CAFFEINE TIMING AND CYCLING PERFORMANCE

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By

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CAFFEINE TIMING AND CYCLING PERFORMANCE (78 pp.)

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PURPOSE: The purpose of the present investigation was to examine the effect of caffeine administration timing on cycling performance. We examined the effects of caffeine administered in chewing gum on metabolic, perceptual, and plasma β -endorphin responses during and after cycling exercise. **METHODS:** Eight male cyclists participated in five separate laboratory sessions. During the first visit, subjects underwent a graded exercise test to determine maximal oxygen consumption (VO_{2max}). During the next four visits, three pieces of chewing gum were administered at three time points (120 min pre-cycling, 60 min pre-cycling, and 5 min pre-cycling). Subjects were instructed to chew the gum for five min and then expectorate the gum. In three of the four visits, at one of the time points mentioned previously, 300 mg of caffeine (Stay Alert™ chewing gum) was administered. During the fourth visit, placebo gum was administered at all three time points. The experimental trials are defined as follows: Trial A (-120), Trial B (-60), Trial C (-5), and Trial D (Placebo). The order in which participants completed the experimental trials was randomized. Following baseline measurements, time allotted for gum administration, and a standard warm-up, participants cycled at 75% VO_{2max} for 15 min then completed a $7 \text{ kJ} \cdot \text{kg}^{-1}$ cycle time trial. Metabolic and perceptual data were collected at baseline and every three min during steady state cycling. Blood samples were obtained via venipuncture at baseline, 10 min pre-cycling, following steady state cycling, and at the end of the cycle time trial. **RESULTS:** Data were analyzed using a

repeated measures analysis of variance. Cycling performance was improved in Trial C (-5), but not in Trial A (-120) or Trial B (-60), relative to Trial D (Placebo). Caffeine did not impact metabolic, perceptual, or plasma β -endorphin responses to exercise.

CONCLUSIONS: Caffeine administered in chewing gum enhanced cycling performance when administered immediately prior, but not when administered one or two hr prior to cycling.

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CHAPTER I

INTRODUCTION

Caffeine is one of the most widely used pharmacologically active substances (Dews, 1984). In moderate doses caffeine is well tolerated and few significant side effects have been reported (Curatolo & Robertson, 1983; Seferin, 1996). Research has shown that caffeine ingestion may enhance physical performance via a variety of mechanisms (Graham, 2001). Several investigators, utilizing a variety of different protocols, have evaluated the effects of caffeine administration on aerobic endurance performance.

The experimental research that has evaluated the effects of caffeine on aerobic performance employing the modality of running has demonstrated that caffeine improves running time to exhaustion (Graham, Hibbert, & Sathasivam, 1998; Graham & Spriet, 1995; McLellan, Bell, & Kamimori, 2004). Furthermore, Bridge and Jones (2006) reported that individuals improved performance time (8 kilometer run) following caffeine ingestion versus placebo. McLellan et al. (2005) reported that performance time (6.3 kilometer run) improved in soldiers receiving caffeine and declined in soldiers receiving placebo following 27 hr of sustained wakefulness.

There has been extensive research examining caffeine ingestion and cycling performance. Several studies have concluded that caffeine ingestion improves cycle time to exhaustion (Bell & McLellan, 2002, 2003; Costill, Dalsky, & Fink, 1978; Flinn, Gregory, McNaughton, Tristram, & Davies, 1990; Graham & Spriet, 1991; Jackman, Wendling, Friars, & Graham, 1996; McLellan & Bell, 2004; Pasman, van Baak,

Jeukendrup, & de Haan, 1995). Other investigators have reported that caffeine ingestion improves total work output (Ivy, Costill, Fink, & Lower, 1979; Jenkins, Trilk, Singhal, O'Connor, & Cureton, 2008) and/or mean power output (Doherty, Smith, Hughes, & Davison, 2004; Foad, Beedie, & Coleman, 2008) during cycling. Research protocols utilizing cycle time trials have found that caffeine ingestion improves (Cox et al., 2002; Foad, et al., 2008; Kovacs, Stegen, & Brouns, 1998; McNaughton et al., 2008) or has no effect (Conway, Orr, & Stannard, 2003; Desbrow, Barrett, Minahan, Grant, & Leveritt, 2009; Hunter, St Clair Gibson, Collins, Lambert, & Noakes, 2002) on cycling performance.

In addition, caffeine ingestion prior to performance has been shown to reduce perceived exertion and pain perception. Research has shown that caffeine ingestion decreases ratings of perceived exertion during submaximal (Demura, Yamada, & Terasawa, 2007; Doherty & Smith, 2004; Hadjicharalambous et al., 2006) and maximal (Doherty, et al., 2004) cycling. Furthermore, it has been reported that caffeine reduces perceived leg pain during submaximal cycling among male (Motl, O'Connor, & Dishman, 2003; O'Connor, Motl, Broglio, & Ely, 2004) and female (Gliottoni & Motl, 2008; Motl, O'Connor P, Tubandt, Puetz, & Ely, 2006) volunteers. Other investigators have concluded that caffeine also reduces perceived pain during repeated maximal voluntary isometric contractions (Maridakis, O'Connor, Dudley, & McCully, 2007). An exacerbated β -endorphin release in response to exercise following caffeine ingestion may, in part, be responsible for the decreases in perceived pain during endurance exercise (Laurent et al., 2000).

In addition to its performance enhancing effects, caffeine has been shown to have a neuroprotective effect (Ritchie et al., 2007). One study, utilizing an animal model, reported that caffeine may block disruption of the Blood Brain Barrier (BBB) (Chen, Gawryluk, Wagener, Ghribi, & Geiger, 2008). Serum s100 β has been suggested as a possible marker of BBB integrity. However, research has not evaluated the effect of caffeine ingestion and exercise on Serum s100 β levels in humans.

Caffeine is available in many forms including coffee, sports drinks, powder forms, capsules, mints, and chewing gum. The most efficacious time to administer caffeine to maximize its performance enhancing effects is poorly understood (Conway, et al., 2003) and is likely dependant on the medium through which it is ingested. Stay Alert™ chewing gum is a product developed by Amurol Confectioners (Specialty gum subsidiary of Wrigley's, Yorkville, IL) that contains 100 mg caffeine/piece. Stay Alert™ has been developed for military personnel to improve physical and cognitive performance and offers a quick and effective delivery of caffeine via the buccal mucosa (Kamimori et al., 2002).

Statement of Problem

It has been customary for researchers to administer oral doses of caffeine (capsule or beverage) one hr prior to performance testing to ensure peak plasma concentrations during exercise. Nonetheless, research has not determined whether or not timing of peak plasma concentration impacts subsequent performance enhancement. Stay Alert™ chewing gum (Mastix Medica LLC, Hunt Valley, MD) has been shown to deliver caffeine significantly faster than other oral formulations due to buccal absorption

(Kamimori, et al., 2002). As the commencement of a drugs' action is limited by the rate at which it reaches target tissues, a faster delivery of caffeine may result in a faster onset of performance enhancement. However, the most efficacious time to administer caffeine in chewing gum or any other oral formulation to maximize its performance enhancing effects is poorly understood (Conway, et al., 2003). Furthermore, the effect of caffeine ingestion (in the form of chewing gum) on plasma β -endorphin and serum s100 β levels at rest and during exercise renders investigation.

Purpose of the Study

The purpose of the proposed investigation was multi-faceted and examined the following:

1. The effects of caffeine administration timing on cycle time trial performance.
2. The effects of caffeine administered in the form of chewing gum on perceived exertion and leg pain during steady state cycling.
3. The effects of caffeine administered in the form of chewing gum on plasma β -endorphin levels during exercise.
4. The effects of caffeine administered in the form of chewing gum on blood markers of Blood Brain Barrier integrity at rest and during exercise.

Summary

Studies have concluded that caffeine ingestion may: improve aerobic performance, decrease perceived exertion and pain experienced during exercise, and alter plasma β -endorphin levels during exercise. The design of the current investigation will

allow for a greater understanding of the performance enhancing and neuroprotective effects of caffeine, and, provide additional insight into caffeine timing and performance.

CHAPTER II

REVIEW OF LITERATURE

Pharmacokinetics

Absorption and Distribution

Caffeine (1,3,7-trimethylxanthine) is a pharmacologically active compound commonly derived from plants for human consumption. Following ingestion, caffeine is rapidly and completely absorbed from the gastrointestinal tract into the bloodstream (Eteng, Eyong, Akpanyung, Agiang, & Aremu, 1997). Complete absorption is typically achieved approximately one hr following ingestion; however, numerous factors such as dosage and dose formulation can impact the absorption rate constant (Bonati et al., 1982). Following the oral ingestion of caffeine, plasma caffeine concentrations rise in a dose-dependent manner (Kamimori, et al., 2002), and peak plasma concentrations are observed anywhere from 15 to 120 min (Blanchard & Sawers, 1983a; Bonati, et al., 1982; Kamimori et al., 1995; Kamimori et al., 2000; McLean & Graham, 2002; Mumford et al., 1996). Furthermore, there exists a linear relationship between dosage and peak plasma concentrations achieved (Bonati, et al., 1982). Due to its hydrophobic properties, caffeine can pass through all biological membranes and is readily distributed throughout all bodily tissues (Carrillo & Benitez, 2000).

Metabolism and Elimination

The catabolism of caffeine occurs mainly in the liver via the cytochrome P-450 system. Demethylation reactions account for the majority of caffeine metabolism in humans, producing three primary dimethylxanthines: paraxanthine

(1,7 dimethylxanthine), 80%; theophylline (1,3 dimethylxanthine), 11%; and theobromine (3,7 dimethylxanthine), 4% (Lelo, Birkett, Robson, & Miners, 1986). Following the aforementioned demethylation reactions, paraxanthine, theophylline, and theobromine are further metabolized into a variety of xanthines, uric acids, and uracils to be eliminated via the kidneys (Kalow & Tang, 1991).

Numerous factors including age (Blanchard & Sawers, 1983b), gender (Callahan, Robertson, Branfman, McComish, & Yesair, 1983) smoking (Benowitz, Hall, & Modin, 1989), and pregnancy (Aldridge, Bailey, & Neims, 1981) may influence caffeine elimination parameters. Numerous investigations indicate that the elimination half-life of caffeine ranges anywhere from 2.5 to 10 hr with plasma clearance rates ranging from 1 to 3 ml·kg⁻¹·min⁻¹ (Magkos & Kavouras, 2005). McLean and Graham (2002) reported that exercise has no effect on the pharmacokinetics of caffeine.

Mechanisms

Central Nervous System

In the central nervous system (CNS) adenosine acts as an inhibitory neurotransmitter. When acting on presynaptic A₁ receptors, adenosine inhibits the release of several neurotransmitters (acetylcholine, GABA, glutamate) acting more efficiently on excitatory neurotransmission (Fredholm & Dunwiddie, 1988) thus decreasing CNS excitation (Phillis & Edstrom, 1976). When acting on presynaptic A_{2a} receptors, adenosine inhibits dopaminergic transmission (Salmi, Chergui, & Fredholm, 2005). Caffeine (1,3,7-trimethylxanthine) is structurally very similar to adenosine and readily crosses the blood brain barrier. In the CNS, caffeine binds with adenosine

receptors without activating them, thus acting as a nonspecific adenosine antagonist via competitive inhibition (Fredholm, 1995). In summary, it has been concluded that caffeine increases CNS arousal via the blockade of CNS adenosine (A₁,A_{2a}) receptors.

