Menthol Mouth Rinsing Improves Cycling Performance in Females Under Heat Stress

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science

Department of Exercise Science
University of Toronto

2019

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University of Toronto, Faculty of Kinesiology and Physical Education, Exercise Science, November 2019

ABSTRACT:

This study investigated the effects of a menthol mouth rinse on performance and physiological responses in female cycling athletes during a 30-km individual time trial (ITT). Participants (n=9) cycled for 30-km in hot conditions (30 ± 0.6 °C, 70 ± 1% relative humidity, 12 ± 1 km/h windspeed) on two test occasions using a menthol (MEN) mouth rinse (MR) occurring every 5 km. Perceptual responses (perceived exertion and thermal perception) were recorded at 5, 10, 15, 20, 25, and 30-km. The MEN MR significantly improved ITT performance by 2.3 ± 2.7% relative to PLA (p= 0.034, d= 0.85, 95% CI= 8.43 to 166.9). There were no significant differences in perceived exertion and thermal perception. These results demonstrate that a non-thermal cooling agent can improve physiological performance in moderately trained female cyclists with no change in perceived exertion or thermal perception.
Acknowledgements

This thesis is dedicated to Lisa Thomaidis, Jacqueline Lavallee, Bruce Craven, Dr. Kent Kowalski, Nancy Lackie, and Dr. Heather Logan-Sprenger. Thank you for believing in me when I did not believe in myself, holding me to high standards, and for showing me that anything is possible through consistent effort and perseverance. I am fortunate to have such tremendous role-models and people to look up too. You have inspired me to challenge myself and pursue my dreams. From the bottom of my heart, thank you.

I sincerely want to thank my supervisor, Dr. Scott Thomas. Thank you for giving me the opportunity to pursue my passion in exercise physiology. It has been an absolute pleasure working with you these past couple of years. Further, thank you for accommodating a busy training and travel schedule. This experience has been everything I have hoped for and more.

Thank you to Dr. Ira Jacobs for the guidance, insight, and encouragement throughout the whole process. I am thankful for our conversations on environmental physiology, ergogenic aids, and physiological performance.

Thank you to Dr. Jenna Gillen for the invaluable insight, mentorship, and friendship. I am especially grateful for the countless hours discussing women’s health and how it may impact physiological performance.

Lastly, thank you to my lab mates, the participants, the University Of Toronto Faculty Of Kinesiology and Physical Education, Ontario Tech University Faculty Of Health Sciences, and the Canadian Sport Institute Ontario for the resources and financial support to pursue this project. This is a dream come true for me; my most sincere gratitude.
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List of Abbreviations

C, Convection
CG, Central Governor
CGT, Central Governor Theory
$T_c$, Core temperature
EMG, electromyography,
E, evaporation
HA, heat acclimation
HR, heart rate
IOC, International Olympic Committee
ITT, independent time trial
K, conduction
$[\text{La}^-]$, lactate ion
M, metabolic rate
MEN, menthol
MR, mouth rinse
OC, oral contraceptive
R, radiation
RPE, rate of perceived exertion
RH, relative humidity
Resp, respiration
S, heat storage
TC, thermal comfort
TS, thermal sensation
$T_{sk}$, skin temperature
TRPM8, Transient Receptor Potential Cation Channel Subfamily M Member 8,
TT, time trial
TTT, team time trial
UCI, Union Cycliste Internationale
USG, urine specific gravity
W, external work done

$W_{\text{max}}$, watt max
Chapter 1

1 Introduction

Every four years, thousands of athletes come together to compete at the Olympic and Paralympic Games. Among the sports showcased at the Olympic and Paralympic Games is cycling. Cycling consists of multiple disciplines such as mountain bike, BMX, track, cyclo-cross, trials, cycle-ball, and road cycling (UCI, 2018). These races are typically performed in environments ranging from 12 to 35°C (UCI, 2018). Whilst bicycles were first developed in the mid-18th century and most popular in Europe, the first road race did not occur until 1868 (Maso et al., 2005), moreover the first World Championships for females was not until 1958 (Cycling, 2019). Over the last 20 years, cycling has experienced movement towards globalization (Olympic cycling, 2018).

Road cycling, a prominent fixture at not only the Olympic and Paralympic Games, but also the Union Cycliste Internationale (UCI) World Championships, and National Championships, can be separated into two categories, road race and time trial (TT). The road race consists of a mass start where riders race against one another to a set finish point; while the TT consists of riders racing against the clock alone, and can be separated into two categories, team time trials (TTT) and independent time trials (ITT).

Typically, the TT is set at distances of ~55-km for men, and ~30-km for women (UCI, 2018). Factors that can influence one’s performance include mechanical variables, thermoregulation, and the environment (Coyle, 1999). Although these factors can affect the cyclist synergistically, independently, each variable can be detrimental. For example, in a 40-km TT study by Racinais et al. (2015) TT performance worsened 14% in hot conditions in contrast to cool conditions.
Throughout a cycling TT, the ability to cope in the heat is imperative. For example, the conditions of the upcoming Tokyo 2020 Olympic and Paralympic Games are expected to be in environments of >30°C and >80% RH (Gerret et al., 2019). During exercise, safe and efficient functioning of the human body is dependent on maintaining a core temperature ($T_c$) of 36.5 to 38.5°C (Byrne et al., 2007), once $T_c$ approaches or exceeds this threshold, the body may start to slow down to prevent catastrophic failure (Noakes et al., 2005). Furthermore, in addition to a high $T_c$, a high skin temperature ($T_{sk}$) is associated with slowing down—perhaps as a result of a decrease in muscle recruitment (Tucker et al., 2004). The central governor theory, a phenomenon which proposes that the subconscious brain regulates motor unit recruitment to prevent catastrophic failure (Weir et al., 2006), can explain the decrease in central drive in regards to high $T_c$ and $T_{sk}$ temperatures. During exercise, the purpose of pacing is to allow completion of the task in the most efficient way possible (St Clair Gibson et al., 2004). The marathon race provides evidence that the athletes race ‘in anticipation’ by setting a pace at the start to maintain homeostasis. An example of this can be displayed in a study by Tucker et al. (2004), in environments of 15 and 35°C, $T_c$, heart rate (HR) and rate of perceived exertion (RPE), were similar up until the end of exercise, however, power output and electromyography (EMG) activity decreased early in the hot condition.

With regard to self-selected output, it is reported sex differences do occur, showing women to have greater resistance to fatigue compared with men (Ettinger et al., 1996; Hunter et al., 2006; Kent-Braun et al., 2002; Salomoni et al., 2008) and reported differences in thermal perception with women having a lower sensitivity to the heat (Mowlavi et al., 2005).

Given that elevated environmental temperature can have a negative effect on performance, cooling interventions have been developed to mitigate fatigue and improve performance.
Examples of this are 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., 2016; Stevens et al., 2017). The purpose of pre-cooling is to drop $T_c$, while the majority of mid-cooling techniques do not drop $T_c$, they drop $T_{sk}$ or change thermal perception. Mid-cooling can be accomplished through application of external or internal methods, such as a cooling garment (Tyler et al., 2011; Hasegawa et al., 2005; Luomala et al., 2012) and ingestion of cold beverages (Riera et al., 2014, Schulze et al., 2015), respectively.

A more recent type of intervention which may act, in part, like mid-cooling, is the menthol mouth rinse. Menthol, a compound which turns on the Transient Receptor Potential Cation Channel Subfamily M Member 8, (TRPM8) receptors (cold) in the mouth and on the skin, has been used to ameliorate fatigue and improve performance (Stevens and Best, 2017). For example, Stevens et al. (2016) observed a 3% improvement in running TT performance with a menthol mouth rinse. In addition to physiological benefit, menthol may also have an “alerting” or “arousal” action, as sensory inputs from thermoreceptors can influence our level of consciousness (Zimmerman, 1989).

It is important to note that the most effective methods are not always most practical. For athletes who do not have an integrated support team, or are logistically challenged, a menthol mouth rinse may be the only feasible option. While a cooling vest may be beneficial, the practicality of it might be very limited. Although the menthol mouth rinse has been shown to improve performance in a number of studies with men (Flood et al., 2017; Stevens et al., 2016; Mundel and Jones, 2010), the influence for women is unknown.
Chapter 2

2 Literature review

This section will review how sex and heat may influence cycling time trial (TT) performance. We will examine physiological and other approaches used to regulate heat balance, mitigate fatigue, and alter perception. Finally, we will examine how high temperatures can degrade cycling performance and affect thermal perception.

2.1 Cycling and Time Trial Performance

Cycling is one of the most efficient forms of locomotion requiring less energy per unit mass per unit distance than any other form of land transportation (Brooks, 1989; Wilson, 1973). When cycling, the power required for locomotion depends on a complex interaction of physiology, the environment, and mechanical variables (Coyle, 1999).

Performance testing allows for a controlled simulation of exercise performance, typically used for research or applied science purposes. Exercise performance is often measured in the laboratory because environmental conditions can be controlled, and physiological parameters can be measured. When investigating race-type events, TT, and time to exhaustion (TTE) are the most common tests. During a TT, riders try to cover a fixed distance as fast as possible, the objective of the TT is to maintain the highest sustainable speed for the distance. In contrast, TTE is exercising at a set intensity for as long as possible. The use of a TTE protocol may be useful when investigating the mechanisms by which an intervention affects performance (Currell and Jeukendrup, 2008). In comparison, TT have greater validity than TTE because they provide good physiological simulation of actual circumstances and correlate with actual performance (Currell and Jeukendrup, 2008). TTs are extensively used in measurement of performance for running
(Paavolainen et al., 1999), cycling (Carter et al., 2004), and rowing (Bruce et al., 2000) based protocols. Furthermore, TTs are commonly used to assess supplementation (Ivy et al., 2009), training interventions (Burke et al., 2000) and physiological responses to exercise (Girard et al., 2013).

Three factors that contribute to a good performance test include: validity, reliability, and sensitivity (Currell and Jeukendrup, 2008). In order to be a valid simulation of performance, the protocol should provide similar physiological responses to actual performance. Foster et al. (1993) showed that there were no physiological differences in a 5-km TT during a laboratory trial and actual performance. Furthermore, Palmer et al. (1996) found a strong relationship between time to completion for a 40-km cycling TT performed on a Kingcycle (Jeukendrup et al., 1996) ergometer compared with time to completion of two outdoor 40-km cycling TT. Moreover, Laursen et al. (2002) investigated the relationship of exercise test variables to Ironman triathlon cycling performance; it was found that when exercising to exhaustion at the power output at the ventilatory threshold, there was no significant correlation with cycling and triathlon performance. These results indicate that the relationship between TTE and cycling ITT performance is not very strong.

Typically, intra-subject variance is the most important reliability measure for measuring performance (Hopkins et al., 2000). Research has shown that TTE protocols have a coefficient of variation (CV) of >10%, whereas TTs are more reliable with a CV of <5%. Small intra-subject variability enhances accuracy in detecting a change in performance. For example, the variability of 20-km and 40-km cycling TT performance have all been established as having relatively low scores of intra-subject variability, CV; <2% in well-trained cyclists.
Another trait that contributes to a good performance test is sensitivity which can be quantified as the change in performance divided by the typical error of measurement, or the “signal-to-noise” ratio. Amann et al. (2008), compared the difference of sensitivity in TTE and TT performance, to conditions of normoxia, hypoxia, and hyperoxia. Sensitivity for the normoxia-hypoxia change in performance in the constant-power test (6.3, 90% confidence interval 4.3-11.4) was similar to that in the time trial (4.5, 3.0-8.2); sensitivities were also similar for the normoxia-hyperoxia changes (3.2, 2.1-6.0; 3.8, 2.5-6.9, respectively). For studies in which the effect of self-selected pace is an issue, TTs are the most viable option, whereas constant-power tests provide more control for studies of physiological correlates of performance (Amann et al., 2008).

2.2 Heat Balance

Safe and efficient functioning of the human body is dependent on maintaining a $T_c$ of 36.5 to 38.5°C, (Byrne and Lim, 2007) with severe consequences for more than a few degrees of deviation— $T_c$ below 33.5°C or above 41.5°C will cause rapid decline in proper body functioning (Hensel et al., 1981) and eventually lead to a catastrophic state. Prolonged exercise in the heat is associated with a significant increase in $T_c$ correlated with a decrease in exercise performance (Nybo and Neilsen, 2001). Peiffer and Abbiss (2011), examined the effects 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 32 to 17°C, moreover TT performance was reduced in the hot condition. Furthermore, in a study by Tucker et al. (2004), of exercise in 15 and 35°C, $T_c$ was similar up until the end of exercise, however, power output and integrated EMG activity began to decrease early in the hot
trial when $T_c$, HR, and RPE were similar in both conditions. This suggests that power output is regulated down to maintain homeostasis.

For thermal balance to be achieved and $T_c$ stabilized, internal heat production must equate to the rate of heat dissipation. Internal heat production can be described as the conversion of metabolic energy into mechanical and thermal energy, while heat dissipation allows heat loss. The heat balance equation which incorporates the four pathways of heat exchange: conduction, convection, radiation, and evaporation, helps explain this equilibrium (Parsons, 2003). A schematic diagram of heat balance is depicted in Figure 2.2.1.

**Heat balance equation:**

$$M - W (W \cdot m^2) = C + R + K + E + \text{Resp} + S$$

Where:

$M =$ metabolic rate, $W =$ external work done, $C =$ convection, $R =$ radiation, $K =$ conduction, $E =$ evaporation, $\text{Resp} =$ respiration $(R_K + R_C)$, $S =$ heat storage
Figure 2.2.1: A schematic showing heat production in active muscle, its transfer from the core to skin, and exchange with the environment (modified from Gisolfi et al., 1984)

Among the four pathways of heat exchange, each variable is characterized in one of two categories: dry heat exchange or wet heat exchange. Dry heat exchange is dependent on the temperature gradient between the body and the environment and is described by conduction, convection, and radiation. Conduction refers to the transfer of heat with solids in direct contact with the body, convection refers to fluid or air movement across a surface resulting in heat exchange, and radiation refers to the transfer of heat in the form of electro-magnetic waves between the environment and the body. Furthermore, all three of these variables can be
influenced by clothing, posture, and surface area exposed (Cheung, 2010). Wet heat exchange, usually known as evaporation, occurs from the conversion of a liquid to vapor. When the secreted sweat is vaporized at the skin, maximal evaporative heat dissipation from the body occurs. To evaporate 1g of sweat 2,427 J of energy is required (Wenger, 1972). Due to the effect of water vapor pressure, the relative humidity (RH) of the environment largely determines the capacity for evaporative heat exchange (Cheung, 2010). When the ambient air temperature approaches the mean $T_{sk}$, the skin-to-air temperature gradient is reduced, thus evaporation becomes the principle pathway for heat dissipation. Similar to dry heat exchange, clothing and exercise intensity also influence the production and evaporation of sweat (Sawka et al., 2000).

During exercise only 20-30% of the produced energy is converted to mechanical work, whereas ~70-80% of the energy is released as heat (Ament et al., 2009). The onset and amount of heat the body produces and stores can be influenced by a variety of variables such as, environmental conditions, exercise intensity, and dehydration, all of which impact the body physically and mentally (Gisolfi et al., 1984). For longer durations, such as continuous exercise, sustaining a high $T_c$ is possible and can be accomplished through the maintenance of a cool $T_{sk}$. The $T_c$ to $T_{sk}$ gradient provides information on both metabolic heat production and dissipation. By maintaining a low $T_{sk}$, the body can function at optimal levels even though the $T_c$ might be high (Ely et al., 2009b; Kenefick et al., 2010; Periard et al., 2011; Sawka et al., 2011). Although the body can function at an increased $T_c$, once the $T_c$ reaches a critical internal temperature, a self-limited value (Deforges & Simon, 1993), hyperthermia will occur. Hyperthermia, a rise in body temperature to a high level, is related to the body heat storage that occurs from an increase in metabolic heat production, a decrease in heat loss, or a combination of the two (Tan et al.,
2.3 Thermoregulation

Thermoregulation is a process by which the body responds to internal and external thermal stimuli such as metabolic heat and environmental conditions, respectively to regulate Tc. The hypothalamus is the control center for thermoregulation processes is responsible for two main functions: the anterior hypothalamus controls heat loss, while the posterior hypothalamus controls vasoconstriction and shivering (Parsons, 2003). Conceptually, as a whole, the hypothalamus acts as a thermostat and initiates blood redistribution responses (Charkoudian, 2010). The human regulatory system combines feedback from baroreceptors, osmoreceptors, and thermoreceptors to maintain a body temperature of ~37 °C (Hensel, 1981). In order to do this, the body loses heat by vasodilation and increased skin blood flow, and preserves heat by vasoconstriction and shivering (Charkoudian, 2003). A large thermal gradient from core to skin allows exercise to continue despite a high rate of heat production. In conjunction with Tsk, once Tc hits a certain threshold, the skin vasodilates for heat dissipation. It has been suggested that the Tc to Tsk gradient is a critical variable for exercise tolerance in the heat. The Tc to Tsk gradient provides information on both metabolic heat production and dissipation, indicating the overall thermal strain being incurred by the combination of the environment and work output (Ely et al., 2009; Kenefick et al., 2010; Periard et al., 2012; Sawka et al., 2011).

Sweating is an important mechanism for maintaining a low Tsk. Under optimal conditions, the skin is cooled by evaporation of sweat which allows heat to dissipate from the blood to the environment. Sweating is controlled in the preoptic and anterior regions of the hypothalamus where thermosensitive neurons are located (Shibasaki et al., 2006). The body has
millions of sweat glands distributed across the human skin, the areas with the highest density
(>250 gland/cm²) include the soles of the feet, scalp, and palms of the hand (Shibasaski et al., 2006). The body has two types of sweat glands, apocrine and eccrine. Apocrine glands are larger
and non-thermoregulatory, while eccrine glands are tiny but very numerous. Eccrine glands
secrete primarily water and electrolytes, important for thermoregulatory mechanisms such as
homeostasis (Cui et al., 2015). Typically, sweat rate is linearly related to internal temperature
and the level of Tsk (Nadal et al., 1971).

In response to thermal stress, skin blood flow can increase substantially from ~1L per
minute to ~6 to 8L per minute (Rowell, 1974). Furthermore, prolonged exposure to hyperthermic
conditions and/or prolonged exercise in the heat can induce water deficits due to profuse
sweating, resulting in hypohydration. The water deficit lowers both intra and extracellular
volumes and results in plasma hyperosmolality and hypovolemia; both of which can impair
sweating and result in an increase of Tc. This impairment stems from a thermal strain-mediated
increase in cardiovascular response (Cheuvront et al., 2009, Periard et al., 2011, Periard et al.,
2015), whereby a thermoregulatory redistribution of blood toward the periphery and a
temperature-mediated increase in intrinsic HR compromise blood volume and the maintenance
of cardiac output (Coyle et al., 2001, Gorman et al., 1984, Johnson et al., 1975, Rowell et al.,
1974). VO2max can be attenuated because of increased skin blood flow (Gonzalez-Alonso et al.,
2008). During exercise, several investigations have shown that water replacement is beneficial
(Walsh et al., 1994; Below et al., 1995; Hargreaves et al., 1996) and that fluid ingestion
ameliorates dehydration attenuating the rise in Tc. Regardless of environmental conditions, the
optimal rate of fluid intake should approximate the sweat rate (Montain et al., 1992; Sawka et al.,
2.3.1 Thermoregulatory sex differences

Several studies have investigated sex differences in thermoregulatory characteristics. These studies have been done with women and men at rest, during controlled hyperthermia, before and after heat acclimation, and in response to heat exposure (Kerslake, 1972). The sex differences are said to be due to a higher set point (Bittel and Henane, 1975), conductance of skin blood flow (Hertig et al., 1963), sweat production per gland (Bar-Or, 1998), circulatory adjustments (Brouha et al., 1960), gland density (Bar-Or, 1998) and sweating responses (Hertig et al., 1963).

