THE EFFECT OF CAFFEINE MOUTH RINSES IN ENDURANCE RUNNING

by

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(Under the Direction of Jamie A. Cooper)

ABSTRACT

Previous studies have shown mouth rinses (MR) containing carbohydrate (CHO) can improve endurance running performance in events lasting approximately 1 hour. Caffeine (CAF) MRs have been shown to have beneficial effects on sprint performance, but the impact on endurance performance is unknown. The objective of this thesis was to determine the effects of CAF MRs, both alone and combined with CHO, on 12.8-kilometer running performance in endurance athletes. 11 trained endurance athletes (5 men, 6 women) completed 12.8-kilometer timed trials on 4 separate occasions to complete 4 treatment conditions. Although there were small performance benefits with CAF alone and CAF+CHO treatments compared to placebo (water), these differences did not reach significance for the whole group or when analyzed by sex. There was also no performance benefit from CHO MR alone versus placebo. In this pilot study, CAF MRs did not have an ergogenic effect on 12.8-kilometer running performance.

INDEX WORDS: MOUTH RINSES, CEPHALIC PHASE RESPONSE, CAFFEINE, CARBOHYDRATE, ENDURANCE PERFORMANCE

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CHAPTER 1

INTRODUCTION

The field of dietary supplements and sports nutrition has expanded greatly in recent decades as athletes use various techniques to gain an edge over their competition. Research has shown that different supplements or sports nutrition products are more beneficial than others depending on the type of sport, duration of event, and acceptability of the supplement to the individual athlete. In 2014, more than 50% of all athletes in the United States use some type of dietary supplement [1]. Furthermore, it has been reported that endurance athletes use supplements to a greater extent than non-endurance athletes [2, 3]. Carbohydrate (CHO) and caffeine (CAF) are two such substances that are frequently used by endurance athletes and can have beneficial effects for performance [4-8].

Ingestion of CHO has been proven to be most beneficial to endurance athletes when glycogen depletion is occurring [9]. Glycogen serves as the storage form of glucose in the human body, and the majority of these stores are found in liver and skeletal muscle [10]. When these stores begin to decline, such as in exercise bouts exceeding 90 minutes, athletes experience fatigue [11], and CHO ingestion is needed to maintain performance levels. However, in endurance events lasting approximately 1 hour, it is unlikely that glycogen stores deplete enough in the body to be a limiting factor on performance [12]. One area of recent interest that is continuing to gain popularity is the use of CHO mouth rinses (MRs) for enhancing performance in athletes for these slightly shorter duration events. In mouth rinsing, an individual swishes a solution around the oral cavity for a period of time before expectorating it. fMRI studies have

revealed exposure to CHO in the oral cavity results in the activation of reward systems in the brain, such as the dopaminergic midbrain and the anterior cingulate cortex [13-15]. This mechanism may explain how CHO MRs have been shown to result in similar, and sometimes greater, performance benefits compared to CHO ingestion [16-18].

CAF can aid performance by acting as an antagonist to adenosine receptors and stimulating the central nervous system [19, 20]. This can produce feelings of alertness while decreasing and individual's sense of fatigue. Unfortunately, CAF exposure can be accompanied by negative side effects, including irritability, elevated respiration, and gastrointestinal (GI) disruptions [21]. It has been shown that long distance runners are more likely to experience GI issues than other endurance-focused athletes, with 30-65% of all long distance runners reporting GI symptoms of some sort [22, 23]. Researchers began exploring ways to provide the performance benefits of CAF to athletes while avoiding the detrimental effects, which led to greater research in the field of CAF MRs. Despite being a relatively new research area, CAF MRs have been shown to improve performance in sprint exercises [24-26] as well as self-paced [27] cycling exercise bouts. The interaction of CAF with adenosine receptors in the oral cavity is believed to provide a motivational and rewarding effect in the brain and may contribute to the ergogenic effects of CAF MRs [28, 29].

To date, however, there has been no research on CAF MRs in endurance running lasting approximately 1 hour in length, the equivalent of a typical 10K, 15K or half marathon race. Furthermore, no one has tested for any synergistic effect of CAF and CHO combined into one MR in endurance running. Lastly, there is no data available on the potential different responses by sex to CAF MRs. Previous research has shown that males are able to metabolize CAF and rid the body of it faster than females, implying that females may be more sensitive to CAF [30-32].

Therefore, the current study had two objectives: (1) determine whether CAF MR, either alone or with carbohydrates, affects endurance running performance and (2) determine whether sex differences exist in the performance response to caffeine MR. In order to test these aims, participants completed a 12.8 kilometer running time trial (TT) on four separate occasions, mouth rinsing at specified times throughout the duration of the run with either CAF alone, CHO alone, CAF + CHO, or water (placebo control). Completion time, as well as heart rate and rating of perceived exertion (RPE) were collected from participants and analyzed both as a whole and by sex. Determining the different effects of CAF MRs, if any, on endurance performance may be beneficial in developing novel ergogenic supplements for both recreational and elite athletes.

The literature review (Chapter 2) provides a synopsis of the current literature surrounding cephalic phase responses, sweet and bitter taste transduction, the nutrition needs of endurance athletes, and the effects of caffeine and carbohydrates on endurance athletes. Chapter 3 contains the manuscript, which examines the effects of CAF, CHO, and CAF + CHO mouth rinses on completion time, heart rate, and rating of perceived exertion in a 12.8 kilometer endurance timed trial. We hypothesized that the CAF + CHO treatment will provide the greatest performance benefit to runners, followed by the CAF alone, then the CHO alone, and finally, the placebo treatment. Chapter 4 includes a summary and conclusions of the research study.

CHAPTER 2

REVIEW OF THE LITERATURE

Nutrient needs for endurance athletes

In order to maximize their performance potential, endurance athletes need to compensate for the increased physical demands being placed on their bodies. Of the three main macronutrients, it has been shown that CHO and fat are the main substrates that are oxidized by the body during endurance exercise [33], but in prolonged high-intensity (i.e., 85% VO_{2max}) exercise, the body begins to depend more on glycogen stores as a CHO source for continued oxidation and lipid oxidation is reduced [12, 33]. Ingestion of CHO in endurance exercise, when glycogen depletion is occurring, has been shown to decrease athletes' perceived exertion and improve overall performance by maintaining plasma glucose levels during high rates of CHO oxidation [6]. However, it does not appear that addition of other macronutrients, such as fat or protein provide a performance benefit. In one study, participants ran a 21-kilometer time trial on three separate occasions and ingested either a CHO-electrolyte-protein solution (CEPS), a CHO-electrolyte solution (CES), or a noncaloric sweetened placebo (PLA). The researchers found improvements in performance time for the CES treatment compared to PLA, but no differences between the CESP and PLA [34], indicating that CHOs aid endurance athletes in performance, and while protein is necessary for healthy recovery, intake of protein is not of great benefit mid-race for endurance athletes.

It has also been shown that although supplementation of dietary fat can alter the availability of fatty acids in the body, the pattern in which bodies oxidize substrates during endurance events typically remains consistent [35]. In endurance exercise, CHOs are the body's preferred source of energy and are more crucial than protein or fat intake to maintain performance level. As far as potential sex differences, it appears that there are no differences in metabolic responses between men and women for CHO ingestion during exercise [36]. In order to maximize CHO availability during exercise, the ideal CHO ingestion rate for endurance exercise has been shown to be approximately 60-90 grams of CHO per hour, depending on the length of the endurance event [4]. Some research has even shown benefits of CHO intake in endurance events lasting between 30 – 75 minutes, when CHO stores and blood sugar levels are not yet depleted [5, 37]. This observation began to fuel research in the area of CHO mouth rinsing as a way of stimulating cephalic phase responses.

Fluid needs also differ for endurance athletes as proper hydration can reduce fluid loss and maintain performance. Hydration protocols for athletes depend on changes in weather, acclimatization state of the athlete, and sweat rate of the athlete (sweating rate = pre-exercise body weight – post-exercise body weight + fluid intake – urine volume/exercise time in hours) [38]. It is recommended that athletes consume approximately 500 to 600 mL of water or sports drink 2-3 hours before exercise and another 200-300 mL 10-20 minutes before exercise [38]. The goal of fluid replacement during exercise is to replace all the water lost through sweating [39]. If this is intolerable to the athlete or impossible due to a high sweat rate, it is recommended to maintain hydration levels so that less than 2% of body weight is lost [38]. For most athletes, this means consuming 200-300 mL every 10-20 minutes of the exercise, but this varies by individual. Post-exercise rehydration, ideally occurring within 2 hours after exercise completion, should

correct any losses in fluid experienced during exercise, calculated based on sweat rate of the athlete or changes in body weight from pre- to post-exercise [38]. Generally, it is recommended to take in 470-700 mL of fluids for every pound of body weight lost during exercise [40]. Major losses in water and electrolytes, such as sodium, chloride, and potassium, can occur for endurance athletes during bouts of extensive sweating. Consuming a beverage with CHO and electrolytes during exercise is recommended to maintain CHO oxidation, blood glucose, and electrolyte balance if the exercise lasts longer than approximately 50 minutes [38, 41]. Such beverages should contain approximately 4% - 8% CHO concentrations/solutions to maintain optimal performance [39]. In order to reduce the risk of hyponatremia in prolonged endurance events, beverages containing salt are recommended [42]. Altogether, CHO, fluids, and electrolytes are the most crucial factors for endurance athletes in order to achieve optimal performance. However, athletes can experience gastrointestinal complications with ingestion of the aforementioned nutrients, so research into other physiologic stimuli for performance enhancement is warranted.

Cephalic phase response

Three phases of bodily responses occur in response to food ingestion: the cephalic/neural phase, the gastric phase, and the intestinal phase [43]. Cephalic phase responses (CPR) are the collection of physiological reactions that occur in response to sensory cues, such as smelling or tasting food [44].These responses, as opposed to those that take place in the gastric and intestinal phases, take place before any actual ingestion of the food has occurred. One of the earliest observations of this was observed in Pavlov's research dogs as the animals began to secrete

saliva in anticipation of receiving food [45]. CPR induces physiological and metabolic changes as the body prepares the gastrointestinal tract, liver, and other organs to receive and metabolize or store the potential nutrients [46]. In a sense, these responses are thought to prime the gut so that optimal digestion and absorption can occur. When food is first placed in the oral cavity, sensory stimulation occurs as chemoreceptors respond to the intake of food and increased salivary flow. The medulla oblongata is then stimulated which, in turn, stimulates the efferent component of the vagus nerve, which then leads to parasympathetic activation of a variety of secretory reflexes [47].

One such reflex is an increase in salivary flow and thus, the release of the enzymes amylase and lingual lipase, as a response to CPR [48]. Salivary amylase plays a role in digestive processes by breaking polysaccharides down via cleavage of the alpha 1,4 glycosidic linkage bonds [48]. The enzyme continues to aid in the breakdown of sugars temporarily after swallowing, but is eventually rendered inactive after it encounters the acidic gastric environment. Lingual lipase functions in digestion to hydrolyze triglycerides into free fatty acids [49]. In terms of CPR, the composition of the saliva secreted is partially dependent on the type of gustatory stimulant. For example, tasting sugars, such as fructose and sucrose, has been shown to initiate secretion of saliva with higher levels of salivary amylase [50].

Another of these CPR reflexes is a secretion of gastric acid whereas gastrointestinal motility increases as a preparatory response for nutrient ingestion [51]. While olfactory stimulation may also elicit these gastric secretions, it has been recently shown that gustatory stimulation produces a greater magnitude of gastric secretions [52]. Thus, both chewing gum and performing MRs, where an individual swishes around a solution in the oral cavity and then expectorates it, have been shown as effective methods to initiate CPR [44, 53]. The magnitude of

these gastric acid secretions is also related to the palatability of the taste present in the oral cavity. It has been shown that the more acceptable the taste is to the individual, the greater amount of gastric acid is secreted [54]. This implies that the volume of gastric acid secretions in response to a highly acceptable taste, such as sweetness, may be greater in magnitude compared to the response elicited by a bitter taste, which has been shown to be less acceptable to humans. In sham feeding trials in humans, gastric acid release starts approximately 4 minutes after the start of sham feeding and lasts for approximately 30-120 minutes after the feeding has concluded [54]. The secretion of pepsinogen from the fundic glands, mediated by the vagus nerve, may also occur in response to cephalic stimulation [55]. Furthermore, leptin may be secreted by the stomach during the cephalic phase, and it has been proposed that this early secretion of leptin contributes to satiation as the body anticipates the digestion process to continue [56].

In addition to stomach secretions, CPR has also been shown to stimulate both endocrine and exocrine pancreatic secretions [57]. In an effort to neutralize the gastric acid release, bicarbonate is also increased up to 35% from the duodenal mucosa during CPR [58]. Further exocrine secretions include digestive enzymes, such as lipase, trypsin, and amylase [59]. As a result of vagal stimulation, endocrine secretions into the bloodstream include insulin, glucagon, and pancreatic polypeptide [60]. Pancreatic polypeptide is controlled almost exclusively by vagal control, and it has been shown to increase up to 100% above baseline levels when participants chew and then expectorate food [47].