Substrate Availability

An increase in free fatty acid (FFA) mobilization and oxidation and subsequent glycogen sparing has been implicated as a possible mechanism by which caffeine improves aerobic endurance performance (Costill, et al., 1978; Essig, Costill, & Van Handel, 1980; Ivy, et al., 1979). It is commonly reported that caffeine ingestion increases epinephrine levels during exercise (Graham & Spriet, 1991, 1995; Greer, Friars, & Graham, 2000; Jackman, et al., 1996; Van Soeren & Graham, 1998). Nevertheless, it is questionable whether or not these increases are of metabolic significance (Graham, 2001).

Several studies have concluded that the ingestion of caffeine stimulates the mobilization of free fatty acids (Bellet, Kershbaum, & Finck, 1968; Costill, et al., 1978; Essig, et al., 1980; Ivy, et al., 1979). Caffeine increases FFA mobilization via competitive inhibition of A₁ adenosine receptors on adipocytes (Greer, et al., 2000; Mohr, Van Soeren, Graham, & Kjaer, 1998) and increasing epinephrine levels (Costill, et al., 1978; Essig, et al., 1980; Ivy, et al., 1979). Essig, Costill, and Handel (1980) suggested that caffeine increases lipid fuel availability and thus fat oxidation. More recently, however, numerous investigations have concluded that fat oxidation may not increase despite increases in FFA availability (Graham, 2001; Graham, Helge, MacLean, Kiens, & Richter, 2000).

Research has revealed that caffeine reduces (Erickson, Schwarzkopf, & McKenzie, 1987; Essig, et al., 1980; Graham & Spriet, 1991) or has no effect on (Chesley, Howlett, Heigenhauser, Hultman, & Spriet, 1998; Graham, et al., 2000; Jackman, et al., 1996; Laurent, et al., 2000) glycogen utilization during exercise. In summary, caffeine has been shown to increase FFA mobilization; however, research has yielded equivocal findings regarding the effects of caffeine on fat oxidation and glycogen utilization.

Excitation Contraction Coupling and Calcium Release

It has been suggested that caffeine may improve endurance performance at the skeletal muscle level via enhancing excitation-contraction coupling (Kalmar & Cafarelli, 1999; Lopes, Aubier, Jardim, Aranda, & Macklem, 1983; Tarnopolsky & Cupido, 2000). During low frequency stimulation, caffeine potentiates skeletal muscle force/tension (Lopes, et al., 1983; Tarnopolsky & Cupido, 2000) and time to fatigue (Kalmar & Cafarelli, 1999). The mechanism underlying the effect of caffeine on excitation-contraction coupling is a potentiated Ca^{++} release from the sarcoplasmic reticulum (SR) (Tarnopolsky & Cupido, 2000). Two studies reported that caffeine potentiates Ca^{++} release from the sarcoplasmic reticulum via activation of ryanodine (RyR) SR Ca^{++} release channels (Rousseau, Ladine, Liu, & Meissner, 1988; Rousseau & Meissner, 1989).

Effort and Pain Perception

The effect of caffeine on effort and pain perception during aerobic endurance exercise has received considerable attention as it may represent a possible mechanism by

which caffeine improves performance. Researchers have reported that caffeine ingestion decreases ratings of perceived exertion during submaximal (Demura, et al., 2007; Doherty & Smith, 2004; Hadjicharalambous, et al., 2006) and maximal (Doherty, et al., 2004) cycling. Furthermore, it has been shown that caffeine reduces perceived leg pain during submaximal cycling among male (Motl, et al., 2003; O'Connor, et al., 2004) and female (Gliottoni & Motl, 2008; Motl, et al., 2006) volunteers. One study concluded that caffeine also reduces perceived pain during repeated maximal voluntary isometric contractions (Maridakis, et al., 2007).

An exacerbated β -endorphin release in response to exercise following caffeine ingestion may, in part, be responsible for the decreases in perceived pain mentioned above (Laurent, et al., 2000). In summary, recent data suggests that caffeine lowers effort and pain perception during exercise, and, this may represent a possible mechanism by which caffeine improves performance.

Caffeine and Running Performance

Several investigators have concluded that caffeine improves run time to exhaustion (Graham, et al., 1998; Graham & Spriet, 1995; McLellan, et al., 2004) and running time trial performance (Bridge & Jones, 2006; McLellan et al., 2005). Graham and Spriet (1995) evaluated the effects of 3, 6, and 9 $\text{mg}\cdot\text{kg}^{-1}$ of body mass of caffeine (capsule form) on run time to exhaustion (85% of $\text{Vo}_{2\text{Max}}$) and the metabolic and catecholamine responses to exercise in well trained athletes. Caffeine doses of 3 and 6 $\text{mg}\cdot\text{kg}^{-1}$ of body mass enhanced run time to exhaustion versus the placebo; however, the 9 $\text{mg}\cdot\text{kg}^{-1}$ dose did not appear to improve performance. Conversely, these data indicated

that plasma epinephrine and serum FFA concentrations were greater in the higher dose trials. It was concluded from these data that the results of this study were not in agreement with the theory that caffeine mediates its performance enhancing effects during exercise by exacerbating the catecholamine response to exercise (Graham & Spriet, 1995).

Graham, Hibbert and Sathasivam (1998) examined the effects of different protocols of caffeine administration on run time to exhaustion and the metabolic responses to exercise. Subjects participated in five experimental trials where they consumed a capsule (caffeine vs. placebo) with water or coffee (decaffeinated with caffeine added, decaffeinated, or regular) one hr prior to running to exhaustion. It was reported that a caffeine dose of $4.45 \text{ mg} \cdot \text{kg}^{-1}$ of body mass increased plasma epinephrine versus placebo, and, caffeine administered in capsule form improved run time to exhaustion (80% of $\text{VO}_{2\text{Max}}$) versus caffeine administered via coffee and placebo.

McLellan, Bell, and Kamimori (2004) evaluated the effects of caffeine ingestion on physical performance during 24 hr of active wakefulness. Caffeine (600 mg) or placebo was administered in a divided dose (400 mg at 14.5 hr and 100 mg at 20 and 22 hr of sustained wakefulness) throughout sleep deprivation. At 15 hr of sustained wakefulness participants began a two hr forced march followed by a sandbag piling task. At 24 hr of sustained wakefulness subjects completed a treadmill run to exhaustion. It was reported that caffeine ingestion significantly reduced perceived exertion during the forced march. Furthermore, these data revealed that caffeine enhanced run time to

exhaustion (85% of $\text{Vo}_{2\text{Max}}$) and maintained performance levels following sleep deprivation.

Bridge and Jones (2006) examined the effects of $3 \text{ mg}\cdot\text{kg}^{-1}$ of body mass of caffeine ingestion on 8 km run performance among male distance runners. Participants completed the run on a standard synthetic track one hr after ingesting a capsule (caffeine vs. placebo) or no supplement. It was concluded that caffeine ingestion improves running performance time in an ecologically valid race setting. Further, McLellan et al. (2005) evaluated the effects of caffeine on vigilance, marksmanship and running performance (6.3 kilometer run) during 27 hr of sustained wakefulness. Thirty-one soldiers were divided into two groups (caffeine vs. placebo) and completed the run before and after sleep deprivation. Placebo versus 200 mg of caffeine was administered at 3 time points during sustained wakefulness. These data showed that performance time improved in soldiers receiving caffeine and declined in soldiers receiving placebo.

Caffeine and Cycling Performance

Cycling to Exhaustion

Several researchers have reported that caffeine ingestion improves cycle time to exhaustion (Bell & McLellan, 2002, 2003; Costill, et al., 1978; Flinn, et al., 1990; Graham & Spriet, 1991; Jackman, et al., 1996; McLellan & Bell, 2004; Pasman, et al., 1995). Costill, Dalsky and Fink (1978) evaluated the effects of caffeine ingestion on cycling to exhaustion and metabolism among competitive cyclists. Subjects cycled at 80% of $\text{Vo}_{2\text{Max}}$ one hr following the consumption of decaffeinated coffee or decaffeinated coffee with 330 mg of caffeine added. Prior to and during cycling metabolic

measurements (blood samples, expired air samples, perceived exertion) were obtained. These data suggested that 330 mg of caffeine enhanced cycle time to exhaustion. Furthermore, it was concluded that caffeine reduced perceived exertion and increased lipid delivery and oxidation during cycling.

Flinn, Gregory, McNaughton, Tristram, and Davies (1990) evaluated the effects of a large dose of caffeine ($10 \text{ mg}\cdot\text{kg}^{-1}$ of body mass) administered three hr prior to performance on incremental cycling to exhaustion in caffeine naive cyclists. It was concluded that caffeine ingestion enhanced time to exhaustion and total work performed. Furthermore, based on blood lactate concentrations and oxygen consumption measures obtained during incremental cycling, it was reported that caffeine ingestion increased lactate threshold in the present investigation.

Graham and Spriet (1991) examined the effects of a high caffeine dose ($9 \text{ mg}\cdot\text{kg}^{-1}$ of body mass) on metabolism and cycle and run time to exhaustion (85% of $\text{V}_{\text{O}_{2\text{Max}}}$). Seven competitive runners participated in four experimental trials one hr following ingesting a capsule containing caffeine or dextrose. Blood and expired air samples were obtained at baseline, following capsule administration, every 15 min during exercise, and at exhaustion. It was reported that a large dose of caffeine: enhanced both cycle and run time to exhaustion, increased plasma epinephrine levels at rest and during exercise, and had no effect on plasma FFA at rest and during exercise.

Pasman, van Baak, Jeukendrup, and de Haan (1995) evaluated the effects of varying dosages (0, 5, 9, $13 \text{ mg}\cdot\text{kg}^{-1}$ of body mass) of caffeine on cycle time to exhaustion (85% of W_{Max}). Nine cyclists exercised to exhaustion one hr following

consuming a capsule containing 0, 5, 9, and/or 13 $\text{mg}\cdot\text{kg}^{-1}$ of body mass of caffeine.

Blood data revealed that caffeine increased FFA and glycerol concentrations relative to placebo similarly among all caffeine doses. Also, it was reported that caffeine enhanced cycle time to exhaustion relative to placebo with no significant difference in performance observed between the various doses.

Jackman, Wendling, Friars, and Graham (1996) evaluated the effects of caffeine on cycling to exhaustion at an intensity equivalent to $\text{VO}_{2\text{Max}}$. Fourteen subjects cycled two min, rested six min, cycled two min, rested six min, and then cycled to voluntary exhaustion one hr following ingesting a capsule containing caffeine ($6 \text{ mg}\cdot\text{kg}^{-1}$ of body mass) versus placebo (dextrose). Eight subjects had muscle and venous blood samples taken before and after each exercise period. These data suggested that caffeine enhanced endurance times when cycling at $\text{VO}_{2\text{Max}}$. Furthermore, it was reported that caffeine had no impact on muscle glycogen stores, and at fatigue, muscle glycogen stores appeared not to be limiting. Thus, Jackman et al. (1996) concluded that, in addition to glycogen sparing, caffeine may improve performance via direct actions on the muscle itself and the CNS.

More recently, Bell and McLellan (2002) examined cycling performance one, three, and six hr after caffeine ingestion ($5 \text{ mg}\cdot\text{kg}^{-1}$ of body mass) among users and nonusers. Thirteen caffeine users and eight nonusers completed exercise rides to exhaustion (80% of $\text{VO}_{2\text{Max}}$) after ingesting a caffeine dose of $5 \text{ mg}\cdot\text{kg}^{-1}$ of body mass or placebo. It was concluded that, following caffeine administration, nonusers experience a more robust improvement in performance. Moreover, Bell and McLellan (2002) showed

that caffeine improves cycling performance in caffeine users at one and three, and nonusers at one, three, and six hr after ingestion.

