cyclists. All subjects performed two maximal exercise tests on a cycle ergometer (separated by 1 weeks) in which they cycled at 50W for the first 4 minutes, 100W for the next 4 minutes, and 150W for the following 4 minutes, after which power output increased by 20W/min until exhaustion. Seven of the participants received the IPC intervention 5 minutes before the first maximal exercise test and 8 participants received the IPC intervention 5 minutes before the second maximal exercise test. The authors found that IPC, whether applied at the first or second test, significantly increased $VO_{2,}$max by ~3%: from 56.8 ml/kg/min to 58.4 ml/kg/min. In addition, maximum power output significantly increased from 366 W without IPC application to 372 W after IPC application (De Groot et al., 2010). The authors speculated that the result of performance enhancement could be attributed to the improved vasodilation in the subject's skeletal muscle. Concretely, such an improvement would be beneficial to exercise performance by increasing the exercise-induced flow of blood and, therefore the delivery of nutrients and O_2, to the muscles highly recruited by a given sport (De Groot et al., 2010). Additional studies have demonstrated that RIPC improves exercise performance by preserving this supply of O_2 and nutrients to skeletal muscle cells even under ischemic conditions, thereby improving resistance to fatigue and maintaining sustained contractile activity over an extended period of time, and consequently, enhancing an athlete's force and power generating capacity. Furthermore, work by Bailey and colleagues (2012), which featured the application of a blood pressure cuff with an inflation pressure of 220 mmHg in the PEIC intervention group and 20 mmHg in the SHAM intervention group for 5 minutes on both lower extremities of healthy males, resulted in a 34 second difference in time to complete a 5-km time trial in favor of the PEIC group compared with

the SHAM group, as well as lower levels of blood lactate accumulation during an incremental running test in the PEIC group compared with the SHAM group (Bailey et al., 2012). In this case, the beneficial effects of IPC were hypothesized to be related to improved vascular function allowing for better clearance of blood lactate levels for uptake and use. The authors stated that ATP production or excitation-contraction coupling efficiency may play a role in the attenuation of performance decline as supported by evidence from previous animal studies, namely one performed on pigs wherein increased content of muscle ATP (25.6% higher at the end of a 4-hour ischemic period and 71% higher after a 1.5-hour reperfusion period) was observed in pre-conditioned porcine compared to non-preconditioned controls (Pang et al., 1995). In addition, Lindsay et al. (2017) examined the effect of IPC when applied in 4 cycles (5-minute/cycle) to each leg with a blood pressure cuff inflated to a pressure of 220 mmHg over 7 consecutive days using the Wingate test and $VO_{2,}$max measurements, which revealed improvements in anaerobic and aerobic capacities, respectively. The results from participants in this group were then compared to those of a SHAM group (placebo control) who received the same number of IPC cycles with a cuff inflation pressure of only 20 mmHg, which is not enough to cause vessel occlusion and ischemia. Specifically, the authors reported that after the study, those in the IPC intervention group experienced a maximal aerobic capacity ($VO_{2,}$max) increase of 12.8% and maximal aerobic power increase of 16.1% (Lindsay et al., 2017). Further research by Patterson and colleagues (2014) on 23 healthy, young (23 years), physically active males participating in either resistance or team sports training reported a higher sprint cycling performance by means of an increase in peak and mean power outputs in the IPC intervention group compared to the SHAM group. Their study protocol consisted of 4 x 5-minute cycles of ischemia followed by a 5-minute reperfusion period using a blood pressure cuff with an inflation pressure of 220 mmHg in the IPC intervention group

and 20 mmHg in the SHAM intervention group on both extremities (Patterson et al., 2015). According to the results of this study, the ergogenic effects of IPC primarily contributed to the more efficient delivery of O_2 to the muscles (Patterson et al., 2015). Another theory, from research conducted by Crisafulli et al. (2011), suggests that the benefits of RIPC on exercise performance may be largely psychological and reflect changes in the athlete's perceived fatigue and pain (Crisafulli et al., 2011). The authors proposed that RIPC may serve to desensitize sensory afferent nerve fibers (specifically type III and IV skeletal muscle afferents) that detect peripheral fatigue and changes in intramuscular metabolite levels responsible for exercise termination due to fatigue signals and feedback to re-establish the body's homeostasis. Extending the sensory detection threshold at which this system terminates exercise increases the ability of these neurons to recruit additional motor units under fatigue, and thus increases muscle force generating capacity (Crisafulli et al., 2011).

Elaborating on the findings of Lindsay et al. (2017), Thijssen et al. (2016) postulated that when repeated, IPC increases the exposure of blood vessels to shear stresses while also providing an influx of signaling molecules (e.g., nitric oxide (NO)), cytokines (e.g., IL-6, IL-10 and stromal-derived factor 1α (SDF-1α)), as well as blood-borne substances (e.g., microRNA-144), all of which serve to improve vascular function and structure (Delves & Roitt, 1998; Dick HJ Thijssen et al., 2016). Furthermore, Clarkson et al. (2002) stated that while muscle damage is initially the result of mechanical stress, the inflammatory response that develops in the days following an exercise stimulus significantly exacerbates muscle damage. Thus, if the effects of this period of inflammation can be mitigated by applying IC after the onset of muscle damage resulting from intense exercise, the degree of sustained insult may be reduced and, therefore, an athlete's recovery rate may increase (Clarkson & Hubal, 2002). In the context of exercise performance, the goal of

IC has progressively shifted from performance enhancement to attenuating performance decline by mitigating future damage, i.e., oxidative damage. In the case of strenuous exercise, the application of IC can be useful in mitigating the elevated levels of indicators for both muscle damage (e.g., plasma CK) and oxidative stress (Justin D. Sprick et al., 2019). Although IPC has been shown to have all of the above ergogenic effects, additional research is needed to fully understand the mechanisms and pathways responsible for these beneficial adaptations. As such, research has highlighted IPC as a promising ergogenic tool, but the lack of consensus on protocols, as discussed in *Section 1.5*, makes its precise application difficult.

1.8. Literature Review of Post-Exercise Ischemic Conditioning on Human Exercise Performance and Recovery. Among the studies on IC and blood flow restriction, its application immediately after a period of exercise is the most recent variation and, therefore, the area in which there is the least amount of research. Beaven et al. (2012) were the first to report on the potentially beneficial effects of post-exercise ischemic conditioning (PEIC) on recovery of power production and sprint performance. In that study, 14 healthy participants (10 men, 4 women) performed an exercise protocol that involved lower-body strength and power tests followed by repeated sprints. Large blood pressure tourniquets were then applied unilaterally to the upper thighs (2 cycles x 3-min per leg) with a tourniquet pressure of either 220 mmHg (PEIC) or 15 mmHg (SHAM). Participants repeated the exercise protocol 24 hours later. Compared to the SHAM control condition, the PEIC intervention elicited delayed beneficial effects after 24 hours in the countermovement and squat jump test outcomes as well as on 10- and 40-m sprint times (Christopher Martyn Beaven et al., 2012).

Page et al. (2017) evaluated the effect of PEIC (3 cycles x 5-min per leg with a tourniquet pressure of 220 mmHg) compared with SHAM (3 cycles x 5-min per leg with a tourniquet pressure of 20 mmHg) on recovery from EIMD caused by 100 drop-jumps. These authors reported that the rate of recovery following EIMD was accelerated in the participants randomly assigned to the PEIC group (n=8) compared to those randomly assigned to the SHAM group (n=8). Specifically, PEIC attenuated the magnitude of decline in maximal isometric voluntary contraction of the quadriceps from baseline at 24 hours, 48 hours and 72 hours post-EIMD; attenuated the magnitude of rise in plasma CK levels from baseline at 24 hours post-EIMD; and decreased subjective ratings of muscle soreness from baseline at 24 hours and 48 hours post-EIMD (Will Page et al., 2017).

A randomized crossover study by Daab et al. (2021) reported that PEIC compared to SHAM (3 cycles x 5-min per leg with a tourniquet pressure of 50 mmHg above systolic blood pressure or 20 mmHg, respectively) accelerated the rate of recovery following a simulated soccer match in 12 male soccer players. Specifically, PEIC almost completely prevented the exercise-induced decrements in squat jump, countermovement jump, maximal isometric voluntary contraction of the quadriceps, and 20-m sprint performance from baseline at 24 hours and 48 hours post-exercise; attenuated the magnitude of rise in plasma CK levels from baseline at 24 hours and 48 hours post-exercise; and decreased subjective ratings of muscle soreness from baseline at 24 hours, 48 hours and 72 hours post-exercise (Daab et al., 2021).