CPR has been shown to result in further anticipatory physiological changes as well, such as an increase in heart rate, changes in blood glucose, and activation of reward centers in the brain [61-63]. Functional magnetic resonance imaging (fMRI) studies have revealed interactions between nutrient exposure in the oral cavity and increased brain activity [13, 14, 64]. It has been

shown that sweet taste alone does not trigger these neural responses to the same degree that a sweet, energy-containing stimulus, such as glucose, does [14, 17]. One study found saccharin, an artificial sweetener, activated only the insula/frontal operculum and left dorsolateral prefrontal cortex (DLPFC) while glucose, an energy-containing sweetener, activated both of these regions as well as the striatum, orbitofrontal cortex, and anterior cingulate cortex [64]. The DLPFC is thought to play a role in the cognitive processing associated with taste, and it can be stimulated by multiple different taste stimuli [65], whereas the dopaminergic circuitry in the striatum as well as the cingulate cortex is associated with rewarding stimuli [66]. These results corroborate the findings from a fMRI study conducted by Frank et al [13], where only an energy-containing sweetener stimulated dopaminergic midbrain areas, while an artificial sweetener did not. This implies that the human brain can differentiate between nutritive and non-nutritive sweeteners, and receives more of a reward from the former, even if humans cannot make this conscious distinction based on taste. Oral exposure to CHO has also been shown to have a priming effect in cortical regions involved in motor control and visual perception [67]. This indicates that these pathways can potentially be manipulated by nutritional intervention so as to minimize motor output losses due to fatigue.

Anatomy and Physiology of Sweet Taste Sensing

Humans generally have a natural preference for sweet tastes. It is thought that, evolutionarily, humans are predisposed to seek out sweet tastes because they are usually indicative of foods higher in energy (kilocalories), which would help humans maintain fat stores in times of potential famine [68]. All sweet tastes, both natural and artificial, are sensed by

heterodimer receptors made up of subunits T1R2 and T1R3. These receptors are primarly found in taste buds in the oral cavity, but have also been observed in the gastrointestinal (GI) tract, pancreas, bladder, and brain [69-72]. Taste buds are distributed throughout the oral cavity, with the majority on the tongue and palate, and are embedded within different forms of papillae. There are circumvallate papillae which are localized near the back of the tongue towards the epiglottis, fungiform papillae which are spread across the front and sides of the dorsal section of the tongue, and foliate papillae, present on either lateral side of the tongue, approaching the posterior. Filiform papillae are also present on the tongue surface, but do not contain taste buds [73]. Contrary to popular belief, all five basic tastes, sweet, sour, bitter, salty, and umami, can be detected by all areas of the tongue [74].

The T1R2 and T1R3 subunits belong to the class C group of G protein-coupled receptors (GPCRs), and the T1R2-T1R3 heterodimer transmits intracellular signals via the heterotrimeric G-protein gustducin [75]. When this occurs, the taste receptor cells are depolarized and ATP is released. After this activation of receptors, the main central gustatory pathways that innervate taste buds - the vagus, glossopharyngeal, and facial nerves – synapse in the nucleus of the solitary tract in the medulla oblongata, which then project to the pons, the thalamus, and finally the primary gustatory cortex [76]. Other areas of the brain that are activated and may result in a "feel good" sensation after sweet stimulation of taste buds include the amygdala, orbitofrontal cortex, and lateral hypothalamus [63, 77, 78].

Despite using similar pathways, it has been shown that the human brain has a different activation response after an individual is exposed to artificial versus a nutritive, energy sweetener, such as glucose [13]. Simple sugars, such as glucose and fructose, are also referred to as monosaccharides and contain one sugar molecule. Sucrose is a disaccharide that results when

a glucose molecule is bound to a fructose molecule. Sucralose, on the other hand, is an artificial sweetener made by removing three hydroxyl groups from sucrose and replacing them with three atoms of chlorine [79]. The T1R2-T1R3 dimer mediates the taste perception of both sugars and artificial sweeteners. However, T1R3 knockout mice have been shown to have no preference for sucralose at all while retaining a reduced preference for sucrose [80]. This implies that artificial sweeteners may only be mediated by T1R3 while sugars interact with additional receptors outside of T1R3 alone. This difference in receptor transduction has been postulated as one of the reasons sucrose exposure results in a greater activation of the brain's reward system, areas such as the anterior insula, frontal operculum, striatum, and anterior cingulate when compared to sucralose exposure [13].

Anatomy and Physiology of Bitter Taste Sensing

While CHO is associated with a sweet taste, CAF itself has a bitter taste [81]. Although the mechanism for taste transduction is similar between them, contrary to sweet taste, bitter compounds are innately less acceptable to humans. It is believed that this distaste serves an evolutionary purpose in helping humans to avoid spoiled, aged, or toxic foods, since many of these substances are known to have a bitter taste [82]. However, bitter taste does not always indicate unsafe foods; brussels sprouts, coffee, and chocolate all have bitter notes, as well. Humans have multiple GCPRs in the T2R family that are stimulated by bitter substances in the oral cavity, but these bitter-taste GPCRs are structurally different from the sweet-taste receptor GCPRs in that none of them form dimers [83]. The T2R GCPRs then interact with α-gustducin to lead to the cascade which ultimately causes calcium influx and depolarization, eventually

leading to taste perception [84]. The T2Rs that have shown to be sensitive to bitterness from CAF include T2R7, 10, 14, 43, and 46 [85]. T2R43 may be particularly important to perceived bitterness, and drinking CAF may increase its expression in humans [86]. Sex also plays a role in the body's ability to detect bitterness. Compared to males, females are better able to identify bitter taste and sense a higher intensity for the same bitter concentration level [87].

Caffeine ingestion in endurance athletes

CAF is a plant-derived methylxanthine stimulant often found in coffee, tea, soda, and chocolate [88]. The human body metabolizes CAF to paraxanthine and theophylline, which are also found in naturally occurring drinks that can act as stimulants [88]. CAF has been shown to produce feelings of alertness and arousal while decreasing the sense of fatigue, all of which may give an athlete an edge during competition [19, 89]. CAF acts on the central nervous system as an antagonist to adenosine receptors A1, A2A, and A2B, which induce sleep when bound to adenosine [19, 20]. However, CAF intake does not come without potential risks as well. Heart palpitations, sleep disruptions, irritability, elevated respiration, and GI disruptions have all been associated with over exposure to CAF [90].

Despite these potential drawbacks of over ingestion, CAF is a commonly used ergogenic aid, with research showing that triathletes, cyclists, and runners have some of the highest usage rates [91]. From 11,361 urinary samples obtained between 1993 to 2002 from in-competition doping tests by the Flemish Community, the International Association of Athletics Federations, Union Cycliste Internationale, and Union of European Football Associations revealed that 74% of athletes were using CAF [92]. In 2004, CAF was removed from the prohibited list of

substances after anti-doping authorities had previously set a urinary threshold of 12 $\mu g \cdot mL^{-1}$ in 1985. Despite the removal of the CAF ban, usage rates have not increased, but rather, remained consistent as 74% of urinary samples from athletes participating in more than 62 sport specialties contained detectable levels of CAF from 2004 to 2008 [91]. Ingestion of 3-9 mg/kg of caffeine approximately 60 minutes prior to exercise is a generally accepted recommendation for athletes [7]. However, ingestion of as little as 30 mg has resulted in improved alertness and reaction times [89]. Research has also shown that there is great variability in the effects of CAF between individual athletes [93], and CAF recommendations should ideally be customized for the individual.

Although the complete mechanism for the rewarding effects of CAF in the brain is unknown, it has been shown that low doses of CAF are rewarding to the human brain while higher doses produce aversions [94]. The aversive effects of CAF are mediated by the central nervous system through dopamine blockade and the dopamine D_2 receptor [95]. CAF is associated with reward in the brain but not directly through the dopaminergic system [96]. This is contrary to reward associated with CHO ingestion. Furthermore, it has been shown that tegmental pedunculopontine (TPP) nucleus does not play a role in CAF reward, implying that mechanism of action for CAF differs from that of other recreational drugs [95]. One study found that the reward associated with CAF is dependent on adenosine A2A receptors in the nucleus accumbens in the basal ganglia [29], as CAF-induced arousal was no longer observed in rats after said A2A receptors were focally removed.

Some of the observed effects of CAF include increased energy expenditure as well as lipolysis. CAF has been shown to increase metabolic rate up to 12% with 200-300 mg doses [97, 98]. Another study revealed a significant thermogenic effect after ingestion of only 100 mg of

CAF in habitual CAF users, and the increase in energy expenditure had not returned to baseline even 3 hours after consumption [98]. Analyses revealed that most of the variation in thermic response was accounted for by lactate, triglyceride, and heart rate levels [98]. CAF also enhances fat oxidation up to 33% by moving fatty acids out of adipose tissue storage cells and into mitochondria to be oxidized for energy during exercise [99, 100].

There have been a number of studies showing improvements from CAF ingestion in both endurance running trials and cyclists. In one study, participants cycled for 120 minutes and were administered a low dose (100 mg) or moderate dose (200 mg) of CAF delivered in a CHO-electrolyte solution at minute 80. Both doses provided significant performance enhancing effects compared to the placebo treatment [101]. In another study, participants ran an 8-kilometer race 1 hour after ingesting a CAF capsule (3 mg/kg body weight), a placebo capsule, or no treatment. The CAF treatment resulted in a 23.8 second improvement in performance overall [102]. Administering CAF treatments to athletes has also been shown to reduce their rating of perceived exertion, a measure of how hard they think their bodies are working [103].

There have also been differences observed in the effects of CAF in male versus female athletes, although the literature is somewhat inconsistent in the findings [8, 30, 104]. One crossover study completed with 26 triathletes revealed that supplementation of 6 mg/kg body weight CAF 45-60 minutes before an Olympic-distance triathlon led to both greater performance improvements and higher cortisol levels in men than in women [8]. Another study found males to have greater changes in both heart rate and blood pressure in response to CAF than did females [104]. However, CYP1A2 activity has repeatedly been shown to be higher in males than females [31, 32]. The CYP1A2 enzyme is what allows for most of the metabolism of CAF to occur [105] so men with increased CYP1A2 function are able to metabolize CAF faster, meaning

it is expected to have less of an effect on their bodies. Additionally, in one crossover study, participants consumed a placebo beverage and a caffeinated energy drink (3 mg/kg body weight) on two separate occasions and then performed a standardized exercise bout. The rearchers found that females showed higher perceived fatigue after intake of placebo beverage compared to the energy drink while the males showed no effect [30]. Taken together, these data indicate that females generally have a higher sensitivity than males to CAF.

Carbohydrate + caffeine ingestion in endurance athletes

Most studies in the current literature focus on either CAF or CHO ingestion for athletes. However, there are some studies that examine the efficacy of combining these substances and testing for a synergistic effect. An analysis of 21 studies found that CHO combined with CAF ingestion provided a small but statistically significant effect to improve endurance performance when compared to CHO alone [106]. Such an effect was observed by one study using low dose CAF. Well trained cyclists were recruited to ride for 2 hours and drank one of 4 treatments at minute 80 and minute 100 of the timed trial. The two drinks were: Coca-Cola (90 mg CAF + CHO), CAF-free Cola (extra CHO), half-strength Cola (90 mg CAF), and half-strength CAF-free Cola (control). There was a 3.3% improvement in timed trial performance from the combined CAF + CHO treatment compared to control. For CAF alone, there was about a 2% improvement from control, and for CHO alone, there was a 0.6% improvement from control [107]. Thus, the magnitude of the ergogenic effect of CAF + CHO outweighed either of these substances used individually. In another study of three separate timed cycling trials, participants ingested one of the following treatments: a placebo, a 6.4% glucose solution, or a 6.4% glucose solution

combined with 5.3 mg CAF/kg body weight [108]. Researchers found that the combined ingestion of CAF + CHO enhanced performance by 4.6% compared with the CHO treatment alone and 9.0% when compared to the placebo treatment. Another study examined the effects of a CAF + CHO-electrolyte beverage (CEB) on cycling performance times and nocturnal sleep. The last treatment on test days was administered 5 h 35 min before bedtime. Ingestion of the CEB resulted in a 4% improvement in performance when compared to the placebo treatment, but the study did find that plasma CAF levels remained elevated by the set bedtime [109]. Thus, CAF + CHO ingestion does appear to provide a significant ergogenic effect, but athletes are encouraged to be cognizant of the timing of their ingestion so as not to interfere with their sleep patterns and recovery.