Bell and McLellan (2003) evaluated the effects of repeated doses of caffeine on repeated bouts of cycling to exhaustion (80% of $\text{Vo}_{2\text{Max}}$). Nine male caffeine users performed exercise rides to exhaustion in the morning and in the early afternoon of the same day on 4 occasions separated by a one week washout period. Caffeine ($5 \text{ mg}\cdot\text{kg}^{-1}$ of body mass) versus placebo was administered one hr before the morning session; an additional $2.5 \text{ mg}\cdot\text{kg}^{-1}$ of body mass of caffeine versus placebo was administered one hr before the afternoon ride. The four experimental treatments consisted of: placebo administered in the morning and afternoon, caffeine administered in the morning with placebo in the afternoon, placebo in the morning and caffeine in the afternoon, and caffeine in the morning and evening. It was concluded that caffeine administered in the morning improved cycle time to exhaustion in the morning and evening, and subsequent dosing throughout the day is unnecessary to improve cycling performance in the evening.

McLellan and Bell (2004) examined the effects of prior coffee consumption on subsequent caffeine (anhydrous) administration and cycle time to exhaustion (80% of $\text{Vo}_{2\text{Max}}$). Thirteen physically active subjects completed six exercise rides to exhaustion after ingesting combinations of coffee (contained $1.1 \text{ mg}\cdot\text{kg}^{-1}$ of body mass of caffeine), decaffeinated coffee, and caffeine (3, 5, or $7 \text{ mg}\cdot\text{kg}^{-1}$ of body mass) and placebo filled capsules. Previous work done by Graham et al. (1998) had suggested that components common in coffee may alter adenosine receptor sensitivity and suppress the ergogenic

effect of caffeine. Nevertheless, these data suggested that caffeine improves cycling time to exhaustion regardless of prior coffee consumption.

Cycle Time Trials

Research examining caffeine ingestion and cycle time trials has yielded inconsistent findings. It has been reported that caffeine ingestion improves (Cox, et al., 2002; Foad, et al., 2008; Kovacs, et al., 1998; McNaughton, et al., 2008) or has no effect (Conway, et al., 2003; Desbrow, et al., 2009; Hunter, et al., 2002) on cycling time trial performance. Kovacs, Stegan and Brouns (1998) evaluated the effects of adding different doses of caffeine to a carbohydrate-electrolyte solution (CES) on metabolism and cycling performance. Fifteen trained triathletes/cyclists participated in five experimental trials in which they consumed a 14 mg·kg⁻¹ of body mass solution (water, CES, CES with 150, 225, 320 mg of caffeine) prior to and during a 20 min warm-up which was followed by a 1h cycling time trial. It was reported that the addition of caffeine (2.1, 3.2, and 4.5 mg·kg⁻¹ of body mass) to a CES improved cycle time trial performance. Also, it was concluded that caffeine ingestion did not enhance free fatty acid availability.

Cox et al. (2002) evaluated the effects of different protocols of caffeine intake on cycling performance (7 kJ·kg⁻¹ cycle time trial) following two hr of steady state exercise (70% of V_{O₂Max}) with CHO supplementation in competitive athletes. In the first portion of the study, caffeine (6 mg·kg⁻¹ of body mass) versus placebo was administered in a single dose prior to cycling or in multiple doses throughout cycling. In the second portion of the study, CHO supplementation was replaced with caffeinated (1.5 mg·kg⁻¹ of body mass) versus decaffeinated cola during the latter stages of steady state cycling and

the cycling time trial. It was reported that caffeine ingestion improved time trial performance regardless of timing of intake, and replacing CHO supplementation with caffeinated cola was equally effective in enhancing cycling performance. Further, McNaughton et al. (2008) evaluated the effects of caffeine ($6 \text{ mg} \cdot \text{kg}^{-1}$ of body mass) on distance achieved in a one hr cycling time trial among well trained male cyclists. It was concluded that caffeine ingestion enhanced distance attained during a cycle time trial.

Hunter, St Clair Gibson, Collins, Lambert, and Noakes, (2002) evaluated the effects of caffeine on cycling performance (100 kilometer time trial) among competitive male cyclists. Eight male cyclists participated in three experimental trials in which they were instructed to cycle 100 kilometers as quickly as possible one hr following caffeine ($6 \text{ mg} \cdot \text{kg}^{-1}$ of body mass) versus placebo ingestion. During cycling, placebo versus a caffeine maintenance dose ($0.33 \text{ mg} \cdot \text{kg}^{-1}$ of body mass) was administered every 15 min until trial completion. It was reported that caffeine ingestion appeared to have no effect on cycling time trial performance. Hunter et al. (2002) concluded that caffeine may not enhance performance during exercise with a predetermined endpoint.

Conway, Orr and Stannard (2003) examined the effects of caffeine administered in a single dose versus multiple doses on (self-paced) cycling time trial performance. Nine trained male cyclists participated in three experimental trials consisting of 90 min of steady state cycling (68% of $\text{Vo}_{2\text{Max}}$) followed by a self-paced performance ride. Caffeine ($6 \text{ mg} \cdot \text{kg}^{-1}$ of body mass) versus placebo was administered in a single dose or in a divided dose 60 min prior to exercise and 45 min following the initiation of exercise. These data showed that caffeine ingestion did not significantly improve time trial

performance nor did dose schedule impact the performance enhancing effects of caffeine (Conway, et al., 2003). Desbrow, Barrett, Minahan, Grant, and Leveritt (2009) evaluated the effects of low doses of caffeine (1.5 and $3 \text{ mg}\cdot\text{kg}^{-1}$ of body mass) on time trial performance. Nine male cyclists participated in 3 experimental trials consisting of two hr of steady state cycling followed by a $7 \text{ kJ}\cdot\text{kg}^{-1}$ time trial. Caffeine or placebo was administered in capsule form one hr before experimental testing. No significant improvements in time trial performance were reported.

Work and Power Output

Research has revealed that caffeine ingestion improves total work output (Ivy, et al., 1979; Jenkins, et al., 2008) and/or mean power output (Doherty, et al., 2004; Foad, et al., 2008) during cycling. Ivy, Costill, Fink, and Lower (1979) examined the effects of multiple doses of caffeine on work production during two hr isokinetic cycling. Seven male and two female cyclists participated in three experimental trials supplemented with caffeine or a glucose polymer versus control. Caffeine (500 mg) enhanced total work production by approximately 7.4% and 5.3% versus control and glucose polymer, respectively. Furthermore, based on respiratory exchange data and plasma free fatty acid concentrations, Ivy et al. (1979) reported that caffeine increased FFA delivery and oxidation in the present investigation.

Doherty, Smith, Hughes, and Davison (2004) examined the effect of caffeine ingestion on mean power output during maximal effort cycling in male cyclists. Eleven male cyclists completed two experimental trials consisting of two min of constant rate cycling at 100% max power output followed by a one min maximal effort. Caffeine (5

mg·kg⁻¹ of body mass) versus placebo was administered one hr pre-exercise in beverage form. It was reported that ratings of perceived exertion were decreased during constant-rate cycling and mean power output was enhanced during one min maximal effort in the caffeine trial. Foad, Beedie, and Coleman (2008) evaluated the effects of caffeine on mean power output during a 40 kilometer time trial. Fourteen male cyclists completed eight performance rides following the administration of caffeine (5 mg·kg⁻¹ of body mass) versus placebo in chilled saline. It was concluded that 5 mg·kg⁻¹ of body mass of caffeine increased mean power output during the cycling time trials.

Jenkins, Trilk, Singhal, O'Connor, and Cureton (2008) examined the effects of low dose caffeine (1, 2, and 3 mg·kg⁻¹ of body mass) on cycling performance, ratings of perceived exertion, and perceived leg pain in male cyclists. Sixty min following caffeine versus placebo administration, participants cycled 15 min at 80% of $\dot{V}O_{2\text{Max}}$ then completed a 15 min performance ride. It was concluded that low dose caffeine did not alter perceived exertion and leg pain during exercise. Nevertheless, the 2 mg·kg⁻¹ of body mass dose resulted in improvements in total work done during the 15 min self-paced performance ride.

Caffeine Timing and Cycling Performance

The pharmacokinetics of caffeine is well understood. Complete absorption is typically achieved approximately one hr following ingestion (Bonati, et al., 1982) and peak plasma concentrations are observed anywhere from 15 to 120 min (Blanchard & Sawers, 1983a; Bonati, et al., 1982; Kamimori, et al., 2002; Kamimori, et al., 1995; Kamimori, et al., 2000; McLean & Graham, 2002; Mumford, et al., 1996). Furthermore,

exercise has no effect on the pharmacokinetics of caffeine (McLean & Graham, 2002). Given the current knowledge of caffeine's pharmacokinetics, it has been customary for researchers to administer oral doses of caffeine approximately one hr prior and caffeine gum five min prior to exercise to enhance performance.

Research examining caffeine dose schedule/timing and performance has concluded that caffeine enhances (Cox, et al., 2002) or has no effect (Conway, et al., 2003) on performance regardless of dose schedule (single dose one hr prior vs. multiple dose paradigm) and/or timing. Cox et al. (2002) evaluated the effects of different protocols of caffeine intake on cycling performance ($7 \text{ kJ} \cdot \text{kg}^{-1}$ cycle time trial) following two hr of steady state exercise (70% of $\text{Vo}_{2\text{Max}}$) with CHO supplementation in competitive athletes. In the first portion of the study, caffeine ($6 \text{ mg} \cdot \text{kg}^{-1}$ of body mass) versus placebo was administered in a single dose prior to cycling or in multiple doses throughout cycling. In the second portion of the study, CHO supplementation was replaced with the cola beverage (caffeinated vs. decaffeinated) during the latter stages of steady-state cycling and the cycling time trial. These data suggested that caffeine ingestion improved time trial performance regardless of timing of intake, and replacing CHO supplementation with the caffeinated cola beverage was equally effective in enhancing cycling performance.

Conway, Orr and Stannard (2003) examined the effects of caffeine administered in a single dose versus multiple doses on cycling time trial performance. Nine trained male cyclists participated in three experimental trials consisting of 90 min of steady-state cycling (68% of $\text{Vo}_{2\text{Max}}$) followed by a self-paced performance ride. Caffeine ($6 \text{ mg} \cdot \text{kg}^{-1}$

of body mass) versus placebo was administered in a single dose or in a divided dose 60 min prior to exercise and 45 min following the initiation of exercise. These data revealed that caffeine ingestion did not significantly improve time trial performance nor did dose schedule impact the performance enhancing effects of caffeine.

Bell and McLellan (2002) intended to determine the duration of caffeine's performance enhancing effects in users and nonusers. Thirteen caffeine users and 8 nonusers completed exercise rides to exhaustion (80% of $\text{VO}_{2\text{Max}}$) one, three, and six hr after ingesting a caffeine dose of $5 \text{ mg} \cdot \text{kg}^{-1}$ of body mass or placebo. It was reported that caffeine improves cycling performance in caffeine users at, one and three, and nonusers at one, three, and six hr after ingestion. Further, Bell and McLellan (2003) concluded that caffeine administered in the morning improved cycle time to exhaustion in the morning and evening, and subsequent dosing throughout the day is unnecessary to improve cycling performance in the evening.

In summary, previous studies have reported that caffeine enhances (Cox, et al., 2002) or has no effect (Conway, et al., 2003) on performance regardless of dose schedule (single dose one hr prior vs. multiple dose paradigm) and/or timing, and a single dose of caffeine may enhance performance up to five to six hr post-ingestion (Bell & McLellan, 2002, 2003). To date, the most efficacious time to administer a single dose of caffeine to maximize its performance enhancing effects remains unclear.

CHAPTER III

METHODOLOGY

The purpose of the current investigation was to evaluate the effects of caffeine administration timing on cycle time trial performance. Furthermore, this study examined how caffeine ingestion impacts markers of Blood Brain Barrier integrity (serum s100 β) and plasma β -endorphin concentrations at rest and during exercise.

