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Can caffeine alter blood potassium concentration or the perception of pain and fatigue after a 1 km cycling sprint?

by

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ABSTRACT

Caffeine is used by some athletes to improve endurance performance, however, the mechanism(s) by which caffeine elicits performance improvements have been unclear. The purpose of this study was to investigate the effects of caffeine on pain perception, fatigue perception, plasma catecholamine concentrations and plasma potassium concentrations to determine whether altered perception related to the central nervous system and potassium ion handling are associated with enhanced performance during a 1 km cycling time trial. A cross-over, double blind design of 13 well trained men (age: 27 ± 6 yrs, height: 180 ± 7 cm, body mass: 76.4 ± 6.4 kg, and VO_2max: 57.5 ± 4.6 $ml \cdot kg^{-1} \cdot min^{-1}$) were randomized to a caffeine (5 $mg \cdot kg^{-1}$) or a placebo condition. Caffeine had no significant effects on the 1 km time-trial performance indicators; time, peak power, or average power. In addition, caffeine had no significant effect on the perception of pain or overall fatigue. There was a significantly greater increase in post-exercise blood lactate, post-exercise catecholamines and lower pre-exercise blood potassium concentrations when caffeine was consumed. The results suggest that although there were no differences in performance time, caffeine caused changes in metabolic markers. In conclusion, caffeine consumption prior to a 1 km simulated cycling time-trial did not improve performance and its use is not warranted.

TABLE OF CONTENTS

List of Figures

List of Appendices

Can caffeine alter blood potassium concentration or the perception of pain and fatigue after a 1 km cycling sprint?

Introduction:

Caffeine has been used as a supplement by some athletes to achieve improved exercise performance that varies in durations and intensity. Caffeine has been previously viewed as an ergogenic aid for aerobic performance (Ganio et al, 2009; Cox et al, 2002; Ivy et al, 2009; Jackman et al, 1996; Graham et al, 1998; Bridge & Jones, 2006), with recent observations finding improvements during anaerobic events (Anselme et al, 1992; Wiles et al, 1992; Bruce et al, 2000; Anderson et al, 2000; Wiles et al., 2006). The two most accepted hypotheses on the mechanisms for the ergogenic properties of caffeine reported in the literature have been changes in potassium ion handling and an altered central nervous system perception. Decreased plasma potassium concentrations during aerobic exercise have been observed with caffeine supplementation (Lindinger et al., 1993), which has been used to indirectly indicate a maintenance of the sodium/potassium electrochemical gradient in the muscle (Davis & Green, 2009; Spriet & Howlett, 2000). Little research, however, has investigated the effects of caffeine on plasma potassium levels after anaerobic exercise (Davis & Green, 2009).

Caffeine has been shown to affect the central nervous system through direct stimulation of the adrenal gland, causing the release of catecholamines (Berkowitz et al, 1970) and also through blocking adenosine receptors (Smith et al., 2003). High-intensity anaerobic exercise has consistently shown increased catecholamine concentrations in blood in response to caffeine supplementation when compared with placebo (Bell et al.,

2001; Greer et al., 1998; Stuart et al., 2005; Doherty et al., 2002). Caffeine supplementation has also been shown to alter central nervous system perception through adenosine receptor blockade (Spriet & Howlett, 2000; Davis & Green, 2009; Davis et al, 2003). Adenosine has been shown to enhance pain perception, reduce arousal and induce sleep (Davis & Green, 2009; Davis et al, 2003). Decreased pain ratings have been observed with caffeine supplementation (Kalmar, 2005) while decreased fatigue ratings have also been hypothesized to be affected (Davis et al., 2003), which may be partly due to a decrease in adenosine receptor activation. The combination of decreased plasma potassium concentrations, which may reflect an enhanced sodium/potassium electrochemical gradient, coupled with diminished perceived pain and fatigue in the muscle from increases in circulating plasma catecholamines and caffeine's antagonistic effect on adenosine receptors, may contribute to enhanced anaerobic performance.

Therefore, the purpose of this study was to investigate the effects of caffeine on pain perception, fatigue perception, plasma catecholamine concentrations and plasma potassium concentrations to determine whether altered central nervous system perception and/or potassium ion handling are associated with an enhanced performance during a 1 km cycling time trial. It was hypothesized that caffeine supplementation would produce a faster 1 km time-trial in conjunction with both a lower pain and fatigue ratings coupled with an increase in plasma catecholamine concentrations, and attenuated increases in plasma potassium concentrations when compared to the placebo condition.

1.1 Significance of Study:

The mechanism through which caffeine exerts its ergogenic effects has not been completely elucidated during anaerobic exercise. This study attempted to determine

whether altered central nervous system perception in combination with potassium ion handling alterations at the cellular level as the primary mechanisms underlying the previously reported improved anaerobic performance with caffeine supplementation (Wiles et al, 2006). These alterations could be distinguished through increases in circulating plasma catecholamine concentrations, caffeine's antagonistic effect on adenosine receptors or indirectly through plasma potassium concentrations.
This study could determine whether caffeine supplementation is beneficial for 1 km sprint cyclists.

1.2 Delimitations:

Recruitment of volunteer subjects was restricted to trained males who had experience cycling. Only male subjects were used because of the effects that the menstrual cycle and oral contraceptive use might have on the elimination and metabolism of caffeine (Kalmar & Cafarelli, 1999) and time required to control for this was not practical for this thesis research. Pre-exercise caffeine consumption was controlled by asking subjects to consume the same meals prior to both test days, which consisted of no caffeine. Caffeine habituation has previously been found not to effect performance following a 24 abstinence from caffeinated products (Wiles et al, 1992). Exercise before the test was controlled through standardized instruction.

1.3 Limitations:

Healthy males between the ages of 18 and 39 were recruited for this study. The sample was calculated to be comprised of 15 volunteers. This is based on an effect size of 0.82 for within group results of a 1 kilometer cycling time-trial with caffeine supplementation (Wiles et al., 2006) and a power of 0.80 as calculated from Cohen

(1977, p 315). The factors of pre-test diet and exercise were logged by the subject for duplication before each test. A limitation was whether the subjects exert a maximal effort and power output during both the placebo and caffeine trails. A change in effort or power output may mask any potential effects of the ergogenic aid.