Daab et al. (2021) proposed that the increase in blood volume resulting from periods of reperfusion following periods of ischemia correlates with increased O_2 availability and distribution, and a faster replenishment of ATP. Further, the presence of blood biomarkers indicative of muscle damage, such as CK, is reduced in athletes who have undergone PEIC compared to those undergoing SHAM intervention protocol, potentially due to the elevated levels

of adenosine that promote dilation of vascular vessels, thereby improving blood flow to the muscles (Daab et al., 2021). Another important marker that was assessed in this study by Daab et al. (2021) was NO, which increased to significant levels in the blood plasma of participants after the PEIC intervention but not the SHAM intervention. NO is thought to play a role in reducing muscle soreness by attenuating the inflammatory response that typically leads to further muscle breakdown (Daab et al., 2021).

As illustrated in **Figure 1.5**, this inflammatory response is reportedly most active 1 to 12 hours after an exercise stimulus, suggesting that this time frame is perhaps the optimal window of opportunity for mitigation of future damage through the application of PEIC. In the setting of major cycling events, where stages are often separated by ≤24 hours, it is likely important to apply PEIC as promptly as possible in an attempt to mitigate the detrimental effects of EIMD and provide an advantage over other fatigued competitors at the onset of each subsequent stage.

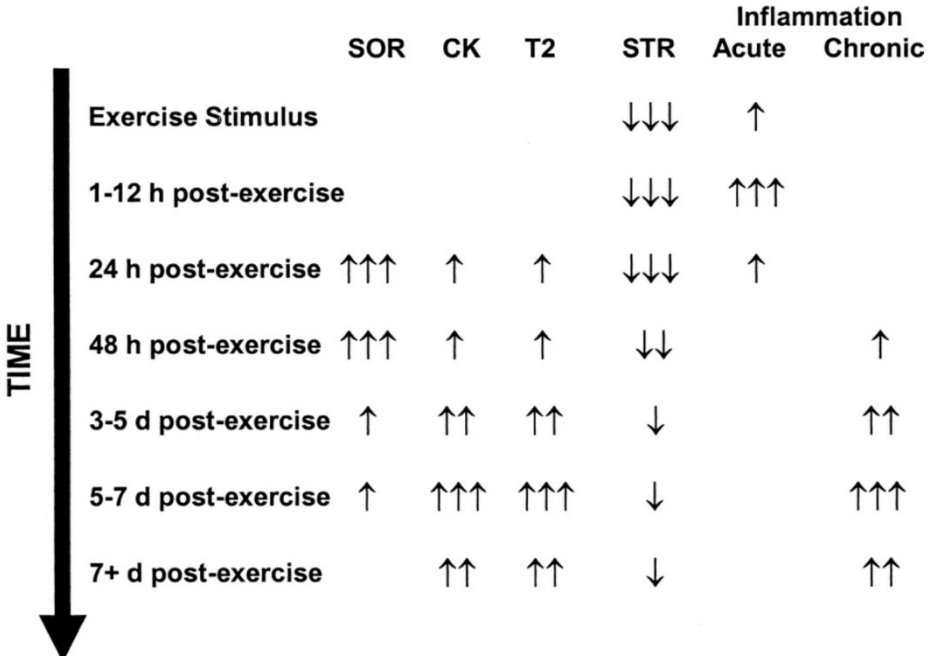

Figure 1.5. Time course of physiological changes after maximal exercise.
One arrow, minor increase/decrease; two arrows, moderate increase/decrease; three arrows, large increase/decrease. Abbreviations: SOR, soreness; CK, creatine kinase; STR, strength. (reproduced from (Clarkson & Hubal, 2002).

Arriel et al. (2018) conducted a study comparing two PEIC protocols on blood CK concentration, muscle soreness and perceived recovery status, heart rate, perceived exertion, power output and aerobic exercise performance in recreational cyclists; 2 cycles x 5 minutes per leg and 5 cycles x 2 minutes per leg, with the PEIC intervention group receiving a tourniquet pressure of 50 mmHg above systolic blood pressure and SHAM receiving 20 mmHg above systolic blood pressure, applied to the thighs resulting in a total of 4 separate groups. Each group received its respective intervention immediately after performing a maximal incremental cycling exercise test (MICT) to obtain baseline values followed by a subsequent MICT performed 24 hours later to compare effect of PEIC and SHAM on the magnitude of performance decline. The authors found promising results in favor of PEIC namely that time to exhaustion during the MICT in the PEIC intervention group was quite consistent at baseline and 24 hours post-intervention, whereas performance in the SHAM intervention groups decreased by 2.2-4.7% at 24 hours post-intervention. In addition, CK levels measured at baseline and 24 hours post-intervention were similar in the PEIC and SHAM intervention groups, with all groups showing an increase in CK concentration at 24 hours compared with baseline levels. The authors also noted that the PEIC intervention group reported higher ratings of perceived pain and perceived fatigue compared with the SHAM intervention groups, although this discrepancy did not appear to affect exercise performance (Arriel et al., 2018).

Arriel and colleagues conducted a systematic review in 2020, analyzing the results of published studies on PEIC. Their analyses showed that athletes from various sports completing multi-day races ranging from 2-7 consecutive days experienced oxidative stress, muscular damage, increased inflammation, and muscle soreness all attributing to decreased performance and posit

that athletes and their teams should prioritize recovery interventions, such as PEIC, that can help mitigate decrements in exercise performance (Rhaí André Arriel et al., 2020).

Arriel et al. (2020) also argued that both protective windows observed in PEIC studies involve NO production, which leads to increased mitochondrial oxidation and thus reduced production of ROS. In addition, PEIC may also lead to decreased leukocyte production, which would reduce the body's occasional hyperinflammatory response following exercise, with the protective effects of this intervention apparently most pronounced 24 hours post-exercise. Despite the lack of consensus on PEIC protocols, as mentioned in *Section 1.5*, the aforementioned effects are reported to be most significant when PEIC is performed using 4-6 cycles, with each ischemic cycle lasting 2 to 5 minutes (Rhaí André Arriel et al., 2020).

However, further research is still required to understand several facets of PEIC as a recovery intervention. Namely, a more in-depth insight into the exact mechanism underlying the protective effects of PEIC, but also the psycho-physiological factors that may contribute to an ergogenic or enhanced recovery effect by PEIC compared to SHAM on multistage cycling performance. In addition, further research is needed to determine whether the effect of PEIC on multistage exercise performance persists beyond two stages of competition, as reported by Arriel et al. (2018). This is particularly relevant to competitive cyclists who often compete in multistage races consisting of more than just two stages wherein the adverse effect of repeated bouts of high-intensity endurance exercise on performance accumulates over time. More importantly, it should be noted that the MICT protocol used in prominent literature does not mimic the time trial nature of a multistage cycling race. Finally, no studies have examined the effect of PEIC on detailed physiological and perceptual parameters at rest and during exercise that might help explain the

relative preservation of multistage exercise performance by accelerating the rate of recovery between races.

1.9. Aim and Hypothesis. The overarching aim of this research project was to examine the role of PEIC as a potential recovery tool by assessing its impact on multistage laboratory-based 20-km cycling TT performance among competitive cyclists. We hypothesized that, compared to a control intervention (SHAM), the PEIC intervention would mitigate the decline in 20-km cycling TT performance over 5 consecutive days.

CHAPTER 2: Effect of Post-Exercise Ischemic Conditioning on Multistage 20-km Cycling Time Trial Performance in Competitive Cyclists

2.1. Abstract

The aim of this study was to examine the role of PEIC as a potential recovery tool by assessing its impact on multistage laboratory-based 20-km cycling TT performance among competitive cyclists. This study compared the effect of post-exercise ischemic conditioning (PEIC) versus a placebo control (SHAM) intervention on multistage laboratory-based bicycle race performance over five consecutive days. Eight cyclists (2 female, 6 male) were recruited and randomly assigned to PEIC (n=3) or SHAM (n=5) intervention groups. The intervention consisted of 4 cycles of 5-min blood flow restriction (PEIC, 50 mmHg above systolic blood pressure; SHAM, 20 mmHg) with 5-min of reperfusion (0 mmHg) applied unilaterally to the upper thighs following completion of a 20-km cycling time trial over 5 consecutive days. Changes in 20-km cycling time trial duration, subjective perceptions of recovery and muscle soreness were compared between PEIC and SHAM. The duration of the 20kmTT decreased (performance improvement) by 0.16% ± 0.57% in the PEIC intervention group from *Visit 3 to 7*, whereas 20kmTT duration increased (performance decline) by 0.58% ± 0.94% in the SHAM intervention group from *Visit 3 to 7*. Mean power output and cycling speed over the 20kmTT remained relatively constant across *Visits 3-7* in both groups, with higher values being observed at each visit in the PEIC compared to SHAM group. Performance readiness scores were slightly higher in the PEIC intervention group compared with the SHAM group for most visits. The preliminary results of this randomized controlled study do not provide evidence to support a potentially beneficial effect of PEIC on multistage 20kmTT performance or indices of recovery and/or performance readiness.