Participants

Eight college aged (25 years $\pm$ 5), trained (50 $\pm$ 5 ml $\cdot$ kg⁻¹ $\cdot$ min⁻¹) male cyclists were recruited via direct contact with the principal investigator. Volunteers were non-to-moderate caffeine users (<300mg/day) who are not taking medication or utilizing dietary supplements of any kind. Participants were excluded from the study if they reported a history of smoking, cardiovascular, metabolic, or respiratory disease on a health history questionnaire. Informed consent (Appendix A) was obtained from each subject prior to participation and this protocol was approved by the Kent State University Institutional Review Board for the Protection of Human Subjects (Appendix B).

Pre-experimental Testing

Participants reported to the Exercise Science Laboratory at which time height and body mass measurements were obtained via a stadiometer and a balance beam scale, respectively. Participants then underwent a graded exercise test (GXT) on a Lode cycle ergometer (Groningen, The Netherlands) until volitional fatigue. To determine maximal oxygen consumption (VO_{2Max}), air samples were analyzed for oxygen and carbon dioxide

concentrations via an indirect open circuit spirometry system (PARVO, Metabolic Cart, Sandy, Utah) A Hans Rudolph three-liter syringe (Hans Rudolph Model 5530, Hans Rudolph, Inc., Kansas City, MO) and known concentration calibration gases were used to calibrate the indirect open circuit spirometry system prior to each $\text{VO}_{2\text{max}}$ test.

Experimental Procedures

Figure 1 provides an overview of the experimental protocol. The current investigation employed a repeated measures, within-subjects design. Subjects participated in four experimental trials. During the experimental trials, three pieces of chewing gum were administered at three time points (120 min pre-cycling, 60 min pre-cycling, and 5 min pre-cycling). Subjects were instructed to chew the gum for five min and then expectorate the gum. In three of the four visits, at one of the time points mentioned previously, 300 mg of caffeine (Stay Alert™ chewing gum) was administered. During the fourth visit, placebo gum was administered at all three time points. The experimental trials are defined as follows: Trial A (-120), Trial B (-60), Trial C (-5), and Trial D (Placebo). The caffeine and placebo gum were virtually identical in shape, color, texture, and taste. Caffeine was administered in double blind manner. The order in which the experimental trials were completed was randomized.

All experimental trials were held between 0600 and 1200. Participants were requested to refrain from exercise and caffeinated or alcoholic beverages for at least 48 hr prior to the experimental trials. Furthermore, subjects reported to the laboratory in the post absorptive state. Upon arrival subjects were weighed via a balance beam scale.

Subjects then consumed a light breakfast (370 calories, 2g fat, 77g carbohydrates, 11g protein, 28g sugar) consisting of a plain bagel and 240 ml of orange juice. Following breakfast, baseline expired air samples and heart rate values were obtained via an indirect open circuit spirometry system (PARVO, Metabolic Cart, Sandy, Utah) and a Polar heart rate monitor (Accurex Plus, Polar Electro, Inc., Woodbury, NY), respectively. Venous blood sampling was then performed via an antecubital venipuncture.

Following baseline measurements, chewing gum was administered at the aforementioned time points. Between administration time points, participants rested in the seated position and were allowed to read, converse with data collectors, use the restroom, and utilize technology. Ten min prior to steady state cycling an additional venous blood sample was obtained. Subjects then completed a standard warm up consisting of cycling at a desired workload.

Steady State Cycling

All cycling was performed on a Lode cycle ergometer (Groningen, The Netherlands). Following time allotted for baseline measurements and gum administration and a standard warm up, participants cycled at constant wattage (workload corresponding to 75% $\text{VO}_{2\text{Max}}$) for 15 min. During steady state cycling, metabolic and perceptual measurements were obtained to allow for comparison between trials.

Metabolic and perceptual measurements during steady state cycling. Expired air samples were collected and analyzed for oxygen and carbon dioxide concentrations via an indirect open circuit spirometry system (PARVO, Metabolic Cart, Sandy, Utah) throughout steady state cycling. Heart rate (HR) was measured via a Polar heart rate

monitor (Accurex Plus, Polar Electro, Inc., Woodbury, NY) and HR values were recorded every three min. Ratings of Perceived Exertion (RPE) recordings were obtained every three min via Borg's RPE 6-20 scale (Borg, 1970). Perceived leg pain was assessed every three min via a 0-10 pain sensation scale (Cook, O'Connor, Eubanks, Smith, & Lee, 1997).

Cycle Time Trial

Following steady state cycling, venous blood sampling, and a five min active cool-down, cycling performance was assessed via a cycle time trial. Participants were instructed to perform a set amount of work ($7 \text{ kJ} \cdot \text{kg}^{-1}$) as quickly as possible. During the cycle time trial, subjects only viewed work achieved and were not informed of exercise time. This type of performance measure has been shown to have high reproducibility and a low coefficient of variation for the outcome measure of performance time (Jeukendrup, Saris, Brouns, & Kester, 1996).

Blood Sampling and Analyses

Blood samples were taken at four time points: 125 min pre-cycling, 10 min pre-cycling, immediately following steady state cycling, and immediately following the completion of the cycle time trial. Venous blood samples were drawn from the antecubital space via venipuncture and collected in three separate 5 ml and one 3 ml tube. An individual who is certified by the University Health Center performed all venipunctures. The purpose of the blood draws was to obtain blood borne hormone (plasma β -endorphin, serum s100 β) concentrations, plasma caffeine concentrations, and

hematocrit and hemoglobin measurements to determine plasma volume shifts (Dill & Costill, 1974).

Samples collected in red top tubes were allotted 30 min for clotting, centrifuged for five min at 3700 rpm (4°C), and aliquoted. Serum samples were stored at -80 °C and later analyzed for s100 β concentrations via a fully automated chemiluminescent assay system (Sangtec assay system, Diasorin, Inc., Stillwater, MN). Samples collected in green top tubes containing sodium heparin were immediately centrifuged for five min at 3700 rpm (4°C), and aliquoted. Plasma samples were stored at -80 °C and later analyzed for caffeine concentrations using a modified high-performance liquid chromatography method (Richard, Leducq, Baty, & Jambou, 1989). Briefly, 250 μ l of 0.8 M perchloric acid containing 25 μ g/ml of the internal standard 8-chlorotheophylline was added to 250 μ l of plasma. The resulting solution was vortexed for 5 sec then centrifuged at 6000 rpm for 5 min. Fifty μ l of the supernatant was injected into the chromatographic system (Agilent 1100 series). Caffeine was eluted with a Waters Resolve C18 (3.9 x 150mm) analytical column. The mobile phase consisted of KH₂PO₄/acetonitrile/tetrahydrofuran (88:9.2:2.8 v:v:v) and was pumped at 1 ml·min⁻¹. Eluted peaks were detected using UV absorption at a wavelength of 274 nm and peak areas were used for quantification utilizing a five point standard curve. The limit of quantification was 500 ng·ml⁻¹.

Samples collected in 5 ml lavender (EDTA) tubes were immediately transferred into centrifuge tubes containing aprotinin (0.6 TIU/ml of blood) and gently rocked several times to inhibit the activity of proteinases. Samples were then centrifuged for 15 min at 1600 rpm (4°C) and aliquoted. Plasma samples were stored at -80 °C and later

analyzed for β -endorphin concentrations via a valid, commercially available enzyme immunoassay (β -endorphin ELISA, M056011, MD Biosciences Inc., St. Paul, MN) (Avrameas, 1992; Porstmann & Kiessig, 1992). Samples collected in 3ml lavender (EDTA) tubes were immediately analyzed for hematocrit and hemoglobin concentrations. Hematocrits were measured in triplicate in a microhematocrit centrifuge (Critspin, Iris sample processing, Westwood, MA) and hemoglobin was measured via the cyanmethemoglobin method (Eagle hemoglobin procedure, Cima Scientific, Dallas, TX). Following the aforementioned analysis and collection of the resulting data, the blood samples were placed in a biohazard materials container and disposed of by Kent State University.

Statistical Analysis

All data were analyzed via SPSS 16.0 software. Cycle time data was assessed using a repeated measures analysis of variance (ANOVA). Metabolic and perceptual data were analyzed using a four (treatment) by six (time) ANOVA with repeated measures on both variables. Percent plasma volume and plasma caffeine data were analyzed using a four (treatment) by four (time) ANOVA with repeated measures on both variables. In the event that the ANOVA revealed a significant main effect or interaction, further exploration via paired samples t-tests with Bonferroni adjustments was performed. β -endorphin data were evaluated using a mixed effects model.

	Resting						WU	Steady State Cycling							Cycle Time Trial								
				CAFF	PLA																		
Trial A (-120)						PLA																	
Trial B (-60)						CAFF																	
Trial C (-5)						PLA																	
Trial D (Placebo)						PLA																	
	-175	-155	-125	-120	-100	-60	-40	-10	-5	0	+3	+6	+9	+12	+15	+20	+30	+40	+50	+60	+70	+80	Post
Meal			Blood VO ₂ RER HR RPE LP					Blood		VO ₂ RER HR RPE LP	VO ₂ RER HR RPE LP	VO ₂ RER HR RPE LP	VO ₂ RER HR RPE LP	VO ₂ RER HR RPE LP	Blood VO ₂ RER HR RPE LP							Blood	

Figure 1. Overview of experimental protocol. Caffeine (CAFF) or placebo (PLA) gum was administered twice at rest and once during the warm-up (WU). Blood sampling (Blood) occurred at -125, -10, +15, and post-cycling (Post). Oxygen consumption (VO₂), respiratory exchange ratio (RER), heart rate (HR), ratings of perceived exertion (RPE), and leg pain (LP) data were obtained at -125 and every 3 min during steady state cycling.

CHAPTER IV

RESULTS

The purpose of the current investigation was multifaceted and primarily examined the effect of caffeine administration timing on performance. In addition, the procedures outlined in Chapter III were selected to determine the effects of caffeine administered in chewing gum on metabolic and perceptual responses to exercise and plasma β -endorphin and serum s100 β concentrations at rest and following exercise. The experimental trials are defined as follows: Trial A (-120), Trial B (-60), Trial C (-5), and Trial D (Placebo).

Participant Characteristics

Participant characteristics are outlined in table 1. A total of eight competitive male cyclists were evaluated. All participants were non-to-moderate caffeine users who were free of disease and not taking medications and/or dietary supplements that could alter physiological responses to exercise.

Table 1. *Participant Characteristics*

Subject #	Age (year)	Mass (kg)	Height (cm)	VO _{2max} (ml·kg ⁻¹ ·min ⁻¹)
1	25.0	85.0	188.0	55.0
2	28.0	79.5	175.0	47.0
3	34.0	80.9	178.0	51.0
4	27.0	80.9	178.0	54.0
5	21.0	72.7	178.0	58.0
6	34.0	102.7	191.0	42.0
7	24.0	93.2	193.0	48.0
8	20.0	90.9	185.0	50.0
Mean $\pm$ SD	26.6 $\pm$ 5.3	85.7 $\pm$ 3.3	183.3 $\pm$ 6.9	50.6 $\pm$ 5.1

Cycle Time Trial Performance

Cycle time trial performance across experimental treatments is depicted in Figure 2. A repeated measures (four treatments) analysis of variance (ANOVA) was conducted to determine differences in cycle time trial performance. The ANOVA revealed a main effect of treatment for performance time ($p = 0.027$). Further analysis via paired samples t-test with Bonferroni adjustments revealed that cycling performance was enhanced in Trial C (-5) ($p = 0.023$) versus Trial D (Placebo). Performance time was not enhanced in Trial A (-120) ($p = 0.899$) or Trial B (-60) ($p = 1.0$) versus Trial D (Placebo).

Figure 2. Cycle time trial performance across experimental treatments. (M ± SEM) * denotes significant difference from Trial D (Placebo).

