Pushing the Limits of Performance. Is it Really Mind Over Matter?

An Investigation into the Effects of Menthol Mouth Rinsing and the Capacity for Fatigue Amelioration in Trained Adolescent Male Cyclists Under Heat Stress

by

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ABSTRACT

This thesis investigated the effects of menthol (MEN) mouth rinsing (MR) on performance responses in trained adolescent male athletes during a modified variable power cycle test (M-VCT). Participants (n=11) cycled for 30-min in hot conditions (31.4±0.9 °C, 23.4±3.7% RH) on two occasions. In a randomized crossover design, (1) menthol MR (0.01%) or (2) placebo (PLA) MR, was administered at 6-min intervals. Power, distance, core temperature, heart rate, surface electromyography, perceptual responses (exertion, thermal stimulation, fatigue, and feeling), and blood lactate were recorded. The MEN MR significantly improved mean power output by 1.81±1.57% relative to PLA (p=<0.001, 95% CI= [1.73-4.46], ES= 1.53). Physiological and perceptual measures did not differ between trials. While individual responses varied to MEN, results demonstrate that a nonthermal cooling agent that acts on the CNS can benefit power regulation during a stochastic cycling task without causing additional decline in perception, as suggested by the central fatigue hypothesis.

Keywords: menthol; cycling; variable cycle test; heat stress; central fatigue

AUTHOR’S DECLARATION

I hereby declare that this thesis consists of original work of which I have authored. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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The research work in this thesis that was performed in compliance with the regulations of Research Ethics Board under **REB Certificate number #**16331.

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I performed the majority of the synthesis, testing of membrane materials, and writing of the manuscript.

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LIST OF ABBREVIATIONS

CGT, Central Governor Theory

Tc, Core body temperature

EMG, electromyography

FS, feeling scale

HR, heart rate

[La^-], lactate concentration

MEN, menthol

MR, mouth rinse

RH, relative humidity

ROF, rating of fatigue

RPE, rate of perceived exertion

TC, thermal comfort

TS, thermal sensation

Tsk, skin temperature; Trec, rectal temperature

TRPM8, Transient Receptor Potential Cation Channel Subfamily M Member 8

TT, time trial

TTE, time-to-exhaustion

UCI, Union Cycliste Internationale (International Cycling Union)

USG, urine specific gravity

VCT, variable cycle test; M-VCT, modified variable cycle test

W, rate of work in watts (absolute); W/kg^{-1}, watts converted to power to weight ratio (relative)

LIST OF KEY DEFINITIONS

Variable cycle test (VCT): a valid and reliable stochastic cycling protocol consisting of 10 x 6-min laps that are completed in a repetitive and continuous fashion. Each lap consists of prescribed and open-ended sections described as ‘recovery’ pace, ‘hard’, ‘accelerating’, and ‘decelerating’ pace (Sharma et al., 2014).

Adolescent: a young person, between the ages of 10-19y who is in the process of developing from a child into an adult (Falk, 1998).

Ergogenic aid: a technique or substance used for the purpose of enhancing performance outcomes, either by affecting energy metabolism or by eliciting an effect on the central nervous system (CNS) (Best et al, 2020).

Heat stress: a process characterized by prolonged heat storage, elevated internal temperature, increased heart rate, and decreased athletic performance (Tucker et al., 2004).

Thermoregulation: a process by which the body responds to external and internal stimuli including environmental conditions and metabolic heat to stabilize core temperature (Tc) (Wendt et al., 2012).

Central Governor Theory: A CNS mechanism that takes information regarding metabolic needs, physiological states, and various motivational drives to regulate physical exertion and protect an organism from catastrophic failure (Noakes et al., 2004).

Central fatigue: a type of fatigue that refers to changes that occur proximal to the motor neuron and involves neural and psychological impairments in exercise performance (Wan et al., 2017).

Peripheral fatigue: a type of fatigue that involves the motor unit and occurs strictly through depletion of the muscle energy supplies (Wan et al., 2017).

Central Fatigue/Serotonin Hypothesis: idea that an increase in the central ratio of serotonin to dopamine is associated with the onset of fatigue, whereas a low ratio can favour improved performance through the maintenance of motivation and arousal (Meeusen et al., 2006).

Rating of perceived exertion (RPE): method of measuring activity intensity level is also referred to as the Borg Rating of Perceived Exertion scale (Borg, 1982).

Thermal sensation: recognized as the perception of one's state during an exercise in a particular environment (Velt & Daanen, 2017).

Thermal comfort: refers to the evaluation of ones' state during an exercise in a particular environment (Velt & Daanen, 2017).

Rating of Fatigue: numerical scale used to rate how tired some is feeling, within an exercise context (Micklewright et al., 2017).

Affective feeling: an emotional feeling or state that can be positive or negative, in response to internal and surrounding stimuli (Ekkekakis et al., 2011).

Electromyography (EMG): an electrodiagnostic technique for evaluating and recording the electrical activity produced by skeletal muscles (Turker & Sozen, 2013).

Menthol: a crystalline compound with a cooling minty taste and odor, found in peppermint and other natural oils, commonly used for flavoring, and in decongestants and analgesics (Bongers et al., 2017).

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CHAPTER 1

1 Introduction

Beginning with the invention of the bicycle in the early 1800s, cycling has since become a popular means of transportation, recreational activity, and now global competition (Mignot, 2015). Bicycle racing is described as a highly demanding sport that can be broadly characterized into disciplines including road, track, mountain bike, BMX, cyclocross, and trials (UCI, 2022). Cycling has been included in the Olympic program since the first modern Games in 1896, but the sport has evolved drastically over past decades (Van Reeth, 2016). Notably, with what started as just six events across two disciplines, has currently grown into the third-largest summer Olympic sport comprised of 22 events that now span five disciplines (UCI, 2022).

According to the International Cycling Union (UCI), the current growth of competitive cycling is driven by the process of globalization (Van Reeth, 2016). In this respect, globalization is defined and measured at three different levels within the competition environment. First, at the individual level. This includes grouping competitors according to their nationality and then comparing across the top 100-ranked riders for each UCI discipline annually to provide a global estimate of participation and individual success in competition (Van Reeth, 2016). Secondly, competitive cycling is analyzed at the group level. This is done by measuring the international diversity both within and amongst cycling teams in hopes of identifying patterns and calculating success rates across different groups (UCI, 2022). And finally, globalization at the tertiary or organizational level is determined by assessing where and how many cycling events are held in a particular location each year (Van Reeth, 2016). This is especially interesting because trends reveal that most professional events are held across the European continent in countries with warm or hot climates. Arguably, it is a combination of these measures which has increased understanding and recognition of the diversity and opportunity that the sport of cycling holds, globally.

From a sports science perspective, cycling performance is understood to be dictated by the diverse interconnection of mechanical, physical, and psychological variables within a certain environment (Coyle, 1999). Each of these factors can serve to either benefit or limit overall performance outcomes. During a maximal effort cycling task the ability to cope with surrounding environmental conditions is intrinsically related to regulatory processes that occur within the human body. This means that in response to ambient temperature during intense exercise, the body will employ a diverse range of protective features that are intended to limit performance and protect an individual from catastrophic failure (Noakes et al., 2005). More precisely, thermoregulation, thermosensation, and fatigue are all important mechanisms known to regulate the homeostatic process when the body is under significant levels of stress. This proposed regulation of exercise has previously been explained by the central governor theory (CGT). This theory suggests that the subconscious brain utilizes input from internal and external stimuli to regulate power output by limiting motor unit recruitment during prolonged or intense exercise to protect the body from biological breakdown (Weir et al., 2006). This means that during exercise, the purpose of pacing is to allow for the completion of a task in the most efficient and safest way (St Clair Gibson et al., 2004). Despite being attractive, this hypothesis has become highly controversial in exercise physiology, with increasing demand to abandon it altogether (Inzlicht & Marcora, 2016). This has arisen from a lack of evidence to explain how the central governor may so easily override important homeostatic concerns during exercise, incomplete understanding surrounding how mental effort consumes inordinate amounts of energy that are not already circulating in the brain, and inconsistencies related to the notion that the model appears as an all-knowing and unfalsifiable principle, thus contributing very little to current understandings of why people tend to disengage from effortful tasks over time (Inzlicht & Marcora, 2016). If this theory was in fact dependable, then this would imply that world athletic records would remain relatively unchanged or unbreakable across time.

To better explain the mechanisms which may regulate effort and influence performance, researchers have turned to the idea that performance breakdown is better explained by ‘central’ factors which control exercise induced fatigue (Gavel et al., 2021; Taylor et al., 2016). While the exact mechanism remains unknown, this observation suggests that extracellular changes in biomarkers, mainly serotonergic and dopaminergic concentrations, innervate the brain during exercise and act on the thermoregulatory centre to contribute to fatigue development at both the central [within the central nervous system (CNS)] and peripheral (voluntary activation of exercising muscle) levels to hinder performance outcomes (Meeusen et al., 2006; Sidhu et al., 2018; Taylor et al., 2016). Since fatigue development is known to emerge as a factor of exercise intensity and duration and is often exacerbated by high ambient temperature conditions; it is imperative that interventions to mitigate fatigue and performance-related decline are explored in athlete populations. Unlike the CGT, this hypothesis reinforces the idea that athletes are continually driven by the desire to extend performance boundaries, but realistically face limitations set by a range of innate and/or acquired characteristics.

If performance can be regulated by the diverse interaction between biological and learned traits, perhaps it is even more interesting to understand how young athletes are able to experience and overcome the same physical and psychological stresses as adults during athletic tasks despite employing different processes to mediate homeostasis. This means that while young athletes may not be as mature or prepared as their adult counterparts for the demands of competition, they are still capable of ‘pushing’ themselves to perform at a comparable level (Falk & Dotan, 2011). While this idea is supported by “NextGen” training models and a desire to prepare young athletes for high end competition much earlier on, it must be recognized that young athletes still employ different thermoregulatory strategies to combat the effects of heat and fatigue during exercise (Falk & Dotan, 2011). Despite higher rates of heat related illness and injury recorded in young athlete populations, this should not give rise to the idea of mental or physical inferiority. Research does suggest that young athletes have the capacity to handle environmental challenges during athletic tasks, but many just have not been adequately prepared to do so (Bontemps et al., 2019). For example, within the sport of cycling, regardless of producing comparative physiological profiles, young, trained riders may be plagued by fatigue much faster than older, more experienced cyclists. This can manifest as power and cadence drop-off, an increased rate of tactical or technical errors made during competition, exaggerated feelings of faintness, intense thirst, and the desire to disengage from the task (Weir et al., 2006). These factors can quickly amalgamate to hinder performance outcomes during competition if not addressed appropriately.

With this information in mind, there appears to be significant value in understanding the relationship between performance outcomes, environmental conditions, and the regulation of homeostatic or protective variables during intense cycling tasks. The literature indicates that performance can be evaluated via a plethora of variables in sports science research. Distinctively, some of the more commonly used measures of athletic function include the rating of perceived exertion (RPE) and thermal scaling, which allow an individual to describe their subjective state within a particular context (ASHRAE, 2004; Borg, 1982). Newer assessments of perceptual function include the rating of fatigue (ROF) and feeling scale (FS). These measures have been specifically developed for exercise and are intended to provide awareness of cognitive processes, offering an athlete the opportunity to quantify their exhaustion and broader emotional experience (Hardy & Rejeski, 1989; Micklewright et al., 2017). In combination, these scales may provide unique insight into an athlete's ability and desire to sustain or even improve performance during a specific task. Additionally, physiological variables including core temperature, sweat rate, blood lactate concentration ($[La^-]$), muscular activity (sEMG), and power output can be used to explain variations in performance across different conditions (Faria et al., 2005). While these are the traditional measures used to understand athletic capacity, when used in combination they may offer a more holistic understanding of the mechanisms which can either limit or benefit performance.

Given that high ambient temperatures can have a negative effect on performance, cooling interventions have been developed to mitigate fatigue and improve athletic outcomes. An example of this is pre-cooling and mid-cooling. Pre-cooling can be described as the rapid removal of heat from the body before exercise (Ross et al., 2013), whereas mid-cooling can be defined as the application of cooling during exercise (Stevens et al., 2017). Pre-cooling typically reduces core temperature prior to engaging in exercise, while mid-cooling techniques simply alter perception during the task. Despite controversy, interests pertaining to the ergogenic properties of menthol (MEN), a mid-cooling intervention, have recently spurred a range of investigations in sports research. Menthol is an organic compound derived from peppermint that often presents as a flavor molecule to target the gustatory system as well as oropharyngeal thermoreceptors (Stevens & Best, 2017). When rinsed in the oral cavity, MEN is shown to activate the transient receptor potential melastatin-8 (TRPM8) (Best et al., 2018; Gavel et al., 2021). Once TRPM8 receptors are stimulated, afferent sensory neurons depolarize and action potentials are transmitted from the dorsal root and trigeminal ganglia to the brain via the spinal cord, where they are integrated by the central nervous system (CNS) to evoke reflexive and cognitive responses (Gavel et al., 2021; Klasen et al., 2012). For example, during moderate to high intensity endurance tasks, particularly involving cycling and running, MEN mouth rinsing (MR) is estimated to trigger reward centers in the brain that amalgamate to decrease the perception of thermal stress and fatigue to provide physical improvements in performance that can range from approximately 0.5 to 6.6% (Gavel et al., 2021). Simply put, MEN is known to elicit a pleasurable cooling sensation, ameliorate fatigue, increase ventilatory drive, and attenuate thirst, all of which create the impression that individuals feel better and therefore can work harder to complete an athletic task (Jeffries et al., 2019; Stevens & Best, 2017). When compared to conventional pre-cooling devices, MEN may be a viable non-thermic alternative that can be reliably used to improve performance during exercise tasks in the heat.

According to the UCI, now more than ever, athletes from all around the world are being recruited to train and race at an elite level at a much younger age (UCI, 2019). While many coaches and programs focus heavily on helping athletes achieve their goals logistically, the sports science community has the responsibility of disseminating knowledge and expertise that will assist in better preparing these athletes for the physiological and psychological demands of exercise and competition across a range of environments. For example, the difference between 1st and 3rd in the junior men’s 2019 World Championships Road race was 0.71% (93 sec) while the difference between 1st and 3rd in the junior men’s 2019 cross-country Mountain Bike World Championships race was only 0.49% (20 sec) (UCI, 2019). As such, strategies to manage exercise-induced fatigue, especially in the heat, are imperative because even small alterations in performance can be critical for event outcomes.

The question(s) guiding this literature review and research inquiry are “what performance testing protocol can be used to reliably predict outcomes in trained adolescent male cyclists?” and “What are the potential effects of utilizing a menthol mouth rinse on adolescent male cyclists during a race-like task in the heat?”. With an increasing demand to better prepare young athletes for the challenges of international level competition, there is an opportunity to study the stochastic nature of high intensity cycling events in adolescent population groups as well as the effectiveness of the water-soluble organic crystalline compound, menthol. With a lack of published research confirming the effects of menthol mouth rinsing during a variable, race-like cycling protocol under hot environmental conditions in adolescent athletes, there is a significant need to contribute to this area of investigation.

CHAPTER 2

2 Review of Literature

This section will review how concepts related to athlete age, environmental temperature, thermoregulation, thermosensation, and fatigue influence athletic performance. This document will also explain various physiological and psychological variables used across research settings to assess performance outcomes in athlete populations. Finally, the use of menthol as a performance enhancing substance in relation to exercise will then be described based on findings provided by relevant publications.

2.1 Evolution of cycling and performance testing

According to historic records, a 1,200-meter bicycle race took place between two fountains in Saint-Cloud Park, Paris in May of 1868, marking the inauguration of cycling as an official sport (Mignot, 2015). Then, over thirty years later, federations from the United States, Switzerland, Italy, France, and Belgium would come together to form the Union Cycliste Internationale (UCI), a global governing body for all cycling disciplines and any individual involved in the sport (UCI, 2019). Currently, the UCI supports the interests of 196 National Federations, five Continental Confederations, more than 1,500 professional riders, one-million licensed competitors, millions of cycling enthusiasts, and over two-billion bicycle users worldwide (UCI, 2019). More specifically, as stated above, the UCI manages and promotes the disciplines of road, track, mountain bike, BMX Racing, BMX Freestyle, cyclo-cross, trials, and indoor cycling across thousands of venues each year.

Broadly speaking, cycling is described as a low-impact fitness activity that benefits muscular strength and improves aerobic fitness across the lifespan, but from the perspective of elite athletes, cycling is often described as an intense and dynamic pursuit that requires an extraordinary level of psychophysical preparedness. With that in mind, exercise researchers and physiologists continue to explore performance outcomes in cycling by investigating the complex interaction that various chemical, mechanical, and environmental stimuli can have on metabolic, cardiovascular, and ventilatory processes during exercise (Faria, 2012). Over the past few decades, a variety of sport-specific laboratory-based testing protocols have been evaluated for their validity, reliability, and applicability to cycling performance. Although standardized-laboratory testing offers the unique ability to isolate and continuously monitor physiological and psychological parameters while strictly controlling ambient temperature conditions (Faria et al., 2005; Paton & Hopkins, 2001), there is doubt surrounding their ability to simulate the stochastic nature of racing events. Perhaps, leading to increased controversy when attempting to identify and describe the interplay of mechanisms that are expected either limit or benefit performance.

Since cycling is encompassed by a variety of on- and off-road events that range in duration from ten seconds to over six hours (Mignot, 2015; UCI, 2019), several types of performance tests have been utilized to quantify a cyclist's experience(s) throughout training and competition (Paton & Hopkins, 2001). The time trial (TT), defined as a performance test in which an individual is successively timed or measured over a set distance or period (Currell & Jeukendrup, 2008), is the most common laboratory protocol employed across cycling performance studies. TT's can be manipulated, in terms of time or intensity to suit the specific endeavours of the researcher while still providing valid physiological results that are reflective of cycling events (Currell & Jeukendrup, 2008). TT's can be classified as constant work tests, which require riders to complete either a set distance (e.g., 40-km TT) or cycle for a pre-determined time (20-min TT), or time-to-exhaustion tests (TTE), which force a rider to maintain a set intensity to the point of exhaustion (e.g., set work rate in terms of power or heat rate) (Laursen et al., 2007). Despite the continued use of both types of protocols across the literature, TT and TTE tests continue to face criticism due to their inherent lack of ecological validity towards actual racing events.

2.1.1 Methodologic considerations for performance testing

Despite controversy, a wide variety of testing protocols have been integrated into performance-based research, in hopes of better simulating realistic physiological profiles, assessing training interventions, and quantifying performance outcomes typical to the demands of different endurance sports (Girard et al., 2013). In theory, this means that a valid, yet domain specific testing protocol should be considered when exploring performance in activities like cycling, running, or rowing (Fisher et al., 2017; Laursen et al., 2007). Consequently, the selection of an inappropriate testing protocol causes inherent limitations when discussing the applicability of results and subsequent performance outcomes within a particular population group. As identified above, performance TT's typically follow one of two formats. Fixed-duration tests require a participant to perform an exercise to the best of their ability for a set time, while a fixed-distance test forces a participant to cover a set distance as fast as possible. While both formats are viable options to consider when designing a study, perhaps adopting a more volatile and demanding protocol, indicative of a race-level effort would offer new insight into the expected boundaries of performance. That said, the validity, reliability, and sensitivity of any performance test should always be considered before use (Currell & Jeukendrup, 2008).

A valid testing protocol is one that generates physiologic responses comparable to the efforts sustained during the actual activity (Paton & Hopkins, 2001; Prins et al., 2007). Since many road and mountain bike events are distance-based or “time capped”, there continues to be a plethora of research examining TT's. Although many endurance athletes prefer to use fixed-duration over fixed-distance protocols to measure improvement because they are easy to implement and can provide comparable physical outcomes without over-taxing the system for too long (Karsten et al., 2017), they still are not replicative of the variable demands of racing. Similarly, TTE protocols can also be valuable, but use is often limited by the need to pre-establish workloads, identify stage durations, and calculate appropriate stage increments to "force" exhaustion in an acceptable timeframe (Currell & Jeukendrup, 2008). Further, the sport of cycling has evolved significantly over the past decades. Courses across most endurance disciplines have now been designed with higher technical difficulty, as well as a shift away from static or long duration fitness driven outcomes towards shorter duration, more volatile demands that challenge physical, mental, and tactical boundaries. This means that although still a useful training tool, the traditional TT likely no longer can be used to reliably predict performance limitations or outcomes for many cyclists. This has led to the recent development of a variable power cycling test (VCT). Not only does this test appear to capture parameters that best relate to the dynamic and repetitive power demands of race events, but it also appears to successfully differentiate between well-trained and untrained cyclists' abilities, making it a useful predictor for the performance-related qualities required for competition (Sharma et al., 2020). Since the contemporary 1-hour VCT (Sharma et al., 2014) has demonstrated high reliability and good validity in adult cyclists, perhaps adopting a similar protocol would be more useful for forecasting performance changes in trained adolescent athlete groups over the traditional models. As such, the use of an ecologically valid fixed-duration variable cycling protocol, modified to suit the race duration and intensity typical of the population of interest, has the potential for widespread benefit and application across sports science research (Fisher et al., 2017, Sharma et al., 2014).

Second, reliability refers to the variation within a performance testing protocol and is highly influenced by intra-subject differences (Currell & Jeukendrup, 2008). Small intra-subject variance increases the accuracy of a test because it means that a particular test is more likely to provide a precise estimate of changes in performance. Investigations show that TTE protocols have a coefficient of variation percent (CV%) typically >10%, whereas TTs are more reliable with a CV% of <5% (Currell & Jeukendrup, 2008). For example, it was determined that a 5-km cycling TT showed a CV of <3%, despite displaying low reliability of physical responses during a performance test (Fisher et al., 2017), whilst Macinnis et al. (2018) concluded that 4-min and 20-min TTs present high reliability of physiological responses and therefore appear useful for assessing performance in trained cyclists. Similarly, McGawley (2017) also showed that a four-minute running TT provides more reliable performance data in comparison to an incremental running TTE. Perhaps of greatest use, is the investigation conducted by Sharma et al., 2014 which demonstrated that the 1-hour VCT produced a CV% of 1.98% in trained cyclists. Since the VCT protocol appears to produce similar results under consistent conditions (or very little random error), it can be assumed that the test would be a very reliable measure for evaluating performance change or improvement in young cyclists.

Finally, a sensitive performance test can accurately detect small, but important changes in performance (Currell & Jeukendrup, 2008). Considering the difference between placing first or second in a sporting event can be <1%, it is of utmost value to be able to detect very small changes in an individual's performance either over time or due to a particular training/experimental intervention. Interestingly, Amann et al. (2008) compared a constant-power TTE to a 5-km TT on a cycle ergometer in conditions of normoxia, hypoxia, and hyperoxia to determine that the TTE has similar sensitivity to that of a TT for the effects of arterial oxygenation and presumably other factors affecting endurance performance. From this information, it was determined that TTE's offer more control for studies of physiological correlates on performance, but a TT is the most viable option when self-selected pace is an issue (Amann et al., 2008). More recently, work by Sharma et al. (2014) has indicated that the VCT is a sensitive testing protocol, with the ability to detect a change as small as 3.9% in athlete performance with 95% certainty. Since significant performance change at the elite level could be equated to an improvement as small as 1%, it is important to select a testing protocol that can detect minimal change with a reasonable level of confidence.

Overall, the literature denotes that a shorter duration performance test reduces the burden associated with laboratory testing without compromising physiological simulation of actual circumstances, especially in younger populations (Inglis et al., 2019). This has encouraged athletes and coaches to focus on a variety of power figures to measure performance – typically ranging from ten seconds all the way up to sixty minutes; depending on the goals of the athlete. Since there is currently no gold standard associated with performance testing, it is reasonable to explore the impact that different protocols can have on performance outcomes. In the past, many studies have utilized TT or TTE testing methodology, but perhaps, further exploration of the variable cycle test - derived from both the sprint and endurance-based requirements of racing- offers a reasonable and interesting alternative for experimental research in cycling.

2.2 The variable-power cycle test

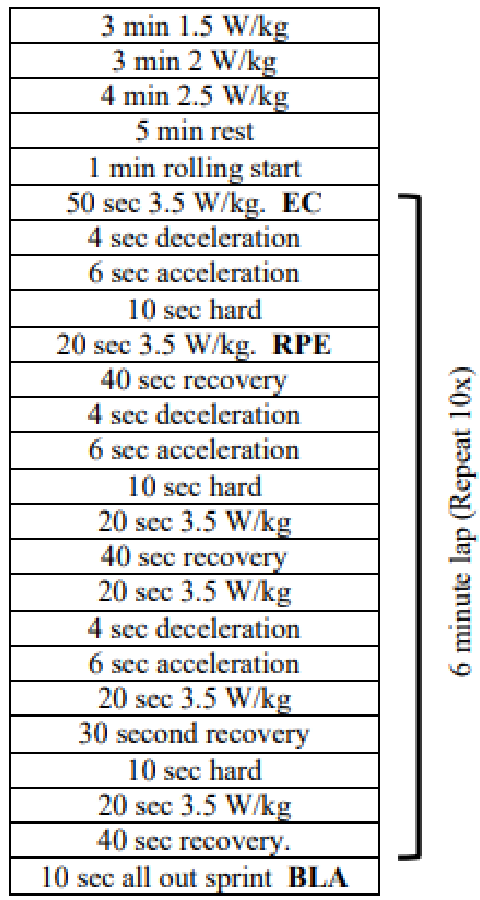
Following the information discussed above, it is important to understand that performance indicators for endurance sports, such as cycling, have traditionally been characterized by incremental maximal tests, where submaximal responses are measured in conjunction with maximal responses during a progressive stepwise protocol. Although these tests do provide invaluable information about the power output associated with physiological indicators such as VO_{2max}, distinct submaximal thresholds, and exercise economy or mechanical efficiency, they do not account for the frequent and substantial variations in power output that modern racecourses demand (Sharma et al., 2014). Now that endurance sporting events take place closer to city centers, in an attempt to attract larger crowds and improve spectator experience, courses have been modified to include shorter, but more variable and demanding tracks (Martin et al., 2012). Consequently, this has altered the physiological demands of cycling across many disciplines.

Rather than evaluating performance using a traditional stepwise or static protocol, there appears to be a need to quantify performance parameters during a “race-like” task that forces an athlete to transition back and forth between submaximal and maximal aerobic capacities. Research indicates that several repeat high-intensity efforts interspaced with a relative workload across a designated distance or time frame has proven to discriminate between top 10 finishes and lower placings in cycling races (Sharma et al., 2020). This is because a variable cycling task appears to better mimic the dynamic patterns experienced during an actual race, a performance indicator that is typically not captured via traditional testing protocols. As sporting events become increasingly competitive worldwide, the need to evaluate and quantify physical and physiological aspects that align with competition demands underpins the increasing diversity of developing laboratory-based testing protocols that better display the qualities of racing.

This has led to the development of a dynamic protocol with open-ended and prescribed sections that give an athlete the opportunity to perform to the best of their ability. The variable cycle test (VCT), a 1-hour cycling protocol developed through pilot testing and modelled on a previous study that quantified the variable (power) nature of racing, is deemed to offer a more flexible and holistic approach to measuring performance in a laboratory setting. The VCT consists of prescribed submaximal power output (40% at 3.5 W/kg) and designated recovery zones that are interspersed with open-ended sections described as “accelerating”, “decelerating”, or “hard” where an athlete’s repeat sprint ability can be measured in a more realistic fashion (Sharma et al., 2014). The frequency and duration of each section in the VCT was determined based on the proportion of time spent in different power zones as well as the typical number and length of sprints performed by elite cyclists during road and criterium races during a professional cycling event. More specifically, the VCT requires an athlete to complete 10 × 6-minute “laps”. The open-ended phases consist of 4 × 30 to 40 seconds of “recovery,” 3 × 10 seconds at “hard” intensity, and 3 × 6-second “sprints” with a final 10-second “all-out” effort at the end of the test (Sharma et al., 2020) (Figure 1).

As discussed previously, past investigations have successfully demonstrated the validity and reliability (CV%=1.98) of the VCT in trained male athletes, as well as the sensitivity of the test in discriminating repeat high-intensity effort performance between 10 national-level male cyclists and 13 club-level male cyclists (Sharma et al., 2020; Sharma et al., 2014). Upon considering that this protocol was developed to mimic the demands of an elite race, perhaps proposing a modified or condensed version of this protocol would offer the opportunity to measure performance parameters in different athlete population groups. Since adolescent or junior level races are typically characterized by a similar intensity profile, but shorter duration, it would be valuable to offer this protocol in a modified 30-min format. With that in mind, this protocol offers the unique opportunity to analyze physiological and psychological parameters typical of a race-level effort, within a controlled laboratory setting.

Figure 1. Schematic representation of the Variable Cycle Test. Displaying the sequence of a single 6 min lap, to be repeated 10 times (Sharma et al., 2014)



2.3 Exercise and environmental temperature

According to past publications, environmental temperature is a significant factor that can contribute to athletic outcomes (Racinais et al., 2017). While environmental conditions are largely unpredictable at outdoor competition venues, indoor settings can be standardized and closely regulated. Regardless of location, environmental conditions can greatly affect both the mental and physical performance of an athlete. Many sporting activities, in particular endurance and maximal effort events, are strongly influenced by climatic parameters. Meteorological variables including increased temperature, wind, precipitation, atmospheric pressure, and high relative humidity have all been shown to negatively impact athletic outcomes during competition

(Olds et al., 1995). Despite this association, cycling events continue to take place all over the world in an array of environmental conditions. Perhaps most interesting is the discipline(s) of mountain bike and/or road racing. Recognized for their grueling intensity, variable lengths, and technical demands; mountain bike and road racing events continue to attract thousands of athletes to a diverse range of venues each year. For example, the 2022 UCI cross country mountain bike series will make eight stops, including venues in Brazil, Germany, the Czech Republic, Austria, Switzerland, Andorra, the United States, and Canada (UCI, 2022), where riders must be prepared to take on whatever the conditions offer. This means that continued evaluation of bioclimatological conditions in sports, especially cycling, has fundamental importance not only for exploring proper preparation but also for assessing changes in performance (Olds et al., 1995). For this reason, understanding the role that cold or very hot conditions can have on athletic performance is highly significant. Since environmental temperatures can fluctuate greatly, regardless of the time of year, athletes must be ready to train and race in temperatures that can range from freezing, all the way up to those that exceed 40°C.

With the above information in mind, it is evident that many athletes are required to train and compete in environmental conditions that can disrupt heat balance. Heat balance refers to a homeostatic process that occurs when heat produced by the body or acquired from the environment equals the body’s rate of heat dissipation (Sawka et al., 1993). During muscular exercise, metabolism increases by five to fifteen times the resting rate to provide energy for muscular contractions. Depending on the type of exercise performed, 70-100% of the metabolism is released as heat and quickly dissipated to maintain heat balance (Tucker et al., 2004). This exercise-induced increase in metabolic rate causes core body temperature (Tc) to initially increase rapidly while the thermoregulatory effector responses for heat dissipation respond more slowly (Sawka et al., 1993). As exercise persists, the rate of heat loss will continue to increase in proportion to the rise in core body temperature. These thermoregulatory mechanisms will upregulate function until heat dissipation is sufficiently balanced with metabolic heat production, essentially allowing for very small increases or even stabilization of core body temperature (Racinais et al., 2017; Sawka et al., 1993; Tucker et al., 2004). However, while exercising in either hot or even thermoneutral (TN) environmental conditions, heat balance cannot always be maintained. When the magnitude of physiological strain imposed by exercise-environmental stress begins to exceed the capacity for heat exchange within the environment, this evokes a disturbance defined as heat stress. Heat stress is characterized by prolonged heat storage, elevated internal temperature (Tc), increased heart rate (HR), and decreased athletic performance (Tucker et al., 2004). In general, muscular exercise and heat stress interact synergistically and can push physiological systems to their limits in simultaneously supporting the competing metabolic and thermoregulatory demands of an athlete.

Although body temperature can fluctuate depending on factors such as fitness level, age, sex, hydration status, and hormone regulation; optimal functionality highly depends on maintaining a core temperature (Tc) of 36.5°C to 38.5°C with significant consequences for both upper and lower range deviations (Sawka et al., 1993). Hensel et al. (1984) showed that a rapid decline in proper functioning occurs below 33.5°C or above 41.5 °C and typically results in devastating outcomes, with Bongers et al. (2017) illustrating that once Tc exceeds 40°C, exercise-induced fatigue may lead to the development of various heat illnesses including heat stroke, heat exhaustion, heat cramps, or heat rashes. Research shows that prolonged bouts of sub-maximal and maximal exercise under either TN (18-24°C) or hot (28-35°C) environmental temperatures can lead to the manifestation of heat stress and therefore impact performance outcomes in athletes (Racinais et al., 2017). This implies that performance is intrinsically related to the body's ability to maintain homeostasis, which is influenced by its ability to mitigate the physiological strain induced by environmental-exercise stress (Nybo, 2010).

To be precise, exercise in hot ambient conditions is associated with a thermoregulatory burden which arbitrates cardiovascular challenges, increases pulmonary ventilation, and alters muscular metabolism, all of which can contribute to fatigue and impair performance outcomes over time (Nybo, 2010; Sawka et al., 1993). Peiffer and Abbiss (2011) examined the impact of environmental temperature on power output, self-selected pacing strategies, and performance during a 40-km cycling TT. Both pace and mean power output were significantly lower when comparing TT performance at 32°C versus 17°C, ultimately demonstrating the consequence of cycling in hot conditions. This also suggests that power output decreases to benefit homeostasis when heat stress begins to overwhelm functionality. Although the body can function at an increased Tc, once Tc reaches a critical level, (Racinais et al., 2017) the body can enter a catastrophic state.

2.4 Thermoregulation

Since a substantial amount of evidence indicates that temperature can have a profound impact on athletic performance, it is necessary to understand thermoregulation. Thermoregulation refers to a process by which the body responds to external and internal stimuli including environmental conditions and metabolic heat to stabilize Tc. Thermoregulatory mechanisms are controlled via the hypothalamus and function to return the body to a state of homeostasis. The anterior hypothalamus controls heat loss, while the posterior hypothalamus controls heat conservation (Wendt et al., 2012). In unison, the hypothalamus acts as an internal 'thermostat' by initiating blood redistribution responses. The regulatory system combines feedback from baroreceptors, osmoreceptors, and thermoreceptors to maintain a body temperature of ~37 °C (Hensel, 1982; Sawka et al., 1993). When body temperature begins to rise, heat is decreased via thermogenic reflexes (a process of vasodilation and sweating) but when body temperature falls, heat is preserved by initiating the thermolytic reflex (vasoconstriction and shivering) (Wendt et al., 2012). These processes ultimately reflect exercise capability within a particular setting and allow for heat to be transferred via pathways of sensible (conduction, convection, and radiation) and insensible (evaporation) heat exchange (Racinais et al., 2017). The rate of exchange from these processes is reliant upon the thermal gradient between the environment and the body, where a large thermal gradient from the core to the skin allows exercise to continue despite a high rate of heat production, while a narrowed gradient is responsible for impaired capacity (Wendt et al., 2012).

When exercising in cool or cold conditions the core-to-skin temperature ratio is often larger than when exercising in hot environments. To describe, in cold or cool environments metabolically generated heat is efficiently dissipated, which allows body temperature to increase to a safe and steady state (Wendt et al., 2012). However, heat dissipation pathways become less efficient in warm or hot conditions due to a reduction in the thermal gradient caused by increased blood flow and a higher internal temperature. This thermoregulatory burden is subsequently linked to a decline in exercise performance over time (Bongers et al., 2017). For example, a 10°C rise in ambient temperature is associated with a 4.5°C decrease in the core-to-skin temperature gradient. This narrowing of the gradient leads to a reflexive rise in skin blood flow which enhances non-evaporative heat loss to the environment (Racinais et al., 2017). Correspondingly, when exercise is performed at 80 to 90% of maximal aerobic capacity, it has the potential to increase body temperature by 1°C every 5 to 8 minutes if heat is not dissipated efficiently

(Periard, 2013). In this case, skin temperature approaches or even surpasses environmental temperature, causing a substantial rise in Tc and narrowing of the thermal gradient. Since core temperature is directly proportional to workload, relative to the intensity of the exercise, and affected by the surrounding environment, this can be a direct indication of exercise tolerance as it suggests that the thermal strain experienced by the combination of climatic conditions and work output largely regulate performance boundaries (Hensel, 1982; Periard, 2013; Racinais et al., 2017). Effective functioning of the thermoregulatory process during exercise is therefore vital to maintain a safe body temperature and allows athletes to avoid entering a hyper- or hypothermic state (Sawka et al., 1993; Wendt et al., 2012).

2.4.1 Thermoregulation and Athlete Age

Interestingly, adults and adolescents (persons between the ages of 10 and 19y) can employ different thermoregulatory strategies to combat the effects of heat stress (Falk, 1998). More specifically, under hot conditions, adults rely on evaporative heat loss to maintain body temperature while young athletes utilize the process of 'dry' heat exchange (Falk & Dotan, 2008; Falk & Dotan, 2011). Dry heat exchange is dependent on the temperature gradient between the body and the environment and is delineated by conduction, convection, and radiation (Falk & Dotan, 2011). This means that thermoregulatory responses in youth can differ slightly from adults when exercising in hot and humid environments (Falk, 1998). Such variations in heat-dissipation strategies typically emerge due to predisposed differences in anatomical, physiological, and psychological functionality between adult and adolescent athletes. Youth tend to have a greater surface area-to-mass ratio, a decreased sweat rate, greater peripheral blood flow in the heat, and a greater capacity for vasoconstriction in the cold when compared to adults (Smith, 2019). Additionally, research shows that young athletes need to divert a greater portion of their cardiac output to the skin (about 10% more), away from the core and working muscles while under heat stress (Falk, 1998; Smith, 2019). Young athletes also tend to acclimate to conditions at a slower rate than adults (Falk and Dotan 2011), meaning they need to be adequately prepared for training and competition in extreme environments. Overall, differences in perceived exertion/thermal strain, cumulative experience in sport, cognitive development, and decision-making capacity all can affect a young athlete's behaviour during competition, which ultimately can subject an individual to greater risk of thermal injury or promote success during competition (Falk & Dotan, 2011). Such differences may imply that young athletes have thermoregulatory inferiorities, yet evidence shows that young athletes are in fact very capable of combating thermoregulatory challenges during exercise.

In particular, a young athlete's smaller body and muscle mass ratio can affect both heat production and dissipation. When compared to adults, youth have about 20% greater surface area relative to their body mass – an implication that allows for higher rates of dry heat exchange when exposed to hot conditions. In this case, youth can dissipate a greater proportion of heat, much faster than via evaporative heat loss, which ultimately delays the onset of heat stress during exercise (Falk & Dotan, 2011). Additionally, sweat rate differs between adults and adolescents because of a decreased secretion rate per gland; perhaps resulting from smaller gland size, lower sensitivity to thermal stimuli, and possibly, lower sweat gland metabolic capacity (Baker, 2019; Falk, 1998). While a lower sweat rate means less evaporative cooling, this does allow young athletes to retain more fluid during exercise, which can mitigate the harmful effects of dehydration during a task and prolong efficiency (Falk & Dotan, 2011). Other physiological changes that occur during growth and maturation that can affect thermoregulation during exercise include metabolic, circulatory, and hormonal disparities (Sawka et al., 1993). For example, lower cardiac output and hemoglobin concentrations seen in young males during exercise are often responsible for the increased cardiovascular strain observed under hot conditions (Sawka et al., 1993). However, as young athletes rely more heavily on peripheral perfusion for heat loss, their cardiovascular and thermoregulatory capacity can be expected to be under increased stress when mitigating the combined effect of muscle metabolism and greater peripheral circulation in the heat. It has also been suggested that the higher metabolic cost of locomotion in youth provides an added strain on the thermoregulatory system during exercise in the heat. While this can be limiting, it is vital to recognize that during competition young athletes perform relative to their own capacity. Due to the chronological age-based category divisions in cycling, young athletes are not required to complete at the level of their adult counterparts but only relative to all other athletes of the same age. This implies that youth are not disproportionally exposed to risks during competition. Finally, it is assumed that hormones such as testosterone and prolactin, which are produced at lesser rates in youth, may also account for thermoregulatory differences and therefore, may alter sweat gland composition and functionality (Falk, 1998).

Nevertheless, the effectiveness of thermoregulation in young athletes is reflected by core temperature stability. Under TN conditions, youth are marked by a higher skin temperature but similar Tc when compared to adults (Falk & Dotan, 2011; Smith, 2019). This likely occurs because of a greater reliance on dry heat loss rather than evaporative cooling. Under hot conditions, research shows that adolescents often have a higher Tc than adults when walking and running but not necessarily while cycling (Falk & Dotan, 2008). Although investigations reveal insight into the mechanisms that affect thermoregulation in adolescents under different conditions, as more young athletes continue to become involved in competitive sport, there is a need to determine if the undesirable association between environmental temperature and performance degradation in this population cohort can be mediated through the use of an external ergogenic mechanism.