Gastrointestinal issues in endurance athletes

It has been observed that 30% to 65% of long distance runners experience adverse GI symptoms [22]. These issues are more commonly observed in runners than in other endurance-focused athletes, such as cyclists [23]. This is most likely due to the repeated and high-impact mechanical jostling experienced by runners. These symptoms, including vomiting, abdominal cramping, nausea, diarrhea, and many others, can have a severely negative impact on performance. The development of GI issues during endurance events is very individualized, and there is evidence suggesting that individuals may be predisposed to experience GI issues based on genetics alone [110]. However, many other factors can induce or exacerbate GI issues in athletes, including timing of feeding [111], reduced GI blood flow [112], and use of non-steroidal anti-inflammatory drugs (NSAID) [113].

CAF may have negative effects on individuals' gastrointestinal systems, with some athletes reporting nausea and queasiness after ingestion [114]. Dehydration, which can often occur in many long-distance events, has been shown to reduce the rate of gastric emptying [115, 116], so the movement of food down the GI tract and absorbed into the body is slowed. This can lead to bloating, nausea, heartburn, vomiting, and all around impaired athletic performance. New techniques are being employed by athletes around the world in attempts to alleviate GI distress without sacrificing performance. Methods include switching over to a temporary low fermentable oligosaccharide, disaccharide, monosaccharide, and polyols (FODMAP) diet [117], ingesting hydrothermally-modified starch supplements [118], and attempting to induce gut adaptations via repetitive GI tract "training" [119]. Researchers have also examined mouth rinsing, in part, as a method of maintaining performance levels without engaging the GI tract at all.

Carbohydrate mouth rinsing

Ingestion of CHO during endurance exercise, where glycogen depletion is occurring, has been shown to decrease athletes' perceived exertion and improve overall performance [6]. However, many athletes experience adverse gastrointestinal issues when consuming CHO during competition [120]. Researchers began to explore potential performance benefits of CHO mouth rinses in situations when glycogen depletion has not yet occurred, such as endurance events lasting about 1 hour in duration where it's unlikely that depleted glycogen stores are a limiting factor [12].

Multiple studies examining CHO MR have shown positive effects on endurance performance lasting approximately 1 hour in duration. In one study, subjects completed a high-intensity timed trial lasting about 1 hour while either rinsing or ingesting a 6% CHO-electrolyte solution (CES) or a placebo equivalent. The researchers saw no differences between the ingestion conditions between CES and placebo, but did observe a significantly faster completion time for the CES rinse when compared to the placebo rinse [16]. This indicates that CHO MR may even be more beneficial than CHO ingestion for a race lasting approximately 1 hour in length. Carter et al [121] conducted a crossover cycling study in which 9 participants were instructed to complete a set amount of work as quickly as possible. Participants mouth rinsed with a 6.4% CHO solution on one occasion and a then with water placebo solution on another visit. The CHO MR improved performance time by approximately 1.5 minutes without having an effect on rating of perceived exertion [121]. This indicates that, while the participants were working harder with the CHO treatment, they did not feel as though they were exerting themselves more than they did with the control treatment. Another study completed with eleven amateur male soccer players found that using a 10% maltodextrin MR significantly increased self-selected jogging speed in a modified version of the Loughborough Intermittent Shuttle Test compared to using a placebo rinse [122]. Similarly, Rollo et al [123] also examined self-selected running speeds, this time on a treadmill, automated to adjust for the participant's self-selected speed without manual input. After being asked to maintain a specific, consistent perceived exertion level, the total distance covered was greater after rinsing with a 6% CHO solution as opposed to a matched placebo. In another study, participants completed three separate self-paced cycling trials, each lasting 30 minutes. The three treatments were a 5-second water placebo MR, a 5-second 6.4% CHO solution, and a 10-second 6.4% CHO solution. Distance cycled was

significantly greater in the 10-second MR group, but there were no significant benefits with the 5-second MR treatment [124]. This indicates that the length of time the MR is in contact with the oral cavity may have an effect on the magnitude of response.

There has also been some evidence that CHO MRs also improve high intensity anaerobic exercise. In one study participants completed a 48 minute cycling session, punctuated by 10 second sprints, while rinsing with either a 6.4% CHO solution or a placebo. The researchers found the CHO MR resulted in a greater mean power output as well as a performance benefit for the final sprint bout [125]. One study assessing the effects of CHO versus placebo MR on countermovement jump height, isometric mid-thigh pull peak force, 10 m sprint time, and bench press and back squat repetitions until failure found significant improvements in countermovement jump height, 10 m sprint time, and both bench and squat repetitions until failure following the CHO MR [18]. Another study measured time to exhaustion in a high-intensity exercise session on a cycle ergometer, preceded by 10 second MR of either maltodextrin or placebo. The investigators found no differences in time to exhaustion, but they did observe lower RPE levels with the CHO MR treatment [126]. This corroborates the idea that the CHO rinses are able to activate reward centers in the brain, but it may not always be enough to result in a performance benefit. Phillips et al [127] examined the effect of repeated CHO MR on cycling sprint performance and found that CHO MR significantly improved peak power output for a single 30-second cycle sprint.

A previous study from our lab showed that CHO MR in a 12.8 kilometer running timed trial improved times by almost 5% compared to placebo [17]. This study also found that the MR treatments needed to contain energy as the beneficial effects of the CHO MRs were not observed in the taste-match artificially sweetened treatments. It has previously been shown through fMRI

that oral exposure to the sugar present in CHO MR activates reward-related brain regions, including the striatum and the anterior cingulate cortex [64]. It is proposed that this is one of the mechanisms behind the endurance-related improvements.

Conversely, not all studies have observed these beneficial performance effects associated with CHO rinsing. Overall, CHO MRs appear to be more useful in terms of improving endurance exercise than sprint or power exercises. In one study, a 5-second MR with either maltodextrin or glucose was not beneficial for maximal sprint performance in cyclists when compared to the control group; there was no difference in maximal power output or mean power output across groups [26]. Another double-blind study examining the effects of maltodextrin MRs on multiple sprint performance found no difference in exercise performance or in rating of perceived exertion compared to the placebo treatment [128]. Another study examined the effect of CHO MRs on maximum strength and strength endurance performance, measured with six sets until failure at 70% of 1 rep max. There were three separate trials, during which participants would MR with either a CHO MR, a placebo MR, or no MR. The investigators found that CHO MR did not result in improved maximum strength or strength endurance performance [129]. One high intensity study examined the effects of CHO ingestion versus a CHO MR with five 15 second max repeat springs on a cycle ergometer, separated by 4 minutes of active recovery. The researchers found overall mean power and total work for the CHO ingestion treatment were significantly greater than the CHO MR treatment. Furthermore, actual ingestion attenuated the fatigue index when compared with the MR [130]. All of these findings again suggest that aerobic endurance athletes are the individuals that would benefit most from CHO mouth rinsing.

Caffeine mouth rinsing

Whereas CAF ingestion is fairly well researched, the area of CAF MR offers opportunity for additional research and understanding. Research is needed in this area since actual ingestion of CAF often causes gastrointestinal distress, which may outweigh the benefits of ingestion [131]. Thus, a CAF MR may be an ideal solution for endurance athletes participating in events lasting about 1 hour in duration, as there is no actual ingestion of the stimulant and smaller doses of CAF MR may have equivalent effects on athletic performance as larger doses of ingested CAF. It has been shown that as little as 30 mg of CAF MR can alter mood, improve alertness, and improve reaction times similar to a 200 mg ingested dose [89, 132]. Furthermore, the length of time that CAF is in contact with the oral cavity has been shown to have a positive correlation to its ergogenic effects. In one study, ingestion of low doses of CAF with a capsule did not produce the same ergogenic effects that were observed from ingestion of a CAF beverage [133], alluding to the necessary role of contact time with the oral cavity for CAF effectiveness. It is believed that interaction of CAF with adenosine A2A receptors in the oral cavity may be part of the mechanism behind these effects [28].

Some studies of CAF mouth rinses have shown beneficial effects on sprint exercises in cyclists, such as improved peak and average power as well as increased distance covered in shorter cycling trials [24, 134]. Beaven et al [24] examined the effects of mouth rinsing on repeated sprint performance on a cycle ergometer with either a noncaloric placebo, 6% glucose, or 1.2% CAF solution. The research personnel found both CAF and CHO to be effective in improving mean power during the first initial sprint of the trial. Another sprint cycling study completed following glycogen depletion sessions found mouth rinsing a 2% CAF solution to

result in significantly greater peak power and mean power compared to the placebo solution, implying that mouth rinsing with a CAF solution may help to combat the performance losses that often occur due to CHO reduction [134]. In one self-paced cycling trial, Bottoms et al [27] observed that administration of a 0.032% CAF MR improved distance cycled by about 1.3 kilometers. Pataky et al [135] also found that genotype and time of day have an effect on the ergogenic impact of CAF MR. Caffeinated chewing gum is now even commercially available on the market because of its observed beneficial effects. Paton et al [25] found that caffeinated gum (240 mg) increased testosterone levels and enhanced performance by 5.4% compared to the placebo treatment. This study provides even further support that the oral cavity plays an important role in the stimulatory effects of CAF.

The current literature in studies testing both CHO and CAF in one MR is limited to date. However, it has been shown that frequent rinsing with a CAF-maltodextrin MR can help to reduce mental fatigue during the Stroop task, an extensive cognitive task, compared to a placebo rinse [136]. This supports the notion that CAF and CHO are able to stimulate dopaminergic "feel good" associations in the brain through a MR. Another study examined the effects of three separate treatments: CHO MR, CAF MR, and CHO + CAF MR on intermittent running performance in collegiate male lacrosse athletes. Although the investigators found lower RPE levels for both the CHO and CHO + CAF treatments, they found no significant improvements in intermittent running performance across the board [137]. No research has been conducted to assess effects of CAF mouth rinsing on endurance performance in distances lasting around a typical 10K or 15K. Furthermore, no research has been conducted to assess a potential synergistic effect of combining both CAF and CHO into one MR for endurance exercise.

Measurements

Borg scale

The Borg scale is an instrument designed to measure one's RPE during a given task. The scale ranges from 6 ("no feeling of exertion") to 20 ("very, very hard") and was determined as an estimate of one's heart rate. Dividing one's perceived heart rate by a factor of ten will provide said individual's RPE [138]. The Borg scale has been validated when utilizing exercise protocols and has been cited by the American Heart Association as being a useful and relatively sound indicator of fatigue [139]. In one study conducted in 2,560 men and women, the participants underwent incremental exercise tests, and RPE reports via the Borg scale were strongly correlated with both heart rate and blood lactate, independent of sex, age, or physical activity status [140]. Another study tested individuals with Parkinson's Disease undertaking an exercise program and showed the Borg scale to be an accurate measure of exertion based on a significant, positive correlation with RPE and heart rate. The researchers also found that RPE values were not associated with age, sex, or disease severity [141]. Another study found strong positive correlations between VO_2 and RPE at three given increments (9, 13, and 17), indicating that the RPE scale is a useful frame of reference for exertion regulation [142]. This was supported by another study that showed the Borg scale to be an effective guidance tool for self-regulation and maintaining one's heart rate within a particular range [143].

Heart rate

Though multiple studies have shown an increase in heart rate (HR) as an effect of CPR [61, 62, 144], one research team did not detect this effect when testing for food exposure [145]. Aside from any potential correlation to CPR, HR is still renowned for being a useful indicator of effort intensity [146, 147]. One study by Noble et al [148] found a positive association between HR and blood and muscle lactate accumulation during a progressive, maximal exercise test on a cycle ergometer. This same association has been confirmed by various groups since this experiment [149, 150]. As HR has been a confirmed marker of exertion, monitoring HR has become popular for many different athletic groups in order to ensure high intensity that provides a challenge to athletes while avoiding overexertion that may cause illness and generalized fatigue [151]. For these reasons, many companies began developing technology to track individuals' HRs. One such company is Polar. One study tested the accuracy of different optically based HR monitors, Polar H7, Scosche Rhythym+, Apple Watch, Fitbit Blaze, Garmin Forerunner 235, and TomTom Spark Cardio, during aerobic exercise. Across all exercise conditions, the Polar H7 monitor had the best agreement with the ECG [152]. This indicates that electrode-containing chest strap HR monitors are the best option when attempting to obtain the most accurate HR measurement.

Visual analog scale

A visual analog scale (VAS) is a continuous measurement instrument often used in questionnaires in order to gauge subjective characteristics or attitudes that can't be directly and

objectively measured [153]. A VAS consists of a question followed by a line that is typically 100 mm long and two anchors on either side that serve as contrasting extremes. For example, a question may read "How much pain are you in right now?" followed by a line anchored with the choices "no pain at all" and "extreme pain" on opposing sides. The participant will mark a horizontal line along the 100 mm line, and the marking will then be measured with a ruler in order to quantify the results. Compared to a Likert scale, which has discrete jumps in the answers to the questions provided, a VAS allows for more nuanced distinctions in participants' subjective states [154]. The VAS has been extensively utilized in the fields of pain and fatigue research [155, 156]. In nutrition research, these scales are often used to assess feelings of satiety, levels of nausea, and taste. The VAS is also often used in clinical settings in order to assess taste preferences and acceptance/tolerance of certain meals in ill patients [157]. Zdilla et al [158] recently found that a taste-intensity VAS provided an improved zinc taste-test assessment as compared to the original Bryce-Smith and Simpson zinc taste test. The VAS provided an expanded range of variables and can now serve as a taste-acuity test for zinc sulfate and zinc taste tests. Therefore, the VAS is a valid tool to assess subjective feelings and is able to do so in a quick and efficient manner.