Metabolic Responses to Steady State Cycling

During steady state cycling, expired air samples and heart rate were continuously monitored via an indirect open circuit spirometry system (PARVO, Metabolic Cart, Sandy, Utah) and a Polar heart rate monitor (Accurex Plus, Polar Electro, Inc., Woodbury, NY), respectively. Oxygen consumption (VO_2), respiratory exchange ratio (RER), and heart rate (HR) measurements were recorded at baseline and every three min. Further, RER data were utilized to originate percent energy drive from carbohydrate data. All metabolic data were analyzed using a four (treatment) by six (time) ANOVA with repeated measures on both variables.

Oxygen Consumption is depicted in Figure 3. Results of the ANOVA revealed a main effect of time ($p < 0.001$). Paired samples t-tests with Bonferroni adjustments revealed that VO_2 increased during exercise and remained elevated relative to baseline ($p \leq 0.001$). Further, VO_2 incrementally increased from min 3 to min 6 ($p = 0.003$) and from min 6 to min 9 ($p = 0.04$), and remained stable thereafter ($p \geq 0.217$). No main effect of treatment ($p = 0.572$) or treatment by time interaction ($p = 0.253$) was found.

Figure 3. Oxygen consumption (VO_2) at baseline (Base) and during steady state cycling across experimental treatments. ($M \pm \text{SEM}$)

RER is depicted in Figure 4. The ANOVA revealed a main effect of time for RER ($p < 0.001$). Further exploration via paired samples t-tests with Bonferroni adjustments showed that individual time points did not significantly differ ($p \geq 0.180$). No main effect of treatment was observed ($p = 1.00$), nor was a treatment by time interaction found ($p = 0.660$).

Figure 4. Respiratory exchange ratio at baseline (Base) and during steady state cycling across experimental treatments. (M ± SEM)

Percent energy derived from carbohydrate data is presented in Figure 5. The ANOVA revealed a main effect of time ($p < 0.001$). Further exploration via paired samples t-tests with Bonferroni adjustments showed that individual time points did not significantly differ ($p \geq 0.180$). No main effect of treatment ($p = 0.830$) or treatment by time interaction ($p = 0.958$) was found.

Figure 5. Percent energy derived from carbohydrates at baseline (Base) and during steady state cycling across experimental treatments. (M $\pm$ SEM)

Heart rate data is presented in Figure 6. Heart rate demonstrated a main effect of time ($p < 0.001$). Further exploration via paired samples t-tests with Bonferroni adjustments revealed that heart rate incrementally increased from baseline to min three ($p < 0.001$), from min 3 to min 6 ($p = 0.003$), from min 6 to min 9 ($p = 0.001$), and from min 9 to min 12 ($p = 0.003$). Heart rate remained stable from min 12 to 15 ($p = 0.220$). No main effect of treatment ($p = 0.682$) or treatment by time interaction ($p = 0.999$) was found.

Figure 6. Heart rate in beats per min (BPM) at baseline (Base) and during steady state cycling across experimental treatments. ($M \pm SEM$)

Perceptual Responses to Steady State Exercise

Perceptual measures were obtained at baseline and every three min during steady state cycling. Ratings of perceived exertion (RPE) and perceived leg pain (LP) were assessed via Borg's RPE 6-20 scale (Borg, 1970) and a 0-10 pain sensation scale (Cook, et al., 1997), respectively. Perceptual data were analyzed using a four (treatment) by six (time) ANOVA with repeated measures on both variables.

Ratings of perceived exertion data is depicted in Figure 6. A main effect of time was observed for RPE ($p < 0.001$). Subsequent analysis via paired samples t-tests with Bonferroni adjustments showed that RPE incrementally increased from baseline to min 3 ($p < 0.001$), from min 3 to min 6 ($p = 0.012$), from min 6 to min 9 ($p = 0.006$), and from min 9 to min 12 ($p = 0.019$). A trend for an increase in RPE was observed from min 12 to min 15 ($p = 0.08$). No main effect of treatment was found ($p = .318$) and no treatment by time interaction was evident ($p = 0.545$).

Figure 7. Ratings of perceived exertion at baseline (Base) and during steady state cycling across experimental treatments. ($M \pm SEM$)

Leg pain is depicted in Figure 7. Leg pain demonstrated a main effect of time ($p < 0.001$). Paired samples t-tests with Bonferroni adjustments revealed that LP incrementally increased from baseline to min 3 ($p = 0.017$), from min 3 to min 6 ($p =$

0.009), from min 6 to min 9 ($p = 0.005$), and from min 9 to min 12 ($p = 0.011$). Leg pain did not significantly increase from min 12 to min 15 ($p = 0.698$). No main effect of treatment ($p = 0.476$) or treatment by time interaction ($p = 0.438$) was found.

Figure 8. Perceived leg pain at baseline (Base) and during steady state cycling across experimental treatments. (M ± SEM)

Blood Analysis

During the experimental trials, venous blood samples were drawn from the antecubital space via venipuncture at baseline (Base), 10 min pre-cycling (-10), following steady state cycling (+ 15), and following the cycle time trial (Post). Percent plasma

volume was calculated from hematocrit and hemoglobin determinations (Dill & Costill, 1974). Plasma β -endorphin concentrations were quantified via a commercially available enzyme immunoassay (β -endorphin ELISA, M056011, MD Biosciences Inc., St. Paul, MN) (Avrameas, 1992; Porstmann & Kiessig, 1992). Plasma caffeine concentrations were determined using a modified high-performance liquid chromatography method (Richard, et al., 1989).

Percent plasma volume data were analyzed via a four (treatment) by four (time) ANOVA with repeated measures on both variables. Percent plasma volume data is presented in Figure 9. Percent plasma volume demonstrated a main effect of time ($p < 0.001$). Further exploration via paired samples t-tests with Bonferroni adjustments showed that percent plasma volume significantly decreased from Base to + 15 ($p = 0.037$) and from Base to Post ($p = 0.006$). No main effect of treatment ($p = 0.934$) or treatment by time interaction ($p = 0.204$) was observed.

Figure 9. Percent plasma volume at baseline (Base), 10 min pre-cycling (- 10), following steady state cycling (+ 15), and following the cycle time trial (Post) across experimental treatments. ($M \pm SEM$)

Only 89 out of 120 (74%) of the β -endorphin samples were evaluable at the completion of the study. Thus, due to sample attrition plasma β -endorphin data were analyzed using a mixed effects model. As depicted in Figure 10, a main effect of time was evident for plasma β -endorphin concentrations ($p = 0.015$). No main effect of treatment ($p = 0.532$) or treatment by time interaction ($p = 0.596$) was observed.

Figure 10. Plasma β -endorphin (Endorphins) concentrations at baseline (Base), 10 min pre-cycling (- 10), following steady state cycling (+ 15), and following the cycle time trial (Post) across experimental treatments. (M $\pm$ SEM)

Plasma β -endorphin data were also analyzed via a mixed effects model following rectification for plasma volume shifts. Corrected plasma β -endorphin concentrations are presented in Figure 11. A main effect of time was evident for corrected plasma β -endorphin concentrations ($p = 0.035$). No main effect of treatment ($p = 0.610$) or treatment by time interaction ($p = 0.716$) was observed.

Figure 11. Plasma β -endorphin (Endorphins) concentrations corrected for plasma volume shifts at baseline (Base), 10 min pre-cycling (-10), following steady state cycling (+15), and following the cycle time trial (Post) across experimental treatments. ($M \pm SEM$)

Plasma caffeine data were analyzed via a four (treatment) by four (time) ANOVA with repeated measures on both variables. Plasma caffeine concentration data is presented in Figure 12. Plasma caffeine concentration demonstrated a treatment by time interaction ($p < 0.001$). Subsequent analysis was performed using paired samples t-tests. Plasma caffeine concentrations did not differ across treatments at baseline ($p \geq 0.505$). At 10 min pre-cycling, caffeine concentrations were increased in Trial A (-120) ($p = 0.03$) and Trial B (-60) ($p < 0.001$) versus Trial D (Placebo). Following steady state cycling, plasma caffeine concentrations were increased in Trial A (-120) ($p = 0.05$) and Trial B (-60) ($p = 0.014$) versus Trial D (Placebo). A trend for an increase in caffeine

concentration in Trial C (-5) ($p = 0.06$) versus Trial D (Placebo) was also observed. Interestingly, plasma caffeine concentrations did not differ between caffeine trials following steady state cycling ($p \geq 0.141$). Following the cycle time trial, plasma caffeine concentrations were increased in Trial A (-120) ($p = 0.05$), Trial B (-60) ($p = 0.01$), and Trial C (-5) ($p = 0.02$) versus Trial D (Placebo). Further, plasma caffeine concentrations did not differ between caffeine trials following the cycle time trial ($p \geq 0.568$). Lastly, plasma caffeine concentrations continued to increase during the cycle time trial in Trial C (-5) ($p = 0.046$), but not in Trial A (-120) ($p = 0.514$) or Trial B (-60) ($p = 0.831$).

Figure 12. Plasma caffeine concentrations at baseline (Base), 10 min pre-cycling (- 10), following steady state cycling (+ 15), and following the cycle time trial (Post) across experimental treatments. ($M \pm \text{SEM}$)

CHAPTER V

DISCUSSION

Caffeine is one of the most widely used drugs in the world and caffeine use is associated with minimal health risks (Curatolo & Robertson, 1983; Dews, 1984; Seferin, 1996). Caffeine is a socially acceptable drug that is readily available for athletes to utilize for performance enhancement both in competition and during training sessions. Research has shown that caffeine improves endurance performance (Graham, 2001) and reduces perceived exertion (Doherty, et al., 2004) and leg pain (Motl, et al., 2003) during exercise. Furthermore, it has been reported that caffeine may exacerbate β -endorphin release in response to exercise (Laurent, et al., 2000) and block disruption of Blood Brain Barrier integrity (Chen, et al., 2008).

Following the oral ingestion of caffeine, complete absorption is typically achieved in approximately one hr (Bonati, et al., 1982) and peak plasma concentrations are observed anywhere from 15 to 120 min (Blanchard & Sawers, 1983a; Bonati, et al., 1982; Kamimori, et al., 1995; Kamimori, et al., 2000; McLean & Graham, 2002; Mumford, et al., 1996). Thus, typically, researchers have administered oral doses of caffeine one hr prior to performance testing to ensure peak plasma concentrations during exercise. Nonetheless, research has not determined whether or not timing of peak plasma concentration impacts subsequent performance enhancement and the most efficacious time to administer caffeine to maximize its performance enhancing effects is poorly understood (Conway, et al., 2003). In the present study, we sought to examine the effects of caffeine administered in chewing gum on metabolic and perceptual

responses to exercise and plasma β -endorphin and serum s100 β concentrations at rest and after exercise.

Cycle Time Trial Performance

The most efficacious time to administer caffeine in any formulation to maximize its performance enhancing effects is poorly understood (Conway, et al., 2003). In the current investigation, cycling performance improved when 255 mg (mean relative dose of 3 mg·kg⁻¹ of body mass) of caffeine was administered in chewing gum immediately prior to cycling versus placebo. Conversely, cycling performance was not enhanced when caffeine gum was administered one or two hr prior to cycling. These data demonstrate that 3 mg·kg⁻¹ of body mass of caffeine administered in chewing gum enhances cycling performance when ingested immediately prior to exercise. Furthermore, these data suggest that timing of caffeine administration profoundly impacts subsequent performance enhancement.