2.2. Introduction

Competitive cyclists participate in several multistage races each season. For example, the Giro d'Italia, Tour de France, and La Vuelta ciclista a Espana consist of 21 stages over 21-23 consecutive days. Thus, elite cyclists often have <24 hours to recover between races, which may compromise subsequent race performance. With the goal of optimizing performance, cyclists and their coaches, performance directors and sports medicine doctors are constantly seeking to identify strategies to accelerate the rate of recovery between and during multistage races.

Evidence suggests that 3-4 brief cycles (3-5 min per cycle) of skeletal muscle ischemia and reperfusion (intermittent vascular occlusion) applied at rest before exercise improves human exercise performance, particularly time trial performance (M. Caru et al., 2019; A. V. Incognito et al., 2016). Although the underlying mechanisms are unclear (Franz et al., 2017; D. H. Thijssen et al., 2016), this *ischemic preconditioning* protects against skeletal muscle injury after ischemic insult and/or strenuous exercise, which is associated with increased biomarkers of muscle damage (e.g., creatine kinase) and oxidative stress (J. D. Sprick et al., 2019). Importantly, many of the same biomarkers are elevated in cyclists during a multistage competition (Cordova Martinez et al., 2015).

Very few studies have tested the hypothesis that ischemic conditioning applied immediately post-exercise (hereafter referred to as *post-exercise ischemic conditioning or PEIC*) accelerates the rate of recovery for optimal performance in subsequent competitions (R. A. Arriel et al., 2020). C. M. Beaven et al. (2012) were the first to report on the potentially beneficial effects of PEIC on recovery of power production and sprint performance. In that study, 14 healthy participants (10 men, 4 women) performed an exercise protocol that involved lower-body strength and power tests followed by repeated sprints. Large blood pressure tourniquets were then applied

unilaterally to the upper thighs (2 cycles x 3-min per leg) with a tourniquet pressure of either 220 mmHg (PEIC) or 15 mmHg (SHAM). Participants repeated the exercise protocol 24 h later. Compared to the SHAM control condition, the PEIC intervention elicited delayed beneficial effects after 24 h in the countermovement and squat jump test outcomes as well as on 10- and 40-m sprint times. A subsequent study of 16 healthy recreationally active men by W. Page et al. (2017) examined the effect of PEIC (3 cycles x 5-min per leg with a tourniquet pressure of 220 mmHg) compared to SHAM (3 cycles x 5-min per leg with a tourniquet pressure of 20 mmHg) on recovery from exercise-induced muscle damage (EIMD). In that study, the rate of recovery following EIMD was accelerated in the participants randomly assigned to the PEIC group (n=8) compared to those randomly assigned to the SHAM group (n=8). Specifically, PEIC attenuated the magnitude of decline in maximal isometric voluntary contraction of the quadriceps from baseline at 24 h, 48 h and 72 h post EIMD; attenuated the magnitude of rise in plasma creatine kinase levels from baseline at 24 h and 48 h post EIMD; and decreased subjective ratings of muscle soreness from baseline at 24 h, 48 h and 72 h post EIMD. In keeping with these observations, a randomized crossover study by Daab et al. (2020) reported that PEIC compared to SHAM (3 cycles x 5-min per leg with a tourniquet pressure of 50 mmHg above systolic blood pressure or 20 mmHg, respectively) accelerated the rate of recovery following a simulated soccer match in 12 male soccer players. Specifically, PEIC almost completely prevented the exercise-induced decrements in squat jump, countermovement jump, maximal isometric voluntary contraction of the quadriceps and 20-m sprint performance from baseline at 24 h and 48 h post-exercise; attenuated the magnitude of rise in plasma creatine kinase levels from baseline at 24 h and 48 h post-exercise; and decreased subjective ratings of muscle soreness from baseline at 24 h, 48 h and 72 h post-exercise. The collective results of C. M. Beaven et al. (2012), W. Page et al. (2017) and Daab et al. (2020)

support the use of PEIC as a potentially novel interventional tool to accelerate recovery, at least from high-intensity, short duration resistance-type exercises. However, these types of exercises do not mimic the skeletal muscle (or physiological) demands of moderate-to-high intensity, long duration endurance-type exercises such as cycling.

Arriel et al. (2018) reported that, compared to SHAM (2 cycles x 5-min per leg or 5 cycles x 2-min per leg with a tourniquet pressure of 20 mmHg), PEIC applied to the thighs of amateur cyclists (2 cycles x 5-min per leg or 5 cycles x 2-min per leg with a tourniquet pressure of 50 mmHg above systolic blood pressure) immediately after completing a maximal incremental cycle exercise test (MICT) prevented declines in subsequent MICT performance 24 h later. Despite these promising results, MICT does not mimic the time trial nature of a multistage cycling race. Moreover, no study has examined whether the effect of PEIC on multistage exercise performance reported by Arriel et al. (2018) persists beyond just two stages of competition, which is particularly relevant to competitive cyclists who often compete in multistage races consisting of more than just two stages wherein the adverse effect of repeated bouts of high-intensity endurance exercise on performance accumulates over time.

The objective of this randomized controlled parallel group study was to examine the efficacy of PEIC on laboratory-based multistage 20-km cycling time trial (20kmTT) performance in competitive cyclists. We hypothesized that, compared to a control intervention (SHAM), the PEIC intervention would attenuate the decline in 20kmTT performance over 5 consecutive days.

2.3. Methods

2.3.a. Experimental study design. Each participant provided written informed consent prior to completing a screening visit (*Visit 1*), a familiarization visit (*Visit 2*), and five experimental visits (*Visits 3-7*) over a period of approximately 14 days (**Table 2.1**). *Visit 1* included screening for

eligibility criteria; completion of the Physical Activity Readiness Questionnaire for Everyone to ensure there were no contraindications to cardiopulmonary exercise testing; and a MICT to determine each participants' peak rate of O_2 consumption (VO_{2peak}) and peak power output (PPO) (**Table 2.1**). Randomization of eligible participants to the PEIC or SHAM intervention group (*see Section 2.3c below*) occurred after *Visit 1* and prior to the start of *Visit 2*. Twenty-four to 72 h after *Visit 1*, during *Visit 2*, participants were familiarized with the experimental test procedures and interventions, including completion of a 20kmTT followed thereafter by application of PEIC or SHAM intervention as per protocol (*see Section 2.3f below*) and the randomization plan (**Table 2.1**). Briefly, familiarization to the PEIC intervention consisted of 4 cycles x 5-min per leg with a tourniquet pressure of 50 mmHg above systolic blood pressure (circulatory occlusion) and of 0 mmHg (reperfusion) applied unilaterally on alternating thighs, whereas familiarization to the SHAM intervention consisted of 4 cycles x 5-min per leg with a standardized tourniquet inflation pressure of 20 mmHg (SHAM occlusion) and of 0 mmHg (reperfusion) applied unilaterally on alternating thighs. Seventy-two to 96 h after *Visit 2*, during *Visit 3*, participants completed a baseline 20kmTT followed within a period of 5 minutes thereafter by the PEIC or SHAM intervention (**Table 2.1**). *Visits 4-7* consisted of a 20kmTT followed within a period of 5 minutes thereafter by the PEIC or SHAM intervention at *Visits 4-6* (**Table 2.1**). All 20kmTTs performed at *Visits 3-7* were separated by 24±2 h.

Table 2.1. Summary of experimental study visits and procedures							
	Experimental Visit						
Procedure	**1**	**2***	**3**	**4**	**5**	**6**	**7**
Informed consent & screening for eligibility	✓						
MICT	✓						
20kmTT		✓	✓	✓	✓	✓	✓
PEIC or SHAM		✓	✓	✓	✓	✓	
PPT		✓	✓	✓	✓	✓	✓
PRSS, BAM+		✓	✓	✓	✓	✓	✓
Participant's expectations of treatment effect			✓	✓	✓	✓	
*, Familiarization; MICT, maximal incremental cycle exercise test; 20kmTT, 20-km cycling time trial; PEIC, post-exercise ischemic conditioning *via* intermittent circulatory occlusion (active intervention); SHAM, placebo control (inactive) intervention; PPT, pain pressure threshold testing; PRSS, perceived recovery status scale; BAM+, brief assessment of mood questionnaire							

At rest before the start of 20kmTTs at *Visits 2-7*: lower limb muscle soreness was assessed by pain pressure threshold testing of the *vastus lateralis*, *rectus femoris*, and *gastrocnemius*; and subjective perception of recovery and performance readiness were collected (**Table 2.1**). Intensity ratings of leg discomfort and perceived exertion (RPE) were assessed at rest prior to the start of exercise and within the last 250-m of every 2-km interval during 20kmTTs at *Visits 2-7*.