As for sweat rate and characteristics, women have a less intense, more delayed, and greater density of heat-activated sweat glands than men (Bar-Or, 1998). The lower sweat response in women may be related to suppression of excessive sweat output, implying that women have a more sensitive feedback from the wetted skin to prevent excessive dripping of sweat (Avellini et al., 1980). The greater sweat gland density of women results in smaller and closer sweat droplets, which allow for a more economical sweating pattern (Bar-Or, 1998).

The menstrual cycle is another factor that can influence $T_c$ and how the body might respond to exercise. During the menstrual cycle the internal body temperature fluctuates in women by 0.3-0.5°C (Kacibua-Uscilko and Grucza, 2001). In a study by Haslag and Hertzman (1965), they showed that $T_c$ was lower during the follicular than luteal phases at rest. It is speculated that both progesterone and estradiol affect $T_c$ (Charkoudian et al, 2014). The follicular phase is characterized by highest levels of estradiol. On the contrary, the luteal phase is characterized by a higher plasma level of progesterone compared to the follicular phase. Kolka et al. (1997)
explained that $T_c$ rhythm reflects changes in the set-point temperature. Moreover, it is plausible that other thermoregulatory processes are also influenced during the menstrual cycle, such as vasoconstriction and non-shivering thermogenesis in cold temperatures. In a study by Grucza et al. (1999), they found that in women performing moderate exercise, the temperature threshold for sweating was shifted to a higher level and gains for sweating were larger in the luteal than in the follicular phase.

Although such findings are of physiological importance, their applicability to the female population is limited given that 47.8% of Canadian premenopausal women use an oral contraceptive (OC) or intrauterine device (IUD) (Abortion Rights Coalition of Canada, 2018). To date, most contraceptives consist of synthetic progesterone and estrogen that are given in a constant dose for 21 days, followed by a 7-day non-medicated period in which menstruation occurs. The alterations are similar in direction and magnitude to those seen when comparing the responses that occur during the luteal vs. follicular phase in eumenorrhoic women (Martin and Buono, 1997).

Similar to endogenous sex hormones (Buxton and Atkinson, 1948) exogenous sex hormones (estrogen and progesterone) administration through OCs have been shown to influence thermoregulatory processes (Charkoudian and Johnson, 1997; Charkoudian and Stachenfeld, 2016). Typically, $T_c$ increases with use and the internal threshold for cutaneous vasodilation is shifted to higher values (Charkoudian and Johnson, 1997). While estrogen alone lowers $T_c$ through an increase in peripheral vasodilation (Charkoudian, 2003), progesterone is thought to induce peripheral vasoconstriction and to increase the thermoregulatory set point (Charkoudian and Stachenfeld, 2016). Given that ~80% of athletic women take OCs (Rechichi et al., 2009), the opposing roles of estrogen and progesterone in maintaining homeostasis are of particular
importance, especially for endurance exercise in the heat (Coyle, 2009). In a study by Minahan et al. (2017), they compared $T_c$, HR, lactate [$La^{-}$], and RPE between women on OCs and naturally menstruating women during exercise performance in the heat. Baseline $T_c$ was higher in the OC, however no other between-group differences occurred. Furthermore, in an exercise endurance study by Joyce et al. (2013), TTE for severe-intensity cycling was similar between control and OC groups.

### 2.3.2 Heat Acclimation

Heat acclimation (HA) is a broad term that can be defined as a complex series of changes of adaptations that occur in response to repeated increases in $T_c$ and heat stress (Periad et al., 2015). Exposure to heat stress has been shown to enhance thermoregulatory functioning (Bruck and Zeisberger, 1987; Sawka et al., 2011), improve submaximal performance (Racinais et al., 2015), improve VO$_{2_{\text{max}}}$ (Sawka et al., 1985) and improve thermal comfort in the heat (Folk, 1974; Gonzalez and Gagge, 1976; Lemaire, 1960). Furthermore, cardiovascular adaptations supporting this challenge include an increase in total body water, plasma volume expansion, better sustainment and/or elevation of stroke volume, reduction in HR, improvement in ventricular filling and myocardial efficiency, and enhanced skin blood flow and sweating responses (i.e. onset and increase of sweating responses) (Periad et al., 2015). Classic indicators of HA can be characterized by a reduction in HR and $T_c$ for a given exercise heat stress (Houmard et al, 1990). Studies show it can take between 7 and 14 days of HA to achieve a plateau for a given stressor, and that approximately 75% of the physiological adjustments occur within the first 4-6 days of heat exposure (Shapiro et al., 1998; Taylor et al., 2014), however in a
study by Peterson et al. (2010) using national level cricket players, four consecutive days of heat exposure did not result in full HA.

When comparing men and women, HA differences are observed that may originate in the different thermoregulatory mechanisms and higher resting $T_c$ of women compared to men. In a study by Mee et al. (2015), they compared sex differences in both short-and long-term HA, 5 and 10 days respectively. The data demonstrated that both males and females achieve partial adaptation after short term heat acclimation, with males demonstrating a reduction in thermal strain and females demonstrating an increased sudomotor function. The results suggested that both males and females respond to short-term HA, however, females require long-term HA to establish thermoregulatory and cardiovascular benefit.

Various HA protocols have been employed for both team and individual sports. For example, HA has shown to be effective in both women soccer players and men’s cycling TT performance (Pethick et al., 2017; Racinais et al., 2015). For both of these groups, HA can be accomplished through natural environments such as outdoor or field training, or artificial environments such as passive heating, a laboratory setting, or controlled hyperthermia. These environments can be separated into four different pathways: constant work rate of exercise (Nielsen et al., 1993; Nielsen et al., 1997), self-paced exercise (Nelms and Turk, 1972), controlled hyperthermia or isothermic HA (Fox et al., 1967; Garret et al., 2009; Patterson et al., 2004; Regan et al., 1996), and controlled HR (i.e. relative intensity) (Periad et al., 2015; Periad et al., 2016), although exercised-induced HA is likely to be most effective in developing sport-specific adaptations (Periad et al., 2015). Additionally, HA can be accomplished with a variety of intensities and durations. For example, Houmard et al. (1990), was able to show HA at 75% $VO_{2max}$ for 30-35 mins/day and 40-50% $VO_{2max}$ for 60-100 min/day. When deciding on the
protocol in which HA should be ascribed in, sport demands and feasibility will have an influence. While natural and artificial HA share physiological adaptations, training in an environment that replicates the competition setting will allow the athletes to experience the exact heat stress of the sport (Hellon et al., 1956; Edholm, 1991; Armstrong and Maresh, 1991).

In contrast to HA, HA decay is a process in which the adaptations made from HA are lost. Past work has found that an individual can expect to lose approximately 2.5% of HA benefit per day (Daanen et al., 2018) with all benefit lost by 6 weeks (Ashley et al., 2015). From 6-21 days’ post HA, the percentage loss of HA appears to be greater for cardiac frequency than $T_c$ (Williams et al., 1967; Pandolf et al., 1977).

### 2.4 Fatigue

In exercise performance, fatigue is commonly described as exercise-induced impairment in the ability to produce muscular force (Gandevia, 2001). Fatigue can be caused by a variety of processes that can be broadly split into peripheral and central origins. Fatigue can be caused by peripheral changes at the level of the muscle, and/or failure of the central nervous system to drive the motor neurons adequately. The extent to which peripheral and central processes contribute to fatigue is dependent on the nature of the task. Typically, early development of contractile impairment is caused by peripheral factors, while central fatigue will be observed toward the end of exercise and results in task failure. For example, in a study by Thomas et al. (2015), they compared fatigue in 4-km, 20-km, and 40-km simulated TTs. The results demonstrated that fatigue after self-paced exercise is task dependent, with a greater degree of peripheral fatigue after shorter, higher intensity TTs and more central after longer, low intensity TTs (>30-min). Fatigue is a complicated sequential process that can be explained in relation to
one or more of the following steps:

Brain ➔ spinal cord ➔ peripheral nerve ➔ neuromuscular junction ➔ sarcolemma ➔ transverse tubules ➔ calcium release ➔ crossbridge formation ➔ contraction ➔ tension

(Edwards et al., 1983).

2.4.1 Peripheral Fatigue

Among the variables of fatigue, each characteristic can be categorized as either peripheral or central fatigue. Peripheral fatigue is described as a decrease in muscular force attributed to problems with neuromuscular transmission down the sarcolemma, calcium release and uptake in the sarcoplasmic reticulum, availability of metabolic substrates, accumulation of metabolites, and actin-myosin cross-bridge interactions (Williams et al., 2009; Roberts et al., 1989). In addition, the cardiovascular system, anaerobic system, energy supply, energy depletion, and biomechanical factors can also play a significant role in peripheral fatigue (Noakes et al., 2000). Peripheral fatigue has been investigated using electromyography (EMG), rate of perceived exertion (RPE) and biomarkers which may reflect particular aspects of fatigue. For example an increase of EMG may reflect increased effort to compensate for muscular contractile impairment while in other circumstances a decrease of EMG may reflect a reduced central drive (Kent-Braun, 1999).

2.4.2 Central Fatigue

Central fatigue, the inability or failure to continue working at a given exercise intensity, is defined as an activity or exercise-induced decline (progressive reduction) in activation of a muscle or muscle group (Gandevia, 2001). During a muscle contraction, the firing of the motor
neuron depends on the appropriate level of descending (efferent output) to the motor neurons in the ventral horn of the spinal cord. In contrast to peripheral fatigue, an important characteristic of the central nervous system is negative feedback, in which a signal is sent from muscle to brain known as an afferent feedback response (Purves et al., 2001). The feedback system plays an essential role in the way muscles maintain their output as they operate locally at the level of the motorneuron or at the supraspinal levels (Gandevia, 2001). Once the brain receives afferent feedback from the working muscle, pulses from the supraspinal motor areas (the motor cortex and subcortex) descend through the spinal cord to the peripheral motor neuron pool, this is known as “central drive”. In regard to the spinal level, central fatigue can alter muscle afferents response time. In an article by Biro et al. (2006), the muscle spindle reflex pathway was recruited 30% sooner in the fatigued muscle when compared to non-fatigue. Central fatigue can be attributed to a number of factors such as an altered centered nervous system transmission, a decrease in motivation, or specific muscle fiber recruitment (Bigland-Ritchie et al., 1982). When measuring central fatigue, it can be quantified using isometric force output, absolute power output, and average power output (Kent-Braun, 1999). With the use of twitch interpolation recording, studies have shown that voluntary activation usually diminishes during maximal voluntary isometric tasks, as central fatigue develops motor unit firing rates decline. Moreover, central fatigue can be estimated from EMG recordings. An example of this was displayed in a study by Nybo and Neilsen (2001). After cycling to exhaustion in the heat, maximal voluntary contraction was measured in both the hand and leg, both measurements showed a decrease in isometric force following exercise, moreover the decrease in hand grip is an example of pure central fatigue. Furthermore, with the use of goats, it has been demonstrated that when hypothalamic temperature is independently increased to 43°C running speed is decreased
(Caputa et al., 1986), this supports the idea that motor activity is inhibited by high $T_c$.

In addition to $T_c$, $T_{sk}$ is another factor that can influence central fatigue. During exercise in hot environments, elevated $T_{sk}$ has been shown to decrease TTE (Galloway et al., 1997), moreover, the perception of the magnitude and quality of thermal stress plays a major role in human behavioral thermoregulation and exercise performance (Cheung, 2010). In a study by Cheung and Sleivert (2004), they investigated the effects of passive hyperthermia on isokinetic maximal force production. Independent of $T_c$, they were able to show skin cooling, even with a warm core of 39.5°C, immediately increased peak torque output.

2.4.3 Fatigue and sex differences

Whether a muscle fiber fatigues will depend on the balance between the rate of energy consumption associated with the contractile activity and the rate at which ATP can be regenerated. Jaworowski et al. (2002), demonstrated men had a higher activity of glycolytic enzymes LDH and PFK than women, while the enzyme activities representing oxidation of fat and CHO's did not differ between men and women. While men are usually stronger than women, evidence supports that women can sustain continuous, as well as intermittent muscle contractions at low to moderate intensities better than men (Fulco et al., 1999; Russ and Kent-Braun, 2003; Hunter et al., 2004, 2006; Russ et al., 2005). On average, men have larger contracting muscles than women. In comparison, when both men and women are working at the same percentage of watt max ($W_{max}$), men will generate more absolute force, occlude circulation to a greater extent, and as a consequence fatigue more rapidly during sustained muscle submaximal contractions. In contrast, the increased fatigability in men during intermittent submaximal isometric contractions is not dependent on maximal strength (Hunter et al., 2004; Gonzales & Scheuermann, 2006), and
even when matched for maximal strength, men are more fatigable than women during both intermittent and sustained muscle contractions (Fulco et al., 1999; Hunter et al., 2004; Gonzales and Scheuermann, 2006). In a study by Hakkinen (1993), after heavy resistance exercise resulting in similar declines in maximal force in both sexes, they found significant decreases in the maximum voluntary EMG in the men subjects but not women. This suggests greater impairment in neuromuscular fatigue in men compared to women. Moreover, in a marathon study comparing first and second half pacing, the mean change in pace was 15.6% and 11.7% for men and women, respectively (Deaner et al., 2015).

Although the menstrual cycle has a significant effect in other areas of physiology, reports on the influence of menstrual cycle on fatigue are ambiguous. For example, while Janse de Jonge et al. (2001) and Oosthuyse et al. (2005) showed no performance decrements in consequence of menstrual cycle phase, work by Sarwar et al. (1996), Redman and Weatherby (2003), and Giacomoni et al. (2000) reported an increase in fatigability during the mid-luteal and luteal phases, phases characterized by higher levels of progesterone. Further, a recent study by Albert (2016) showed isokinetic peak strength was significantly lower during the follicular (151.6 ± 26.8 NM) than the ovulatory phases (157.5 ± 27.1 NM, \( P<0.05 \)).

A reason for this variation can be attributed to nutritional status and verification of menstrual cycle phase (Oosthuyse and Bosch, 2010; Janse de Jonge, 2003). For example, a high estrogen and progesterone concentration in the luteal phase is characterized by an increase in glycogen storage capacity (Oosthuyse and Bosch, 2010). Hence, in order to attain equal carbohydrate levels depicted in the luteal phase, carbohydrate consumption during the follicular phase will need to increase (Oosthuyse and Bosch, 2010). In regard to methodology, four common ways to verify the menstrual phase include: counting days from the onset of menses,
basal body temperature, urinary luteinising hormone, and the measurement of estrogen and progesterone (Janse de Jonge, 2003). Early studies relied on counting days since menses, but this was a problem as the follicular phase is much more variable than the luteal phase, unless the days are counted backwards it is difficult to determine which phase the individual is in (Janse de Jonge, 2003). The second method, basal body temperature charting, is determined by an increase in body temperature by ~0.3 °C, this increase occurs right after ovulation and is sustained during the luteal phase, however this method does not give information about hormonal levels. The third method, urinary luteinising hormone concentration is determined by the colorimetric enzyme immunoassays of the urinary luteinising hormone, moreover progesterone and estrogen levels can also be identified in the urine. Lastly, the fourth method is the measurement of estrogen and progesterone, through urine or blood. In contrast, the ‘gold standard’ of determining menstrual phase is through the concentration of progesterone. While determination of menstrual phase via luteinising hormone is convenient, this method is only 85-92% accurate (Venners et al., 2006). The menstrual phase influences short duration and maximal exercise intensities (Brutsaert et al., 2002), time to exhaustion (Kendrick et al., 1987), and time trial performance (Campbell et al., 2001). In a time trial study by Campbell et al. (2001), they compared time to expend a given amount of energy after completing a 2-hour submaximal session at 70% VO_{2max} in the mid-follicular and mid-luteal phase following an overnight fast. In comparison, the mid-follicular phase was characterized by a 2.3-fold of estrogen to progesterone and translated to an improvement of 13% versus the mid-luteal phase. They believe the better performance was associated with higher carbohydrate use (hepatic glucose production and rate of disappearance). Conversely, two other time trial studies by McLay et al. (2007) and Oosthuyse et al. (2005) did not find a significant difference between the mid-follicular vs. mid-luteal, and early-follicular vs.
mid-luteal phase, respectively. While Mc Lay et al. (2007) used moderately trained athletes, Oosthuyse et al. (2005) used both a trained and untrained population. Furthermore, both studies followed a normal diet and observed a 1.4-fold and 4-fold increase of estrogen to progesterone in the follicular phases. Unfortunately, Mc Lay et al. (2007) allowed the subjects to view power output throughout TT which may have influenced pacing strategy. Moreover, although the Oosthuyse et al. (2005) study did not reach significance with Bonferroni correction, the results suggest a positive influence of estrogen on performance with a positive effect of \(5.3 \pm 2.9\%\) \((p=0.027)\).

Moving forward, both applied sport scientists and exercise researchers need to account for nutritional status and menstrual phase. Furthermore, developing a ‘gold standard’ will be imperative for future work. If this does not happen, results will continue to differentiate as a consequence of inconsistent methodology and study design (Oosthuyse and Bosch, 2010; Gordan et al., 2018).

2.5 Central activity and physiological performance

The central governor is proposed to be some central nervous system mechanism that takes input information about energetic needs, current physiological states, and various motivational drives to regulate physical exertion and save the organism from catastrophic homeostatic failures (Noakes et al., 2005) (Fig. 2.5.1). Regardless of intensity, duration, or the athletes’ biological state (Tucker et al., 2006), the central governor model proposes that the subconscious brain regulates power output by modulating motor unit recruitment to preserve whole body homeostasis (Weir et al., 2006). The marathon race provides evidence that athletes’ race ‘in anticipation’ by setting a variable pace at the start of exercise, dependent in part on the
environmental conditions and the expected difficulty of the course (Noakes, 2007). Furthermore, the subconscious brain sets the exercise intensity by determining the number of motor units activated (i.e. the amount of skeletal muscle mass recruited). The subconscious brain informs the conscious brain of an increasing neural effort, perhaps related to an increased difficulty in maintaining homeostasis at that exercise intensity. As such, the purpose of pacing is to allow completion of the task in the most efficient and safe way possible, this is accomplished by maintaining internal homeostasis and a metabolic or physiological reserve capacity (St Clair Gibson et al., 2004).

In addition to the ‘anticipatory response’, another challenge occurs when exercise is open-ended, this has been observed during VO$_{2\text{max}}$ tests or when the athlete is unaccustomed to the task (Noakes, 2008). People have tested this concept using a variety of experimental designs, an example of this can be observed in a study by Farra et al. (2017), measuring arterial oxygenation. Cycling on a cycle ergometer with saturation the same across trials, the rate of change in saturation resulted in an earlier and greater decrease in voluntarily chosen power output. Relative the central governor theory, these results suggest that the body slows down in order to maintain homestasis. Furthermore, during an open-ended task, the pace is modified contraction by contraction. Factors that can decrease motor output and increase RPE include an increase in T$_c$ (Castle et al., 2012), fluid loss (Edwards et al., 2007) and variables related to the rate of heat accumulation (Marino et al., 2000; Tucker et al., 2004, 2006; Morante and Brotherhood, 2008; Altareki et al., 2009; Flouris and Cheung, 2009; Schlader et al., 2011). In addition, T$_{sk}$ is another factor that can influence self-selected pace without any influence on T$_c$. In a 10-sec repeated cycling sprints study by Matsuura et al. (2015), under hot-dry and thermoneutral conditions, the mean 2-sec power output over the 1-4$^{\text{th}}$ sprints was significantly
lower under the hot-dry condition than the thermoneutral condition. Therefore, the power output at the onset of a cycling sprint can be decreased in different environmental conditions with no influence on $T_c$.