Literature Review:

2.1 Caffeine Supplementation & Performance:

Caffeine (1,3,7-trimethylxanthine) is a lipid soluble compound which is metabolized by the liver, resulting in the metabolites paraxanthine, theophylline and theobromine (Goldstein et al, 2010). Caffeine is a drug found in many everyday foods and drinks. The consumption of caffeine has continually increased over time with the increased popularity of energy drinks, sports drinks and caffeine enhanced consumables (Burke, 2008). The increased use of these substances may be attributed to a desire to enhance performance by increasing energy and alertness. Caffeine consumption has slowly shifted from social use to athletic supplement, to try and improve physical performance (Burke, 2008). The ergogenic effects of caffeine on endurance performance are well recognized, but the effects on anaerobic performance are still somewhat unclear (Davis & Green, 2009).

2.2 Caffeine & Aerobic Exercise

There has been considerable research showing that caffeine improves endurance performance (Ganio et al, 2009; Cox, et al 2002; Ivy et al, 2009; Jackman et al, 1996; Graham et al, 1998; Bridge & Jones, 2006; Irwin et al, 2011), with fewer publications reporting no effect (Hunter et al, 2002; Jacobson et al, 2001). It has been observed that

during endurance activities lasting 30-60 minutes, caffeine acts as an ergogenic aid which can enhance training and performance time.

2.3 Caffeine & Anaerobic Exercise

Research on the effects of caffeine supplementation on anaerobic performance is less conclusive (Davis & Green, 2009). Studies have shown that caffeine improves anaerobic power output, time to fatigue and time-trial times (Anselme et al, 1992; Wiles et al, 1992; Bruce et al, 2000; Anderson et al, 2000; Wiles et al., 2006; Doherty et al, 2004), while other studies suggest that there is no improvement (Crowe et al, 2006; Williams et al, 1988; Greer et al, 1998; Woolf et al, 2009; Glaister et al, 2012). A possible rationale for these varied results may be a lack of consistency between procedures, such as the administration time and dose of caffeine, testing protocol; and fitness level of participants.

2.4 Dose & Side Effects

The optimal timing for administration and dosage of caffeine has not been elucidated. Graham (2001) has reported that maximum plasma concentrations peak approximately 1 hour after ingestion. According to Graham (2001), a dose of caffeine between 3 and 6 mg/kg of body mass is recommended for improved aerobic performance times. The dose of 6 mg/kg seems to be the upper limit, with 9 mg/kg showing no additional decreases in aerobic performance time (Spriet & Howlett, 2000). Side effects that have been associated with higher doses of caffeine include jitters and increased anxiety (Yeomans et al., 2002) although these effects may be amplified in individuals with a lower caffeine tolerance (Pasman et al., 1995). Previous research has shown a trend in which both maximal and sub-maximal exercise protocols lasting longer than 30

seconds have shown performance improvements from caffeine (Anderson et al, 2000; Bruce et al, 2000; Wiles et al, 1992; Wiles et al, 2006) while the maximal protocols lasting 30 seconds or less have not (Woolf et al, 2009; Greer et al, 1998; Williams et al, 1988, Glaister et al, 2012). Trained cyclists, runners and rowers have shown greater improvements in maximal and sub-maximal aerobic performance than untrained individuals in the same events (Collomp et al, 1992). The training may have allowed the subjects to maximize the benefits from the caffeine.

2.5 Potential Mechanisms

Spriet and Howlett (2000) have organized various theories on how caffeine improves performance into three categories: 1) metabolic mechanisms, 2) skeletal muscle ion handling, and 3) altered central nervous system perception (CNS).

2.5.1 Metabolic Mechanism

The metabolic mechanism theory explains glycogen sparing. It suggests that the stimulation of adrenaline secretion by caffeine increases plasma free fatty acid (FFA) concentrations at the onset of exercise, which in turn spares glycogen stores (Goldstein, 2010). This theory, however, has limitations. Circulating catecholamines have been shown to increase, but plasma free fatty acid (FFA) which was increased at rest did not show further increases at the onset of steady state exercise (Graham et al., 2000). Contradicting these findings, Mohr and colleagues (1998) found that when tetraplegic subjects were administered caffeine and performed stimulated exercise on a modified cycle ergometer, there were no adrenaline increases, as observed in previous research, but there were increases in plasma FFA levels. The increase in plasma FFA does not explain the improved performance observed during short duration maximal anaerobic exercise

when glycogen does not seem to be a limiting factor (Graham T., 2001). Graham et al (2000) and Fletcher and Bishop (2012) have shown that the respiratory exchange ratio (RER) was unaltered with caffeine supplementation and that carbohydrate oxidation remained the dominant substrate. The unchanged RER between placebo and caffeine trials observed by Graham et al (2000) was explained by the increase in arterial blood glucose with caffeine supplementation. One aspect of carbohydrate metabolism that has consistently responded to caffeine supplementation is blood lactate levels, which have consistently increased during and following aerobic and anaerobic exercise (Graham et al, 2001; Anselme et al, 1992; Greer et al, 1998; Collomp et al, 1992; Jacobson et al, 2001; Jackman et al, 1996; Ivy et al, 2009; Cox et al, 2002; Graham et al, 1998; Bridge & Jones, 2006; Graham et al, 2000; Davis & Green, 2009). This increase in lactate may be due to caffeine inhibiting adenosine receptors altering CNS perception, thus allowing the subject to increase their exercise intensity (Davis & Green, 2009).

2.5.2 Skeletal Muscle Ion Handling

Skeletal muscle ion handling involves increased calcium release from the sarcoplasmic reticulum, or a decrease in plasma potassium concentration after caffeine administration (Graham, 2001). Electrochemical gradients must be maintained to preserve membrane potentials (Magkos & Kavouras, 2005). Fatigue could be caused by a decreased resting membrane potential from the efflux of potassium to the plasma or decreased calcium release from the sarcoplasmic reticulum (Graham, 2001). A blockage in stimulus conduction is thought to be caused by high concentrations of potassium in the transverse tubule which could lead to muscular fatigue (Tarnopolsky, 2008). Following potassium accumulation in the transverse tubule during muscle activity, potassium has

been found to diffuse into capillaries, thereby increasing plasma potassium concentrations (Nielsen & de Paoli, 2007). Plasma potassium concentrations have been used to indirectly indicate sodium/potassium fluxes at the cellular level (Davis & Green, 2009; Nielsen & de Paoli, 2007). A better maintained sodium/potassium electrochemical gradient has been found to result in a more forceful muscle contraction (Spriet & Howlett, 2000). Exercise induced plasma potassium concentrations have been observed to attenuate with caffeine supplementation during aerobic work (Lindinger et al., 1993). Four studies have been conducted investigating plasma potassium concentrations during caffeine supplemented anaerobic work (Crowe et al, 2006; Doherty et al., 2002; Greer et al., 1998; Mohr et al., 2011). Davis and Green (2009) noted that there were mixed results in regards to plasma potassium levels with caffeine consumption and future research was recommended. Potassium efflux from muscle tissue into blood plasma has been found to increase with increased exercise intensity (Nielsen & de Paoli, 2007), which may allow for larger drops in plasma potassium concentrations during maximal anaerobic exercise with caffeine supplementation.