2.5 Thermosensation

Thermosensation is the ability to detect temperature changes in the environment through sensory neurons that project into the outer layers of the skin, oral, and nasal cavity (Stevens & Best, 2017). Afferent sensory neurons transmit signals via the spinal cord to the brain where they are integrated to evoke reflexive and cognitive responses (Digel et al., 2008). Sensory receptors in the mouth and nose are responsible for detecting the temperature of food and beverages, while external surface receptors influence thermoregulation (Digel et al., 2008). Thus far, six transient receptor potential (TRP) ion channels are known to constitute an important component of the sensory system as they play a specific role in the detection of thermal stimuli (Wang & Siemens, 2015). TRPV1, TRPV2, TRVP3, and TRVP4 are characterized as heat thermo-sensors, while TRMP8 and TRPA1 are activated via cold stimuli (Tominaga, 2007). Interestingly, each TRP channel is triggered by distinct temperature ranges and act as receptors for ligands that elicit distinct psychophysical sensations (Wang & Siemens, 2015). For example, TRPV1 channels respond to heat >43°C and capsaicin (an ingredient of hot chili peppers). During this process, the ligand binds to the open channel which depolarizes the neuron and causes an action potential to fire. The brain stem then interprets this input as a change in ambient temperature and initiates sweating and vasodilation (Tominaga, 2007). On the other hand, TRPM8 responds to cold temperatures <22°C and menthol (a derivative of peppermint). Overall, TRP channels are now known to constitute important components of sensory systems, which allows for the detection or transduction of osmotic, mechanical, or thermal stimuli. This topic will be expanded on in *section 2.11.*

2.6 Fatigue and Athlete Age

Exercise-induced fatigue (EF) is a commonly experienced phenomenon defined as a reduction in voluntary muscular force that results from intense or prolonged exercise (Enoka, & Duchateau, 2008). EF is a complex experience that is influenced by peripheral and central factors. Central fatigue refers to biochemical changes that occur proximal to the motor neuron and leads to an attenuated response in neural excitation causing physiological and psychological impairments in exercise performance, while peripheral fatigue involves the motor unit, and occurs strictly via depletion of the muscle energy supplies (Wan et al., 2017). The extent to which peripheral and central processes contribute to fatigue is highly related to the exercise task performed. Typically, peripheral fatigue emerges quickly during maximal contractions of a single muscle group, while central mechanisms become a limiting factor when the duration of exercise increases (Wan et al., 2017). A study conducted by Thomas et al. (2015) demonstrated this concept by comparing levels of fatigue during self-paced, fixed-distance cycling tasks. Researchers determined that peripheral fatigue was highest following a 4-km TT, the shorter, high-intensity task, while more central fatigue emerged following a long, submaximal 40-km TT. Interestingly, Amann et al., (2011) propose the idea that peripheral muscle fatigue following high-intensity endurance exercise never exceeds an individual's critical threshold because these tasks are regulated by a "centrally mediated" restriction that prevents excessive homeostatic disruption. This arises from the finding that the level of peripheral fatigue incurred during exercise does not depict the muscles' ultimate limit, suggesting that exercise is controlled centrally to preserve muscular capacity or reserve functionality, even at exhaustion or at voluntary termination of exercise.

Although the central and peripheral origins of fatigue are not fully elucidated in youth, significant differences in levels of EF have been successfully recorded between adults and youth during exercise. Current literature shows that children typically experience less peripheral fatigue and more central fatigue than adults, regardless of the muscle group employed (Taylor et al., 2016). This has been attributed to a greater relative energy contribution derived from oxidative rather than anaerobic sources during high-intensity exercise, combined with a potentially greater proportion of fatigue-resistant slow-twitch muscle fibers seen in youth (Wan et al., 2017). This concept was demonstrated by a study that measured fatigue development in youth compared to trained and untrained adult endurance athletes during isometric and isokinetic maximal voluntary contractions as well as during whole-body dynamic exercises using amplitude changes in electromyography (Bontemps et al., 2019). In general, the results indicated that youth fatigued more slowly than untrained adults and as much as trained athletes. They also developed less peripheral and more central fatigue than adults and, although central fatigue appeared somewhat higher in youth than endurance athletes, both youth and endurance athletes experienced greater decrements than untrained adults. Hence it was concluded that youth (especially young, trained athletes) exhibit more comparable neuromuscular fatigue profiles to trained adult athletes than untrained adults (Bontemps et al., 2019). Several other investigations have also compared the accumulation of fatigue across various major muscle groups between youth and adult populations to conclude that untrained adult’s fatigue much faster than young athletes (Bontemps et al., 2019; Taylor et al., 2016). Muscle typology and exercise type combined with capacity for force production may explain the differences seen between muscle groups between youth and adults (Wan et al., 2017). Therefore, trained adolescent athletes likely exhibit a neuromuscular fatigue profile very similar to those of trained endurance athletes.

2.6.1 Peripheral fatigue

As stated above, during strenuous exercise the force-generating capacity of working skeletal muscle progressively declines; that is, fatigue develops until the task is terminated. Peripheral fatigue refers to changes in the motor units and involves processes associated with cellular and mechanical alterations in the muscular system (Thomas et al., 2015). Basically, peripheral fatigue is determined by the type and intensity of the exercise performed and occurs chiefly through exhaustion of the muscle energy supplies, which results in the accumulation of lactic acid and other metabolites within the muscle (Poole et al., 2016). Research suggests that this is attributed to problems with neuromuscular transmission down the sarcolemma, calcium release and uptake, and actin-myosin cross-bridge interactions (Wendt et al., 2007). Additionally, this pathway is shown to interact with cardiovascular and ventilatory responses to exercise. Essentially, exercise-induced increases in ventilation and peripheral hemodynamics promote oxygenation and muscle perfusion which together assure that oxygen demand and delivery are matched in working muscles (Bontemps, 2019; Wan et al., 2017). Important in this context is the fact that muscle blood flow and oxygen delivery depict key components in the rate of development of peripheral fatigue during exercise. Consequently, the circulatory and ventilatory responses to exercise play a role in adequate muscle blood flow and oxygen delivery thereby preventing premature fatigue of the contracting muscle groups (Thomas et al., 2015).

Peripheral fatigue has been quantified using electromyography (EMG), rate of perceived exertion (RPE), and biomarkers. For example, publications indicate that fatigue can be measured using surface electromyography (sEMG) during many types of movements (Beretta-Piccoli et al., 2015). Surface EMG assesses muscle function by recording neuromuscular activity from the surface of the skin above the working muscle. Fatigue is known to be reflected in the EMG signal as an increase in its amplitude and a decrease in its characteristic spectral frequencies, specifically during constant load tasks (Enoka & Duchateau, 2008). The main limitation associated with sEMG-based indexes is their lack of sensitivity in differentiating between central and peripheral aspects of fatigue during variable activities (Beretta-Piccoli et al., 2015). Thus, more sensitive parameters should be developed to study sEMG signals during dynamic fatiguing contractions.

2.6.2 Central fatigue

Central nervous system (CNS) fatigue, or central fatigue (CF), is a form of fatigue that is defined as a reduction in the ability to voluntarily activate a muscle during exercise due to a decline in motoneuronal output (Enoka, & Duchateau, 2008). Current literature presents the idea that neural feedback from fatiguing muscles is a key determinant of endurance exercise performance yet the methods that have been used to assess central fatigue are limited and do not appear sensitive enough to detect failure in central drive (Amann & Dempsey, 2008). Findings suggest that afferent muscle neurons alter endurance performance via two pathways (Taylor et al., 2016). Firstly, neural feedback might enable exercise performance to prevent premature fatigue by optimizing muscle oxygen delivery via its influence on circulation and pulmonary ventilation (Taylor et al., 2016). Second, neural feedback may limit exercise performance via restricting central motor output to muscles at both reflexive and cognitive levels (Taylor et al., 2016).

To explain, during exercise an afferent feedback response occurs where a signal, mediated by intramuscular by-products, is sent from the working muscle to the brain (CNS). This signal is then transmitted back down the spinal cord to the peripheral motor neuron pool, which creates a process known as central motor drive (Amann & Dempsey, 2008). Central motor drive is a mechanism that acts to improve muscle performance by regulating peripheral fatigue development and by avoiding excessive muscle impairments (Amann & Dempsey, 2008). Essentially, this central projection reflexively augments circulation, ventilation, and muscle activation for exercise maintenance. However, when central fatigue is present, the muscles' afferent response time at the spinal level becomes altered, progressively reducing muscle activation. Research shows that the muscle spindle reflex pathway is typically recruited 30% sooner in a fatigued muscle when compared to non-fatigued (Biro et al., 2007). Since various excitatory and inhibitory inputs on the spinal motoneurons recruit or de-recruit motor units (MU) based on motor neuron size during exercise, slowing or cessation of MU firing contributes to the loss of muscular force that marks fatigue (Amann & Dempsey, 2008). Hence, CF likely presents as a result of a decrease in the size of the excitatory input to the motor neuron, an increase in cortical inhibitory input to the motor neuron, and a decrease in the responsiveness of the motor neuron itself (Biro et al., 2007). Furthermore, CF is associated with changes in the synaptic concentration of neurotransmitters within the CNS which affects exercise performance and muscle function (Wan et al., 2017; Zając et al., 2015). Central fatigue can occur from prolonged exercise and is associated with neurochemical changes in the brain, primarily involving serotonin (5-HT), noradrenaline (NA), and dopamine (DA) (Zając et al., 2015). Existing experiments show that an increase in synaptic DA is strongly ergogenic, while an increase in serotonin or NA can impair exercise performance (Zając et al., 2015).

Central fatigue has been assessed by measuring isometric or isokinetic force output (using a dynameter), absolute power output, and average power output (Kent-Braun, 1999).

Despite controversy, investigations indicate that voluntary muscle activation usually diminishes during maximal voluntary tasks due to a decrease in motor unit firing that presents with CF, this is why an activity such as a maximal hang grip test or quadricep contraction may offer a pure measurement of CF (Gavel et al., 2019). Likewise, CF may also be estimated from sEMG recordings. This was exemplified in a study that had riders' cycle to exhaustion in the heat and under thermoneutral conditions (Nybo & Neilsen, 2001). Results from this investigation indicate that exercise in the heat was specifically marked by a lower percentage of voluntary muscle activation when compared to TN temperatures, which implies that CNS fatigue forms as a factor of exercise length, intensity, and environment strain (Taylor et al., 2016). This finding was further supported by the idea that increased Tc, typical of exercising in the heat, may further inhibit the brain's ability to provide adequate neural drive to the muscles, and this may be the explanation as to why people fatigue faster when a higher body temperature is reached (Kent-Braun, 1999; Nybo & Neilsen, 2001). This suggests that the thermoregulatory system plays an important role in exercise performance. Overall, fatigue is a very complex concept, involving both psychological and physiological factors. Consequently, fatigue cannot be viewed as an individual process, but rather as a highly complex phenomenon comprised of different components that act at many sites within both the central nervous system as well as the muscle cells to dictate athletic capacity (Amann et al., 2008; Taylor et al., 2016; Zając et al., 2015).

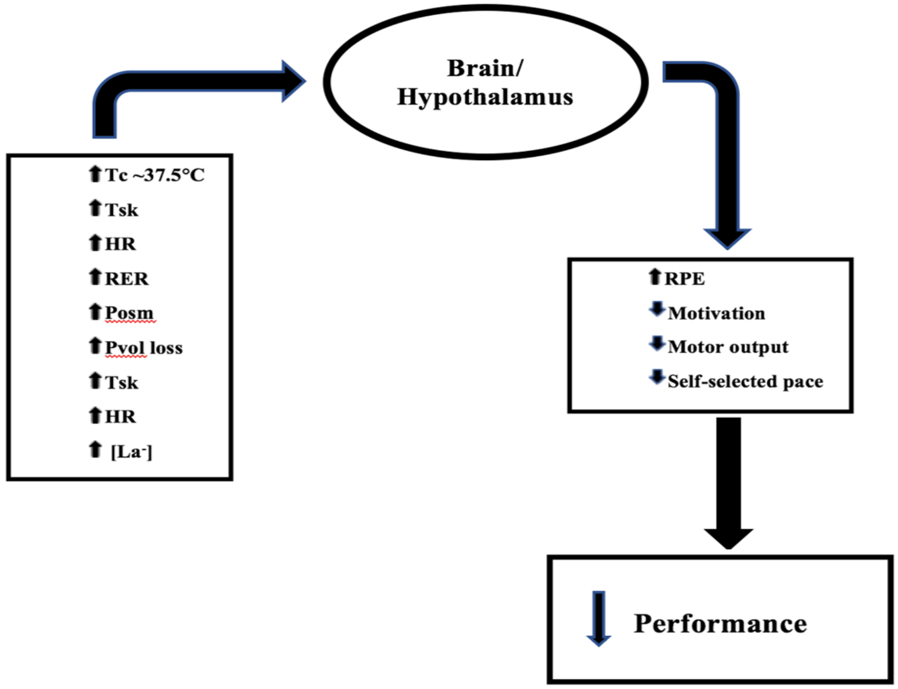
2.7 Central governor theory

The central governor theory (CGT) is proposed to be a central nervous system mechanism that takes information regarding metabolic needs, physiological states, and various motivational drives to regulate physical exertion and protect an organism from catastrophic failure (Noakes et al., 2004). Irrespective of intensity, duration, or biological state (Tucker et al., 2006), the central governor model proposes that the subconscious brain adjusts power output by modulating motor unit recruitment to safeguard homeostasis (Weir et al., 2006). Running, cycling, and rowing races provide substantial evidence that athletes ‘pace’ efforts in anticipation of expected difficulty and surrounding environmental conditions.

It has been said that during maximal exercise the subconscious brain will signal the conscious brain of increasing physical strain, which can then lead to an alteration in pacing strategy during prolonged strenuous activity. This is said to allow for the completion of a task in an efficient and safe manner. Interestingly, this phenomenon has also been observed during open-ended exercises such as VO_{2max} tests (Noakes, 2004). Research shows that when told to cycle at maximal capacity for as long as possible, athletes often show a greater decrease in voluntarily chosen power output. Relative to the central governor theory, this suggests that the body slows down to maintain homeostasis. Furthermore, pace is influenced by variables related to the rate of heat accumulation (Tucker et al., 2004). In a study conducted by Matsuura et al. (2015), a comparison between 10-sec repeated cycling sprints under hot-dry and TN conditions showed a significant decrease in average power output under the hot-dry condition versus the TN condition. These results suggest that athletic performance is intrinsically linked to perception and anticipation of internal and external variables. Finally, and perhaps most noteworthy is work done conducted by Kay et al. (2000) which showed that when participants are instructed to complete a 1-minute maximal sprint at 10-minute intervals during a 60-minute self-paced cycling event in a hot environment, power output and the integrated electromyography signals declined during sprints 2 thorough 5 when compared to sprint 1. However, during the 6th and final sprint, subjects were able to re-adopt the power output and associated EMG signal to near sprint 1 values. This data was then interpreted to imply that efferent drive is subconsciously controlled by a mechanism which preserves muscle functionality during sprints 2-5 as part of a regulatory process that limits physiological damage or prevents premature fatigue during exercise. This is supported by the increase in power and EMG activity during the final effort when it is known that the exercise will be terminated shortly.

Although it seems logical for performance outcomes to depend on a balance between (1) physical preparedness and biological processes, (2) emotional characteristics, including pain tolerance and motivation, and (3) mental awareness or self-preservation, the inherent mechanisms which support this theory do not appear to function quite as cohesively outside of theory. Despite being attractive, this hypothesis has become highly controversial in exercise physiology, with increasing demand to abandon it altogether (Inzlicht & Marcora, 2016). This has arisen from a lack of evidence to support the idea that important homeostatic processes can be regularly overturned by incentives and implies that other functions are responsible for fatigue during activity. Additionally, even though the central governor is thought to use information about the metabolic needs of the body, there continues to be no credible evidence that mental effort utilizes inordinate amounts of energy that are not already circulating in the brain. Finally, more recent modifications of the CGT imply that the model functions as an all-knowing homunculus and therefore is unfalsifiable in principle. Hence, the CGT has contributed very little to the current understanding of why people disengage from effortful tasks over time (Inzlicht & Marcora, 2016).

Figure 2. A schematic of the "central governor theory" (Gavel et al., 2019)



2.8 Serotonin Hypothesis

To better explain the process by which individuals disengage from activity or fatigue over time, researchers have moved towards a concept supported by the neurochemical relationship between physical exertion and central fatigue. This idea is founded on the idea that the central nervous system (CNS) is linked to mechanisms that control motor drive and motivation (Nybo, 2007). While the exact mechanism remains unknown, research suggests that central fatigue can be influenced by inhibitory signals sent to the hypothalamus and via neurotransmitter networks affecting brain activity (prefrontal, lateral, orbitofrontal, and anterior cingulate cortexes), specifically the serotonergic and dopaminergic systems (Pitsiladis, Strachan, Davidson & Maughan, 2002), during conditions of exercise-induced heat-stress (Nybo & Nielsen, 2001). This information has led to the replacement of the CGT with a concept known as the serotonin (5-HT) or central fatigue hypothesis. This hypothesis suggests that an exercise-induced increase in the ratio of serotonin to dopamine within the brain causes feelings of exhaustion, whereas a lower ratio improves motivation and helps to improve or maintain performance over time (Meeusen et al., 2006). The premise of this hypothesis was developed upon the knowledge that serotonin is a chemical messenger that influences feelings of tiredness and lethargy while also having the ability to modulate mood, emotion, sleep, appetite, control, and numerous physiological functions (Nybo & Nielsen, 2001). On the other hand, dopamine is a messenger that is suggested to correlate with feelings of motivation, memory, reward, and attention meaning it has been linked to movement and cognitive pathways (Meeusen et al., 2006). For example, research involving running rats shows that serotonin increases during acute exercise and remains high at the point of fatigue, while dopamine is marked by an increase followed by a steep drop once exhaustion sets in. This combined response is predicted to be important in the fatigue process (Meeusen & Watson, 2007).

Further, serotonin and dopamine synthesis and transportation into the brain largely depend on the peripheral availability of the amino acids tryptophan (TRP) and tyrosine (TYR). Work conducted over the last few decades has focused on the possibility that pharmacological manipulation of these circulating neurotransmitter precursors may delay the onset of fatigue (Meeusen & Watson, 2007). While there is evidence to suggest that TYR (used to elevate brain DA) ingestion can influence perceived exertion and some measures of mental performance, the results of several laboratory studies have not yet demonstrated a clear positive effect on exercise capacity or performance (Meeusen & Watson, 2007). Hence, nutritional manipulation of these systems has proven largely unsuccessful.

While the cause of fatigue is undoubtedly complex, serotonin and dopamine may not be acting alone. Additional work to support this hypothesis arose from investigations that studied physiological responses to amphetamines (Meeusen et al., 2006). Amphetamines are CNS stimulants that broadly function to increase catecholamine (CAT) release and inhibit reuptake (of dopamine, epinephrine, and norepinephrine). While manipulation of central catecholamines using amphetamines is not a reasonable solution to improving exercise performance, increasing the circulation of these stress molecules does appear to influence exercise capacity, especially in the heat. This may be because increases in CAT concentrations play a role in the acute expression of strength and power – chiefly demonstrated by the "fight or flight" response. This is thought to occur because these hormones act as central motor stimulators and peripheral vascular dilators that enhance enzyme systems to increase calcium release in muscles (Messan et al., 2017; Zouhal et al., 2008). It has been reported that concentrations of CAT can increase by approximately 1.5 to >20 times basal rates depending on exercise characteristics and intensity (Zouhal et al., 2008), with norepinephrine increasing at a much faster rate than epinephrine. This exponential increase activates the CNS and ultimately evokes cardiovascular and respiratory adjustments that are shown to play a role in enhanced performance (Messan et al., 2017). Additionally, it is known that variability in CAT concentrations, specifically norepinephrine, at a constant volume of oxygen consumption during exercise is intrinsically related to characteristics including physical training status, sex, age, and the emotional state of an individual (Zouhal et al., 2008). This implies that differing CAT concentrations could be moderately responsible for performance differences observed between men and women or between trained and untrained athletes (Zouhal et al., 2008). An experimental investigation conducted by Messan et al (2017) measured catecholamine levels in 86 professional cyclists prior to and following a maximal effort exercise test. Findings from this study indicate that increased CAT secretion during physical activity helped to prevent bronchospasm and aided in the preservation of performance capacity.

Finally, as serotonergic and catecholaminergic systems innervate areas of the hypothalamus (thermoregulatory centre), the activity of these neurons is expected to contribute to the control of body temperature and perceived exertion (Meeusen & Watson, 2007). For example, work by Soares, Lima, Coimbra, and Marubayashi (2004) used rats to demonstrate that an injection of TRP, the serotonin precursor, triggered a rise in core body temperature and resulted in a subsequent decline in performance. From the alternate perspective, work by Watson et al. (2005) demonstrated that a dopamine/noradrenaline reuptake inhibitor (bupropion) allowed male cyclists to maintain higher power output in the heat with the same perception of effort and thermal stress reported during the placebo trial, despite the attainment of a higher core temperature. While uncertainty still exists, a summation of evidence does offer the idea that mediating changes in the ratio of serotonin to dopamine may influence pathways that affect central fatigue. While little is known about how this can be done on a human model, this hypothesis brings forward novel information about the ability of neurotransmitters to act on pleasure or reward centers of the brain to benefit voluntary motor functions.

2.9 Measures of Function

2.9.1 Rating of perceived exertion

The Borg rating of perceived exertion (RPE) scale is commonly used to evaluate and regulate exercise intensity (Borg, 1982; Tucker et al., 2006). According to the central governor theory, RPE is also used to prevent homeostatic failure during intensive exercise (Weir et al., 2006). RPE is based on the physical sensations a person experiences during physical activity, including increased heart rate, increased respiration, increased sweating, and muscle fatigue (Tucker et al., 2006). Although this is a subjective measure, it is shown to provide a good estimate of actual heart rate during exercise (Borg, 1982). Factors that influence RPE are thought to be multifaceted and highly interconnected with perceptual, peripheral, and environmental sensory cues (Hampson et al., 2001). For example, level of exertion is understood to be generated by input from local physiological factors (skin, muscles, and joints), central physiological factors (pulmonary and cardiovascular organs), and psychological factors (conscious thoughts) (Hampson et al., 2001).

The RPE scale continues to be the most well-known method utilized (Borg, 1982; Muyor, 2013). The original 6-20 Borg scale was developed in healthy individuals to correlate with exercise heart rates (e.g., RPE 15 would approximate a HR of 150 bpm), and enables subjects to better understand terminology (Borg, 1982). Since then, this scale has been revised to include a category-ratio 0-10-point scale which has been modified to record symptomatic breathlessness. Both scales estimate the linear relationship between perceived intensity, HR, and oxygen consumption during exercise, but the 0-10 version has specifically shown high reliability and validity in healthy athletic populations (Chen et al., 2002; Muyor, 2013). For example, a study conducted by Herman et al. (2006), had cyclists and runners perform six randomized 30-minute constant-load exercise bouts at easy, moderate, and hard intensity. Results demonstrated a strong and significant correlation between variables and no significant differences between test and retest values of $\%VO_{2peak}$, $\%HR_{peak}$, and RPE ratings (easy: 47 vs. 47%, 65 vs. 66%, and 2.0 vs. 1.9; moderate: 69 vs. 70%, 83 vs. 84%, and 4.2 vs. 4.3; and hard: 81 vs. 81%, 94 vs. 94%, and 7.3 vs. 7.4 bouts of exercise).

A meta-analysis published in 2002 indicates that the strength of the relationship between RPE and physiological measures such as heart rate, blood lactate concentration, percent maximal oxygen uptake (%VO_{2max}), oxygen uptake (VO_2), ventilation and respiration rate is highly dependent upon the sex of participants, fitness level, type of RPE scale used, type of exercise, exercise protocol, and study quality (Chen et al., 2002). This analysis indicated that validity may not be as high as previously thought (r = 0.80-0.90), except under certain conditions, such as when used with cycling or swimming (Chen et al., 2002). With that in mind, a review published in 2017 assessed 36 studies that examined the validity and reliability of this method using the modified CR-10 scale (Haddad et al., 2017). These studies confirmed the validity, reliability, and internal consistency of the Borg RPE method across different sports and physical activities with men and women of different ages (children, adolescents, and adults) across various sport experience levels (Haddad et al., 2017). It was concluded that this can be used as a stand-alone method for determining exercise load, although it is recommended to combine it with other physiological parameters (Haddad et al., 2017; Soriano-Maldonado et al., 2014). Despite these findings, several sport-specific investigations tend to report that RPE offers a more practical value for measuring and prescribing exercise intensity, however, athletes with less experience with RPE scales may benefit from a learning-based protocol (Soriano-Maldonado et al., 2014).

2.9.2 Thermal scaling

Thermal scales have been widely used to assess subjective experiences of thermal conditions within built environments. In general, thermal scaling can be divided into two categories, “thermal sensation” (TS) and “thermal comfort” (TC) and refers to the use of a numerical rating system capable of predicting sensations evoked by thermal stimulation. Thermal scales often depict thermal stimulation as a conscious feeling that can be graded into seven categories, ranging from cold to neutral to hot (Velt & Daanen, 2017).

Of the existing scaling models, 7-point thermal sensation (TS) and thermal comfort (TC) scales continue to be the most common methods used in unison within research-based settings (Karjalainen, 2012). This is related to the underlying assumption that TS is a one-dimensional sensory experience that can be used for the sufficient description of a state of mind or sensations (TC) within the physical environment. More specifically, TS is recognized as the subjective perception of ones' state (ASHRAE, 2004), while TC is defined as the subjective evaluation or condition of mind that expresses satisfaction with the thermal environment (Fanger, 1970). From the viewpoint of heat transfer, TS is largely understood to be a psychological response to the state of thermoreceptors within the body (e.g., *you feel* 'slightly warm'), while TC may be predominantly influenced by contextual factors that dictate heat exchange with the environment (e.g., *you find it to be* 'comfortable'). Although seemingly straightforward, a longstanding criticism in the assessment of thermal scaling is that the relationship between TS and TC would actually benefit from multidimensional conceptualization.

At present, ASHRAE scales, based on Fanger's model (1970), are the most cited in thermal standards. This model is most applicable to uniform environments where ratings can be mediated by four environmental parameters (air temperature, radiant temperature, humidity, and air speed), and two personal parameters (clothing and activity level, or metabolic rate) (ASHRAE, 2004). This makes their use seem overwhelmingly appropriate across different contexts yet, it should be noted that thermal scales were not originally developed for use during exercise, but instead have been adapted to suit this purpose (Koelblen et al., 2016). Not only does this raise concerns about the validity and reliability of models, but also has led to inconsistencies and challenges for interpretation.

For example, an investigation conducted by Schweiker et al. (2016) identified that people typically do not perceive the categories on the scale as equidistant sensory experiences and therefore have a tendency to report different intensities of sensation for the same exposure condition. This discrepancy becomes even more pronounced during maximal bouts of prolonged exercise in a thermally demanding environment. Other individual factors including personality traits, sex, age, and body mass index (BMI) can all cause inter- and intra-individual variation in subjectively reporting TC and TS. Consequently, ratings given on any scale should be interpreted with respect to not only differences between individual preferences of thermal conditions but also individual differences in the interpretation of the scale provided (Schweiker et al., 2016).

Another example of the lack of validity in thermal scaling can be observed from a preliminary model by Gagge et al. (1967). This research showed that differences in TC and TS are not perfect correlates for increased core and skin temperatures, and that the verbal descriptors used to explain sensations can greatly influence a participant's number rating. This was further supported by a study conducted by Flouris and Cheung (2009) which showed that during exercise under hot conditions, differences in thermal sensation and thermal comfort were not observed, while an increase in core and skin temperature was present. Again, this contradicts existing empirical findings in the sense that increasing Tc should parallel increased reporting on thermal scales, thus demonstrating that their use may be unreliable during exercise protocols (Flouris & Cheung, 2009). With this information in mind, the use and interpretation of thermal scaling should be approached with caution.

Despite the information presented above, it is reasonable to discuss the pertinence of continuing to use thermal scales in exercise-based research, specifically investigations involving non-thermal cooling interventions, such as menthol. This is largely related to the fact that when administered, menthol does not prevent heat gain or reduce Tc, but instead, aids in athletes feeling perceptually cool or fresh and being able to perform for longer or at a higher power output (Crosby et al., 2022). Moreover, as long as a critical Tc (≥40°C) is not reached and other heat illness factors are minimized, improvements in thermal stimulation, specifically TC, have shown to enhance performance independent of an athlete's thermal state (Crosby et al., 2022). For example, Gibson, et al. (2019), reported improvements in TC in a study that compared the effects of menthol to capsaicin, placebo, and control rinses during repeated supramaximal sprints under laboratory conditions. Although performance was not altered via menthol, these findings reinforced the significance of perceptual alterations in exercise-based heat studies involving menthol. On the contrary, Riera et al. (2014) reported no changes in perceptual measures (TS, TC, or RPE), despite measuring substantial performance improvements when menthol was added to beverages that were administered at neutral, cold, and iced temperatures throughout a 20-km time trial. This finding highlights the idea that perceptual changes may become negated once improvements in performance reach a particular physiological threshold. As indicated above, there have been a relatively equal number of menthol studies published that have observed lower thermal perceptual measures compared to those who have reported no change at all. Independently, it is known that menthol can induce elevated localized sensations of oral cooling which can impart acute improvements in TC and TS but in contrast, TC and TS may remain unchanged because of increased heat storage and presumably metabolic heat production from ATP degradation during prolonged exercise in the heat (Crosby et al., 2022). Hence, further investigation into the use of perceptual scaling is warranted during exercise investigations involving menthol.

2.9.3 Rating of Fatigue

As indicated in sections above, fatigue has been described as a ubiquitous, complex, and multifactorial phenomenon that is often defined based on the dichotomy between its peripheral or central origin. Although this separation continues to elucidate valuable information in exercise and performance-oriented contexts, it does little to consolidate the theoretical understandings of the mechanisms, causes, and prevention behind fatigue and its effects. With that in mind, a recent distinction has been made between fatigue, described as a subjective sensation, and fatigability, identified as the objective change in physical or motor performance. While past investigations have attempted to illustrate the directional relationship between perceptual (fatigue) and physiological fatigue (fatiguability) within various contexts, perhaps more useful advancements to sports research can arise from acknowledging the interactive psychophysiological nature of fatigue.

Past approaches to measuring perceptions of fatigue often utilized instruments and scales designed for specific populations or diseases, like elders or cancer patients, making their use impractical in situations involving concentration, skilled motor tasks, or even exercise. While these validated scales are pragmatic and clinically significant, they are not generalizable and therefore do not provide global progress towards a common understanding of fatigue. Alternatively, researchers have now proposed to measure fatigue using a general scale that quantifies the intensity of the subjective feeling state during an activity, regardless of qualitative variations in the actual feelings of fatigue. This measurement is not intended to disregard qualitative differences in fatigue, but rather offer the potential to both identify and better understand the interactions between fatigue, fatiguability, and behaviours that are common to a variety of situations.

The rating-of-fatigue (ROF) scale is one of the newest developments in holistic fatigue monitoring and was constructed with the intent of providing researchers with a universal method for measuring perceived fatigue. The scale consists of 11 numerical points ranging from 0 to 10 that describe an individual's state of feeling to be "0 - not fatigued at all" all the way to "10 - totally exhausted – nothing left" (Micklewright et al., 2017). These descriptions are presented in combination with simple diagrams, making this scale viable for a wide range of users. In practice, this scale offers a simple yet effective way of tracking sudden changes in subjective perceptions of fatigue intensity during exercise, as well as slower changes across a longer timeframe in various contexts. While in theory, this scale offers valuable insight into intra-individual and situational variations that may further the scientific community's understanding of the relationship between fatigue, fatigability, and the inherent limits to athletic performance.

Within the context of this thesis, it is relevant to discuss that although the ROF scale appears similar in nature to the rating of perceived exertion scale (BORG), key distinctions have been made. First, a perceived exertion scale operates on the definition of "how hard or laborious the physical task feels", while the ROF scale encourages individuals to rate "how diminished their [mental and physical] capacity is in completing the task". In this respect, perceptions of exertion and fatigue should correlate highly during an exercise task (i.e. ROF=10, RPE=20), but once exercise terminates, perceived exertion should immediately drop to its lowest point on the scale (approximately 6) whereas perceived fatigue is expected to gradually diminish over time (Micklewright et al., 2017). Moreover, it can be argued that the ROF scale offers distinct information from the RPE scale in the sense that it allows users to incorporate the impact that either real or imagined stressors within the physical environment have on fatigue intensity, rather than independently measuring the intensity of the "training load" during the task itself. With that in mind, ROF can be viewed as a continuous construct that is experienced at all moments in time (i.e., work and recovery periods) with consistent correlation to other physiological markers, while RPE acts as a discrete construct that only exists to quantify episodes of physical work (Micklewright et al., 2017).

As of late, research indicates that not only does the ROF scale present with good face validity, but it also has a high degree of convergent validity during incremental or ramped cycling to exhaustion tasks, resting recovery, and during daily living activities. Micklewright et al. (2017) conducted a series of experiments that allowed for the development and validation of the current ROF scale. The first investigation was utilized to provide the evidential foundation for the construction of the scale. To do this, eighteen male participants performed a graded cycling test to volitional exhaustion followed by 30-min of rest while being asked to rate their level of fatigue on a general 11-point numerical scale as well as select written descriptors and diagrams that best represented how they felt. This empirical evidence allowed for the alignment of numerical, descriptive, and diagrammatic components of the ROF. Experiment two was then conducted to investigate the face validity of the newly derived ROF scale by asking participants (n=103) to rate what they thought the ROF scale measured using both open and closed questionnaire methods. Third, the convergent validity of the ROF scale was measured during a ramped cycling protocol followed by resting recovery (n=20). And finally, the scale was validated using a 7-day longitudinal study (n=50) which looked at daily and weekly living activity cycles. Results from these investigations indicate that the ROF scale offers a high level of both face and convergent validity, as well as extends theoretical and applied potential in understanding changes in fatigue in a variety of contexts (Micklewright et al., 2017). Upon considering the dynamic and exhaustive nature of the M-VCT, utilizing the ROF scale as a valid descriptor of perceived fatigue within an exercise context offers novel insight into understanding the limits to performance capacity in trained athlete populations during race-like simulations.

2.9.4 Feeling Scale

Affective response is broadly defined as the general psychological state of an individual, including but not limited to emotions and mood, within a given situation. When considered in an exercise context, affective response is known to be dynamic; frequently fluctuating between positive and negative valence (pleasure/displeasure) based on a multitude of factors including exercise intensity, duration, and individual characteristics (Magnan et al., 2013; Parfitt & Hughes, 2009). For example, the relationship between exercise intensity and affective response has been modelled as an inverted-U curve. This implies that mid-range exercise intensities should result in optimal affective changes, whereas intensities that are 'too low' or 'too high' are less effective (Ekkekakis et al., 2011). Although this model has been criticized for providing an overly simplistic view of the relationship, it does adequately express the idea that variable affective response during an exercise task can influence motivation levels and therefore dictate performance outcomes. Still, it should be recognized that athletes may not respond uniformly in terms of affective response to the same repeated exercise stimulus. This is suggested because the relationship between exercise and affective response is likely an active process, where internal (e.g., ventilation, muscular strain) and external (e.g., environment, distractions) factors can interact to influence perceptions at any given point (Ekkekakis et al., 2011; Hardy & Rejeski, 1989). This also means that affective response to a particular exercise stimulus can evolve with time or repetition. Based on an exposure effect, affective response can either become more positive over time due to increased familiarization or comfort, or a negative shift may occur as participants become bored with increased repetition to a particular stimulus (Ekkekakis et al., 2011). Despite the dynamic nature of affective response, exercise-induced increases or decreases in pleasure likely contribute to the formation of behavioral and memory tendencies during exercise tasks.

The feeling scale (FS), an 11-point rating scale, ranging from (I feel) 'very good' (+5) to 'very bad' (-5), has been used across the literature to assess and describe changes in affective valence during exercise (Hardy and Rejeski, 1989). Past investigations have predominantly focused on the role that affective response has in exercise program compliance, clearly indicating that self-determination of exercise intensity and duration illustrates higher affect and improves motivation in completing exercise tasks while any prescribed form of 'high-intensity training' typically evokes a higher degree of negative affect and an avoidant response for participation in future sessions (Frazao et al., 2016; Magnan et al., 2013). Previous work by Parfitt & Eston (1995) examined differences in rating of perceived exertion (RPE) and affect (FS) during cycle ergometry in a steady state (60% VO_{2max}) versus a high-intensity exercise condition (90% VO_{2max}) in active and inactive individuals. Results from this investigation showed that RPE increased for both intensity levels, but there was a greater increase in RPE during the 90% than the 60% work rate. Rated feeling (FS) was more positive for both groups during the 60% work rate sessions, and although affect declined significantly during the 90% work rate sessions, active subjects remained more positive than inactive subjects. Conclusions from this study present the idea that despite similar trends in affective response during a demanding exercise task, active individuals appear to be more accustomed to the discomfort of high-intensity training and therefore can maintain a higher affect, for longer than those who are not trained. Another study investigated the influence of cycling cadence (rpm) on affect (FS), perceived exertion (RPE), and physiological responses in 15 males when performing a 30-min constant workload (50% of peak power) test at either 60 rpm or 100 rpm (Agricola et al., 2017). Findings indicate that pleasure (FS) was higher during the later portion of the exercise task when participants cycled at 60 rpm, whereas RPE, heart rate, and oxygen uptake were lower. It was also found that the rate of decrease in pleasure and increase in RPE was less dramatic when cycling at 60 rpm compared to 100 rpm. In this regard, it was assumed that the improved affective response at a lower cadence would correlate to better exercise adherence behavior.

Despite a common trend indicating that a more positive affective response during low-to-moderate intensity exercise tasks is associated with improved task compliance, little is known about the influence that affect has on performance in elite level athletes. Unsurprisingly, it appears that trained athletes are just as likely as untrained athletes to report low or declining affective valence during high-intensity tasks when compared to low-intensity steady-state tasks despite repeated exposure. However, the difference may be that despite affective changes, trained athletes appear to maintain performance standards for longer. It is also likely that increased vigor and reduced fatigue typical of trained athlete profiles, correlates to a reduction in mood disturbance during demanding tasks, therefore improving affective balance towards the end of high-intensity tasks (Magnan et al., 2013; Parfitt & Hughes, 2009). While theoretical work by Ekkekakis and Acevedo (2006) highlights the complexity and multifaceted nature of affective responses to exercise, suggesting that it is continuously driven by a variety of underlying biological, cognitive, and social mechanisms, less is known about the potential relationship between fatigue, decreased affective responses and improved performance outcomes in trained athletes. With this information in mind, there is novelty in investigating the relationship between affective ratings and performance in trained athletes during high-intensity tasks.

2.10 Physiological Measurements

2.10.1 Core Temperature

Core body temperature (Tc) is the optimal state at which internal organs and bodily systems function. Humans possess different physiological factors that contribute to the variability of a healthy core temperature, including basal metabolic rate, physical conditions, the ingestion of medications, and exercise-induced stressors (Osilla et al., 2020). When core temperature approaches either the upper or lower limit, the body will take corrective measures through thermal regulation. If the body enters a hypothermic state, the hypothalamus will initiate a reduction in the volume of blood circulating near the surface of the body in order to retain a greater volume of warm blood near the internal organs (Transey & Johnson, 2015). Conversely, if core temperature reaches hyperthermic conditions, the opposite occurs, and warmed blood is directed towards the surface of the skin to promote its cooling (Transey & Johnson, 2015).

In general, Tc increases during continuous exercise because about 80% of the energy produced through muscular contractions is converted to heat and therefore increases core temperature (Osilla et al., 2020). Accurate and convenient measurement of internal body temperature is an essential component of exercise-based research and observing core temperature change is a critical diagnostic tool for preventing exertional heat stroke, measuring environmental acclimatization, and monitoring various medical conditions in athlete populations (Transey & Johnson, 2015). When measuring temperature changes three vital components must be considered: (1) ease of measurement, (2) the external environment must not influence the measurement, and (3) the measurement must remain consistent and accurate throughout the exercise and cool-down period (Bongers et al., 2015).

Currently, rectal and gastrointestinal (GI) temperature monitoring systems are the most accurate internal body temperature assessment methods that have been applied in sports research and medicine (Gagnon et al., 2010). On the contrary, research suggests that peripheral thermometers such as the oral, skin, temporal, aural, and axillary measures result in a high degree of variability and therefore should not be used to evaluate the internal temperature of an individual during exercise (Casa et al., 2007). Rectal thermometry is considered the 'gold standard' method for temperature assessment during exercise. This technique involves the use of a rather invasive and slightly uncomfortable malleable probe. It also presents a challenge in the sports research setting as wires between the thermistor and the measuring device can easily become entangled in moving limbs (Buono et al., 2007). Some studies involving cycling or running in hot conditions have employed this method (Casa et al., 2007; Ganio et al., 2009; Hosokawa et al., 2017). On the other hand, gastrointestinal thermometry is another method that has been gaining traction among sports researchers (Bongers et al., 2018; Hosokawa et al., 2016). GI thermometry involves a wireless, ingestible telemetric pill that contains a thermistor that transmits internal body temperature readings to a receiver (Bongers et al., 2018; Casa et al., 2007; Ganio et al., 2009; Hosokawa et al., 2016). This less invasive system has demonstrated negligible mean bias (-0.1–0.2°C) when compared to rectal thermometry during exercise and the post-exercise period, meaning that data extracted using this system can be deemed precise and valid (Casa et al., 2007; Hosokawa et al., 2016).