With regard to fatigue and self-selected output, it is reported that sex differences do occur, showing women to have greater resistance to fatigue compared with men (Ettinger et al., 1996; Hunter et al., 2006; Kent-Braun et al., 2002; Salomoni et al., 2008). Furthermore, these differences include thermal perception (Denegar et al., 2012; Matos et al., 2011; Mowlavi et al., 2005; Sarlani et al., 2003). Typically, women have a lower sensitivity to the heat than men (Mowlavi et al., 2005). An example of this is derived from a resistance exercise temperature study by Kwon et al. (2015) where participants were instructed to lift as many repetitions as possible. Following the reps, participants either heated or cooled their palms. Hand cooling was associated with a significant increase in performance for both females and males, while palm heating decreased performance in women but not men (Kwon et al., 2010). These results suggest that women are more sensitive to elevated temperatures compared to men. Furthermore, the literature shows us that thermal perception is individually regulated and can significantly affect performance.
2.6 Measures of Function and Physiology

2.6.1 Rating of perceived exertion

The RPE scale is commonly used to evaluate and regulate exercise intensity (Borg, 1982; Tucker et al., 2006). Furthermore, according to the central governor theory, RPE is also used to prevent homeostatic catastrophic failure (Smits et al., 2014). Factors that determine RPE are thought to be multifaceted which include central integration of perceptual, peripheral, and environmental sensory cues (Hampson et al., 2001). Perceived exertion can be regarded as a “Gestalt” made up of perceptions from physiologically “local” factors, such as skin, muscles, joints, and “central” factors, such as cardiovascular and pulmonary organs (Ekblom and Goldbarg, 1971), and also psychological factors (Morgan, 1973; Weiser and Stamper, 1977).
The most well-known is the RPE scale for Rating of Perceived Exertion (Borg, 1970; Borg 1985; Borg, 1998). The 6-20 RPE scale was constructed to show linear growth with stimulus intensity, HR, and oxygen consumption. The linear growth function of RPE data during incremental work has been confirmed in several studies (Robertson and Noble, 1997; Borg 1998). Other studies have evaluated the validity and reliability of RPE in elite swimmers and Australian footballers (Psycharakis, 2011; Scott et al., 2013). As for determining validity, both studies used %HR\textsubscript{max} as one of the criterion measures. In the swimming study, RPE was found to be a valid method for monitoring exercise intensity with a correlation coefficient of \( r = 0.85 \) for HR, whereas the football was \( r = 0.81 \) (Psycharakis, 2011; Scott et al., 2013). In regard to reliability, while the study by Psycharakis (2011) observed no significant differences in the values of %HR\textsubscript{max} or [La\textsuperscript{-}] among four separate trials, the results from Scott et al. (2013) demonstrated poor reliability RPE scales for all speeds of running (31.9% CV, 0.66 ICC). All of these studies reported that RPE is of practical value for measuring and prescribing exercise intensities, however athletes with less experience of RPE may benefit from a learning-based protocol (Soriano-Maldonado et al., 2014). Furthermore, a 0-10 version of the scale was developed and has excellent measurement properties (Borg, 1998). The 0-10 version is a category-ratio scale based upon similar psychophysical function between the perceptual response and physiological variable. The 0-10 scales have shown high reliability and validity in healthy athletic populations. For example, in a cycling and running study by Herman et al. (2006), participants performed 6 randomly ordered 30-minute constant-load exercise bouts at 3 different intensities, there were no significant differences between test and retest values of %VO\textsubscript{2peak}, %HR\textsubscript{peak}, and Session RPE for 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).
Furthermore, the correlations between repeated bouts for $%\text{VO}_2\text{peak}$ ($r = 0.98$), $%\text{HR}_{\text{peak}}$ ($r = 0.98$) and session RPE ($r = 0.88$) were strong and significant.

2.6.2 Thermal Scaling

Thermal sensation (TS) models, a rating system capable of predicting a human’s perception of thermal sensation, can be defined as “a conscious feeling graded into the categories of cold, cool, slightly cool, neutral, slightly warm, warm, and hot” (ASHRAE, 2004). Of those models, two common scaling systems include thermal sensation and thermal comfort (Mcintyre, 1984; ASHRAE, 2004). Thermal sensation is the perception of ones’ state, while thermal comfort is the evaluation.

In a systematic review by Koelblen et al. (2016), they compared seven thermal sensation models which showed discrepancies between the predictions of the models. The main discrepancies included different intensities of sensation for the same exposure; this was more pronounced with increased metabolic rate and clothing thermal insulation values. When deciding on a scale, the model of choice can strongly influence the assessment of ones’ state, therefore ratings must be used with caution. For example, thermal sensation and mean thermal votes models were developed for the prediction of TS in vehicles, not during exercise or higher metabolic rates (Koelblen et al., 2016). Therefore, during exercise the predictions of these two models might be less valid. In addition to the environment, geographic location, season of the year, and size of the individual (Koelblen et al., 2016) can influence scaling and the way one perceives the environmental conditions. As of late, no standardized approach or protocol to this topic has been developed (Koelblen et al., 2016). Moreover, a protocol which integrates exercise performance has not been developed. Another example of a lack of validity in thermal scaling
can been observed in a behavioral thermoregulation study by Flouris and Cheung (2009). Using a model modified from Gagge et al. (1967), during exercise under a hot condition, differences in thermal sensation and thermal comfort were not observed while an increase in $T_c$ and $T_{sk}$ was present.

The thermosensory system encompasses an array of perceptions such as coolness and warmth, cold pain and heat pain, and is divided in two dimensions; affect and perceived intensity. For example, heat pain has been documented as a close relationship between perceived intensity and unpleasantness of extreme heat (Price et al., 1992; Price et al., 1999). Typically, the thermosensory system is studied separately from nociceptive system (Kenshalo, 1970; Stevens et al., 1974; Green, 1986), and the affective system has rarely been studied outside of environmental comfort (Stevens et al., 1969; Mower, 1976; Hensel, 1982). In a study by Greenspan et al. (2003), the thermosensory system was evaluated psychophysiologically spanning a range of tolerable temperatures. The participants provided ratings of: (1) perceived thermal intensity; (2) perceived pleasantness or unpleasantness; and (3) perceived pain intensity. The relationship between perceived intensity and (un)pleasantness was different between the hot and cold conditions. Furthermore, cold stimuli were rated as more unpleasant than hot conditions. As cold temperatures decreased, and warm temperatures increased, stimuli were perceived as being unpleasant before painful. Furthermore, in a hand water immersion study by Sarlani et al. (2002), they used the same model when comparing gender differences in thermosensation. Given that several studies indicate females perceive noxious stimuli as more painful than males (Fillingim and Maixner, 1995; Berkley, 1997; Riley et al., 1998; Dao and LeResche, 2000), the aim of the study was to evaluate the gender differences in sensory and affective dimensions of the entire thermosensory system. During the study, participants immersed their hands in water ranging
from 10-45°C, and their perceived thermal intensity, (un) pleasantness, and pain intensity were recorded. No gender differences were evident for the thermal intensity ratings, however, a significant sex effect was present for the pain intensity rating ($P < 0.01$), and a significant sex X temperature interaction for the affective ratings ($P < 0.01$).

After reading the literature, it can be concluded that there is no reliable or valid scale for thermal perception (Koelblen et al., 2016). Although the Gagge et al. (1967) scale has been used by Flouris and Cheung (2009), these results were not reliable as thermal perception did not change with deviations in $T_c$ and $T_{sk}$. Given that the model used by Greenspan et al., (2003) and Sarlani et al., (2002) has been used with females and encompasses different dimensions of the thermosensory system, both affect and perceived intensity, and showed differences in how temperature influences pain and pleasantness, (Sarlani et al., 2002), allows the researcher to analyze thermal perception from different angles compared to a standardized 1-10 or 7 point scale (Gagge et al, 1967; ASHRAE, 2004). Furthermore, with all of the scales developed in a non-exercise setting, the Greenspan et al. (2003) model allows the researcher to separate affect and perceived intensity. The model used by Greenspan et al. (2003) and Sarlani et al. (2002) is depicted below (Fig 2.6.2.1).
Figure 2.6.2.1 (a) pleasantness, (b) pain intensity, and (c) temperature perception model modified from Greenspan et al, (2003).
2.6.3 Physiological measurements

2.6.3 a Temperature

As both the applied and research setting use temperature as an indicator of thermoregulatory response, the accurate measurement of $T_{sk}$ and $T_c$ is critically important. In the past, this usually required the attachment of hard-wired thermal sensors to the skin surface (Buono et al., 2007) and a mercury thermometer (Moran et al., 2002), respectively. Currently, a variety of invasive and non-invasive methods are used to track and collect $T_{sk}$ and $T_c$.

While each technique has its own methodological limitations, $T_{sk}$ can be measured through contact and non-contact. As of late, wired thermistors are the ‘gold standard’ and used as the criterion when validating new tools (Kelechi et al., 2011; Buono et al., 2007; Burnham et al., 2006), however wires getting entangled in moving limbs or slow response times can be an issue and difficult to use in the sport research setting (Buono et al., 2007). To avoid these issues, a common method of measurement includes the infrared thermometer—a non-contact measurement tool. In contrast, Buono et al. (2007) showed that infrared thermometry is a valid measure of $T_{sk}$ during rest and exercise in both hot and cold environments 35, 25, and 15°C. As expected, $T_{sk}$ increased significantly ($P < 0.05$) with an increase in environmental temperature. Furthermore, at rest and exercise, there was a non-significant difference between the infrared and contact thermometry. The correlation of the resting mean temperature using the infrared and contact thermometry was $r= 0.95$, while the correlation during exercise was $r= 0.98$.

As with $T_{sk}$, the wired thermistor is the ‘gold standard’ when measuring $T_c$, specifically the rectal area in scientific research (Moran and Mendal., 2002). In comparison, the wire connection between the thermistor and the measuring device makes this technique problematic.
and may not be practical for research, particularly in a field setting. In addition to the rectal area, the wired thermistor can be used through the oral, axilla, tympanic membranes, or at the body surface. Invasively, measurement sites include the pulmonary artery, the rectal area, through the esophagus, and at the urinary bladder (Moran and Mendal., 2002). The purpose of the measurement will determine which modality should be used.

An alternative approach is the telemetry pill. The telemetry pill is a device used to monitor \( T_c \) via a radio wave signal transmitted from the ingested pill sent to a small external receiver. In comparison, Lee and colleagues (2000) analyzed measurements of intestinal temperature (\( T_{in} \)) to esophageal (\( T_{es} \)) to rectal temperatures (\( T_{rec} \)), respectively. Peak temperature tended to be different, however, not significant (\( p = 0.07 \)). During exercise \( T_{rec} \) was less than the \( T_{es} \) and \( T_{in} \) at the end of the 40% (\( T_{rec} \): 37.20 ± 0.10; \( T_{es} \): 37.38 ± 0.11; \( T_{in} \): 37.35 ± 0.06°C) and 65% VO\(_2\)peak stages (\( T_{rec} \): 37.63 ± 0.08; \( T_{es} \): 37.83 ± 0.10; \( T_{in} \): 37.75 ± 0.05°C). The results suggest that \( T_{in} \) may be an acceptable alternative to \( T_{es} \) and \( T_{rec} \). During exercise, \( T_{in} \) were similar to \( T_{es} \), however, in recovery \( T_{es} \) (-0.030 ± 0.002°C/min) was lower than \( T_{in} \) (-0.023 ± 0.003°C/min) and \( T_{rec} \) (-0.010 ± 0.003°C/min). Therefore, given that the differences are small and not significant when comparing peak temperature in \( T_{in}, T_{es}, \) and \( T_{rec} \), makes \( T_{in} \) a viable option when measuring \( T_c \).

### 2.6.3 b Sweat rate

There are four typical physiological signals representing the human thermal status: \( T_c \), mean \( T_{sk} \), peripheral blood flow, and sweat. Several studies have reported that the amount of sweat is highly related to the thermal status (Wilke et al., 2007; Cheuvront et al., 2009). When measuring sweat, the most widely used methods are sweat rate and skin conductance. While
sweat rate measures the humidity evaporation rate and sweat generation on the skin (Wilke et al., 2007), skin conductance measures the change in electrolyte content—accomplished with two electrodes attached on the skin. A sweat rate sensor is a type of device that can measure both at the same time (Sim et al., 2017). Furthermore, through calculating the difference in body weight from pre-to-post exercise, measuring fluids during, and monitoring urine/fecal output, sweat rate can be determined without advanced technology. Although this method is commonly used in exercise physiology, error may arise and lead to misleading results. Factors that influence error include substrate oxidation, endogenous oxidation stores, and changes in the water content of the bladder and the gastrointestinal tract (Maughan et al., 2007), therefore, 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 being used (Cheuvront and Montain, 2017). Given that estimates of sweat loss from changes in body mass should adjust for non-sweat losses, we propose the use of the following equation:

\[
\text{Sweat loss (g)} = [\text{change in body weight (g)} + 0.20 \text{ g kcal}^{-1} \text{ fluid intake (g)} - (\text{urine + fecal output})(g)]
\]

(Broad et al., 1996; Cheuvront and Montain, 2017)

2.7 Interventions to Improve Performance in the Heat:

2.7.1 Cooling Devices

Strategies to improve performance in the heat include pre-cooling, which involves the rapid removal of heat from the body before exercise to create a larger heat storage capacity (Ross et al., 2013), can be accomplished passively or through substance ingestion. The purpose of pre-cooling is to lower the core-to-skin temperature gradient that is associated with a decrease in exercise performance (Cuddy et al., 2014; Kenefick et al., 1985). As of late, cooling strategies
have been used to counterbalance the effects of exercising under heat stress and drop $T_c$ through a variety of ways (Tyler et al., 2015). Most recently, Bongers et al. (2017) did a comprehensive review on pre-cooling techniques and the characteristics of each method. Usually, differences between each intervention include temperature, total covered body surface area, and the applicability in a field based setting. Some of these techniques include cooling vests, ice vests, cold water ingestion, ice slurry ingestion, menthol cooling, facial wind/water spray, cooling packs, cold water immersion, and cryotherapy. Furthermore, core temperature will respond to each cooling technique quite differently (Bonger et al., 2017), thus, external cooling may not always elicit a reduction in core temperature prior to exercise (Minett et al., 2011). In addition, a dichotomous approach toward pre-cooling is apparent with interventions either cooling externally or internally, a cooling vest and slurry ingestion are examples of these, respectively.

As of late, the mixed-methods approach is gaining popularity, the purpose of this is to increase total body surface area being cooled, hence, a combination of internal and external cooling will impact the splanchnic region thermosensitive receptors and reduce skin temperature (Rowell et al., 1968; Duffield et al., 2009). Ultimately, the type of cooling technique will be determined by accessibility, practicality, and the rules and regulations of the sport. In addition to passive cooling, the ambient temperature also has the potential to be performance enhancing. Most recently, Periard et al. (2016) demonstrated that individuals who exercised in cool environments were able to maintain a higher power output for a greater amount of time, this was compared between cold, hot, and hypoxic environments. Moreover, in a study by Gonzalez-Alonso et al. (1999), participants partook in a cycle to exhaustion study under three different pre-exercise conditions; submersion from the neck down at 17, 36, or 40°C, initial $T_c$ was $35.9 \pm 0.2$, $37.4 \pm 0.1$, and $38.2 \pm 0.1^\circ$C, respectively. Furthermore, TTE was related to initial body temperature: 63
± 3, 46 ± 3, and 28 ± 2 min with initial $T_c$ and ~36, 37, and 38°C, respectively (all $P < 0.05$). Ultimately, core temperature can be a major concern for athletes competing at all levels, therefore, to mitigate the effects of exacerbated heat strain, pre-cooling is a viable option.

In contrast to pre-cooling, mid-cooling is another technique that can decrease thermal stress. Mid-cooling can be defined as the application of cooling during a test of endurance performance or capacity (Stevens et al., 2017). The philosophy of mid-cooling is similar to pre-cooling as the purpose is to increase time to fatigue. Moreover, the mechanisms contributing to the ergogenic benefits of mid-cooling include the combination of cardiovascular and thermoregulatory adjustments (increased HR, skin blood flow, and $T_{sk}$), central nervous system adjustments (skeletal muscle activation and neurotransmitter activity), and psychophysiological adjustments (TS and TC), all largely responsible for heat-mediated fatigue (Nybo et al., 2014; Cheung et al., 2004). Although pre and mid-cooling are quite similar, differences do occur. While pre-cooling is fixated on dropping $T_c$, most mid-cooling interventions do not drop $T_c$, they drop $T_{sk}$ or change thermal perception. Moreover, the effects of mid-cooling differ from pre-cooling since such strategies cool the body already under heat stress—which usually translates to an immediate impact on performance (Tyler et al., 2011). Mid-cooling induced improvements have varied between 9 and 18% (Stevens et al., 2017). As with pre-cooling, mid-cooling can be accomplished through the application of internal and external methods, such as a cooling garment (Tyler et al., 2011; Hasegawa et al., 2005; Luomala et al., 2012) and ingestion of cold beverages (Riera et al., 2014, Schulze et al., 2015), respectively. Mid-cooling can be done with a variety of techniques that include cooling packs (Minniti et al., 2010; Tyler et al., 2011; Tyler et al., 2010), cooling vests (Eijsvogels et al., 2015; Luomala et al., 2011), cold/ice slurry ingestion (Byrne et al., 2011; Mundel et al., 2006), facial wind or water spray (Schlader et al., 2011;
Stevens et al., 2016), and MEN cooling (Mundel et al., 2010; Barwood et al., 2015; Stevens et al., 2016). During exercise, the beneficial effects of pre-cooling normally attenuate after 20-25 minutes of exercise (Bolster et al., 1999). Moreover, new comparisons utilizing robust and externally valid experimental designs suggest that the advantage gained from mid-cooling can outweigh that of pre-cooling (Stevens et al., 2016; Stevens et al., 2017). In addition, the thermal strain during exercise is typically higher compared to rest or warm-up conditions, therefore, mid-cooling could have a greater benefit on thermoregulation.

The basis of pre-cooling and mid-cooling strategies is to reduce heat stress of the thermoregulatory system prior to and during exercise by increasing heat storage capacity (Marino et al., 2002; Wegmann et al., 2012). In intermittent running work by Price et al. (2009), they showed that both pre-cooling and mid-cooling protocols are beneficial, however, pre-cooling + mid-cooling was most effective in reducing heat storage. In addition, it is important to note that the most effective methods are not always most practical (Marino, 2002). The characteristics of the sport, competition level, and competition venue will influence what cooling strategies should be used. For example, in the sport of cycling, strategies that are practical and have shown to improve performance include pre-cooling with an ice-vest (Cuttell et al., 2016), and mid-cooling with an ice-slurry ingestion or menthol mouth rinse (Riera et al., 2014; Schulze et al., 2015; Flood et al., 2017). However, if the participant does not have an integrated support team or the venue is in a hot place without deep freeze access, the easily transportable menthol mouth rinse may be the only viable option.
2.7.2 Mouth Rinses

2.7.2 Carbohydrate

In humans and other types of organisms, the purpose of taste is to enable appropriate use of chemical cues; these are used in the selection of nutritive, non-nutritive, and toxic foods (Galef, 1981). Taste perception, the sensation produced when a substance reacts chemically with a taste receptor, starts on the tongue and soft palate where the brain processes the stimuli. When comparing men and women, sex differences occur at the level of the receptor and are processed differently, women detect basic taste stimuli at lower concentration than men. (Glanville et al., 1964; Fikentscher et al., 1977; Weiffenbach et al., 1982; Mojet et al., 2001; Heft and Robinson, 2010; da Silva et al., 2014; Yoshinaka et al., 2015).