Fatigue has been shown to occur when calcium movement is altered and causing an increased concentration within the sarcoplasmic reticulum (Tarnopolsky, 2008). Increased mobilization of calcium, which has been observed after caffeine supplementation, decreased the required membrane potential and in turn, lowered the mechanical threshold (Magkos & Kavouras, 2005). This may positively affect excitation-contraction coupling, thus postponing muscular fatigue (Davis & Green, 2009). Excitation-contraction coupling is the process by which the surface membrane events cause a release of calcium from the sarcoplasmic reticulum (Magkos & Kavouras,

2005). An action potential depolarizes the transverse tubule which stimulates the release of calcium from the sarcoplasmic reticulum. The resulting calcium binds to troponin in the actin filaments, which stimulates the actin to combine with myosin ATPase, causing myosin cross-bridge movement (Magkos & Kavouras, 2005). Caffeine supplementation has been reported to affect the sarcoplasmic reticulum calcium release channel (Magkos & Kavouras, 2005). The increases in calcium mobilization from the sarcoplasmic reticulum have been observed in a lab setting (Davis & Green, 2009). The dose of caffeine required to observe this effect would, however, be toxic to humans (Davis & Green, 2009). This makes it unlikely that calcium mobility plays a critical role in the ergogenic effects of caffeine.

2.5.3 Altered Central Nervous System Perception

The central mechanism theory is the most accepted explanation of the ergogenic effects of caffeine. According to this theory, caffeine affects the central nervous system through direct stimulation of the adrenal gland (Berkowitz et al, 1970) and by blocking adenosine receptors (Smith et al., 2003). High-intensity exercise has consistently shown increased catecholamine concentrations in blood in response to caffeine supplementation when compared with placebo (Bell et al., 2001; Greer et al., 1998; Stuart et al., 2005; Doherty et al., 2002). In a controlled state, 30 second Wingate tests have elicited increased norepinephrine and epinephrine concentrations from 2.70$\pm$ 0.21 nmol/L pre-exercise to 7.26$\pm$ 0.50 nmol/L post-exercise and 348.3$\pm$ 24.6 pmol/L pre-exercise to 647.8$\pm$ 79.4 pmol/L post-exercise, respectively (Bell et al, 2001). In contrast, 30 second Wingate tests supplemented with caffeine have elicited norepinephrine and epinephrine concentration increases from 3.30$\pm$ 0.20 nmol/L pre-exercise to 9.52$\pm$ 0.63 nmol/L post-

exercise and 451.6± 31.3 pmol/L pre-exercise to 895.8± 118.4 pmol/L post-exercise, respectively (Bell et al, 2001). These significant increases in catecholamine levels are indicative of the effect of caffeine on the central nervous system. In addition, caffeine's lipid solubility characteristic permits easy travel across the blood-brain barrier, allowing caffeine to reach the central nervous system and adenosine receptors, and thus stimulating and blocking adenosine receptors at the brain (Spriet & Howlett, 2000; Davis & Green, 2009; Davis et al, 2003). Adenosine receptors are also found throughout the body in smooth muscle and skeletal muscle (Lynge & Hellsten, 2000). Adenosine has been known to enhance pain perception, reduce arousal and induce sleep (Davis & Green, 2009; Davis et al, 2003). Caffeine, having a structure similar to adenosine, may increase time to fatigue by blocking adenosine receptors (Davis et al., 2003). Davis and Green (2009) noted that the breakdown of adenine nucleotide regulates adenosine release, which, in turn, increases adenosine concentration during exercise (Davis et al, 2003). Caffeine has been reported to inhibit adenosine receptors, increasing wakefulness and decreasing pain perception (Davis et al, 2003). Spriet and Howlett (2000) noted that the antagonism of adenosine receptors causes an increase in the concentration of neurotransmitters. Adenosine generally causes decreases in motor activity, leading to fatigue and decreased wakefulness, and decreased neurotransmitter concentrations (Spriet & Howlett, 2000). The two main adenosine receptors that caffeine is thought to work through are A_1 and A_2, depending on the concentration of caffeine (Spriet & Howlett, 2000). The extent to which caffeine can reduce the perception of pain and fatigue can be measured by numerical scales which assess exertion, pain, fatigue and other mood states following exercise (Gliottoni & Motl, 2008; LeMura et al, 2001; Hampson et al, 2001).

Perceived pain has been speculated to reduce muscular power output. This speculation is supported by Davis and Greene (2009) who observed that pain decreased motor unit firing rate. Therefore, it has been hypothesized that the decreased RPE (rate of perceived exertion) scores observed with caffeine supplementation during aerobic exercise may be the result of a decreased firing threshold, meaning more motor units were recruited, increasing the muscular force and power output for any given stimulus (Spriet & Howlett, 2000). RPE has been used as an indirect method to measure afferent and efferent neural activity (LeMura et al, 2001; Hampson et al, 2001; Doherty & Smith, 2005). Hampson et al (2001) listed sources of afferent information that could influence RPE scores. The list included heart rate, blood lactate and pH changes, muscle pH changes, sensation of mechanical strain and damage to exercising muscles. Caffeine, due to its inhibitory effects on adenosine receptors, may alter the perceived stimuli received by the brain during exercise. The stimuli may be altered at the muscular level, before the signal is transported to the brain or at the level of the brain since adenosine receptors have been observed at both locations (Lynge & Hellsten, 2000; Fredholm et al, 1999).