Notably, in exchange for the convenience of wireless measurement, the use of an ingestible thermistor pill is expensive and entails planning to confirm the suitable settlement of the pill within the GI tract. The ingestion of the pill should occur several hours prior to any exercise to minimize the chance of prematurely measuring gastric temperature and minimize the chance of the pill passing through the body (Casa et al., 2007; Ganio et al., 2009; Hosokawa et al., 2016). Furthermore, the ingestion of cold fluids may also influence temperature readings, which can also be impacted by differences in gut motility in athletes (Savoie et al., 2015).

Although methodological limitations are present, the telemetry pill is shown to be a reasonable alternative. A study conducted by Hunt et al., (2017) examined the range in the systematic bias of ingestible temperature sensors compared to a certified and traceable reference thermometer. In this protocol, 119 ingestible temperature sensors were immersed in a circulated water bath at five water temperatures along with a certified traceable reference thermometer. Results showed that using an uncalibrated ingestible temperature sensor may provide inaccurate data that still appears to be statistically, physiologically, and clinically meaningful. Correction of sensor temperature to a reference thermometer by linear function eliminates this systematic bias. Perhaps most applicable is an investigation conducted by Bongers et al., (2018). This was an investigation that examined the accuracy and responsiveness of available ingestible telemetric temperature capsule systems (CorTemp, e-Celsius, myTemp, and VitalSense) through immersion of ten capsules from each system in a temperature-controlled water bath during three trials. Trials 1 and 2 assessed validity and reliability by gradually increasing water bath temp from 33°C to 44°C. Trial 3 assessed inertia by increasing temp from 36°C to 42°C. Results showed that although differences in temperature and inertia were observed between capsule systems, an excellent level of validity, test-retest reliability, and inertia was found for each system between 36°C and 44°C after removal of outliers. When applied in an exercise setting, Lee et al., (2000) analyzed measurements of intestinal temperature (Tin) to esophageal (Tes) to rectal temperatures

(Trec), respectively. During exercise Trec was less than the Tes and Tin at the end of the 40% (Trec: 37.20 ± 0.10; Tes: 37.38 ± 0.11; Tin: 37.35 ± 0.06°C) and 65% VO_{2peak} stages (Trec: 37.63 ± 0.08; Tes: 37.83 ± 0.10; Tin: 37.75 ± 0.05°C). Peak temperature also tended to be different, however, not significant between methods. The results suggest that Tin may be an acceptable alternative to Tes and Trec, but it should be noted that calibration is needed prior to steady-state or intermittent exercise.

Interestingly, recent advancements in technology have allowed for the development of a wearable device that continuously and wirelessly measures core body temperature (CORE, greenTEG, Rümlang, Switzerland). CORE is a Swiss-made thermal energy transfer (TET) sensor which integrates greenTEG's miniaturized, highly sensitive body temperature sensor for the non-invasive measurement of core temperature across a variety of contexts. According to manufacturers, the TET sensor, or heat flux sensor, is a device created on the Seebeck effect – a phenomenon in which a temperature difference between two dissimilar electrical conductors or semiconductors produces a voltage difference between the two substances. When the two conductors or semiconductors are exposed to a variance in temperature, heated electrons flow toward the cooler conductor (high to low temperature gradient). When heated electrons pass through the sensor, this generates a voltage signal proportional to the energy passing through it that can be measured and tracked in real-time. During exercise, heat is either lost or gained between the human body and the surrounding environment, this means that the TET sensor can be placed on the surface of the skin to measure the energy (not skin temperature) that is being transferred from the body to it.

This apparatus allows for the measurement of TET in real time and therefore provides users with a seamless way of monitoring and recording heat flux changes <0.01 W/m^2. The accuracy of the CORE device is described by the manufacturer to be ± 0.26 °C and is measured using the equation:

$$HF = V / S$$

HF = Heat Flux, in W/m2
V = Voltage, in µV
S = Sensor sensitivity, in µV/(W/m²)

Where: a voltage signal, logged by the tracker in the µV range, which is proportional to the heat that passes through the sensor, is converted into the heat flux value by dividing it by the sensor sensitivity.

Due to the novelty of this device, only one study has reported on both the reliability and validity of this lightweight, non-invasive sensor. Verdel et al. (2021) conducted a two-part investigation that compared Tc temperature values indicated by the CORE sensor to corresponding values obtained via rectal thermometry (Trec). During the first investigation, 12 males completed two identical 60-min steady-state cycling tasks in a thermoneutral laboratory (19°C, 30%RH). The second investigation subjected 13 males to a moderate-to-high heat load by performing 90-min of steady-state cycling in a heat laboratory (31°C, 39% RH). Results indicate that CORE has an acceptable level of reliability since the mean bias between temperature-matched trials was not significantly different, but low validity given that significant deviation (poor agreement) in core body temperature values were found between devices. More specifically, analysis showed that approximately 50% of all paired measurements (between CORE and the "gold standard" Trec) differed by more than the predefined threshold for validity of ≤0.3 °C. Concerningly, CORE appeared to overestimate temperature at lower heat loads (36°C to 38.5°C) and then underestimate body temperature at higher heat loads (≥39°C), perhaps when heat-related illnesses are of greatest concern during exercise. Although systematic differences did present between the CORE and rectal systems at specific time points, it should be noted that the total temperature increase during the entire exercise task was not significantly different. Furthermore, a notable limitation of this investigation was the use of a continuous steady-state cycling protocol. Published work by Taylor et al. (2014) indicates that rectal thermometry is precise and acceptable during steady-state athletic tasks, yet it appears to be inadequate, impractical, and far too invasive for certain dynamic events. With that in mind, it should be acknowledged that uncertainties also exist when monitoring core temperature via rectal thermometry for race-like simulations.

Although current findings do not support the manufacturer's claim that the CORE sensor provides a valid measure of core body temperature, CORE has proven to be more accurate than other non-invasive devices (i.e., devices to assess forehead, oral, temporal, aural, and axillary) used in sports (Ganio et al, 2009), which warrants its continued use in exercise research settings. Since each core temperature system appears to present with both benefits and limitations, additional factors including cost, accessibility, practicality, as well as the variability and duration of the exercise task/protocol should be considered before selecting a method for core temperature measurement. While CORE's validity and reliability continues to be investigated via collaborations between clinical researchers and global medical research teams, specific care should still be taken when assessing or monitoring elevated core temperature (above 39.5 °C).

2.10.2 Sweat rate

Thermal status is represented by four physiological parameters, core temperature, mean skin temperature, peripheral blood flow, and sweat rate (Baker, 2019). Sweat rate is related to overall thermal status and is typically studied in relation to factors such as intensity of exercise, secretory capacity of the sweating mechanism, and body temperature (Baker, 2017). Sweat rate measures humidity evaporation rate and sweat generation rate on the skin (Wilke et al., 2007).

This can be quantified simply by calculating the difference in body weight from pre-to-post exercise, measuring fluid intake, and monitoring urine/fecal output (Araki et al., 1979). Although various technological devices are available for this measure, this method provides a cost-effective and useful alternative in exercise research.

Although this method has been used extensively, it is important to recognize that errors can occur that may mislead results. For example, dehydration is the process of losing body water (e.g., during exercise). Dehydration leads to a decreased rate of sweat loss during exercise and can impair the body's ability to control the thermoregulatory process (Cheuvront et al., 2009). Sweating is an important thermoregulatory defense that can prevent overheating during maximal and submaximal exercise bouts. With this information in mind, it is imperative that subjects participating in exercise research are first classified by a state of euhydration (urine osmolarity or USG < 1.020). Additionally, substrate oxidation, endogenous oxidation stores, and changes in the water content in the bladder and gastrointestinal tract (Maughan et al., 2007) can influence results. Hence, research shows that acute body mass losses in response to exercise can represent a close prediction for body water losses. However, the difference between body mass and sweat loss becomes increasingly inaccurate as more energy is used (Cheuvront et al., 2009). Given the information above, it is proposed that sweat rate is calculated using the following equation: *Sweat loss (g)= [change in body weight (g) + fluid intake (g)*] *(*Cheuvront et al., 2009*).*

2.10.3 Blood lactate

Blood lactate concentration ([La^-]) is one of the most common metabolic biomarkers measured during clinical exercise testing as well as during performance testing of athletes. While elevated [La^-] may be indicative of ischemia or hypoxemia, it is also a "normal" physiological response to exertion. With that in mind, lactate is a normal product of glycolysis and glycogenolysis, and it is a precursor for glucose and glycogen resynthesis. Rather than a dead-end metabolite that accumulates as the result of muscle anoxia during exercise and waits until the recovery period to be returned to glucose and glycogen, lactate is actually an important contributor to skeletal muscle energy metabolism. Once formed, lactate can function as an acidic buffer, fuel source, or hormone in a diverse range of processes (Jacobs, 2012).

Through the process of glycolysis, one molecule of glucose is broken down to form two molecules of pyruvate. Depending on the microcellular environment, specifically, oxygen availability and energy demand, pyruvate can (1) in the presence of oxygen enter the citric acid cycle and undergo oxidative phosphorylation to produce 32 ATP per glucose molecule, or (2) in anaerobic conditions pyruvate can be converted to lactate via the enzyme lactate dehydrogenase, providing 2 ATP per glucose molecule. To explain, when oxygen is insufficient, pyruvate will accept a hydrogen ion, a circulating product of the simultaneous process of ATP hydrolysis, to form lactate. It is important to understand that lactate is functioning to buffer hydrogen ions from the bloodstream and return blood pH to electroneutrality, rather than acidifying it.

These processes occur simultaneously when the body is at rest, with more energy being contributed through aerobic glycolysis. This allows the body to stabilize $[La^{-}]$ around 1-2 millimoles per litre (mmol/L). During exercise, the same processes occur but the body begins to shift its reliance away from aerobic metabolism and moves towards anaerobic metabolism. In this case, the proportion of energy contributed from each pathway becomes a factor of exercise intensity, duration, and individual characteristics of the athlete. This can cause lactate to accumulate with concentrations surpassing 20 mmol/L during high-intensity tasks (Goodwin et al., 2007).

Once concentration builds up, the body will employ different mechanisms for clearance. First, systemic lactate can be cleared via the activity of oxidative muscle fibres (type I). These muscles utilize lactate by reducing it back to pyruvate and then using it as a fuel source in the presence of oxygen. Lactate-rich blood can also be shunted to the liver where it will go through the Cori cycle to regenerate glucose, which then can be used in glycolysis. Finally, systemic lactate can also be utilized by cardiac muscle fibres and the brain.

While there continues to be debate surrounding the effects of lactate accumulation, its relationship to fatigue, and how that affects athletic performance, the $[La^-]$ relative to the exercise performed is a relevant marker of exercise capacity. Work by Goodwin et al. (2007) indicates that during maximal exertion tasks lasting 30-120 seconds, peak $[La^-]$ values of about 15–25 mmol/L may be observed 3–8 minutes post-exercise. On the other hand, $[La^-]$ will rise gradually in response to the intensity of a progressive incremental task. The work rate at which $[La^-]$ increases exponentially is known as the lactate threshold. The lactate threshold has been described as a better predictor of performance than VO_{2max} and as a better indicator of exercise intensity than heart rate (Inglis et al., 2019). It is particularly useful in research settings as it correlates with the transition from aerobic to anaerobic metabolism as well as a subsequent decline in affective state during a task, but it can also be used to assess fitness or performance levels, training adaptations, and to assess the effects of a prescribed training intervention (Bonaventura et al., 2015). Although the lactate response to exercise is reproducible under standardized conditions it can be influenced by the site of blood sampling, ambient temperature, changes in the body's acid-base balance prior to exercise, dietary manipulations, or pharmacological interpretation (Heuberger et al., 2018; Jacobs, 2012).

Blood lactate is often measured from a finger prick capillary sample using a portable, handheld analyzer. A recent study by Bonaventura et al. (2015) demonstrated the Edge Lactate Analyzer, among four others, provided sufficient validity and reliability. It was determined that the reliability of common portable blood lactate analyzers was generally <0.5 mM for concentrations in the range of ~1.0-10 mM but increased slightly for concentrations of 15-23 mM. While no analyzer was perfectly accurate, the Edge analyzer demonstrated low bias for [La^-] both above and below 15 mM, making it the preferred device for athlete testing where peak lactate is important for understanding performance capacity (Bonaventura et al., 2015).

2.10.4 Electromyography

Electromyography (EMG) is a diagnostic procedure that evaluates the health condition of muscles and the nerve cells that control them (Turker & Sozen, 2013). EMGs provide information on the muscular electrical activity that occurs during contraction and relaxation phases and reveals insight into what a muscle does at any moment during various movements and different postures (scaling of the intensity and velocity of muscle contraction) (Jorge & Hull, 1986). Currently, two kinds of EMGs are used in research and clinical practice. An intramuscular EMG (iEMG) allows for signals to be detected via inserting needles or wires into specific muscles while a surface EMG (sEMG) assesses muscle function by recording muscle activity via placing electrodes above the muscle on the skin (Smith & Hargrove, 2013). Since iEMG's are rather invasive and typically painful, sEMG's continue to be the preferred modality in healthy voluntary sedentary subjects and athletes, despite specific limitations and drawbacks. Obtaining information produced by an active muscle provides information about the activities of motor control centers (Smith & Hargrove, 2013). For example, single channel sEMG signals provide average information on the activity of many concurrently active motor units. This often makes reproducibility challenging and exposes why standard recording procedures are still confined to laboratories, therefore limiting comparisons among results obtained by clinical research (Smith & Hargrove, 2013).

Skeletal muscles are composed of individual muscle fibres that can be classified as either slow twitch (type I) or fast twitch (type II & III) (Turker & Sozen, 2013). Slow-twitch muscle fibres are fatigue resistant (aerobic in nature), and therefore play a large role in sustaining smaller movements. Fast-twitch muscle fibres provide bigger and more powerful forces, but for shorter durations and fatigue rather quickly (anaerobic in nature). Since fast twitch fibres can shorten twice as quickly as slow-twitch fibres while generating the same tension, research shows that cyclists with a high percentage of fast-twitch muscle fibres in their leg muscles will be able to produce more power, and at higher cadences (da Silva et al., 2016). Dominant lower limb muscles used throughout the pedal stroke include vastus lateralis, vastus medialis, rectus femoris, biceps femoris, semitendinosus, semimembranosus, and tibialis anterior (Prilutsky & Gregory, 2000). Broadly speaking, the gluteal muscles are largely slow-twitch dominant, while the hamstrings and quadriceps are largely fast-twitch dominant (about 70%) (Smirmaul et al., 2010). This combination allows for the completion of both long bouts of cycling activities and high-intensity events but means that some muscles are more prone to fatigue, and fatigue faster than others.

In current research, surface-detected signals (sEMG) are preferably used to obtain information about the time or intensity of superficial muscle activation. During this process, electrodes are placed along the body of the muscle and the signal collected represents the anatomical and physiological properties of the muscle in question (Chowdhury et al., 2013). However, sEMG recordings can become contaminated by various surrounding internal noises.

This means that analyzing and classifying EMG signals is difficult and complicated (Chowdhury et al., 2013). Nevertheless, analyses may look at amplitude or, more rarely, frequency in hopes of measuring muscle activation and coordination during exercise (Turker & Sozen, 2013). Increasingly, signal patterns may even be compared across different conditions and at variances in speed in hopes of gathering more information. It has been predicted that sEMG's even produce slightly different results when used on youth rather than adult populations (Wan et al., 2017). Youth are characterized by having less peripheral fatigue and greater central fatigue, possibly due to a greater capacity to supply energy from oxidative metabolism (Bontemps et al., 2019).

Signal characteristics during an EMG show that despite the natural recruitment of new motor units, fatigue in an individual muscle is characterized by a marked reduction in the conduction velocities (frequency) of action potentials along the recruited muscle fibres and an increase in its amplitude (Enoka & Duchateau, 2008). This suggests that mean power frequency decreases linearly as a function of fatigue time during muscular contractions. For example, a study conducted by da Silva et al., (2016) described thigh muscle activation in nine cyclists during an incremental test using iEMG recordings of eight thigh muscles. From the results of this investigation, researchers describe the complex coordination pattern of said muscles involved in cycling as well as identify the different degrees and times of activation of each muscle throughout the pedal stroke. In another example, sEMG was used to determine and then compare the fatigue threshold (EMG(FT)) in the Vastus Lateralis (VL), Rectus Femoris (RF), Biceps Femoris (BF), Semitendinosus (ST), and Tibialis Anterior (TA) during a maximum incremental cycling test in 13 trained cyclists and 11 non-cyclists (Smirmaul et al., 2010). For the five muscles analyzed, EMG(FT), marked by a decrease in frequency and increase in the amplitude of the signal, showed no between group differences for RF, BF and ST, however VL and TA were lower for cyclists than non-cyclists. This implies that EMG(FT) is more easily identified in RF and VL muscles for both groups and may provide an interesting method to evaluate the adaptive responses from aerobic and anaerobic metabolisms during cycling programs (Enoka & Duchateau, 2008; Smirmaul et al., 2010). Additionally, Prilutsky and Gregory (2000) used sEMG to predict muscle patterns during cycling in hopes of providing insight into the functional significance of coordination. It was shown that muscle coordination strategies in cycling may emerge from rate of perceived effort and be used to minimize fatigue during an activity.

2.11 Exercise and ergogenic aids

In the context of sport, an ergogenic aid can be broadly defined as a technique or substance used for the purpose of enhancing performance outcomes, either by affecting energy metabolism or by eliciting an effect on the CNS (Best et al., 2020). Ergogenic aids have been classified as pharmacologic, physiologic, psychologic, or nutritional and range in use from accepted methods, such as carbohydrate loading, to illegal and unsafe approaches including anabolic-androgenic steroid use (Barwood et al., 2020). Although the efficacy of many techniques remains controversial, the use of supplements in sport continues to be widespread. For example, creatine is perhaps the most widely used supplement in sport. Its use can increase creatine phosphate levels and improve short-term high-intensity performance, but its use is associated with a significant risk of gastrointestinal side effects (Tarnopolsky, 2010). Similarly, caffeine is a supplement commonly used to influence performance via increasing muscular activity and influencing the perception of fatigue and effort (Tarnopolsky, 2010). Although these products do not violate the International Olympic Committee regulations on doping in sports, these supplements have not necessarily been proven to be beneficial across a broad range of sport modalities (Barwood et al., 2020).

Likewise, cooling interventions can be loosely classified as performance aids since they have been shown to mitigate fatigue. More specifically, pre-cooling is described as the removal of heat from the body before exercise (Ross et al., 2013), while mid-cooling is defined as the application of cooling during exercise (Stevens et al., 2016; Stevens et al., 2017). Pre-cooling techniques function to decrease Tc, while mid-cooling interventions alter thermal perception. Mid-cooling can be undertaken through external or internal methods, such as by using cooling garments or ingestion of cold beverages (Riera et al., 2014). This approach to improving performance perhaps offers a more realistic option for athletes.

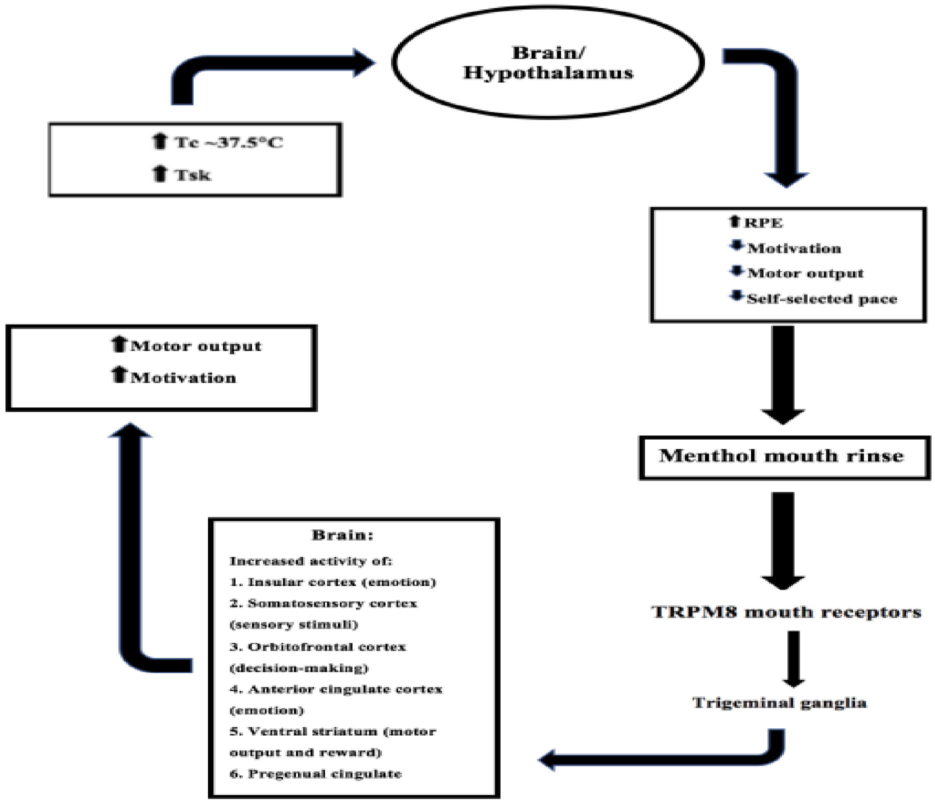
2.11.1 Menthol as an ergogenic aid

Research on ergogenic aids has led to investigations involving the use of menthol as a performance-enhancing substance for distinct aspects of athletic performance. Menthol ($C_{10}H_{20}O$) is a naturally occurring plant compound that is classified as a nonthermal perceptual cooling intervention; meaning it can be used to alter behavior in different environments without necessarily causing thermoregulatory or cardiovascular changes (Bongers et al., 2017). Cold stimuli often have an excitatory effect on the human body. This means that when applied to the skin or mucosal surfaces, menthol stimulates the cold receptor TRPM8 to exert a cooling "sensation" (McKemy, 2007) or "alerting" action (Barwood et al., 2020) that works to improve conscious perception of exercise and temperature via its action on sensory nerve endings (Digel et al., 2008; McKemy, 2007; Stevens & Best, 2016). The use of menthol as an intervention technique to alleviate symptoms of heat stress and improve exercise performance in hot environments has increased in recent years.

Despite not actually cooling the body, menthol is a mind-cooling technique that works to improve the perception of exercise and temperature (McKemy, 2007; Stevens & Best, 2016). TRPM8 is a relatively unexplored cold-sensitive thermoreceptor that is known to bind menthol, a cooling agent present in many toothpastes, vapor-rubs, chewing gums, candy, mouth wash, cold medications, and aromatherapies. To date, various application methods have been tested on moderate to well-trained adult athletes in the heat including mouth rinses, flavoured beverages, external gels or sprays, and immersion tanks (Flood, 2018; Jeffries et al., 2018; Stevens & Best, 2016). Several studies indicate that menthol appears to have the greatest benefit on endurance performance when taken internally, while others demonstrate no performance benefit at all when used externally (Stevens & Best, 2016). More specifically, menthol has the potential to increase self-selected cycling power output and to extend cycling time when rinsed in the mouth (Mundel and Jones, 2010; Schlader et al., 2011) as it is assumed to incite performance-enhancing effects via mechanisms related to thermal, ventilatory, analgesic and arousal properties (Bongers et al., 2017; Flood, 2018). To describe, menthol activates TRPM8, a non-selective, temperature-sensitive, voltage-dependent ion channel this is predominantly expressed in a subpopulation of thermoceptive/ nociceptive neurons found in the dorsal root and trigeminal ganglia (Jeffries & Waldron, 2019). Once TRPM8 receptors are stimulated, afferent sensory neurons depolarize, and action potentials are transmitted to the brain via the spinal cord, where they are integrated by the CNS to evoke responses in reward centers of the brain that amalgamate to provide cognitive and reflexive improvements in exercise performance (Gavel et al., 2022; Klasen et al., 2012).

Furthermore, the perception of temperature has been explored using thermal comfort and sensation scales since the 1960s where an increase in thermal comfort (TC) or sensation (TS) is commonly associated with a higher Tc (Flood, 2018). Most cooling intervention techniques cannot reduce thermal perception without also decreasing Tc; however, menthol is an agent that allows for a separation between the perception of temperature and exercise, therefore facilitating behavioral changes (Bongers et al., 2017). This is shown to be an important factor that correlates with performance during aerobic activities (Barwood et al., 2015). Current studies suggest that a menthol mouth rinse can improve TT performance by ~3-9% in adult female and male populations, respectively (Gavel et al, 2019; Mundel & Jones, 2010). This is intrinsically related to the idea that thermal state and sensation appear to influence performance in the heat (Cheung, 2010). Muscle activation may also be impaired due to the manifestation of fatigue; hence, menthol has been used to ameliorate these effects, yet temperature, frequency, and concentration remain unevaluated. This means the use of menthol continues to warrant further investigation as athletes and federations intend to explore the benefits it may bring to elite sport performance, specifically for Olympic or international sporting events that are to be held in hot environments (Barwood et al., 2020).

Figure 3. A proposed mechanism of the influence of menthol on perception and motor output (Gavel et al., 2019).



2.12 Menthol and performance-based research

Preliminary research conducted by Mundel and Jones (2010) demonstrated that swilling menthol while cycling in the heat significantly affects endurance capacity, ventilation, and the (central) sense of effort. Nine, non-acclimated males cycled to exhaustion at 65% of their peak aerobic power output in a 34°C environment, swilling 25 ml of either menthol or orange-flavoured placebo solution every 10 minutes. Results from this trial showed that eight out of nine subjects cycled for longer under the menthol condition which resulted in a 9 ± 12% improvement in endurance capacity. No differences between trials were observed for heart rate (HR), oxygen uptake or carbon dioxide production, blood lactate concentration, sweat rate, or volume of water ingested. The most likely reason for these changes is that menthol stimulates the oral thermal receptors and thus provides a pleasant cooling sensation that influences exercise capacity.

Trong et al. (2015) had ten, heat acclimated males complete three trials consisting of 4-km of cycling and 1.5-km of running. Subjects drank 190 ml of aromatized menthol beverages prepared at either neutral (28.7°C), Cold (3.1°C), or Ice-slurry (0.17°C) prior to, during, and post-trial. Investigators confirmed that the ice-slurry/menthol beverage increased performance by 6.2% and 3.3% compared with neutral/menthol and cold /menthol beverages, respectively. No between-trial differences were noted for Tc, HR, rating of perceived exertion (RPE), and TC but TS was lower with ice-slurry/menthol and cold/menthol compared with neutral/menthol ingestion. This research was the first of its kind to be conducted in an outdoor stadium in hot and humid conditions. It also was one of the first investigative studies to employ a short distance cycling TT protocol, which revealed insight into the possible benefits of menthol on short duration aerobic events. Similarly, Stevens et al. (2016) conducted menthol research with male runners that had eleven athletes complete 10-minutes of self-paced running on a non-motorized treadmill followed by a 5-km running TT on a non-motorized treadmill under hot conditions. Stevens et al. then furthered their preliminary research by performing a study in 2017 that required the same eleven male athletes to complete a 20-minute running TT paced at 70% of VO_{2max} followed by 5-minutes of seated rest, and then a 3-km maximal running TT in the heat. Results showed that the menthol mouth rinse improved performance time by 3% and 4%, respectively, while also decreasing TS without influencing HR, Tc, and TC. These conclusions closely align with Trong et al. (2015). Interestingly, only Stevens et al., (2017) has utilized EMG technology in combination with menthol thus far. During this investigation, the EMG integrals of every muscle (Vastus Lateralis, Tibialis Anterior, and gluteus maximums) were added together to form the parameter of sum-iEMG, representing the general behavior of muscle electrical activity during the running time trial across each condition. Findings were largely inconclusive between trials and therefore, no further insights into the possible central mechanisms of performance improvements from cooling could be determined. Therefore, further research pertaining to this measure of function and physiology is required.

Recent work completed by Flood et al. (2017) measured the effectiveness of a menthol mouth rinse versus a placebo in a fixed RPE cycling protocol with an isokinetic sprint before and after the trial in eight male amateur cyclists. Findings showed that both exercise time and average power output increased in the menthol trial which equated to an improvement of ~7%. In this protocol, participants perceived cycling at an RPE of 16 as harder during the placebo trial when compared to the menthol trial. When rinsing with menthol, participants also voluntarily adopted a higher work rate, thus completing the trial faster. Furthermore, a significant decrease in sprint power was recorded during the menthol condition but not in the placebo trial. This is assumed to be due to a diminished capacity to generate peak power after the RPE protocol, suggesting participants accumulated greater levels of peripheral fatigue from the higher rate of work. This investigation also showed a decrease in TS with the use of menthol, despite no changes in T_c and skin temperature (Flood et al., 2017). This confirms the findings of other researchers but under the premise of employing a different testing protocol.

Likewise, Riera et al. (2014) compared the effects of beverage temperature with and without menthol during a 20-km cycling TT in hot/ humid conditions in twelve trained men. Athletes drank 190mL of either aromatized (menthol) or non-aromatized beverage before warm-up, prior to the trial, and every 5-km throughout the trial. Results showed that both beverage aroma and temperature have positive effects on performance, with considerably better effects being seen with ice-slush than neutral temperature beverages, and with menthol versus non-menthol. This investigation also reported that HR, Tc, and RPE did not differ between trials, while a reduction in TS was recorded in protocols with menthol (Riera et al., 2014).

Interestingly, research conducted by Rinaldi et al., (2018) had eight male cyclists perform two 20-minute cycling TTs in the heat that were separated by 10-minutes of cold-water immersion. This study compared the effects of cold-water immersion, with and without menthol, on the recovery of cycling performance. Measurement parameters included power output, RPE, Tc, Tsk, TS, and TC. Results from this study appeared to be rather unclear and inconclusive as researchers indicated that menthol immersion probably improves 20-minute cycling performance, most likely decreases TS, and seems to impair thermoregulatory processes. This was one of the first investigations to publish ambiguous conclusions. But even more interesting, is work completed by Gibson et al. (2019) that concluded that swilling menthol for 6-seconds every 10-minutes during 40-minutes of intermittent sprinting in fourteen moderately trained men results in no difference between peak power, work done, TS, or overall performance in the heat. Best et al. (2020) then also found that menthol produced only a trivial benefit for male and female participants on strength and power performance tests under TN conditions. These investigations raise questionability related to the impact that menthol has on athlete performance and behavior across different aerobic and anaerobic events. Thus, there is a need to further investigate the effect that a mouth rinse may have in different timed events in the heat and under TN conditions.

More applicable is work completed by Jeffries et al. (2018) and Gavel et al. (2019). While both investigations concluded that a menthol mouth rinse improves exercise time by 6% and 2.3%, respectively, each study utilized very different testing protocols and population groups. Jeffries et al. (2018) had ten men use a menthol mouth rinse at 85% of individualized baseline during a TTE cycling exercise at 70% W_{max} in hot conditions. On the other hand, Gavel et al. (2019) had nine women complete a 30-km cycling TT while swilling either a 25 mL placebo (crystal-light) or a menthol mouth rinse every 5-km for 5-seconds. This was the first study to investigate the unique impact that menthol has on performance in female athlete populations (Gavel et al., 2019). Both studies determined that cooling oral stimuli have an immediate behavioral, rather than physiological influence on aerobic cycling performance, which was used to highlight the perceptual influence that menthol may have on athletes during athletic tasks under hot conditions.

Most recent, is work completed by Crosby et al., (2022) which demonstrates that menthol mouth rinsing allows for the maintenance of relative power production during a 3-minute maximal cycling test in the heat when compared to both cold water and placebo rinsing. During this investigation, 11 participants (6 males, 5 females) performed three modified maximal tests, where each trial included a different pre-exercise mouth rinse: either menthol (MEN), cold water (WAT), or placebo (PLA). During the test, participants rated their thermal comfort (TC), thermal sensation (TS) and rating of perceived exertion (RPE), while HR, Tc, and cycling power variables were monitored continuously. Blood lactate was collected pre- and post-test. Results from this investigation showed small to moderate effects between solutions MEN, WAT and PLA towards the end of the test in relation to relative power output. Analysis revealed that participants produced higher relative power for longer durations with the addition of the menthol mouth rinse, compared to cold water or placebo, which allowed the researchers to conclude, that the use of menthol (0.1%) as a mouth rinse showed small performance benefits for short duration high-intensity exercise in the heat with little to no change in any perceptual measures (Crosby et al., 2022). This study provides novelty for the use of menthol as an ergogenic intervention, as this was the first experiment to demonstrate some benefit to using menthol during a short duration, high intensity cycling protocol.

(See ***Table 1***. *Summary of research determining the effect of menthol on exercise performance*)

2.13 Themes across menthol-based research

Upon reviewing each of the thirteen randomized control trials individually, specific themes related to menthol and its effects can be discerned and analyzed. First of all, the effect of menthol on various physiological and psychological variables can be summarized across investigations. Furthermore, similar conclusions across the literature lend themselves to the specific effect of menthol applications when used under different environmental temperatures. And finally, information about the impact of menthol on various populations can be summated. The following section of this literature review offers a summary analysis of the current information available on this topic.

2.13.1 Menthol and performance variables

According to most of the published literature, the internal application of menthol does have a significant effect on performance-related variables. More specifically, of the thirteen articles included in this review, eleven used menthol as an oral stimulus in the form of either a mouth rinse or an aromatized beverage (Best et al., 2020; Crosby et al., 2022; Flood et al., 2017; Gavel et al., 2019; Gibson et al., 2019; Jeffries et al., 2018; Mundel and Jones, 2010; Riera et al., 2014; Stevens et al., 2017; Stevens et al., 2016; Trong et al., 2015). One study utilized menthol as an external spray (Barwood et al., 2015), while another used a cold-water immersion bath containing menthol (Rinaldi et al., 2018). Since all investigations were focused on quantifying both the physiological and psychological effects of fatigue and the benefit of using menthol during exercise, specific performance variables can be compared across investigations. Perhaps most significant is the finding that menthol influences perception during exercise, despite high levels of fatigue which has been associated with improved physical performance over time. For example, the measures of TC and TS are used across all investigations, often indicating that menthol reduces TS and improves TC to some degree. This relates to the idea that menthol acts on thermoreceptors to elicit a cooling sensation or state of arousal. This then translates to the perception that an individual is more comfortable in a warm or hot environment, despite not actually impacting Tc. This supports the finding that menthol applied as a mouth wash or aromatized beverage is an effective mid-cooling technique. Furthermore, these articles also indicate that no meaningful differences across several physical variables can be detected. Measures of core body temperature, heart rate, and sweat rate were included across most investigations. Despite ingesting menthol, researchers consistently agreed that internal use had no significant impact on these physical parameters. However, all investigations that applied menthol internally, rather than externally, did indicate that after ingesting menthol participants generally adopted a higher rate of work (W). Thus, the interconnection between physiological and psychological variables plausibly accounts for the overall improvement in performance times seen across multiple studies.

2.13.2 Menthol and environmental temperature

Of the studies included in this review, almost all (twelve) were conducted in warm/hot ambient temperature conditions (28°C-40°C) (Barwood et al., 2015; Crosby et al., 2022; Flood et al., 2017; Gavel et al., 2019; Gibson et al., 2019; Jeffries et al., 2018; Mundel and Jones, 2010; Riera et al., 2014; Rinaldi et al., 2018; Stevens et al., 2017; Stevens et al., 2016; Trong et al., 2015). On the other hand, only one study took place under thermoneutral temperature conditions

(22°C-23°C) (Best et al., 2020). As described earlier, menthol has been classified as a mid-cooling agent, meaning it can be used to activate the Transient Receptor Potential cation channel subfamily M member 8, (TRPM8) receptors (cold) in the mouth and on the skin which has been used to ameliorate fatigue and improve performance under hot conditions. This is because sensory inputs from thermoreceptors are transmitted to the brain via the spinal cord, where they are integrated by the central nervous system (CNS) to evoke reflexive and cognitive responses (Gavel et al., 2021; Klasen et al., 2012). During endurance exercise, especially in the heat, this cascade of events is assumed to evoke responses in reward centers of the brain that amalgamate to reduce the perception of heat stress, generating the impression that individuals feel better and therefore can work harder to complete an athletic task (Jeffries & Waldron, 2019; Stevens & Best, 2017).

An investigation by Best et al (2020) is the only study thus far, to assess the effects of repeated MEN use in thermoneutral conditions on strength and power performance in both males and females. This randomized crossover investigation compared MEN swilling to a control (no swill) before completing three anaerobic exercise tasks (isometric mid-thigh pull, a vertical jump, and a 6 second sprint on a bike) on two separate occasions. Unclear differences were noted between MEN and control conditions across all activities, leading researchers to presume that MEN MR does not improve anaerobic strength or power performance under neutral temperature conditions. Despite uncertainty, it cannot be overlooked that these findings further support the idea that MEN acts predominately on the CNS, only generating significant impacts for longer-duration aerobic tasks that are linked to increases in central fatigue rather than peripheral fatigue (Gavel et al., 2021; Thomas et al., 2015).

Although it might be clear to assume that menthol has a significant impact on exercise performance when external temperature is high, one must consider that type of testing protocol selected to explore this relationship is an important contributing factor to observed outcomes. For example, research insinuates that menthol is most effective during endurance-type sporting events, yet when reviewing the methodology employed by each study, it is evident that no gold standard in performance testing currently exists. Past investigations have used several popular models, such as the TT and TTE, because of a high rate of validity and reliability, but it is obvious that several different exercise protocols have been explored. Based on the studies included in this review, three employed a TTE (Flood et al., 2017; Jeffries et al., 2018; Mundel and Jones, 2010), seven used a TT based protocol (various distances) (Barwood et al., 2015; Gavel et al., 2019; Riera et al., 2014; Rinaldi et al., 2018; Stevens et al., 2017; Stevens et al., 2016; Trong et al., 2015), one used a short duration or modified maximal TT (3-min) (Crosby et al., 2022), one investigated intermittent sprint performance (Gibson et al., 2019), and one measured strength and power performance from a standardized fitness test (isometric mid-thigh pull, vertical jump, six second cycling sprint) (Best et al., 2020).

Interestingly, the most recent investigation involving MEN, conducted by Crosby et al. (2022), presents novel findings surrounding MEN efficacy during a short duration cycling task that exceeds maximal aerobic capacity. This randomized crossover-controlled study assessed the effects of MEN MR upon a modified three-minute maximal test in the heat. While findings did not indicate significant performance improvement, participants did produce higher relative power for longer durations with the addition of MEN, compared to cold water or a placebo. This suggests that even when heat exposure is brief, athletes may benefit from perceptual cooling despite not experiencing harmful alterations in physiological temperature.

With this information in mind, it is relevant to assume that menthol positively impacts endurance performance while also possessing the ability to maintain performance or power output during short, intense tasks, due to its ability to act on the CNS, without compromising warmth-defence responses during exercise. This implies it can be safely applied from a thermoregulatory point of view to alter performance in hot environments. Therefore, a gap continues to exist in determining how menthol impacts variable (race-like) cycling tasks that combine maximal and submaximal efforts over an extended period, under hot temperature conditions.

2.13.3 Study methodology and menthol

Based on the articles included in this review, it appears relevant to differentiate between the various methodologies used. A major factor that may influence the effectiveness of menthol relates to the specific population investigated. This correlates with the age, sex, experience level, and number of training years of a recruited participant group. Ten studies involved a moderate-to-well trained adult male population, including anywhere from 8-14 individuals (Barwood et al., 2015; Flood et al., 2017; Gibson et al., 2019; Jeffries et al., 2018; Mundel and Jones, 2010; Riera et al., 2014; Rinaldi et al., 2018; Stevens et al., 2017; Stevens et al., 2016; Trong et al., 2015), one study investigated the effects on menthol on an adult all-female well-trained cohort (n=9) (Gavel et al., 2019), and two studies compared the intervention between moderately-trained women (n=9 and n=5) and men (n=10, n=6), respectively (Best et al., 2020, Crosby et al., 2022). Not only can it be noted that all participants included thus far in MEN based research are considered adults (19y+), but description of their characteristics indicate that only healthy individuals with moderate-to-well training status have been recruited. As interest in MEN uses across different sporting environments grow, it becomes even more important that its potential advantage is understood for elite or highly trained populations. Since elite athletes are generally accustomed to pushing their physical and/or mental boundaries during competition, it would be interesting to know if a CNS-based ergogenic intervention offers the potential for more podium finishes or greater achievements in the world of high-performance sport. Now combine this prospect with the recent push to prepare younger generations of athletes for the requirements of high-end sport much earlier on, exploring the effects of MEN on highly trained adolescent athletes appears to be warranted. While it is known that youth employ a slightly different thermoregulatory process and respond to fatigue in a slightly different manner than their older counterparts, it is assumed that activation of the TRPM8 thermoreceptor and stimulation of the CNS remains relatively consistent regardless of age. With this information in mind, it could be hypothesized that the effect of MEN in trained adolescent athletes would present much like its ability to improve performance in moderately trained adult athletes. This is largely based on the idea that adolescents, despite training status, are not yet equipped with the same experience or mental poise that older elite athletes typically display during competition.