Participants were asked to avoid any strenuous exercise unrelated to the study for 48 h prior to *Visit 1*, and throughout the remainder of the study. Similarly, alcohol and cannabis consumption were not permitted 48 h prior to *Visit 1*, and throughout the remainder of the study. Participants were asked to avoid heavy meals and caffeine ≥4 h before testing at *Visits 1-7*. Participants using dietary supplements (e.g., creatine, beta-alanine, fish-oil, multivitamin) were asked to maintain the

same daily dietary supplement regimen throughout the course of study. Participants were asked not to engage in any exercise recovery strategies throughout the course of the study (e.g., oral or topical cannabidiol, melatonin, heat or cryotherapy, massage therapy, non-steroidal anti-inflammatories). Participants were instructed to wear the same pair of their own undergarments (underwear, bra, socks), cycling shoes, cycling shorts and shirt at each visit; and to wash and dry their undergarments, cycling shorts and shirt between each visit.

2.3.b. Participants. Participants included ostensibly healthy men (n=6) or women (n=2) aged 18-40 years without any self-reported health condition. Participants were included if they (i) competed as a cyclist and/or triathlete in a regional, provincial, national and/or international event and placed in the top 50% of all competitors in the field; and (ii) had a VO_{2peak} on MICT >90^{th} percentile by age and sex (Kaminsky et al., 2017). Participants were excluded if they: self-reported having any personal experience with ischemic conditioning, either as part of their training / recovery program and/or as part of their prior participation in a research study on ischemic conditioning; self-reported being current or ever smokers; self-reported having a personal or family history of blood clots, deep vein thrombosis or embolism; self-reported having a history of cardiac, vascular, pulmonary, renal, liver, musculoskeletal, endocrine, neurologic, metabolic, humoral, menstrual cycle or sleep-related disease/disorder/dysfunction; were pregnant (confirmed by urine pregnancy test at *Visit 1*) or attempting to become pregnant; self-reported taking any doctor prescribed medication(s), other than oral contraceptives.

Participants were recruited: from La Fédération Québecoise des Sports Cyclistes; Canada Cyclisme; Centre National de Cyclisme de Bromont; *via* contact with coaches of cycling and triathlon teams/clubs in the Montreal and surrounding areas; and *via* posted announcements in the

greater Montreal area. Initial contact consisted of a thorough explanation of the study procedures and pre-screening for the abovementioned inclusion/exclusion criteria prior to study consent by a member of the research team, either in person or by telephone or by email.

2.3.c. Randomization and blinding. Using an online randomization plan generator (www.radomization.com), male and female participants were separately and randomly assigned in a 1:1 ratio to the PEIC or SHAM intervention group.

Given the nature and obvious differences of the PEIC and SHAM interventions (i.e., PEIC has high tourniquet pressure of 50 mmHg above systolic blood pressure and SHAM has low standardized tourniquet pressure of 20 mmHg; *see Section 2.3f below*), blinding participants to the intervention is very difficult because the use of SHAM occlusion is not very convincing. However, the potential for participant unblinding to have a placebo/nocebo effect on our primary outcome (magnitude of change in 20kmTT duration from *Visit 3-7*) was minimized, at least in part, by our use of a randomized parallel group study design. Briefly, a *placebo effect* represents positive expectations of the participant that the SHAM intervention will have a positive effect on subsequent 20kmTT performance, whereas a *nocebo effect* represents negative expectations of the participant that the PEIC intervention will have a negative effect on subsequent 20kmTT performance. To further minimize the possibility of any placebo/nocebo effects on our primary outcome (magnitude of change in 20kmTT duration from *Visit 3-7*), participants were provided only general information about PEIC and SHAM interventions in the informed consent form. Participants, all of whom were required to have no prior personal experience with ischemic conditioning (*see Section 2.3.b above*), were also told that neither intervention causes harm, despite potentially uncomfortable circulatory occlusion sensations. If a participant asked the

investigator(s) about the hypothesized effect of either PEIC or SHAM on recovery and multistage 20kmTT performance, they were told that little is definitively known and that the effects of each intervention on recovery and exercise performance are unclear. All of this was done to avoid influencing recovery and subsequent 20kmTT performance expectations (or predispositions) of each intervention.

To determine the participants' expectations (or predispositions) of the potential effect of PEIC and SHAM interventions on (i) 20kmTT performance 24 h later and (ii) speed of recovery from 20kmTT over the next 24 h, each participant was questioned immediately after application of PEIC or SHAM interventions at *Visits 3, 4, 5 and 6*. Specifically, using a 100-mm visual analog scale (VAS) anchored by the descriptors "*far worse*" and "*much better*" (for 20kmTT performance 24 h later) or "*very slow*" and "*very fast*" (for speed of recovery from 20kmTT over the next 24 h), participants were asked to indicate how they expected the intervention they received to affect: (i) their 20kmTT performance at *Visit 4* relative to *Visit 3*, at *Visit 5* relative to *Visit 4*, at *Visit 6* relative to *Visit 5*, and at *Visit 7* relative to *Visit* 6; and (ii) their speed of recovery from 20kmTT between *Visits 3 and 4*, between *Visits 4 and 5*, between *Visits 5 and 6*, and between *Visits 6 and 7* (**Table 2.1**).

2.3.d. Pain pressure threshold. Lower limb muscle soreness was assessed by pain pressure threshold (PPT) testing of the *vastus lateralis*, *rectus femoris*, and *gastrocnemius* (Vaegter et al., 2018) (**Table 2.1**). Using a handheld electronic pressure algometer (Wagner Instruments, Greenwich, CT, USA), PPT was assessed by manually applying a constant rate of increasing pressure with a padded mechanical foot plate of 1 cm^2 to the *vastus lateralis*, *rectus femoris*, and *gastrocnemius* while the participant was semi-recumbent on an examination table. The participant

was instructed to signal (by raising their hand) the moment at which the pressure sensation first started to become painful; the pressure reported on the algometer at this point was then recorded and identified as the PPT. This procedure was performed three times per location. The average of the two most reproducible PPT measurements per location was used for analysis.

2.3.e. Subjective assessments of recovery and performance readiness. Subjective perception of recovery was assessed using the perceived recovery status scale (PRSS), which ranges from "*0 – Very poorly recovered / Extremely tired*" to "*10 – Very well recovered / Highly energetic*" (Laurent et al., 2011). Performance readiness was assessed using the Brief Assessment of Mood (BAM+) questionnaire (Shearer et al., 2017), which consists of 10 questions related to subjective wellbeing, each of which is scored by marking a line on a 100-mm VAS anchored with "*not at all*" and "*extremely*" at opposing ends. The BAM+ score was calculated by subtracting the mean score for the 6 negatively associated questions (e.g., "How angry do you feel?") from the mean score for the 4 positively associated questions (e.g., "How confident do you feel?"), as per the following equation: ((vigor + sleep quality + confidence + motivation) ÷ 4) – ((anger + confusion + tension + depression + fatigue + muscles soreness) ÷ 6). Performance readiness is positively related to the BAM+ score; that is, as the BAM+ score increases, performance readiness increases (Shearer et al., 2017).

2.3.f. Post-exercise ischemic conditioning (PEIC) and SHAM control protocols. An interventionist applied an 11.5-cm wide tourniquet (PTS BFR Easi-Fit; Delfi Medical Innovations Inc, Vancouver, Canada), secured using Velcro straps, to the sub-inguinal region of the

participants' upper left and upper right thigh. Tourniquet inflation pressure was controlled automatically. The PEIC intervention consisted of 4 cycles x 5-min of circulatory occlusion at a tourniquet inflation pressure of 50 mmHg above systolic blood pressure (recorded within 5-min of completing 20kmTT at each visit) with 5-min of reperfusion at a tourniquet deflation pressure of 0 mmHg. SHAM and PEIC protocols were the same, with the exception of using a standardized tourniquet inflation pressure of 20 mmHg for the SHAM intervention. PEIC and SHAM were applied unilaterally on alternating thighs with participants in a semi-recumbent position on an examination table within 5-min of completing the 20kmTT. Intensity ratings of leg discomfort were assessed within the last 15-sec of each minute during the 5-min circulatory occlusion period using Borg's modified 0-10 category scale, which ranged from "*0 – Nothing at all*" to "*10 – Very, very severe (almost max)*" (Borg, 1982). For each participant and study visit, the multiple intensity ratings of leg discomfort (n=40, equivalent to 8 cycles of circulatory occlusion [4 cyclers per right and left leg] x 5 ratings of leg discomfort per cycle) were averaged and used for analysis.