Davis and colleagues (2003) injected rats with caffeine (an adenosine antagonist) or 5'-N-ethylcarboxamidoadenosine (NECA), an adenosine agonist. The doses of both caffeine and NECA were administered intracerebroventricularly, and the rats were run to fatigue. Davis et al (2003) observed that the rats injected with caffeine demonstrated significantly increased run times to fatigue and spontaneous locomotor activity, while the rats injected with NECA reached fatigue much faster. These researchers also found that, in the rats receiving caffeine and NECA, the caffeine neutralized the NECA inhibitory

effects. Davis et al (2003) found no significant difference, in concentrations of muscle glycogen, plasma glucose or FFA, between rats injected with either caffeine or NECA.

The current research supports the notion that caffeine is an effective ergogenic aid for aerobic performance. The exact mechanism through which caffeine works to improve performance and delay fatigue has not yet been completely elucidated, but it appears that the central nervous system and potassium ion handling at the cellular level may be involved. It is unlikely that caffeine acts through the metabolic theory of glycogen sparing, because the theory does not explain the improvements observed during short duration anaerobic activity, where levels of muscle glycogen is not a limiting factor. Increased catecholamine levels, secondary to the ingestion of caffeine and reduced fatigue and pain scores support a CNS mechanism theory, possibly through adenosine antagonism, and result in enhanced performance during a 1 km cycling time-trial. The literature indicates that performance time may be improved by attenuated plasma potassium levels with caffeine supplementation.

Methods & Procedures

3.1 Subject selection:

Thirteen trained males (age: 27 ± 6 yrs, height: 180 ± 7 cm, body mass: 76.4 ± 6.4 kg, and VO_2max: 57.5 ± 4.6 $ml \cdot kg^{-1} \cdot min^{-1}$) with cycling experience were recruited from the University of Alberta and various cycling clubs. The subjects were screened for medical conditions that would prevent participation in the study. The study was approved by the University of Alberta Research Ethics Board for human subject research and all participants provided verbal and written consent. Subjects varied greatly in caffeine habituation.

3.2 Purpose and Hypothesis:

The purpose of this study was to investigate the effects of caffeine ingestion on pain perception, fatigue perception, plasma catecholamine concentrations and plasma potassium concentrations after a 1 km cycling time trial. It was hypothesized that caffeine supplementation would produce a faster 1 km time trial in conjunction with both a lower pain and fatigue rating coupled with an increase in plasma catecholamine concentrations and attenuated increases in plasma potassium concentrations when compared to the placebo condition.

3.3 Variables:

The independent variable was the type of supplement ingested, either caffeine or placebo. In order to reduce researcher bias, each treatment was administered in a double blind fashion. The caffeine was masked in a drink that was the same volume and flavor as the placebo. The blinding was done by an individual who was not directly involved with the study.

The main dependent variables were 1km performance time, pain perception, fatigue perception, plasma catecholamine concentrations and plasma potassium concentration, assessed before and after a 1 km cycling time-trials in both experimental conditions. Other secondary dependent variables were blood lactate concentrations and blood pH since previous research has observed increased blood lactate concentrations (Graham et al, 2001; Anselme et al, 1992; Greer et al, 1998; Collomp et al, 1992; Jacobson et al, 2001; Jackman et al, 1996; Ivy et al, 2009; Cox et al, 2002; Graham et al, 1998; Bridge & Jones, 2006; Graham et al, 2000; Davis & Green, 2009). .

3.4 Research design:

This research study used a randomized double-blind, crossover design with repeated measurements made over time. The within subject design was preferable as it required fewer subjects, increased statistical power, and reduced biological variability between individuals.

3.5 Procedure:

On day one, subjects arrived at the lab and signed a written consent form which was provided to them prior to this date by e-mail to allow the subject to familiarize themselves with the risks and procedures. The risks were explained verbally to ensure clarity. If a subject accepted the risks and signed the consent form, they then completed a PAR-Q form. Provided the PAR-Q was negative, a graded exercise test (GXT) to exhaustion to determine aerobic power (VO_{2max}) was performed on a Velotron stationary cycle (Racer Mate, Seattle, WA, U.S.A.). The GXT was done in order to determine a power output of 20% of the subject's VO_{2max} which was used as the power output during exercise recovery. The subjects began the GXT at a power output of 100 watts which was increased by 25 watts every minute until volitional exhaustion (Elliott & Grace, 2010). Expired gases were collected and analyzed for O_2 and CO_2 with a metabolic cart (Parvo Medics TrueOne 2400, Utah) that was calibrated before and after each test with known gas concentrations. Heart rates were determined every minute using a heart rate monitor (Polar Electro, Finland). VO_{2max} was determined as the point at which there was a peak and plateau in oxygen uptake (< 100mL/min) with increasing workload that was associated with secondary criteria including a respiratory exchange ratio greater than 1.1, a heart rate equal to or greater than age-predicted maximum and volitional exhaustion (Syrotuik et al, 2005). The participants were assisted with setting up the Velotron cycle

by adjusting the vertical and horizontal position of the seat and handle bars to mimic their own racing bicycle. These measurements were recorded so that they could be replicated during subsequent experimental trials. The participants were also allowed to use their own pedals. Each participant was instructed on how to perform the 1 kilometer time-trial protocol and performed one practice trial. On departure from the familiarization session, subjects were instructed to avoid all forms of caffeine, alcohol and intense physical activity for 24 hours prior to the experimental trials. The subjects were asked to consume the same meals the day prior to both testing sessions.

On days 2 and 3, subjects arrived at the lab between 7 and 10am. A resting blood sample of 10 ml was taken using veinipuncture, by an individual trained in this procedure. Each subject then consumed a randomly assigned treatment of either placebo (500ml of water with "Crystal Light') or caffeine at a serving of 5mg/kg body mass (Graham, 2001) dissolved in 500ml of water containing "Crystal Light". Following consumption of the caffeine, each subject rested 1 hour, which has been shown to be an effective duration for caffeine absorption into the blood stream (Wiles et al., 2006). The Velotron cycle was set-up according to the previously determined measurements and preferred riding position determined on day 1. Thirty minutes before the 1 km time trial began, an intravenous catheter was inserted into a forearm vein by a registered nurse and a 10 ml sample was collected. The site was kept patent with 0.5 ml of sterile saline (0.9% NaCl) and prior to each blood sample, a small (2-3 ml) blood sample was drawn and discarded to remove any saline present. A second 10 ml blood sample was withdrawn prior to a 10 minute warm-up consisting of a self-selected intensity with three 5 second sprint intervals, followed by a 5 minute period for the subjects to stretch and

recover with an additional 5 minute period for set up and preparation. This warm-up was monitored and remained the same for both trials. During both 1 km time-trials participants were provided with consistent verbal encouragement to provide an all-out effort. Subjects were also provided with a visual marker on a computer monitor indicating the distance through the 1 km time-trial. The participants were not provided with the time at any point throughout or following the 1 km time-trial.