While a difference in taste preference is inconsistent (Dessor et al., 1975; Conner and Booth, 1988; Frye et al., 1994; Frye and Demolar, 1994; Jamel et al., 1996), several reports have confirmed enhanced food consumption and preference for sweetness (Bowen and Grunberg, 1990; Dalvit, 1981) and decrease of gustatory sense (Glanville and Kaplan, 1965; Bajaj et al., 2001) in women just prior to menstruation. It has been speculated that the involvement of the steroid hormones (estrogen and progesterone) may be one of the causes of this phenomenon. Moreover, it has been shown that women tested during the luteal or menstrual weeks displayed greater preference for taste stimuli compared to those tested during the follicular and ovulatory weeks (Fryre et al., 1994). Glanville and Kaplan (1965) found that taste thresholds during the menstrual period were significantly more sensitive than those in pre-menstrual phase; he speculated that fluctuations in taste sensitivity at the time of menstruation correlate with fluctuations in the endocrine balance.
Carbohydrate (CHO) tastants, known as the gustatory system, provides sensory input that is critical for ingestive behavior and avoidance of toxic compounds, the sense of taste interfaces extensively with neural substrates of reward and motivation (Chambers et al., 2009). Once a gustatory stimulus is evoked, a two-dimensional response discriminative at the cortical level and affective (emotional) at the hypothalamo-limbic level occurs. The discriminative dimension corresponds to the intensity and chemical and physical properties of tastes (Norgren, 1985). The sense of taste can be differentiated in four sensory qualities: sweet, salty, sour, and bitter. Furthermore, the umami taste has been widely accepted with the Japanese being first. Among the sensory qualities, each tastant can be denoted as pleasant or unpleasant stimuli, pleasant stimuli will elicit approach and acceptance, while unpleasant ones induce rejection (Smith and Vogt, 1997). Moreover, each tastant will affect the autonomic nervous systems differently. In a study by Rousmans et al. (2000), the pleasant-connoted sweet taste induced the weakest electrodermal, thermovascular, and cardiac responses whereas unpleasant-connoted tastes (salty, sour, and bitter) induced the strongest responses.

Through physiological testing in endurance-based sport, a plethora of research has been conducted on CHO sensory receptors and the impact they may have on exercise performance. The presence of CHO in the mouth has been shown to attenuate declines in motor function associated with fatigue, but also increase motor performance without fatigue (Gant et al., 2010). Carter et al. (2004a) first studied the effect of CHO mouth rinse on performance after results demonstrated CHO ingestion improved performance during high intensity exercise and was not accompanied by an increase in CHO oxidation. Following this, a subsequent study took place where they demonstrated a positive mouthwash effect was eliminated when glucose was infused
instead of ingested (Carter et al., 2004b). These results indicate that an oral CHO mouth rinse may exert its effects during high intensity action via central nervous system.

CHO mouth rinsing is defined as a CHO fluid distribution around the mouth for 5 to 10-sec with subsequent expulsion by spitting. The duration of mouth rinse can significantly impact performance as Sinclair et al. (2014) showed greatest results at 10-sec compared to 5-sec. Typically, a low concentration of 6.0% or 6.4% glucose (Chambers et al., 2009) or partially hydrolyzed maltodextrain are the most common CHO used (Carter et al., 2004). Most recently, Devenney et al. (2016) studied the effects of various concentrations of CHO mouth rinse on cycling performance in a fed state, when comparing a 6% to 16% CHO solution, no significant difference was observed. The effects of a CHO mouth rinse have been proven in euthermic and hot environments (Carter et al., 2004a; Carter et al., 2004b), trained and untrained individuals (Fares and Kayser, 2011; Whitham and McKinney, 2007), cycling and running (Rollo et al., 2008), and in fasted and fed states (Lane et al., 2013). In a study by Carter et al. (2004), they investigated the effects of a CHO mouth rinse (6.4% maltodextrin solution) or a non-CHO flavored matched placebo rinse on a 1-hr cycling time-trial performance. The researchers reported that the CHO-containing mouth rinse significantly improved performance (~3%, \(p<0.05\)).

It has been suggested that oral receptors within the mouth directly stimulate reward centers in the brain which increase “central drive” and improve work capacity (Carter et al., 2004; Chambers et al., 2009; Fares and Kayser, 2011; Jeukendrup and Chambers, 2010; Pottier et al., 2010; Rollo et al., 2010). The activation of reward areas in the brain, such as the insula/frontal operculum, orbitofrontal cortex, and striatum are suggested to lower perception of exertion during exercise (Chambers et al., 2009; Backhouse et al., 2007), and potentially feelings...
of displeasure (Backhouse et al., 2005). However, evidence suggests that the magnitude of performance increment is dependent on several factors and must be taken with caution. In a study by Lane et al. (2013), the CHO mouth rinse improved mean power to a greater extent after an overnight fast (282 vs. 273 W 3.4%; p<0.01) compared with a fed state (286 vs 281 W, 1.8%; p< 0.05). Therefore, even though CHO mouth rinse improved performance to a greater extent in fasted compared to fed, optimal performance was achieved in the fed state. A proposed mechanism of the CHO mouth is depicted below in Figure 2.7.2.1

Figure 2.7.2.1. A proposed mechanism of the influence of CHO perception and motor output.
2.7.3 Menthol:

In humans, temperature is sensed through primary afferent sensory neurons whose cell bodies are located in the dorsal root and trigeminal ganglia. The signals from these cells are transmitted to the brain via spinal cord where they are integrated to evoke reflexive and cognitive responses. These sensory neurons are found on the external surface of the body and in the oral cavity and the nose (Eccles et al., 1994). The external surface receptors are involved with thermoregulation, while the receptors in the mouth and nose are involved with temperature of food and drink (Bruck, 1989). The principle molecular thermosensors in the sensory neurons belong to the family of transient receptor potential (TRP) channels. So far, six TRP channels have been identified with four belonging to the TRPV subfamily heat sensing (TRPV1, TRPV2, TRVP3, and TRVP4) and TRMP8 and TRPA1 cold sensing. Some of these channels are also sensitive to compounds that mimic temperatures (Peier et al., 2002; McKemy et al., 2002). The TRPV1 channels in sensory nerves respond to heat and capsaicin, an alkaloid from “hot” peppers, which binds to open the channel and thus depolarizes the neuron and fires action potentials (Caterina et al., 1999). The brain interprets this information as an increase in ambient temperature and initiates vasodilation and sweating. TRPM8, which binds ligands like MEN or icilin and elicits a cold sensation, is a non-selective cation channel predominantly expressed in a subpopulation of thermoceptive/nociceptive neurons found in the dorsal root ganglia and in trigeminal ganglia. From the trigeminal ganglia, increased activity in the insular taste cortex, somatosensory cortex, orbitofrontal cortex, anterior cingulate cortex, ventral striatum, and pregenual cortex is observed (Guest et al., 2007). Stimulation of this thermoreceptor can lead to shivering, a mechanism to raise body temperature. TRPM8 is activated by cold temperature with a threshold of ~22°C (Voets et al., 2004; Brauchi et al 2004) and by MEN (Hensel and
Menthol, a cooling agent which stimulates the TRPM8 receptor, can be found in an array of products such as candy, chewing gum, toothpastes, common cold medications, vapo-rubs, cigarettes, aromatherapy medications, and mouth wash. Furthermore, menthol has been proven as an ergogenic aid in exercise performance and can be used internally and externally (Stevens and Best, 2017). The use of menthol as a non-thermal cooling agent has the capability to increase self-selected cycling power output when applied topically to the face (Schlader et al., 2011) and increase cycling TTE when rinsed in the mouth (Mundel and Jones, 2010). Given that menthol triggers stimulation of the TRPM8 thermoreceptor, recent research has demonstrated that the use of menthol as a non-thermal cooling agent can reduce thermal sensation and/or state during exercise, which may contribute to increased skeletal muscle activation (Duffield, 2009). Moreover, menthol may also have an “alerting” or “arousal” action. Cold stimuli can influence our level of arousal as sensory inputs from thermoreceptors and influence our level of consciousness. Our level of consciousness and the excitability of the cerebral cortex is controlled by the brainstem reticular formation (Zimmerman, 1989). When feeling drowsy and about to fall to sleep, cold water or air applied to the face will stimulate trigeminal nerves serving the sensation of touch or cold. Moreover, it is possible to arouse someone who has temporarily lost consciousness and “fainted” by administering smelling salts to the nose; smelling salts contain strong smelling substances such as ammonia and menthol which stimulates the trigeminal nerves (Eccles, 2000).

Sex differences in responses of TRPM8 receptors on skin are observed. Typically, females report discomfort in cool environments, while their male counterparts find the same temperature as comfortable or even a bit warm (Karjalainen, 2012). In contrast, the differences between sexes
in the perception of cool temperature could be manifested in the primary afferent fibers in the expression of TRPM8, or some sort of ion channel that resulted in a difference in the frequency or pattern of their action potentials in response to low temperatures. In a study by (Caudle et al., 2017), behavioral experiments demonstrated greater sensitivity to 18°C in female mice compared to male. While the menthol mouth rinse improves athletic performance in male athletes (Mundel and Jones, 2010; Stevens et al, 2016; Flood et al, 2017; Jeffries et al, 2018), no study to date has examined women in menthol sensitivity and impact on the TRPM8 receptor in the mouth.

During exercise, both thermal state and sensation appear to have an influence on endurance performance in the heat (Cheung, 2010). Muscle activation during dynamic exercise may be reduced in hot conditions because of central fatigue (Nybo and Nielsen, 2001) or anticipatory afferent signals to the brainstem (Tucker et al., 2004). Menthol has been used to ameliorate these effects and can be used to improve performance (see Table 2.1). During exercise, a novel strategy for implementing the menthol mouth rinse is to simply rinse the mouth with a liquid menthol solution for 5-sec (Stevens et al., 2016). As of late, no research has quantified the effects of duration of menthol mouth rinse. The menthol mouth rinse has shown performance improvements in hot environments (Stevens and Best, 2017), at different drink temperatures and frequencies (Tran Trong et al., 2015; Stevens and Best, 2017), in time trial and endurance performance (Stevens et al., 2015; Mundel and Jones, 2010) and at concentrations of 0.01 and 0.05% (Mundel and Jones, 2010; Riera et al., 2014). Similar to menthol mouth rinse duration, no study has evaluated the impact on performance of menthol mouth rinse concentration. Tran Trong et al. (2015) compared menthol beverages at three temperatures: neutral, cold, or ice-slurry. No difference was noted for T_c, HR, RPE, thermal comfort, or thermal sensation, although the greatest increase of performance was observed in the
menthol/ice-slurry beverage of 6%. A proposed mechanism of the menthol mouth rinse is depicted below, Figure 2.7.3.1.

Figure 2.7.3.1. A proposed mechanism of the influence of menthol on perception and motor output.
<table>
<thead>
<tr>
<th>Investigation</th>
<th>Ambient conditions</th>
<th>Subjects</th>
<th>Menthol Application method</th>
<th>Protocol</th>
<th>Performance Outcome</th>
<th>Thermal Sensation Outcome</th>
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<tbody>
<tr>
<td>Mundel and Jones (2010)</td>
<td>34°C, 27% relative humidity</td>
<td>9 men, VO₂max = 54 ± 5 mL·kg⁻¹·min⁻¹</td>
<td>Menthol mouth rinse (25 mL at 0.01% every 10 min)</td>
<td>-Cycling TTE at 65% VO₂max</td>
<td>-Increased TTE by 5 min (9%)</td>
<td>-Not reported</td>
</tr>
<tr>
<td>Stevens et al, (2016)</td>
<td>33°C, 46% relative humidity</td>
<td>11 men, 5-km run time of 18–22 min</td>
<td>Menthol mouth rinse (25 mL 0.01% at 0.2 of every 1 km)</td>
<td>-10-min walk/run on non-motorized treadmill then running TT of 5 km on non-motorized treadmill</td>
<td>-Improved performance time by 0.7 min (3%)</td>
<td>-0.3 pt decrease in TS at across trial</td>
</tr>
<tr>
<td>Stevens et al, (2017)</td>
<td>33°C, 47% relative humidity</td>
<td>11 men, 3-km run time of 17–23 min</td>
<td>Menthol mouth rinse (25 mL 0.01% at every 0.2 of 1-km of the preload trial, and every 0.1 km of every 1 km)</td>
<td>-Preloaded running time trial consisting of 20-min at 70% VO₂max, 5-min of seated rest, and a 3-km maximal self-paced TT</td>
<td>-Improved performance by 4%</td>
<td>-0.3 pt decrease in TS at across trial</td>
</tr>
<tr>
<td>Flood et al, (2017)</td>
<td>35°C, 48% relative humidity</td>
<td>8 men, VO₂max= 55.4 ± 6.0 ml/min/kg), minimum 5h general fitness training per week</td>
<td>Menthol mouth rinse (25mL at 0.01%) 1.5 min prior to exercise, and at regular 10 min interval</td>
<td>-TTE cycling exercise at an RPE of 16</td>
<td>-Improved trial duration by 7%</td>
<td>- 0.4 pt decrease in TS at across trial</td>
</tr>
<tr>
<td>Study</td>
<td>Temperature</td>
<td>Relative Humidity</td>
<td>Participants</td>
<td>Exercise Details</td>
<td>Findings</td>
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<tr>
<td>Jefferies et al., (2018)</td>
<td>35 ± 0.2°C, 40 ± 0.5%</td>
<td></td>
<td>10 men, (age 33 ± 9 years; peak oxygen uptake [( V_{O2peak} ) 52.4 ± 5.3 ml kg(^{-1}) min(^{-1}); maximal aerobic power output (W(_{max}) 371 ± 27 W)]</td>
<td>Menthol mouth rinse (25mL at 0.01%) at 85% of the participants baseline time to exhaustion</td>
<td>-TTE cycling exercise at 70% W(_{max})</td>
<td>-Exercise time was increased by 6%</td>
</tr>
<tr>
<td>Gibson et al., (2018)</td>
<td>40.2 ± 0.6°C, 42 ± 2%</td>
<td></td>
<td>14 men participant s, (age 33 ± 9 years; ( V_{O2peak} = 3.30±0.90 \text{L min}^{-1} ))</td>
<td>Menthol (0.01% L-menthol) or capsaicin (0.2% capsaicin) every 10 min</td>
<td>-40-mins of intermittent sprinting</td>
<td>-No difference in intermittent sprint performance between trials</td>
</tr>
</tbody>
</table>
Chapter 3

3 Study Rationale, Objectives, and Hypothesis

The literature has provided us with tangible evidence describing the influence of environmental temperature on fatigue and cycling TT performance. By changing the perception of how one feels, an individual can mitigate fatigue resulting in an improvement of performance.

During exercise in the heat, the thermoregulatory system is under severe stress to keep the body in homeostasis. Furthermore, women may experience greater stress compared to their male counterparts. Menstrual fluctuations in thermoregulation and corticomotor activity have been described with increases of $T_c$ and attenuation of motor neuron excitability observed during the luteal phase (Haslag and Hertzman, 1965). Moreover, for the 47.8% of Canadian premenopausal women using an OC or IUD, they can expect similar responses to women during the luteal phase (Martin and Buono, 1997).

During exercise, the body relies on the central governor, a central nervous system mechanism that takes input information about energetic needs, current physiological states, and various motivational drives to regulate physical exertion and save the organism from catastrophic homeostatic failures during physical exertion (Noakes et al., 2005). A plethora of research has been done to override the central governor and improve performance. One intervention which shows promise is a menthol mouth rinse. Menthol, a cooling agent which stimulates the TRPM8 receptor in the mouth, reduces thermal sensation and may increase self-selected power output (Mundel and Jones, 2010); this has translated to an improvement of performance between 3-9% (Jefferies and Waldron, 2019).

As of late, no research has studied the effects of a menthol mouth rinse in female athletes. Given that many competitions (Provincial, National, and Olympics) are hosted in hot
environments, strategies to handle and mitigate fatigue are imperative. Even small alterations in performance can be critical. At the 2016 Rio de Janeiro Olympic Games during the women’s 29.8 km cycling TT, the difference between 1-4th was 0.83%, while the difference between 1-8th was 2.06% (Olympic, 2018).

Findings from this study will help describe the physiological and psychological differences in 30-km TT performance under hot conditions, with and without menthol. The applicability of this research is to help female athletes improve performance and mitigate fatigue in hot conditions. Moreover, we hope to help female athletes at the 2020 Tokyo Olympic and Paralympic Games. Considering a menthol mouth rinse has been shown to increase performance in male athletes, postulates the menthol mouth rinse could benefit females as well. To date, there are no published studies quantifying the effect of a menthol mouth rinse in female athletes.

3.2 Objectives

3.2.1 Primary Objectives

To characterize the effect of a menthol mouth rinse on cycling 30-km TT performance by simultaneously measuring power output, $T_c$, HR, sweat loss, fatigue, and perception of the environment, to:

I. Determine if a menthol mouth rinse can improve TT performance

II. Determine if a menthol mouth rinse can alter perceptual responses during exercise in the heat

III. Determine if a menthol mouth rinse can alter thermoregulatory responses during exercise in the heat
IV. Determine if a menthol mouth rinse can improve isometric strength and a singular bout of maximal exercise

3.3.1 Primary Description 1. TT performance (covering a fixed distance as fast as possible) will be quantified and measured through:
   a) Total time
   b) Average power output

2. Thermoregulatory responses will be quantified and measured through:
   a) Average HR
   b) Average $T_c$
   c) Maximal $T_c$
   d) Sweat loss

4. Fatigue will be quantified through:
   a) Handgrip strength
   b) 5 sec maximal sprint
   c) Power output at 5km, 10km, 15km, 20km, 25km, and 30km
   d) Average power output

5. RPE and thermal scaling will be quantified through:
   a) Borg 20-point scale
   b) Thermal pleasantness
   c) Thermal pain
   d) Thermal sensation
3.4 Hypotheses

3.4.1 Primary hypothesis:
A menthol mouth rinse will improve 30-km TT performance in female athletes under hot conditions.

3.4.2 Secondary hypothesis:
1. A menthol mouth rinse will increase power output during the later stages of a 30-km TT in the heat.
2. A menthol mouth rinse will increase power output during a 5-sec sprint
3. A menthol mouth rinse will increase hand grip strength following a 30-km TT
4. A menthol mouth rinse will improve RPE, thermal pleasantness, thermal sensation, and decrease thermal pain at a given point of exercise

Chapter 4

4 Methods and Study Design

4.1 Study Participants

Participants, specifically premenopausal women between the ages of 25-35 yr were recruited from cycling clubs in the Greater Toronto Area.