A final methodological consideration in current MEN research applies to variability in the use of MEN during exercise. Based on the papers discussed thus far, it is clear that no standardized or best practice approach to the frequency and timing of using a MEN MR has been established. Some investigations apply the mouth rinse/ beverage prior to and at regular intervals during the exercise task (time or distance dependant), while others only allowed a single dose that occurred either prior to or towards the final quarter (time based) of the effort. Time spent swilling also varied from 5-10 seconds across investigations. Although timing and frequency remain controversial, 25mL of mouth rinse with a menthol concentration of either 0.01% or 0.1% was consistently administered. With the imminent transition of using MEN out of the laboratory and into the field of competition, it is relevant to consider the plausibility of repetitive and continuous rinsing. Since many race disciplines have now adopted course structures to accommodate spectators, races often consist of multiple laps of a shorter course. This perhaps presents a unique opportunity for athletes to utilize MEN on multiple occasions throughout a race, and therefore timing and swill duration should be further explored.

2.14 Current research gaps and significance

While each investigation presents specific methodologic strengths and limitations, it can be concluded that menthol applied as a mouth rinse can influence perception, which subsequently allows for an alteration in skeletal muscle activity, and therefore improves performance under certain conditions. Yet, questionability surrounding testing protocol, environmental settings, rinse timing and frequency, modality of use, and population demographic remain inconsistent. This means that results are not directly comparable across studies. Interestingly, no investigation, to date, has compared the effects of a menthol mouth rinse under hot environmental conditions on a highly trained cohort during a variable cycle test that simulates race-like performance (extended duration and intensity). Moreover, no study has explored the impacts of menthol on an adolescent athlete population group.

With the above information in mind, there is a need to understand the current research surrounding the use of menthol in sports. By assessing the literature available on this topic and the supplementary peer-reviewed information published related to its underlying effects, we see that there are connections between the consumption of menthol and improved performance outcomes. This information is significant across the research community because it reveals insight into the individual mechanisms that can either limit or benefit performance, but also offers practical importance to athletes and coaches. From a team or club perspective, this information may be beneficial in developing better training programs for athletes, and from an organizational standpoint, the findings from this study could help sporting organizations plan and prepare for events, such as Provincial Championships, National Championships, and Junior UCI World Championships. Although there are several unanswered questions pertaining to this topic, since cycling continues to attract many young athletes annually, it is of utmost value to understand the ergogenic properties of menthol on cycling performance across various age groups.

Table 1: Summary of research determining the effect of menthol on exercise performance

Investigation	Ambient Conditions	Subjects	Menthol Application	Protocol	Physiological/ Psychological Measures	Performance Outcomes
Mundel and Jones (2010)	34 ± 1C and a relative humidity (RH) of 27 ± 4%	9 men, age = 25 ±7 years; VO_{2max} = 54±5 ml kg-1 min-1; W_{max} = 302±38 W	Orange flavoured placebo or Menthol mouth rinse (25 mL at 0.01% every 10 min, swill 10 sec	Cycling TTE at 65% VO_{2max}	-RPE -HR -O_2 uptake -CO_2 production - [blood lactate] - [blood glucose] -sweat rate	Increased TTE by 5 min (9%)
Riera et al. (2014)	30.7°C±0.8°C and 78%±0.03% relative humidity.	12 men, heat acclimated, age=42±13 years; VO_{2max}=59.9±10. 4 ml kg-1 min-1; W_{max}=340±42 W	190mL aromatized drink (0.5 mL of menthol) or non-aromatized drink (neutral temperature: 23°C, cold: 3°C, or ice-slush: −1°C) every 5km	Cycling 20km TT against the clock	-heart rate (HR) -core temp -thermal comfort -thermal sensation -rate of perceived exertion (RPE)	-beverage aroma and temperature had positive effects on performance -best performances with ice-slush/menthol and cold/menthol -No differences in HR and *Tc*.
Trong et al. (2015)	Outdoor cycling stadium in Guadeloupe (outdoor temp: 27.6°C±0.8°C, dry bulb temp: 32.5±1.2°C; 57%±0.05% RH and wind: 25.9 $km.h^{-1}$)	10 males, heat acclimated, age= 41±17, VO_{2max}= 59±11mL.min-1.kg-1, W_{max}= 335±48 W	WU, each block, CD; athletes drank 190 ml of aromatized (0.05 mL of menthol) at three temperatures: Neutral (28.7°C), Cold (3.1°C), or Ice-slurry (0.17°C)	3 trials, 5 blocks: 4-km cycling TT, 1.5-km running TT	-trial time -Tc -HR -RPE -TS -TC	Ice-slurry/menthol increased performance by 6.2% and 3.3% No differences were noted for Tc, HR, RPE, TC and TS was lower with ice-slurry/menthol and cold water/menthol
Barwood et al. (2015)	33.5 °C, 33% relative humidity	8 men, not trained cyclists per se. (age=22±2 years)	t-shirt was sprayed with control-spray or menthol-spray after 10 km	16.1-km cycling TT, self paced	-TS -TC -RPE -Tc -T_{skin} -HR -power -TT time	Menthol-spray improved heat perception and comfort but did not alter performance
Stevens et al. (2016)	33°C, 46% relative humidity	11 men, 5- km run time of 18–22 min	Menthol mouth rinse (25 mL 0.01% at 0.2 of every 1 km)	-10-min walk/run on non-motorized treadmill followed by 5-km running TT on a nonmotorized treadmill	- TS -TC -RPE -Tc -T_{skin} -HR -power -TT time	Menthol Improved performance time by 0.7 min (3%)
Stevens et al. (2017)	33°C, 47% relative humidity	11 men, 3- km run time of 17–23 min	Menthol mouth rinse (25 mL 0.01% at every	Preloaded running time trial	- TS -TC -RPE	-Improved performance by 4%

			0.2 of 1-km of the preload trial, and every 0.1 km of every 1 km)	consisting of 20-min at 70% VO_{2max}, 5-min of seated rest, and a 3- km maximal self paced TT	-Tc -T_{skin} -HR -power -TT time	
Flood et al. (2017)	35°C, 48% relative humidity	8 men, VO_{2max}= 55.4 ± 6.0 ml/min/kg)	Menthol mouth rinse (25mL at 0.01%) 1.5 min prior to exercise, and at regular 10 min interval	-TTE cycling exercise at an RPE of 16 -isokinetic sprints immediately following trial	-Power output -$\dot{V}O2$ -HR Tc, T_{skin} -TS, TC	Improved trial duration by 7%, power output increased
Rinaldi et al. (2018)	Hot and humid environment	8 men, heat acclimated (age= 24.1 ± 4.4 years)	compared the effects of cold-water immersion, with (CMWI) and without (CWI) menthol, on the recovery of cycling performance	-20-min cycling trial (T1) followed by 10 min of immersion during recovery and then a second 20-min cycling trial (T2).	-power output -RPE -Tc, T_{skin} -TS, TC	menthol immersion probably (i) improves the performance of a repeated 20-min cycling bout, (ii) decreases TS, and (iii) impairs thermoregulation processes.
Jeffries et al. (2018)	35 ± 0.2 °C, 40 ± 0.5% relative humidity	10 men, (age 33 ± 9 years; V O_{2peak}=52.4 ± 5.3 ml kg^{-1} min^{-1}; W_{max}=371 ± 27 W	Menthol mouth rinse (25mL at 0.01%) at 85% of the participants baseline time to exhaustion	TTE cycling exercise at 70% W_{max}	-Tc -T_{skin} -HR -RPE -TC -TS	Exercise time was increased by 6% thermally cooling oral stimuli have an immediate behavioral, rather than physiological influence
Gibson et al. (2019)	40.2 ± 0.6 °C, 42 ± 2% relative humidity	14 men (age= 33 ± 9 years), VO_{2peak} = 3.30±0.90 L/min^{-1}	Menthol (0.01% L-menthol) or capsaicin (0.2% capsaicin), 25 mL for 6 sec every 10 min	-40-mins of intermittent sprinting (peak power and work done)	- HR -Tc -T_{skin} -RPE -TS -TC -sweat rate	- No difference in intermittent sprint performance between trials -No difference in TS between trials
Gavel et al. (2019)	30 ± 0.6 °C, 70 ± 1% relative humidity, 12 ± 1 km/h windspeed	9 women (age= 26.7 ± 1.4, VO_{2max} (ml. kg-1.min-1): 50.8 ± 6.0)	25 mL Placebo (crystal-light) mouth rinse or Menthol mouth rinse every 5-km for 5-sec	30-km cycling TT, Handgrip (HG) and a 5-sec sprint (SPR) were measured	-Tc -HR -Sweat rate -RPE -TS -TC -TP	MEN MR significantly improved ITT performance by 2.3 ± 2.7% relative to PLA Average power output was higher in the MEN trial

				as indicators of central and peripheral fatigue		
Best et al. (2020)	thermoneutral conditions (22 ± 1 °C)	19 participants; 10 males (age= 24.6 ± 3.9 years), 9 females (age= (20.2 ± 1.0 years)	25 mL repeated menthol mouth swilling (0.1% concentration) or control (no swill)	strength and power performance: isometric mid-thigh pull, vertical jump, six second cycling sprint	-isometric mid-thigh pull (Newtons), vertical jump (height-cm), six second cycling sprint (W)	Menthol mouth swilling only trivially affected performance, relative to control trials, across all performance tests undertaken, in normothermic conditions
Crosby et al. (2022)	33.0 ± 3.0 ◦C; 46.0 ± 5.0% relative humidity	11 participants. 6 males, 5 females (25 ± 5 years)	25 mL of either placebo - 4 mL of synthetic nonmenthol containing peppermint, cold water (4 ◦C), or 0.1% menthol swilled for 5-sec 1-min prior to the completion of the test	3-min maximal TT	-HR -Tc (rectal) -VO_2, VE, RER -TC, TS, RPE -W, W/kg^{-1} -blood lactate	-Small to moderate effects between solutions towards the end of the test in relation to relative power (higher relative power for longer durations) -No difference in RPE, TC, or TS

CHAPTER 3

3 Research Proposal

3.1 Study Rationale

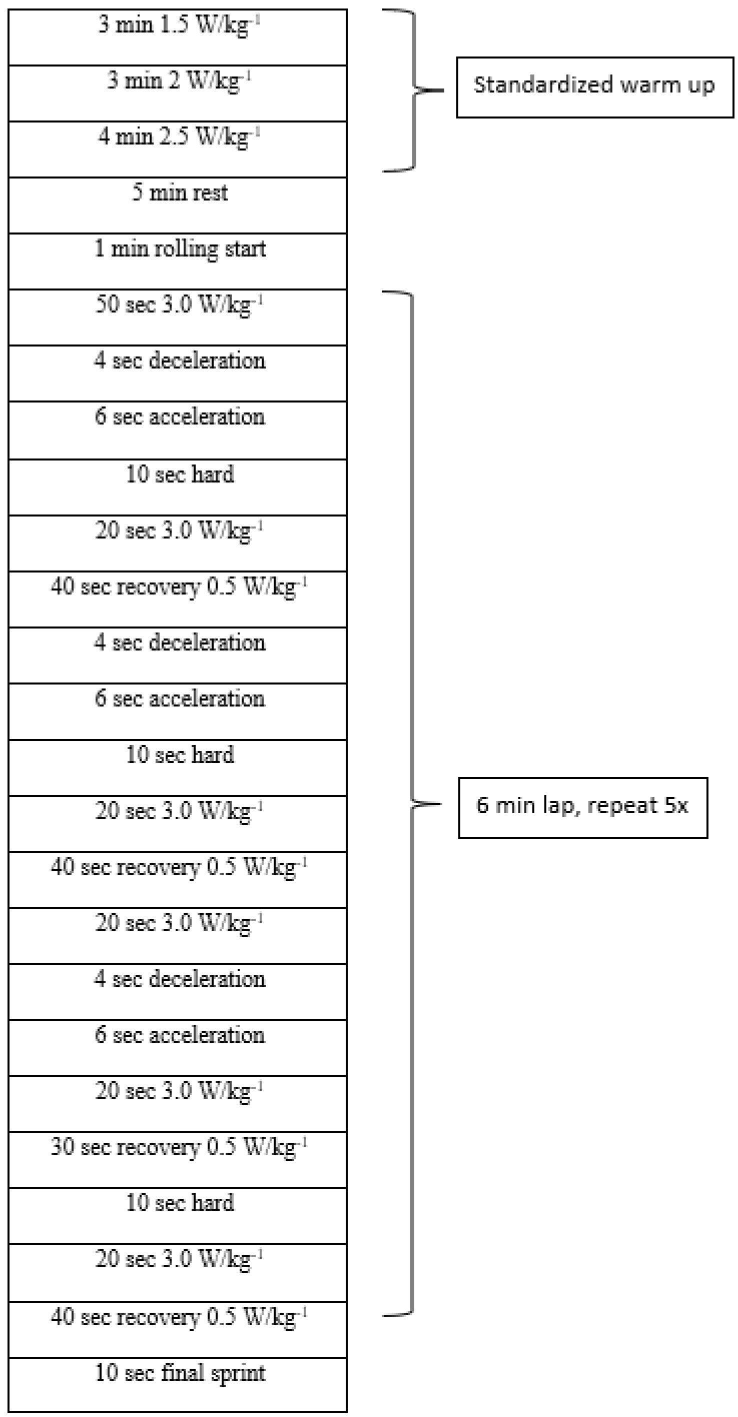
Previous investigations have successfully demonstrated the efficacy of MEN MR at different concentrations, frequencies, and temperatures when used during steady-state endurance tasks in hot environments (≥30°C) (Gavel et al., 2021; Mundel & Jones, 2010; Stevens et al., 2016; Flood et al., 2016), yet little is known about its effectiveness during a variable, race-like task in a well-trained adolescent population group. To explain, MEN efficacy is predicted to be associated with central fatigue (CF), a process within the CNS that reduces neural drive to the exercising muscle and leads to a decrease in voluntary activation. Since CF emerges as a factor of exercise intensity and duration (Thomas et al., 2015), it is reasonable to estimate that MEN will benefit any athletic activity characterized by the accumulation of CF.

With this information in mind, the proposed investigation will characterize the effect of a MEN MR on M-VCT performance in adolescent male cyclists in the heat. On the individual level, the findings of this study will advance knowledge of how environmental conditions and variable cycling efforts can affect various aspects of physiology and cycling performance in adolescent athletes. Coupled with that, the results of this study will elucidate the variability in individual response and help affirm and support the recommendation of customized solutions for athletes based on response. From a team or club perspective, this information may be beneficial in developing better training programs for competing athletes and provide strategies for combating obstacles to performing in hot environments. Lastly from an organizational standpoint, the findings from this study may help sporting organizations plan and prepare for events in the heat, such as Provincial Championships, National Championships, and Junior UCI World Championships.

3.1.1 Rationale for a modified VCT

According to Sharma et al. (2014), the variable cycle test (VCT) was developed through pilot testing and modelled on a previous study that quantified the variable (power) nature of elite level cycling and criterium (circuit) style racing. The VCT required participants to complete 10 x 6 min blocks or laps in a repetitive and continuous fashion (60-min). Each 6 min block consisted of sections of varying duration and intensity with approximately 40% of the trial being prescribed at a submaximal intensity of 3.5 $W \cdot kg^{-1}$ to moderate total power output during the test to be replicative of a race. The remaining portions of the test are described as self-paced periods, where the participant cycled at levels of exertion prescribed to be ‘recovery’ pace, ‘hard’ or ‘accelerating’ and ‘decelerating’. The frequency and duration of each of these sections in the VCT were assigned based on the typical proportion of time spent in different power zones by elite cyclists during racing events. It has been proposed that by modifying the existing VCT, whose validity and reliability has been previously established (Sharma et al., 2014; Sharma et al., 2020), the test will be more representative of the duration and intensity that is characteristic of youth/adolescent level events. The *modified* variable cycle test (M-VCT) will follow the same sequence pattern, with athletes being required to complete 5 x 6-minute laps for a total of 30 minutes (half the duration of the entire VCT; hence, modified). Submaximal prescribed work sections will also be reduced to 3.0 W/kg^{-1} so that the total work performed during the test better replicates the duration and intensity of cycling events for adolescent age categories across various cycling disciplines.

Figure 4. Schematic representation of one lap of the modified variable cycle test M-VCT. This 6-min lap will be repeated 5x (figure adapted from Sharma et al., 2014).



3.2 Participants, Overview, and Objectives

Adolescent males between the ages of 15-19 years were recruited from cycling clubs and racing teams across Durham and the Greater Toronto Area (GTA). To be included in the study participants must have trained a minimum of three sessions per week, had experience performing interval training on a bike, and had race experience in road, mountain bike, or track cycling within the last 6-months.

This thesis project consisted of a two-part investigation. Part 1 included test-retest pilot testing of the M-VCT protocol in trained adolescent male athletes to determine the coefficient of variation (%CV), a measure of reliability, of the M-VCT in this population. Part 2 consisted of the randomized control trial (RCT) characterizing the physiological and psychological effects of menthol versus placebo mouth rinsing during the M-VCT in the heat (in the same group of trained adolescent athletes from Part 1). Power output, Tc, HR, sweat rate, blood lactate concentration, sEMG, and perception (RPE, TC, TS, ROF, & FS) were the key variables measured which are described in more detail in the forthcoming sections. The objectives of this thesis project (Part 1 and 2) are listed below:

I. *To e*stablish the test-retest reliability (CV% and least significant change (LSC)) of the M-VCT protocol for trained adolescent male cyclists

II. *To* determine if a MEN MR improves M-VCT performance (mean power output and perceptual responses) in a hot environment with race-simulated intensity in a trained adolescent male population.

III. *To* further explore menthol’s mechanisms of action by measuring sEMG and adding two novel (to MEN research) perceptual scales; rating of fatigue and feeling scale to potentially delineate the effect of MEN on perception.

IV. *To* determine the individual responsivity to MEN MR on stochastic cycling performance and provide support for a customized approach (e.g., frequency and concentration) to race-day use of MEN MR.

3.3 Identification of Experimental Measures

- M-VCT (5x6-min laps) performance will be quantified and measured through:

1. Power output ($W \cdot kg^{-1}$)
    a) Total trial mean power
    b) Mean power per lap (1,2,3,4,5)
    c) Mean power during “acceleration” (6sec) and “hard” (10sec) sections
    d) Maximum and mean power during the final 10-sec sprint
2. Cadence (rpm)
    a) Total trial mean cadence
    b) Mean cadence per lap
    c) Mean cadence during “acceleration” (6sec) and “hard” (10sec) sections
    d) Mean cadence during the final 10-sec sprint
3. Thermoregulatory and cardiovascular responses will be quantified and measured through:
    a) Maximal and mean HR (total trial and per lap)
    b) Maximal and mean Tc (total trial and per lap)
    c) Sweat loss (total body mass changed during the trial, where 1kg = 1L)
4. Biomarker activity (fatigue) will be quantified through:
    a) Blood [La^-]
5. Muscular activation and fatigability will be measured through:

a) Surface electromyography (sEMG) (MG, TA, VL, VM, RF) (mean and peak)

6. Perception will be measured and quantified through:

   a) Borg RPE 20-point scale (RPE)

   b) Thermal sensation 7-point scale (TS)

   c) Thermal comfort 7-point scale (TC)

   d) Rating of Fatigue scale (ROF)

   e) Feeling Scale (FS)

3.4 Hypotheses

3.4.1 Primary hypothesis

I. Test-retest performance (power output and physiological variables) during the M-VCT will be reliable, with <2% variance, in trained adolescent male athletes.

II. Menthol mouth rinsing will improve mean power output, and therefore performance, of the M-VCT in trained adolescent male athletes.

III. A menthol mouth rinse will increase mean power ($W \cdot kg^{-1}$) during the final 10sec sprint despite increased fatigue.

3.4.2 Secondary hypothesis

IV. A menthol mouth rinse will help maintain muscular activation (sEMG) and power ($W \cdot kg^{-1}$) in the latter laps of the M-VCT

V. A menthol mouth rinse will not alter thermoregulatory or cardiovascular activity during the protocol

VI. A menthol mouth rinse will improve ROF and FS during the latter laps of the M-VCT

CHAPTER 4

4 Menthol Improves Stochastic Cycling Performance in Trained Adolescent Athletes Under Heat Stress

4.1 Abstract

The purpose of this study was to investigate the effect of a menthol (MEN) mouth rinse (MR) on cycling performance during a modified variable cycling test (M-VCT) in trained adolescent male athletes under hot conditions (31.4±0.9°C, 23.4±3.7% RH). Eleven trained males (n=11, 16.7 ± 1.3 years, height 176.6 ± 8.8 cm, weight 65.8 ± 11.6 kg, VO_{2max} 62.97 ± 7.47 mL·kg^{-1}·min^{-1}, $VO_{2\,max}$ peak power output 5.36 ± 0.79 W·kg^{-1}) voluntarily completed three trials (familiarization and 2 experimental trials) of a 30-minute M-VCT which included 5 laps of 6 minutes in duration with three 6 second (s) accelerations and three 10s sprints throughout each lap. In a randomized crossover design, (1) menthol MR (0.01%) or (2) placebo MR (crystal-light sucralose sweetener), was swilled for 5s before the start of each lap (total of 6 MR). Power output (PO), distance (m), core temperature (Tc), heart rate (HR), perceptual responses (rating of perceived exertion (RPE), thermal stimulation (TS & TC), fatigue (ROF), feeling (FS)), surface electromyography (sEMG), and blood lactate (BLa) were recorded. MEN MR significantly improved M-VCT mean PO by 1.81±1.57% relative to PLA (p= <0.001, 95% CI= [1.73 to 4.46], ES= 1.53). In the MEN trial, 6s and 10s mean PO was significantly higher than PLA (6 sec, p=0.041, ES=0.71; 10 sec p=0.002, ES=1.29). There was no significant difference in Tc, HR, BLa, sEMG or any perceptual measure between trials (p>0.05) despite work being significantly higher in MEN versus PLA. 7/11 athletes (64%) significantly improved M-VCT performance with the use of MEN MR. While individual response rates varied, the results demonstrate that a nonthermal cooling agent that acts on the CNS can benefit power regulation during a stochastic cycling task without causing additional decline in perception.

4.2 Introduction

Scientific literature provides us with substantial evidence describing the influence that environmental temperature has on physiological and psychological parameters during an athletic task with strong evidence demonstrating the harmful relationship that often emerges between performance and hot temperature conditions (≥28°C). Specifically, the multi-disciplinary sport of cycling (road, track, BMX, mountain, etc.) includes a range of demands from short sprints to long endurance rides with complex interactions between biological, cognitive, biomechanical, and environmental factors. Due to this diversity, sports science researchers have long been discussing the concept of affective perception, fatigue, and the ability to overcome various limitations to cycling performance in extreme temperature conditions (Amann et al., 2011; Ekkekakis et al., 2011; Micklewright et al., 2017). More precisely, prolonged and/or intense exercise in the heat is shown to disrupt biological processes and force activation of protective thermoregulatory mechanisms to return the body to a state of homeostasis. Although it is assumed that adults and young athletes employ similar strategies to combat the effects of heat stress to maintain performance during strenuous exercise; level of physical maturity (i.e., not fully developed sweat glands) and less training experience, combined with reduced mental preparedness can result in performance differences. While adolescent athletes are described as being physically equipped to perform intense exercise, many still lack adequate “readiness” to cope with the demands of elite level competition under intense conditions. This can cause a young athlete to “push beyond” their known limitations which may increase the likelihood of experiencing heat-related illness or injury, ultimately, threatening performance outcomes at significant events (Inge & Bass, 2001).

Despite a rather limited amount of research surrounding fatigue and limits to performance in adolescents, it is known that the neurophysiological characteristics and metabolic mechanisms associated with fatigue manifestation can cause young athletes to (1) reach a state of mental and physical ‘exhaustion’ much faster during an athletic task than their older counterparts, (2) increase the frequency of technical and/or tactical errors made during the competition event, and (3) cause a rapid decline in affective response which can lead to higher rates of frustration and dissatisfaction (Falk & Dotan, 2011). Although physiological profiles between young athletes and adult athletes often appear quite similar, age-related disparities in the ability to cope with fatigue during an athletic event typically contribute to feelings of weakness and tiredness and therefore impair performance outcomes (Falk & Dotan, 2011). Many performance-based tests derived from time-trial and time-to-exhaustion protocols have been used to describe performance and fatigue in the literature, yet few have been shown to simulate the actual dynamic demands of racing. The recent development and validation of the variable cycle test (VCT) in trained athletes (Sharma et al., 2014) provides a unique opportunity to investigate and quantify the variable nature of cycling and other performance parameters using a modified version of the VCT (M-VCT) protocol (half the duration of the VCT). The M-VCT will reflect the time and intensity profile typical of an adolescent-level race.

Perhaps even more interesting is the recent finding that menthol (MEN), an organic compound derived from peppermint that often presents as a flavor molecule to target the gustatory system as well as oropharyngeal thermoreceptors, may be used to evoke responses in reward centers of the brain that amalgamate to deliver improvements in exercise performance (Gavel et al., 2021). From a mechanistic point of view, research shows that when rinsed in the oral cavity, MEN activates the transient receptor potential melastatin-8 (TRPM8). This is a non-selective, temperature-sensitive, voltage-dependent ion channel that is predominantly expressed in a subpopulation of thermoceptive/nociceptive neurons found in the dorsal root and trigeminal ganglia (Klasen et al., 2012). When TRPM8 receptors are stimulated by MEN, afferent sensory neurons depolarize, and action potentials are transmitted to the brain via the spinal cord, where they are integrated by the central nervous system (CNS) to evoke reflexive and cognitive responses during athletic tasks (Gavel et al., 2021; Klasen et al., 2012). This proposed explanation relates to the idea that MEN acts solely on the CNS, only benefiting performance endeavours that are characterized by greater central fatigue rather than peripheral fatigue (Gavel et al., 2021; Thomas et al., 2015). Alternatively, some research has linked MEN success to its proposed ability to override the central governor, a CNS mechanism that regulates 'pace' during physical activity to prevent catastrophic failure (Noakes et al., 2005). Although still disputed, this theory relies on the idea that when MEN elicits a pleasurable cooling sensation, rewards centres in the brain are activated which triggers the perception that thermal strain and fatigue have been ameliorated, which is then thought to 'overrule' the central governor and provide the individual with the ability to upregulate performance (Gavel et al., 2019; Flood et al., 2017; Mundel and Jones, 2010). Upon considering the popularity of using natural supplements during sporting competitions, it is essential that one understands the purpose of MEN and whether its use safely fits the goals and context of the environment. Thus, further research is warranted.

According to the world governing body for cycling (UCI), an exponential increase in cycling's popularity over past decades has not only resulted in the sport becoming more competitive across racing disciplines but also led to an increase in the number of youth riders entering the sport. Furthermore, it has been recognized that these athletes are beginning to take part in high level sporting events at progressively younger ages. Although many young riders now aspire to compete at either the national or international level in cycling, only the select few who receive adequate training during adolescence will be likely to fulfill such goals. Thus, there is now a growing demand to prepare the next generation athletes for the physiological and psychological demands of high-performance sport earlier on. For example, the difference between 1^{st} and 3^{rd} in the junior men's road race at World Championships in 2019 was 0.71% (93 sec) while the difference between 1^{st} and 3^{rd} in the junior men's cross-country World Championships Mountain Bike race in 2019 was only 0.49% (20 sec) (UCI, 2019). Once the basics are satisfied chasing marginal gains may be the difference in a podium position or a mere finishing result, thus even small alterations in performance can be critical. Finally, given that many road, track, and mountain bike competitions (Provincial, National, and International) take place in the summer months across different venues that typically expect temperatures to range from 18-34°C with moderate to high humidity, strategies to manage exercise-induced environmental fatigue are now even more imperative.

As of late, no research has explored cycling performance using a modified version (M-VCT) of the recently validated VCT protocol under hot environmental conditions, nor have investigators explored the impact that menthol may have on performance for trained adolescent male athletes. Thus, this project is novel in several areas of performance-based research. Findings from the first part of this study will be used to establish the test-retest reliability of the M-VCT protocol in a homogenous subpopulation of the recruited adolescent athletes. Findings from the second part of this investigation will describe and contrast the physiological and psychological impact of utilizing menthol versus a crystal light "placebo" mouth rinse during the M-VCT in the heat on the larger homogeneous group of adolescent athletes. This research is intended to help young athletes improve cycling performance by making use of a variable protocol that reliably simulates the stochastic nature of racing performance (Sharma et al., 2014) and to mitigate fatigue when training or competing under hot conditions. Considering a menthol mouth rinse has been shown to increase performance in the heat during submaximal endurance efforts in moderately to well-trained adults by 0.5-6.6% (Gavel et al., 2019; Mundel and Jones, 2010), it is reasonable to hypothesize that a menthol mouth rinse can benefit young athletes as well. To date, there are no published studies quantifying the effect of a menthol mouth rinse in adolescent athlete populations while performing the M-VCT in the heat hence, this investigation offers novel objectives and applicability for advancements in sports science research.

The primary purpose of the current study was to characterize the effect of a MEN MR on M-VCT performance in the heat among adolescent male cyclists, while simultaneously measuring power output, Tc, HR, sweat loss, muscle activity (surface electromyography (sEMG)), blood lactate concentration, perception of the task, and level of exertion. A secondary purpose of this investigation was to further investigate MEN mechanistic pathway in this population by adding novel measurements including sEMG and perceptual scales (rating of fatigue (ROF) and feeling scale (FS)). We hypothesize that MEN will improve M-VCT performance due to its ability to act on reward centers in the brain (*see section 3.4 for detailed description of hypotheses*). Since exercise-induced CF is a contributing factor to performance decrement, overriding feelings of exhaustion will improve power output and therefore performance. Given that MEN is easily transportable, low in cost, and highly accessible (Gavel et al., 2021), findings from this study could extend the use of MEN across a wider range of sporting events that take place globally.

4.3 Methods

4.3.1 Study Participants

Study participants were informed both verbally and in writing of the experimental purpose, protocol, and potential risks prior to giving their verbal and written consent to participate. Since deception was utilized to conceal the true purpose of the investigation, participants were debriefed verbally and then in writing after completing the study. The Research Ethics Board of Ontario Tech University provided approval for the study (REB#16331).

Part 1: Pilot Testing – Test-retest reliability of the M-VCT in this population (n=4)

Four trained adolescent males (n=4), mean age 16.5 ± 1.0 years (yrs), height (ht) 179.5 ± 5.3 cm, weight (wt) 69.8 ± 1.9 kg, and VO_{2max} 68.57 ± 7.17 $mL \cdot kg^{-1} \cdot min^{-1}$, VO_{2max} peak power output (PPO) 6.00 ± 0.93 $W \cdot kg^{-1}$), participated in Part 1 of this study. All athletes were members of cycling teams in the Durham Region or Greater Toronto Area, trained at least 3 days a week (12.8 ± 4.3 hr/week total training and 10.0 ± 5.7 hr/week bike specific training), had experience doing interval training sessions on a bike, and had pervious racing experience (Table 2).

Part 2: Performance Testing - With randomized mouth rinse intervention (n=11)

Eleven trained adolescent males (n=11), including and expanding on the participants from Part 1 (age 16.7 ± 1.3 yrs, ht 176.6 ± 8.8 cm, wt 65.8 ± 11.6 kg, and VO_{2max} 62.97 ± 7.47 $mL \cdot kg^{-1} \cdot min^{-1}$, VO_{2max} PPO 5.36 ± 0.79 $W \cdot kg^{-1}$) participated in Part 2 of this study. Again, all athletes were members of cycling teams in the Durham Region or Greater Toronto Area, trained at least 3 days a week (11.9 ± 3.2 hr/week total training and 8.6 ± 4.1 hr/week bike specific training), had experience doing interval training sessions on a bike, and had pervious racing experience (Table 3).

Prior to participation, participants filled out a “Physical Activity Readiness Questionnaire-PARQ”. Following the questionnaire, if the participant answered “yes” to one or more of the follow-up questions, they were advised to seek a health care practitioner and return an “ePARmedX+Online” form before they were cleared to participate. Each participant was asked to fill out a 24-hr food and exercise log and instructed to closely repeat food intake and weekly training load from one trial to the next. Participants were advised to avoid strenuous activity 24 hrs prior to testing and to replicate their sleep schedule. Prior to attending each session, participants completed an online COVID-19 screening questionnaire, as dictated by Ontario Tech University. On days of acute illness or injury, participants were rescheduled.

Table 2. Part 1: Reliability Testing: Descriptive Characteristics of the Male Participants (n=4).

Variable	**Mean ± SD**
Age (yrs)	16.50±1.00
Weight (kg)	69.77±1.98
Height (cm)	179.50±5.26
VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	68.57±7.17
VO_{2max} peak power output ($W \cdot kg^{-1}$)	6.01±9.33
Total training hr/week	12.75±4.27
Bike specific training hr/week	10.00±5.66

Data are means±standard deviation (SD). Yrs, years; kg, kilogram; cm, centimeter; $mL \cdot kg^{-1} \cdot min^{-1}$, (mL) milliliter per ($kg^{-1}$) kilogram per ($min^{-1}$) minute; $W \cdot kg^{-1}$, (W) watt per (kg^{-1}) kilogram; hr, hours

Table 3. Part 2: Performance Trials: Descriptive Characteristics of the Male Participants (n=11)

Variable	**Mean ± SD**
Age (yrs)	16.66±1.29
Weight (kg)	65.67±11.60
Height (cm)	176.60±8.80
VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	62.97±7.47
VO_{2max} peak power output ($W \cdot kg^{-1}$)	5.36±0.79
Total training hr/week	11.91±3.21
Bike specific training hr/week	8.55±4.08

Data are means±SD. Yrs, years; kg, kilogram; cm, centimeter; $mL \cdot kg^{-1} \cdot min^{-1}$, (mL) milliliter per ($kg^{-1}$) kilogram per ($min^{-1}$) minute; $W \cdot kg^{-1}$, (W) watt per (kg^{-1}) kilogram; hr, hours

4.3.2 Sample Size and Recruitment

The critical outcome of this investigation was to complete a 30-min M-VCT under hot environmental conditions (>28.0°C). While it is suggested that menthol exerts considerable ergogenic effects, its impact seems to differ from one study to the next. Individual investigations demonstrate that menthol mouth rinsing can improve performance in hot environments, for both cycling and running in females and males by ~0.5-6.6% but it should be noted that findings from a current meta-analysis (in press, Gavel & Sprenger et al. 2022) highlight negligible performance change across all published group data. Considering this and the subsequent G-power calculation (statistical power analyses), the determined sample size for this study was 12 participants.

A total of 26 cycling clubs/teams were contacted by the principal investigator, with 18 people expressing interest. 12 participants were initially recruited and committed to the study; however, one participant withdrew from the study due to an opportunity to train/race with the Canadian National Cycling team. Therefore, 11 participants completed the study and were included in analysis.

4.3.3 Data Collection

This investigation was conducted in the laboratory at Ontario Tech University. Data collection commenced in the Fall semester of 2021 and was concluded by the end of the Winter semester in 2022. All participants were given an alpha-numerical assignment for confidentiality reasons. All data was stored encrypted on a password-protected computer belonging to the principal investigator.

4.3.4 General Study Design

This experimental investigation consisted of two parts, divided across 7 laboratory visits. Part 1 consisted of pilot testing which was used to validate and describe the test-retest reliability of the M-VCT protocol in a homogeneous group of well-trained, adolescent male cyclists (n=4). Testing took place over a two-month period in the Fall, of 2021. In Part 2 of this study, a larger homogenous sample of trained adolescent male athletes (including the athletes from part 1) (n=11) completed two single-blind randomized crossover performance trials of the M-VCT under two different conditions: hot environmental conditions with a placebo mouth rinse, and hot environmental conditions with a menthol mouth rinse. Part 2 sessions were completed over a 3-month period during the Winter/ Spring, of 2022.

Part 1: Reliability Testing – Test-retest reliability of the M-VCT in this population (n=4)

- Visit 1: preliminary testing (VO_{2max}) and M-VCT protocol familiarization (2-3 laps)
- Visit 2-3: M-VCT test-retest sessions

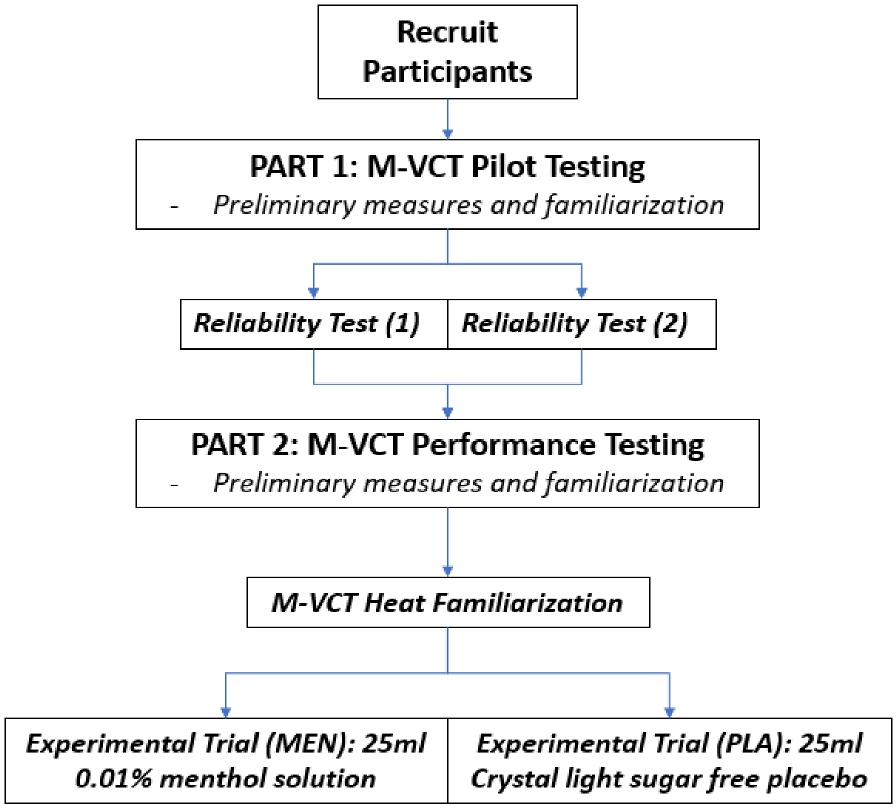
Part 2: Performance Testing - With randomized mouth rinse intervention (n=11)

- Visit 1: preliminary testing (VO_{2max}) and M-VCT protocol familiarization (2-3 laps)
- Visit 2: M-VCT familiarization in the heat
- Visit 3-4: Randomized experimental trials
    - Hot + placebo
    - Hot + menthol

Participants performed all trials (Part 1 and 2) at the same time each day to reduce the effect of circadian variation and on the same cycle ergometer (LODE Excalibur Sport, Quinton Instrument, Groningen, the Netherlands). Participants were instructed to arrive at the laboratory hydrated, whereby they were reminded 2 hours prior to their session via email/text message to consume 500 millilitres (mL) of water (Tarnopolsky et al., 2005). Pre-trial euhydration was established upon arrival by identifying urine specific gravity (USG < 1.020) with a portable refractometer (Atago, Bellevue, WA, USA) that was calibrated with distilled water. If the participants concentration of urine (urine osmolarity) was above 1.020 USG (hydration status threshold), they were deemed dehydrated (Casa et al., 2000; Sawka et al., 2007) and instructed to consume 500-mL of water over a ~30–45-minute period (to prevent diuresis) (Logan-Sprenger & Spriet, 2012). If the participant was still deemed dehydrated, their session was rescheduled. Before and after each trial, participants were weighed in semi-nude attire to determine body mass (BM) loss for the calculation of sweat rate. Participants refrained from ingesting any fluid during the testing period. Prior to beginning any exercise in the laboratory, participants were fitted with a heart rate monitor (Polar® H7 heart sensor, USA) and a wireless core temperature (Tc) monitoring device (CORE, greenTEG AG, Switzerland) to assess cardiovascular and core temperature dynamics throughout the M-VCT.

Prior to riding, participants were set up on the cycle ergometer to determine their appropriate positioning. Saddle height and fore/aft position was obtained by measuring for approximately a 30-degree bend in the knee, followed by alignment of the hip, knee, and ankle joints at the bottom of the pedal stroke, respectively. Handlebar height and fore/aft position was adjusted according to preference. Position values were saved to the LODE ergometer database for future sessions. Testing sessions were separated by 7 days.

Figure 5. Schematic displaying the progression of the testing for both Part 1 and Part 2.