2.3.g. Exercise testing. Exercise tests were conducted on an electronically braked Velotron Pro cycle ergometer (RacerMate Inc., Seattle, WA, USA) according to published methods (Zavorsky et al., 2007). The vertical height and horizontal distance of both the cycle ergometer seat and handlebars were adjusted to each participant's preference prior to the MICT at *Visit 1* and kept constant for all subsequent 20kmTTs.

The MICT consisted of a steady-state pre-exercise baseline period of ≥3-min, followed by 25 watt/min increases in power output (starting at 150 watts for men and 75 watts for women): peak power output (PPO) was defined as the highest power output the participant was able to sustain for ≥30-sec, whereas VO_{2peak} was taken as the average of the last 30-sec of loaded pedaling.

Breath-by-breath measures of VO_2 were recorded using a Vmax Encore® metabolic cart (Trudell Healthcare Solutions, London, ON, Canada) while participants breathe through a rubber facemask (Hans Rudolph Inc, Shawnee, KS, USA) and low-resistance flow transducer (Muscat et al., 2015).

The 20kmTT included a steady-state pre-exercise baseline period of ≥3-min, followed by 1-km of cycling at a constant power output corresponding to 20% of PPO (warm-up), and then 20kmTT. Participants were instructed to complete each 20kmTT as fast as possible by maintaining the highest possible cycling speed and power output. With the exception of evaluating symptom responses (*see Section 2.3.g.i. below)*, no verbal feedback, encouragement and/or instruction was provided to the participant by the experimenter(s). During each 20kmTT, participants were provided with real-time visual feedback on their distance, pedal cadence, and gear, but were otherwise blinded to their speed, power output and test duration.

2.3.g.i. Symptom Responses. Using Borg's modified 0-10 category ratio scale (Borg, 1982), participants rated the intensity of their perceived exertion (RPE) as well as the intensity of their perceived leg discomfort prior to the start of exercise and within the last 100-m of every 2-km interval during 20kmTTs.

2.3.g.ii. Analysis of 20kmTT end-points. Cycling power output and speed were averaged over the entire duration of each 20kmTT and used for analysis. Similarly, the multiple RPE and intensity ratings of perceived leg discomfort (n=10 per symptom, equivalent to 1 rating per symptom within the last 100-m of every 2-km interval) were averaged over the entire duration of each 20kmTT and used for analysis.

2.3.h. Analysis of data. Due to the small sample size of 8 participants, including 3 randomized to the PEIC group and 5 randomized to the SHAM group, outcome variables were compared within- and between-groups qualitatively (descriptively) and not using statistical (quantitative) methods. Data are presented as mean ± SEM and visualized for qualitative interpretation.

2.4. Results

2.4.a Participant Characteristics. Six men and two women were recruited for participation in this study, and their characteristics are summarized in **Table 2.2**. Three male participants were randomized to the PEIC group, and five participants (3 male, 2 female) were randomized to the SHAM group. On average, participants in the PEIC compared to SHAM group were slightly younger (by 4 years) and had a higher PPO (by ~14 watts) and VO_{2peak} (by ~6 ml/kg/min).

Table 2.2. Participant characteristics

Parameter	All Participants	PEIC (n=3)	SHAM (n=5)
Sex, male:female	6:2	3:0	3:2
Age, yrs	29 ± 4 [22 – 36]	28 ± 6	32 ± 2
Body height, cm	176.3 ± 4.4 [167 – 184.5]	177.7 ± 4.3	175.5 ± 7.2
Body mass, kg	73.1 ± 2.9 [65.5 – 78.6]	74.4 ± 1.9	72.3 ± 4.9
Peak power output, watts	365.6 ± 31.2 [275 – 425]	253.4 ± 3	239.1 ± 4.1
VO_{2peak}, ml/kg/min	56.5 ± 3.5 [45.8 – 63.2]	53.9 ± 1.9	48.2 ± 0.8

Values are means ± Standard Error [range]

2.4.b. PEIC and SHAM Interventions. As illustrated in **Figure 2.1A**, all participants in the SHAM group received, by design, a tourniquet pressure of 20 mmHg. The personalized tourniquet pressure (equivalent to the lowest circulatory occlusion pressure + 50 mmHg) for PEIC group ranged from an average of 234 mmHg to 257 mmHg across *Visits 3-6*. As illustrated in **Figure**

2.1B, the ~10-fold higher tourniquet pressures during PEIC compared to SHAM was associated with uniformly higher intensity ratings of leg discomfort across *Visits 3-6*. Interestingly, despite no occlusion causing lower limb blood flow restriction and ischemia created by the SHAM intervention, participants nevertheless reported mild-to-moderate leg discomfort during the periods of cuff inflation to just 20 mmHg.

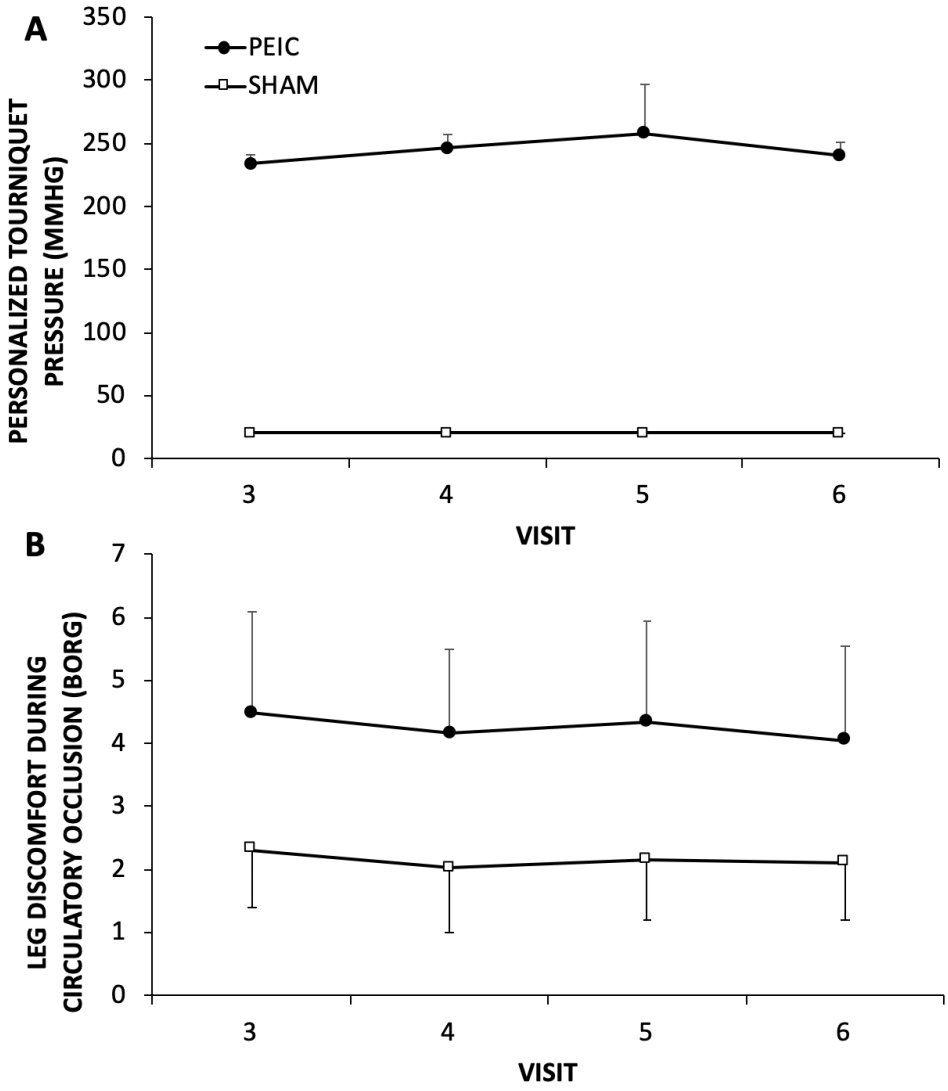

Figure 2.1. (A) Personalised cuff pressure values and (B) corresponding ratings of perceived leg discomfort in both the PEIC (n=3) and SHAM (n=5) intervention groups. Values are Mean ± Standard Error

2.4.c. Participants' expectations of the potential effect of PEIC and SHAM interventions on 20kmTT performance and speed of recovery from 20kmTT. The results presented in **Figure 2.2A** suggest/demonstrate that: (i) participants in both groups expected the intervention they received to improve their subsequent (next day) 20kmTT performance with mean ratings approximating 60% of full scale across *Visits 3-6*; (ii) there is greater variability in the expected effect of PEIC compared to SHAM on subsequent 20kmTT performance across *Visits 3-6*; and