Conclusion

In summary, while MR techniques are receiving more attention and credibility recently, there are still a multitude of questions to be answered. It is important to unravel the role of CAF MRs in endurance performance and whether CAF MRs have a potential synergistic effect on endurance performance when combined with CHO into one MR. Furthermore, there has been no

research to date that looks for potential sex differences in response to CAF MRs. This could have major implications for athletes trying to obtain a competitive edge in endurance competitions; these findings could potentially contribute to the development of a novel ergogenic aid for both recreational and elite athletes, especially those with a history of GI distress issues.

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CHAPTER 3

THE EFFECT OF CAFFEINE MOUTH RINSES IN ENDURANCE RUNNING: A PILOT STUDY[1]

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ABSTRACT

Studies have shown that caffeine (CAF) mouth rinses (MRs) can improve sprint performance. ***Purpose:*** To investigate the effects of CAF MRs alone, and combined with carbohydrate, on endurance running performance. ***Methods:*** Eleven runners (6 women, 5 men) completed 4 testing visits (for 4 treatments) in this randomized, double-blind, crossover study. At each visit, participants MR with a specific solution at the start and every 12.5% of during a 12.8-kilometer running time trial (TT). The 4 MR solutions were carbohydrate alone (CHO), caffeine alone (CAF), combined CHO+CAF, and water to serve as the placebo (PL). Completion times, heart rate (HR), and rating of perceived exertion (RPE) were collected. ***Results:*** There were no significant differences in completion time between CHO+CAF, CAF, CHO, or PL treatments (61.41 ± 3.54 min vs. 62.05 ± 3.49 min vs. 60.73 ± 3.15 min vs. 62.59 ± 3.66 min, respectively). Furthermore, there were no differences in percent change from control in CHO+CAF (-1.72 ± 1.4%), CAF (-0.74 ± 1.2%), or CHO treatments (0.76 ± 1.3%). Men completed the TTs faster than women ($p<0.01$), but there were no treatment differences within each sex. There were no differences in average or max HR across or within sexes. Men reported higher max RPE values than women ($p<0.001$). There were no other differences in average or max RPE. There were some "responders" as six participants had faster completion times for CAF vs. placebo (-4.22 ± 0.62% faster, $p<0.001$), six had faster completion times for CHO+CAF vs. placebo (-5.47 ± 0.93% faster than placebo, $p<0.001$), and five participants had faster run times for CHO vs. placebo (-2.34 ± 0.66% faster, $p=0.008$). ***Conclusion:*** Although use of CHO+CAF MRs resulted in a 1.72% improvement in TT performance for all participants, this did not reach significance.

Some individuals did respond strongly to the MR treatments; however, so further work is needed to examine responder vs. non-responder effects.

INTRODUCTION

Ingestion of carbohydrate (CHO) during endurance exercise, when glycogen depletion is occurring, has been shown to decrease an athlete's perceived exertion and improve overall performance by maintaining plasma glucose levels during high rates of carbohydrate oxidation [1]. It has been observed that consuming between 60-90 grams of CHO aids athletes in maintaining their performance in endurance events lasting over 1 hour in length [2]. However, there is emerging evidence to show that merely rinsing the oral cavity with CHO can be beneficial in delaying central fatigue during shorter endurance events that last about one hour in length, before CHO ingestion becomes crucial [3-5]. One study reported performance benefits with CHO mouth rinse (MR) compared to ingestion during a 1-hour cycling time trial [6]. This ergogenic benefit from MR is thought to be due to cephalic phase responses (CPR). CPR is the collection of physiological reactions that occur in response to sensory cues, such as tasting food, before any actual ingestion of the food has occurred [7]. Cephalic phase response (CPR) induces physiological and metabolic changes as the body prepares the gastrointestinal tract, liver, and other organs to receive and metabolize or store the potential nutrients [8]. Based on previous studies utilizing functional magnetic resonance imaging (fMRI), CHO MRs are thought to improve endurance performance by activating reward centers in the brain related to motor control [5, 9, 10]. Multiple studies in recent years have shown beneficial effects of using CHO MR in both sustained endurance and sprint events [11-13].

While CHO MRs have been shown as beneficial for endurance performance, research incorporating caffeine (CAF) MRs is lacking. Actual ingestion of CAF has long been utilized as an ergogenic method for its ability to decrease fatigue and increase alertness [14-17]. However, one of the common issues with CAF ingestion is gastrointestinal (GI) disturbances. It has been observed that 30% to 65% of long distance runners experience GI symptoms [18]. These issues are more commonly observed in runners than in other endurance-focused athletes, such as cyclists [19], likely due to the repeated and high-impact mechanical jostling experienced by runners. For many runners, GI discomfort can be exacerbated by CAF consumption. Other potential side effects of CAF ingestion include anxiety, irregular heartbeat, inability to focus, and insomnia [20]. The potential ergogenic benefits of CAF, coupled with common negative side effects with ingestion, make it an ideal candidate for MR. It is hypothesized that interaction of CAF with adenosine receptors in the oral cavity may be part of the mechanism behind its ergogenic effects, and this interaction would occur with a MR protocol [21]. Furthermore, CAF may also activate reward centers in the brain similar to those activated by CHO MR [22].

Initial studies that have examined CAF MRs have shown positive associations with performance benefits in high-intensity sprint exercises [17, 23-25]. To date, however, there is no research on the effects of CAF MRs on endurance running performance. Furthermore, it is unknown if there is a synergistic effect from combining CHO and CAF into one MR for endurance athletes. Lastly, no studies have tested for potential sex differences in response to CAF MRs. The purpose of this study was to examine the potential ergogenic effects of a CAF MR on a running time trial (TT) in endurance-trained men and women. We also sought to determine if there was a synergistic effect of CAF + CHO and whether sex differences existed for TT performance. In addition to identifying the presence of sex differences, we sought to

analyze all data by each sex separately. We hypothesized that the CAF + CHO MR would provide the greatest performance benefit to the runners, followed by the CHO alone and then CAF alone compared to the placebo control. We also hypothesized that women would receive a greater performance benefit from the CAF-containing MRs than men based on females' heightened sensitivity to CAF ingestion in previous research [26, 27].

METHODS

Study design

This was a randomized, double-blind, placebo-controlled crossover design. All subjects completed five visits: one screening visit (visit 1) and 4 testing visits (visits 2-5). Visits 2-5 were completed in a random order, were separated by at least five days, and each visit took place at the same time of day for each subject (between 0600 – 0900 hours). At the testing visits, subjects completed a 12.8-kilometer running time trial (TT) with the only difference between trials being the different MR solutions administered. This study was approved by the Institutional Review Board for Human Subjects at the University of Georgia and informed written consent was obtained prior to beginning study procedures. Figure 1 depicts the timeline of study events.

Participants

Eleven (n = 6 women; n = 5 men) healthy, endurance trained athletes between the ages of 18-45y with a body mass index (BMI) between 18.0–24.9kg/m^2 were included in the study. To qualify, athletes had to perform endurance exercise at least 4 days per week for at least 1 hour per day. Exclusion criteria included changes in the participant's current exercise program, any type of low carbohydrate diet, chronic diseases, medication use, nicotine use, supplement use

other than a daily multivitamin, pregnancy, or nursing. Individuals were excluded if they had a sensitivity or allergy to red food dye FD&C Red No. 40. Furthermore, individuals were excluded if they consumed more than 300mg of caffeine per day to eliminate habitually high caffeine consumers. Participants were compensated $5 for completing the consent visit and $10 for each testing visit thereafter for a total of $45 for completing the entire study. Women completed the running trial visits only during the follicular phase (days 3-9) of their menstrual cycle to control for hormone fluctuation. Subjects were asked to arrive to each testing visit after an 8- to 12-hour overnight fast and having abstained from vigorous exercise, caffeine, and alcohol for 24 hours prior to testing visits. Finally, participants avoided brushing their teeth with toothpaste the morning of testing trials.

Protocol

Screening Visit (Visit 1):

At the baseline visit, height, weight, waist and hip circumference, blood pressure, and body composition were measured. Body composition was assessed with bioelectrical impedance analysis (RJL Systems, Clinton Township, MI). Participants then underwent a familiarization protocol which consisted of running on the indoor track at the Ramsey Recreational Center at the University of Georgia for 10 minutes and completing two MRs with water to become adjusted to the testing environment and the MR protocol for the TT. At the conclusion of this visit, participants were instructed to consume a 1-day lead-in diet (standard diet consisting of 55–60% carbohydrate, 15–20% protein, and 20–25% fat) for a 24-hour period prior to each subsequent testing visit. A food diary was used to assess compliance. They were also instructed to avoid consuming alcohol or caffeine during this 24-hour period. The exact same lead-in diet prior to visit 2 was then repeated before each subsequent testing visit. Subjects also kept a training log

for 7 days before each testing visit, and were then instructed to follow the same training prior to all subsequent study visits. Finally, participants avoided vigorous exercise for 24 hours before all testing visits.

Testing Visits (visits 2-5)

For visits 2-5, participants reported to the indoor track between 0600 – 0900 hours to complete a 12.8-kilometer running TT. Each subject was fitted with a heart-rate (HR) monitor (Polar Electro Inc, Bethpage, NY) and was given 10 minutes to warm up before the TT began. The only instruction they were given was related to the distance of the TT and that they should try to complete the TT as quickly as possible. Times for individual laps completed, split times at every 12.5% completion mark, and total completion time were all recorded. All information regarding their completion times and their HR during the TT was withheld from participants so as not to influence their performance during future visits. Participants were not permitted to listen to music or to wear their own devices to record their results during the trials.

The MR solutions were administered by research personnel throughout the TT. Subjects were instructed to rinse the solution (25mL) in their mouth and swish it around their oral cavity for a 5-second period before expectorating the solution so that none was ingested. The amount of expectorated solution was measured by research personnel to ensure that none of the solution was swallowed. This rinsing and swishing protocol was incorporated at the following time points: immediately before starting the TT and every 12.5% of the 12.8-kilometer TT completed, for a total of 8 times during each TT. The only communication between the subjects and the researchers other than the administration of the MR every 12.5% of the TT was informing the subject of the distance completed thus far in the TT. This information was provided only during each MR to stay consistent between trials. No encouragement or additional communication was

provided. Subjects were also asked to rate their perceived exertion (RPE) based on the Borg scale (ranging from 6 – 20) at every 12.5% of the TT. After the TT, HR data were recorded from the HR watches, which were programmed to record average and maximum HR.

MR Solutions

Each of the MRs were administered to the participant in 25mL boluses. The 4 MR treatments were: (1) water to serve as the placebo (PL), (2) a caffeine alone solution that had 20 mg of caffeine per bolus (CAF), (3) a 6.4% glucose solution per bolus (CHO), and (4) a solution of both 20mg of caffeine and 6.4% glucose per bolus (CAF + CHO). Both the CHO and the CAF + CHO solutions consisted of 160 grams of glucose powder (Modernist Pantry) dissolved in 2500mL of water with a bolus of 25mL to be used for the MR protocol. Both the CAF and the CAF + CHO solutions consisted of 2 grams of caffeine powder (Sigma-Aldrich) dissolved in 2500mL of water with a bolus of 25mL to be used for MR protocol. Each MR also had 1mL of red food dye FD&C Red No. 40 to ensure the same sensory response through appearance.

Statistics

Statistical analyses were conducted using JMP version 13.2 (SAS, Cary, NC, USA). Descriptive statistics including mean, range, standard deviation, standard error, and percent change (difference) from control were calculated for all outcome variables. A two-way repeated measures ANOVA was used to determine if there were any main effects of treatment, time, or sex as well as any interactions of these effects on TT performance and RPE over the course of the trial. In addition to determining differences between sexes with the repeated measures ANOVA, we sought to analyze all data by each sex separately to examine effects of the MR treatments independently in men and in women. A two-way ANOVA was used to determine if there were effects of treatment or sex on max HR, average HR, max RPE, and visual analogue

scale (VAS) data. HR, VAS data, and max RPE were also examined by each sex separately to examine within group differences. If significance was found, post hoc analyses were completed using a Tukey's test. Statistical significance was set at $p \leq 0.05$, and data are presented as mean ± SEM, unless otherwise stated.