4.1.1 Inclusion/Exclusion Criteria

To be included in the study, participants were between the ages of 25-35y, furthermore, participants trained five sessions per week and included winter training. Prior to participation, participants filled out a “Physical Activity Readiness Questionnaire-PAR-Q”. Following the questionnaire, if the participant answers “yes” to one or more of the follow-up
questions, they were advised to seek a health care practitioner and return an “ePARmed-X+Online” form before they could participate. On days of menstruation, acute illness, and in case of injury, participants were rescheduled.

4.1.2 Recruitment and Consent

Candidate participants were recruited by the principal investigator via email, flyers, and phone calls. A total of 33 cycling clubs were contacted, 26 people expressed interest, however only 11 signed up for the study. Each participant was informed of the purpose, procedure, potential risks of the study. Participants were deceived and told the purpose was to compare mouth rinse preference, they were then debriefed the actual purpose following the study. Participants were informed of their right to withdraw from any point of the study. After the purpose and procedures were explained and questions were answered, participants were asked to provide oral and written consent.

4.2 Study Design

- An interventional randomized order cross over study took place to determine 30-km TT performance under two different conditions: hot with a placebo, and hot with menthol mouth rinse (Fig 4.1.1). The participants were provided no encouragement throughout the trials. However, the participants were told of distance left every 2.5 km. Once the participant reached 20km, they were told distance left every 1 km. Each mouth rinse contained blue food colouring.
- Visit 1: Preliminary testing
- Visit 2: Familiarization trial
Visit 3-4: Randomized experimental trials

- Hot + placebo
- Hot + menthol

Participants performed their trials at the same time each day to reduce the effect of circadian variation. Participants were asked to follow their normal diet and to refrain from any form of intense physical activity for the 24h prior to testing. Four to six hours prior to each trial, participants ingested a telemetry pill. Upon arrival to the laboratory, hydration status was measured. Euhydration was established by identifying urine specific gravity of <1.020. Before and after each trial, participants were weighed in nude attire to determine sweat rate. Upon entering the environmental chamber, RPE and thermal scaling was recorded. An incremental cycling test to volitional fatigue was conducted to measure maximal values. The values served as a reference point throughout the additional trials. During the familiarization trial, participants were allowed to drink freely, the volume of water ingested was adjusted in the first subsequent trial and replicated in the second.

Within a week, but no less than 72 hours before the familiarization trial, each participant completed preliminary testing, hand grip strength, a 5-sec maximal sprint, dual-energy X-ray absorptiometry (DEXA) scan, and a maximal oxygen uptake (VO\textsubscript{2max}) incremental test to volitional exhaustion on a cycle ergometer. The incremental test determined each participant’s maximum aerobic capacity in relation to RPE, HR, and power output. These maximum values served as reference data throughout the following trials.
4.2.1. **Warm-up**

Before each trial, participants warmed-up for 10 mins on a cycle ergometer at an intensity of ~10/20 Borg RPE scale (~40% VO\textsubscript{2max}), and then rested at least 3-min prior to performing the 5s sprint (Mendez-Villanueva et al., 2007).
4.2.2 Preliminary Testing

4.2.2 a Handgrip strength

In a sitting position with arms by the side, each participant squeezed a Jamar Hydraulic Hand Dynamometer in the dominant hand. Three trials were made with a pause of 20s between each trial to avoid the effects of muscular fatigue. The highest measurement was categorized as peak kilograms ($K_g_{peak}$).

4.2.2 b 5-sec Maximal Sprint

Subjects were instructed to perform a 5s sprint at maximum effort from a sitting start on the ergometer (Velotron, RacerMate, Inc., Seattle, Washington USA). All sprints were performed from the same initial pedal position with a resistance of 100W. These efforts were repeated three times with a 5-min break between each set. Strong verbal encouragement was provided during each trial. Peak power ($W_{max}$) and mean power output ($W_{ave}$) were recorded for each maximal 5-sec cycling bout.

4.2.2 c Incremental Cycling Test

Participants began the incremental test on a cycle ergometer to determine maximal oxygen uptake ($VO_{2max}$). The incremental test started at 25W with 20W increments every 1-min until voluntary exhaustion. Participants cycled at ~90 rpm, At the conclusion of each stage, RPE and thermal sensation, were recorded, furthermore, HR was monitored throughout. For the incremental cycle test, the 30s average VO$_2$ ($VO_{2ave}$) from each stage was used in analysis.
Maximal oxygen uptake (VO$_{2\text{max}}$) was defined as the highest VO$_2$ value in the last 30s of the test. Once the participant could not hold ~90 rpm for a total of 30 consecutive seconds, the session was ended. At the completion subjects cooled down for 5 minutes at 100W.

4.2.2 d Sample size

The critical outcome is time to complete 30-km. Previous research would suggest the impact of menthol is a 3 minute reduction in time with a standard deviation of 3.5 minutes. This would result in a sample size of 13. Due to time constraints and changing of seasons (spring to summer), only 11 participants were recruited, one participant dropped out and one participant was an outlier (> 3SD away from the mean change in time to complete independent time trial), 9 participants were included in analysis.

4.2.3 Experimental trials

4.2.3 a ACE Environmental Climatic Chamber

Each participant performed a 30-km TT. A full familiarization trial and a total of 2 randomized experimental trials separated by 7 days in an environmental chamber (MTS, 248.05) took place. Each trial was separated by 7 days to prevent heat acclimation. The familiarization and experimental trials were in environments of $30 \pm 0.6 ^{\circ}C$, $70 \pm 1\%$ relative humidity, and $12 \pm 1 \text{ km/h}$ windspeed. The participants conducted one experimental trial of “hot with placebo”, and one experimental trial of “hot with menthol mouth rinse”. Throughout the trial, participants were notified of km completed and km left, the reason of this was to simulate real life performance, however, power output, time, HR, and $T_c$ were not shared with the participant. Furthermore, before entering the chamber, participants rested for 20-mins and baseline measures
were recorded. Further, participants entered the environmental chamber and conducted a standardized 10 min warm-up. Refer to Figure 4.3.4.1 for a schematic of study design.

4.2.3 b  Hand Grip Strength

In a sitting position, each participant squeezed the dynamometer in the dominant hand. This effort was conducted twice at the beginning, and once at the end of each trial. The first effort was before the start of the TT immediately followed by the 5-sec maximal sprint, 5-min later participants swilled a menthol mouth rinse and repeated the hand grip exercise and 5-sec maximal sprint to characterize the acute effects of a menthol mouth rinse without fatigue. The second hand grip exercise was immediately followed (within 20 s) by the 5-sec maximal sprint, respectively. $K_{\text{g peak}}$ was recorded as an indicator of fatigue after each trial.

4.2.3 c  5-sec maximal sprint

Following the standardized warm-up and hand grip portions, participants performed a 5-sec sprint at maximum effort from a sitting start on the cycle ergometer against 15 kiloponds. These were repeated a total of 3 times, at the very beginning, 5-min prior to, and immediately following 30-km TT. $W_{\text{peak}}$ was recorded as an indicator of fatigue after each trial.

4.2.3 d  Mouth rinse

Participants were given 25 ml solution to rinse 4:40 min following first hand grip and 5-sec sprint, to and at regular 5-km intervals. They were instructed to swill around the mouth for 5-sec before spitting into a bowl without swallowing. L-Menthol solution (Sigma Aldrich, Canada)
was at a concentration of 0.64 mM (0.01%). A sucralose sweetener, “Crystal Light- Sugar Free”, was used as a placebo. All liquid ingested in the time trial was > 22 °C to prevent TRMP8 receptor disturbance (Voets et al., 2004; Brauchi et al 2004).

Menthol mouth rinse 30-km TT study design:

![Menthol mouth rinse 30-km TT study design](image)

**Figure 4.3.4.1**: Menthol mouth rinse 30-km TT study design. Each participant participated in a total of three “HOT” trials (30 ± 0.6 °C, 70 ± 1% relative humidity, 12 ± 1 km/h windspeed), one familiarization and two experimental. Each mouth rinse occurred at 5, 10, 15, 20, 25, and 30-km.

### 4.3 Measurements

#### 4.3.1 Core temperature, heart rate, and sweat

Rectal temperature was recorded every 10-min during the familiarization and experimental trials using an ingestible telemetry pill (HQ Inc., Palmetto, FL). Participants ingested the pill at least 4-6 hours prior to each trial to avoid any interaction with fluid ingestion (Bongers et al., 2015). HR was recorded by the second using a Polar H7 (Polar® H7 heart sensor). Sweat rate was determined by:
Sweat loss (g) = \[\text{change in body weight (g) + 0.20 g kcal}^{-1} + \text{fluid intake (g)} - (\text{urine + fecal output})(g)\]

4.3.2 RPE and Thermal sensation/comfort scales

In line with American College of Sports Medicine guidelines (ACSM, 1998), participants were instructed to pay close attention to how difficult the exercise felt, combining total exertion, and the physical stress of the environment, this was done with the 20 point RPE Borg Scale. Furthermore, thermal scaling was measured through thermal sensation, thermal pain, and thermal pleasantness. RPE and thermal scaling were recorded at the beginning, immediately after each mouth rinse, and at the end of each trial.

4.4 Data Collection

All preliminary testing (handgrip strength, 5-sec maximal sprints, and incremental cycling tests) was conducted at the Canadian Sport Institute Ontario (CSIO). All familiarization and experimental trials were conducted at Ontario Tech University. 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.

4.4.1 Statistical Description and Analysis

Data analysis was performed using SPSS (version 24; IBM Corp., Armonk, NY, USA) statistical software. The data was described using means and standard deviations if normally distributed. A paired sample t-test was carried out to assess differences in time trial performance and average power output. Furthermore, a two-way (trial X time) repeated measures ANOVA was used to examine changes in heart rate, core temperature, watt peak, hand grip, and the 5-sec
sprint. If statistically significant, a Bonferroni correction was applied, this was set at $p=0.008$ for heart rate, core temperature, and watt peak, and $p=0.025$ for the hand grip and sprint. Moreover, RPE, thermal sensation, thermal pain, and thermal pleasantness, were evaluated with the Freidman Test. If statistically significant, a Wilcoxon Signed Ranks Test was applied, this was set at $p=0.008$. 
Chapter 5- Menthol mouth rinsing improves cycling performance in females under heat stress

This chapter is a modified version of a manuscript to be submitted for review and publication

ABSTRACT

Introduction: This study investigated the effects of a menthol (MEN) mouth rinse (MR) on performance, physiological and perceptual variables which may relate to performance in female cycling athletes during a 30-km independent time trial (ITT). Further, this study provided an opportunity evaluate an application of the Central Governor Theory (CGT)

Methods: Participants (n=9) cycled for 30-km in hot conditions (30 ± 0.6 °C, 70 ± 1% relative humidity, 12 ± 1 km/h windspeed) on two test occasions: with a placebo (PLA) mouth rinse (MR) and with a menthol (MEN) MR, the MR occurred every 5 km. Handgrip (HG) and a 5-sec sprint (SPR) were measured as indicators of central and peripheral fatigue before, following the first MR, and after the ITT. Rate of perceived exertion Borg 6-20 (RPE), thermal sensation (TS), thermal pain (TP), and thermal pleasantness (PL) were recorded at 5, 10, 15, 20, 25, and 30-km. Tc and HR were recorded throughout.

Results: The MEN MR significantly improved ITT performance by 2.3 ± 2.7% relative to PLA (p= 0.034, d= 0.85, 95% CI= 8.43 to 166.9). Average power output was significantly higher in the MEN trial (p= 0.031, d= 0.87, 95% CI= 0.92 to 15.09). There were no differences in HG and SPR between and within each trial (HG, p= 0.581, $\eta^2 = 0.04$; SPR, p= 0.365, $\eta^2 = 0.103$). Heart rate, core temperature, and sweat loss did not differ between trials. There were no significant differences in RPE, TS, and TP between trials. PL significantly differed at 5-km, however there were no differences between PLA and MEN at 10, 15, 20, 25, and 30-km.

Conclusion: These results suggest that a non-thermal cooling agent can improve 30-
km ITT performance in female cyclists, the improved performance with MEN MR is not due to altered thermal perception or to reduced effort. Further, observed improvement in 30-km ITT performance with no change of HG or SPR with MEN MR supports CGT regulation of performance.
INTRODUCTION

Cycling time trials (TT) have become popular at the club and international competition levels, and can be separated into two categories, team time trials (TTT) and independent time trials (ITT). The TT consists of individual riders racing as a team (TTT) or independently (ITT). The purpose is to cover a fixed distance as fast as possible, maintaining the highest sustainable speed for distance and is completed at close to maximal intensities (Padilla et al., 2000). While competitions take place all over the world, often these races occur in hot environments, which can be detrimental to performance when compared to thermoneutral environments (Tatterson et al., 2000; Peiffer and Abbiss, 2011; Altareki et al., 2009). Relative to a thermoneutral environment (20-25°C), cycling in the heat (>25°C) is associated with a higher core temperature (T_c), elevated heart rate (HR) at a submaximal workload, and greater rate of perceived exertion (RPE) and thermal discomfort (Gisolfi and Wenger, 1984). Interventions to improve performance can be prior to (e.g. heat acclimation, ice baths, ice slurry ingestion) or during competition (e.g. cold water ingestion, cooling vest, menthol mouth rinse) (Stevens et al., 2016 Mundel et al, 2006; Karlsen et al., 2015; Jones et al., 2012; Stevens et al., 2016).

Using a menthol (MEN) mouth rinse (MR) throughout or during the latter stage of exercise is a practical strategy that has been shown to improve running (Stevens et al., 2016; Stevens et al., 2017), and cycling (Mundel and Jones, 2010; Jefferies et al., 2018; Flood et al., 2017) performance in the heat. Stevens et al. reported that MEN MR on its own, or with a concurrent water facial spray each km during running led to improvements of approximately 2.7% and 3.5% in 5 km and 3 km time trials, respectively (Stevens et al, 2016; Stevens et al, 2017). Similarly, when cycling at 65% to 70% of maximal power output from an incremental to
maximum exercise test, time to exhaustion was extended by between 4.6% and 9% compared to a placebo MR (Mundel and Jones, 2010; Jefferies et al, 2018).

Rinsing of MEN stimulates oropharyngeal cold receptors, which has been suggested to improve arousal, reduce ventilatory drive, and decrease thirst (Eccles, 2000). Specifically, MEN activates the Transient Receptor Potential Cation Channel Subfamily M Member 8 (TRPM8) (McKemy et al, 2002; Peier et al, 2002). As well, this receptor responds to cold stimuli and is believed to be responsible for the cold sensation experienced from MEN shown to improve thermal comfort, reduce RPE, and improve performance (Mundel and Jones, 2010; Flood et al, 2017). Furthermore, stimulation of the oropharyngeal cold receptor also increases activity in the reward centers of the brain and can improve the perception of ones’ state (Guest et al, 2007), which may be linked to a reduced sense of effort. The sense of effort can be related to the ‘central governor theory’ (CGT), a phenomenon which proposes that the subconscious brain regulates power output by modulating motor unit recruitment to preserve whole body homeostasis. For example, although not statistically significant, work by Mundel and Jones, (2010) showed that global RPE was lower during exercise when swilling MEN (14.6 vs. 15.1). In addition, in a study by Flood et al. (2017) moderately trained participants completed cycling trials at a RPE of 16 (hard to very hard) with a MEN or placebo MR (Flood et al., 2017). They found that for an RPE of 16, when given MEN, participants self-selected a higher power output, and their time to exhaustion was longer before dropping below 70% of their initial power output (Flood et al., 2017). Despite maintaining a higher power output and the same RPE, participants reported a lower thermal sensation (TS) throughout the trial (Flood et al., 2017). Participants also had a greater reduction from baseline in peak power output during an isokinetic sprint, following the MEN trial compared to the placebo trial (9.0% vs. 3.4% decrement, respectively) (Flood et
al., 2017). This suggests that the MEN trial led to greater peripheral fatigue, because participants exerted themselves more.

To date, the available research has only been conducted in male participants; therefore, the ergogenic benefits of a MEN MR in females is unknown. Several sex-based differences have been observed suggesting males and females may respond differently to exercise in the heat and menthol. In general, at 31 body locations, Gerrett et al, (2014) found that females reported higher thermal sensations to 40°C heat, at rest and during exercise than males (Gerrett et al, 2014). A higher sweat rate has also been consistently reported in males than females (Gagnon and Kenny, 2012). Furthermore, in rats, TRPM8 responsiveness was altered by the presence of testosterone or estradiol (Kondrats et al, 2009). Finally, sex-based differences have also been observed in the gustatory system (Glanville et al., 1964; Fikentscher et al., 1977; Weiffenbach et al., 1982; Mojet et al., 2001; Heft and Robinson, 2010; da Silva et al., 2014; Yoshinaka et al., 2015). Therefore, it is unknown whether females would respond differently to a MEN MR.

The primary purpose of the current study was to characterize the effect of a MEN MR on cycling 30-km ITT performance by simultaneously measuring power output, $T_c$, HR, sweat loss, fatigue, and perception of the environment, to determine if a MEN MR (1) can improve ITT performance in the heat, (2) can alter perceptual responses during exercise in the heat, (3) can alter thermoregulatory responses during exercise in the heat, and (4) can improve isometric strength and a singular bout of maximal exercise.
METHODS

Study Participants: The nine females who participated in the study were all members of cycling clubs from across the Greater Toronto Area, trained at least 5 days per week in the summer, and participated in winter training (Table 4.1). Study participants were informed both verbally and in writing of the experiment protocol, potential risks, and told the purpose of the study was to compare mouth rinse preference before giving their written informed consent. As the purpose was an act of deception, the participants were debriefed after the study. The Research Ethics Boards of the University of Toronto and the Ontario Tech University approved the study. Participants were tested at a time other than menstruation (monophasic intrauterine device, n=7; triphasic oral contraceptive, n=1, days 11 and 18; natural menstruation, n=1, days 6 and 13). Testing occurred at the same time each day to reduce the impact of circadian rhythm fluctuations. Each participant was asked to fill out a 24h food log and repeat similar food intakes from one trial to the next.