Blood analysis showed that plasma caffeine concentrations did not differ between the three caffeine trials immediately before and after performance testing. Moreover, plasma caffeine concentrations continued to rise during the cycle time trial in Trial C (-5) but remained stable during the cycle time trial in Trial A (-120) and Trial B (-60). These data suggest that the ergogenic effect of caffeine was not evident when caffeine gum was administered one or two hr prior to exercise even though plasma caffeine levels were maintained. Furthermore, it is concluded that performance enhancement with caffeine was evident only when plasma caffeine levels were still

rising during the cycle time trial and not when peak plasma concentrations were achieved before the cycle time trial began.

Few studies have investigated the duration of caffeine's performance enhancing effect. In the present study, cycling performance was not enhanced when caffeine gum was administered one or two hr prior to cycling. Contrary to the present findings, Bell and McLellan (2002) reported that caffeine improves cycling performance in caffeine users at, one and three, and nonusers at one, three, and six hr after ingestion. These equivocal findings can be explained by methodological differences. Bell and McLellan (2002) had subjects ingest a larger dose ($5 \text{ mg} \cdot \text{kg}^{-1}$ of body mass) of caffeine than was utilized here. Also, in this study caffeine was administered in chewing gum where as in the study conducted by Bell and McLellan (2002) caffeine was administered in capsule form.

Metabolic Responses to Steady State Cycling

In the present study, heart rate responses during steady state cycling did not differ between trials. These results are in agreement with early studies that have reported that caffeine has little effect on heart rate at rest and during exercise (Graham, 2001). Further, these data showed that caffeine did not impact oxygen consumption during steady state cycling. This was expected as metabolism is regulated by adenosine triphosphate (ATP) availability. When working muscles utilize ATP and ATP stores begin to decline, oxygen consumption increases to allow for ATP replenishment. Thus, during physical activity, oxygen consumption is directly proportional to workload.

Several studies have concluded that the ingestion of caffeine stimulates the mobilization of free fatty acids (Bellet, et al., 1968; Costill, et al., 1978; Essig, et al., 1980; Ivy, et al., 1979). Essig et al. (1980) suggested that caffeine increases lipid fuel availability and thus fat oxidation. In the current investigation, caffeine did not influence fat oxidation during steady state cycling as indicated by respiratory exchange ratio. These results are in agreement with those of more recent studies (Graham, 2001; Graham, et al., 2000) that have reported fat oxidation is not increased with caffeine ingestion.

Perceptual Responses to Steady State Cycling

Researchers have reported that caffeine ingestion decreases ratings of perceived exertion (Demura, et al., 2007; Doherty, et al., 2004; Doherty & Smith, 2004; Hadjicharalambous, et al., 2006) and leg pain (Gliottoni & Motl, 2008; Motl, et al., 2006; Motl, et al., 2003; O'Connor, et al., 2004) during cycling exercise. Based on the results of these studies, it has been suggested that caffeine may improve endurance performance by decreasing perceived exertion and pain during exercise allowing athletes to work harder and longer.

In the present study, caffeine did not impact ratings of perceived exertion and leg pain during steady state cycling; although, performance enhancement with caffeine was evident. Jenkins et al. (2008) also reported that caffeine improved cycling performance but did not decrease ratings of perceived exertion and leg pain during steady state cycling. Subjects in the study conducted by Jenkins et al (2008) consumed 3 mg·kg⁻¹ of body mass of caffeine then cycled 15 min at 80% V_{O₂Max}. Participants in the current investigation consumed a mean relative dose of caffeine equivalent to 3 mg·kg⁻¹ of body

mass then cycled 15 min at 75% $\text{VO}_{2\text{Max}}$. Based on these findings, it is concluded that a caffeine dose in excess of $3 \text{ mg}\cdot\text{kg}^{-1}$ of body mass is necessary to reduce perceived exertion and pain during cycling exercise.

Blood Analysis

As expected, plasma β -endorphin concentrations increased with exercise in the present study. However, caffeine had no effect on plasma β -endorphin concentrations at rest and immediately after cycling exercise. Contrary to these findings, Laurent et al. (2000) reported that caffeine increased β -endorphin concentrations following two hr of cycling at 65% $\text{VO}_{2\text{Max}}$. One likely explanation for these equivocal findings is that a much larger dose of caffeine ($6 \text{ mg}\cdot\text{kg}^{-1}$ of body mass) was administered in the study conducted by Laurent et al. (2002) relative to present study.

In the present study, serum samples were analyzed for s100 β concentrations via a fully automated chemiluminescent assay system (Sangtec assay system, Diasorin, Inc., Stillwater, MN). The s100 β assay was inconclusive as virtually all of the serum samples contained s100 β concentrations below that of the detectable limit. Interestingly, other investigators have reported using the same assay for quantification of s100 β in serum from older adults (Benitez et al., 2009; Keary et al., 2008). Based on these findings, it is postulated that serum s100 β levels are higher in older adults relative to the group of younger adults who participated in the present study. Furthermore, a more sensitive method of quantifying serum s100 β concentrations than was utilized here is required for younger adults.

Summary and Conclusions

Few studies have investigated the effect of caffeine administration timing on performance enhancement. In the present study, caffeine ($3 \text{ mg}\cdot\text{kg}^{-1}$ of body mass) administered in chewing gum improved cycling performance when administered immediately prior to a bout of cycling exercise lasting approximately one hr. Plasma caffeine concentrations were comparable between all caffeine treatments before and after performance testing; however, cycling performance was not enhanced with caffeine when the caffeine gum was administered at one or two hr prior to cycling. It is important to note that plasma caffeine concentrations continued to rise during performance testing only when caffeine gum was administered immediately prior to cycling. Based on these results, it is concluded that caffeine gum should be administered immediately prior to exercise to enhance cycling performance during an event lasting approximately one hr. Furthermore, it is recommended that researchers administer caffeine in a manner that allows for an increase in plasma caffeine concentrations during performance to obtain the best ergogenic effect.

In the present study, $3 \text{ mg}\cdot\text{kg}^{-1}$ of body mass of caffeine did not impact metabolic and perceptual responses to steady state cycling. These results are in general agreement with those previously reported (Graham, 2001; Jenkins, et al., 2008). Plasma β -endorphin concentrations increased with exercise; however, caffeine had no effect on plasma β -endorphin concentrations at rest and immediately after cycling exercise. Work done by Laurent et al. (2000) suggests that a larger dose of caffeine than was utilized here

may be necessary to observe increases in plasma β -endorphin concentrations after exercise.

Limitations

Numerous factors such as dosage, dose formulation, and route of administration can impact the pharmacokinetics of caffeine (Bonati, et al., 1982; Kamimori, et al., 2002). Thus, the results presented here are specific to caffeine ($3 \text{ mg}\cdot\text{kg}^{-1}$ of body mass) administered in chewing gum. Future research is necessary to determine the effect of caffeine administration timing on cycling performance when caffeine is administered in larger doses and other formulations. Moreover, pharmacokinetic modeling using plasma caffeine data was not performed in the present study. The estimation of caffeine pharmacokinetic parameters would have provided further insight into the effect of caffeine administration timing on cycling performance.

APPENDICES

APPENDIX A

Letter of Informed Consent

Appendix A

Letter of Informed Consent

CONSENT TO PARTICIPATE AS A VOLUNTEER IN A RESEARCH STUDY

TITLE: Caffeine Timing and Cycling Performance

DESCRIPTION: This study is being undertaken to examine the following: caffeine timing and cycling performance, caffeine ingestion and effort and pain perception during cycling, and caffeine ingestion and blood markers of pain reducing hormones (β -endorphins) and blood brain barrier integrity (serum s100 β) at rest and during exercise. The qualifications to participate include:

1. Male gender
2. Age: 18-32 years
3. Competitive cyclists
4. Non-to-moderate caffeine usage (<300mg/day)

The exclusion criteria include:

1. Supplement use
2. Smoking
3. Signs or symptoms of cardiovascular, metabolic, or respiratory disease
4. Known cardiovascular, metabolic, or respiratory disease

A determination regarding eligibility will be made following the completion of a health history questionnaire.

Your participation in the study will include:

1. Five visits to the Exercise Science Laboratory (1 familiarization session allowing you to practice cycle endurance testing and 4 experimental trials)
2. Completion of a 7 kJ·kg⁻¹ cycle endurance test (4 separate experimental trials)
3. Completion of 15 min of cycling at 75% capacity (4 separate experimental trials)
4. Chewing 3 pieces of gum at 3 time points (120 minutes pre-exercise, 60 minutes pre-exercise, and 5 minutes pre-exercise) during each of the 4 experimental trials. In 3 of the 4 visits, at one of the time points mentioned previously, the 3 pieces of chewing gum will contain 100 milligrams of caffeine (total of 300 milligrams)
5. Measurement of your oxygen consumption during maximal and submaximal cycling via an automated open circuit system
6. Having your blood drawn (antecubital venipuncture) four times in each of the four experimental trials (125 minutes pre-exercise, 10 minutes pre-exercise, immediately post steady state cycling, and immediately following the completion of the cycle endurance test) for a total of 16 blood draws

Prior to the four experimental trials, you will be asked to not eat for 8 hours before each trial and to not consume any alcohol or caffeine containing drinks for 24 hours before each trial.

RISK AND BENEFITS

Although the caffeine doses used in this study are similar to those encountered in everyday life, all neutraceuticals have the potential for minor side effects. Side effects for caffeine may include the possibility of headache, dizziness, nausea, and muscle tremor. Individuals who are caffeine naïve may be more prone to side effects than those who are habitual caffeine users. In addition, chewing gum may cause dental fillings to become loose. Additional risks associated with this investigation are related to the venipuncture. Following the venipuncture, there is the potential for the development of an infection, the development of a bruise and/or blood clot, and localized pain and discomfort; furthermore, you may experience some dizziness. These risks associated with caffeine ingestion and venipunctures are extremely rare and proper safeguards are in place.

The benefit from participating in this study is learning your aerobic fitness level.

CONFIDENTIALITY

All information from this experiment, and all records related to this experiment will be held confidential.

RIGHT TO WITHDRAW

You are free to refuse to participate in this study and you are free to withdraw at any time for any reason that you feel is necessary. The principal investigator may ask you to withdraw from the study if you fail to comply with the requirements of the investigation, or if you do not meet the basic qualifications of the study.

COMPENSATION

Upon qualification for the investigation, you will be compensated \$125, broken down into the following increments based on the completion of each trial:

- \$25 for completion of trial 1
- \$25 for completion of trial 2
- \$25 for completion of trial 3
- \$25 for completion of trial 4

COMPENSATION FOR ILLNESS OR INJURY

Due to the nature of the experimental procedure, there is little chance of physical injury or illness to you. However, emergency medical treatment, within the limits of that normally offered to students and KSU employees by the University Health Center (tel. 330-672-2322), will be provided for injury or illness occurring during normal working hours. No other medical treatment or financial compensation for injury or illness to the participants involved in this project is available.

If you would like to know more about this research project, please contact me at the School of Exercise, Leisure, and Sport at 330-672-2930.

The Kent State University Human Subjects Review Board has approved this project. If you have questions about Kent State University's rules for research, please call Dr. John West, Vice President for Research and Dean of Graduate Studies at 330-672-2704.

You will receive a copy of this consent form.

Sincerely,

Edward Ryan M.S.
Principal Investigator

CONSENT STATEMENT (S)

I agree to participate in this project. I have read and fully understand my role in this project and the instructions given to me. I understand that I will have to comply fully with these instructions in order to participate in this experiment. I understand that I can stop at any time and for any reason.