A blood sample of 10 ml was taken immediately before the 1 km time-trial began. The selected cycle gear ratio was 48/14, which elicited a speed of 40 kilometers per hour at 90 rpm. This speed has been shown to elicit improved 1 km time-trial times with caffeine supplementation by Wiles et al. (2006). The time trial was recorded to one tenth of a second. Following the 1km cycle, blood samples of 10 ml were taken immediately after or as soon as feasibly possible (0), 5, 10 and 15 minutes of recovery (Appendix A). The subjects completed a standardized recovery that consisted of cycling at an intensity equivalent to 20% VO_{2max} for 5 minutes followed by a passive recovery while sitting on a chair. Following the completion of the time-trial, the subjects completed the categorical Pain Perception Scale (Appendix B) (Cook et al., 1998) and visual analogue fatigue scales (Appendix C) (Egerton et al., 2009; Leung et al., 2004; Lee et al., 1991).

The placebo and caffeine trials were performed once per week with a minimum of seven days in between, allowing for appropriate washout and recovery. Magkos & Kacouras (2005) found the half-life of caffeine to be 2.5-10 hours.

3.6 Special Equipment & Tools:

The subjects performed the test on an electrically braked cycle ergometer **(**Velotron Electronic Bicycle Ergometer, Racer Mate, Seattle, WA) to allow for a cycling

position that closely represented racing bicycles. A numerical pain perception scale (Appendix B) was used to assess perceived leg pain following the test (Gliottoni & Motl, 2008). This numerical pain perception scale has been used previously and has been found to be reliable and valid to assess leg pain during and following high intensity cycling (Cook et al., 1997). Visual analogue scales were used to assess feelings of fatigue (Appendix C) and have been shown to be valid and reliable to assess overall fatigue in a resting state (Lee et al, 1991). This visual analogue scale has also been used to assess fatigue following physical activity in healthy older adults (Egerton et al 2009) as well as older adults of various disease states following exercise (Pickard-Holley, 1991; Egerton et al, 2009). One scale assessed overall fatigue anchored by the points "Not at all fatigued" and "Extremely fatigued" (Underwood et al, 2006). Another scale assessed leg fatigue anchored by the points "Not at all fatigued" and "Extremely fatigued".

3.7 Blood Procedures and Assays

Blood lactate levels were measured in the lab in duplicate spectrophotometrically (Gutmann & Wahlefeld, 1974). Blood pH and electrolyte analysis was performed in single using an Abbott point of care i-STAT hand held analyzer with $EG7^{+}$ cartridge (Abbott Laboratories, Princeton, New Jersey). The blood samples were collected by the nurse in two 10 ml syringes. A few drops of blood were immediately placed in a cartridge for pH and electrolyte analysis while the remaining blood was placed in a test-tube coated in Ethylenediaminetetraacetic acid (EDTA). Two-hundred and fifty (250) ul of whole blood was removed from this tube and placed in 1ml of 8% perchloric acid for deproteinization and vortexed. The deproteinized and EDTA treated blood tubes were then centrifuged for 10 minutes at 1500 x gravity and then decanted into microcentrifuge

tubes and frozen immediately at -20ºC and then transferred to a -80ºC ultra low freezer until the lab analyses were performed. Epinephrine and norepinephrine was determined in duplicate using an enzyme-linked immunosorbent assay kit (ELISA) (Rocky Mountain Diagnostics Inc, Colorado Springs, CO).

3.8 Data analysis:

Data from the 2 experimental trials were compared using a two factor repeated measures ANOVA. A one-way repeated-measure ANOVA provided an overall test of significance for the mean differences between performance times. If a significant F-ratio was determined, a Newman-Keuls multiple comparison procedure was performed to determine which mean differences were significantly different. An alpha level of $p \leq 0.05$ was set to determine significance for all statistical analyses. All data are means $\pm$ standard deviation unless otherwise noted. Statistica, version 8.0 (StatsSoft Inc., Tulsa, OK) was used to perform all statistical analysis.

Results:

All 13 subjects were able to complete the study, 12 of which correctly guessed which condition they were given based on feelings of "jitters" and "readiness". Four subjects had missing data points for potassium and pH concentrations due to instrument and/or user error and were removed from analysis of these variables.

4.1 Performance Parameters:

Caffeine ingestion did not improve the simulated 1 km cycling performance time (caffeine vs. placebo: 82.1±2.4 vs. 81.9±3.9 seconds), peak power output (caffeine vs. placebo: 633.0±83.6 vs. 638.7±110.1 watts) or average power output (caffeine vs.

placebo: 466.0±37.3 vs. 467.5±59.9 watts). Individual values for 1 km finish time under placebo and caffeine conditions are shown in (Figure 1).

4.2 Perceived Pain and Fatigue:

The numerical pain scale revealed that caffeine did not reduce perceived pain immediately following a 1 km cycling time-trial (caffeine vs. placebo: 5.6±2.4 vs. 5.5±2.6). The fatigue visual analogue scales revealed that caffeine ingestion did not attenuate leg fatigue (caffeine vs. placebo: 7.1±1.4 vs. 7.4±2.0) or overall fatigue (caffeine vs. placebo: 7.1±1.8 vs. 7.1±1.8) compared to the placebo condition.

4.3 Blood Variables:

Blood lactate concentrations were significantly increased post exercise in both the caffeine and placebo condition when compared to pre-1 km cycling time-trial concentrations ($p<0.001$). Caffeine ingestion caused a significant increase ($p<0.05$; Figure 2) in blood lactate concentrations 0, 5 and 15 minutes post-1 km cycling time-trial compared to the placebo condition.