Table 4.1: Anthropometric and performance descriptors of the female participants (mean ± SE)

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr):</td>
<td>26.7 ± 1.4</td>
</tr>
<tr>
<td>Height (cm):</td>
<td>165 ± 5.4</td>
</tr>
<tr>
<td>Weight (kg):</td>
<td>60.7 ± 6.3</td>
</tr>
<tr>
<td>Monophasic hormonal contraceptive:</td>
<td>n=7</td>
</tr>
<tr>
<td>Triphasic hormonal contraceptive:</td>
<td>n=1</td>
</tr>
<tr>
<td>Natural (no hormonal contraceptive):</td>
<td>n=1</td>
</tr>
<tr>
<td>Hours of training per week (hr):</td>
<td>14.3 ± 2.9</td>
</tr>
<tr>
<td>% body fat:</td>
<td>26.2 ± 6.4</td>
</tr>
<tr>
<td>% lean body mass:</td>
<td>71.0 ± 7.0</td>
</tr>
<tr>
<td>VO$_{2\text{max}}$ (ml/kg$^{-1}$min$^{-1}$):</td>
<td>50.8 ± 6.0</td>
</tr>
<tr>
<td>5-sec sprint (watts):</td>
<td>493.4 ± 100.5</td>
</tr>
<tr>
<td>Handgrip (kg):</td>
<td>12.3 ± 2.3</td>
</tr>
</tbody>
</table>
Study Design:

Preliminary Measurements

Participants visited the laboratory on one occasion. Upon arrival to the laboratory, each participant was fasted and received a Dual Energy X-ray Absorptiometry (DEXA) scan to determine body composition. Following a meal of choice, subjects then partook in three different physiological tests to determine, handgrip strength (HG), 5-sec maximal sprint (SPR), and an incremental VO$_{2\text{max}}$. Before the tests, participants warmed-up for 10-min on an electromagnetically-braked cycle ergometer (Velotron, RacerMate, Inc., Seattle, Washington USA) at an intensity of ~10 on the 6-20 Borg RPE scale (~40% VO$_{2\text{max}}$), followed by 3 bouts of maximal acceleration (approximately 2-sec) (Mendez-Villanueva et al., 2007). Each subject then rested for 3-mins before performing the HG and SPR tests. Sitting on the bike with arms by side, each participant maximally squeezed a Jamar Hydraulic Hand Dynamometer in the dominant hand three times, each squeeze was separated with a pause of 20-sec. After the third HG, each subject performed three SPR on the cycle ergometer, separated by 5-mins of rest. Following the third SPR, participants sat for 5-mins before performing the VO$_{2\text{max}}$. VO$_{2\text{max}}$ was determined during an incremental cycling test to exhaustion on the cycle ergometer. During the incremental test, participants cycled at ~90 rpm starting at 25W with 20W increments every 1-min until voluntary exhaustion. At the conclusion of each stage, RPE and TS, were recorded, furthermore, HR was monitored throughout. For the incremental cycle test, the 30-sec average VO$_2$ (VO$_{2\text{ave}}$) from each stage was used in analysis (Zuniga et al., 2012). Maximal oxygen uptake (VO$_{2\text{max}}$) was defined as the highest single breath VO$_2$ value in the last 30-sec of the test. Once the participant could not hold ~90 rpm for a total of 30 consecutive seconds, the session was ended.
At the completion subjects cooled down for 5-min at 100W. Respiratory gases were collected and analyzed using a metabolic cart (MOXUS metabolic system; AEI Technologies, Pittsburgh, PA).

**Experimental protocol**

Each participant performed three 30-km ITT in an environmental chamber (30 ± 0.6 °C, 70 ± 1% relative humidity, 12 ± 1 km/h windspeed). A full familiarization trial and a total of 2 randomized experimental trials separated by 7 days took place. Each trial was separated by 7 days to prevent heat acclimation, normal training was resumed. Upon arrival, subjects provided their 24h food log, a urine sample, and were weighed nude. During the weigh, participants entered a private room containing a body weight scale, the scale reader was located on the outside of the room to assure privacy. After the familiarization trial, each subject was assigned in a randomized crossover design to perform two self-paced 30-km ITT on an electromagnetically-braked cycle ergometer (Velotron, RacerMate, Inc., Seattle, Washington USA). Furthermore, each subject performed HG and SPR tests after warm-up, following the first mouth rinse (MR1), and at the end of the trial. The trials were performed at the same time of the day to reduce circadian rhythm effects. Hydration strategy was replicated from one trial to the next, mouth rinse temperature was kept above 22°C to prevent TRPM8 receptor stimulation by cold liquid (Voets et al., 2004; Brauchi et al., 2004). The experimental trials involved either a placebo (PLA), or a menthol (MEN) mouth rinse (MR). The MR occurred a total of 7 times (pre-2nd HG and SPR, 5, 10, 15, 20, 25, and 30-km). After each trial, subjects’ towel dried and were weighed for body mass change. Sweat loss was determined by:

\[
\text{Sweat loss (g)} = \left[ \text{change in body weight (g)} + 0.20 \text{ g kcal}^{-1} + \text{fluid intake (g)} - (\text{urine + fecal output})(g) \right]
\]

(Broad et al., 1996; Cheuvront and Montain, 2017)
Trial conditions

Environmental chamber temperature (°C), relative humidity (%), and wind speed (km/h) were measured with a heat stress tracker (Kestral 5400, Boothwyn, PA; accuracy, ± 0.5 °C, ± 2% RH, windspeed ± 3%). Participants provided their 24h food log and replicated for both experimental trials. Urine specific gravity (USG) was measured with a portable refractometer (Atago, Bellevue, WA, USA) and calibrated with distilled water. If the participants concentration of urine was above 1.020 USG, they were deemed dehydrated (Casa et al., 2000; Sawka et al., 2007) and instructed to consume 500 mL of water and wait 1-hr. If the participant still deemed dehydrated, they would have been rescheduled.

Mouth rinse

Subjects were given 25 mL solution to rinse prior to the second HG, and at 5, 10, 15, 20, 25, and 30-km. They were instructed to swill the solution around the mouth for 5-sec before expectorating into a container, and not to swallow the solution. L-Menthol solution was formulated from menthol crystals (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) dissolved in de-ionized water heated to 40°C (Flood et al., 2017). The solution was stored for a maximum of 3-wks at 5°C. A placebo solution was made using a non-caloric berry flavoured sweetener consisting of sucralose (Crystal Light, Don Mills, ON).

Hand grip and 5-sec sprint

In a sitting position, each subject squeezed the Jamar Hydraulic Hand Dynamometer in the dominant hand. This effort occurred 5-mins after the warm-up, 5-mins following the first SPR and after first MR, and at the end of the ITT. Kg_peak was recorded as an indicator of central fatigue.
Participants performed a 5-max sprint in a sitting position on the Velotron with resistance loading of 15 kiloponds. W_{peak} was used as an indicator of combined central and peripheral fatigue.

**RPE, thermal sensation, thermal pain, and thermal pleasantness**

RPE was measured using a 6-20 scale (where 6= no exertion and 20= maximal exertion; Borg, 1982). TS was measured using a 0 to 100 point scale (where 0= not warm at all and 100= extremely hot), thermal pain (TP) was measured using a 0 to 100 point scale (where 0= not at all intense and 100= extremely intense), and thermal pleasantness (PL) was measured using a 0 to 100 point scale (where 0= not pleasant at all and 100= extremely pleasant) (Greenspan et al., 2003). RPE, TS, TP, and PL were recorded after the MR at 5, 10, 15, 20, 15, and 30-km.

**Heart rate and core temperature**

HR was collected using a downloadable Polar® H7 heart sensor, and T_c was determined using an individually calibrated ingestible thermistor (HQ Inc., Palmetto, FL) that was ingested 4-6h before each trial. HR and T_c, were recorded every 5-km.

**Statistical analysis**

The mean and standard deviation were calculated for all data and tested for normality of distribution. Time versus condition data were tested using a 2-way repeated measures ANOVA, to detect singular differences a Bonferroni post-hoc was performed. A paired t-test was used to compare single parameter differences. Time verses condition categorical data were tested using the Friedman Test, to detect singular differences a Wilcoxon Signed Ranks Test was used. Statistical significance was accepted at P < 0.05. Correlations between variables were assessed using a Pearson’s correlation analysis. Exact p-values, Cohen’s D, and 95% confidence intervals are presented to show magnitude of effect.
RESULTS

Trial conditions

No significant differences existed between the PLA and MEN trials for environmental conditions (PLA, Temperature 30.2 ± 0.2 °C, Relative Humidity 69.9 ± 0.9 %, Air Speed 12.2 ± 1.2 km/h; MEN, 30.2 ± 0.6°C, 68.6 ± 3.2 %, 12.5 ± 0.9 km/h, \( p = 0.919 \)), or pre-ITT USG levels (PLA, 1.011 ± 0.005 USG; MEN, 1.009 ± 0.005 USG, \( p = 0.546 \)).

Effect of L-Menthol on exercise performance

All data sets were normally distributed, and no order effect was observed between first and second trials (\( p = 0.961 \)). Trial duration was significantly shorter in the MEN condition compared to the PLA (1:02:34 ± 5:40 min; 1:04:02 ± 4:54 min, \( p = 0.034, d = 0.85, 95\% CI= -166.9 to -8.43 \) sec, Figure 4.1), respectively, representing a 2.3 ± 2.7% improvement in ITT performance. We used correlational analysis to examine possible determinants of time trial performance. There were no correlations between time trial performance and % BF (\( r = 0.28, p = 0.462 \)), % LBM (\( r = 0.33, p = 0.385 \)), and VO\(_{2}\)max (\( r = 0.38, p = 0.309 \)).

\( W_{ave} \) was significantly higher in the MEN condition and improved by 6.0 ± 6.7 % between trials (PLA, 134 ± 27 W, vs. MEN, 142 ± 32 W; \( p = 0.031, d = 0.87, 95\% CI= 0.92 to 15.09 \), Figure 4.2). While \( W_{pk} \) was slightly higher at 5, 10, 15, 20, and 25-km, the difference was not significant, further, no difference was observed at 30-km (\( p = 0.347, d = 0.33, 95\% CI= 9.01 to 22.79; p = 0.346, d = 0.33, 95\% CI= 11.42 to 28.98; p = 0.142, d = 0.54, 95\% CI= 8.15 to 47.26; p = 0.376, d = 0.31, 95\% CI= 11.03 to 26.14; p = 0.491, d = 0.24, 95\% CI= 14.13 to 27.02; p = 0.954, d = 0.02, 95\% CI= 29.16 to 30.72 \).
There were no differences in HG strength between trials ($p = 0.581$, $\eta^2 = 0.04$, Figure 4.3a). Furthermore, no significant differences were found in the SPR performance between each trial ($p = 0.365$, $\eta^2 = 0.103$, Figure 4.3b)

**Figure 4.1:** The effect of MEN in time trial performance. Data are means ± SE. Time trial performance was significantly faster in the MEN condition ($P = <0.05$). * Significantly less in the MEN trial ($P < 0.05$).
Figure 4.2: The effect of MEN on average power output. Data are means ± SE. Average power output was significantly higher in the MEN condition ($P < 0.05$). * Significantly greater in the MEN trial ($P < 0.05$).
Figure 4.3: The effect of MEN in handgrip strength (kg) (A) and 5-sec sprint (Watts) (B) Pre-MR, Post-MR, and Post ITT. Data are means ± SE (n=9). Handgrip strength was not significantly greater in the MEN condition at any time point ($P > 0.017$). 5-sec sprint was not significantly greater in the MEN condition at any time point ($P > 0.017$).

Sweat loss and fluid intake

Sweat loss was similar between both trials (PLA, 1.18 ± 0.28 L; MEN, 1.08 ± 0.16 L, $p=0.218$), There were no significant differences in fluid intake during (PLA, 0.786 ± 0.208 L vs. MEN, 0.876 ± 214 L, $p=0.232$) and body mass % change (PLA, 0.71 ± 0.47 vs. MEN, 0.61 ± 0.48, $p=0.279$) between trials.

Cardiovascular and thermoregulatory responses

HR increased over time, however the change from 5 to 30-km was not significant in both trials (PLA, 158.2 ± 19.5 vs. 168.7 ± 13.3 bpm, $p=0.081$; MEN, 160.7 ± 21.8 vs. 168.7 ± 17.7 bpm, $p=0.397$). HR did not significantly differ between trials at 5, 10, 15, 20, 25, and 30-km (Figure 4.4a). The average HR did not significantly differ between trials (PLA, 163.2 bpm; MEN, 164.0 bpm). Participants exercised at an average of 74% of their Heart Rate Reserve. $T_c$
significantly increased from 5 to 30-km in the PLA, however the change in $T_c$ from 5-km to 30-km in MEN was not significant (PLA, 38.0 ± 0.30, vs. 38.63 ± 0.42, $p = 0.015$; MEN, 38.21 ± 0.41, vs. 38.48 ± 0.75, $p = 0.273$). $T_c$ did not significantly differ between PLA and MEN between trials at 5, 10, 15, 20, 25, and 30-km (Figure 4.4b). Moreover, the change over time in $T_c$ did not differ from one trial to the next.

(A)
Figure 4.4: The relationship between HR (A), $T_c$ (B), and kilometers. Data are means ± SE (n=9). Heart rate (HR), $T_c$ significantly increased throughout the time trial. There were no significant differences between PLA and MEN at 5, 10, 15, 20, 25, and 30-km ($P > 0.008$).

Rate of perceived exertion, thermal sensation, thermal pain, and thermal pleasantness

RPE significantly increased from 5 to 30-km in both trials (PLA, 11.0 ± 1.5, vs. 16.8 ± 1.4, $p=0.007$; MEN, 11.6 ± 2.1, vs. 17.3, $p = 0.008$). The average RPE was significantly higher in the MEN (PLA, 14.05 ± 2.09, vs. MEN, 14.45 ± 2.0, $p=0.022$). RPE was not significantly different between trials at 5, 10, 15, 20, 25, and 30-km (Figure 4.5a). TS significantly increased from 5 to 30-km in both trials (PLA, 41.1 ± 12.7, vs. 83.3 ± 7.1, $p = 0.007$; MEN, 42.2 ± 15.6, vs. 79.4, $p = 0.007$). The average TS between PLA and MEN did not differ (68.0 ± 15.4, vs. 67.1 ± 13.5, $p = 0.455$). TS was not significantly different at 5, 10, 15, 20, 25, and 30-km (Figure 4.5b). TP significantly increased from 5 to 30-km in both trials (PLA, 56.9 ± 13.7, vs. 62.4 ± 15.8, $p = 0.007$; MEN, 34.4 ± 11.3, vs. 77.2 12.0, $p = 0.007$). The average TP was significantly higher in the MEN trial (PLA, 56.9 ± 13.7, vs. MEN, 62.4 ± 15.8, $p = 0.009$). TP was not significantly different at 5,
10, 15, 20, 25, and 30-km (Figure 4.5c). PL significantly decreased from 5 to 30-km in both trials (PLA, 45.6 ± 13.3, vs. 20.6 ± 7.3 \( p = 0.002 \); MEN, 56.7 ± 11.2, vs. 22.2 ± 19.2, \( p = 0.001 \)). The average PL did not significantly differ (PLA, 32.0 ± 9.4, vs. MEN, 33.2 ± 13.5, \( p = 0.623 \)). PL was significantly different at 5-km (\( p = 0.024 \)), but did not differ at 10, 15, 20, 25, and 30-km (Figure 4.5d). There was a significant correlation between RPE and TS in the MEN trial (\( r = 0.78, p = 0.014 \)), however the correlation in the PLA trial was not significant (\( r = 0.45, p = 0.221 \)).
Figure 4.5: Relationship between RPE (A), TS (B), TP (C), PL (D) and kilometers. Data are means ± SE (n=9). RPE gradually increased from the beginning of the trial to the end. There were no significant differences in RPE between PLA and MEN at 5, 10, 15, 20, 25, and 30-km (P > 0.008). TS gradually increased from the beginning to the end of each trial. There were no significant differences in TS between PLA and MEN at 5, 10, 15, 20, 25, and 30-km (P > 0.008). TP gradually increased from the beginning to end of each trial. There were no significant differences in TP between PLA and MEN at 5, 10, 15, 20, 25, and 30-km (P > 0.008). PL gradually increased from the beginning to end of each trial. PL significantly differed at 5-km, however there were no differences between PLA and MEN at 10, 15, 20, 25, and 30-km (P > 0.008).
DISCUSSION

Cycling performance was enhanced with a menthol mouth rinse during a 30-km time trial in active females. Results showed that when swilling menthol, ITT and average power output improved in young adult, female cyclists, 2.3 ± 2.7% and 6.0 ± 6.7%, respectively. Regardless of the improvement in ITT, perceptual (RPE, TS, TP, and PL) and thermoregulatory responses (HR, Tc, and sweat loss) did not differ across the TT between placebo and the menthol mouth rinses. Surprisingly, the menthol mouth rinse did not significantly improve HG or SPR; these findings suggest that maximal isometric muscle force generation, or a supramaximal intensity cycle exercise is not impacted by a menthol mouth rinse. To our knowledge, this is the first menthol mouth rinse study focused on cycling time trial performance and female cyclists.

MEN and improved ITT performance sex-based differences

The improvement in ITT performance was slightly less than observed for male athletes (Jeffries and Waldron, 2019). While this is the first study to use the MEN MR during a cycling ITT, recent work by Stevens et al. (2017) showed an improvement of performance of 4% during a 3-km running TT, whereas Stevens et al. (2016) showed an improvement of 3% during a 5-km running TT. Differences in modality and distance among studies make it difficult to directly compare the effects of menthol on TT performance between men and women. Moreover, environmental conditions, mouth rinse frequency, mouth rinse temperature, and hydration status entering the trial also varied among the present and running studies. Notably, the present study ensured the participants entered the trial hydrated, whereas the other two did not. As such, hydration status may have influenced the time to reach the ‘critical core temperature’, the point at which an increase \( T_c \) causes a decrease in performance (Nybo and Neilsen, 2001). However, the average effect of a thermoneutral menthol mouth rinse on TT performance we observed (2.3%) is
lower than that calculated (approximately 3.5%) in a systematic review by Jeffries and Waldron (2019).

The central governor theory, a central nervous system mechanism that takes input information about energetic needs, current physiological states, and environmental circumstances, proposes that the subconscious brain regulates power output by modulating motor unit recruitment to preserve whole body homeostasis (Weir et al., 2006). The marathon provides evidence that depending on environmental conditions, current physiological state, and difficulty of the course, an athlete will race ‘in anticipation’ by setting a variable pace at the start of exercise (Noakes, 2008). Relative to the present study, this phenomenon could be related to sex-differences as well, as women are typically more sensitive to the heat (Gerrett et al., 2014), have a lower sweat rate (Gagnon and Kenny, 2012; Gagnon and Kenny, 2014; McLellan, 1998), can exhibit a faster rise of $T_c$ (McLellan, 1998) and differ in gustatory responsiveness (Glanville et al., 1964; Fikentscher et al., 1977; Weiffenbach et al., 1982; Mojet et al., 2001; Heft and Robinson, 2010; da Silva et al., 2014; Yoshinaka et al., 2015), all factors that can influence endurance performance in hot environments.

**MEN and improved ITT performance**

As of late, a correlation between the menthol mouth rinse and decrease in TS has been observed, however the findings across multiple studies are equivocal (Jeffries and Waldron, 2019). While Mundel and Jones (2010) and Flood et al. (2017) showed a relationship between the menthol mouth rinse, thermal perception, and performance, the present study, Jeffries et al. (2018), and Stevens et al. (2016) did not. In terms of the environment, all studies were similar with an average of $34°C$ and RH 40%, given that environmental conditions can modulate motor output and change thermal perception through a change in skin temperature, suggesting that this was not
a factor that differentiated among each study (Matsuura et al., 2015). Factors not consistent between each study were the frequency, concentration, and duration at which the mouth rinse was administered. While the swilling duration, frequency, and concentration of the menthol mouth rinse has never been tested, we can speculate that the length, number, and intensity of cooling exposures could impact performance (Best et al, 2018). Relative to the carbohydrate mouth rinse, both mouth rinses’ cause an acute response in brain activity (Smeets et al, 2005; Guest et al, 2007; Eccles, 2000), the hypothesized mechanism responsible for the improvement of performance. Examples of this can be observed in fMRI work by Smeets et al. (2005) and Guest et al. (2007). In comparison, both carbohydrate and drink temperature fMRI studies have shown differences in brain activity at varying concentrations and temperatures of solution. For example, in the work by Smeets et al. (2005), they were able to show that glucose ingestion at a variety of concentrations (0, 8.3, and 25%) differentiated hypothalamic response, whereas work by Guest et al, (2007) saw an increase in activity among the reward centers of the brain with cold water compared to warm.