RESULTS

Participants

Twenty-four participants started the study and thirteen dropped out over the course of the study. Therefore, eleven participants (6 women, 5 men) completed all four TT exercise visits. Subject characteristics can be found in **Table 1**. As expected, height and body fat percentage were significantly different between the sexes. There were no differences between men and women for age, weight, BMI, and daily CAF intake. For women, the daily average CAF intake ranged from 0-175 mg/day, and for men, the daily average CAF intake ranged from 0-190 mg/day. For the racial breakdown, 8 participants were Caucasian, 1 participant was Indian, 1 participant was Hispanic, and 1 participant was Asian. Analysis of the training logs revealed no statistically significant treatment differences in weekly total exercise time or intensity. Weekly averages for aerobic activity total time was 265.7 ± 32.5 min before CHO+CAF treatment, 305.3 ± 57.5 min before CAF treatment, 276.9 ± 57.4 min before CHO treatment, and 303.3 ± 59.2 min before PL treatment (p=ns). Weekly averages for aerobic activity intensity on a scale of 1-10 was 6.2 ± 0.4 before CHO+CAF treatment, 6.2 ± 0.4 min before CAF treatment, 6.1 ± 0.3 min before CHO treatment, and 6.4 ± 0.3 min before PL treatment, p=ns. Additionally, there were no treatment or sex differences for the total energy intake or macronutrient composition of the diet

based on the food diaries filled out for a 24-hour period before each trial (average intake across all trials was 1,973 ± 90 kcals/day for men and 1,859 ± 65 kcals/day for women).

Time Trial Performance

The average completion time for all subjects was 61.41 ± 3.54 minutes for CHO + CAF, 62.05 ± 3.49 minutes for CAF, 60.73 ± 3.15 minutes for CHO, 62.59 ± 3.66 minutes for PL (**Figure 2**). No significant treatment or time effects were found, but there was a significant effect of sex ($p<0.05$). As expected, men completed each TT faster than the women (52.07 ± 2.20 vs 69.25 ± 3.98 minutes for CHO + CAF, 52.35 ± 2.08 vs 70.15 ± 3.62 minutes for CAF, 53.52 ± 1.88 vs 67.93 ± 3.88 minutes for CHO, 52.83 ± 1.82 vs 70.73 ± 4.22 minutes for PL for men vs women; $p<0.01$). When analyzed by each sex independently, there were no treatment differences for completion times between solutions for either men or women (**Figure 3A and 3B,** respectively).

In order to assess the magnitude of difference for each treatment against the control/placebo treatment, percent change from control was also calculated (each subject's completion time of interest – control completion time/control completion time). There were no significant treatment or sex differences in percent change from control (**Figure 4A**). When analyzed by each sex separately, there were no significant differences in percent change from control in either men (**Figure 4B**) or women (**Figure 4C**).

Heart Rate

Figure 5A shows the average HR as a percent of predicted max HR for each TT, and **Figure 5B** depicts recorded max HR as a percent of predicted max HR for each TT. Each individual's predicted max HR was calculated by the Center for Disease Control's formula for predicted max HR (220 – subject's age) [28]. The percentages were then calculated by dividing

each subject's average and max HR for each trial of interest by this previously calculated predicted max HR. There were no main effects of treatment or sex for average HR as a percent of predicted max HR. For recorded max HR as a percent of predicted max HR, there were also no main effects of treatment or sex. When analyzed by each sex separately, there were no significant within-group differences for average HR or max HR for either men or women.

Rating of Perceived Exertion

The average RPE and the maximum RPE for all subjects for the duration of the TT is shown by sex in **Figure 6A** and **Figure 6B**, respectively. For the time course of RPE, there was a significant effect of time ($p<0.001$), showing that all participants' RPE levels increased over the course of the trials. There were no treatment effects or sex effects for the time-course RPE data. For max RPE, there was a main effect of sex which was for higher RPE for men compared to women (19.10 ± 0.68 vs 15.21 ± 0.62, respectively; $p<0.001$). There was no treatment effect or treatment by sex interaction for max RPE. When analyzed by each sex separately, there were no significant within-group differences in max RPE for either men or women (**Figure 6B**).

Visual Analog Scales

The data collected from the VAS immediately after the completion of each TT are displayed in **Table 2**. There were no treatment or sex differences for thirst, nausea, perceived saltiness of the rinses, or perceived sourness of the rinses. For hunger, there was a main effect of sex with men having a higher score for hunger immediately post trial compared to women ($p<0.001$). There were no treatment effects or interactions for hunger ratings. There was also a main effect of sex for nausea ratings with men reporting a higher score for nauseous at the end of the trials compared to women ($p=0.02$). There were no treatment effects or interactions for nausea ratings. For perceived sweetness of the rinses, there was a main effect of treatment. The

two CHO-containing solutions were rated sweeter than CAF and PL ($p<0.001$ and $p=0.001$, respectively). Similarly, the CAF+CHO solution was rated sweeter than CAF and PL ($p=0.003$ and $p=0.02$, respectively). There were no sex effects or interactions for perceived sweetness ratings. There was also a significant treatment effect for the perceived bitterness of the mouth rinses. The CAF solution was rated more bitter than the CHO solution ($p=0.007$), and there was a trend for the CAF to be rated more bitter than the PL solution ($p=0.07$). There were no differences between the CAF and the CHO+CAF solution in perceived bitterness, and there were no sex effects or interactions for perceived bitterness ratings. Finally, when asked what treatment they thought they had been administered immediately following the trial, 70% of participants correctly guessed the CHO treatment, 55% of participants correctly guessed the PL treatment, 45% of participants correctly guessed the CAF treatment, and only 9% of participants correctly guessed the CHO+CAF treatment.

DISCUSSION

The purpose of this pilot study was to determine the effects of caffeinated MRs, both alone and combined with CHO, on endurance running performance as measured by a 12.8-kilometer TT. In this study, we found no significant effects of CAF, CAF+CHO, or CHO MRs on exercise performance. Furthermore, contrary to our hypothesis that CAF MRs would have a more pronounced ergogenic effect in women, there were no significant differences in TT performance in either men or women for any treatment. There were also no differences in HR or RPE within either sex for any treatment. Finally, there were no differences in HR or average

RPE between sexes, but men completed all trials faster than women and rated their max RPE levels higher than women.

For all participants, the CHO+CAF treatment resulted in a non-significant 1.72% improvement in performance times as compared to PL, which would translate to approximately 1-minute faster completion time. Furthermore, the CAF treatment resulted in a non-significant 0.74%, or approximately 30-second, improvement compared to PL. However, the CHO treatment resulted in 0.76% slower performance times, or approximately 30-second increase in time, on average. Although the faster completion times observed with both CAF alone and CHO+CAF were not statistically significant, for competitive runners, similar improvements in performance time could be the difference between finishing first in a race and not placing at all. Hence, continuation of this pilot study is needed to elucidate potential effects that went unobserved in the current study due to the small sample size and insufficient power. Since the current study is underpowered, a secondary analysis using effect sizes (ES) with Cohen's d was conducted in order to determine the magnitude of difference in performance between treatments. Cohen's d represents the difference in two groups' means divided by the average of their standard deviations. Thresholds of 0.2, 0.5, and 0.8 were used for small, medium, and large effect sizes, respectively [33]. Effects observed between -0.19 and 0.19 were deemed trivial. For all subjects, it was found that there were small improvements in performance time compared to the placebo treatment with both the CAF+CHO treatment (ES, 0.47) and CAF alone treatment (ES, 0.32). This means that the CAF+CHO treatment performance times differed from placebo performance times by 0.47 of a standard deviation, and the CAF treatment performance times differed from placebo performance times by 0.32 of a standard deviation. No effect was observed with the CHO alone treatment compared to placebo treatment.

The results observed in the current study are similar to some previous research. In an endurance TT conducted with cyclists, Doering et al [29] found that a 10- second CAF MR did not significantly enhance performance. Furthermore, it has been shown that neither CAF alone nor CHO+CAF MRs have any significant effect on muscular endurance performance [30]. However, multiple studies have found CAF MRs to have beneficial effects on sprint cycling performance [22, 25]. Those findings, along with the results from the current study, indicate that CAF may be a more effective ergogenic aid for high-intensity, sprint exercise than for endurance exercise. Although the majority of the available literature indicates that CHO MRs provide performance benefits to endurance athletes for exercise lasting approximately one hour in length [3, 6, 12], the CHO treatment in the current study did not yield any significant improvements in performance times. However, this lack of ergogenic effect has been previously reported and supports results from some prior findings [31, 32]. Two previous studies in endurance men [31] and endurance women [32] both found no significant performance benefits from CHO MRs in endurance running performance lasting about one hour in length in fasted participants. Therefore, it is possible that there are responders and non-responders to either CAF or CHO MRs. Although we did not observe any statistically significant effects on running performance for the CAF or CHO MRs, a more in-depth analysis revealed that there may be some responders vs. non-responders to the MRs. Responders to each treatment were classified as participants that experienced performance benefits as measured by the average percent change from baseline while non-responders were those participants that did not experience performance benefits with a particular treatment. Only participants whose group average for a treatment was statistically significantly different from placebo remained in the "responder" category. For the CAF alone, six participants had faster run times compared to placebo ($-4.22 \pm 0.62\%$ faster, $p<0.001$) and

could be considered “CAF responders” while five did not and could be considered “CAF non-responders” (2.69 ± 1.46% slower than placebo, p=0.14) (**Figure 7A**). CAF responders also experienced significantly greater improvements in performance compared to CAF non-responders as analyzed by percent change from placebo treatment (p=0.01). Furthermore, six participants experienced performance benefits with the CHO+CAF treatment (-5.47 ± 0.93% faster than placebo, p<0.001) while five did not and were significantly slower when using the CHO+CAF treatment (2.77 ± 0.70% slower than placebo, p<0.001) (**Figure 7B**). CHO+CAF responders also experienced significantly greater improvements in performance compared to CHO+CAF non-responders as analyzed by percent change from placebo treatment (p<0. 001). Finally, five participants had faster run times for CHO vs. placebo (-2.34 ± 0.66% faster, p=0.008), while five individuals did not and were significantly slower when using the CHO treatment (3.87 ± 1.39% slower than placebo, p=0.03) (**Figure 7C**). CHO responders also experienced significantly greater improvements in performance compared to CHO non-responders as analyzed by percent change from placebo treatment (p=0.004). Of the “responders” for each treatment, there were four participants who experienced the performance benefits with all three treatments. Conversely, three participants did not experience benefits with any of the three treatments. One participant experienced performance benefits with both CAF and CAF+CHO treatments but not CHO treatment, and the remaining “responder” participants responded to only one of the three treatments. Upon further assessment of the responders to the three different treatments, no factors could be found to separate them from the non-responders. Neither sex, age, max RPE levels, intensity for weekly aerobic training, VAS bitter taste ratings nor daily CAF intake was different between any of the responder vs. non-responder groupings (**Table 3**). However, maximum HR as a percentage of predicted maximum HR was significantly

higher in CAF+CHO responders versus non-responders. Furthermore, CAF+CHO responders performed fewer minutes of weekly aerobic exercise compared to CAF+CHO non-responders. However, there were no differences in maximum HR or weekly aerobic exercise between responders and non-responders for either of the other treatments, CAF alone and CHO alone (**Table 3**). The differences in maximum HR as a percentage of predicted maximum HR as well as weekly aerobic exercise may have potentially affected responder status in some participants. However, since these differences were only observed with the CAF+CHO treatment, it remains unknown what accounted for the responder vs. non-responder status in the participants.

Since we are unable to study mechanism in this study, we can only speculate as to the reasons for responders vs. non-responders. For CHO, it may be possible that some of the participants have downregulated sweet taste sensing pathways. Different factors have been shown to lead to desensitization of sugar-stimulated receptors, such as high-sugar diets [34] and high leptin levels [35, 36]. One fMRI study also found that, when exposed to either sucrose or saccharin, diet soda drinkers had more dopaminergic midbrain activation [37]. Furthermore, in rats, sensitivity to sweet taste has been linked to differences in gene expression of taste receptors, specifically T1Rs [36], implying that the magnitude of reward response from CHO MRs may depend on an individual's genetic makeup. For CAF, it has been shown that both mice and humans have distinct within-species differences in how sensitive they are to bitter compounds, and this is mediated by gene expression of T2Rs [38]. Humans have been shown to have many T2R genes that aid in bitter perception. Allelic variation of these could result in differences in bitter perception and thus, differences in the activation of reward pathways associated with CAF [39]. It is possible that the relatively low dose of 20mg CAF used in the MRs in the current study was not enough to elicit an ergogenic effect in participants with certain T2R genotypes.

Conversely, potential genotype differences for the T2R in humans could explain why we had some responders and some non-responders for the CAF and CHO MRs. Unfortunately, we did not genotype our participants for the T2R taste receptor, so we are unable to determine if genetic differences in taste receptor could account for differences in impact performance times for CHO or CAF MRs.