4.3.5 Part 1: Reliability Testing Design and Procedures

The purpose of Part 1 was to determine the test-retest reliability of a modified version of the VCT protocol (M-VCT) in a trained adolescent male population. This data would be used to inform the least significant change (LSC) needed in Part 2 of the study for this protocol, to establish the true effect of a MEN vs PLA mouth rinse. This part consisted of 3 visits to the laboratory

- *Visit 1:* A maximal oxygen uptake test (VO_{2max}) to volitional exhaustion on a cycle ergometer was used to determine each participant's maximum aerobic capacity in relation to RPE, HR, and power output. After self-directed recovery, participants completed a familiarization session with a shortened version of the M-VCT protocol. Participants also received instruction/practice on the use of perceptual scales (rating of perceived exertion (RPE), thermal comfort (TC), thermal sensation (TS), rating of fatigue (ROF), and feeling scale (FS)).

- *Visit 2 & 3:* Participants returned to the lab on two separate occasions to complete a standardized warm-up followed by the full M-VCT protocol in thermoneutral environmental conditions (temperature, 20.3 ± 2.2 °C; relative humidity, 63.3 ± 3.9 % RH). During these sessions power output, cadence, HR, and Tc was recorded continuously. Blood lactate concentration was collected at 3 time points. Perceptual scales were used to monitor exertion level, fatigue, thermal perception, and feeling throughout the sessions.

4.3.6 Part 2: Performance Testing Design and Procedures

After establishing the reliability of this M-VCT in Part 1, the same homogenous group of adolescent male athletes returned to the laboratory for performance-based testing of the M-VCT under hot environmental conditions. These sessions were used to establish if a true change in performance could be attributed to the MEN intervention. This part of the study consisted of 4 visits to the laboratory.

- *Visit 1:* A maximal oxygen uptake test (VO_{2max}) to volitional exhaustion on a cycle ergometer under thermoneutral conditions was used to determine each participant's maximum aerobic capacity in relation to RPE, HR, and power output. After self-directed recovery, participants completed a familiarization session with a shortened version of the M-VCT protocol. Participants also received instruction/practice on the use of perceptual scales (rating of perceived exertion (RPE), thermal comfort (TC), thermal sensation (TS), rating of fatigue (ROF), and feeling scale (FS)).

- Visit 2: Within a week, participants returned to the lab to complete a full (all 5 laps) familiarization session in the heat (31.2 ± 0.8 °C, 23.0 ± 2.0% RH). At this time, participants were also fitted with five wireless EMG electrodes (Delsys Inc., Natick, MA, USA). Sensors were placed superficially on the lower right limb. During these sessions power output, cadence, HR, and Tc was recorded continuously. Blood lactate concentration was collected at 3 time points. Perceptual scales were used to monitor exertion level, fatigue, thermal perception, and feeling throughout the sessions. Participants received detailed instructions as well as the opportunity to practice using a water mouth rinse during the familiarization session.
- Visit 3 & 4: The final two visits to the laboratory consisted of single blind randomized crossover experimental sessions used to determine the influence of MEN compared to a PLA MR on M-VCT performance in the heat (31.4 ± 0.9 °C, 23.4 ± 3.7% RH). Five wireless EMG electrodes (Delsys Inc., Natick, MA, USA) were attached to the lower right limb. During these sessions power output, cadence, HR, and Tc was recorded continuously. Blood lactate concentration was collected at 3 time points. Perceptual scales were used to monitor exertion level, fatigue, thermal perception, and feeling throughout the sessions. Sessions were separated by 7 days to prevent heat acclimation and to provide adequate recovery.

The following section is an extension of the previous section, Section 4.3 General Study Design, with a more detailed description of the methods and measurements used during Part 1 and Part 2 of this thesis.

4.4 Detailed Experimental Procedures

4.4.1 Standardized warm-up protocol for the M-VCT

Before each trial, participants rested for 20-min in the laboratory (thermoneutral or heat) while baseline measures were recorded. Once ready, participants warmed-up for 10 minutes using a standardized protocol (at an intensity of ~10/20 Borg RPE scale) on the cycle ergometer. The warm-up progressed from 1.5 Watts/kilogram ($W \cdot kg^{-1}$) for 3-min, to 2.0 $W \cdot kg^{-1}$ for 3-min and finished with 2.5 $W \cdot kg^{-1}$ for 4-min (figure 4). Participants then rested for 5 minutes prior to starting the M-VCT (Sharma et al., 2014).

4.4.2 Incremental Cycling Test (VO_{2max})

Participants began the incremental test on a cycle ergometer (LODE Excalibur Sport, Quinton Instrument, Groningen, the Netherlands) to determine maximal oxygen uptake (VO_{2max}), peak power output (PPO), and ventilatory thresholds. The incremental test started at 25 Watts (W) and increased by 25 W every 1-minute (min) until voluntary exhaustion. Participants cycled at ~85-95 rpm. HR was monitored continuously while RPE and Tc were recorded at the conclusion of each stage. The session was terminated at volitional exhaustion or once the participant could no longer maintain a minimum cadence of ~80-85 rpm for the assigned workload for a total of 30 consecutive seconds (sec). Cardiorespiratory-metabolic variables were measured throughout the test using a fully automated spiroergometry system (METAMAX 3B, Cortex, Germany), the reliability of which has been established (Vogler, 2010). The analyzer was calibrated before each test using gases of known concentration and a known volume of air, according to the manufacturer's instructions. The test was analyzed using 30 sec average VO_2 (VO_2avg) from each stage (Zuniga et al., 2012). Maximal oxygen uptake (VO_{2max}) was then defined as the highest 15-sec average in the final 30-sec of the test. Results from the test were described according to predefined VO_{2max} criteria including: (1) a plateau or a decrease in VO_2 despite an increase in workload, (2) a RER ≥ 1.10, (3) ±10% of age predicted max heart rate, and (4) a rate of perceived exertion (RPE) >17-18 on the Borg scale. If two or more of the criteria were not satisfied, results were defined as a VO_{2peak} (Beltz et al., 2016). PPO was recorded as the maximum power sustained for the final 30-sec of the test. Power output (W) and oxygen uptake (VO_2) corresponding to VT2 was defined as the point where there was a simultaneous non-linear increase in VE/VO_2 and VE/VCO_2 (Sharma et al., 2014). At completion, subjects cooled down for 5-min at 0.5 $W \cdot kg^{-1}$.

4.4.3 The Modified Variable Cycle Test (M-VCT)

The M-VCT, a modified version of the previously validated VCT protocol (Sharma et al., 2014), consisted of 5x6-min laps that were completed in a continuous and repetitive fashion. Each 6-min lap consisted of sections of varying duration and intensity, with approximately 40% of the trial prescribed submaximal intensity of 3.0 $W \cdot kg^{-1}$, while the remaining 60% of the test consisted of self-paced periods where the participant cycled at levels prescribed to be 'recovery', 'hard', 'accelerating' or 'decelerating' (figure 4). Participants were instructed to closely follow the autonomous M-VCT protocol displayed on the computer screen placed in front of the experimental bike which prompted them to work, sprint and/or rest when needed during each lap. The participants were provided no verbal encouragement throughout the trials; however, participants were told of the number of laps and number of "hard" work sections remaining at regular intervals throughout the testing protocol.

Short M-VCT familiarization sessions were completed following adequate recovery from the incremental exercise test (during the same visit). This aimed to introduce participants to the dynamic nature of the M-VCT protocol. During this visit, participants completed 2-3 laps (12-18-min) of the protocol before cooling down for 5-min at 0.5 $W \cdot kg^{-1}$. Published work by Abbiss et al. (2008) indicates that a single familiarization session is sufficient to obtain a high reliability of mean power output during a dynamic time-trial, where subsequent trials are performed within 7 days of each other. Further, work by Sharma et al. (2014) shows that a single familiarization session of the VCT was enough to demonstrate good reliability for power output during the protocol. Therefore, the subsequent sessions for this investigation were completed within a week, but no less than 72 hours after completing a short familiarization session.

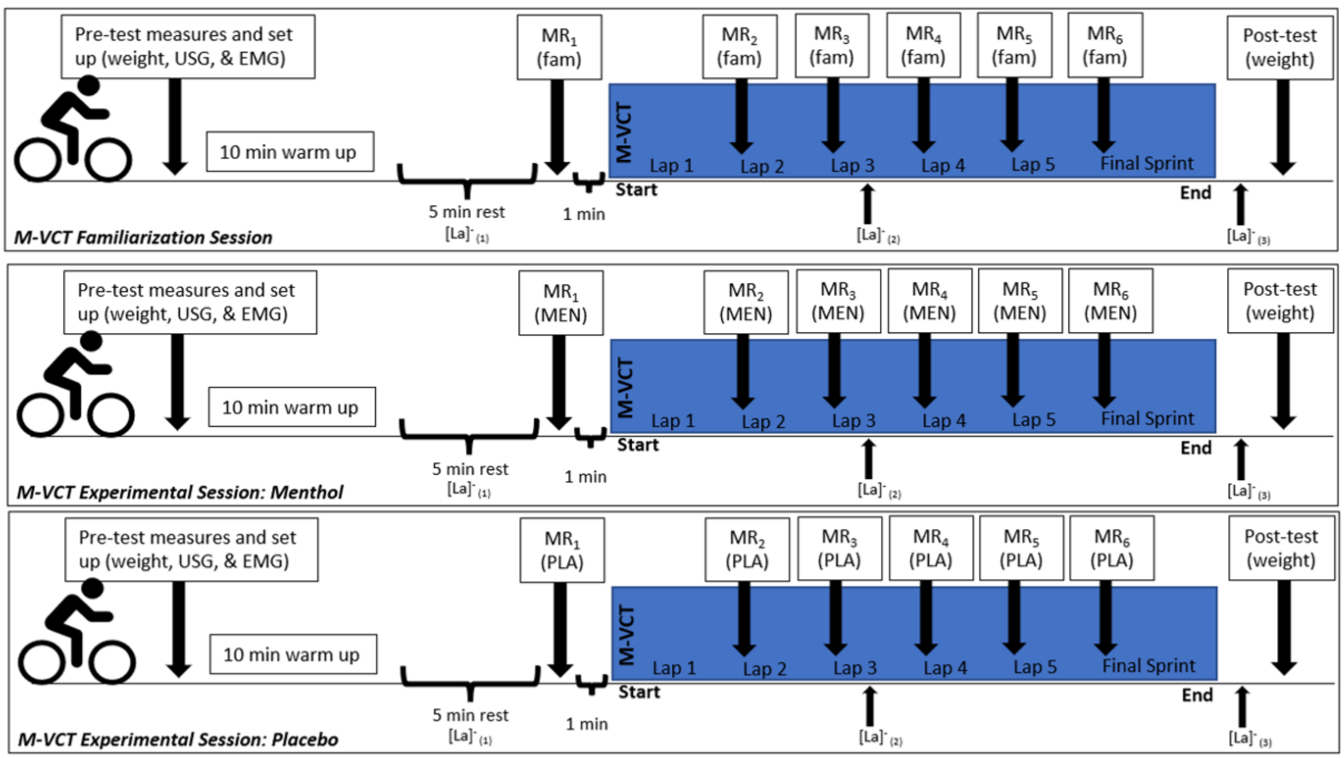
The full M-VCT was performed a total of five times during this study (1x heat familiarization, 2x during reliability testing, 2x during experimental testing). During these sessions, participants completed 5 laps (31-min) of the protocol before cooling down for 5-min at 0.5 $W \cdot kg^{-1}$. Physiological and psychological parameters were assessed throughout.

4.4.4 Mouth Rinse Intervention

Participants were given 25 millilitres (mL) of solution to rinse on six (6) occasions during the experimental sessions. The first rinse occurred 60-sec prior to beginning the M-VCT, at regular 6-min intervals (beginning of lap 2,3,4,5), and finally 30-sec prior to the final sprint at the end of lap 5. Subjects were instructed to swill the rinse in the oral cavity for 5-sec before spitting it into a container. No solution was swallowed. Distilled water was utilized as a rinse during the heat familiarization session. L-Menthol solution was formulated from 0.1-g of menthol crystals (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) dissolved in 1-L of de-ionized water heated to 40°C (Flood et al., 2017) to form a rinse at a concentration of 0.64 mM.

The 0.01% L-menthol solution was stored for a maximum of 3-wks at approximately 20°C. A placebo solution was made using 1-L of de-ionized water and a non-caloric berry flavoured sweetener consisting of sucralose (Crystal Light, Don Mills, ON). The solutions were colour matched using a blue non-caloric edible food colouring (Club House, McCormick & Company, CA). At time of use, solutions were warmed to room temperature or >22°C to prevent TRPM8 receptor stimulation by cold liquid (Brauchi et al., 2004; Voets et al., 2004).

Figure 6. Menthol mouth rinse M-VCT study design. Each participant participated in a total of three experimental heat trials (31.4 ± 0.9 °C, 23.4 ± 3.7% relative humidity); one familiarization and two experimental. A mouth rinse was used at six points in time throughout each trial.



4.5 Measurements

4.5.1 Power output (watts and W·kg^{-1}) and cadence (rpm)

Power output and cadence were measured continuously throughout the test using LODE ergometry manager software (version 10, LODE, Groningen, the Netherlands). Total trial mean power and cadence, mean power and mean cadence per lap, and mean power and mean cadence during “acceleration” and “hard” sections of the M-VCT were utilized in analysis of work rate.

4.5.2 Core temperature, heart rate, and sweat loss

Core temperature was measured continuously throughout the trial in real-time using a non-invasive, wearable body temperature sensor (CORE, greenTEG AG, Switzerland). Heart rate (HR) was recorded by the second using a downloadable chest sensor, Polar H7 (Polar® H7 heart sensor, Polar, USA). Sweat loss was calculated as change from pre- to post-exercise body mass with adjustment for fluid intake or urine production and under the assumption that 1 kg of body mass change was equal to 1 L of sweat loss. Sweat loss was determined by: *Sweat loss (g)= [change in body weight (g) + 0.20 g kcal*$^{-1}$ *+ fluid intake (g) – (urine + fecal output) (g)]*

4.5.3 RPE, Thermal Sensation and Thermal Comfort

In line with American College of Sports Medicine guidelines (ACSM, 1998), participants were instructed to pay close attention to how difficult the exercise activity felt during the M-VCT. RPE was measured using a 6–20-point scale and was applied to assess the participants subjective effort level during the exercise task (where 6= no exertion and 20= maximal exertion; Borg, 1982). TS was measured using a 7-point numerical scale (-3 to +3; where -3= cold and +3= Hot) and required participants to rate how they felt about the thermal environment in the laboratory (ASHRAE, 2004). TC was also measured using a 7-point numerical scale (-3 to +3; where -3= much too cool and +3= much too warm) and was used to rate how the environmental temperature impacted a participant’s physical response to the exercise task (ASHRAE, 2004; Fanger, 1970). RPE, TS, and TC were recorded prior to starting the exercise protocol, directly after each MR, as well as immediately following completion of the M-VCT.

4.5.4 Rating of Fatigue and Feeling Scale

ROF was measured using a 0–10-point scale (where 0= not fatigued at all and 10= total fatigue and exhaustion – nothing left) and was used to assess how tired participants felt within an exercise context (Micklewright et al., 2017). FS was measured using an 11-point numeric scale (-5 to +5; where -5= very bad and +5= very good) where participants were instructed to rate their affective state during the exercise task within the environment (Hardy & Rejeski, 1989). ROF and FS were recorded prior to starting the exercise protocol, directly after each MR, as well as immediately following completion of the M-VCT.

4.5.5 Blood Lactate

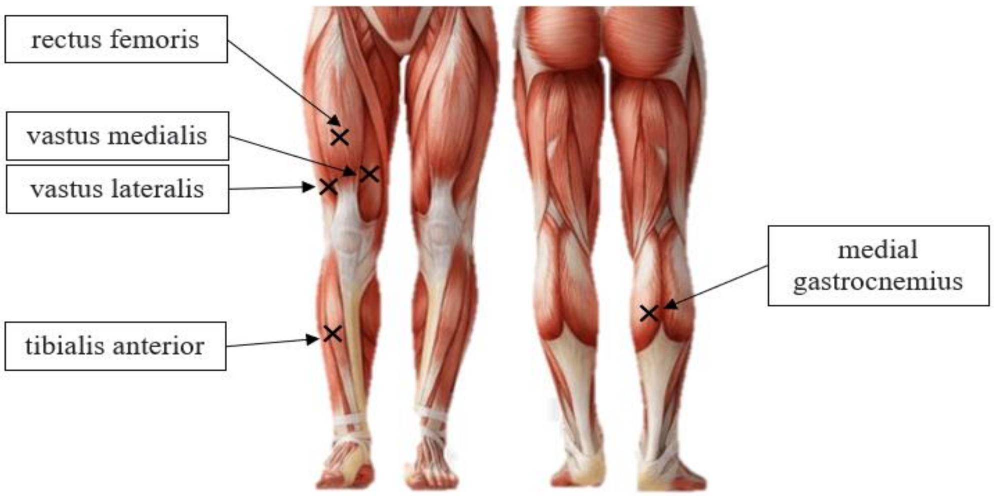
Blood lactate concentration was measured at three points during each M-VCT test (Figure 6) from a small (3μL) capillary blood sample using a portable lactate analyzer (EDGE Lactate Analyzer, The EDGE, USA), whose reliability has been previously established (Bonaventura et al., 2015). A sample was collected following warm-up after a 5 min rest (prior to starting the testing procedure), at the mid-point of the protocol (16-min mark during a recovery segment), and finally, 3-min after finishing the test (to ascertain peak values) (Goodwin et al., 2007). Lactate levels were assessed relative to power and HR, as a biomarker marker for fatigue within the exercising muscle.

4.5.6 Surface Electromyography (sEMG)

Wireless surface electromyography was used to assess neuromuscular function throughout the M-VCT (Liu et al., 2019). Prior to mounting the bike, while in a seated position, each participant's right leg was prepared for the placement of 5 surface electrodes. First, the skin was landmarked, and muscle location(s) were identified. Excess hair that may occlude the muscles signal was then removed, followed by using an alcohol swab to eliminate surface oils and other contaminants from the skin. Each sensor was then placed along the longitudinal midline of the desired muscle with the arrow parallel to the muscle fibers and secured using hypafix self-adhesive tape. Surface EMG was collected unilaterally from 5 muscles using wireless bipolar electrodes from a Delsys Trigno system (Natick, MA, USA). Signals were sampled at 2000Hz through Delsys EMGworks software (Natick, MA, USA) before export for analysis in a custom LabVIEW program (National Instruments, Austin, TX, USA). Sensors were placed on the tibialis anterior (TA), medial gastrocnemius (GA), rectus femoris (RF), vastus medialis (VM), and vastus lateralis (VL) on the right limb (Figure 7). Muscular activity was measured continuously throughout the protocol to assess the relationship between muscular activation and fatigue during the 10 sec "hard" work sections per lap.

The data was band pass filtered with a dual pass 2nd order Butterworth filter from 20-500Hz. EMG amplitude (aEMG) was calculated using a 0.15s RMS window and averaged over the duration of the time trial or sprint. Although muscle activation naturally trends on and off during cycling, averaging over the entirety of the trial was a desirable way to investigate slow time-series and fatigue-related changes rather than intra-limb activation times (Kajee et Al., 2010). This resulted in smoother data for analysis between participants. aEMG was normalized to each participant's individual peak activation level during the sprint at the end of the session (Rouffet & Hautier, 2008). aEMG amplitudes were compared between PLA and MEN.

Figure 7. Identification of EMG sensor sites on the right limb. Anterior muscles include rectus femoris (RF), vastus medialis (VM), vastus lateralis (VL), and tibialis anterior (TA). Posterior muscle is medial gastrocnemius (GA).



4.5.7 Statistical Analysis

Data analysis was performed using SPSS (version 28; IBM Corp., Armonk, NY, USA) statistical software, with statistical significance set a priori at $p \leq 0.05$. Following assessment for normal distribution, all data were described using means and standard deviations (SD). Paired samples t-tests were then used to assess single parameter differences between trial conditions as well as between performance variables for both Part 1 and Part 2 of this study.

Test-retest data were analyzed using a statistical model recommended for reliability studies (Stewart & Hopkins, 2000), in which the typical error of measurement is estimated after changes in the mean between repeated measurements have been controlled for. Data were log-transformed for the analysis because this approach yields variability as a percent of the mean (coefficient of variation, CV%), which is the natural metric for most measures of athletic performance (Hopkins, 2000). The CV% was then used to calculate the least significant change (LSC) with 95% confidence per variable using the equation $1.96\ (CV/\sqrt{n}) \times \sqrt{2}$, where n = number of trial replicates. This value served as the reference point to be exceeded before a raw change in performance value can be considered true (Jeffries et al., 2018). Changes in performance were then considered practically meaningful if the mean response exceeded the variability of the test (meaning the magnitude of change is large enough to be considered meaningful in real life). Pearson's correlation coefficient with 95% confidence intervals was also used to determine relationships between variables from the test-retest session including mean power ($W \cdot kg^{-1}$), peak power ($W \cdot kg^{-1}$), mean cadence (RPM), total trial distance (km), heart rate, core temperature, blood lactate concentration, and each perceptual scale.

Two-way repeated measures analyses of variance (ANOVA) were used to assess the effects of time, trial condition, and time x trial condition interactions for all physiological variables. Multivariate analysis of variance (MANOVA) was used to assess the relationship between condition and time, and condition x time on each muscle for EMG activity. Where sphericity could not be assumed a Greenhouse–Geisser correction was applied. Significant differences in main effects were further analyzed through a comparison of simple main effects with the Bonferroni post-hoc correction factor. Time versus condition ordinal data were evaluated using the Friedman Test, and singular differences were detected using the Wilcoxon Signed Ranks Test, set at $p= 0.008$. Exact *p*-values, standardized mean differences (Cohen's d), and 95% confidence intervals are presented to show magnitude of effect. Effect sizes were interpreted as per the recommendations outlined by Hopkins et al. (2009), where trivial, small, moderate, and large effects were <0.2 SD, between 0.2–0.6 SD, 0.6–1.2 SD and >1.2 SD, respectively. Finally, individual responses were calculated via the difference between the mean response rate and the variability of the test, thus representing the threshold for improved performance. Responses were classified as no response (0) (≤0.00%), minimal (1) (0.01-0.99%), moderate (2) (1.0-2.0%), or high (3) (≥2.1%).

4.6 Results

4.6.1 Part 1: Reliability Testing

M-VCT test-retest reliability (n=4)

Trial Conditions

During the test–retest M-VCT sessions, there was no significant difference in laboratory temperature (°C) (Trial 1 (T1), 20.5 ± 1.7°C; Trial 2 (T2), 20.0 ± 1.4°C, p=0.495) and relative humidity (%RH) (T1, 62.0 ± 3.7%; T2, 64.5 ± 1.3%, p=0.312). All athletes arrived in a euhydrated state for both sessions with mean USG <1.020 (T1, 1.010 ± 0.006; T2, 1.010 ± 0.006, p=0.84). Athletes also arrived at the sessions 'well rested' based on self-reported sleep quality, with no significant differences found in the number of hours spent sleeping the night prior to the test day (T1, 8.75 ± 0.96 hrs; T2, 8.88 ± 1.44 hrs, p=0.789).

Sweat Rate

Sweat loss was significantly different between trials, with greater pre-to-post body mass loss during T2 (T1, 0.750 ± 0.041 L; T2, 1.000 ± 0.147 L, p=0.038).

M-VCT Reliability

The means±SD for each variable measured during the M-VCT is presented in Table 4. Mean power output ($W \cdot kg^{-1}$) was not significantly different between trials (T1, 2.99 ± 0.50 $W \cdot kg^{-1}$; T2, 3.01 ± 0.48 $W \cdot kg^{-1}$, p=0.083). The calculated coefficient of variability (CV) between the two trials was 0.46%, with low random error (Figure 8). Calculation of the LSC (smallest change needed for the effect to be considered real) revealed that the M-VCT could detect a 0.90% change in mean power with 95% confidence based on the CV observed over two trials. Analysis of average power output ($W \cdot kg^{-1}$) during "acceleration" (6-sec) and "hard" (10-sec) work sections revealed no significant difference between trials and demonstrated high reliability (T1-Accel, 3.62 ± 0.58 W·kg^{-1}; T2-Accel, 3.64 ± 0.55 W·kg^{-1}, r=0.99, *p*=0.295, CV%=0.67%; T1-Hard, 7.48 ± 1.50 W·kg^{-1}; T2-Hard, 7.51 ± 1.50 W·kg^{-1}, r=0.98, *p*=0.646, CV%=1.55%). Additionally, no significant difference was found in mean power output (W·kg^{-1}) between trials during the final sprint (T1-sprint, 8.67 ± 1.44 W·kg^{-1}; T2-sprint, 8.86 ± 1.37 W·kg^{-1}, r=0.76, *p*=0.719, CV%=9.03%). Furthermore, there was no significant difference between test and re-test sessions for any other performance variable including total mean cadence (RPM) (*p*=0.109), heart rate (bpm) (*p*=0.086), core temperature (°C) (*p*=0.971), lactate concentration (mmol/L) (*p*=0.269), total distance covered (km) (*p*=0.234), or perceptual measurements (RPE, *p*=0.082; TS, *p*=0.527; ROF, *p*=0.299; FS, *p*=0.278). The highest CV was found for rating of thermal sensation between trials (CV%=13.11%), whilst the lowest Pearson's correlation was found for core temperature between trials (r=0.45).

Table 4. Mean values (n=4) of physiological and performance variables measured during test and re-test of the M-VCT

Variable	Trial 1	Trial 2	Mean difference (T2-T1)	r *. P<0.05 **. P<0.01	ICC	TEM	Mean Δ (%)	CV%	LSC (95%)	*p* value
Total mean power ($W \cdot kg^{-1}$)	2.99 ± 0.50	3.01 ± 0.49	0.02 ± 0.02	1.00** (1.00, 1.00)	1.00 (0.99,1.00)	0.01 (0.01-0.04)	0.76	0.46	0.90%	0.083
"acceleration" mean power ($W \cdot kg^{-1}$)	3.62 ± 0.57	3.65 ± 0.55	0.03 ± 0.03	1.00** (0.93, 1.00)	0.99 (0.97,1.00)	0.02 (0.01-0.08)	0.67	0.67	1.31%	0.295
"hard" mean power ($W \cdot kg^{-1}$)	7.48 ± 1.50	7.51 ± 1.50	0.03 ± 0.14	0.98* (0.19, 1.00)	0.98 (0.74, 1.00)	0.1 (0.05-0.36)	0.47	1.55	3.04%	0.646
Final sprint mean power ($W \cdot kg^{-1}$)	8.67 ± 1.44	8.86 ± 1.37	0.19 ± 0.98	0.76 (-0.79, 0.99)	0.76 (-0.36, 0.98)	0.69 (0.39-2.57)	2.27	9.03	17.69%	0.719
Total mean cadence (RPM)	82.24 ± 7.43	86.00 ± 6.94	3.76 ± 3.32	0.90 (-0.58, 1.00)	0.89 (0.07, 0.99)	2.35 (1.33-8.76)	4.66	2.92	5.72%	0.109
Trial distance (km)	10.17 ± 0.60	10.22 ± 0.55	0.05 ± 0.06	0.99** (0.88, 1.00)	0.99 (0.92, 1.00)	0.04 (0.02-0.16)	0.47	0.45	0.88%	0.234
Blood Lactate (mmol/L)	9.41 ± 2.25	10.43 ± 0.79	1.02 ± 1.50	0.97* (-0.99, 1.00)	0.60 (-0.59, 0.97)	1.06 (0.60-3.97)	12.99	2.56	5.02%	0.269
Heart Rate (bpm)	158.25 ± 5.50	159.67 ± 10.86	1.42 ± 6.36	0.90 (-0.56, 0.99)	0.73 (-0.42, 0.98)	4.50 (2.55-16.76)	0.77	2.77	5.43%	0.686
Core Temperature (°C)	37.86 ± 0.42	37.88 ± 0.13	0.02 ± 0.37	0.45 (-0.91, 0.98)	0.26 (-0.80-0.93)	0.26 (0.15-0.98)	0.05	0.70	1.37%	0.971
RPE (Borg)	15.08 ± 2.14	14.28 ± 1.54	-0.80 ± 0.62	0.99** (0.74, 1.00)	0.95 (0.39, 0.99)	0.44 (0.25-1.64)	4.99	2.66	5.21%	0.082
Thermal Sensation	2.11 ± 0.36	1.98 ± 0.35	-0.13 ± 0.35	0.52 (-0.90, 0.99)	0.52 (-0.66, 0.96)	0.25 (0.14-0.92)	6.09	13.11	25.69%	0.527
Rating of Fatigue	6.73 ± 0.67	6.33 ± 0.54	-0.40 ± 0.64	0.46 (-0.91, 0.98)	0.45 (-0.71, 0.95)	0.45 (0.26-1.68)	5.85	7.37	14.44%	0.299
Feeling Scale	1.05 ± 2.41	1.43 ± 2.47	0.38 ± 0.68	0.98** (0.82, 1.00)	0.99 (0.96, 1.00)	0.13 (0.08-0.50)	6.24	7.45	14.60%	0.278

The data and mean difference are expressed as means±SD, Pearson's r values are expressed as mean (95% confidence intervals), mean change (Δ) expressed as a %, and CV is expressed as a %. LSC calculated as 1.96 (CV/√n) x √2, where n = number of trial replicates. r, Pearson coefficient; CV, coefficient of variation; ICC, intraclass correlation; TEM, typical error of measurement; RPE, rating of perceived exertion; LSC, least significant change at 95% confidence; exact *p* values, $p < 0.05$*; W/kg^{-1}, watts per kilogram; RPM, revolutions per minute; km, kilometers; mmol/L, millimoles per litre blood; bpm, beats per minute; °C, degrees Celsius.

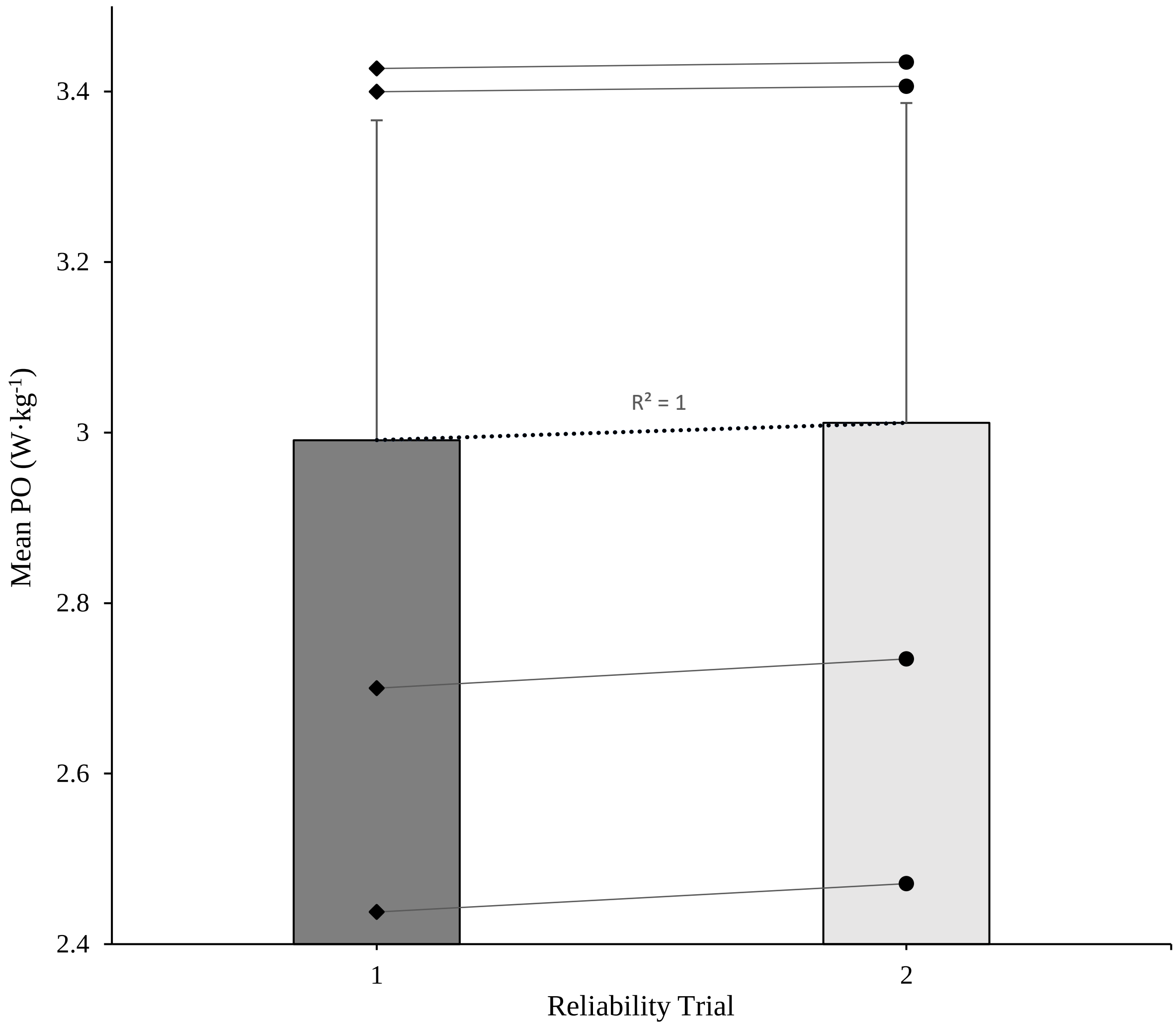


Figure 8. Reliability of power output (W/kg^{-1}) in the modified variable cycling test (M-VCT) pilot sessions. Data are means±SD. Total trial mean power was not significantly different between trial 1 and trial 2 (*p*=0.102). Individual mean power (n=4) is overlaid on group mean power data. Individual variability (coefficient of variation (CV) percentage (%)) in M-VCT performance ranges from 0.09 to 0.68%, or a mean % change of 0.18 – 1.36%.

4.6.2 Part 2: Performance Testing

M-VCT performance in the heat with a MEN MR in adolescent male athletes (n=11) *(Table 5)*

Trial Conditions

No significant differences existed in laboratory environmental conditions between the MEN and PLA trials (MEN, 31.34 ± 0.68°C, PLA, 31.35 ± 0.61°C *p*=0.961; MEN, relative humidity 23.55 ± 2.46%, PLA, 23.27 ± 2.76%, *p*=0.588). Additionally, there was no significant difference in mouth rinse temperature between MEN and PLA conditions (MEN, 25.16 ± 1.26 °C, PLA, 25.96 ± 1.73 °C, *p*=0.182). All athletes arrived at the laboratory in a euhydrated state with USG<1.020 (MEN, 1.004 ± 0.004; PLA, 1.005 ± 0.004, *p*=0.012). Athletes self-reported no differences in the quality and amount of sleep obtained the night prior to the MEN and PLA trials (MEN, 7.64 ± 1.03 hrs, 7.64 ± 1.14 hrs, *p*>0.05).

Sweat Rate

Total body sweat loss, measured via pre-to-post trial body mass change (in kg), indicated no significant difference between MEN and PLA conditions (MEN, 0.88 ± 0.43 L; PLA, 0.83 ± 0.38 L, *p*=0.402).

M-VCT Performance and Menthol

Distance Completed

All data sets were normally distributed, and no order effect was observed between first and second trials (*p*=0.763). Total distance (m) completed during the M-VCT increased over time and was significantly greater in the MEN condition compared to PLA (MEN, 21,810 ± 3,809 m; PLA, 21,477 ± 3,693 m, *p*=0.002, 95% CI= [156.51 to 508.34], ES=1.27), respectively, representing a 1.1 ± 1.0% increase in total distance covered when corrected for test-retest reliability. Sub-analysis of cumulative distance covered per lap (m) revealed a significant difference during lap 3 (p=0.046), lap 4 (p=0.008), and lap 5 (p=0.002) between conditions, with greater distance completed for MEN.

Power Output (PO) – Mean, 6 and 10 second averages

Mean power output (PO_{mean}) for the entire M-VCT was significantly higher in the MEN condition by 1.81 ± 1.57% (this is the corrected % change based on the reliability data in Part 1) when compared to PLA (MEN, 177.8 ± 31.4 W; PLA, 174.7 ± 30.5 W, p<0.001, 95% CI= [1.73 to 4.46], ES=1.53, Figure 9). Mean power output was further analyzed according to 6-second "acceleration" (PO_{avg_6}) and 10-second "hard" (PO_{avg_10}) work sections throughout the M-VCT under both conditions. PO_{avg_6} for MEN was significantly higher when compared to PLA (MEN, 272.8 ± 73.5 W; PLA, 258.8 ± 61.8 W, p=0.041, 95% CI= [0.73 to 27.19], ES=0.71, Figure 9). Similarly, PO_{avg_10} was significantly different between MEN and PLA, with higher power output for MEN (MEN, 710.3 ± 157.8 W; PLA, 687.1 ± 150.8 W, p=0.002, 95% CI= [11.08 to 35.22], ES=1.29, Figure 9).

A significant difference was found between mean power per lap (PO_{lap_avg}) between MEN and PLA conditions (MEN, 173.4 ± 29.7 W; PLA, 170.4 ± 28.8 W, p<0.001, 95% CI= [1.66 to 4.40]). Post-hoc testing revealed that PO_{lap_avg} was significantly higher for MEN during lap 3 of the M-VCT (p=0.003, 95% CI= [1.69 to 6.41], ES=1.15), but not significantly different during any other laps. Further analysis of PO_{lap_avg} during "acceleration" (PO_{lap_6}) and "hard" work sections (PO_{lap_10}) indicate that mean power output was significantly higher for MEN when compared to PLA; specifically, PO_{lap_6} was significantly higher in lap 3 (p=0.029, 95% CI= [2.48 to 36.16]) and lap 4 (p=0.019, 95% CI= [3.59 to 32.43]) for MEN, while PO_{lap_10} was only significantly higher in MEN during lap 3 only (p=0.008, 95% CI= [11.42 to 59.05]) (Figure 10). PO_{lap_avg} was not affected by time under either condition (MEN, p=0.614; PLA, p=0.264).

Lastly, there was no significant difference in mean power during the final sprint of the M-VCT following lap 5 (PO_{sprint}) between conditions (MEN, 972.9 ± 234.9 W; PLA, 952.9 ± 216.5 W, *p*=0.191, 95% CI= [-11.79 to 51.79], ES=0.42) suggesting that MEN MR may have more of a central rather than peripheral effect on fatigue amelioration.

In Part 2, the performance difference between MEN and PLA for PO_{avg} was determined to be an unadjusted improvement of 2.24 ± 1.67%. By applying Part 1 test-retest M-VCT CV% results (0.46%) to the improvement change found in Part 2, the true change in performance between MEN and PLA conditions in Part 2 is 1.81 ± 1.57%. Since this change is greater than the LSC% (0.90%) of the M-VCT, it can be concluded with 95% confidence that this is a meaningful change that occurred in response to MEN. When analyzed further, the difference during PO_{avg_6} and PO_{avg_10} sections between MEN and PLA were 6.77 ± 9.43% and 3.64 ± 3.03%, respectively. Following a correction for the test-retest CV% of "acceleration" (0.67%) and "hard" (1.55%) sections, the true change in performance between conditions is 6.19 ± 9.53% in PO_{avg_6} and 2.39 ± 2.95% in PO_{avg_10}. Since the observed true change is greater than the calculated LSC% (1.31%) in the M-VCT for "acceleration" in the group, it can be concluded with 95% confidence that this was also a meaningful change in response to MEN. On the other hand, because the group LSC% was determined to be 3.04% for "hard" sections, it cannot be determined with confidence that this performance change really resulted from MEN.