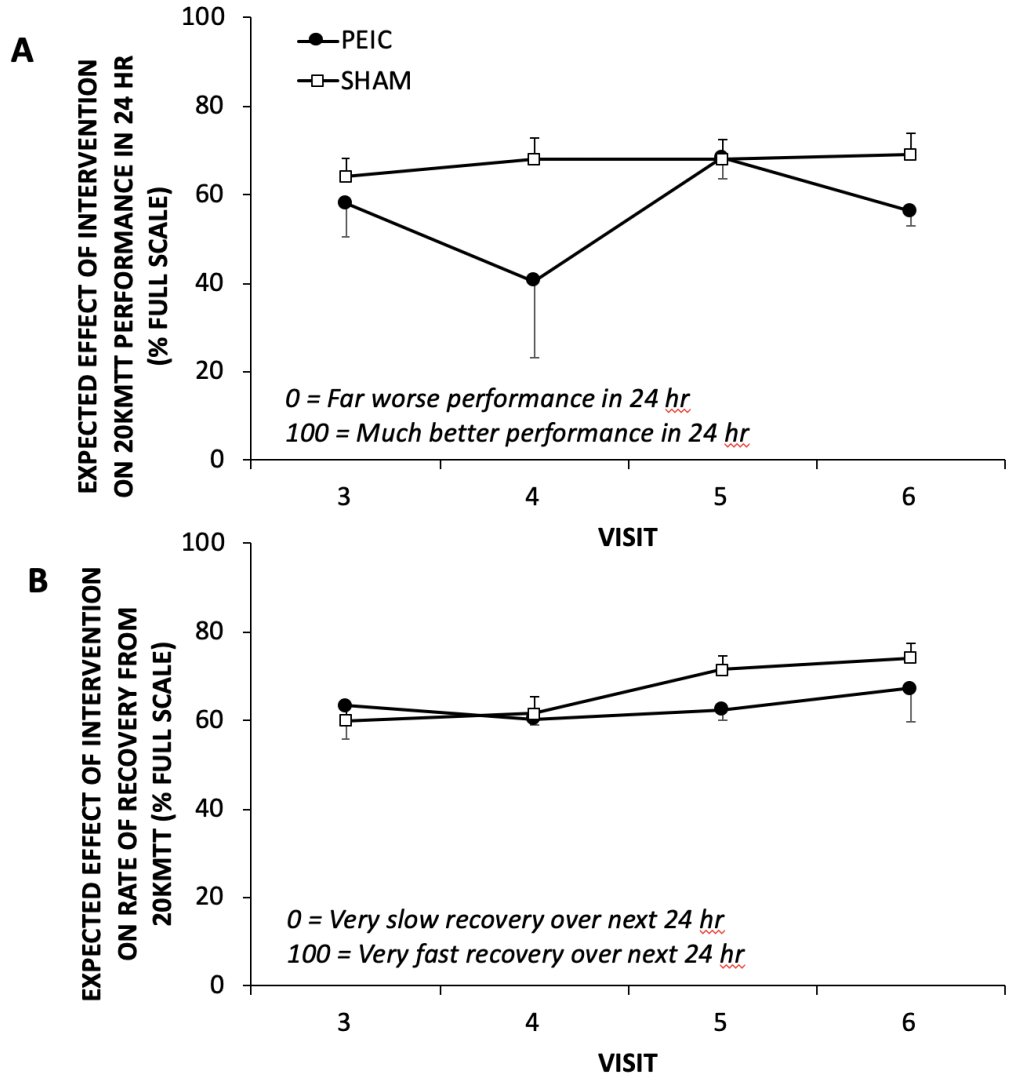

Figure 2.2. Expected effect of the intervention in both the PEIC (n=3) and SHAM (n=5) intervention groups on (A) 20kmTT performance 24 hours later and (B) speed of recovery from 20kmTT over the next 24 hours. Values are Mean ± Standard Error

(iii) despite this variability, expectations for better subsequent performance were broadly similar between groups. The data presented in **Figure 2.2B** indicated that participants in both PEIC and SHAM groups had similar expectations that the intervention they received would facilitate recovery from 20kmTT over the next 24 hours, as indicated by mean ratings approximating 60-70% of full scale.

2.4.d. Subjective assessments of recovery and performance readiness. As illustrated in **Figures 2.3A,** ratings of perceived recovery status were similar in PEIC and SHAM groups across *Visits 3-7*, with ratings in both groups starting high (reflecting being well recovered and energetic) at *Visit 3* and progressively declining each subsequent visit. As illustrated in **Figure 2.3B**, BAM+ ratings of performance readiness decreased progressively from *Visit 3* to *Visit 7* in both groups; and were slightly higher (except at *Visit 5*) in the PEIC compared to SHAM group, with the greatest mean differences (of ~2 BAM+ score units) in performance readiness observed at *Visits 6 and 7.*

2.4.e. Pain pressure threshold. Pain pressure thresholds of the *vastus lateralis* (**Figure 2.4A**), *rectus femoris* (**Figure 2.4B**) and *gastrocnemius* (**Figure 2.4C**) remained largely unchanged within both PEIC and SHAM groups across *Visits 3-7* (i.e., unaffected by multistage 20kmTT), with no notable differences between groups at any measurement time point.

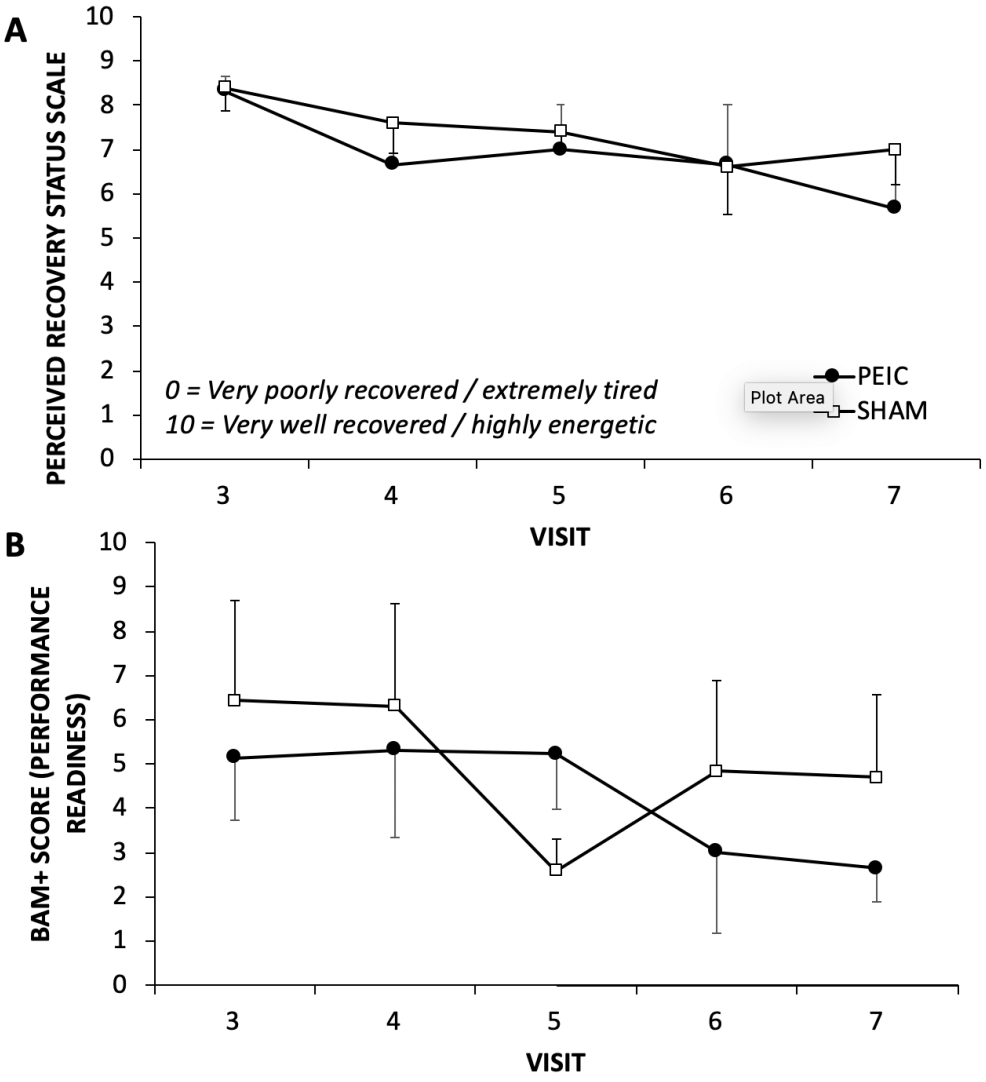

Figure 2.3. Subjective assessments in both the PEIC (n=3) and SHAM (n=5) intervention groups of (A) perceived recovery and (B) perceived performance readiness. Values are Mean ± Standard Error