There were no differences in average RPE by treatment, indicating that subjects felt as though they were exerting the same amount of effort across the duration of the trial for each treatment. The lack of treatment differences in average and max HR as a percentage of predicted max HR corroborates this. There were also no differences in max RPE by treatment, but there was a difference by sex. Men reported higher RPE ratings than females for all treatments, despite the fact that there were no differences between sexes for either average or maximum HR. This is somewhat unexpected since RPE has been shown to be a reliable indicator of HR [40]. However, multiple other factors may have been inflating the men's max RPE levels, such as psychological differences or different physiological markers. For example, it has been shown that individuals experiencing more stress and anxiety have more difficulty in perceptual processing of work intensity [41], and extroverts tend to underrate work intensity compared to their more introverted counterparts [42]. Although we did not assess them, it is possible that psychological factors acted as a confounding variable for RPE levels between sexes. Furthermore, the VAS data collected revealed some potential reasons for men's higher max RPE levels. Men rated themselves as hungrier than women at the conclusion of the trial, which is a reasonable outcome because men tend to expend more kilocalories compared to women for the same exercise. However, men also reported higher levels of nausea following the trials. This could indicate that males had lower blood sugar levels following the trials than women since hunger and nausea are both indicators

of hypoglycemia [43]. Perhaps men rated their max RPE levels higher due to both these physiological differences compounded with the exertion of the TT. Unfortunately, we do not have blood glucose data to examine physiological markers.

Limitations

There were some limitations to the current study. We had a relatively small sample size due to the unexpectedly high drop-out rate (54%). The most common reasons reported for drop-outs were timing issues, inconsistent menstrual cycles, and compliance issues. Using an alpha of 0.05 and power of 0.80, it was determined that a sample size of 26 (13 males, 13 females) would be needed to detect significant treatment differences (SISA Program). Therefore, we were slightly underpowered in this study and also did not have enough participants for the sex subgroup analysis. Since we did not use fMRI, we are also unable to examine potential central mechanisms underlying the performance results between treatments. Lastly, the fact that the CAF treatment was perceived as the most bitter and the CHO treatment was perceived as the sweetest show that, although the study was double-blinded, the participants may have actually been able to discern these treatments from the others. This may have resulted in some potential bias in completion times. Since it has already been determined that energy is required for sweet MRs to have a beneficial impact on endurance performance and artificial sweeteners such as sucralose do not act as an ergogenic aid in MRs [12], future studies may benefit from adding a CAF+artificial sweetener treatment in order to mask treatments better. This would also help researchers to understand if merely masking the bitter taste of CAF with a sweet taste provides greater performance benefits than CAF alone, since humans have been shown to have aversions to bitter tastes [38].

Conclusion

In conclusion, the current pilot study showed that mouth rinsing with CAF alone, CAF combined with CHO, or CHO alone did not improve performance time in a 12.8-kilometer running TT in endurance-trained athletes compared to a water placebo. HR and RPE were also not affected by any of the treatment conditions. When analyzed by each sex separately, there were still no differences between treatments for completion times, HR, or RPE. Further research is needed to gain knowledge about the mechanism and the effects of both MRs containing CAF alone as well as CHO+CAF MRs on athletic endurance performance and potential responder vs. non-responder differences. For this reason, a follow-up study with more participants is warranted.

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Table 1 - Participant Characteristics

	Overall (n=11)	Women (n = 6)	Men (n=5)
Age (y)	22.7 ± 6.1	21.7 ± 4.4	24 ± 8.0
Height (cm)	172.4 ± 8.6	167.6 ± 6.1	178.2 ± 7.9*
Weight (kg)	64.9 ± 9.8	62.1 ± 10.2	68.1 ± 9.1
Body fat (%)	20.6 ± 7.4	26.5 ± 3.1	13.6 ± 3.6*
BMI (kg/m^2)	21.7 ± 1.9	22.0 ± 2.3	21.4 ± 1.5
Daily caffeine intake (mg)	67.7 ± 72.4	92.5 ± 55.5	38.0 ± 85.0
Range of daily caffeine intake (mg)	0 – 190	0 – 175	0 – 190

Table 1. Data are presented as Mean ± SD. * Indicates significant difference between sexes at $p<0.05$. % = percentage; BMI = body mass index; mg = milligrams.

Table 2 – Visual Analog Scale Responses

	CHO+CAF	CHO	CAF	PL
Hunger				
Overall (n = 11)	32.5 ± 10.0	24.2 ± 8.5	24.8 ± 10.5	30.7 ± 8.1
Men (n = 5)[A]	55.6 ± 14.2	39.2 ± 13.6	50.4 ± 17.4	39.2 ± 15.4
Women (n = 6)	13.2 ± 8.2	9.2 ± 5.3	3.5 ± 1.6	23.7 ± 7.8
Thirst				
Overall (n = 11)	55.9 ± 10.3	66.1 ± 8.0	59.9 ± 8.1	56.7 ± 8.7
Men (n = 5)	48.2 ± 13.7	64.2 ± 8.1	58.8 ± 8.1	51.6 ± 14.3
Women (n = 6)	62.3 ± 15.6	68.0 ± 14.8	60.8 ± 13.9	61.0 ± 11.6
Nausea				
Overall (n = 11)	17.8 ± 7.6	17.4 ± 6.0	16.8 ± 4.9	20.1 ± 6.8
Men (n = 5)[B]	23.0 ± 14.3	22.4 ± 8.0	25.6 ± 8.8	33.8 ± 11.2
Women (n = 6)	13.5 ± 8.4	12.4 ± 9.3	9.5 ± 3.8	8.7 ± 5.3
Saltiness				
Overall (n =11)	11.3 ± 5.1	8.3 ± 3.9	14.7 ± 6.6	10.5 ± 3.7
Men (n = 5)	11.0 ± 11.0	3.8 ± 2.9	17.0 ± 13.6	13.8 ± 7.7
Women (n = 6)	11.5 ± 3.6	12.8 ± 7.0	12.8 ± 5.7	7.8 ± 2.5
Sweetness				
Overall (n = 11)	51.1 ± 10.2[C]	64.3 ± 10.3[C]	6.2 ± 3.6	15.1 ± 6.2
Men (n = 5)	51.4 ± 16.7	64.8 ± 13.6	6.2 ± 5.7	12.8 ± 10.5
Women (n = 6)	50.8 ± 13.9	63.8 ± 17.2	6.2 ± 5.2	17.0 ± 8.2
Sourness				
Overall (n = 11)	16.6 ± 9.0	16.1 ± 4.6	9.7 ± 4.2	11.6 ± 4.5
Men (n = 5)	13.4 ± 7.1	11.6 ± 6.0	5.2 ± 4.7	7.0 ± 6.8
Women (n = 6)	19.3 ± 16.2	20.6 ± 6.9	13.5 ± 6.5	15.5 ± 6.2
Bitterness				
Overall (n = 11)	28.6 ± 8.6	12.2 ± 4.3	55.5 ± 10.1[D,E]	25.2 ± 8.8
Men (n = 5)	39.2 ± 16.6	13.8 ± 8.1	59.6 ± 14.9	19.2 ± 11.6
Women (n = 6)	19.8 ± 7.3	10.6 ± 4.3	52.0 ± 14.7	30.2 ± 13.6

Table 2. Subjective ratings of the mouth rinses. Data are presented as Means ± SEM. [A] indicates significantly higher hunger ratings versus women ($p<0.001$). [B] indicates significantly higher nausea ratings versus women ($p=0.02$). [C] indicates greater sweetness versus CAF and PL ($p<0.05$). [D] indicates significantly higher levels of bitterness versus CHO ($p<0.01$). [E] indicates a trend for higher bitterness versus PL ($p=0.07$). CHO+CAF = carbohydrate plus caffeine treatment; CHO = carbohydrate treatment; CAF = caffeine treatment; PL = placebo treatment.

Table 3 – Responder vs. Non-Responder Characteristics

	Responders	Non-Responders
CAF+CHO Treatment		
Sex	3 male, 3 female	2 male, 3 female
Age (y)	22.8 ± 1.5	22.6 ± 3.9
Daily caffeine intake (mg)	60.8 ± 29.7	76.0 ± 35.5
Max RPE	17.7 ± 0.8	16.4 ± 1.5
Max HR as a % of predicted HR	97.4 ± 0.01%[A]	92.4 ± 0.02%
Total weekly aerobic training time (mins)	193.4 ± 14.7[B]	338.0 ± 44.0
Weekly aerobic training intensity	5.5 ± 0.4	6.9 ± 0.6
VAS bitter taste ratings	29.0 ± 9.8	28.2 ± 16.1
CAF Treatment		
Sex	2 male, 4 female	3 male, 2 female
Age (y)	22.3 ± 1.6	23.2 ± 3.8
Daily caffeine intake (mg)	76.7 ± 27.3	57.0 ± 38.0
Max RPE	17.0 ± 1.0	16.8 ± 1.6
Max HR as a % of predicted HR	99.0 ± 0.03%	95.4 ± 0.01%
Total weekly aerobic training time (mins)	259.6 ± 46.9	351.0 ± 107.8
Weekly aerobic training intensity	6.4 ± 0.3	6.1 ± 0.8
VAS bitter taste ratings	56.7 ± 13.3	54.0 ± 17.0
CHO Treatment		
Sex	2 male, 4 female	3 male, 2 female
Age (y)	20.4 ± 0.8	25.6 ± 3.7
Daily caffeine intake (mg)	73.0 ± 33.2	57.0 ± 38.0
Max RPE	17.0 ± 1.2	17.4 ± 1.2
Max HR as a % of predicted HR	92.0 ± 0.01%	94.5 ± 0.02%
Total weekly aerobic training time (mins)	238.8 ± 28.1	307.4 ± 104.3
Weekly aerobic training intensity	5.8 ± 0.4	6.3 ± 0.6
VAS bitter taste ratings	11.0 ± 6.5	13.4 ± 6.5

Table 3. Potential factors distinguishing responders from non-responders. Data are presented as Means ± SEM. Four participants were considered responders for all three treatments, while three were considered non-responders for all three treatments. One participant was a responders for only CAF and CAF+CHO treatments, and the remaining responder participants responded to only one of the three treatments. [A] indicates significantly higher maximum heart rate as a percentage of predicted heart rate versus non-responders for the CAF+CHO treatment ($p=0.04$). [B] indicates significantly lower weekly total time spent in aerobic exercise versus non-responders for the CAF+CHO treatment ($p=0.01$). CAF+CHO = carbohydrate plus caffeine treatment; CHO = carbohydrate treatment; CAF = caffeine treatment; RPE = rating of perceived exertion; HR = heart rate; VAS = visual analogue scale.

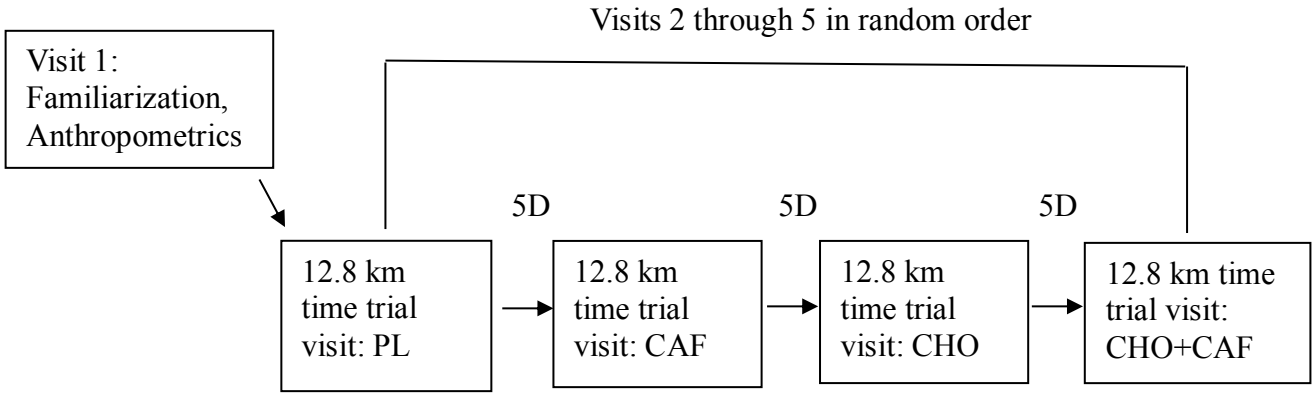


Figure 1. Experimental Protocol. Following visit 1, visits 2 through 5 were completed in a random order. Abbreviations: PL = placebo; CAF = caffeine; CHO = carbohydrate; 5D = 5-day washout period between trials.