Caffeine significantly attenuated blood potassium concentrations immediately prior to the 1 km cycling time-trial compared to the placebo condition ($p<0.05$; Figure 3). Blood potassium levels were significantly elevated ($p<0.001$) immediately following the 1 km cycling time-trial in both the caffeine and placebo conditions.

Blood pH was significantly higher pre-exercise compared to all time points post-exercise ($p<0.001$) in both the caffeine and placebo conditions. Caffeine consumption did not alter pH levels at any time point when compared with the placebo condition ($p>0.05$; Figure 4).

Epinephrine concentration in the blood was significantly increased immediately post-exercise as well as 5 minutes post-exercise compared to all pre-exercise times, the 10 minute post-exercise and the 15 minute post-exercise time points in both placebo and caffeine conditions ($p<0.001$). Caffeine consumption significantly increased epinephrine concentrations immediately following the 1 km cycling time-trial as well as 5 minutes post-exercise when compared to the placebo condition ($p<0.05$; Figure 5).

Immediately post-exercise, as well as 5 minutes and 10 minutes post-exercise, norepinephrine concentrations were significantly increased in the blood when compared to all pre-exercise time points in both the caffeine and placebo conditions ($p<0.001$). Caffeine consumption significantly increased norepinephrine concentrations immediately following the 1 km cycling time-trial as well as 5 minutes post-exercise when compared to the placebo condition ($p<0.05$; Figure 6).

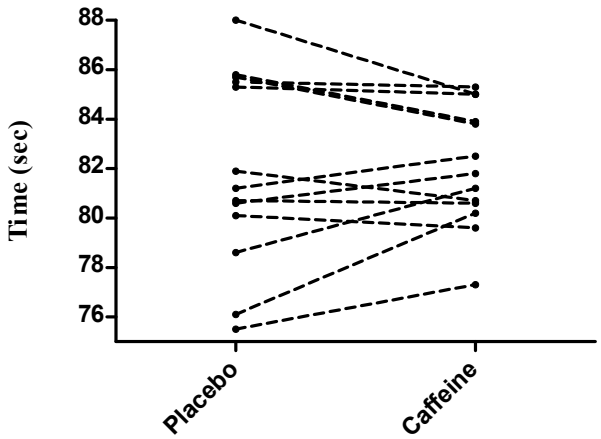

Fig. 1. Individual values for 1 km time-trial finish time under placebo and caffeine conditions.

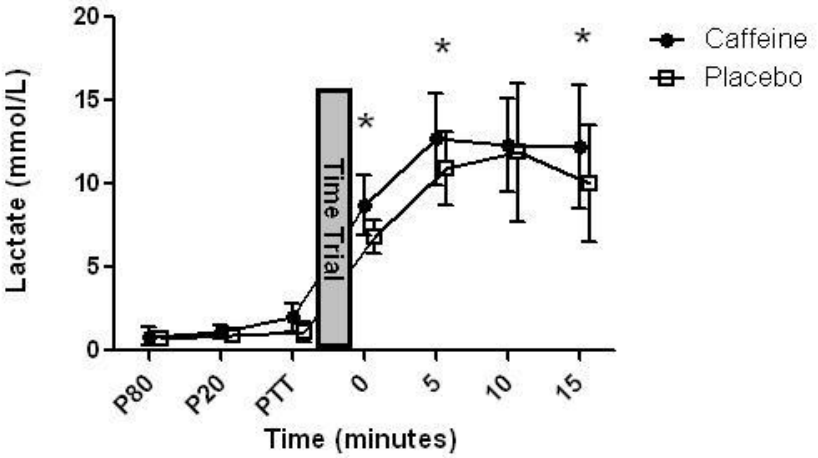

Figure 2. Blood lactate responses (means ±SD; n=13) prior to and during recovery from cycling 1 km after consuming caffeine or placebo. P80 = 80 minutes pre-time trial; P20 = 20 minutes pre-time trial; PTT=immediate pre-time trial; TT= 1 km time trial. * Significant difference between caffeine condition and placebo, $p<0.05$

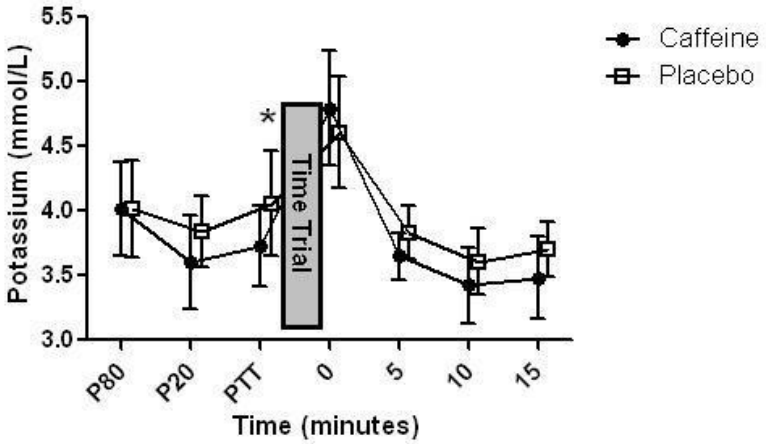

Fig. 3. Blood potassium responses (mean±SD; n=9) prior to and during recovery from cycling 1 km after consuming caffeine or placebo. P80 = 80 minutes pre-time trial; P20 = 20 minutes pre-time trial; PTT=immediate pre-time trial; TT= 1 km time trial. * Significant difference between caffeine condition and placebo, $p<0.05$

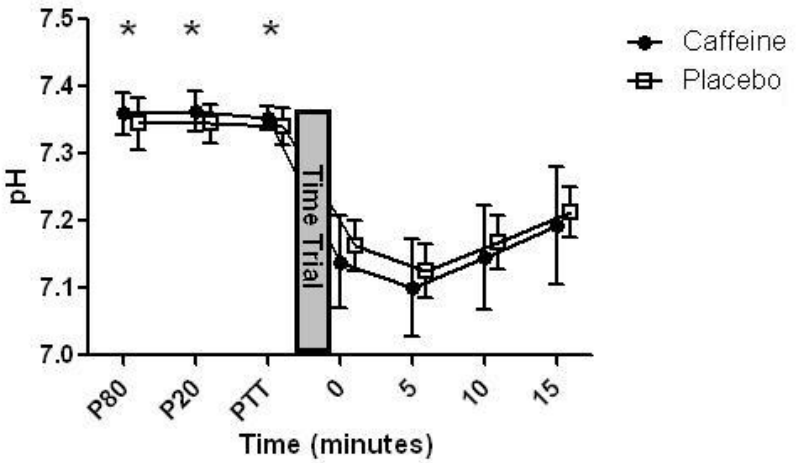

Fig. 4. Blood pH responses (mean±SD; n=9) prior to and during recovery from cycling 1 km after consuming caffeine or placebo. P80 = 80 minutes pre-time trial; P20 = 20 minutes pre-time trial; PTT=immediate pre-time trial; TT= 1 km time trial.*, both caffeine and placebo conditions are significantly different from all post time trial measures.