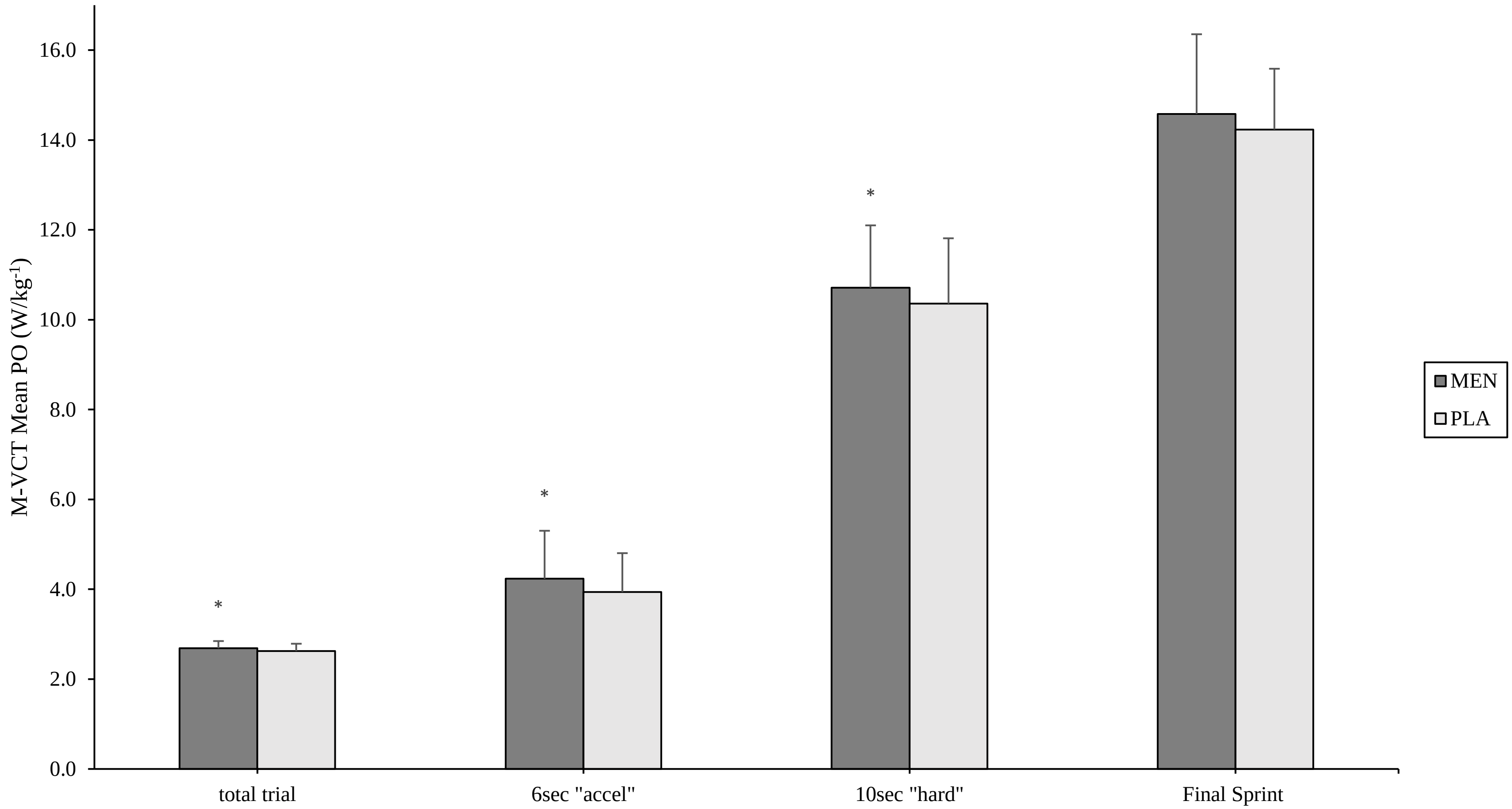


Figure 9. Mean power output (PO) during the M-VCT displayed according to key performance sections. Data are means ± SD (n=11). Total trial PO (p=<0.001), total "acceleration" PO (p=0.041), and total "hard" PO (p=0.002) were significantly greater in the MEN condition. No significant difference was found during the final sprint (p=0.191). **Significantly greater in the MEN trial (p<0.05).* MEN, menthol condition; PLA, placebo condition; W, watts; kg, kilogram.

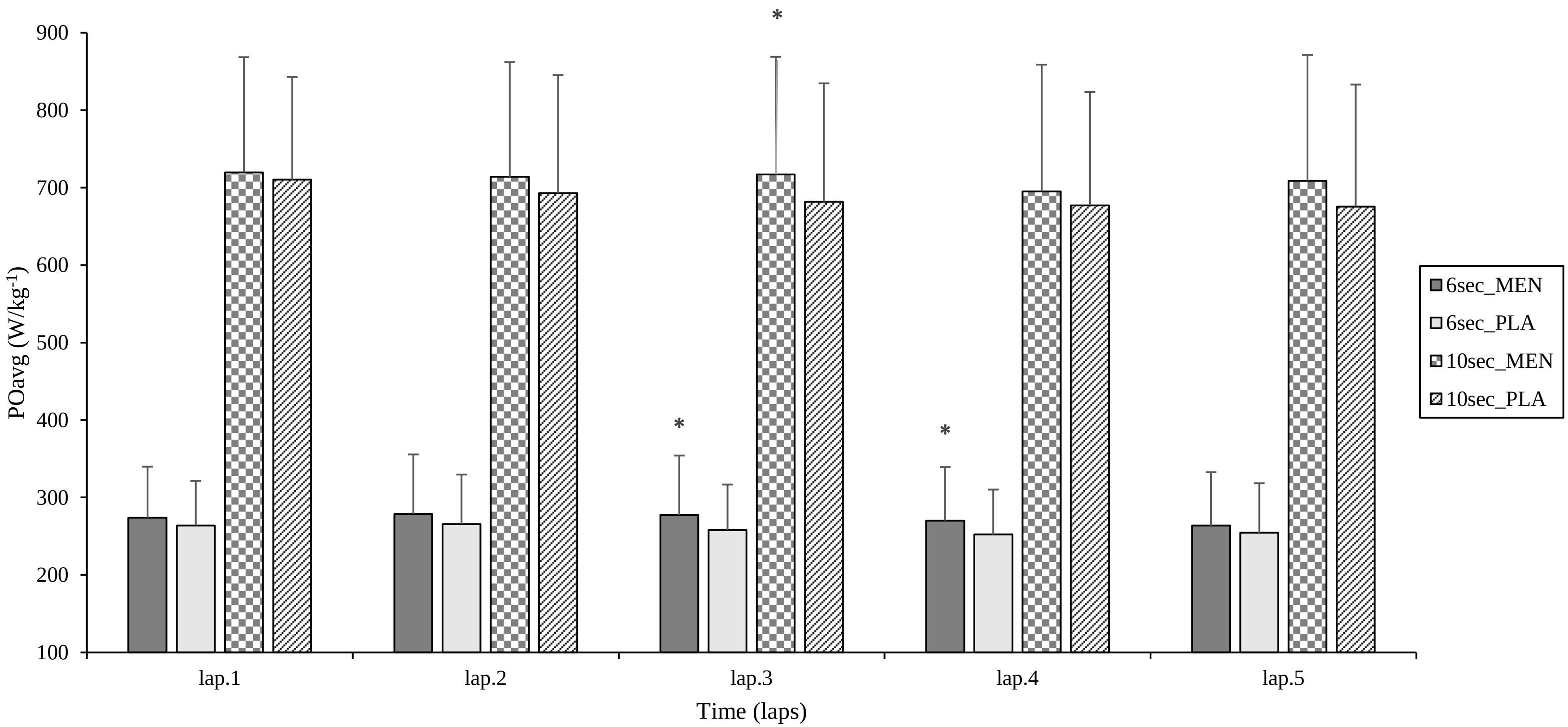


Figure 10. Mean PO during key performance sections per lap of the M-VCT. Data are means ± SD (n=11). PO was significantly greater in the MEN condition for "acceleration" sections during lap 3 and 4 (p=0.029, p=0.019). PO was significantly greater in the MEN condition for "hard" sections during lap 3 (p=0.008). **Significantly greater in the MEN trial (p<0.05).*

Peak Power Output (PPO) – 1 second

While absolute 1-second peak power output across the entire trial (PPO_{trial}) was slightly higher for MEN, the difference was not significant (MEN, 1032.5 ± 217.4 W, PLA, 964.2 ± 178.7 W, *p*=0.095, 95% CI= [-14.23 to 150.77], ES=0.56). PPO was also not significantly different during "acceleration" (PPO_6), "hard" (PPO_{10}), or the final sprint (PPO_{sprint}) between conditions (PPO_6. MEN, 459.0 ± 144.4 W, PPO_6. PLA, 428.4 ± 95.8 W, *p*=0.576, 95% CI= [-87.34 to 148.61], ES=0.174; PPO_{10}. MEN, 1032.5 ± 217.4 W, PPO_{10}. PLA, 964.2 ±178.7 W, *p*=0.095, 95% CI= [-14.23 to 150.77], ES=0.56; PPO_{sprint}. MEN, 1156.3 ± 269.8 W, PPO_{sprint}. PLA, 1146.4 ± 284.9 W, *p*=0.561, 95% CI= [-26.83 to 46.65], ES=0.18), respectively.

Cadence (RPM)

Despite a decline in average cadence over time for both conditions (*p*>0.05), total mean cadence (RPM_{avg}) was significantly different between conditions, with higher cadence being maintained during the MEN trial (MEN, 87.7 ± 4.8 rpm; PLA, 84.7 ± 5.0 rpm, *p*=0.010, 95% CI= [0.90 to 5.10], ES= 0.96). When compared per lap between conditions, cadence was significantly higher during lap 4 (*p*=0.003) and lap 5 (*p*=0.050) for MEN. Average cadence was also significantly higher for MEN during "acceleration" (RPM_{avg_6}) and "hard" (RPM_{avg_10}) sections ($RPM_{avg\_6.}$ MEN, 92.6 ± 11.7 rpm; PLA, 90.8 ± 10.8 rpm, *p*=0.045, 95% CI= [0.05 to 3.59], ES=0.69; $RPM_{avg\_10.}$ MEN, 107.55 ± 9.46 rpm; PLA, 106.00 ± 8.85 rpm, *p*=0.005, 95% CI= [0.58 to 2.51], ES=1.07). Post-hoc analysis revealed a significant difference between conditions for mean cadence per lap during "acceleration" (RPM_{lap_6}) and "hard" (RPM_{lap_10}) sections. RPM_{lap_6} was higher in lap 4 (*p*=0.033) while RPM_{lap_10} was higher in lap 3 (*p*=0.032) for MEN. No significant difference was found in average cadence during the final sprint between conditions (MEN, 124.46 ± 10.36 rpm, PLA, 124.64 ± 10.34 rpm, *p*=0.877, 95% CI= [-2.73 to

2.36], ES=0.05). Moreover, the use of MEN demonstrates a significant effect on the maintenance of cadence in the later stages of the M-VCT, despite lower neuromuscular activation and higher power output.

Cardiovascular and thermoregulatory responses

HR increased over time during each trial (MEN, $p<0.001$, PLA, $p=0.002$), however total mean HR was not significantly different between conditions (MEN, 164.6 ± 14.9 bpm, PLA, 163.4 ± 15.6 bpm, $p=0.208$, 95% CI= [-0.83 to 3.38], ES=0.41). HR also did not differ between trials when analyzed by lap averages ($p=0.128$, 95% CI= [-0.55 to 3.75]) (Figure 11). Maximum HR, although slightly higher for MEN, did not differ significantly between conditions (MEN, 186.5 ± 12.3 bpm, MEN, 185.8 ± 11.6 bpm, $p=0.451$, 95% CI= [-1.17 to 2.45], ES=0.24).

Similarity, Tc increased over time during each trial (MEN, $p=0.003$, PLA, $p<0.001$) with a significant change occurring across all laps except between the start and lap 1 ($p=0.122$), the start and lap 2 ($p=0.100$), and lap 1 and lap 2 ($p=0.360$) for both conditions. Trial mean Tc was not significantly different between conditions (MEN, 37.58 ± 0.25 °C, PLA, 37.69 ± 0.35 °C, $p=0.258$, 95% CI= [-0.34 to 0.10], ES=0.36) and Tc did not differ between trial conditions when analyzed by lap averages ($p=0.244$, 95% CI= [-0.34 to 0.10]) (Figure 11). Although maximum Tc was slightly higher during the PLA trials when compared to MEN, there was also no significant difference (MEN, 38.06 ± 0.30 °C, PLA, 38.11 ± 0.48 °C, $p=0.647$, 95% CI= [-0.31 to 1.20], ES=0.14).

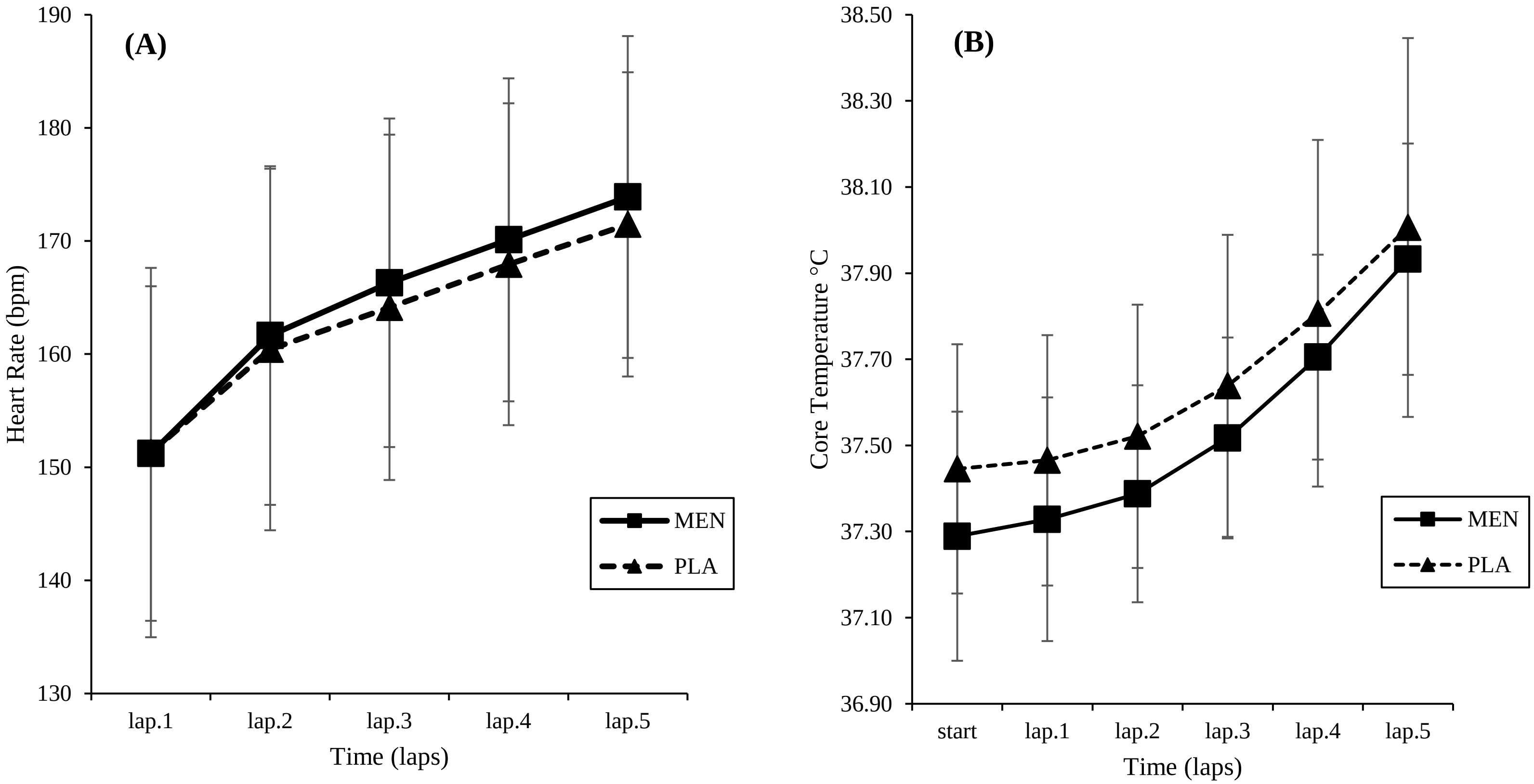


Figure 11. The relationship between heart rate (HR) (A), core temperature (Tc) (B), and laps completed in the modified variable cycling test (M-VCT). Data are means ± SD (n=11). HR increased over time in both conditions, but no significant difference was found between trials (p=0.208). TC increased over time in both conditions, but no significant difference was found between trials (p=0.258).

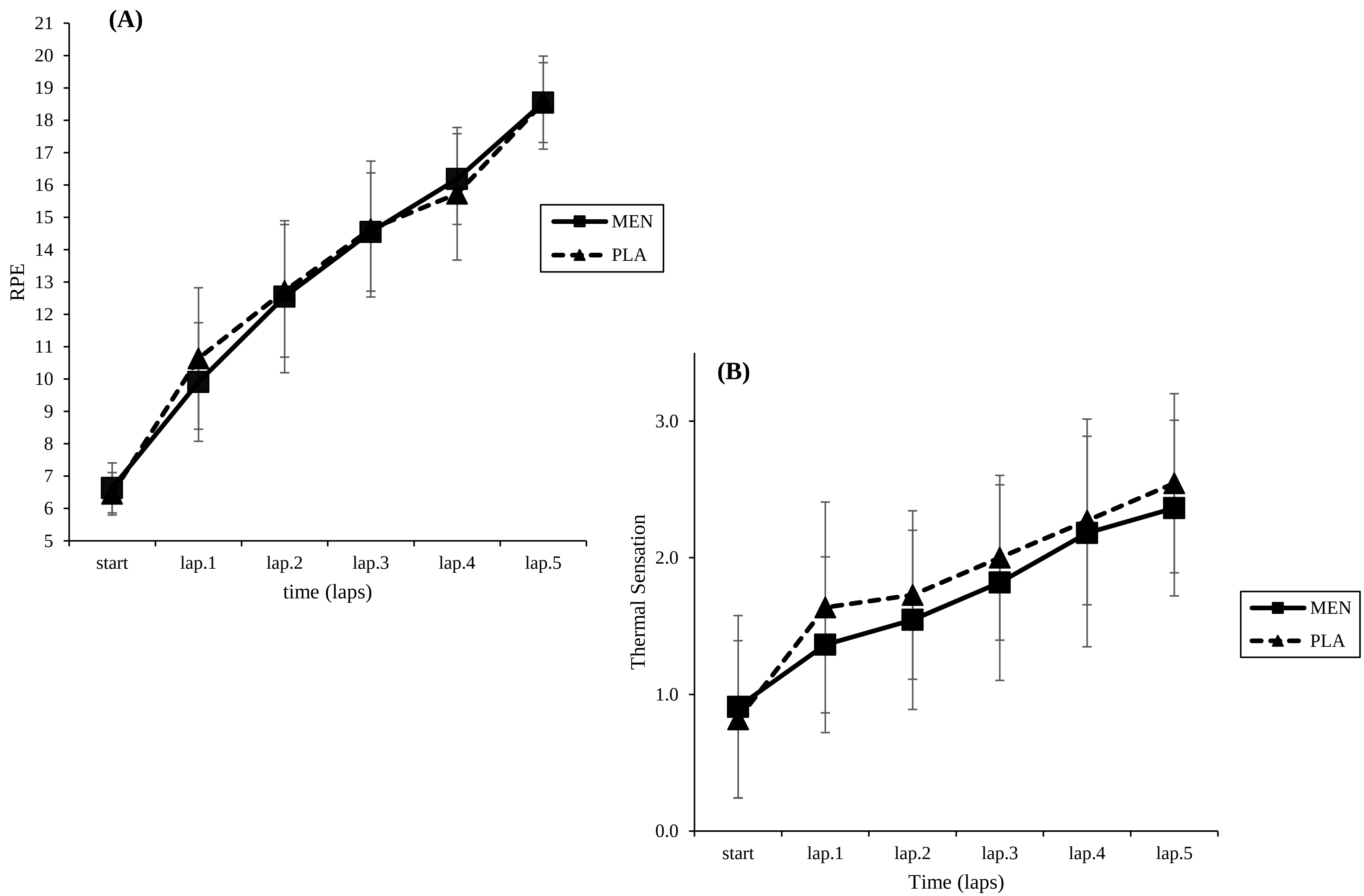
Blood Lactate Concentration

Blood lactate concentration increased significantly from the pre- to mid-trial measurement during both the MEN and PLA trials (pre-MEN, 1.71 ± 0.77 mmol, mid-MEN, 9.21 ± 2.31 mmol, p<0.001, 95% CI= [-8.91 to -6.08], ES=3.56; pre-PLA, 1.61 ± 0.55 mmol, mid-PLA, 8.09 ± 1.75 mmol, p<0.001, 95% CI= [-7.65 to -5.32], ES= 3.73), but no significant change occurred between the mid- to post-trial measurement for either condition (mid-MEN, 9.21 ± 2.31 mmol, post-MEN, 8.87 ± 2.37 mmol, p=0.685, 95% CI= [-1.46 to 2.13], ES=0.13; mid-PLA, 8.09 ± 1.75 mmol, post-PLA, 8.76 ± 2.45 mmol, p=0.291, 95% CI= [-2.02 to 0.67], ES= 0.34). When lactate was compared between MEN and PLA at pre-, mid-, and post-trial measures, no significant difference was found between pre- (p=0.576, 95% CI= [-0.29 to 0.48]) and post- (p=0.871, 95% CI= [-1.35 to 1.57]), but a significant difference in mid-lactate was found between trials, with mid-lactate reaching a higher concentration for MEN (MEN, 9.21 ± 2.31 mmol, PLA, 8.09 ± 1.75 mmol, p=0.041, 95% CI= [0.59 to 2.18], ES=0.71). Furthermore, no significant difference in total mean lactate concentration existed between conditions (p=0.158, 95% CI= [-0.21 to 1.09]).

Perceptual Scales

RPE increased significantly from the start of the trial to the end of lap 5 for both trial conditions (MEN, 6.6 ± 0.8 vs 18.6 ± 1.3, p=<0.001; PLA, 6.5 ± 0.7 vs 18.6 ± 1.5, p=<0.001). Mean RPE was not significantly different between trials (MEN, 13.06 ± 1.47, PLA, 13.12 ± 1.66, p= 0.965) and a pairwise comparison per lap showed no significant difference in RPE measurement at any time point between conditions (p>0.05 across all laps) (Figure 12). Thermal comfort (TC) significantly increased from the start of the trial to the end of lap 5 for both conditions (MEN, 0.27 ± 0.47 vs 2.09 ± 0.83, p=<0.001; PLA, 0.36 ± 0.51 vs 2.59 ± 0.49,

$p=<0.001$). Average TC was not significantly different between trials (MEN, 1.38 ± 0.52, PLA, 1.61 ± 0.38, $p= 0.123$) and a pairwise comparison per lap showed no significant difference in TC measurement at any time point between conditions ($p>0.05$ across all laps) (Figure 12). Likewise, TS significantly increased from the start of the trial to the end of lap 5 under both conditions (MEN, 0.91 ± 0.70 vs 2.36 ± 0.67, $p=<0.001$; PLA, 0.82 ± 0.60 vs 2.55 ± 0.69, $p=<0.001$). Average TS was not significantly different between trials (MEN, 1.69 ± 0.59, PLA, 1.84 ± 0.49, $p=0.138$) and again, a pairwise comparison per lap revealed no significant difference in TS measurement at any time point between conditions ($p>0.05$ across all laps) (Figure 12). Rating of Fatigue (ROF) significantly increased from the start of the trial to the end of lap 5 under both conditions (MEN, 2.00 ± 1.00 vs 7.73 ± 1.35, $p=<0.001$; PLA, 2.09 ± 1.38 vs 7.55 ± 1.44, $p=<0.001$). Mean ROF was not significantly different between trials (MEN, 5.06 ± 1.10, PLA, 4.95 ± 1.29, $p=0.878$) and a pairwise comparison per lap showed no significant difference in ROF measurement at any time point between conditions ($p>0.05$ across all laps) (Figure 12). Finally, Feeling Scale (FS) decreased significantly from the start of the trial to the end of lap 5 under both conditions (MEN, 2.64 ± 1.75 vs 0.00 ± 3.26, $p=<0.001$; PLA, 2.91 ± 1.45 vs 0.18 ± 3.22, $p=<0.001$). Mean FS was not significantly different between trials (MEN, 1.37 ± 2.19, PLA, 1.46 ± 2.06, $p=0.562$) and a pairwise comparison per lap revealed no significant difference in FS measurement at any time point between conditions ($p>0.05$ across all laps) (Figure 12).


(A)
RPE
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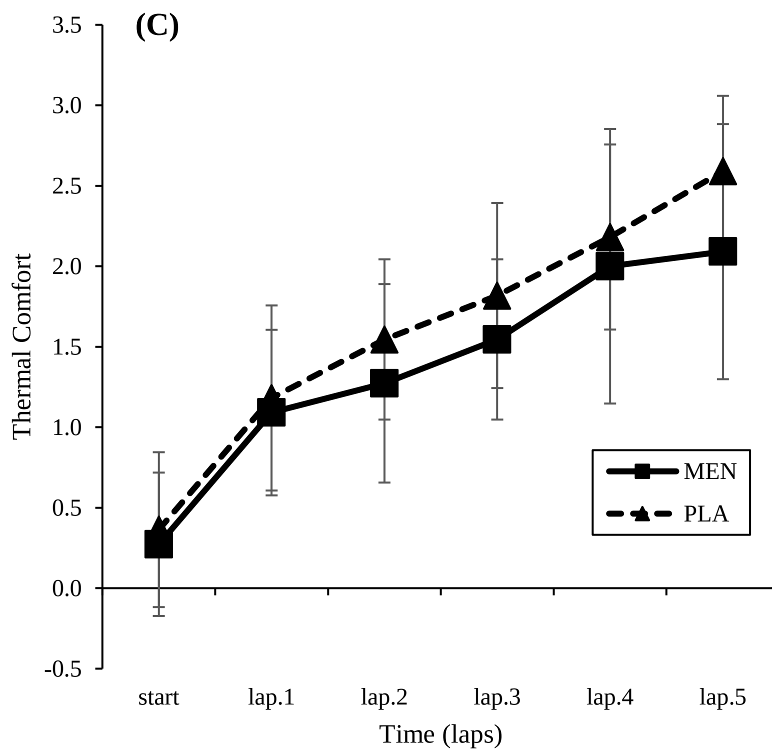
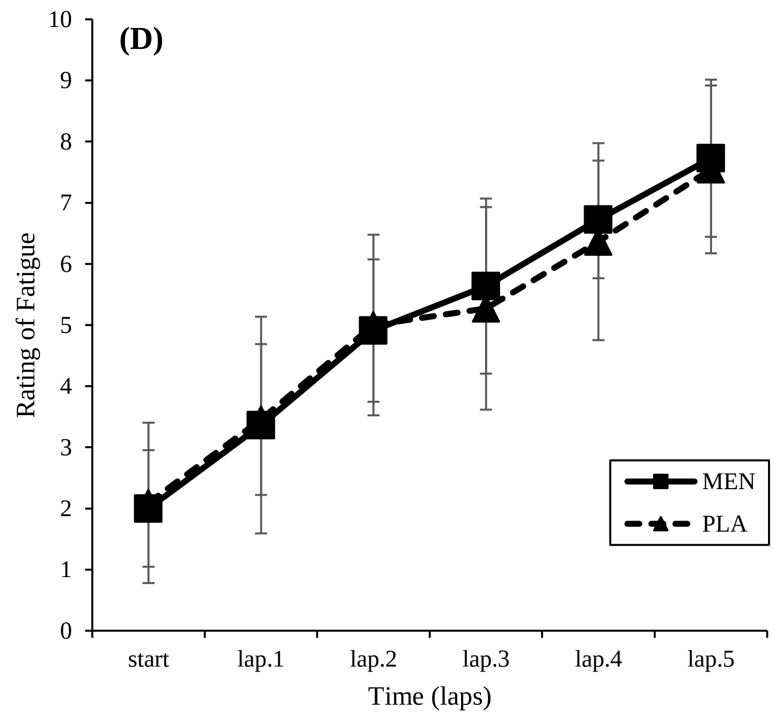
lap.3
lap.4
lap.5
time (laps)
MEN
PLA
(B)
Thermal Sensation
3.0
2.0
1.0
0.0
start
lap.1
lap.2
lap.3
lap.4
lap.5
Time (laps)
MEN
PLA


(C)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
Thermal Comfort
start
lap.1
lap.2
lap.3
lap.4
lap.5
Time (laps)
MEN
PLA


(D)
10
9
8
7
6
5
4
3
2
1
0
Rating of Fatigue
start
lap.1
lap.2
lap.3
lap.4
lap.5
Time (laps)
MEN
PLA

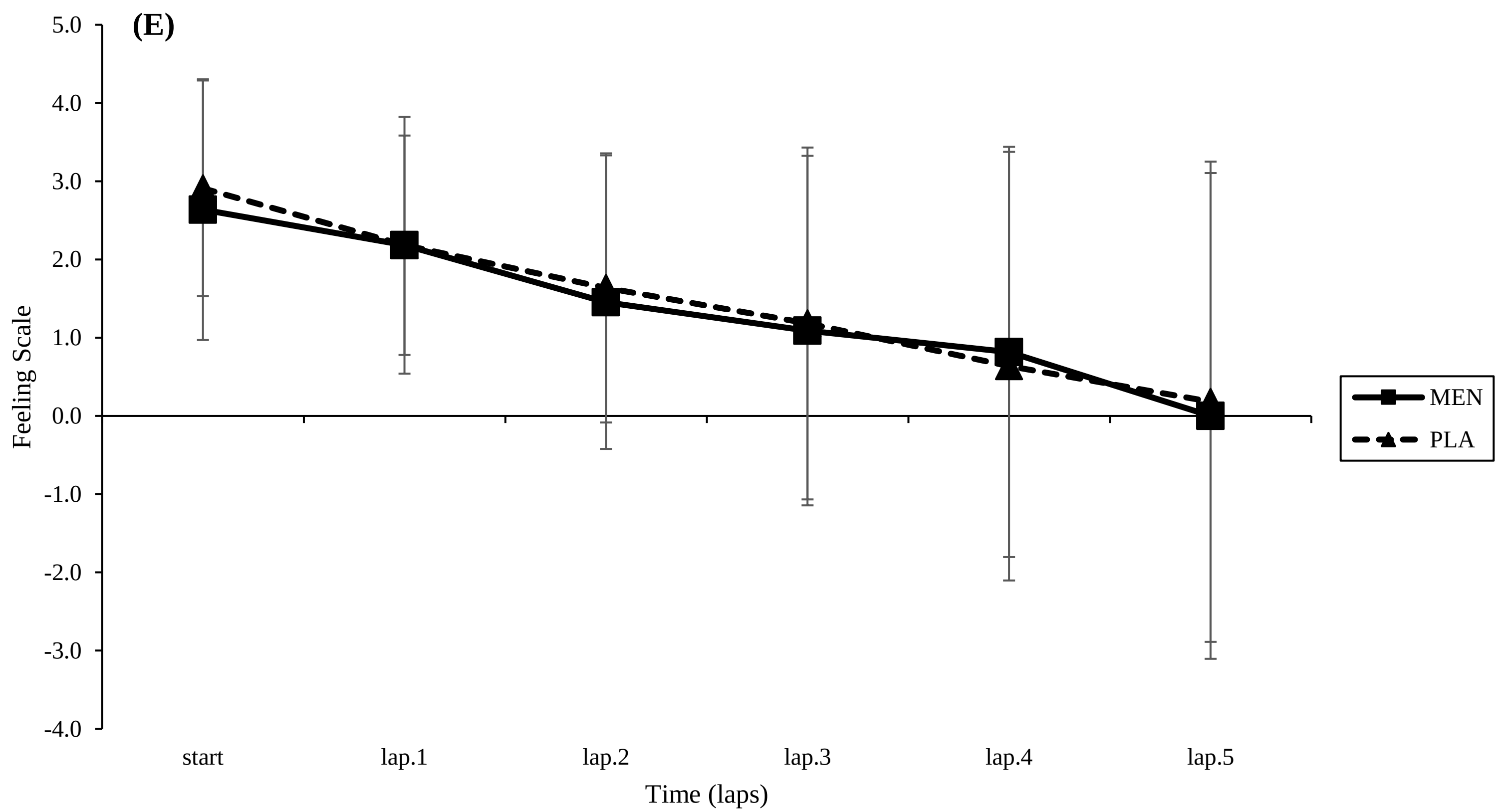


Figure 12. Relationship between rating of perceived exertion (RPE) (A), thermal sensation (TS) (B), thermal comfort (TC) (C), rating of fatigue (ROF) (D), feeling scale (FS) (E) and laps (time) during the modified variable cycling test (M-VCT). Data are means ± SD (n=11). RPE increased significantly from the beginning of the trial to the end. There were no significant differences in RPE between MEN and PLA across laps (p>0.008). TS gradually increased from the beginning to the end of each trial. There were no significant differences in TS between MEN and PLA across laps (p>0.008). TC gradually increased (increased discomfort) from the beginning to the end of each trial. There was no significant difference in TC between MEN and PLA across laps (p>0.008). ROF increased from the beginning to the end of each trial. ROF was not significantly different between MEN and PLA across laps (p>0.008). FS gradually decreased from the beginning of the trial to the end, however there were no differences between MEN and PLA (p>0.008).

Surface Electromyography (sEMG)

Five lower extremity derived EMG measurements (rectus femoris (RF), vastus medialis (VM), vastus lateralis (VL), tibialis anterior (TA), and medial gastrocnemius (GA)) were obtained during MEN and PLA trials. Mean and peak amplitude values per lap were derived by combining all 10 sec "hard" work sections per lap and final sprint data (10 sec x 3 "hard" efforts for 5 laps + 10 sec final sprint).

A significant main effect was observed for mean EMG amplitude ($aEMG_{mean}$) across all laps (p=0.002, ES=0.20), whereby $aEMG_{mean}$ decreased over time, relative to the starting point for both conditions when compared to work rate. $aEMG_{mean}$ did increase during the final sprint, but this change was not significant when compared between conditions (p=0.240, ES=0.66). Post-hoc analysis revealed a significant effect of lap time on all muscles (VL, p=0.010; RF, p=0.017; GA, p=0.003; TA, p=0.002), except VM (p=0.088). No significant difference was found for $aEMG_{mean}$ between MEN and PLA conditions (p=0.388, ES=0.20) for each muscle measurement (VL, p=0.254; RF, p=0.180; VM, p=0.652; GA, p=0.993; TA, p=0.988). Finally, the interaction effect between conditions across laps for each muscle was also not significant (p=0.381, ES=0.11) (Figure 13). Visually, $aEMG_{mean}$ data presents a trend between MEN and PLA conditions. Although not significant, neuromuscular activation appears to be slightly lower with MEN across each muscle despite higher cadence and increased power production (figure 13). This result demonstrates the complex interconnection between peripherally and centrally mediated factors known to influence performance and confirms that fatigue was higher in the PLA trial.

A significant main effect was observed for peak EMG amplitude ($aEMG_{peak}$) across all laps (p=0.001, ES=0.21), whereby $aEMG_{peak}$ decreased over time, relative to the starting point for both conditions. Again, $aEMG_{peak}$ increased during the final sprint, but this change was not significant when compared between conditions (*p*=0.311, ES=0.62). Post-hoc analysis revealed a significant effect of lap time on all muscles (VL, *p*=0.013; RF, *p*=0.005; GA, *p*=0.025; TA, *p*=0.011), except VM (*p*=0.240). No significant difference was found for $aEMG_{peak}$ between MEN and PLA conditions (*p*=0.639, ES=0.42) for each muscle measurement (VL, *p*=0.262; RF, *p*=0.303; VM, *p*=0.560; GA, *p*=0.972; TA, *p*=0.547). Finally, the interaction effect between conditions across laps for each muscle was also not significant (*p*=0.698, ES=0.09).

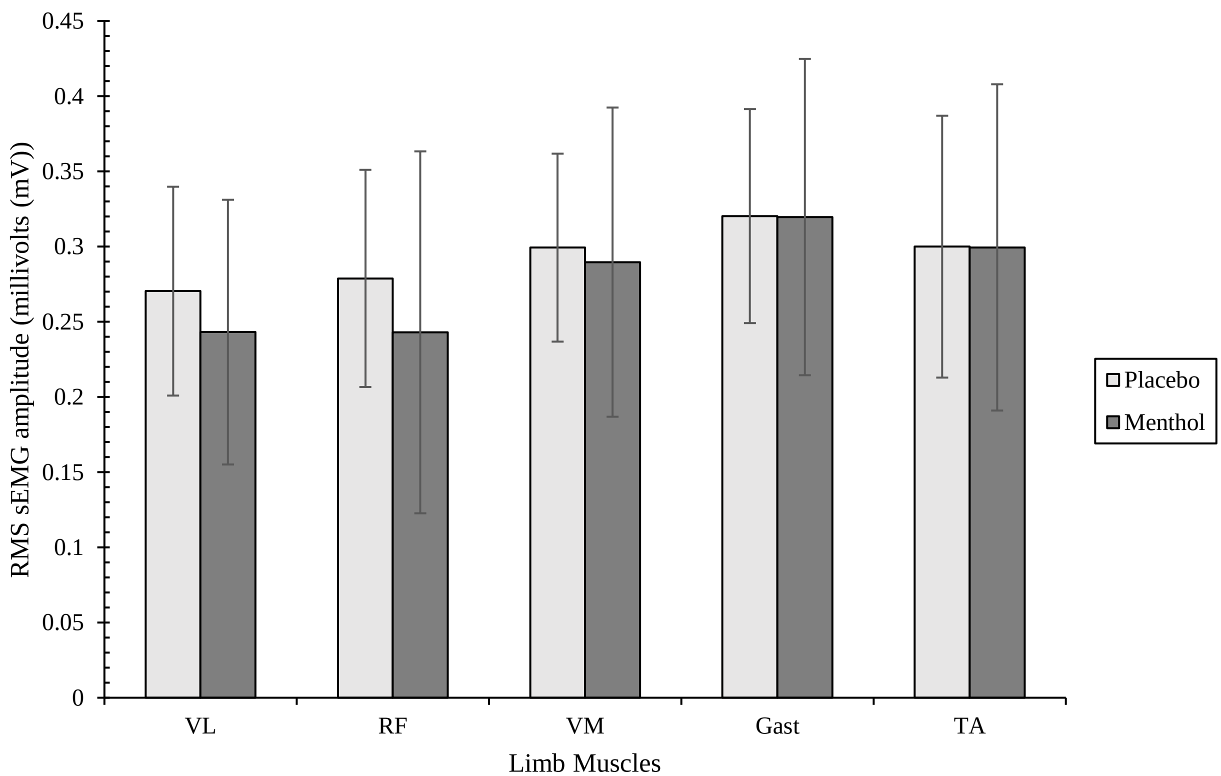


Figure 13. Mean surface electromyography amplitude (aEMG) during hard work sections of the M-VCT per muscle. Data are means ± SD (n=10). aEMG was not significantly different between conditions for any muscle (*p*=0.240).

Individual Responses to Menthol

Pilot testing of the M-VCT protocol demonstrated high reliability in performance outcomes between test-retest sessions in this population group. Mean CV% for total average PO ($W \cdot kg^{-1}$) between reliability sessions was 0.46%, while "acceleration" and "hard" sections were determined to be 0.67% and 1.55%, respectively. CV% values were then used to calculate the least significant change (LSC%), or the percent threshold for real change. If an athlete's true or corrected performance change (%Δ) between MEN and PLA trials was found to exceed this a priori LSC% value, then the improvement in performance was concluded to be real. To explain, individual response rates to menthol in this homogenous population sample were analyzed by comparing the percent mean change between the MEN and PLA trials to individual test-retest CV% (n=4) and mean test-retest CV% (n=7) corrected values. This allowed for the determination of each participant's true difference in performance for the M-VCT. This corrected value was then compared against the LSC% to determine if the true %Δ could be attributed to MEN. The true %Δ in performance for each athlete was then compared against the mean %Δ of 1.8%. Athletes were classified as being above or below this average. Finally, participants were then grouped according to the magnitude of change in their performance (Δ) as no response (≤0.00%), minimal (0.01-0.99%), moderate (1.0-2.0%), or high (≥2.1%).

Based on the information above, analysis of total trial average PO ($W \cdot kg^{-1}$) per athlete indicates that all individuals were positive responders to MEN to some degree. To be precise, each athlete's true percent change in performance (%Δ) between MEN and PLA was found to exceed either their individual test-retest CV% (n=4) or the group mean test-retest CV% of 0.46% (n=7). While all values were positive, it cannot be said with confidence that this change resulted from MEN for all athletes (because the true %Δ needs to also exceed the LSC%). Comparison of the true (corrected) %Δ values (which ranged from a minimum of 0.29% to a maximum of 5.06%) to the LSC% clarified that 7/11 (64%) participants were "true" positive responders to the intervention. Of these 7 true responders, 5 participants demonstrated %Δ greater than the mean change of 1.8% and 2 fell below. Lastly, based on the magnitude of true %Δ, 4/11 participants were high responders, 2/11 were moderate, 1/11 was minimal, and 4/11 were "non-responders" to MEN (Table 6, Figure 14).

This calculation process was then repeated for 6 sec "acceleration" and 10 sec "hard" work sections as follows. Individual response rates during the "acceleration" and "hard" sections of the M-VCT were corrected for, using individual CV% (n=4) or mean CV% (n=7), to determine the true difference in work capacity between MEN and PLA. Analysis of average "acceleration" PO ($W \cdot kg^{-1}$) demonstrated that 9/11 athletes were true positive responders to MEN, with improvement ranging from 2.0% to 23.4% in 6 second acceleration sections. Of these 9 athletes, 6 exceeded the mean improvement of 6.2%, while 3 fell below this average for 6 second accelerations. Analysis of average "hard" PO ($W \cdot kg^{-1}$) revealed that 8/11 athletes were positive responders to MEN, with improvement ranging from 0.2% to 6.7%. Of these 8 athletes, 5 were true positive responders and exceeded the mean improvement of 2.4%, while 3 fell below the average for 10 sec hard sections. Moreover, this section highlights the large individual variability in the response to MEN mouth rinsing during the stochastic nature of the M-VCT in trained adolescent male athletes. In totality, this analysis shows that MEN appears to be beneficial to some athletes while also being ineffective or harmful to others.