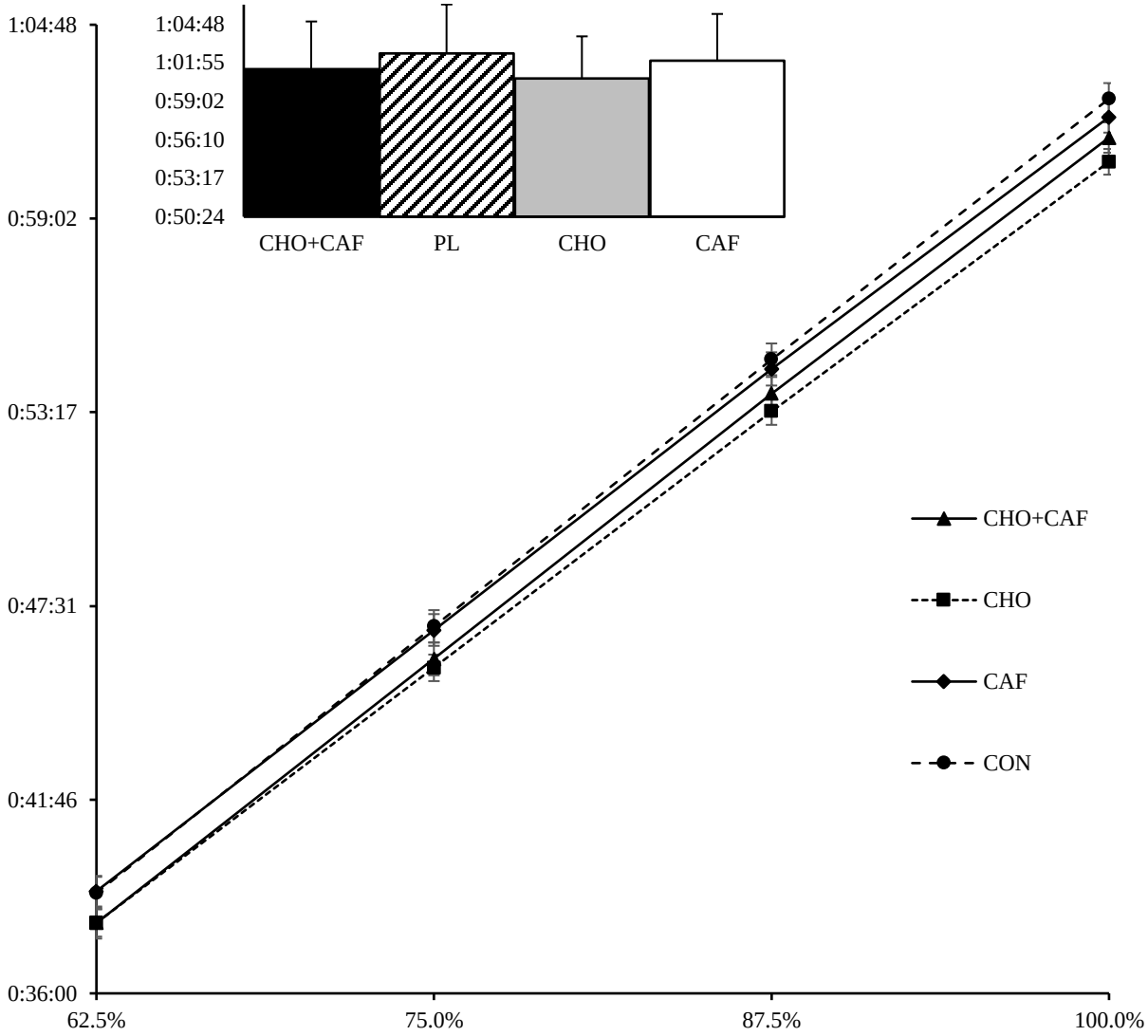


Figure 2. Performance times for the final four 12.5% markers of the time trial. Embedded bar graph shows average completion time for all subjects for each solution. There were no significant differences in completion time for any of the treatments. Abbreviations: PL = placebo; CAF = caffeine; CHO = carbohydrate.

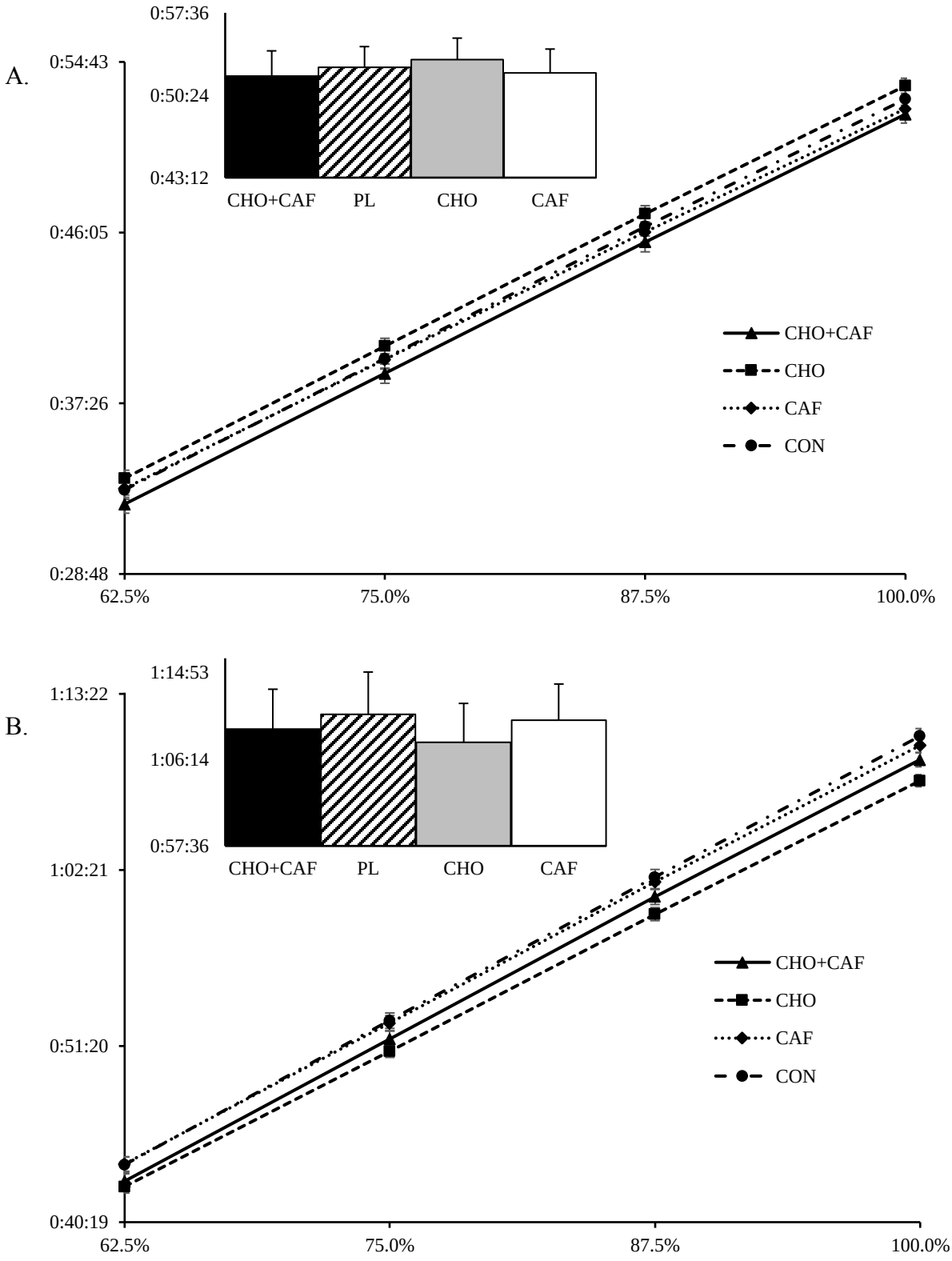


Figure 3. Performance times for the final four 12.5% markers of the time trial for men (Figure 3A) and women (Figure 3B). Embedded bar graph shows average men (3A) and women (3B) completion time for each treatment. There were no significant differences in completion time for any of the treatments for either men or women. Abbreviations: PL = placebo; CAF = caffeine; CHO = carbohydrate.

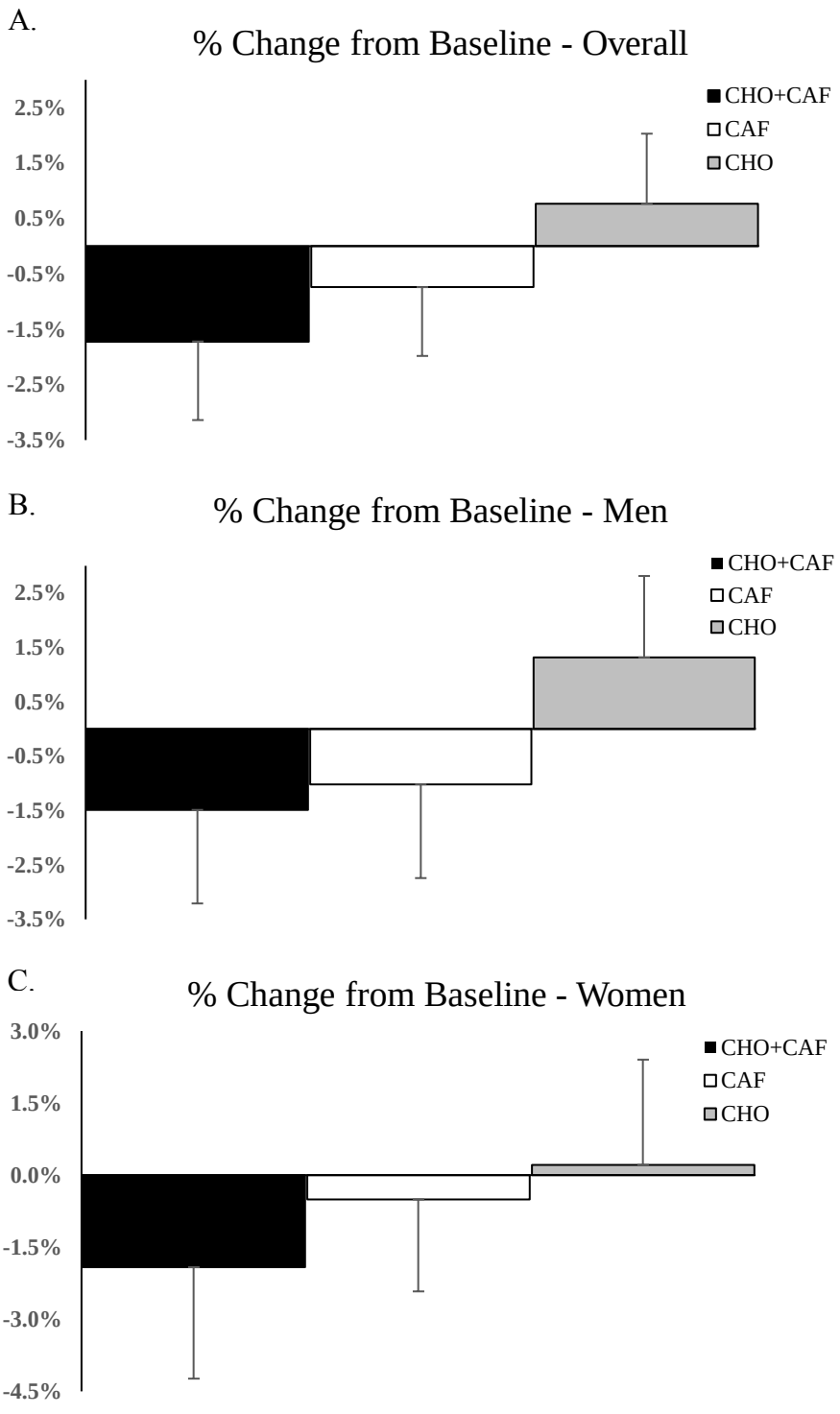
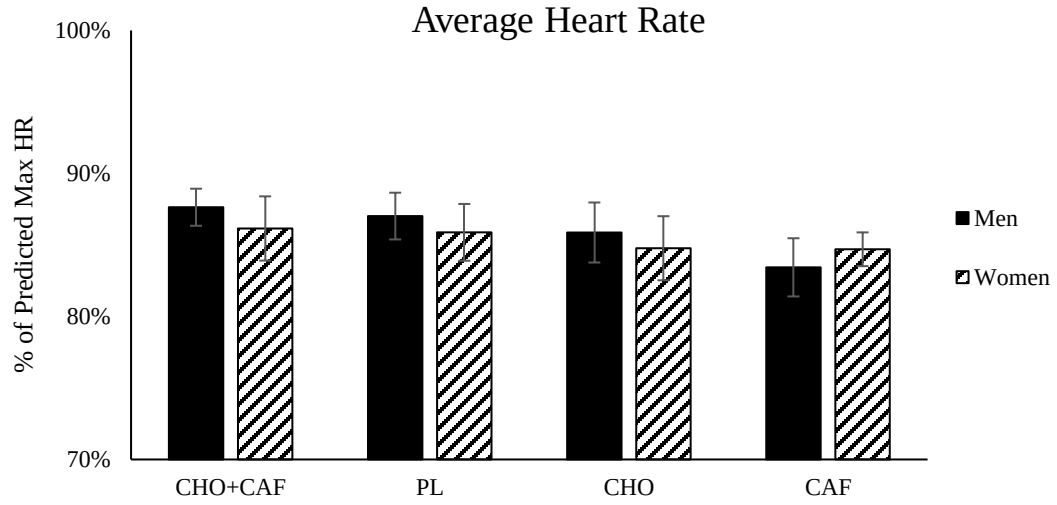


Figure 4 - A) Overall percent change in completion time from control for all subjects for all treatments. B) Percent change in completion time from control for all men for all treatments. C) Percent change in completion time from control for women for all treatments. There were no significant treatment differences within or between sexes. Abbreviations: CAF = caffeine; CHO = carbohydrate.