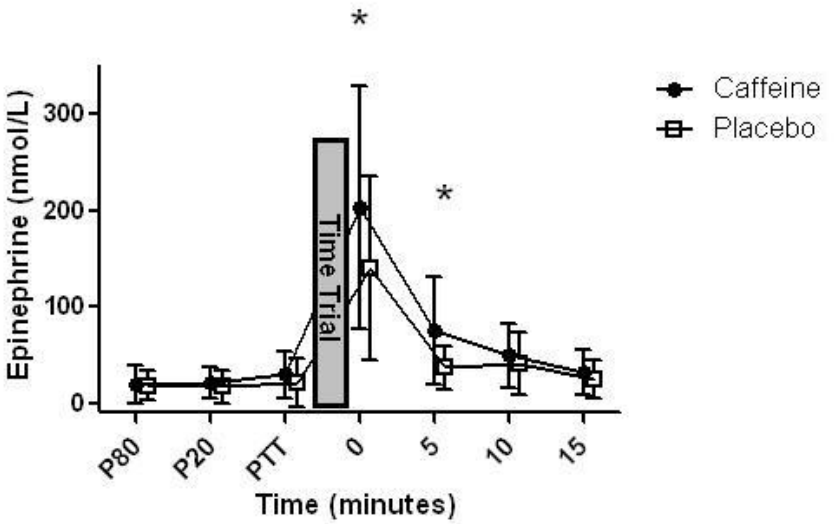

Fig. 5. Blood epinephrine (Epi) responses (mean±SD; n=13) prior to and during recovery from cycling 1 km after consuming caffeine or placebo. P80 = 80 minutes pre-time trial; P20 = 20 minutes pre-time trial; PTT=immediate pre-time trial; TT= 1 km time trial. * Significant difference between caffeine condition and placebo, $p<0.05$

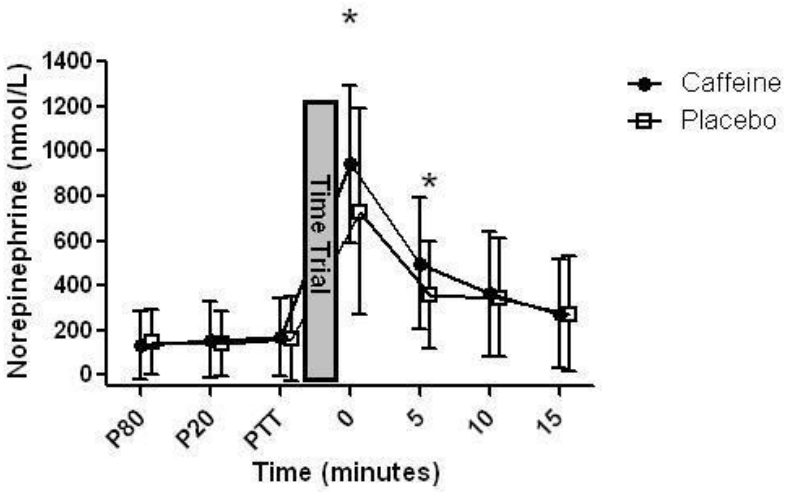

Fig. 6. Blood norepinephrine (Nor) responses (mean±SD; n=13) prior to and during recovery from cycling 1 km after consuming caffeine or placebo. P80 = 80 minutes pre-time trial; P20 = 20 minutes pre-time trial; PTT=immediate pre-time trial; TT= 1 km time trial. * Significant difference between caffeine condition and placebo, $p<0.05$

Discussion:

Athletes in a variety of sports consume caffeine and products containing caffeine to enhance their aerobic performance (Burke, 2008). Caffeine's ability to improve aerobic activities is well established while caffeine's effects on anaerobic activities are less conclusive (Davis & Green, 2009). This study examined the hypothesis that caffeine supplementation would produce a faster 1 km time trial in conjunction with both a lower pain and fatigue ratings coupled with an increase in plasma catecholamine concentrations and attenuated increases in plasma potassium concentrations, when compared to the placebo condition. The findings of this study demonstrated that caffeine ingestion, at a dose of 5 $mg \cdot kg^{-1}$ body mass, did not elicit improved performance time, peak power output, or average power output during a 1 km cycling time-trial. These findings corroborate previous research which also found no performance improvements in anaerobic activities combined with caffeine supplementation (Crowe et al, 2006;

Williams et al, 1988; Greer et al, 1998) but contradict other research which found improved 1 km cycling time-trial performance following caffeine ingestion (Wiles et al, 2006). Wiles et al (2006) may have observed improved 1 km time-trial performance with caffeine supplementation due to the high fitness level of their subjects. Crowe et al (2006) and Williams et al (1988) used subjects not specifically trained in cycling, and Greer et al (1998) used subjects not accustom to intense exercise. Collectively, the fitness status of the subjects seems to be a very important factor in observing the beneficial effects of caffeine on performance measures.

There was no difference between caffeine and placebo conditions for post-exercise pain rating. These findings corroborate the research conducted by Astorino et al (2011), which used the same pain scale as the current study and found that caffeine did not decrease the perception of pain during a high intensity knee extension protocol. Overall fatigue and fatigue localized to the legs were unaltered with caffeine supplementation in the current study. A possible explanation for this lack of apparent changes in perceived pain or fatigue with the caffeine condition may have been related to the timing of when subjects completed the pain and fatigue scales, post-time trial. This data was collected 5 minutes post-time-trial or as soon as possible thereafter. The collection of this data was delayed for logistical issues as a result of the participant actively cooling down on the Velotron while still hooked up the metabolic cart. This made the recording of the visual analogue scale challenging. This time delay in data collection may not accurately reflect the pain and fatigue subjects were experiencing at the very end of the 1 km time-trial.