From a performance or behavioral standpoint, the same can be observed in recent carbohydrate mouth rinse research. For example, in work by Sinclair et al. (2014), and Stellingwerff and Cox (2014), they were able to show that swilling duration and mouth rinse frequency were both factors that contributed to an improvement of performance, respectively. As such, a greater improvement of performance was observed with 5-sec of swilling vs. 10-sec (Sinclair et al, 2014), whereas similar improvements of performance were observed when swilling 6% of glucose for 5-secs every 12-15-mins (Stellingwerff and Cox, 2014). Further, in a behavioral study by Treesukosol et al. (2011) where rodents were allowed free access to different solutions, a dose-response effect was present in glucose, with 9% being the optimal concentration and no response for concentrations less than 4.5%. While a dose-response relationship has not been
depicted with the CHO mouth rinse in a fed state (Devenney et al., 2016) results from Smeets et al., (2005) and Treesukosol et al. (2011) suggest that a dose-response could be prevalent under different circumstances.

Relative to the carbohydrate mouth rinse, similar trends are observed with the menthol mouth rinse. For example, in cycle to exhaustion work by Mundel and Jones et al. (2010) and Flood et al. (2017), both administered the mouth rinse at 10-min intervals with average trial lengths of 63 ± 14 min and 23:23 ± 3:36 min and saw an improvement of performance of 9 and 7%, respectively. In comparison, in running TT work by Stevens et al. (2016) and Stevens et al. (2017), they administered the mouth rinse every 0.2 and 0.1 of 1-km from baseline, with a total trial length of 25.3 ± 3.5 min and 13.7 ± 1.2 min and observed an improvement of performance of 3 and 4%, respectively. Furthermore, in a 20-km TT cycling study by Trong et al. (2015), they were able to show that ingestion of menthol combined with ice-slurry was more beneficial than menthol with neutral water (6.2 vs. 3.3%, respectively). This could indicate that modality, mouth rinse frequency, protocol, and concentration are factors which influence the response of menthol.

Relative to the present study, none of the previous work replicated both modality and frequency, however the present study was most comparable to time trial performance in the work by Stevens et al. (2016) and Stevens et al. (2017). We previously hypothesized that the improvement of performance would be related to a decrease in thermal perception, however that was not the case as the present study did not show any differences in thermal sensation, thermal pain, or thermal pleasantness with mouth rinses’ every 5-km.

Variance in ITT performance response to menthol were evident but not correlated with VO$_{2\text{max}}$, % body fat, and % lean body mass. Moreover, while five people preferred the placebo and four preferred menthol, the improvement of performance does not seem to be related to mouth
rinse preference as the improvement among responders is equivalent between menthol and the placebo. Factors not measured with potential impact include gustatory responsiveness (Williams et al., 2016), thermoreceptors in the mouth (Cliff and Green, 1996), and circulating hormone levels (Kolka et al., 1997). Ethnicity could also be linked to heat and menthol mouth rinse sensitivity. While ethnic background was not included in analysis, daily eating patterns and exposure to “hot” or “spicy” food is another factor that may be related to thermoreceptor responsiveness (Williams et al., 2017).

**Central activity and physiological performance**

During exercise, the mechanisms which cause hyperthermia induced fatigue, appear to be related to the central nervous system (CNS) (Nybo, 2007). Although the CNS is generally involved with feelings in tiredness, mood disturbance, and lethargy, hyperthermia induced CNS fatigue can lead to a decrease in motor output and a decrement in endurance performance (Meeusen et al., 2006). Further, CNS fatigue can be influenced by inhibitory signals from the hypothalamus arising secondary to an increase in brain temperature (Nybo, 2007) and neurotransmitter networks activated during exercise—affecting the preoptic area and the anterior hypothalamus (Meeusen and Meirleir, 1995; Meeusen et al., 2006). Brain activity, specifically the prefrontal cortex, lateral prefrontal cortex, orbitofrontal cortex, and anterior cingulate cortex, is another factor influenced by exercise and hyperthermia (Nybo and Nielsen, 2011; Robertson et al, 2015; Robertson et al, 2016) as it indirectly communicates with motor output to regulate exercise performance. For example, in a RPE, $T_c$, and brain activity study by Nybo and Nielsen (2001), they were able to show a linear correlation among $T_c$, electroencephalogram activity (frontal, central, and occipital
cortex), and RPE, indicating that cerebral activity may be associated with hyperthermia induced central fatigue.

Unfortunately, the present study did not measure brain activity during the TT, however work by Guest et al. (2007) showed that intra-oral thermal stimulation also activates a network of taste- and reward-responsiveness regions of the human brain, these are associated with dopaminergic pathways within the primary and secondary cortices, the same regions engaged with positive self-talk (Cascio et al, 2016). These neural components are especially important as hyperthermia reduces dopamine, arousal, and motivation (Meeusen et al, 2006; Bridge et al, 2003; Neilsen et al, 2003). Furthermore, although the work with menthol and performance has only been trialed in hot environments, it can be hypothesized that menthol increases brain and dopaminergic activity given that an increase in brain activity is observed in thermoneutral environments (Guest et al, 2007). Similar to motivational self-talk work by Wallace et al, (2016), although neither study measured brain activity, the improvement of performance could be linked to an increase in dopaminergic activity among the reward centers of the brain.

**Effects of MEN on an isometric contraction and 5-sec maximal sprint**

It is well established that hyperthermia affects central fatigue and results in an impairment of neuromuscular performance (Meeusen et al., 2003). Surprisingly, the same is not true for an isometric contraction or a singular bout of maximal exercise, as higher temperatures will improve performance (Asmussen and Boje, 1945). During exercise, a moderate increase in body temperature, specifically the skeletal muscles benefit from the rise by increasing the speed of chemical reactions, metabolic processes, nerve conduction, and changes involved with muscle contractions. Further, the temperature quotient for biochemical process is in the order of ~2, suggesting that an increase of 10°C in muscle temperature will double the speed of processes
related to mechanical and metabolic reactions (Nybo, 2008). For example, in muscle temperature work by (Asmussen and Boje, 1945), they demonstrated that performance during a singular sprint on a cycle ergometer improved 5% following a warm-up. Passive heating studies have confirmed these results with the use of electrical nerve stimulation (Meeusen et al., 2006; Todd et al., 2005). In contrast, the same is not true for high $T_c$ and prolonged exercise, where reductions have been observed at intensities varying from 40 to 80% of maximal oxygen uptake, continuous exercise $>1h$ (Gonzalez-Alonso et al, 1999) maximal exercise lasting $\sim$3-10mins (Arngrimsson et al, 2003; Nybo and Nielsen, 2001), repeated isometric contractions (Martin et al, 2005; Morrison et al, 2004; Nybo and Nielsen, 2001; Sabolsky et al, 2003; Thomas et al, 2006), and repetitive sprints (Drust et al, 2005). Primarily, these decrements in performance are related to the central nervous system. A systematic review by Guy et al. (2014) on performance and environmental conditions supports these findings. While endurance performance was worse in hot conditions ($\sim$3% reduction in performance, Cohen’s $d > 0.8$; large impairment), performance in short-duration sprint events was augmented in the heat ($\sim$1% improvement, Cohen’s $d > 0.8$; large performance gain). We had hypothesized that increased activity in the reward centres (Guest et al., 2007) with menthol would increase HG and SPR performance but we observed no change.

The central governor theory suggests that athletes’ race ‘in anticipation’ by setting a variable at the start of the race, this is dependant in part on environmental circumstances and difficulty of the task. Furthermore, the purpose of the central governor is to maintain homeostasis and prevent catastrophic failure, by modulating the number of motor units activated in exercising muscle (Noakes, 2007). As such, given that the pace one takes is dependent upon environmental conditions, difficulty of task, and duration of the event, it can be hypothesized that regardless of the temperature of the environment and central fatigue, the brain allows for maximal contractions.
knowing it will be finished in a minimal amount of time (Noakes, 2007). Of interest, we can postulate that while the menthol mouth rinse increased activity among the reward centers of the brain, the lack of improvement in both the HG and SPR can be related to the central governor theory. Whilst the menthol mouth rinse has shown ergogenic benefit in endurance exercise, given that the duration of the task for HG and SPR is short and does not put the body at physiological harm, the brain is already recruiting close to maximal outputs.

**CONCLUSION**

This was the first study to observe the effects of a menthol mouth rinse in 30-km ITT performance and perceptual responses in trained female cyclists. This study demonstrated that a menthol mouth rinse improves time trial performance and average power output in trained women. Further, the effect does not appear to relate to thermal perception given absence of significant change in thermal sensation, thermal pain, and thermal pleasantness between trials. Therefore, we attribute the improvement of performance to the central governor theory and an increase in activity among the reward centers of the brain.

**ACKNOWLEDGEMENTS**

The authors would like to thank the participants for their time and effort, and acknowledge the Canadian Sport Institute Ontario, Ontario Tech University Faculty of Health Sciences, and the General Motors Centre of Excellence engineers and staff for their assistance with data collection.
6 Conclusion

6.1 Study Limitations

It is recognized that the present study has limitations. Although the majority of the participants were on a monophasic hormonal contraceptive, we did not measure estrogen and progesterone levels, which would have helped elucidate individual variation among each participant. While 8 of 9 participants were on hormonal contraceptives, one participant was on a triphasic contraceptive, and the other was on nothing, these participants were tested on days 11 and 18, and 6 and 13, respectively. In addition, testing the participants on the same day of their menstrual cycle would have been beneficial as this can help stabilize hormone levels. Moreover, hormonal levels have been shown to influence glucose mobilization, this was not accounted for as the hormonal levels were unknown. However, participants were asked to repeat their 24h food log. Further, given that the study recruited females between the ages of 25-35yrs limited recruitment. The inclusion of females from the age of 18yrs would have yielded undergraduate students from both the University of Toronto and Ontario Tech University, this could have resulted in a larger sample size. The exclusion of male participants limits the ability to directly compare between-sex differences. Whilst the majority of menthol mouth rinse research has only been studied in male athletes, we can not compare as the mouth rinse frequency, protocol, and modality are not consistent among each study.

We did not measure brain activity, which would have helped interpret the effect of the menthol mouth rinse. Also, the present investigation did not measure thermal or gustatory sensitivity, it could be hypothesized that the receptors in the mouth can influence the change of performance or how one perceives a stimulus. In addition, motivation or psychological state was not measured at the beginning of each trial. Given that positive self-talk can influence brain activity
and improve physiological performance, this may have contributed to the difference between MEN and the placebo.

Lastly, it should be mentioned that the majority of the participants did not have experience with 30-km TT in an environment of 30°C and 70% RH. Despite partaking in a familiarization trial, participants commented on the inability to view power output and time left. While the participants were told distance accomplished every 2.5-km, and every 1-km at the 20-km time point, a continuous visual of distance left competitive type of environment could have motivated the participants to cycle harder.

6.2 Future Directions

This thesis answered pertinent questions regarding a menthol mouth rinse and physiological performance during heat stress, however several questions remain. While the menthol mouth rinse improved performance in hot environments, the effect of menthol in a thermoneutral or cold environments is unknown. Whilst the improvement of performance has been correlated with a change in thermal perception and brain activity during exercise in the heat, it can be speculated that menthol would improve performance under thermoneutral circumstances given that fMRI at different drink temperatures has shown a differentiation of brain activity in thermoneutral conditions (Guest et al, 2007). Moreover, we can postulate that a menthol mouth rinse would improve performance under an array of conditions given that menthol may have an altering or arousal effect. Ultimately, the temperature of the environment should not influence the physiological impact of a menthol mouth rinse. It would be of interest to repeat the present study under multiple environmental conditions and different modalities.

From this thesis we have identified that rinsing of menthol is an effective ergogenic aid for female cyclists. To date, this is the only menthol mouth rinse research conducted in female athletes.
Considering that there are several differences between men and women, 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 outcomes and performance, there is little known knowledge of the potential impact a menthol mouth rinse may have in women. It is speculated that during menstruation, men and women would portray a similar rate of improvement with the menthol mouth rinse, when both estrogen and progesterone are low. However, the comparison during the follicular or luteal phase may differ when estrogen is high, or when both estrogen and progesterone are high, respectively. While the majority of menthol mouth rinse work has been done in male athletes, we are not able to compare as protocol, mouth rinse frequency, mouth rinse duration, and exercise modality all differ. Moving forward, it would be beneficial to directly compare the effect of a menthol mouth rinse in both female and males given the same protocol.

In addition, the effect of menthol combined with other substances is unknown, particularly the addition of carbohydrates or caffeine. Through the receptors in the mouth, it is speculated that menthol, carbohydrates, and caffeine enter the brain via different pathways (Guest et al., 2007; De Pauw et al., 2014). While menthol activates the oropharyngeal TRPM8 thermoreceptors, carbohydrates and caffeine interact with the gustatory chemoreceptors, T1R2/T1R3 sweet and TAS2R bitter receptors, respectively. It was originally proposed the caffeine mouth rinsing elicited its effects by allowing caffeine molecules to inhibit adenosine through binding of the adenosine receptors (Beaven et al., 2013; Clarke et al., 2015), however a study by Doering et al. (2014) they were able to show that a caffeine mouth rinse does not increase blood caffeine concentrations. Moreover, the same response is observed with the carbohydrate mouth rinse (Murray et al., 2018). While electroencephalography recording has shown that a caffeine mouth rinse increases activity among the orbitofrontal and dorsolateral prefrontal cortex, whereas a carbohydrate mouth rinse
only increases activity in the orbitofrontal cortex (De Pauw et al., 2015) suggests that there could be a summation effect. However, in a high intensity running study by (Germaine et al., 2019), the summation effect of carbohydrate and caffeine did not significantly improve performance compared to caffeine alone. Moreover, given that the carbohydrate mouth rinse has been deemed more beneficial in fasted vs. fed participants (Lane et al, 2013) suggests nutrition status of the participants could have influenced the results. Further, in light of fact that both carbohydrate and caffeine bind to chemoreceptors, could have impacted these results. Notably, as menthol activates thermoreceptors, suggests that menthol may have a performance enhancing synergistic effect with either carbohydrate or caffeine. Moreover, one could determine if the synergist effect influences different types of exercise, as such these outcomes could either refute or support the central governor theory.

### 6.3 Conclusion

The present results extend previous findings by demonstrating a positive effect of a menthol mouth rinse in ITT for trained women. While menthol proved to elicit performance-enhancing effects during the 30-km ITT to the majority of participants, there was individual variation amongst the group. Moreover, the menthol mouth rinse did not alter thermal perception (thermal sensation, thermal pain, and thermal pleasantness), and thermoregulatory properties (HR, Tc, and sweat loss) relative to the placebo trial. Further, the menthol mouth rinse did not improve hand grip or 5-sec sprint performance which would be expected from a non-specific ergogenic aid. As such, these results support the Central Governor Theory regulation of performance.
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Appendix A: Recruitment Poster

MOUTH RINSE AND CYCLING PERFORMANCE IN FEMALE ATHLETES

Want to improve your time trial performance? If yes…….

Are you eligible????
- Are you female?
- Are you between the ages of 25-35 years?
- Are you using hormonal contraceptives?
- Do you partake in 5 training sessions/wk during the summer, and train throughout the winter?

What would you have to do???
- Participate in a 30-km time trial
- Attend a total of four visits:
  - First visit: ~1.5h
  - Visits 2-4: ~2h

What are the benefits:
- Determination of your aerobic capacity (VO2max) and lactate threshold, providing you with accurate training zones
- Performance time trials to get you fit for the spring season and provide recommendations to optimize your performance
- Body composition assessment to determine your lean mass & bone mineral density
- Have training questions answered by a sport scientist

Contact information:
If you are interested in participating in the study, please contact the principle investigator, Erica Gavel at erica.gavel@mail.utoronto.ca or (306)-491-5634
Appendix B: Informed Consent

Informed Consent - The Influence of Mouth Rinse Preference During a 30-km Time Trial in Female Cyclists

This consent is only part of the process of informed consent, participants will receive a verbal explanation as well. If you would like more information on anything you see here, please do not hesitate to get in contact with Erica Gavel. Please take the time to read this form carefully.

**Study Name:**
Mouth Rinse Preference During a 30-km Time Trial in Female Cyclists

**Researchers:**
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**Purpose of Research:**
The purpose of this study is to compare mouth rinse preference between menthol and Crystal Light solutions. Flavour preference may be different during demanding exercise and during heat exposure.

**Study Information:**
In order to test your preference for one of two mouth rinse solutions during a 30-km time trial, the following procedures will take place:

The study will consist of four visits: preliminary testing, one familiarization, and two experimental trials. The preliminary and familiarization trial will be separated by at least 3 days, while the familiarization and experimental trials will be separated by about 7 days. The familiarization and experimental trials will be conducted in an environmental chamber with conditions of 30 °C, relative humidity of 70%, and a fan providing 30 km/hr of wind. Throughout the study, power output, hand grip strength, heart rate, core temperature, and blood markers will be measured. To access the core temperature of the individual, a core temperature pill will be ingested ~3h before each trial. Further, for blood sample collection, the investigator will make a small cut to collect a small amount (less than a teaspoon) and analyze hemoglobin, hematocrit, and lactate; the purpose is to measure hydration status and lactate production from the beginning to end of each trial. At the end of each exercise trial we will ask you which mouth rinse you preferred.

Preliminary testing will include bone mineral density and lean body mass measurements, an aerobic fitness (VO$_{2\text{max}}$) measure, lactate threshold measurements, finger capillary blood analysis, hand grip strength, and a maximal cycle sprint. The preliminary testing will take place at the **Canadian Sport Institute Ontario** (875 Morningside Ave, Toronto, ON M1C 0C7).

Familiarization and experimental trials will consist of a 30-km time trial with a Crystal-Light or menthol mouth rinse every 5-km. In addition, hand grip strength, power output, and blood analysis will occur at the beginning and end of each trial. The familiarization and experimental trials will take place in an environmental chamber at the **University of Ontario Institute of Technology** (2000 Simcoe Street North, Oshawa, ON L1G 0C5).

To participate in this study, you must be a woman between the ages of 25-35 years. The 30-km time trial is demanding, so you should be used to rides of at least 30-km long. Prior to preliminary testing, you will complete a basic “PAR-Q: Physical Activity Readiness Questionnaire” and be asked to declare any pre-existing conditions of heat related illness or menthol allergies. If needed, you will then follow-up with a medical practitioner. Furthermore, your normal training should be 5 sessions/week and you should participate in winter training. In addition, you must actively be taking hormonal contraceptives. On days of menstruation, acute illness, and in case of injury, you will be re-scheduled. Taste can change through your cycle and we want to make fair comparisons between mouth rinses.
**Risks and Discomforts:**
During the 30-km time trial, if you feel like you are about to faint, are delirious, or nauseated, or your head is throbbing, cooling therapy will begin immediately. Cooling therapy will consist of cool water, ice packs, and fans. If needed, we will take you to the nearest hospital.

**Benefits to Participants and Benefits to Research:**
With involvement in this project, you will receive an interpretation and have access to your fitness testing data. Furthermore, you will have opportunity to practice time trial performance and train in a hot environment with careful monitoring. Moreover, you will be contributing to an understudied research topic.

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 specifically for female cyclists. With the majority of the literature focusing on male athletes and cycling performance, the findings from this research will benefit women in both the recreational and high performance athletic population.

**Voluntary Participation:**
Your participation in the research is completely voluntary. If you chose not to participate, this will not affect your relationships with the principal investigator, co-investigators, the University of Toronto, the Canadian Sport Institute Ontario, or the University of Ontario Institute of Technology.

**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, the University of Toronto, the Canadian Sport Institute Ontario, or the University of Ontario Institute of Technology. The participant can withdraw at any point during the study. If you decide to withdraw, your data will be deleted and removed. The participants will have no consequence by withdrawing from the study.