Signature

Date

Witness

Date

APPENDIX B

Institutional Review Board Application

Appendix B

Institutional Review Board Application

**KENT STATE UNIVERSITY
INSTITUTIONAL REVIEW BOARD
APPLICATION FOR APPROVAL
TO USE HUMAN RESEARCH SUBJECTS**

*Please type all information. HANDWRITTEN FORMS WILL NOT BE ACCEPTED.
Submit completed form with original signatures and all required attachments to the IRB
Reviewer associated with your Department or College, or to: Office of Research Safety and
Compliance, Research and Graduate Studies, 137 Cartwright Hall, Phone: 330-672-2704.*

Project Title: Caffeine Timing and Cycling Performance

Principal Investigator

Name: Edward Ryan
Address: 163 Gym Annex
Phone: 724-730-0130

Department: Exercise Science
Email: eryan4@kent.edu

Status: ☐ Faculty
☒ Doctoral Student
☐ Graduate Student
☐ Undergraduate Student

☐ Other: (Specify:)

Project: ☐ Faculty Research
☒ Student Dissertation
☐ Student Thesis
☐ Course Requirement:
(Course #:)
☐ Other:
(Specify:)

KSU Faculty Co-Investigator(s) (Use additional sheets if necessary)

Name:
Address:
Phone:

Department:
Email:

Status: ☐ Faculty
☐ Doctoral Student
☐ Graduate Student
☐ Undergraduate Student
☐ Other: (Specify:)

Faculty Advisor (If PI is a student)

Name: Ellen Glickman
Phone: 330-672-2930

Department: Exercise Science
Email: eglickma@kent.edu

Protocol Funding: ☒ Not-applicable ☐ Pending ☐ Awarded

Federal: ☐ Yes ☐ No

Funding Agency:

Estimated Project Duration: Starting Date: October 1, 2009
(But not before approval is obtained)

Ending Date: October 1, 2010

Part I: Please answer the following questions by checking the correct response.

- ☐ Yes ☐ No 1. Will participants be identifiable to anyone other than the researchers through records, responses, or identifiers linked to the participants?
- ☐ Yes ☒ No 2. Could participants be at risk of criminal or civil liability, damage to employability or to financial standing, or undue embarrassment, if responses became known outside this research project?
- ☐ Yes ☒ No 3. Does research deal with sensitive aspects of participants' behavior, such as illegal conduct, drug use, sexual behavior, use of alcohol, or potential harm to self or others?
- ☐ Yes ☒ No 4. Does research involve the study of existing data?
(If yes, please specify.)
- ☐ Documents, archives, and/or records
☐ Biological specimens
- ☐ Yes ☒ No 5. Does the research involve audio, video, digital, or image recordings of participants? (If yes, please specify.)
- ☐ Video-taped ☐ Audio-taped
☐ Photographed ☐ Other: (Specify:)
- ☒ Yes ☐ No 6. Are participants free to withdraw at any time without penalty?
- ☐ Yes ☒ No 7. Is there deception of participants? (If so, answer questions in Part VII, #35-44)
- ☐ Yes ☒ No 8. Does the research deal with participants under the age of 18?
- ☐ Yes ☒ No 9. Will identifiable medical information be collected?

10. Does the research deal with any of the following vulnerable Populations:

☐ Yes ☒ No Legally incompetent adults	☐ Yes ☒ No Traumatized or Comatose
☐ Yes ☒ No Cognitively/Mentally impaired	☐ Yes ☒ No Economically Disadvantaged
☐ Yes ☒ No Physically challenged	☐ Yes ☒ No Terminally ill
☐ Yes ☒ No Pregnant women	☐ Yes ☒ No Prisoners

11. Does the project involve: (If yes, also answer question #20 on page 4).

☐ Yes ☒ No Administering drugs	☐ Yes ☒ No Medical devices
☐ Yes ☒ No Administering alcohol	☐ Yes ☒ No Invasive procedures
☒ Yes ☐ No Administering nutritional supplements	☒ Yes ☐ No Drawing blood
☐ Yes ☒ No Taking tissue samples	☐ Yes ☒ No Giving injections

12. Are you collecting any portion of your data on-line? ☐ Yes ☒ No

13. Are you requesting a waiver of any elements of the consent process?

☐ Yes ☒ No

(If yes, answer questions in Part VIII, #45-46.)

Part II: Summary of Research

14.) Describe the purpose and significance of the proposed research; include sufficient background information and the specific objectives of the study. Summarize the major hypotheses. (Use non-technical language that can be understood by someone outside the discipline.)

Caffeine is one of the most widely used nutraceuticals in the world (Dews, 1984). In moderate doses (<300 mg) caffeine is well tolerated and few significant side effects have been reported (Robertson, 1984). Stay Alert™ chewing gum is a product developed by Amuro Confectioners (Specialty gum subsidiary of Wrigley's, Yorkville, IL) that contains 100mg caffeine/piece. Caffeine in this form is considered a nutritional supplement and requires no additional FDA approval for use in this form. Research has revealed that caffeine ingestion may: improve aerobic performance, decrease perceived exertion and pain experienced during exercise, exacerbate plasma β -endorphin levels during exercise and protect against neurological disorders.

The most efficacious time to administer caffeine to maximize its performance enhancing effects is poorly understood (Conway et al., 2003) and may depend on the medium through which it is ingested. Furthermore, to date, the effects of caffeine ingestion (in the form of chewing gum) on plasma β -endorphin and serum s100 β levels at rest and during exercise renders investigation. Thus, the purpose of the proposed investigation is multi-faceted and will examine the following:

1. The effects of caffeine administration timing on cycle time trial performance.
2. The effects of caffeine administered in the form of chewing gum on perceived exertion and leg pain during cycling.
3. The effects of caffeine administered in the form of chewing gum on plasma β -endorphin levels during exercise.
4. The effects of caffeine administered in the form of chewing gum on blood markers of Blood Brain Barrier integrity at rest and during exercise.

15.) Describe the study design, research methods and procedures. (Please append copies of the consent form and all measures, including interview questions and self-report questionnaires, to this form.) What are the qualifications of the individual(s) who will be collecting the data?

Participants:

Ten apparently healthy, competitive male cyclists between 18 and 32 yrs of age will be recruited via direct contact with the principal investigator. Volunteers need to be non-to-moderate caffeine users (<300mg/day) who are not currently taking medication or utilizing dietary supplements of any kind. Participants will be excluded from the study if they have a history of smoking, have signs or symptoms of cardiovascular, metabolic, or respiratory disease, or if they are known to have any cardiovascular, metabolic, or respiratory disease as determined via a health history questionnaire. Informed consent will be obtained from each subject prior to participation and this protocol will be approved by the Kent State University IRB.

Pre-experimental testing:

Participants will report to the Exercise Science Laboratory at which time height and body mass measurements will be taken. Participants will then undergo a graded exercise test (GXT) on a Lode cycle ergometer (Groningen, The Netherlands) until volitional fatigue at which time expired air samples will be analyzed for oxygen and carbon dioxide concentrations via an indirect open circuit spirometry system (PARVO, Metabolic Cart, Sandy, Utah) to determine maximal oxygen consumption (VO_{2max}). A Hans Rudolph three-liter syringe (Hans Rudolph Model 5530, Hans Rudolph, Inc., Kansas City, MO) and known concentration calibration gases will be used to calibrate the indirect open circuit spirometry system prior to each VO_{2max} test.

Experimental Procedures:

The current investigation will employ a counterbalanced, within-subjects design. Subjects will participate in five experimental trials. All experimental trials will be held between 0800 and 1200. Participants will be requested to refrain from exercise and caffeinated or alcoholic beverages for at least 24 hours prior to experimental trials.

Furthermore, subjects will need to report to the laboratory in the post absorptive state. The first trial will allow for participants to become familiarized with the cycle Time Trial (TT). For the next four visits, 3 pieces of chewing gum will be administered at 3 time points (120 minutes pre-cycling, 60 minutes pre-cycling, and 5 minutes pre-cycling). Subjects will be instructed to chew the gum for 5 minutes and then expectorate the gum. In 3 of the 4 visits, at one of the time points mentioned previously, 300 milligrams of caffeine (Stay Alert™ chewing gum) will be administered. During the fourth visit, placebo gum will be administered at all three time points. Caffeine will be administered in a randomized, counterbalanced, double blind manner.

Steady State Cycling

All cycling will be performed on a Lode cycle ergometer (Groningen, The Netherlands). Following time allotted for baseline measurements and gum administration and a standard warm up, participants will cycle at constant wattage (workload corresponding to 75% $\text{VO}_{2\text{max}}$) for 15 minutes. During steady state (SS) cycling, metabolic measurements will be obtained to allow for comparison between trials.

Metabolic Measurements during SS Cycling

Expired air samples will be collected and analyzed for oxygen and carbon dioxide concentrations via an indirect open circuit spirometry system (PARVO, Metabolic Cart, Sandy, Utah) throughout SS cycling. Heart rate will be measured via a Polar heart rate monitor (Accurex Plus, Polar Electro, Inc., Woodbury, NY) and HR values will be recorded every 3 min. Ratings of Perceived Exertion (RPE) recordings will be obtained every 3 min via Borg's RPE 6-20 scale (Borg, 1970). Perceived leg pain will be assessed every 3 min via a 0-10 pain sensation scale (Cook, et al., 1997).

Cycle Time Trial

Following SS cycling and a 5 min active cool-down, cycling performance will be assessed via a cycle time trial (TT). Participants will be instructed to perform a set amount of work ($7 \text{ kJ} \cdot \text{kg}^{-1}$) as quickly as possible. During the cycle TT, subjects will only view work achieved and will not be informed of exercise time.

Blood Sampling

Blood samples will be taken at four time points: 125 minutes pre-cycling, 10 minutes pre-cycling, immediately following steady state cycling, and immediately following the completion of the cycle TT. Venous blood samples will be drawn from the antecubital space via venipuncture and collected in 4 separate 5ml and one 3ml tubes. An individual who is certified by the University Health Center will perform all venipunctures. The purpose of the blood draws is to obtain blood borne hormone (plasma β -endorphin, catecholamines, and serum s100 β) concentrations, plasma caffeine concentrations, and hematocrit and hemoglobin measurements to determine plasma volume shifts (Dill & Costill, 1974).

Samples collected in red top tubes will be allotted 30 min for clotting, centrifuged for 5 min at 3700 rpm (4°C), and aliquoted. Serum samples will be stored at -80 °C and later analyzed for s100 β concentrations via a fully automated chemiluminescent assay system (Sangtec assay system, Diasorin, Inc., Stillwater, MN). Samples collected in chilled green top tubes containing reduced glutathione, sodium heparin, and EGTA will be immediately centrifuged for 5 min at 3700 rpm (4°C), and aliquoted. Plasma samples will be stored at -80 °C and later analyzed for epinephrine (EPI) and norepinephrine (NE) concentrations using alumina extraction (Chromsystems, Germany) and HPLC with electro-chemical detection as previously described (Schneider, McGuiggin, & Kamimori, 1992). Samples collected in chilled green top tubes containing sodium heparin only will be immediately centrifuged for 5 min at 3700 rpm (4°C), and aliquoted. Plasma samples will be stored at -80 °C and later analyzed for caffeine and caffeine metabolite concentrations.

Samples collected in 5ml lavender (EDTA) tubes will be immediately transferred into centrifuge tubes containing aprotinin (0.6 TIU/ml of blood) and gently rocked several times to inhibit the activity of proteinases. Samples will then be centrifuged for 15 min at 1600 rpm (4°C) and aliquoted. Plasma samples will be stored at t -80 °C and later analyzed for β -endorphin concentrations via an enzyme Immunoassay kit (β -endorphin ELISA, M056011, MD Biosciences Inc., St. Paul, MN). Samples collected in 3ml lavender (EDTA) tubes will be immediately analyzed for hematocrit and hemoglobin concentrations. Hematocrits will be measured in triplicate in a microhematocrit centrifuge (Critspin, Iris sample processing, Westwood, MA) and hemoglobin will be measured via the cyanmethemoglobin method (Eagle hemoglobin procedure, Cima Scientific, Dallas, TX). Following the aforementioned analysis and collection of the resulting data, the blood samples will be disposed of in a biohazard materials container and disposed of by Kent State University.