A.



B.

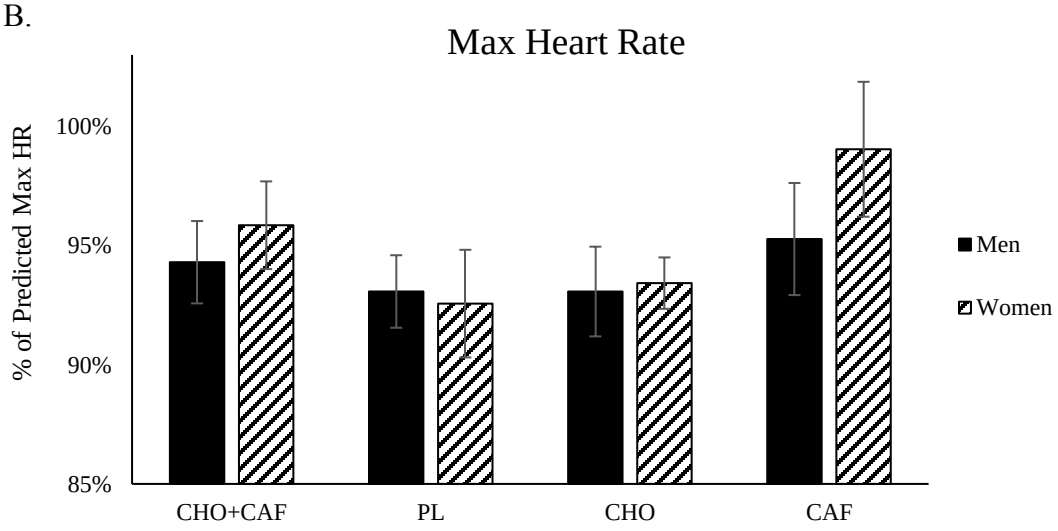


Figure 5. A) Average heart rate as a percent of predicted max HR across duration of each time trial by treatment for men and women. No significant differences were found by treatment, across sexes, or within sexes. B) Maximum heart rate as a percent of predicted max HR by treatment for men and women. No significant differences were found by treatment, across sexes, or within sexes. Abbreviations: PL, placebo; CAF, caffeine; CHO, carbohydrate.

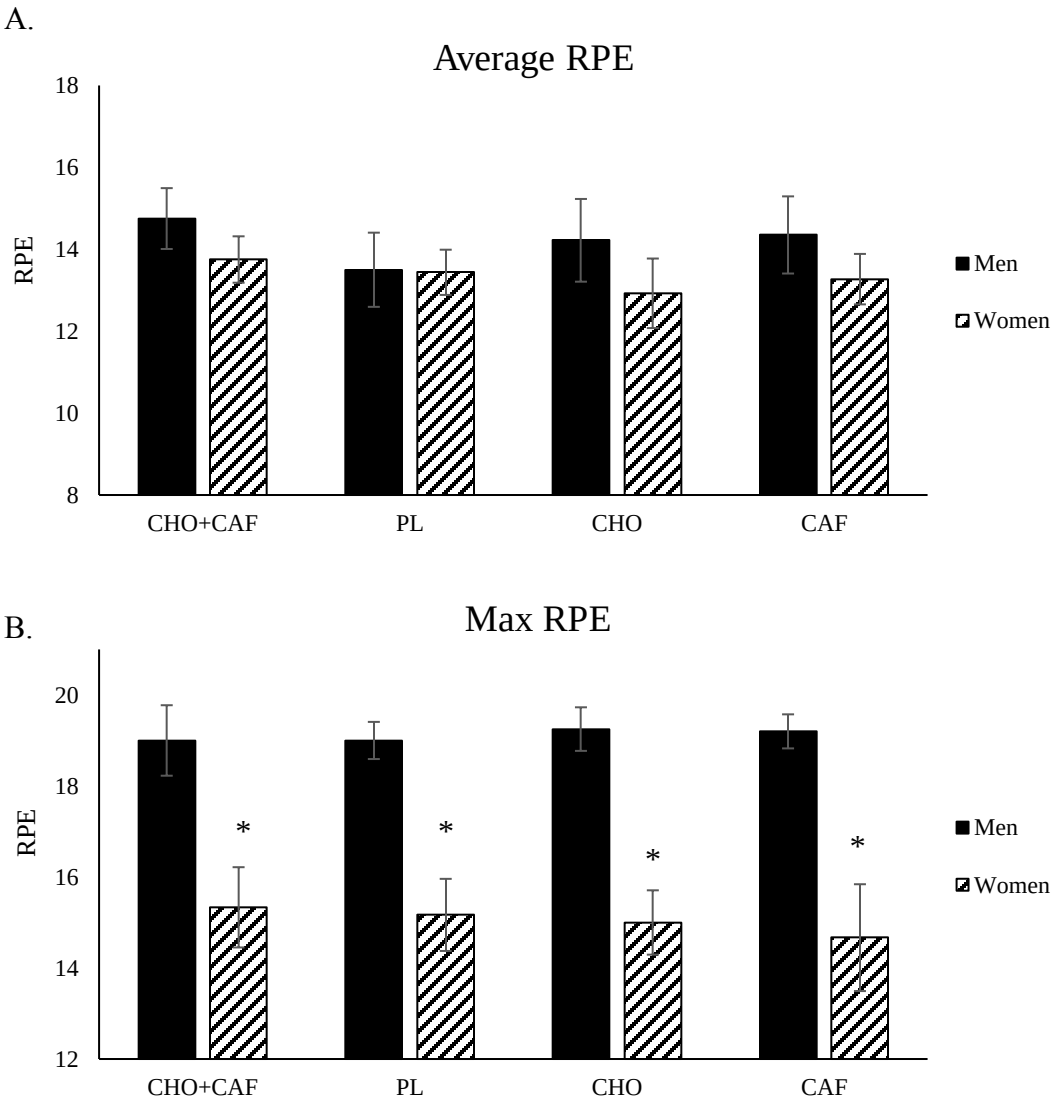
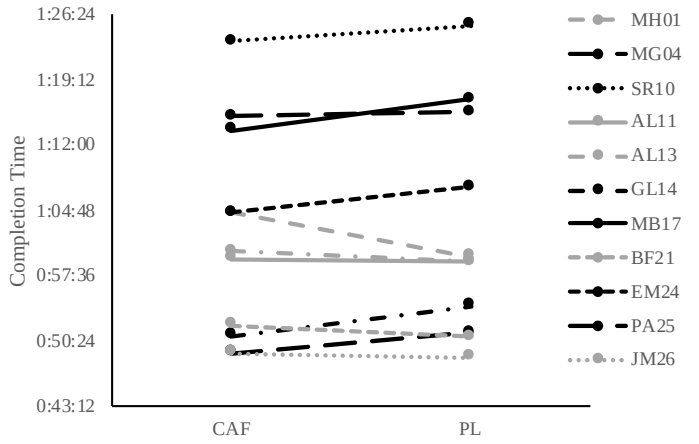
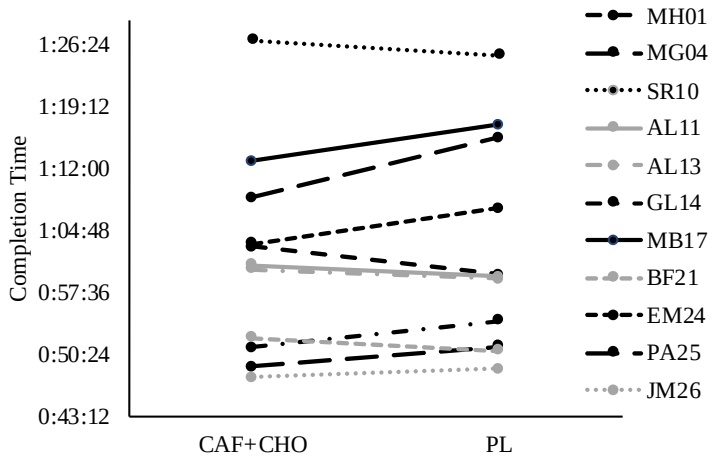


Figure 6. A) Average RPE for women and men across duration of the time trial by treatment. No significant differences were found by treatment, across sexes, or within sexes. B) Maximum RPE for women and men by treatment. Men reported significantly higher RPE levels for all treatments. * indicates significant difference between men and women across all treatments ($p<0.0001$). No other differences were found. Abbreviations: PL, placebo; CAF, caffeine; CHO, carbohydrate.

A.



B.



C.

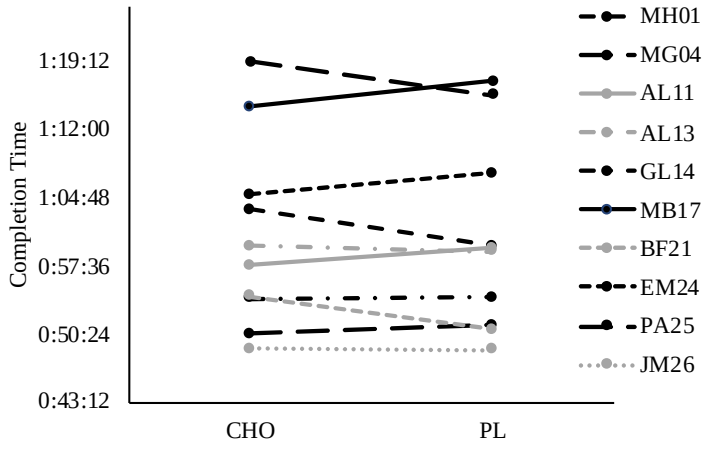


Figure 7. A) Completion time for CAF vs. PL treatments by individual participant. Six participants had faster run times compared to PL; five did not. B) Completion time for CAF+CHO vs. PL treatments by individual participant. Six participants had faster run times compared to PL; five did not. C) Completion time for CHO vs. PL treatments by individual participant. Five participants had faster run times compared to PL; five did not. PL = placebo; CAF = caffeine; CHO = carbohydrate.

CHAPTER 4

SUMMARY AND CONCLUSIONS

The main purpose of this thesis was to determine the effects of CAF MRs on12.8-kilometer running performance in endurance athletes. We also wanted to determine if there was a synergistic effect of combining CAF and CHO into one MR. Furthermore, we sought to examine any sex differences in response to the MR treatments. Research has shown that GI disturbances are a problem for many endurance athletes, especially runners due to the mechanical jostling that occurs during endurance events. There is a need for research to test for novel ergogenic aids that can provide athletes performance benefits without any accompanying detrimental GI effects. Previous research has shown CHO MRs to be an effective ergogenic aid in endurance performance for both runners and cyclists while CAF MRs have been shown to provide performance benefits in sprint cycling bouts. However, prior to this study, there was no literature available on the effectiveness of CAF MRs in endurance runners.

The results presented in Chapter 3 revealed that although there were small performance benefits with CAF alone and CAF+CHO treatments compared to the placebo treatment, these differences were not statistically significant for the whole group or within each sex. There was also no performance benefit from CHO MR alone versus placebo, which is contrary to many previous studies. There were, however, responders and non-responders for each treatment, indicating that CAF MRs may provide an ergogenic effect to a subset of the endurance running population. However, based on the current study alone we were unable to ascertain why specific individuals responded positively to the MRs while others did not. Future studies should consider

genotyping participants and include a VAS scale to measure hedonic liking of the MRs. This data can be compared to responders vs. non-responders to determine which populations receive the greatest performance benefits from CAF MRs.

One of the main possibilities for the lack of significant differences in completion times between treatments may be attributed to an insufficient statistical power. This was a pilot study, so we were slightly underpowered with a sample size of only 11 participants. We also had several challenges with participant adherence and retention, which led to a high drop-out rate. This, in combination with the responder vs. non-responder effect, may have prevented us from detecting significant differences. Furthermore, the CAF alone MRs contained only 20 mg of CAF per MR. This may not have been sufficient to provide stimulation to the central reward system typically associated with CAF. Despite this relatively low dose, participants still rated CAF MRs as significantly more bitter than all other treatments. Research has shown that humans innately avoid bitter taste, and it is possible that this natural avoidance overpowered any rewarding effects of the CAF.

Altogether, this study was the first to examine the impact of CAF MRs, either alone or in combination with CHO, on endurance running performance as well as the first to examine sex differences in response to said MRs. The results of the study indicate that CAF MRs, both alone and combined with CHO, do not provide significant endurance performance benefits in either men or women and cannot be classified as an ergogenic aid for this type of event. However, given the small sample size of the current pilot study and the responder vs. non-responder phenomena, future studies are warranted to elucidate any potential effects that went unobserved in the current study.

APPENDICES

A

CONSORT Flow Diagram