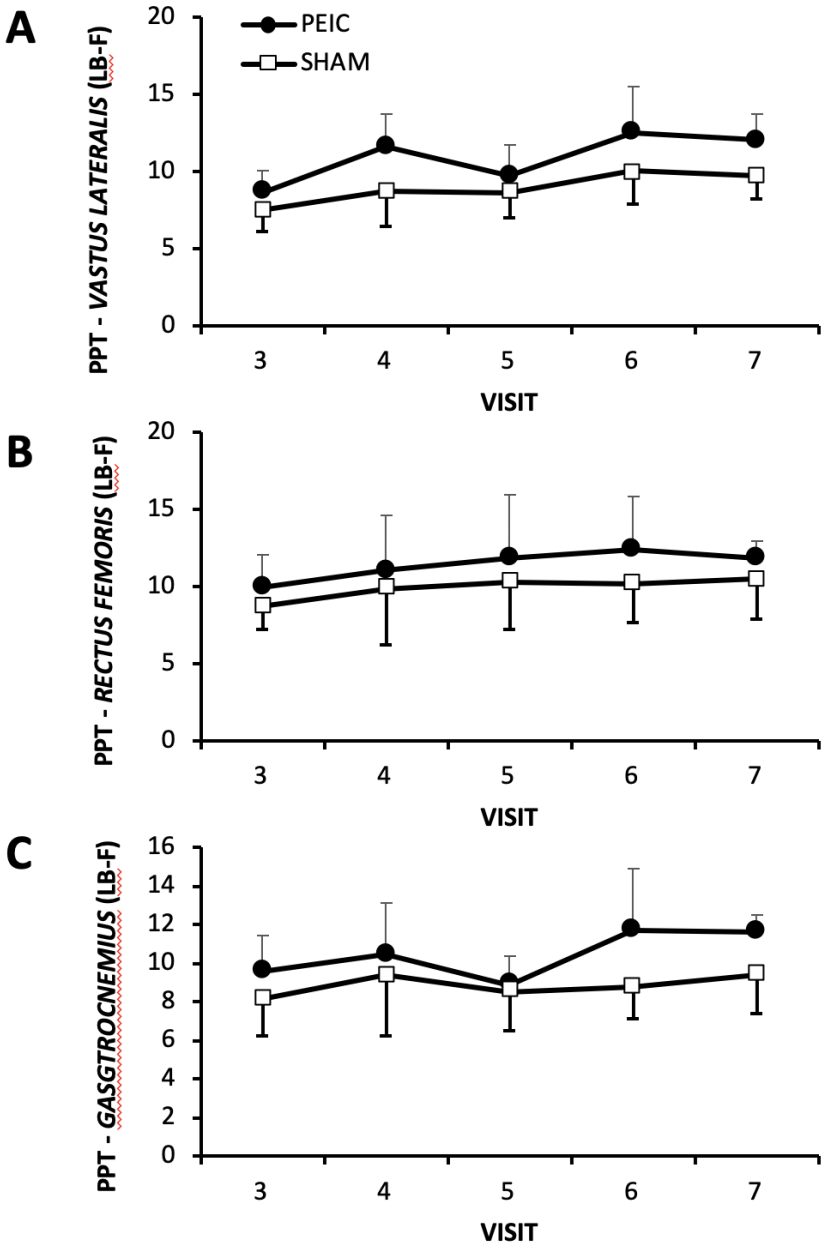

Figure 2.4. Perceived pain pressure thresholds prior to the 20kmTT in both the PEIC (n=3) and SHAM (n=5) intervention groups on muscles (A) Vastus Lateralis, (B) Rectus Femoris, and (C) Gastrocnemius. Values are Mean ± Standard Error

2.4.f. 20-km Cycling Time Trials. When comparing the duration of the 20km time trial between the intervention groups, it was found that for the totality of all 7 visits, the PEIC intervention group completed the task slightly faster than its SHAM counterpart as demonstrated in **Figure 2.5A.** Between *Visits 3 and 7*, the time required to complete the 20kmTT by the cyclists in the PEIC intervention group decreased by 0.16%; that is, these participants covered the 20-km distance 3 seconds faster at *Visit 7* than at *Visit 3*. By comparison, participants in the SHAM group experienced a 0.58% increase in time required to complete the 20kmTT; that is, it took these participants 11 seconds longer to cover the 20-km distance at *Visit 7* compared to *Visit 3*. In addition, **Figure 2.5F** depicts the change in 20kmTT performance at *Visits 4, 5, 6 and 7* in both groups compared to their respective 20kmTT performance at *Visit 3*. This data suggests that 20kmTT performance from *Visit 3* to *Visit 7* was well maintained in the PEIC group, where the mean difference in 20kmTT duration was 0.05 minutes or 3 seconds faster at *Visit 7* compared to *Visit 3*. However, 20kmTT performance was more variable across visits in the SHAM group, where the mean difference in 20kmTT duration was 0.18 minutes or 11 seconds slower at *Visit 7* compared to *Visit 3*.

As shown in **Figures 2.5B-C,** participants in both the PEIC and SHAM intervention groups demonstrated similar mean cycling power output and speed throughout the 20kmTT, though the PEIC group did generate slightly higher values for both outcomes at each visit. **Figure 2.5D-E** represent the average ratings of perceived exertion and perceived leg discomfort respectively, expressed by each group throughout the duration of the 20kmTT at each visit. These figures indicate that the PEIC and SHAM groups consistently report very similar values for both variables at all visits, although the ratings provided by the PEIC group appear to have greater variability.