**Confidentiality:**
All data collected and stored will be deemed as confidential. All personal identification will be removed and the data stored using identification numbers. We are asking your consent to have use your anonymous data in a thesis, peer-reviewed publications, presentations, and conferences. All quantitative and qualitative data will be stored encrypted on a password protected computer belonging to the principal investigator. Data will be stored for 5 years after which point it will be destroyed.
Participants Concerns and Reporting:
If you have concerns in regards to the study, please contact the researcher Erica Gavel at (306)-491-5634 or erica.gavel@mail.utoronto.ca.

If you do not feel comfortable contacting a research team member, any questions regarding your rights as a participant, complaints about the study, or adverse events can be directed to the Research Oversight and Compliance Office- Human Research Ethics Program at ethics.review@utoronto.ca, or (416)-946-3273.

Signatures:

I ________________________________ consent to participate in The Influence of Mouth Rinse Preference During a 30-km Time Trial in Female Cyclists research project conducted by Erica Gavel. I have understood the purpose and protocol of this project. The signature below indicates my consent.

Signature ________________________________ Date: ____________________
Participant:

Signature ________________________________ Date: ____________________
Witness:

Appendix 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.

<table>
<thead>
<tr>
<th>Question</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Has your doctor ever said that you have a heart condition OR high blood pressure?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2) Do you feel pain in your chest at rest, during your daily activities of living, OR when you do physical activity?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>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).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? PLEASE LIST CONDITION(S) HERE:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5) Are you currently taking prescribed medications for a chronic medical condition? PLEASE LIST CONDITION(S) AND MEDICATIONS HERE:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>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:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7) Has your doctor ever said that you should only do medically supervised physical activity?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.
- 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**

**DATE**

**WITNESS**

**SIGNATURE OF PARENT/GUARDIAN/CAFE 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 ePARmedX at [www.ePARmedx.com](http://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)

   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 spondyloysis/pars defect (a crack in the bony ring on the back of the spinal column)?

   1c. Have you had steroid injections or taken steroid tablets regularly for more than 3 months?

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?

   2b. Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)?

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)

   3b. Do you have an irregular heart beat that requires medical management?
   (e.g., atrial fibrillation, premature ventricular contraction)

   3c. Do you have chronic heart failure?

   3d. Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 12 months?

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)

   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)

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?

   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.

   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?

   5d. Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)?

   5e. Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future?
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 or 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 ____________________________

SIGNATURE ____________________________

DATE ____________________________

WITNESS ____________________________

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER ____________________________

For more information, please contact www.eparmedx.com
Email: eparmedx@gmail.com

The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+ Collaboration chaired by Dr. David E.R. Warburton with Dr. Norman Gladhill, Dr. Veronica Janvrin, and Dr. Donald C. McIvor (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:

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Appendix D: Physical Readiness Questionnaire 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

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.
Appendix E: Preliminary Trial Data Collection Form

Participant: PAR-Q? Y/N    Date: 

The Effects of a Menthol Mouth Rinse During 30-km TT Performance in Female Cyclists

Preliminary Testing:
Upon Arrival: Actively taking HC or IUD? Y / N    Fasted? Y / N

Body weight  USG  Height

Handgrip strength (Kg\text{peak}): 1) 2) 3)
Isokinetic sprint (W\text{peak}): 1) 2) 3)

Seat height/position:
Hand grip width:

Incremental cycling test:

<table>
<thead>
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<th>Min</th>
<th>HR</th>
<th>W\text{peak}</th>
<th>RPE</th>
<th>TS</th>
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<td>345W</td>
<td>17-min</td>
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</table>
Appendix F: Preliminary Trial Checklist

Trial Checklist- Preliminary Trials CSIO

Day Before Trial: Pre-trial setup

CSIO Lab

- MOXUS Metabolic Cart
  - Mask, headstrap (check for good velcro), two-way rebreathing valve, blue tube
- Heart rate
  - Polar monitor, watch/app, strap
- Table
  - Labeled USG cups
  - Portable Refractometer
  - Disposable pipettes
  - Permanent marker
  - Gauze
  - Gloves
  - Glucose strips
  - Glucometer
  - Disposable lancets
  - Capillary tubes
  - Alcohol pads
  - Paper bags
- Other
  - Position of bike in relation to MOXUS
  - Garbage can
  - Biohazard bin
  - Sharps bin
  - Hand dynamometer
  - Disinfectant wipes
  - Get two-way valves set-up (# of valves= # of participants)
**Trial Day: Pre-trial setup – Chamber Prep and Drug Mixing**
- Turn on Moxus pump power and pneumotach at least 30 min before first participant
- Turn on computer and open Wingate Software → create protocol (5 sec sprint) → input athletes
- Set-up wet-lab station (urine and blood)
- Place hand dynamometer beside Velotron
- Calibrate MOXUS while athlete is getting blood taken

**Trial Day: Pre-trial setup—Calibrating MOXUS (during athletes’ stations 1 and 2)**
- Open MOXUS
- **Calibrate**
  - Turn on **Calibrate Port**
  - Beside O2mix and CO2mix, select the open boxes
  - Calibrate **Low O2** found under **Gas/User** (once the empty box at the bottom reads “Press SAVE when status indicates **Calibrated**) move onto calibrating **High O2**
  - Beside O2mix and CO2mix, select the open boxes
  - Calibrate **High O2** found under **Gas/User** (once the empty box at the bottom reads “Press SAVE when status indicates **Calibrated**)
- Calibrate Pneumotach
  - **Pneumotach**
  - **Calibration**
  - Take 5 strokes (keep each stroke consistent)
  - Next screen
  - Take 5 strokes (<1% average standard error)…. If error is higher than 1%, redo the process

**Trial Day: Lobby – Participant Arrival**
- Meet participant at entrance
- Tell participant to put belongings into locker and provide urine sample
  - Collection instruction:
    - Urinate a small amount in the toilet, then stop
    - Urinate into the cup until ¼-½ full (essentially, midstream)
    - Stop urinating into the cup before you are finished
    - Finish urinating into the toilet
    - Put the lid on the cup and place in brown bag
    - Wash hands and give to administrator

**Trial Day: Lab – Urine, blood glucose, and Hb/ Hct**
- Athlete drops off urine sample
- With a clean alcohol swab, wipe off either pointer or middle finger
Make small cut on finger
Collect blood with capillary tube
Analyze for Hb and Hct
From same cut, analyze blood glucose with glucometer
Apply gauze until the cut has stopped bleeding

**Trial Day: Lab – DXA Scan**
- Prior to the scan, athletes will be asked to adequately hydrate (500mL of water during lunch, dinner, mid-day snacks, and right before the fast)
- Check that they have no metal (prosthesis or piercings)
- Participant will have voided their bladder (section #1)
- Explain nature of scan – low level radiation—like taking a flight to California

**Trial Day: Lab – Warm-up:**
- Warm-up
  - Open Velotron CS Software
    - Start- Manual
  - Place HR strap + monitor, verify working with watch
  - Adjust Velotron cycle ergometer accordingly: seat height, bar height/reach, etc – record position values
    - ~15 to 25 degrees of knee flexion at the 6 o’clock position of pedal stroke
    - Make sure the knee does not track ahead of the foot when pedaling
    - Allow the athlete to take ~5 pedal strokes to ensure they are comfortable
  - At an intensity of ~10/20 Borg RPE scale, take opportunity to explain Borg scale is exertion not fatigue – rate overall feeling etc. participants will cycle for 10min
  - Following the 10min, participants will perform 3 bouts of maximal acceleration (approximately 2sec)
  - Participant will rest for 3min prior to performing handgrip and 5sec sprint test

**Trial Day: Lab – Hand grip (all measurements will take place sitting on bike):**
- A total of three trials will take place
- Allow for a 20sec break between each trial
  - Set to desired spacing (make note—will use for subsequent trials)
  - Place hand dynamometer in dominant hand
  - Ask participant to squeeze the hand dynamometer maximally—the peak-hold needle will automatically record the highest force exerted
  - Record the reading and reset the peak-hold needle to zero

**Trial Day: Lab— 5sec Maximal Sprint:**
- Record the reading and reset the peak-hold needle to zero
Open Velotron Wingate Software

Start the program by going to Start- Programs- Velotron Wingate

Select New Database in the Client menu. Create a Database name

Select New Client in the Client menu, input the following information:
- Weight
- Age
- Sex

Select Create New Protocol from the Wingate menu

Make sure Velotron is plugged into PC and is turned on

Select Load Client in the Client menu

Select Load Protocol in the Wingate menu

Select Run Wingate from the Wingate menu to launch the Wingate screen

The Velotron will be in Warm-Up Mode. When the test is ready to start, press the Start Wingate button to begin test

Trial Day: Lab—Incremental Test:

Open MOXUS

Adjust Velotron cycle ergometer accordingly: seat height, bar height/reach, etc.)

Equip athlete with heart rate monitor and connect to both iPad app and MOXUS software

Equip athlete with mask/headgear, ensuring the T-shape rebreathing valve is properly assembled and true seal is formed

Input Subject Data
- Do not need to worry about BP (rest), HR (rest), or MVV

Study Information
- Pressure (mmHg)
- Temp (degrees C)
- Humidity
- FiO2 (%)= values on analyzer
- FiCO2(%)= values on analyzer

Start Study

NOTES:
- Make sure values are reasonable
  - VO2= 200-600ml/min
  - RER= -0.8
  - HR= 40-90 bpm
- Following test keep mask on for at least 30 seconds
- Collect 2min of resting metabolic data

Protocol:
- Warm-up: prior to incremental test, athlete should be warm from maximal sprint and handgrip exercises
- **Incremental step test** - each athlete will start at 25W with 20W increments every minute until volatile fatigue, when the athlete gives up, or when they are no longer able to maintain ~90 RPM for 30secs.
  - Leading up to predicted exhaustion, give strong verbal encouragement to prevent the athlete from ending prematurely

- **Completion of test** - once the test has been completed, drop the wattage very low (30-70W)—this will prevent a rapid drop in blood pressure which can lead the athlete to synoscope
  - Keep the gas exchange mask on the athlete for 30secs following completion of the test to ensure final data points are not skewed by ambient air
Appendix G: Familiarization and Experiment Trial Trial Data Collection Form

**Familiarization/ Experimental Trial:**

Upon arrival: Actively taking HC or IUD? Y/N    Replicated meal? Y/N  Tc pill? Y/N

*Body weight*:  

*Urine specific gravity*:  

*Hours sleeping*:  

*Phase of menstrual cycle*:  

*Type*:  

**Environmental Conditions:**

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<th>Temp</th>
<th>Humidity</th>
<th>Wind speed</th>
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</table>

**Hand grip and isokinetic sprint:**

1): $Kg_{peak}$ _________  $W_{peak}$ _________

2): $Kg_{peak}$ _________  $W_{peak}$ _________

**Environmental Chamber:**

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<thead>
<tr>
<th>HR</th>
<th>Tc</th>
<th>$W_{Peak}$</th>
<th>RPE</th>
<th>TS</th>
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</tr>
</tbody>
</table>

**Total time trial time:**

**Hand grip and isokinetic sprint:**

**Post: $Kg_{peak}$ _________  $W_{peak}$ _________**

**Sweat Rate and Hydration:**

<table>
<thead>
<tr>
<th>Post Body weight: _________</th>
<th>Water consumed during: _________</th>
</tr>
</thead>
</table>

Sweat rate: $Sweat loss (g) = | change in body weight (g) + fluid intake (g)| = _________
Appendix H: Familiarization and Experiment Trial Checklist

**Trial Checklist: Menthol 30-km Time Trial**

**Day of the trial:**
- Core temp
  - Tc pill→ find location and deliver to each participant (watch participant ingest)
- Bring towels for the participant

**Trial Day: Pre-trial setup – Chamber Prep**
- Set up environmental reader
- Check environmental chamber is (30°C, 70% RH, wind speed 20km/h)
- Pre-measure and set out mouth rinses’
- Set disposable water bottles into chamber water bottles into chamber
- Turn on computers for Velotron
- Open Wingate and Velotron CS Software
  - Wingate→ Load Client→ Load Protocol→ Run Wingate
  - Velotron CS Software→ Charts→ 30 km TT (Erica Research) v2.crs
- Calibrate Velotron: when calibrating, make sure to stop pedaling at 23 mph (do not let the pedals keep spinning)
- In Velotron CS software, ensure gears are set to:

![Gear Definitions](image)
• Load Participant:
  a. **Source**
  b. **New**
     i. Fill in characteristics (do not touch HR low/high and drag)
     ii. **OK**
  c. **Source**
  d. **Open**
     .Click on the client
• FYI: The Wingate and Velotron CS software cannot be open at the same time

**Pre-trial setup**
• Heart rate
  • Polar monitor, watch, strap… make sure HR monitor and cell phone is charged
• Hand dynamometer
  • Working fine
• Table
  • Menthol/Crystal Light shot glasses
  • Expiratory container
  • Clip boards
  • RPE and Thermal Charts
  • Pens
  • Familiarization and Experimental Workflow Protocol Sheet
• Other
  • Position of bike (measurements from preliminary trial)
  • Garbage can

**Blood Glucose**
• Blood glucose: insertions
  • Gauze
  • Gloves
  • Glucose strips
  • Glucometer
  • Disposable lancets
• Other
  • Garbage can
  • Biohazard bin
  • Sharps bin
  • Clean room

**Urine**
• Urine specific gravity, body mass, and sweat rate
  • Label USG cups
• Portable Refractometer
• Disposable pipettes
• Water bottle scale
• Body weight scale
• Other
  • Clean room

**Trial Day: Pre-Experimental Protocol Setup**

- Environmental Chamber
  - Table
    • Menthol/Crystal Light shot glasses
    • Expiratory container
    • Clip boards
    • RPE and Thermal Charts
    • Pens
    • Familiarization and Experimental Workflow Protocol Sheet
    • Water bottles
  - Garbage
  - Water bottle→ make sure water is >20 degrees C

- Seat participant
  - Before entering environmental chamber, ask if they need to pee
  - Record baseline numbers for Tc and HR
  - Testing clock is started when participant enters the chamber (time in chamber to start of warm-up noted as it will be kept consistent for testing sessions)
  - Guide participant to sit on bike
  - Adjust bike if needed (should be fine—going off of preliminary measurements)

**Experimental Protocol:**

- Work Flow:
  - Participant starts 10-min warm-up (40% VO\(_{2\max}\))
  - 5-min break sitting on bike
  - Initial ratings of RPE, thermal scaling, resting core temp, and HR
  - Hand grip + sprint
  - 4:40-min- BREAK
  - Menthol mouth rinse
  - Hand grip + sprint
  - 3-min-BREAK
  - START 30-km TT
  - Menthol mouth rinse: 5, 10, 15, 20, 25, 30-km’s
  - Miles: (3.1, 6.2, 9.3, 12.4, 15.5, 18.6)
  - Manual recording of HR, Tc, \(W_{pul}\), RPE and thermal scaling: 5, 10, 15, 20, 25, 30-km’s
- Miles: (3.1, 6.2, 9.3, 12.4, 15.5, 18.6)
- At 30-km: menthol→hand grip + sprint
- “Cool-down” riding at 100W for 5min

**Trial Day: Checks During Experimental Protocol**
- Start trial in order
- Cycle to perform
  - Record every 5-km:
    - Menthol mouth rinse
    - RPE
    - Thermal scaling
    - HR
    - Tc
    - Wpeak
  - Let the participant know every 2.5km…. Once the participant hits 20km, tell participant every km until 30-km is reached

**Trial Day: Post-Trial Procedures**
- Participant taken from environmental chamber to prep room
- Equipment removed
- Post-ride nude body weight taken (without equipment)

**Cleaning Tasks**

Download files and Exporting Data
- HR data (found on Polar account)
  - Username: [ericaheartrate1@gmail.com](mailto:ericaheartrate1@gmail.com)
  - Password: Saskatchewan#03
  - Username: [ericaheartrate2@gmail.com](mailto:ericaheartrate2@gmail.com)
  - Password: heartrate
- Tc data
  - Collected manually
- Wingate data

**Exporting:**
- **Step 1** -- In the Tree-View select the database and then the client the performance file resides. Then right-click on the performance file. Choose Export. The program will export the performance file in comma separated text format into the same database folder the original performance file was found. The file will have the same file name, but with a TXT file extension. These files can be used with any spreadsheet program.
- CS data
  - In the home screen
    - Export Options- select:
• **Time**
• **Speed**
• **Power**
• **RPM**
• **HR**
• **Miles**
• **Grade**
• **Load**

**Auto export**
• **Export:** click on file (course_name.crs-date)
  • Information box will appear (Exported C:\VelotronCS2008\perfs\course….)
  • Open the PC document folder
    • **Local disk C:**
    • **Velotron CS 2008**
    • **Perfs**
    • **Text document**

**Other:**
• Place equipment back into storage locker
• Put away equipment properly
• Wipe down all equipment
• Wipe down all surfaces
• Thoroughly wash heart rate strap and expiratory container with hot soapy water
• Check rooms after participant leaves
Appendix I: Deception and Debrief Letter

The Effects of a Menthol Mouth Rinse During 30-km Time Trial Performance in Female Cyclists

Thank you for agreeing to participate in this study! Initially, you were told the purpose of this study was to determine which mouth rinse you preferred, the menthol or Crystal-Light flavour. With that being said, the actual purpose of this research is to explain the effects of a menthol mouth rinse during a 30-km time trial in female cyclists. The reason you were deceived into thinking the study was for something else, is to prevent altered motivation from one trial to the next. Previous literature supports that motivational self-talk has the capability to improve performance.

Throughout the trials, the experimenter did not know whether you were using the placebo (Crystal-Light) or the menthol solution. In the study, total 30-km time trial time, average power output, and power output at each mouth rinse was captured and analyzed. To date, this method is well established in the male population, however, hasn’t been proven with females. The results from the study indicate that the menthol mouth rinse improves time trial performance in female athletes.

If you feel especially concerned about the protocol, results, or anything related to this study, feel free to contact Erica Gavel at erica.gavel@mail.utoronto.ca, Dr. Scott Thomas at scott.thomas@utoronto.ca, or Dr. Heather Sprenger at heather.sprenger@uoit.ca. In addition, if you have any concerns about the aspect of the study or rights as a participant, you can contact the Research Oversight and Compliance Office- Human Research Ethics Program at ethics.review@utoronto.ca, or (416)- 946- 3273.
Additional Reading:


Appendix J: Thermal Scaling

(a) a temperature perception, (b) pain intensity, and (c) temperature perception model modified from Greenspan et al, (2003).
Pain Intensity Scale

- Extremely Intense
- Highly Intense
- Moderately Intense
- Slightly Intense
- Not at all Intense

Pain

NO Pain
### Appendix K: Borg 6-20 Scale

<table>
<thead>
<tr>
<th>Rating</th>
<th>Descriptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>No exertion at all</td>
</tr>
<tr>
<td>7</td>
<td>Extremely light</td>
</tr>
<tr>
<td>8</td>
<td>Very light</td>
</tr>
<tr>
<td>9</td>
<td>Light</td>
</tr>
<tr>
<td>10</td>
<td>Somewhat hard</td>
</tr>
<tr>
<td>11</td>
<td>Hard (heavy)</td>
</tr>
<tr>
<td>12</td>
<td>Very hard</td>
</tr>
<tr>
<td>13</td>
<td>Extremely hard</td>
</tr>
<tr>
<td>14</td>
<td>Maximal exertion</td>
</tr>
</tbody>
</table>