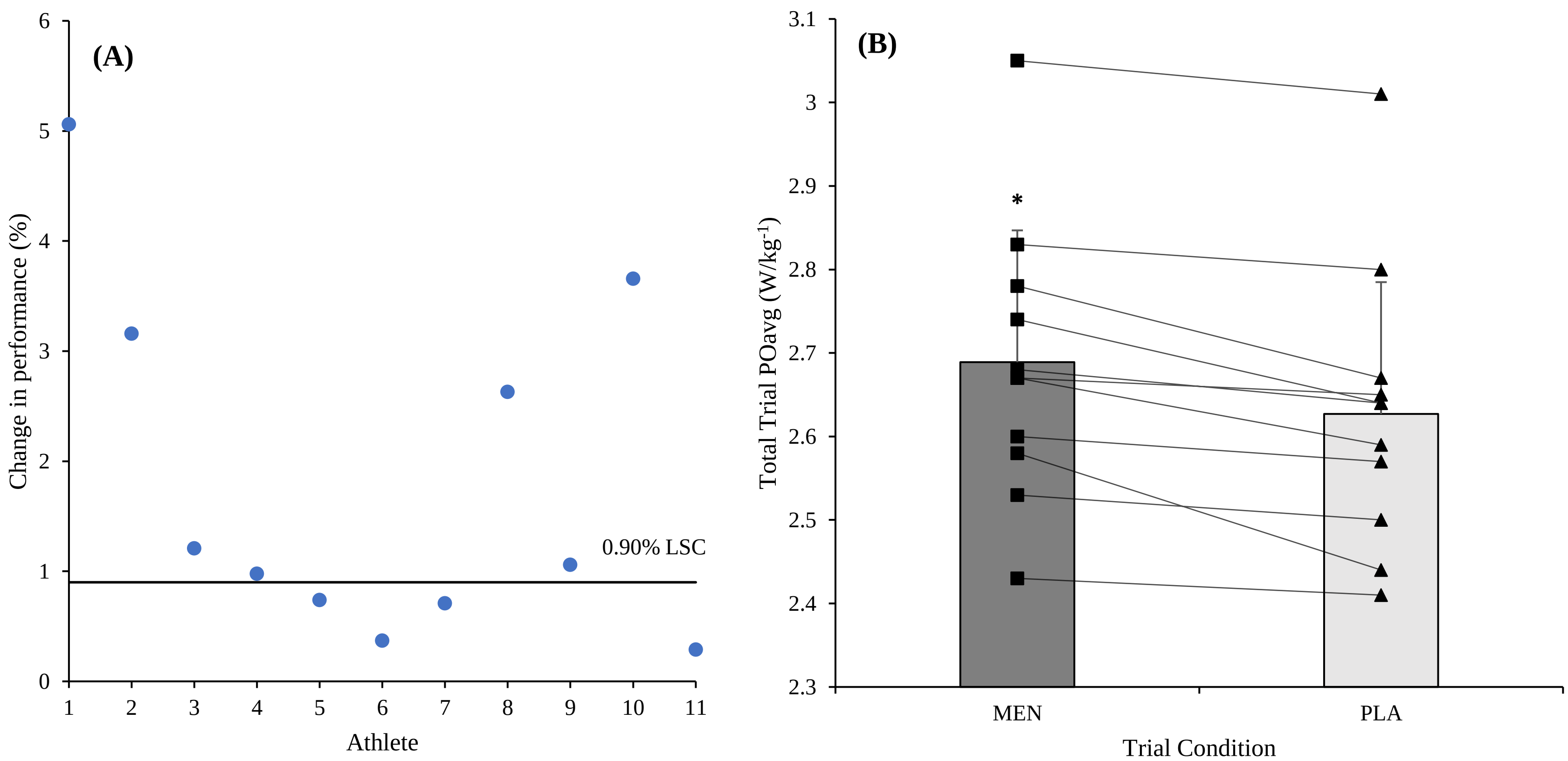


Figure 13. Individual (A) and Group (B) responses to MEN. Displays individual true % improvement between MEN and PLA, relative to the 0.90% LSC threshold. 7/11 athletes surpassed this threshold, indicating with 95% confidence that the improvement was due to MEN. (B) Displays individual and group responses to MEN vs PLA. Mean PO was significantly higher with MEN MR (p<0.001). **Significantly greater in the MEN trial (p<0.05).*

Table 5. Total mean values of physiological and performance variables measured during the menthol (MEN) and placebo (PLA) trials of the modified variable cycling test (M-VCT) (n=11).

Variable	MEN	PLA	*p* value	95% CI	ES
Total mean power (W)	177.8 ± 31.4	174.7 ± 30.5	<0.001*	[1.73 to 4.46]	1.53
6 sec “acceleration” mean power (W)	272.8 ± 73.5	258.8 ± 61.7	0.041*	[0.73 to 27.19]	0.71
10 sec “hard” mean power (W)	710.3 ± 157.8	687.1 ± 150.8	0.002*	[11.08 to 35.22]	1.29
Final sprint mean power (W)	972.9 ± 234.8	952.9 ± 216.5	0.191	[-11.79 to 51.79]	0.42
1-sec Peak Power (PPO) (W)	1032.4 ± 217.3	964.2 ± 178.7	0.095	[-14.23 to 150.77]	0.56
6 sec “acceleration” PPO (W)	459.0 ± 144.4	428.4 ± 95.8	0.576	[-87.34 to 148.61]	0.17
10 sec “hard” PPO (W)	1032.5 ± 217.4	964.2 ±178.7	0.095	[-14.23 to 150.77]	0.56
Final sprint PPO (W)	1156.3 ± 269.8	1146.4 ± 284.9	0.561	[-26.83 to 46.65]	0.18
Total mean cadence (RPM)	87.7 ± 4.8	84.7 ± 5.0	0.010*	[0.90 to 5.10]	0.96
6 sec “acceleration” mean cadence (RPM)	92.6 ± 11.7	90.8 ± 10.8	0.045*	[0.05 to 3.59]	0.69
10 sec “hard” mean cadence (RPM)	107.6 ± 9.5	106.0 ± 8.9	0.005*	[-2.51 to -0.58]	1.07
Final sprint mean cadence (RPM)	124.5 ±10.4	124.6 ± 10.3	0.877	[-2.73 to 2.36]	0.05
Trial distance (m)	21810.4 ± 3808.9	21477.9 ± 3693.1	0.002*	[1.73 to 4.46]	1.27
Blood Lactate (mmol/L)	6.6 ± 1.5	6.2 ± 1.3	0.158	[-0.21 to 1.09]	0.46
Heart Rate (bpm)	164.6 ± 14.9	163.4 ± 15.6	0.208	[-0.83 to 3.38]	0.41
Core Temperature (°C)	37.6 ± 0.2	37.7 ± 0.4	0.258	[-0.34 to 0.10]	0.36
RPE (Borg)	13.1 ± 1.5	13.1 ± 1.7	0.965	N/A	N/A
Thermal Sensation	1.7 ± 0.6	1.84 ± 0.5	0.138	N/A	N/A
Thermal Comfort	1.4 ± 0.5	1.6 ± 0.4	0.123	N/A	N/A
Rating of Fatigue	5.1 ± 1.1	4.95 ± 1.29	0.878	N/A	N/A
Feeling Scale	1.4 ± 2.2	1.5 ± 2.1	0.562	N/A	N/A

Data are means ± SD. *$p<0.05$. MEN, menthol; PLA, placebo; 95% CI, 95% confidence interval; ES, Cohen’s D effect size estimate; W, watts; PPO, peak power output; Sec, seconds; RPM, revolutions per minute; m, meters; mmol/L, millimoles per litre blood; bpm, beats per minute; °C, degrees Celsius.

Table 6. True difference in the modified variable cycling test (M-VCT) performance and individual responsivity to menthol (MEN) using total mean power output (Watts (W)/kilogram (kg)) (n=11).

Athlete	CV% T1 vs T2	M-VCT LSC%	%Δ MEN vs PLA	True %Δ	True %Δ Relation to LSC% (+ / -)	↑ / ↓ 1.8% mean performance Δ	MEN Response
1	0.68	1.33	5.74	5.06	+	↑	3
2	0.63	1.23	3.79	3.16	+	↑	3
3	0.12	0.24	1.33	1.21	+	↓	2
4	0.09	0.18	1.07	0.98	+	↑	1
5	0.46	0.90	1.20	0.74	-	↓	0
6	0.46	0.90	0.83	0.37	-	↓	0
7	0.46	0.90	1.17	0.71	-	↓	0
8	0.46	0.90	3.09	2.63	+	↑	3
9	0.46	0.90	1.52	1.06	+	↓	2
10	0.46	0.90	4.12	3.66	+	↑	3
11	0.46	0.90	0.75	0.29	-	↓	0

Data is expressed as percent difference (%) between trial 1 and trial 2, and MEN and PLA. True percent change (%Δ) is calculated as [(%Δ MEN vs PLA) – (CV% T1 vs T2)]. Above (↑) or below (↓) 1.8% mean performance Δ indicates if individual change in performance is greater than or less than the mean improvement value. MEN response is classified for true %Δ as no response (0) (≤0.00%), minimal (1) (0.01-0.99%), moderate (2) (1.0-2.0%), or high (3) (≥2.1%). White rows = athletes who completed test retest (n=4) and have and individual CV% for T1 vs T2. Grey rows = athletes who did not complete reliability testing and have the mean CV% applied for T1 vs T2. CV%, Coefficient of Variation percent. Mean Reliability CV% = 0.46%. MEN, menthol; PLA, placebo; CV, coefficient of variation; %, percent; Δ, change; T1, trial 1; T2, trial 2; LSC, least significant change; +, above the LSC; -, below the LSC; ↑, above mean value; ↓, below mean value.

4.7 Discussion

The current study aimed to assess and compare the effects of menthol (MEN) mouth rinsing (MR) (0.01%) on performance and perception during a modified variable cycle test (M-VCT) compared to a placebo (PLA) rinse, after defining the reliability and threshold required for significant performance change in this population group. To our knowledge, not only was this the first investigation to determine the test-retest reliability of mean power output and other physiologic parameters during a stochastic cycling protocol in a small homogenous group of trained adolescent male athletes, but this was also the first investigation to then apply the predetermined correction factor to the performance change measured during heat testing with the use of a likely ergogenic intervention within the same homogenous athlete sample. Principal results of this two-part study suggest that (1) the M-VCT provides a reliable measure of mean power output in adolescent cyclists (p=0.083, CV=0.46%) and (2) M-VCT performance in the heat is improved by MEN when compared to a PLA MR (p<0.001), as demonstrated by moderate to large differences in power output between conditions in the later stages of the M-VCT (ES=0.71 to 1.53). The results also demonstrate that (3) thermoregulatory responses (HR and Tc) along with perceptual measures (RPE, TS, TC, ROF, FS) are not different between conditions despite a higher mean power output with MEN (p>0.05). Lastly, (4) neuromuscular function measured by EMG is not affected by MEN even with the adoption of a higher work rate (p>0.05).

Test Reliability of the M-VCT

The present study demonstrates that the M-VCT is a reliable predictor of mean power output in trained adolescent athletes. The coefficient of variation (CV%) between test-retest sessions for the total trial was determined to be ~0.5%, while variation increased slightly to

0.67% and 1.55% following isolation of power output during 6 sec “acceleration” and 10 sec “hard” work sections, respectively. Importantly, pilot testing of the protocol in this athlete population highlights the ability of this test to detect small (>0.9%) changes in total trial performance with a high level of confidence. Although the reliability of variable power protocols has not been extensively studied, these results are consistent with findings from Sharma et al. (2014) who reported a CV of 1.24% for total mean power output for the 1-hour VCT in trained adult athletes as well as increased variability during self-selected open-ended sections (CV=1.98% and 3.38%) of the protocol. Since it has been reported that power output CV during a testing protocol tends to decrease as intensity increases but increases as a function of testing time, perhaps this justifies a CV for the M-VCT of approximately half of that for the original 1-hour VCT protocol. These findings are also supported by work completed by Abbiss et al. (2008) who indicated that the test-retest mean CV of 30 km dynamic time trial following a full familiarization session was 2.2% or 2.4% following a half familiarization session. While a preliminary familiarization session identical to the two subsequent test-retest trials would have been ideal, a single short familiarization session conducted no less than 72 hours before the next sessions, was adequate to demonstrate high reliability of the M-VCT in young, trained athletes. It should be noted that the impact of multiple familiarization sessions on M-VCT reliability remains unknown, but irrespective of this limitation, the reliability measured can be accredited to the participants’ experience in road and mountain bike race events – as such, participants were already accustomed to the intensity and demands of the M-VCT. While completing test-retest reliability sessions for the M-VCT protocol is an important advantage for determining the actual performance change that occurred from the experimental intervention, it should be recognized that pilot testing was limited by a small sample size. While this may cause some uncertainty, it is relevant to state that Part 1 testing was carried out on a subpopulation of the larger homogenous athlete population that participated in Part 2. This lends support to characterizing the true effect of MEN in this adolescent athlete population, as well as the potential value in investigating individual response rates to MEN. In this case, results from this investigation provide preliminary insight into the variable responses that athletes may exhibit in response to MEN, ranging from harmful to beneficial, based on the establishment of individual or group CV% during Part 1 testing. Cadence (r=0.90, CV=2.9%), HR (r=0.90, CV=2.8%), Tc (r=0.45, CV=0.7%), and blood lactate concentration (r=0.97, CV=2.6%) all demonstrated low variability with CV's less than 3%. While trained athletes are not immune to physiological variations in performance, it is well known that athletes who are familiar with intense exercise tasks are more likely to have established pacing strategies that allow for better alignment of physiological parameters across tasks (Hibbert et al., 2017). Tc (r=0.45) and perceptual ROF (r=0.46) displayed weakest correlation between trials. Rating of thermal sensation showed the highest variability of all measures between trials (CV=13.1%), a finding in agreement with those of de Korte et al. (2021), who highlights that thermal sensation can fluctuate independent of core temperature changes. Mechanistically, it is assumed that body composition and skin temperature are the primary drivers of thermal comfort during exercise, whereby increased sweating will occur in response to higher skin (and core) temperatures to preserve function. Since a significant difference was found in sweat loss between test-retest trials, it can be assumed that inconsistency in TS ratings between sessions is driven by differences in the thermoregulatory potential to provide heat loss through sweat activity (Xu et al., 2021). In this case, it can be noted that greater sweat loss during trial 2 equates with improved ratings of thermal sensation. This finding demonstrates that TS can fluctuate as a factor of sweat activity, and measurements may be largely unreliable across time under certain conditions (i.e., hypohydration).

Menthol and M-VCT Performance

The present study supports previous findings suggesting that MEN MR improves cycling performance in the heat by ~1.8% in trained athletes, with a specific ability to increase relative power production during the latter stages of a 30 min variable power cycling task. While this investigation offered novel insight into the ability of repetitive MEN MR to benefit performance parameters during an effort characterized by stochastic fluctuations between maximal and submaximal aerobic capacity work rates, findings are in line with Jeffries et al (2018), despite differences in investigative methodology. More specifically, Jeffries et al (2018) indicate that administration of a single MEN MR during the latter stages (~ 85%) of baseline exercise duration for a TTE task in the heat increased performance by ~6%. While this change in performance is significantly greater than the "uncorrected" 2.2% change observed between MEN and PLA conditions in this study, Jeffries et al. do indicate that since this 6% change exceeded the typical error of the TTE (CV%=4.3), then a true change of approximately 1.7% could be observed. Hence it is reasonable to assume that MEN ergogenic effects are likely lower than what has been reported in the literature, especially in trained athlete populations who are already better accustomed to the demands of strenuous athletic tasks. Perhaps by offering a more conservative or "corrected" estimate of typical change, expectations surrounding an athlete's ability to realistically improve beyond day-to-day fluctuations while coping with high stress situations can be better managed.

Additionally, more recent work by Crosby et al. (2022) demonstrates that rinsing MEN prior to a 3 min maximal cycling task in the heat allowed for the maintenance of relative power output for a longer duration when compared to cold water or placebo. While only small to moderate differences were observed, results highlight the potential for MEN MR to benefit higher intensity, short duration cycling bouts (Crosby et al., 2022). Comparably, findings from this investigation suggest that MEN MR improved power output during the mid-to-final stages of a variable cycling task, as expressed by a moderate to large significant effect for "acceleration" (p=0.041, ES=0.71) and "hard" (p=0.002, ES=1.29) work sections during laps 3 and 4 of the M-VCT. No changes in power output were observed during the final lap of the M-VCT, nor in the final sprint (p=0.191, ES=0.42). One second peak power output was also not significantly different between conditions (p=0.095, ES=0.56). In theory, this lends support to the current hypothesis linking MEN's performance improvement properties to increased drive within the central nervous system during tasks characterized by higher levels of central fatigue. Mechanistically, MEN's activation of TRPM8 oral receptors promotes action on reward centers in the brain via intracellular signaling cascades within the CNS. This is expected to create a signal discrepancy which causes acute pleasurable sensations that may disrupt the heat perception process, increase ventilatory drive, and attenuate thirst. In combination, this may help to ameliorate the effects of impaired muscle activation caused by fatigue and/or interrupt the protective anticipatory afferent signals sent to the brainstem to decrease effort during strenuous activity (Gavel et al., 2021; Klasen et al., 2012; Thomas et al., 2015). As a result, MEN appears to promote increased central drive to skeletal muscle to sustain power output (work rate) for longer periods of time (Flood et al., 2017; Thomas et al., 2015), but not necessarily promote increased neuromuscular activation during highly anaerobic sections of work. These findings may offer a combined perspective on the potential applicability of using MEN during fatiguing exercise tasks characterized by both submaximal and maximal workload zones over an extended period, much like the characteristic demands of a road or mountain bike race.

It is also relevant to discuss that mean cadence was significantly higher in the MEN condition compared to PLA (p=0.01, ES=0.96) during both the "acceleration" (p=0.045, ES=0.69) and "hard" (p=0.005, ES=1.07) work sections of the protocol. While this observation was largely expected as a direct result of increased power output during these sections, the mechanisms behind this change remain rather elusive. Recent work by Mater et al. (2021) provides theoretical insight into the relationship between pedalling rate and torque in the production of power. While cadence selection is highly individualized, it is known that for a given power output, cadence can be increased to reduce the amount of torque applied to the pedal or vice versa. Although it is unclear how much of a role cadence plays in the onset of fatigue, it has been reported that cycling at non-preferred cadences can impact neuromuscular function and cause increased perceived exertion. More specifically, maintaining a high cadence at a sufficiently high exercise intensity is known to impair dynamic neuromuscular performance more than low or preferred cadences (Mater et al., 2021). In this study, participants were able to adopt a higher cadence under the MEN condition compared to PLA, perhaps allowing for the optimization of power output while minimizing neuromuscular fatigue and reducing the amount of torque or stress applied to the pedal. It is assumed that participants were able to adopt a slightly higher cadence without over-taxing the system due to a stabilization in the sense of perceived effort, which essentially allowed for greater mechanical efficiency and ultimately aided in performance improvement. Another investigation conducted by Sarre et al. (2003) was able to demonstrate that cadence manipulations did not result in neuromuscular alterations of the knee extensor muscles (VM, VL, RF). These results also align with the finding of this study as no significant difference was found in neuromuscular activation of 5 limb muscles (VL, VM, RF, TA, Gast), despite a difference in cadence between conditions (to be discussed below). In totality, these findings reaffirm the idea that MEN produces a centrally mediated effect to benefit performance.

Menthol and Perceptual Responses

While different perceptual models have been developed to assess the influence of environmental conditions on the human body, no specific model has been validated with the inclusion of exercise in athletes. This study measured perception using five different scales and aimed to provide investigators with a more holistic view of the cognitive mechanisms which may influence performance following the use of MEN. Although Gagge et al. (1967) demonstrates that differences in perception are not perfect correlates for changes in physiological parameters, and Flouris and Cheung (2009) show that perception can vary for the same stimulus during exercise in a consistent environment, perceptual scales do offer the unique opportunity to quantify an athlete's subjective response to the prescribed task within a particular environment.

Secondary findings from the current study demonstrated that there was little to no change in perceptual measures between conditions, despite significant changes across time. More specifically, RPE, TS, TC, and ROF were all found to increase linearly with time, while FS decreased per lap throughout the M-VCT. Although it was originally hypothesized that MEN MR would benefit perception, the observed consistency in responses between MEN and PLA trials may be related to the duration of heat exposure as well as the variable nature of the cycling test itself. While differences in experimental methodology make it difficult to directly compare across the literature, it should be recognized that some studies report significant differences across perceptual measurements in the heat with the use of MEN (Flood et al., 2017; Gibson et al., 2019; and Jeffries et al., 2018), while there also appears to be an equal number of reports that indicate no change at all (Crosby et al., 2022; Gavel et al. 2019; Riera et al., 2014; Stevens et al., 2017). For example, work by Gibson, et al. (2019) reported improvements in perceptual measures, specifically thermal comfort, in a study that utilized MEN MR while performing repeated supramaximal sprints under hot laboratory conditions. Although performance itself was not improved with the use of MEN compared to capsaicin, placebo and control rinses, these findings reinforce the value of perceptual alterations in heat studies, particularly during high-intensity exercise efforts, and thus indicate that further investigation is justified. Likewise, Flood et al., (2017) showed a reduction in thermal perception by comparing MEN to a PLA MR during a 'hard' time-to-exhaustion cycling test in the heat. Not only was exercise time extended under the MEN condition, but athletes also adopted a higher average power out despite reporting a decreased thermal effect. While long bouts of moderate to intense exercise may override alterations in thermal perception because of exercise associated heat storage, MEN still appears to elicit a pleasurable response that, in some part, benefits or stabilizes thermal perception during the latter stages of exercise efforts in the heat. On the other hand, Riera et al. (2014) found no changes in perceptual measures (TC, TS, RPE) despite considerable enhancements in performance when menthol was added to beverages of varying temperatures. As reported, MEN was added to neutral, cold, and iced beverages and ingested prior to a warmup, at the beginning of the test, and every 5 km of a 20 km trial. Results showed no significant differences between beverages with all perceptual measures increasing over time. Similarly, Crosby et al (2022) indicates that TC, TS, nor RPE differed significantly between MEN, PLA, and cold-water rinse conditions during a maximal 3 min cycling test in the heat. In this case, investigators presume that MEN did induce elevated localized sensations of oral cooling, but due to the very short high-intensity testing protocol, the hyperthermic potential of the exercise may have been limited, resulting in no changes in the participants interpretation of the perceptual measures. While ambiguity continues to present across MEN based research, it should be recognized that MEN's action on reward centers in the brain appears to trigger several intracellular signaling cascades within the CNS known to some extent, disrupt the effort and/or heat perception process to create a signal discrepancy between actual effort/temperature and perceived effort/temperature. Depending on the duration, intensity, and characteristics of the exercise task as well as the preferences of the individual athlete, this pleasurable oral sensation may begin to subconsciously mitigate the effects of exercise-induced fatigue to provide acute alterations in perception. These alterations may manifest as an improvement or simply as the maintenance of perceptual state. Since this was the first investigation to include two additional perceptual measures, rating of fatigue and affective feeling, comparisons cannot be made across the literature. Nevertheless, results from these scales support the same trend as those discussed above to reiterate that MEN central effect is largely linked to perceptual stability.

Within the context of this investigation, it can be noted that participants were able to adopt a higher work rate and perform better under the MEN condition without reporting a higher level of perceived effort, no additional thermal discomfort or strain, no elevation in subjective fatigue, and no further deterioration in their negative affective state. In its entirety, findings from the perceptual scales utilized indicate that MEN MR during a dynamic race-like task in the heat may help to maintain, rather than improve, an athlete's perceptual state, which still appears to benefit work capacity. This is of particular interest because high-intensity exercise is typically associated with decreased affective valence and reduced motivation which has been shown to correlate with avoidance behaviors or total disengagement from the task (Ekkekakis et al., 2011;

Micklewright et al., 2017). Similarly, it is assumed that perceived exertion is intrinsically linked with the central drive command (Crosby et al., 2022; Gavel et al., 2021). This implies that as tasks begin to feel harder or more fatiguing, an individual will subconsciously and consciously decrease effort for the completion of the task. If MEN offers the unique opportunity for athletes to upregulate or even maintain performance without experiencing additional decline across perceptual states during difficult tasks, perhaps further investigation into MEN’s applicability for race-like tasks is necessary.

Menthol and Physiological Parameters

Exercising in the heat increases thermoregulatory stress and often causes an athlete to fatigue more quickly. Within the competition setting, fatigue hinders performance and can increase the likelihood of exercise-induced illness or injury. While exercise itself can induce similar physiological responses over time, exercise-induced heat stress often causes physiologic parameters like core temperature, heart and ventilatory rate, and neuromuscular fatigue to rise exponentially. This investigation monitored changes in core body temperature, heart rate, blood lactate concentration, and neuromuscular activation of lower limb muscles over time, to provide a comparison of responses between MEN and PLA trial conditions. Findings indicate that core temperature and heart rate increased linearly across time, but no differences in mean or maximum values occurred between conditions (Tc: p=0.258, ES=0.36; HR: p=0.208, ES=0.41). Correspondingly, surface electromyography (sEMG) indicated a decline in mean and peak amplitude over time, yet no significant differences were measured between conditions (p=0.388, ES=0.20). Despite non-significance, a visual trend does appear across this data (figure 13), displaying slightly lower neuromuscular activation in the MEN condition compared to PLA for all muscles. Blood lactate increased over time, with a significant difference found between MEN and PLA conditions at the mid-point of the cycling test (p=0.041, ES=0.71).

These findings are in line with reports across the literature as it has been established that MEN acts as a non-thermal ergogenic intervention. This means it can be used without causing significant changes to core body temperature or heart rate responses (Jeffries & Waldron, 2019). While a variety of testing protocols, athlete populations, and experimental methodologies have been employed across this type of research, investigators including Crosby et al. (2022), Gavel et al., (2019), Gibson et al., (2019), Jeffries et al (2018), and Riera et al., (2014) collectively report that Tc and HR increase in proportion to either the work performed or the heat load experienced by the athlete, but not in relation to the use of different mouth rinse interventions. While it is known that young athletes employ different thermoregulatory processes in the heat when compared to their adult counterparts, this was not expected to produce diverging results from the published literature as trained adolescent athletes have been characterized by physiologic profiles that are comparable to trained adults (Birat et al., 2018). This is largely because young athletes become efficient at performing in different environments by employing the thermoregulatory strategies that their bodies are equipped with at that point in development. For instance, adolescent athletes have greater reliance on dry heat exchange compared to evaporative heat loss, which accounts for considerably lower sweat rates when contrasting adolescent and adult males (Falk & Dotan, 2011). While this may be seen as a disadvantage, it should be assumed that consistent training at this age increases efficiency and ultimately promotes development of a thermoregulatory strategy that takes advantage of their greater surface-area-to mass ratio and therefore minimizes susceptibility to the ill-effects of heat and fluid loss during exercise (Falk & Dotan, 2011). Perhaps it can be assumed that training status (hours/years) is largely responsible for physiological, cognitive, and behavioral similarities between trained adolescent males and trained adult males during intense and demanding activity. Additionally, while blood lactate concentration tends to be variable across time, likely due to changes in production and subsequent diffusion rates from the skeletal muscle to blood, no significant differences were measured when comparing pre- and post-trial samples between MEN and PLA, but differences were seen at the midpoint measure. Lactate accumulates exponentially during exercise once the aerobic system becomes insufficient in proving energy alone, forcing the body to begin recruiting more energy from the anaerobic systems. Not only does a higher mid-lactate measure in the MEN condition directly reflect the upregulation of power output during laps 3 and 4 during the M-VCT, but these findings are also consistent with the results from Crosby et al., (2022) during the 3-min maximal cycling test.

sEMG was collected in this study for the purpose of describing an exercise-induced decline in force generation capacity during intense exercise. Neuromuscular activation (amplitude) of the rectus femoris (RF), vastus medialis (VM), vastus lateralis (VL), tibialis anterior (TA), and medial gastrocnemius (GA) in the right leg were measured simultaneously throughout the M-VCT. Past investigations have used sEMG during stationary exercise tasks to discuss the relationship between exercise duration, reduced neuromuscular functionality, and the subsequent increase in the perception of effort because these factors are suggested to play a combined role in the emergence of peripheral and central fatigue (Best, 2020; Tucker et al., 2004). While it can be expected that neuromuscular, or peripheral fatigue increases as a factor of exercise time and intensity, less is known about central fatigue mechanisms. Work by Thomas et al. (2015) assessed the contribution of central and peripheral processes to fatigue while trained male cyclists completed 4-, 20-, and 40-km self-paced time trials. Findings indicate that the magnitude of peripheral fatigue was greater during high-intensity, short-duration exercise tasks, but central fatigue was exacerbated by lower-intensity longer exercise bouts. This conclusion was generated by measuring the percent of force reduction from pre- to post-trial neuromuscular function in the vastus lateralis and biceps femoris in the right leg. This investigation allowed Thomas et al. (2015) to suggest that the threshold responsible for controlling the emergence of peripheral and central fatigue is both athlete and situation dependent. It was also proposed that an increase in core body temperature may be responsible for a reduction in EMG activity, suggesting endurance capacity, but not anaerobic capacity is impaired by internal and external temperature fluctuations. Findings from the present study largely align with this idea. While the M-VCT is a 30-min endurance task, it also consists of repetitive maximal work segments. While core temperature increased during the trial, sEMG demonstrated a subsequent decline in neuromuscular capacity. This trend continued throughout the M-VCT, except during the final sprint, whereby Tc and sEMG were both high. This emphasizes that elevated temperature exacerbates a reduction in central output leading to diminished endurance capacity but not necessity maximal effort contractions during exercise.

One study involving MEN, conducted by Stevens et al. (2017), compared the effects of applying pre-cooling (cold-water immersion and ice slurry ingestion), mid-cooling (facial water spray and menthol mouth rinse), or a combination of all methods to a control for 3km running time trials. sEMG was measured from the GA, TA, VL, and RF in the right limb during the task but findings from the data collected were rather inconsistent. Stevens et al. (2017) indicate that EMG activity was significantly higher during trials involving pre-cooling, while only small effect sizes were generated for the trials involving MEN – despite observing the largest performance benefit for the MID-MEN condition when compared to the control. While it has been suggested that physiological cooling, rather than perceptual cooling interventions can increase neuromuscular activation (likely due to its ability to reduce core temperature to delay the onset of fatigue), little is known about the possible central mechanisms that must play a role in fatigue reduction and power output improvement.

Another study, as touched upon above, demonstrated that alterations in cadence produced no differences in neuromuscular activity (EMG) in the knee extensor muscles (VL, VL, RF) during cycling tasks performed at 60, 80, and 100% of maximal aerobic power output (Sarre et al., 2003). While mean power output was significantly different across trials, cadence manipulations of 70, 85, 100, 115, and 130% of a freely chosen baseline did not appear to impact neuromuscular functionality. In combination, these sEMG findings can be directly compared to what was observed in this investigation. More precisely, no significant differences in sEMG activity were observed between muscles compared across conditions, despite displaying a general decrease in activity across time. The visual representation of these findings also gives rise to the general trend that sEMG activation from the measured muscles was lower in the MEN condition than the PLA, despite adoption of an increased work rate in the MEN trial. Although power production requires work from many muscles throughout the body, cycling is a very leg dominant task. Further, the biomechanics of the pedal stroke consists of a specialized pattern of contraction and relaxation from various limb muscles to maintain constant power output, with the quadriceps functioning as the primary "push" along the downstroke and hamstrings as the "pull" throughout the upstroke of the rotation (da silva et al., 2016). Interestingly, it is also known that the proportion of muscular contribution is mediated by task length and intensity, as well as fatigue. For example, when feeling 'fresh' athletes typically recruit power from the quadriceps and hamstrings equally, but when 'tired' a quadricep dominant approach will ensue.

While this investigation collected sEMG measurements from 5 muscle locations with a prime focus on quadricep activity, it is recommended that further research begins to specifically investigate the role that the hamstrings may have in the observed performance outcomes. Based on these findings it is hypothesized that the hamstrings could play a more significant role in maintaining or improving neuromuscular activity once fatigue emerges (da silva et al., 2016; Sarre et al., 2003). With that in mind, investigating the effect that MEN may have on various muscle groups could be useful in better describing the influence that peripheral and central mechanisms of fatigue have on performance outcomes in athletes. Perhaps the upregulation in power output measured during the MEN trials can be justified via the maintenance of greater efficiency in the biomechanics of muscle recruitment during the pedal stroke.

An Individualized Advantage and the Central Fatigue Hypothesis

Previous investigations have successfully demonstrated the efficacy of MEN MR at different concentrations, frequencies, and temperatures when used during various anaerobic and aerobic cycling tasks in hot environments (≥30°C) (Crosby et a., 2022; Gavel et al., 2019; Flood et al., 2017; Mundel & Jones, 2010; Trong et al., 2015). While no past investigation has attempted to describe the response range measured from rinsing MEN in the heat, this investigation offers a unique perspective into MEN usefulness at the individual level. More specifically, by determining the test-retest reliability or typical error of performance (CV%) within a small subpopulation of the larger homogeneous athlete population who completed this study, we were not only able to compute a more conservative and accurate value for mean performance change using a MEN, but we were also able to classify individual athletes according to their response rate, based on a predefined threshold for least significant change (LSC%). To explain, mean and individual athlete CVs were first determined from the test-retest sessions of the M-VCT. These values were then used to define the limit for LSC%, or the value that must be exceeded before a change can be considered meaningful or true. Since even small alterations in athletic performance can be critical for event outcomes, the LSC% was calculated with 95% confidence. At the group level, this means that any change in performance between MEN and PLA conditions exceeding 0.9% (mean) can be considered a true change influenced by the intervention with 95% confidence. While the mean CV% for the M-VCT was computed to be 0.46%, individual CV% ranged from 0.1 to 0.68%. The mean threshold for LSC% was set at 0.90% and individual changes ranged from 0.18 to 1.33%.

Participants then completed Part 2 - experimental testing for the calculation of performance change between MEN and PLA trials. The mean, uncorrected percent change between conditions was 2.2%, while individual differences ranged from 0.75 to 5.75%. These values were then adjusted using the typical error of measurement from part 1 to provide a more realistic interpretation of performance benefit. This revealed that MEN provided a mean benefit of 1.8%, while individual limits ranged from 0.29 to 5.06%. After obtaining the corrected values of change, this was compared against the LSC% threshold from part 1. If an athlete exceeded this threshold, it was concluded that a true change in performance occurred, beyond day-to-day variation from rinsing MEN. If the athlete fell below the boundary, we could not be certain their improvement was due to the experimental intervention. With this information, athletes were then grouped according to the magnitude of their responses. While all athletes demonstrated a positive response, only 7/11 (64%) athletes were deemed ‘real’ responders (performance improvement > LSC%). Furthermore, of these 7, 4 (57%) were classified as high responders (>2.1% change), 2 (29%) as moderate (1.0-2.0% change), and 1 (14%) as minimal (0.1-0.99% change), leaving 4 of the original 11 to be ‘non-responders’. As this was the first investigation to provide insight into individual responses to MEN, it should be acknowledged that approximately 64% of the trained adolescent athletes benefited in some capacity from utilizing a MEN MR repetitively in the heat.

While controversy continues to surround MEN mechanistic pathways and varied impact on aerobic and anaerobic athletic tasks, current findings have been consolidated using the central fatigue hypothesis. This theory suggests that MEN effectiveness lies in its ability to act on reward centers in the central nervous system (CNS) to disrupt the central fatiguing process during a demanding task. Neurochemical underpinnings of this idea are based on findings indicating that exercise induced increases in extracellular serotonin concentrations, compared to dopamine, contribute to fatigue, and ultimately impair performance, especially in the heat (Meeusen et al., 2006). No investigation has been able to precisely measure ratio changes in these biochemical markers during exercise but it has been suggested through current physiological and perceptual measurements, that when MEN activates the TRPM8 oral receptor, afferent sensory neurons depolarize and action potentials are transmitted to reward centers in the brain, where they are integrated by the central nervous system (CNS) to evoke cognitive and reflexive responses (Gavel et al., 2021; Klasen et al., 2012). As a result, MEN is expected to promote increased central or neural drive to the exercising muscle which allows for the maintenance or improvement of performance during athletic tasks (Flood et al., 2016; Thomas et al., 2015). Despite recognition of the limitations within this investigation, perhaps there is a new need to understand personal response rates to menthol and to quantify its potential capacity to hinder, stabilize, or benefit athletic performance. In its entirety, this study reaffirms findings surrounding the use of MEN in the heat.

4.8 Practical applications

The results of Part 1 of this study suggest that performance tests that simulate the stochastic nature of road and/or mountain bike racing may be conducted in a reliable way in young athletes. This protocol allows for the assessment of cycling parameters that cannot be quantified by traditional tests, highlighting its potential usefulness when used as a training and prediction model. It is recommended that this protocol is utilized in future studies to further examine the relationship between different interventions and factors that dictate performance capacity. The results from Part 2 of this study indicate that menthol mouth rinsing may be used to benefit performance during variable cycling tasks in the heat in trained adolescent male athletes. While menthol is known to benefit aerobic capacity and help maintain anaerobic performance independently, this investigation provides novel insight into its impact on race-like tasks. It is suggested that individualized responses to menthol are further analyzed before liberally transitioning its use to competition environments while also considering the possibility of noticing athlete desensitization to this intervention over time.

4.9 Conclusion

The findings of this investigation suggest that 0.01% menthol used as a mouth rinse, at regular intervals during a 30-min variable cycling test in the heat, increases power production during the latter stages of a race-like task, in comparison to a placebo mouth rinse. It is speculated that this is due to MEN ability to act on reward centers in the CNS, once central fatigue emerges, to increase central drive to the exercising muscle. However, no improvements in perceptual measures of thermal comfort, thermal sensation, RPE, rating of fatigue, or affective feeling were observed—likely due to the duration of heat exposure and taxing nature of the exercise bout. In this case, MEN appeared to benefit performance without initiating a subsequent decline in perceptual state. Transitioning the use of menthol out of the laboratory and into the field may highlight more appropriate means of using menthol as a tool to combat heat-related performance decrements during stochastic tasks. Future research assessing menthol should consider how individual athletes may respond to this intervention and even postulate about how desensitization to this intervention could impact stochastic cycling efforts at the professional level.

CHAPTER 5

5 Thesis Conclusion

5.1 Limitations

Sample size/ Participants

Although this study was able to provide critical information to further our understanding of the effects of utilizing menthol as a nonthermal perceptual cooling intervention to improve performance during a variable cycling task, only 11 athletes completed testing which resulted in a small sample size. While an n=11 is comparable to other published investigations, a G-power sample size calculation for a study of this nature lends itself to a population of 12. While this means that this study is slightly underpowered, it should be recognized that there are very few adolescent male cyclists across Durham and the GTA between 15-19 years of age who fit the inclusion criteria of this study. Twelve athletes were recruited to complete this study, but one dropped out due to an opportunity to train with the Canadian National Team for the upcoming season. Due to the novelty and specialized nature of this investigation, findings should still be considered meaningful and applicable.

While 11 adolescent males took part in experimental testing, only 4 athletes (from within this homogenous population sample) were involved in reliability testing. While establishing test-retest reliability of the M-VCT protocol is a significant advantage of this investigation, individual performance variability was only determined in 36% of the population sample, warranting the use of a calculated mean value for all other athletes. While investigations of this nature are time consuming and highly subjective to participant dropout, future inventions should attempt to conduct reliability testing on all participants recruited for the experimental portion of the study. Not only will this improve the power of statistical findings, but this will also allow for the identification and classification of individual responses to a particular ergogenic intervention.

Whilst the exclusion of female athletes limits the ability to further investigate sex-based differences, the majority of menthol mouth rinse research has been studied in male athletes, making findings more comparable across the literature. It can be noted that this was the first investigation to recruit trained adolescent athletes, therefore filling a very specific gap in sports science research. Future investigations may also benefit from differentiating use across various cycling disciplines. While this study focused on athletes with road and mountain bike racing experience, participants could also be recruited from track cycling and triathlon events in hopes of improving the sensitivity and specificity of conclusions.

Protocol Familiarization

A second limitation of this investigation was the use of a shortened M-VCT familiarization session during Part 1 of testing. Although this session was only intended to introduce and educate athletes on the dynamic and repetitive nature of the testing protocol, a full preliminary familiarization session identical to the two subsequent test-retest trials would have been ideal. While this may have inflated/increased base-line variability in M-VCT performance, a single short familiarization session conducted no less than 72 hours before the next sessions, was still adequate in demonstrating high reliability of the M-VCT in young, trained athletes. It should be noted that the impact of multiple familiarization sessions on M-VCT reliability remains unknown, but irrespective of this limitation, the reliability measured (<1%) can be accredited to the participants' experience in road and mountain bike race events. As such, it can be assumed that participants were already accustomed to the intensity and demands of a variable cycling task and did not necessarily require further adaptation to the protocol.