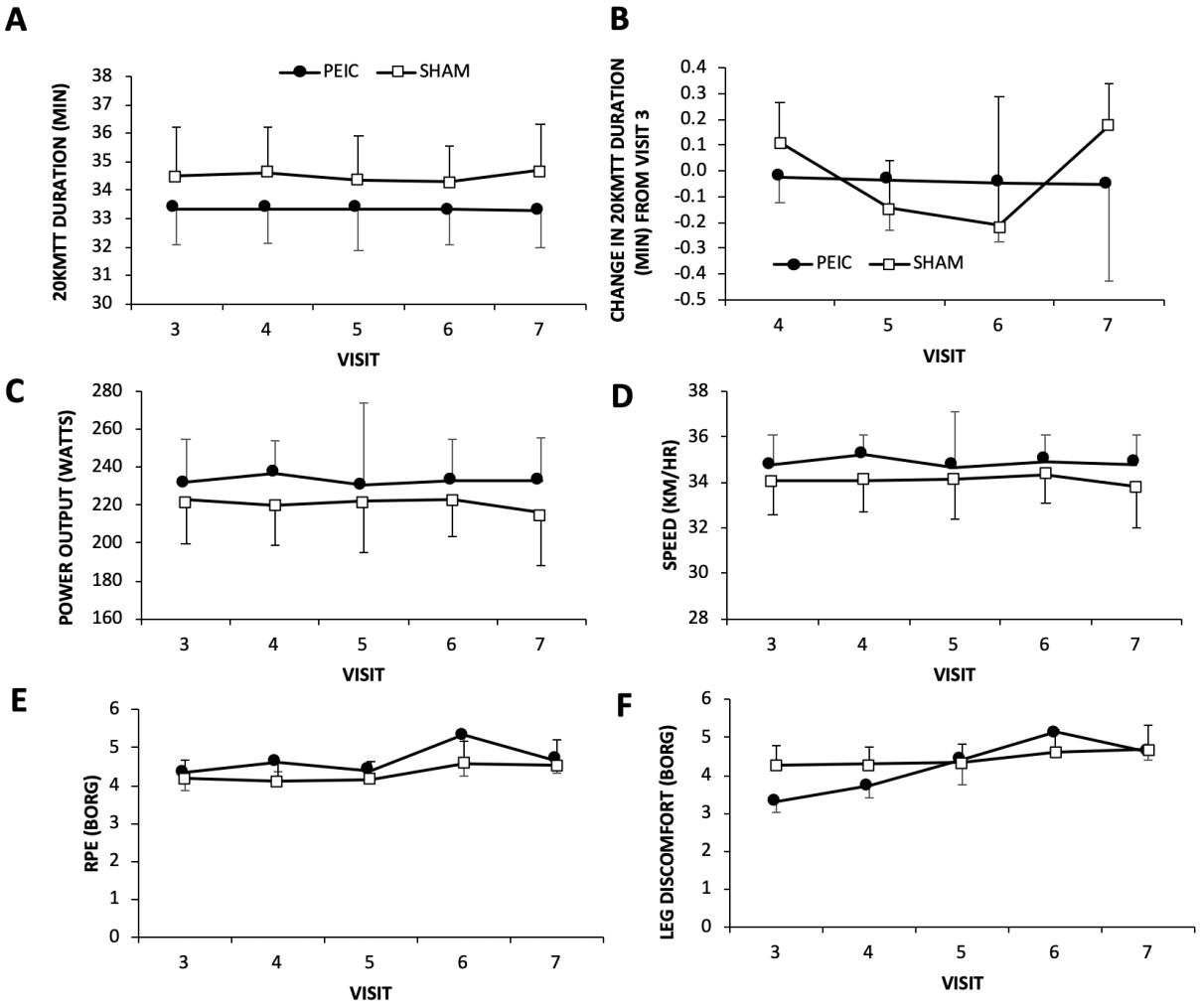

Figure 2.5. 20-km Cycling Time Trial assessments of performance and perceptual parameters in both the PEIC (n=3) and SHAM (n=5) intervention groups. (A) Average of 20km Time Trial duration. (B) Average Change in 20km Time Trial duration from Visit 3. (C) Average Power Output throughout the 20km Time Trial. (D) Average Speed throughout the 20km Time Trial. (E) Average Ratings of Perceived Exertion throughout the 20km Time Trial. (F) Average Ratings of Perceived Leg Discomfort throughout the 20km Time Trial. Values are Mean ± Standard Error.

2.5. Discussion

As expressed by Paton et al. (2005; 2006), the smallest incremental change in laboratory-based cycling time trial performance needed to achieve better placement in major cycling competitions is 0.5% (Paton & Hopkins, 2005; Paton & Hopkins, 2006). This study revealed a 0.16% decrease (performance improvement) in 20kmTT duration from *Visits 3 to 7* in the PEIC group and a 0.58% increase (performance decline) in 20kmTT duration during the same stretch of visits in the SHAM group. However, despite subtle differences in the magnitude of change in 20kmTT duration between *Visit 3 and Visit 7* in the PEIC compared to SHAM group, the preliminary results of this study do not support a beneficial effect of PEIC on laboratory-based 20kmTT performance and perceptual indices of recovery and performance readiness.

The results seem to indicate no significant variations in speed, and power output throughout the 20kmTT. In addition, there were virtually no differences in the ratings of perceived pain thresholds between the PEIC and SHAM intervention groups in any of the 3 muscles measured (*vastus lateralis, rectus femoris and gastrocnemius)*. This lack of significant difference across multiple variables between intervention groups observed in the preliminary results of this research does not suggest that PEIC application will be significant for high-level athletes as seen in studies involving low-fitness participants (Rhaí André Arriel et al., 2020).

2.5.a. Qualitative measures. In the present study, certain trends emerged: Both the PEIC (who received a cuff inflation pressure that ranged from approximately 235-260 mmHg across visits) and SHAM (who received a cuff inflation pressure of 20mmHg across visits) intervention groups reported similar ratings of expected effect on performance and expected rate of recovery at each visit, with the exception of Visit 4 (primarily) and Visit 6 (secondarily) as per the results reported

in Figure 2.2A. Despite the significantly higher pressures experienced by the PEIC intervention group, its participants reported average ratings at approximately 60% of full scale for both variables just like the participants from the SHAM group. However, both the perceived recovery scale ratings and performance readiness scores decreased progressively from *Visit 3 to Visit 7* in both groups, suggesting that the multistage study design was associated with a progressive decline in perceived recovery and performance readiness, even though 20kmTT duration, mean power and speed, RPE and leg discomfort, and PPT responses were broadly similar within and between PEIC and SHAM groups across visits.

It should also be noted that the progressive decline in perceived recovery and performance readiness across visits in the SHAM group was accompanied by a slight increase in 20kmTT duration (by 0.58% or 0.18 minutes or 11 seconds) from *Visit 3 to Visit 7*, reflecting an overall decrease in 20kmTT performance. In comparison, 20kmTT duration was well preserved across *Visits 3-7* in the PEIC group, despite ratings of perceived recovery status and performance readiness decreasing similarly to the SHAM group from *Visit 3 to Visit 7*. This could indicate that PEIC may potentially maintain cycling performance in the face of decreasing ratings of perceived recovery and performance readiness, although it is too early to draw conclusions and further research is needed.

2.5.b. Methodological considerations. The purpose of this study was to determine the effects of PEIC on the performance of competitive cyclists as well as to determine its effects on the athlete's perceived recovery and performance readiness, as well as their expectations of treatment effects. Although this research was conducted with only 3 participants in the PEIC intervention group and 5 participants in the SHAM intervention group, it had many methodological strengths. Namely, it was a randomized, parallel-group study design in which placebo and nocebo effects were

accounted for and addressed through the application of either a PEIC or SHAM condition without informing the athletes of what they were receiving or, the effects of either intervention. Furthermore, the use of a laboratory 20kmTT, which has been deemed as a good predictor of real-world cycling performance represents a valid and reliable test of athletic performance that best mimics the sport of cycling over other testing methods such as incremental cycling tests (Paton & Hopkins, 2005; Paton & Hopkins, 2006). The study design also involves more than two stages of cycling, which distinguishes it from research conducted by Arriel et al. (2018) that only measured the effects of PEIC over 2 consecutive days, and thus better recreates the demands of multi-stage races such as those in high-level cycling.

2.5.c. Study limitations. The most notable limitation of this study is the limited number of participants. The sample size with which this research was conducted is indeed small, although several external barriers played an important role in both the initiation of this study and the recruitment of participants. There were several delays due to the COVID-19 pandemic and government-mandated health restrictions. Also compounding the difficulty of recruiting participants was finding competitive cyclists and/or triathletes willing to avoid training for approximately 2 weeks to participate in this study. A total of 26 potential participants were contacted by e-mail and 14 expressed interest in participating in the study. Ultimately, 9 individuals were recruited, and 8 cyclists completed the study. Competitive cyclists were also very reluctant to participate in such a time-consuming study, during their time-sensitive competitive season. Typically, there is a pre-competition phase from January to May, with the competitive phase beginning in May and ending anywhere from July to September depending on an athlete's geographic location (Hopker et al., 2009). Despite these challenges, we successfully recruited 8

competitive cyclists/triathletes, with each participant completing a MICT and six 20kmTTs across seven laboratory visits with each visit lasting ~2.5 hours (equivalent to a total of 56 laboratory visits, ~140 hours of experimental testing, and more than 300 hours data reduction and analysis).

2.5.d. Potential study improvements. Throughout the recruitment process, several potential participants expressed hesitation about the demand and commitment of 7 visits. Perhaps, as recruitment for this study continues to progress, *Visit 2*, the familiarization visit, could be removed from the protocol. The exclusion of *Visit 2* is a possibility demonstrated by analysis of the difference between the 20kmTT duration recorded at *Visit 2* and *Visit 3*. **Table 2.3** shows that these differences in 20kmTT duration are not significant, yielding a p-value of 0.600 (by paired T-test) and a difference in performance of only 0.34%, which is less than the significant difference of 0.5% first proposed by Paton et al. (2005). Thus, it can be seen that eliminating *Visit 2* would not likely harm the integrity of this study and that there is apparently little need to familiarize participants with the 20kmTT. Given these data, the 0.58% increase in 20kmTT duration (decreased performance) in the SHAM group might be potentially meaningful (i.e., may indicate impaired performance in this group) seeing as it is greater than the day-to-day within-subject measurement variability.

	Visit 2 (Familiarization)	Visit 3	Diff between V3 and V2	% diff. between V3 and V2
001_JG	35.08	34.81	-0.27	-0.77
002_DC	32.45	32.72	0.27	0.84
004_LC	39.86	40.87	1.01	2.53
005_FG	31.58	30.90	-0.67	-2.13
006_MB	31.11	31.51	0.40	1.29
007_MR	32.18	32.86	0.68	2.11
008_AB	33.60	33.10	-0.50	-1.49
Average	***33.69***	***33.83***	***0.13***	***0.34***
Ttest (p-value)		***0.600***	***(Mean Diff of 0.13 min or 7.8 sec)***	***(Mean % diff of 0.34%)***

Table 2.3. Analysis of 20kmTT duration (performance) between the familiarization visit (*Visit 2*) and first experimental visit (*Visit 3*).

2.5.e. Conclusion. Under the experimental conditions of the current study, we observed that, over 5 consecutive days, the results do not provide significant preliminary evidence to support a potentially beneficial effect of PEIC vs. SHAM on multistage 20kmTT performance or indices of recovery and/or performance readiness.

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