Part III: Research Participants

16.) Briefly describe the characteristics of your population(s). Describe the ethnic background, sex, age, state of health, and the criteria for inclusion or exclusion of participants. (Include rationale for use of special classes of participants such as pregnant women, children, institutionalized mentally disabled, prisoners, or those whose ability to give voluntary informed consent may be in question.) If your population is all one gender or ethnic group, please explain.

Ten apparently healthy, competitive male cyclists between 18 and 32 yrs of age will be recruited via direct contact with the principal investigator. Volunteers need to be non-to-moderate caffeine users (<300mg/day) who are not currently taking medication or utilizing dietary supplements of any kind. Participants will be excluded from the study if they have a history of smoking, have signs or symptoms of cardiovascular, metabolic, or respiratory disease, or if they are known to have any cardiovascular, metabolic, or respiratory disease as determined via a health history questionnaire. Informed consent

will be obtained from each subject prior to participation and this protocol will be approved by the Kent State University IRB.

17.) Indicate the anticipated sample size.

10

18.) Explain the recruitment process. State how potential participants will be identified and who will make the initial contact. Explain how you will ensure that recruitment and selection of participants is equitable. (Please include all recruitment materials, including scripts, flyers, and advertisements as attachments to this form.)

Initial contact will be made directly by the principle investigators, Edward Ryan. An explanation of protocol procedures, and potential risks and benefits, as well as monetary reward will be included in the consent form.

Part IV: Risks/Benefits

19.) Identify any expected or potential risks or discomforts (including physical, psychological, social, or legal) to which participants may be exposed as a result of participation in the research project (beyond those encountered in everyday life).

Although the caffeine doses used in this study are similar to those encountered in everyday life, all neutraceuticals have the potential for minor side effects. Side effects for caffeine may include the possibility of headache, dizziness, nausea, and muscle tremor. Individuals who are caffeine naïve may be more prone to side effects than those who are habitual caffeine users. In addition, chewing gum may cause dental fillings to become loose. Additional risks associated with this investigation are related to the venipuncture. Following the venipuncture, there is the potential for the development of an infection, the development of a hematoma, localized pain and discomfort, dizziness, and possible thrombosis of the vein. These risks associated with caffeine ingestion and venipunctures are extremely rare and proper safeguards are in place.

a.) What safeguards will you use to protect the participants from these risks, as well as to protect their rights, welfare, and privacy? (Must provide a response; never answer "N/A".)

Precautions to minimize the risks associated with caffeine ingestion include the pre-screening, thorough health history questionnaires, and the monitoring of heart rate during each trial. Regarding the venipunctures, to minimize bruising and

discomfort, personnel who are certified by the Kent State University Health Center or the State of Ohio will do the venipunctures.

- 20.) Describe the anticipated benefits to individual subjects and to society expected to be gained from this project.** (This should include any direct benefits to the participants as well as any generalized gain in knowledge. If there are not direct benefits to individual subjects, state that.)

In addition to the monetary reward (\$100), each participant will learn their aerobic fitness level.

- 21.) Describe the qualifications of the person administering drugs, alcohol, or nutritional supplements, or drawing blood, taking tissue samples, or giving injections.**

Certified personnel who have completed the appropriate training and blood-borne pathogens course will conduct venipunctures. Caffeine will be administered by the principal investigator under the supervision of a member of the graduate faculty.

- 22.) Describe any form of compensation to participants. (i.e., money, extra credit, etc. If money, extra credit, or grade is given to students who participate in the project, what opportunity for extra credit or grade is provided to students who choose not to participate?)**

Upon qualification for the investigation, compensation will consist of \$100, broken down into the following increments based on completion of each phase:

- a) \$25 for participation in trial 1
- b) \$25 for participation in trial 2
- c) \$25 for participation in trial 3
- d) \$25 for participation in trial 4

- 23.) Research participants will be informed of the risks and benefits through:**

- ☒ Consent form (Include copy with application)
- ☐ Verbal Script (Include with application materials)
- ☐ Assent (children 12 years of age and under) and consent from parents/guardians

Part V: Informed Consent *(Please include a copy of the informed consent document with application materials. See ICD template for assistance.)*

24.) Describe the consent process. Explain when and where consent will be obtained and identify who will be obtaining informed consent.

Initial contact will be made directly by the principal investigator, Edward Ryan. An explanation of protocol procedures, and potential risks and benefits, as well as monetary reward will be included in the consent form. All consent forms will be completed by potential volunteers in room 163 of the gym annex. The principal investigator as well as a witness will be present at all times.

25.) If you will be using children under 18, explain in detail how you will obtain parental consent and assent (for children under 12) or consent (for children 12 to 18). If assent/consent will be obtained orally, supply a script of what you will say and how you will give the children the opportunity to agree to participate or decline.

NA

26.) Explain how the possibility of coercion or undue influence will be minimized in the consent process (e.g., if employer is approaching employees, instructors are approaching students, physicians are approaching patients, if compensation is involved, etc.).

No extra incentive beyond what is listed in the consent form will be provided.

Part VI: Privacy and Confidentiality of Records

27.) Where will the signed consent forms be kept? *(Consent forms must be kept in a secured location on campus, not in a private home or office.) If the study does not involve consent forms, answer "N/A".*

The consent forms and all data related to this investigation will be locked in a cabinet in Room 163E of the Memorial Gym Annex

28.) Describe specifically how you will maintain the confidentiality of the data.

Each subject will be assigned a number and the number assigned will also be locked in a cabinet in room 163E of the Memorial Gym Annex.

29.) How will the data/results of the research be disseminated?

☐ Thesis	☒ Publication
☐ Dissertation	☐ Public presentation
☐ Other:
(Specify:)	☐ Course Requirement:
(Course #:)

30.) How will the data be stored after study completion? *Please be specific as to the retention or destruction of audio/video data or cell lines.*

The consent forms and all data related to this investigation will be locked in a cabinet in Room 163E of the Memorial Gym Annex.

31.) a) If the participants' personal files (school, medical, etc.) will be read, where are the files kept (name the place, e.g. doctor's office, hospital, clinic, etc.) and who will gather the information?

NA

b) Has permission been obtained to gather this information? (Attach documentation)

NA

c) Do the participants (and/or their parents or guardians) know that these files will be read? If no, explain.

NA

32.) a) Will individual results or other data be disseminated to the participants (and/or their parents or guardians)?

No

b) If so, explain the qualifications of the person(s) interpreting the results.

NA

33.) Does the proposed study involve deception?

☒ No ☐ Yes

(Please complete Part VII)

Part VII: Projects Involving Deception

- 34.) Describe the type of deception being used. Consider in your answer both deception by omission (an important aspect of the research is withheld from the subject) and deception by commission (the subject is misled about the true purpose of the research).
- 35.) Why is deception a necessary and unavoidable component of the experimental design? (Does the deception improve the internal or external validity of the study?)
- 36.) Has this research protocol (involving deception) been previously used? If "Yes," please provide information on any actual harms to the participants and reactions of the participants to the use of deception in this research.
- 37.) What alternative procedures were considered that did not involve deception and why were these alternatives rejected?
- 38.) Since deception precludes informed consent by the subject prior to participation:
- a.) How will participants be debriefed?
 - b.) Who will debrief them?
 - c.) Will the debriefing of participants be:
 - ☐ Immediate
(immediately following the experimental session in which deception occurs)
 - ☐ Delayed
 - ☐ Full (all deceptive aspects of the study will be revealed)
 - ☐ Partial (some deceptive aspects of the study will remain unexplained)
- 39.) If debriefing is delayed, why is delayed debriefing necessary and when will debriefing occur?

- 40.) If debriefing is partial, why is the partial debriefing necessary? Why is unexplained deception necessary? Would the subject be harmed in any way by full debriefing?
- 41.) Even if the subject is partially debriefed during the study, will full debriefing occur later?
- 42.) Does the presence of deception increase the risk of harm to the subject?
- 43.) Is the respondent free to withdraw his/her data after being fully debriefed? (e.g., form like audio/video taping).

Part VIII: Request for Waiver of Elements of Informed Consent

- 44.) Are you requesting a waiver of the documented informed consent form for each participant?

☐ Yes ☒ No

Please indicate the justification for requesting this waiver:

- ☐ The only record linking the subject to the research would be the signed consent document and the principal risk of the research would be breach of confidentiality.
- ☐ The research involves only minimal risk to the subjects and involves no procedures for which written consent is normally required outside of the research context (e.g. anonymous surveys of adults).

Note: Participants must still be provided with a written statement regarding the research that contains the required elements of informed consent.

- 45.) Are you requesting a waiver or alteration of any of the other required elements of informed consent? ☒ No ☐ Yes

(An IRB may, on occasion, approve a consent process that alters some or all of the required elements of informed consent or waive the requirement for informed consent. The following criteria must be met: 1) the research involves no more than minimal risk, 2) waiver or alteration will not adversely affect the rights and welfare of subjects, 3) the research could not practicably be carried out without waiver or

alteration, and 4) when appropriate, the subjects will be provided with additional pertinent information after participation.)

- a.) **Provide justification for the waiver.**
- b.) **Indicate why the proposed research presents no more than minimal risk to participants.**
- c.) **Explain whether or not a waiver of written informed consent would adversely affect the rights and welfare of participants.**
- d.) **Explain why it would be impracticable to carry out the research without a waiver or alteration of informed consent.**
- e.) **How will pertinent information be provided to participants, if appropriate, at a later date?**

Part IX: Conflict of Interest

- 46.) Do the researchers conducting this protocol have any potential conflicts of interest?** *Conflicts of interest may include financial or personal interest, or any condition in which the investigator's judgment regarding a primary interest may be biased by a secondary interest. Examples include speaking and consultation fees, travel expenses, stock options, royalties, company ownership or equity, etc.)*

☒ **No** ☐ **Yes**

(If yes, conflict of interest must be disclosed)

Investigator Assurance

I certify that the information provided in this application is complete and correct. I understand that as Principal Investigator, I have ultimate responsibility for the protection of the rights and welfare of human research subjects, the conduct of the study, and the ethical performance of the project.

I agree to comply with all Kent State University policies and procedures on research involving human subjects (KSU policy #3342-3-03.2), as well as with all applicable federal, state, and local laws regarding the protection of human subjects in research. I agree that:

- The project will be performed by qualified personnel, according to the KSU approved protocol.

- Approval from the Institutional Review Board will be obtained prior to implementing any changes to the protocol.
- If the project involves approval/permission from other institutions, the research will not begin until permission has been obtained from these institutions.
- Legally effective informed consent will be obtained from human subjects if applicable, and documentation of informed consent will be retained in a secure environment for three years after termination of the project.
- Injuries, adverse events, and/or unanticipated problems involving risks to subjects or others will be reported in writing to the Kent State University IRB promptly, and no later than within 5 working days of the occurrence.
- A Continuing Review and Progress Report will be completed and submitted before the review deadline, as determined by the IRB appropriate to the degree of risk (but not less than once per year). All protocols are approved for a maximum period of one year. Research must stop at the end of the approval period unless the protocol is re-approved for another term.
- All research staff, employees, and students assisting in the conduct of the research will be informed of their obligations and responsibilities in the above commitments.

I further certify that the proposed research will not begin until approval has been obtained. A signed approval letter from the Office of Research Safety and Compliance communicates IRB approval.

Signature of Principal Investigator

Date

Signature of Co-Investigator

Date

Faculty Advisor Assurance:

I have reviewed and approved the research project described in this application. I agree to meet with the student on a regular basis to monitor study progress and assure that the well-being of subjects is adequately safeguarded. I agree to be available to assist the student investigator should any problems arise in the study.

Signature of Faculty Advisor

Date

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