Experimental Intervention and Blinding

While all athletes were blinded to the experimental interventions until after completing all testing, it cannot be stated with absolute certainty that they were unaware of the experimental interventions used. Despite taking steps to colour, volume, and temperature match the experimental interventions, the rinses presented with very distinct flavours. Since it is unclear if a synthetic mint-flavoured placebo rinse would activate TRPM8 receptors, as menthol does, a crystal-light non-caloric sweetener was used as a placebo (which does not activate TRPM8 receptors). Since menthol and the placebo had very distinct tastes, athletes may have been aware that deception was utilized to conceal the true purpose of the study. To reduce or prevent the possibility of observer bias, future investigations may also benefit from utilizing a double-blind experimental design.

Although concentration and frequency of menthol rinsing remain debated across the literature, utilizing menthol at a 0.01% concentration may not be 'potent' or strong enough to elicit substantial activation of TRPM8 receptors in some individuals. While considerable success has been reported at this concentration for frequent rinsing, receptor sensitivity may vary, altering perception towards a given stimulus.

Physiological and cognitive measures

Finally, this study did not measure neurochemical biomarkers, which would have aided in understanding menthol's proposed mechanistic pathways. Since the central fatigue/serotonin hypothesis suggests that menthol's ability to disrupt the fatiguing process lies in altering the concentration of serotonin to dopamine in the brain, future investigations should explore measures that may advance this theory. While it has been suggested that venous blood samples, motion eye tracking devices (to measure pupil dilation) and measuring brain activity could help support this hypothesis – these methods are invasive, costly, time consuming, and difficult to utilize during a dynamic exercise task. While any of these methods of measurement may have contributed to a better understanding of central and peripheral factors which explain fatigue, their efficacy has not yet been proven in conjunction with the administration of menthol. Notwithstanding, future investigations may first benefit from determining the sensitivity of collecting a venous blood sample for the measurement of systemic serotonin/dopamine levels to provide an estimate of cerebral concentrations, or maybe by identifying the validity and reliability of utilizing pupillary dilation (sympathetically driven) to predict the amount and timing of dopamine influx into the brain and its correlation to power production during an activity - before extending these methods of measurement to investigations involving menthol.

While the continuous measurement of cardiorespiratory-metabolic variables may also have benefited discussion on anticipated differences in ventilatory drive and perception associated with menthols' use, these parameters were not measured due to the frequent administration of rinses (every 6-min) as well as the variable demands of the M-VCT. Potential investigations may choose to quantify the efficacy of a single dose of menthol during a race-like task, during which the measurement of cardiorespiratory-metabolic variables or cycling economy may become much more viable.

5.2 Future Directions

This thesis answered pertinent questions regarding the repetitive use of a menthol mouth rinse during a stochastic cycling task in the heat in trained adolescent male athletes; however, additional questions remain.

Perhaps one of the most important inquiries emerging from this investigation not only pertains to menthol's effectiveness during different variable tasks across various environments but also – to what degree does the individual response rate vary to this ergogenic aid? To explain, each athlete appears to have a specific level of sensitivity to utilizing menthol. While some athletes enjoy the minty essence left behind from swilling, others express that the taste is unfavourable and that they would not like using it during competition, regardless of the benefit. Past research indicates that the most beneficial and tolerable rinse concentrations range from 0.01 to 0.1%, with anything greater than 2% causing detrimental thermoregulatory effects. For example, at higher concentrations, menthol appears to act as a severe vasoconstrictor, hindering the thermoregulation process and causing core temperature to rise (Barwood et al., 2020). While 2% is expected to represent the general response threshold, perhaps some athletes are actually more or less sensitive to this intervention than predicted. With that in mind, there is a growing recommendation to test oral stimulation via MEN at different concentrations in individual athletes prior to competition to hopefully find the optimal balance between performance improvement and hindrance.

While the use of menthol in hot environments has gained significant traction, especially following the Olympic Games in Tokyo, more information needs to be gathered surrounding reward center activation and the duration that positive impacts can be felt. It has been previously discussed that athletes may become desensitized to menthol's ergogenic properties over time. Not only does this mean that use needs to be timed appropriately prior to and during competition for an athlete to receive the most significant benefit, but it also indicates that more information needs to be gathered about the duration of its effectiveness. In this investigation, menthol was used six times (at 6 min intervals) over the course of 30-min. Despite effectiveness, perhaps rinsing on fewer occasions, separated by more time would be most beneficial and realistic in a race setting. Or, maybe preserving one rinse for the final stage or lap of a difficult task, once central fatiguing processes sufficiently develop, will provide all the benefit needed. While many questions remain unanswered about menthol, perhaps the next step is to promote its transition out of the laboratory setting and onto modern day racecourses. Although time trial, time to exhaustion, and now, the variable cycle test offers distinct advantages and disadvantages to monitoring physical, cognitive, and behavioral responses to exercise tasks, the ultimate goal is to understand menthol's capacity to stimulate performance benefits beyond day-to-day variability within a competition environment. To do this, it is suggested that future investigations establish the longitudinal validity of the VCT or M-VCT in monitoring changes in performance.

Further, menthols' effectiveness has now been demonstrated for moderate to well-trained male and female athletes as well as very well-trained adolescent males in hot environments. While questions surrounding its usefulness across different athlete classifications – chiefly at the elite or professional level – there is also questionability surrounding its use in thermoneutral temperature conditions. Much of the effect of menthol has been attributed to action on the CNS, creating meaningful changes in performance, especially in the heat, because an individual is able to increase power output without feeling 'worse'. Since these changes may correlate with alterations in neurochemical concentrations in the brain, this outcome should occur independent of temperature conditions, if the activity itself induces central fatigue. Therefore, we can postulate that a menthol mouth rinse would improve performance under an array of conditions, independent of the temperature of the environment. Further, considering that there are several differences between adolescent and adult males and females, such as (1) thermal perception and thermo-behavior, (2) the effect of the menstrual cycle and response to exercise, (3) taste preference, and (4) pacing strategies/cognitive preparedness, there is less information surrounding the potential range of its 'true' effect. This points back to sex and age-based sensitivity differences becoming a key mediator in validating the effectiveness of menthol as well as emphasizes applicability as we push to prepare the next generation of athletes for elite competition much earlier on.

Finally, the effect of menthol combined with other substances, particularly the addition of carbohydrates or caffeine, is unknown. While menthol activates TRPM8 thermoreceptors, carbohydrates and caffeine interact with gustatory chemoreceptors, T1R2/ T1R3 sweet and TAS2R bitter receptors, respectively, and enter the brain via different pathways (Gavel et al., 2021; Guest et al, 2007). Activation in areas, such as the insula/frontal operculum, orbitofrontal cortex, and striatum, is suggested to lower the perception of exertion during exercise and potentially, feelings of displeasure, which aids in increased central drive and an improved capacity for work (Gavel et al., 2019). While caffeine and carbohydrates do not appear to work synergistically (Germaine et al., 2019), given that they both bind to chemoreceptors, perhaps menthol's ability to act on thermoreceptors provides the key to determining if the combined effect of these interventions is both useful and appropriate among different sporting environments.

5.3 Conclusion

Interventions that solely act on the central nervous system (CNS) are gaining considerable interest, particularly products consumed through the oral cavity. The oropharyngeal cavity contains a wide array of receptors that have been shown to improve physiological performance when stimulated by specific substances. Of late, the ergogenic benefits of menthol (MEN) have been explored using various methodologies, but this investigation extends previous findings and provides novelty by demonstrating the positive effect of a menthol mouth rinse during a highly reliable stochastic race protocol in trained adolescent males. While menthol proved to elicit performance-enhancing effects for most athletes, the response rate did vary. Moreover, the menthol mouth rinse did not alter perception (perceived exertion, thermal sensation, thermal comfort, fatigue, and feeling), or thermoregulatory properties (HR, Tc, sweat loss, sEMG) relative to the placebo trial. As such, these results support the underpinnings of the central fatigue hypothesis and reveal greater insight into menthols' ability to act acutely on the central nervous system to stabilize or even benefit performance.

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Appendices

A – Recruitment Poster

MOUTH RINSE AND CYCLING PERFORMANCE

We are conducting a study to better understand the way the body responds to different mouth rinsing flavours during a new variable power performance test under hot environmental conditions. Previous research has shown that during endurance exercise, mouth rinse flavour has been linked to an improvement of performance. What you ingest greatly influences how you might perform during exercise.

Would you like to participate in a study examining the influence of **mouth rinse preference on cycling performance in the heat?** If yes…….

SUBJECTS: Looking for male cyclists who are between 15-19 years old. All information collected will be strictly confidential.

With participation you will be eligible to receive a $25 gift card and access to/interpretation of current fitness levels in cycling.

If you have any questions concerning the research study, please contact the researcher. Kierstyn Hawke at kierstyn.hawke@ontariotechu.net

This study has been reviewed by the Ontario Tech Research Ethics Board [#16331]. If you would like to participate in this study, please send an email to the below contact information: Kierstyn Hawke at kierstyn.hawke@ontariotechu.net

B- Informed Consent Form

Consent Form to Participate in a Research Study

Title of Research Study: Investigating the effects of mouth rinse preference on adolescent male cyclists during a modified variable cycling test (M-VCT) in the heat

Name of Principal Investigator (PI): Dr. Heather Logan-Sprenger

PI's contact number/email: 905.721.8668 ext. 3605/ heather.sprenger@ontariotechu.ca

Name of Student Lead, Faculty Supervisor, and contact number/email: Kierstyn Hawke, kierstyn.hawke@ontariotechu.net. Supervisor: Dr. Heather Logan-Sprenger, 905.721.8668 ext. 3605/ heather.sprenger@ontariotechu.ca

Departmental and institutional affiliation(s): Canadian Sport Institute Ontario (CSIO) and Ontario Tech University, Faculty of Health Sciences/ Faculty of Science

Introduction

You (if between the ages of 15-19 years of age) are invited to participate in a research study entitled ***Investigating the effects of mouth rinse preference on adolescent male cyclists during a modified variable cycling test (M-VCT) in the heat*** operating out of ***Ontario Tech University in Oshawa, ON***. If you/your parents/guardians have symptoms of COVID-19, or feel that you are in or live with a vulnerable group with respect to COVID-19 effects (e.g. senior, immunocompromised, living with individuals that may be susceptible to COVID-19), it may be best that you do not participate in the study. Please read the information about the study presented in this form. The form includes details on the investigation's procedures, as well as risks and benefits that you should be aware of before making the decision to take part or making the decision to take part. You should take as much time as you need to make your decision. You should ask the Principal Investigator (PI) or study team to explain anything that you do not understand and make sure that all your questions have been answered before signing this consent form. Before you make your decision, feel free to talk about this study with anyone you wish including your friends and family. Participation in this study is voluntary.

All aspects of this study have been revised to include all possible risk mitigation strategies against COVID-19. Please see additional documents for specific procedures and protocols regarding COVID-19.

This study has been reviewed by the University of Ontario Institute of Technology (Ontario Tech University) Research Ethics Board.

Purpose and Procedure:

Purpose:

You have been invited to participate in this study because you are between the ages of 15-19 years, participate in a minimum of two regular training sessions per week, and are familiar with the demands of performing high intensity efforts on a bike. This investigation has two purposes. First is to determine the accuracy and usefulness of the modified variable cycling testing (M-VCT) protocol, and secondary, to compare mouth rinse flavour preference between minty and fruity solutions during the M-VCT when performed in hot and humid conditions. Since temperature and flavour preference may be different during demanding exercise, this can lead to differences in performance.

Procedures:

Visit	**Study procedure/tests/interventions**	**Duration of visit**
Visit 1	Preliminary testing/ short familiarization trial	~1.5h
Visit 2	Full Familiarization trial (in heat)	~1.5h
Visit 3	Experimental trial 1 (in heat)	~1.5h
Visit 4	Experimental trial 2 (in heat)	~1.5h

Please refer to the attached documents for additional information on COVID-19 risk and mitigation pertaining to this study. Specific protocols exist for entering and exiting the building, wearing non-medical face masks, and pre-screening for COVID-19 symptoms. Please review this document carefully before also considering the following procedures that are specific to this experimental study. Furthermore, it is important to note that physical distancing will not occur at all times. Given that the researcher will need to interact with the participant, the researcher will be closer than 2m at multiple points throughout the study. As such, the researcher will wear personal protective equipment (mask, face shield, lab coat, and gloves) from commencement to the end of the study. The researcher will interact with the participant a total of 7 times (every ~6 minutes) for ~30secs per contact. Throughout the rest of the study, physical distancing will occur.

What to Expect?

This study will consist of 4 visits to the laboratory at Ontario Tech University. Visit 1 will include preliminary testing which involves body size measurements and an aerobic fitness test (VO_{2max} test), and then will be followed by a short familiarization M-VCT. Visit 2 will consist of a full familiarization trial in the heat so that you are accustomed to all aspects of the cycling test during the following two experimental sessions. Finally, sessions 3 and 4 will be experimental M-VCT trials. Each trial will be separated by at least 3 days, but no more than 7 days. The M-VCT is demanding, so you should be familiar with the effects of hard exercise. Prior to preliminary testing, you are requested to complete a basic "PAR-Q: Physical Activity Readiness Questionnaire". If your responses indicate that follow-up with a medical practitioner is advisable and /or if you have recently experienced an acute illness or injury, your participation in the trial will be reviewed.

Visit 1: Preliminary and Short Familiarization

- VO_{2max} test followed by 3x6min "laps" of the M-VCT protocol. Power output, cadence, heart rate, core temperature (using a wearable, non-invasive device) will be measured, and a small blood sample (finger pinprick) will be collected to analyze lactate levels (on 2 occasions).

Visit 2: Full Familiarization

- Full M-VCT (5x6min "laps") under hot condition (≥28°C). Power output, cadence, heart rate, core temperature (using a wearable, non-invasive device) will be measured, and a small blood sample (finger pinprick) will be collected to analyze lactate levels (on 2 occasions). Surface electromyography (sEMG) will be collected periodically during the test (small electrode patches will be placed on the legs) to record information about muscle activation and strength. Pupil Labs core eyeglasses (non-invasive eyewear) will be worn throughout the trial. A mouth rinse will be used on 5 occasions throughout the trial.

Visit 3 & 4: Experimental Sessions

- Full M-VCT (5x6min "laps") under hot condition (≥25°C). Power output, cadence, heart rate, core temperature (using a wearable, non-invasive device) will be measured, and a small blood sample (finger pinprick) will be collected to analyze lactate levels (on 2 occasions). Surface electromyography (sEMG) will be collected periodically during the test (small electrode patches will be placed on the legs) to record information about muscle activation and strength. Pupil Labs core eyeglasses (non-invasive eyewear) will be worn throughout the trial. A mouth rinse will be used on 5 occasions throughout the trial. At the end of the experimental exercise trials, we will ask you which mouth rinse you preferred.

On an additional note, all wearable, reusable devices will be cleaned (using a soap and water solution) and disinfected (using a medical grade disinfectant effective against COVID-19) prior to and following use. Proper cleaning and sanitization procedures will ensure participant safety.

Potential Benefits

Research in recreational and high-performance sport is highly important for long-term athlete development and athlete preparation for performance. In addition, this research will be useful for nutritional companies as they develop sport drinks.

With involvement in this project, the participant will receive an interpretation and have access to their fitness testing data (VO_{2max} data, fatigue/lactate profiles, muscle recruitment and force during the pedal stroke). Furthermore, the participant will have opportunity to practice a variable power performance test and train with careful monitoring. The participant will also be contributing to a valuable research topic which is understudied. Finally, all athletes will receive at $25 gift card for their participation, regardless of level of completion or withdrawal from the study.

Potential Risk or Discomforts:

Participation in any research study is associated with some risks. The potential risks of this study include, feelings of shortened breath, quickened heart rate, light headedness, and muscular discomfort during and following exercise. During the M-VCT, if the participant feels like they are about to faint, are delirious, or nauseated, or their head is throbbing, cooling therapy will begin immediately. Cooling therapy will consist of cool water, ice packs, and fans. If needed, after those cooling therapies we will take the participant to the nearest hospital. In the event of an adverse reaction, the researcher will call 911 and the participant will be taken to the hospital. The closest hospital is Lakeridge Health, Oshawa, which is roughly 5km away from the University. Furthermore, given the state of COVID-19 and the current pandemic, there is added risk of acquiring COVID-19 during data collection.

COVID-19 Related Information

Please find information below regarding the risk mitigation strategies we will be employing to minimize the risk of COVID-19 exposure during this research study.

- Access to the University is currently limited. As such, before each visit, you will be asked to fill out and submit a “COVID-19 Screening Survey”. The survey can be accessed here https://ssbp.mycampus.ca/apex/r/banner/covid19-prescreen168/login.
- If you present with any symptoms, your study participation will be suspended.
- Please note, all researchers will wear a mask and face shield at all times. You will be asked to bring a mask or face covering from home and will be asked to wear it while walking through the facility.

Laboratory Session: Entering and Exiting the Lab

- A member of the research team will escort you from the north entrance of the lab.
- You will be required to wash and sanitize your hands and wear a mask at that time.
- Once inside the laboratory you may remove your mask or face covering.
- To maximize your safety, upon arriving in the laboratory a member of the research team will spray and wipe down all contact surfaces with a 70% alcohol solution.
- Once you have completed your laboratory session, all contact surfaces will be sprayed and wiped down with a 10% bleach solution.
- Prior to exiting the laboratory, you will be asked to put your mask or face covering back on and wash and hand sanitize one more time.
- A member of the research team will escort you out of the building.

Preliminary, Familiarization, Experimental and Sessions

- You will be asked to arrive at the lab dressed in active wear that you comfortable exercising in.
- You will be greeted by a member of the research team, wearing a mask and face shield.
- You will be asked to wear a mask or face covering that you have brought from home and use the hand sanitizer provided.
- To maximize your safety, just prior to beginning your session a member of the research team will spray and wipe down the cycle ergometer, heart rate monitor, and CORE device down with a 70% alcohol solution.
- As you begin the trial, you will be able to remove your mask or face covering.
- Once you have completed your trial, you will be asked to remove the heart rate monitor, CORE device transfer to your day chair, and wear your mask or face covering.
- The cycle ergometer, heart rate monitor, and CORE device will then be sprayed and wiped down with a 10% bleach solution.

Use and Storage of Data:

All electronic data will be stored encrypted on a password protected computer belonging to the principal investigator (PI). Participants will be linked to a numeric code on electronic files. The linking key will be kept on a different password protected computer that only the PI has access to. The encryption key used for different folders will be unique and all files/data which has either identifiers or which contains the linking code will be kept separate from other study data. The folders used to store study data will have their own independent encryption keys and therefore will be independently protected. This will ensure that stored information is treated with the utmost care and confidentiality. This code along with all collected data will be deleted from the hard drive of the protected devices after 5 years. All printed forms (consent, ParQ, and ParMedX) that contain any personal or medical information will be stored in a locked cabinet at Ontario Tech University that only the PI will have access to. These forms will not be scanned or copied in any way. All print files will be kept for 1 month following the last participant's data collection in this study. After this, they will be shredded and disposed of. If there is a security breach of the participant data, the participants will be informed that there was a breach of data. All information collected during this study, including your personal information, will be kept confidential and will not be shared with anyone outside the study unless required by law. The participant will not be named in any reports, publications, or presentations that may come from this study.

Confidentiality:

We will be collecting personal contact information that we must retain to follow up with you and/or conduct contact tracing if you have been exposed to COVID-19 in coming to the research site. As a result, we cannot guarantee privacy and confidentiality of your participation in the study. All personal contact information will be stored in a locked filing cabinet within the laboratory and will be destroyed 1 month following the last participant's data collection in this study. Furthermore, we cannot guarantee anonymity, as the personal contact information allows identification of you as a participant. Contact information is stored separately from data collected during the research study to allow for de-identification of the research data.

Voluntary Participation:

Participation in this study is voluntary and you may partake in only those aspects of the study in which you feel comfortable. You may also decide not to be in this study, or to be in the study now, and then change your mind later. You may leave the study at any time without affecting your relationship between the principal investigator, co-investigators, or Ontario Tech University.

Right to Withdraw:

The decision to stop participating, or refusal to answer any particular question will not affect the relationship between the principal investigator, co-investigators, or Ontario Tech University. If you decide to withdraw, your data will be deleted and removed. There will be no consequence associated with withdrawing from the study. You will have one-week following your last trial to withdraw. However, if you do withdraw, we will continue to maintain your contact information and will only give it Durham Public Health and the University if required for contact tracing.

Conflict of Interest:

Researchers have an interest in completing this study. Their interests should not influence your decision to participate in this study.

Compensation, Reimbursement, Incentives:

There is no compensation for participating in the study. However, upon completion of the study, you will have access to your fitness testing results with interpretation by a sport scientist, worth ~$700. Participants will also receive a gift card/ prepaid card valued at $25, as a token of appreciation for their time.

Debriefing and Dissemination of Results:

Following the study, you will be informed and receive a copy when the study is published. If you are interested in learning of the results, you will be provided with an opportunity to contact the researcher.

Participant Rights and Concerns:

Please read this consent form carefully and feel free to ask the researcher any questions that you might have about the study. If you have any questions about your rights as a participant in this study, complaints, or adverse events, please contact the Research Ethics Office at (905) 721-8668 ext. 3693 or at researchethics@ontariotechu.ca

If you have any questions concerning the research study or experience any discomfort related to the study, please contact the researcher Kierstyn Hawke at kierstyn.hawke@ontariotechu.net.

By signing this form, you do not give up any of your legal rights against the investigators, involved institutions, nor does this form relieve the investigators or involved institutions of their legal and professional responsibilities.

I agree to participate in this study taking place at OTU during the current COVID-19 pandemic. I understand that my participation is optional. I confirm that I have read and understood the consent form and have been advised on the potential risks related to in-person face-to-face research involving human participants at this time.

By checking each of the boxes below, I acknowledge and agree with the statements as follows:

- ☐ I have either been fully vaccinated with an approved government vaccine, or I have chosen not to be vaccinated.
- ☐ I acknowledge and accept that there is a risk that I could be exposed to COVID-19 while participating in this research project, despite the approved precautions and protocols that have been put in place.
- ☐ I acknowledge and accept that while participating in the study, the researchers may need to be closer than the recommended social distancing guidelines in order to carry out the experimental protocols and/or procedures.
- ☐ I acknowledge and confirm that I am willing to accept this risk as a condition of attending to participate in research.
- ☐ I acknowledge and understand that there may be unknown risk related to COVID-19.
- ☐ I confirm that the study team has answered all my questions about the study and has advised me of all the risks related to in-person face-to-face research for this study.
- ☐ I acknowledge that participating in this study may involve third party risks to others where I may expose individuals that I live with or am in close contact with.

Consent to Participate:

1. I have read the consent form and understand the study being described;
2. I have had an opportunity to ask questions and those questions have been answered. I am free to ask questions about the study in the future;
3. I understand that there may be additional risks to participating in this research during the COVID-19 pandemic that are currently unforeseen and, therefore, not listed in this consent form. If you should develop any symptoms of COVID-19 up to 2-wks following completion of this study, please contact Kierstyn Hawke (kierstyn.hawke@ontariotechu.net) at your earliest convenience;
4. I freely consent to participate in the research study, understanding that I may discontinue participation at any time without penalty. A copy of this consent form has been made available to me.

☐ I have discussed/shared information about the study with my parents/legal guardian, but I recognize that I have the authority to consent on my own behalf.

Print Participant's Name	Signature	Date

Optional Parent/Guardian Signature

This consent form is addressed to the participant. However, this parental signature remains optional for participants who have the capacity to consent on their own behalf.

☐ I have discussed/shared information about the study with my child, but I recognize that he/she/they have the authority to consent on their own behalf.

Name of Parent/Guardian	Signature	Date

Researcher's Signature

My signature means that I have explained the study to the participant named above. I have answered all questions.

Print Name of Person Obtaining	Signature	Date

C- Physical Activity Readiness Questionnaire (PAR-Q)

2018 PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

GENERAL HEALTH QUESTIONS

Please read the 7 questions below carefully and answer each one honestly: check YES or NO.	**YES**	**NO**
1) Has your doctor ever said that you have a heart condition ☐ OR high blood pressure ☐?	☐	☐
2) Do you feel pain in your chest at rest, during your daily activities of living, **OR** when you do physical activity?	☐	☐
3) Do you lose balance because of dizziness **OR** have you lost consciousness in the last 12 months? Please answer **NO** if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? **PLEASE LIST CONDITION(S) HERE:** ____________	☐	☐
5) Are you currently taking prescribed medications for a chronic medical condition? **PLEASE LIST CONDITION(S) AND MEDICATIONS HERE:** ____________	☐	☐
6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer **NO** if you had a problem in the past, but it ***does not limit your current ability*** to be physically active. **PLEASE LIST CONDITION(S) HERE:** ____________	☐	☐
7) Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐

If you answered NO to all of the questions above, you are cleared for physical activity.
Please sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2 and 3.

- Start becoming much more physically active – start slowly and build up gradually.
- Follow International Physical Activity Guidelines for your age (www.who.int/dietphysicalactivity/en/).
- You may take part in a health and fitness appraisal.
- If you are over the age of 45 yr and NOT accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
- If you have any further questions, contact a qualified exercise professional.

PARTICIPANT DECLARATION
If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness centre may retain a copy of this form for records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME ____________________ DATE ____________

SIGNATURE ____________________ WITNESS ____________

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER ____________________

If you answered YES to one or more of the questions above, COMPLETE PAGES 2 AND 3.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at **www.eparmedx.com** before becoming more physically active.
- Your health changes - answer the questions on Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.

2018 PAR-Q+

FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

1.	**Do you have Arthritis, Osteoporosis, or Back Problems?** If the above condition(s) is/are present, answer questions 1a-1c — If **NO** ☐ go to question 2	
1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	**YES** ☐ **NO** ☐
1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	**YES** ☐ **NO** ☐
1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	**YES** ☐ **NO** ☐
2.	**Do you currently have Cancer of any kind?** If the above condition(s) is/are present, answer questions 2a-2b — If **NO** ☐ go to question 3	
2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and/or neck?	**YES** ☐ **NO** ☐
2b.	Are you currently receiving cancer therapy (such as chemotheraphy or radiotherapy)?	**YES** ☐ **NO** ☐
3.	**Do you have a Heart or Cardiovascular Condition?** *This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm* If the above condition(s) is/are present, answer questions 3a-3d — If **NO** ☐ go to question 4	
3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	**YES** ☐ **NO** ☐
3b.	Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction)	**YES** ☐ **NO** ☐
3c.	Do you have chronic heart failure?	**YES** ☐ **NO** ☐
3d.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	**YES** ☐ **NO** ☐
4.	**Do you have High Blood Pressure?** If the above condition(s) is/are present, answer questions 4a-4b — If **NO** ☐ go to question 5	
4a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	**YES** ☐ **NO** ☐
4b.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer **YES** if you do not know your resting blood pressure)	**YES** ☐ **NO** ☐
5.	**Do you have any Metabolic Conditions?** *This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes* If the above condition(s) is/are present, answer questions 5a-5e — If **NO** ☐ go to question 6	
5a.	Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies?	**YES** ☐ **NO** ☐
5b.	Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness.	**YES** ☐ **NO** ☐
5c.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, **OR** the sensation in your toes and feet?	**YES** ☐ **NO** ☐
5d.	Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)?	**YES** ☐ **NO** ☐
5e.	Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future?	**YES** ☐ **NO** ☐

2018 PAR-Q+

6. Do you have any Mental Health Problems or Learning Difficulties? *This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome*

If the above condition(s) is/are present, answer questions 6a-6b — If **NO** ☐ go to question 7

6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
6b.	Do you have Down Syndrome **AND** back problems affecting nerves or muscles?	YES ☐ NO ☐

7. Do you have a Respiratory Disease? *This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure*

If the above condition(s) is/are present, answer questions 7a-7d — If **NO** ☐ go to question 8

7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
7b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	YES ☐ NO ☐
7c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	YES ☐ NO ☐
7d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	YES ☐ NO ☐

8. Do you have a Spinal Cord Injury? *This includes Tetraplegia and Paraplegia*

If the above condition(s) is/are present, answer questions 8a-8c — If **NO** ☐ go to question 9

8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
8b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	YES ☐ NO ☐
8c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	YES ☐ NO ☐

9. Have you had a Stroke? *This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event*

If the above condition(s) is/are present, answer questions 9a-9c — If **NO** ☐ go to question 10

9a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
9b.	Do you have any impairment in walking or mobility?	YES ☐ NO ☐
9c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	YES ☐ NO ☐

10. Do you have any other medical condition not listed above or do you have two or more medical conditions?

If you have other medical conditions, answer questions 10a-10c — If **NO** ☐ read the Page 4 recommendations

10a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months **OR** have you had a diagnosed concussion within the last 12 months?	YES ☐ NO ☐
10b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	YES ☐ NO ☐
10c.	Do you currently live with two or more medical conditions?	YES ☐ NO ☐

PLEASE LIST YOUR MEDICAL CONDITION(S) AND ANY RELATED MEDICATIONS HERE: ____________________

GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.

2018 PAR-Q+

If you answered NO to all of the FOLLOW-UP questions (pgs. 2-3) about your medical condition, you are ready to become more physically active - sign the PARTICIPANT DECLARATION below:

- It is advised that you consult a qualified exercise professional to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually - 20 to 60 minutes of low to moderate intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate intensity physical activity per week.
- If you are over the age of 45 yr and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.

If you answered **YES** to **one or more of the follow-up questions** about your medical condition:

You should seek further information before becoming more physically active or engaging in a fitness appraisal. You should complete the specially designed online screening and exercise recommendations program - the **ePARmed-X+ at www.eparmedx.com** and/or visit a qualified exercise professional to work through the ePARmed-X+ and for further information.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ **at www.eparmedx.com** before becoming more physically active.
- Your health changes - talk to your doctor or qualified exercise professional before continuing with any physical activity program.

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The authors, the PAR-Q+ Collaboration, partner organizations, and their agents assume no liability for persons who undertake physical activity and/or make use of the PAR-Q+ or ePARmed-X+. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

PARTICIPANT DECLARATION

- All persons who have completed the PAR-Q+ please read and sign the declaration below.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME ______________________________ DATE ______________________________

SIGNATURE ______________________________ WITNESS ______________________________

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER ______________________________

For more information, please contact

www.eparmedx.com
Email: eparmedx@gmail.com

Citation for PAR-Q+

Warburton DER, Jamnik VK, Bredin SSD, and Gledhill N on behalf of the PAR-Q+ Collaboration. The Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) and Electronic Physical Activity Readiness Medical Examination (ePARmed-X+). Health & Fitness Journal of Canada 4(2):3-23, 2011.

The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+ Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or the BC Ministry of Health Services.

Key References

1. Jamnik VK, Warburton DER, Makarski J, McKenzie DC, Shephard RJ, Stone J, and Gledhill N. Enhancing the effectiveness of clearance for physical activity participation; background and overall process. APNM 36(S1):S3-S13, 2011.
2. Warburton DER, Gledhill N, Jamnik VK, Bredin SSD, McKenzie DC, Stone J, Charlesworth S, and Shephard RJ. Evidence-based risk assessment and recommendations for physical activity clearance; Consensus Document. APNM 36(S1):S266-s298, 2011.
3. Chisholm DM, Collis ML, Kulak LL, Davenport W, and Gruber N. Physical activity readiness. British Columbia Medical Journal. 1975;17:375-378.
4. Thomas S, Reading J, and Shephard RJ. Revision of the Physical Activity Readiness Questionnaire (PAR-Q). Canadian Journal of Sport Science 1992;17:4 338-345.

D- PAR-Q Practitioner Follow-up

ePARmed-X+Online

ePARmed-X+ Physician Clearance Follow-Up

This form is separated into three main sections:

A) Background information regarding the PAR-Q+ and ePARmed-X+ clearance process,
B) A brief history and demographic information regarding the participant, and
C) The physician's recommendations regarding the participant becoming more physically active.

At the end of this process, the participant is recommended to take this signed clearance form to a qualified exercise professional or other healthcare professional (as recommended in the ePARmed-X+) before becoming more physically active or engaging in a fitness appraisal.

A BACKGROUND INFORMATION REGARDING THE PAR-Q+ AND ePARMed-X+ CLEARANCE PROCESS

The ePARmed-X+ is an easy to follow interactive program (www.eparmedx.com) that can be used to determine an individual's readiness for increased physical activity participation or a fitness appraisal. The ePARmed-X+ supplements the paper and online versions of the new Physical Activity Readiness Questionnaire for Everyone (PAR-Q+).

Individuals who use the ePARmed-X+ have had a positive response to the PAR-Q+, or have been directed to the online program by a qualified exercise professional or another healthcare professional, owing to his/her current medical condition. At the end of the ePARmed-X+, it is possible that the participant is advised to consult a physician to discuss the various options regarding becoming more physically active. In this instance, the participant will be required to receive medical clearance for physical activity from a physician. Until this medical clearance is received, the participant is restricted to low intensity physical activity participation.

This document serves to assist both the participant and physician in the physical activity clearance process.

B PERSONAL INFORMATION

NAME: ______________________________ SEX: ☐ M or ☐ F

ADDRESS: ______________________________ BIRTHDATE (mm/dd/yy): ____________
____
____

TELEPHONE: ______________________________ HEALTH/MEDICAL NUMBER: __________

REASON FOR REFERRAL (SELECT ALL THAT APPLY):

☐ QUALIFIED EXERCISE PROFESSIONAL REFERRAL
☐ HEALTH CARE PROFESSIONAL REFERRAL
☐ ePARmed-X+ RECOMMENDATION

ePARmed-X+Online

C ePARmed-X+ PHYSICAL ACTIVITY READINESS PHYSICIAN REFERRAL FORM

Based on the current review of the health status of ______________________________(name)
I recommend the following course of action:

- ☐ The participant should avoid engaging in physical activity at this time.
- ☐ The participant should engage in only a medically supervised physical activity/exercise program involving the supervision of a qualified exercise professional (or other appropriately trained health care professional) and overseen by a physician.
- ☐ The participant is cleared for intensity and mode appropriate physical activity/exercise training under the supervision of a qualified exercise professional.
- ☐ The participant is cleared for intensity and mode appropriate physical activity/exercise training with limited supervision (i.e., unrestricted physical activity).

The following precautions should be taken when prescribing exercise for the aforementioned participant:

- With the avoidance of: ______________________________
- With the inclusion of: ______________________________

NAME OF PHYSICIAN: ______________________________

ADDRESS: ______________________________

TELEPHONE: ______________________________

Date of Medical Clearance (mm/dd/yy): ____________________

PHYSICIAN/CLINIC STAMP AND SIGNATURE

NOTE: This physical activity/exercise clearance is valid for a period of six months from the date it is completed and becomes invalid if the medical condition of the above named participant changes/worsens.

E- Preliminary Trial Data Collection Form

Participant: PAR-Q? Y/N Date:

Preliminary testing M-VCT

Preliminary Testing: Incremental cycling test

Fasted? Y / N Hydrated? Y / N 24hr Diet Recall? Y / N

Body weight: Height: USG($\leq$ 1.020):

WATTS	MIN	HR	RPE	CORE TEMP
25W	1-min			
50W	2-min			
75W	3-min			
100W	4-min			
125W	5-min			
150W	6-min			
175W	7-min			
200W	8-min			
225W	9-min			
250W	10-min			
275W	11-min			
300W	12-min			
325W	13-min			
350W	14-min			
375W	15-min			
400W	16-min			
425W	17-min			
450W	18-min			
475W	19-min			
500W	20-min			
525W	21-min			

F- Familiarization/ Experimental Trial Data Collection Form

OntarioTech UNIVERSITY

M-VCT + Data Collection: ID: Visit#. Date.

Body weight: **kg** **Urine specific gravity:** **Hours sleeping:** **h**

Age:

Environmental Conditions:

Temp: **Humidity:** **3.0 w/kg = w**

	HR	Watts	Core Temp	RPE	ROF	FS	TS	$[La^-]$	Time
START									0.00
End lap 1									7.00
End lap 2									13.00
End lap 3									19.00
End lap 4									25.00
End lap 5									31.00

Total time trial time: 30-minutes → 5 x 6 min laps

Avg PO per lap:

1. ______ 2. ______ 3. ______ 4. ______ 5. ______/10-sec max sprint ______

Total trial PO avg: _______

Sweat Rate and Hydration: Post Body weight:

Sweat rate: *Sweat loss (g)= [change in body weight (g) + fluid intake (g)]=*

Trials:

- Preliminary (VO2max
- Familiarization (M-VCT in heat)
- Experimental$_1$ (menthol)
- Experimental$_2$ (crystal-light placebo)

Warm-up:

- **3-min at 1.5 w/kg**
- **3-min at 2 w/kg**
- **4-min at 2.5 w/kg**
- **5-min rest**
- **1-min rolling start: 100W**

G- Debrief and Deception Letter

The Effects of Mouth Rinse Preference and Modified Variable Cycle Test (M-VCT) Performance in the Heat

Researchers:

Kierstyn Hawke, M.HSc. (Student)
Faculty of Health Science
Ontario Tech University
Kierstyn.hawke@ontariotechu.net

Dr. Heather Logan-Sprenger, PhD
Assistant Professor
Faculty of Health Sciences
Ontario Tech University
heather.sprenger@uoit.ca

Purpose of Research:

Thank you for taking the time to participate in this study! Initially, you were told the purpose of this study was to determine which mouth rinse you preferred; however, the actual purpose of this research is to determine if a menthol mouth rinse changes your responses and performance during a power performance test in the heat. The reason you were deceived into thinking the study was for something else, was to prevent altered motivation (a placebo effect) from one mouth wash trial to the next.

Throughout the trials, information related to power output, heart rate, core temperature, and sEMG with either a menthol mouth rinse or a crystal light mouth rinse was recorded and analyzed. Using menthol to improve performance has been researched in adults under hot conditions, but we did not know if adolescent athletes would gain the same benefits. The results from the study indicate that…………….

If you feel especially concerned about the protocol, results, anything related to this study, or would not like your data used in the published research, feel free to contact Kierstyn Hawke at kierstyn.hawke@ontariotechu.net, or Dr. Heather Logan-Sprenger at heather.sprenger@uoit.ca. In addition, if you have any concerns regarding your rights as a participant, complaints or adverse events may be addressed to Research Ethics Board through the Ethics and Compliance Officer - researchethics@uoit.ca or 905.721.8668 x. 3693.

Withdrawal from Study:

The decision to stop participating, or refusal to answer any particular question will not affect the relationship between the principal investigator, co-investigators, or Ontario Tech University. If you decide to withdraw, your data will be deleted and removed. The participants will have no consequence by withdrawing from the study.

Signatures:

I _____________________________________, consent that the data collected in *Investigating the effects of mouth rinsing on adolescent male cyclists during a modified variable cycling test (M-VCT) in the heat* research project conducted by Kierstyn Hawke can be used for publications. I have understood the purpose and protocol of this project. The signature below indicates my consent.

Signature Participant: ____________________________. ………………...**Date:**____________________

Signature Witness : ____________________________. . **Date:**____________________

Additional Reading:

Gavel, E., Hawke, K., Bentley, D., Logan-Sprenger, H. (2021). Menthol Mouth Rinsing Is More Than Just a Mouth Wash—Swilling of Menthol to Improve Physiological Performance. Frontiers in Nutrition. https://doi.org/10.3389/fnut.2021.691695.

Stevens, C. J., & Best, R. (2016). Menthol: A Fresh Ergogenic Aid for Athletic Performance. *Sports Medicine*, *47*(6), 1035–1042. http://doi.org/10.1007/s40279-016-0652-4

Stevens CJ., Thoseby B., Sculley DV., Callister R., Taylor L., Dascombe BJ. (2015). Running performance and thermal sensation in the heat are improved with menthol mouth rinse but not ice slurry ingestion, *26*(10), 1209–1216. http://doi.org/10.1111/sms.12555

H- Thermal Scaling Charts (TC and TS)

Scale value	Thermal comfort descriptor	Thermal sensation descriptor
+3	Much too warm	Hot
+2	Too warm	Warm
+1	Comfortably warm	Slightly warm
0	Comfortable	Neutral
-1	Comfortably cool	Slightly cool
-2	Too cool	Cool
-3	Much too cool	Cold

I- Rate of Perceived Exertion Borg 6-20 Scale

Rating	Perceived Exertion
6	No exertion
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

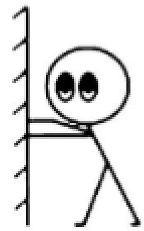
J- Rating of Fatigue Scale

10 TOTAL FATIGUE & EXHAUSTION – NOTHING LEFT

9

8

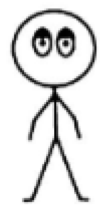
VERY FATIGUED



7

6

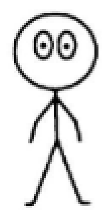
5 MODERATELY FATIGUED



4

3

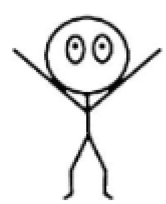
A LITTLE FATIGUED



2

1

0 NOT FATIGUED AT ALL



K- Feeling Scale

FEELING SCALE

+5	Very Good
+4	
+3	Good
+2	
+1	Fairly Good
0	Neutral
-1	Fairly Bad
-2	
-3	Bad
-4	
-5	Very Bad