Caffeine ingestion caused significant time and interaction effects for blood lactate concentrations but did not influence resting pre-exercise blood lactate levels. This is consistent with previous findings (Bridge et al, 2006; Glaister et al, 2012). Lactate concentrations were significantly increased immediately after and 5 and 15 minutes post-exercise with caffeine ingestion compared to placebo. The results from this study corroborate previous studies, which found caffeine to increase post-exercise lactate concentrations greater than placebo (Graham et al, 2001; Anselme et al, 1992; Greer et al, 1998; Collomp et al, 1992; Jacobson et al, 2001; Jackman et al, 1996; Ivy et al, 2009; Cox et al, 2002; Graham et al, 1998; Bridge & Jones, 2006; Graham et al, 2000; Davis & Green, 2009). Graham (2001) suggested that the increase in blood lactate may be due to caffeine inhibiting lactate clearing. This may help explain the observed increase in blood lactate without increased performance in the present study.

The findings of the present study confirmed that plasma potassium levels increase with exercise with the greatest increases occurring immediately following the 1 km simulated cycling time-trial. Prior to consuming either the caffeine or the placebo and one hour post-consumption of either treatment, there was no significant difference between plasma potassium concentrations. Following the standardized 10 minute warm up, caffeine consumption elicited a significantly attenuated plasma potassium concentration compared to the placebo condition. This may have been caused by caffeine's ability to accelerate muscle potassium handling (Mohr et al, 2011). Mohr et al (2011) suggested that caffeine may stimulate the Na^+-K^+ pump indirectly through increased catecholamine response and increased glucose concentrations, or directly at the muscle. Mohr et al (2011) hypothesize that caffeine may accelerate the Na^+-K^+ pump

activation at the start of exercise. The ability to maintain forceful muscle contractions at a high frequency is dependent on the capability to return potassium from the interstitial space back into the muscle cell following a muscle contraction (Green, 1997). The initial attenuation of potassium efflux with caffeine supplementation should allow for increased cellular concentrations during the early stages of sprint performance. The decreased plasma potassium levels prior to exercise may enhance the potential for an improved sprint performance, however, this was not observed in the current study.

Blood pH levels were significantly higher prior to the 1 km simulated cycling time-trial than post-exercise in both treatments. Due to the breakdown of glycogen in working muscle during exercise, there is an increase in H^+ concentrations which decreases pH levels following exercise (Medbø et al, 2009). Caffeine ingestion did not produce different pH concentrations at any time point compared to placebo. The increased blood lactate concentrations, found in this study, without an associated decrease in blood pH support the hypothesis that the increase in blood lactate could be due to caffeine inhibiting lactate clearance rather than increased production (Graham, 2001).

Plasma catecholamine levels were significantly higher immediately following the 1 km simulated cycling time-trial and 5 minutes after in both the caffeine and placebo conditions compared to resting values. Norepinephrine was also significantly higher 10 minutes post-exercise compared to resting values. These findings were consistent with the results of Zouhal et al. (2008), who reported an increase in catecholamine concentrations with exercise and suggested that the increases were closely related to intensity. Caffeine supplementation significantly increased both epinephrine and

norepinephrine concentrations immediately following the 1 km simulated cycling time-trial, as well as 5 minutes post-exercise greater than placebo. These findings support previous research which found caffeine consumption induced increased catecholamine concentrations, compared to placebo, following high intensity anaerobic exercise (Bell et al., 2001; Greer et al., 1998; Stuart et al., 2005; Doherty et al., 2002; Collomp et al., 1991). The increase in both epinephrine and norepinephrine plasma concentrations with caffeine supplementation indicate caffeine stimulated the central nervous system. These physiological changes in hormones levels cannot, however, be used to differentiate whether the increased epinephrine and norepinephrine concentrations were due to caffeine directly stimulating the adrenal gland, thus enhancing production (Berkowitz et al, 1970), or from the suggested blocking mechanism of the adenosine receptors (Smith et al, 2003).

Although positive changes were observed at both the central nervous system and cellular levels, which might have allowed for a better performance in the 1 km cycling time trial, there were no performance improvements documented. A possible explanation is that the subjects were not highly trained at sprint cycling, which could have led to a level of learning during each trial. Each trial may have been paced differently by the subjects, as previously discussed.

Although the mean 1 km cycling performance time was not significantly improved, there appears to be a person-by-treatment effect with caffeine ingestion with 8 of the 13 subjects (62%) performing a faster 1 km sprint under the caffeine condition (1.1± 1.0 seconds). The documented improvement in 1 km sprint times in this sub-set of 8 individuals would support that some participants, due to their biological variability,

might be considered responders to caffeine supplementation (Syrotuik and Bell, 2004). Certainly further analysis and follow-up research would be required to confirm this responder phenomenon.

In addition, although subjects had an orientation for familiarization to the 1 km simulated cycling time-trial, and subjects were instructed to perform an all-out effort, pacing may have confounded the results of this study and other sprint studies (Glaister et al, 2012). Following the first trial, subjects may have altered their strategy if they believed it would achieve a faster time or avoid physiological consequences, such as hydrogen ion accumulation and associated perceived pain within the muscles, experienced the first day (Zajac et al, 1999). Changes in pacing could, thus, mask the ergogenic effects of caffeine and its ability to improve all out sprint performance that a 1 km cycling time trial demands. An order effect analysis revealed that there was not a significant difference between 1 km finish time between experimental day 1 and day 2. If the same effort and pacing strategy was implemented during both trials, the changes in the central nervous system and in potassium ion handling, associated with acute caffeine supplementation, might have resulted in improved performance.

Although the participants in this study were all physically fit individuals with a range of experience cycling (1-15 years), only one subject had specific sprint cycling experience which may have accounted for the lack of improved performance.

Strengths and Shortcomings

6.1 Strengths

This study used a 1 km cycling time-trial and trained subjects to closely simulate a sporting event making the results more relevant to athletic performance. The dose of caffeine administered to the subjects (5 mg/kg body mass) has previously been shown to

improve anaerobic performance (Wiles et al, 2006) with even lower doses showing improvements in aerobic performance (Bridge & Jones, 2006). The study design was a double blind cross-over to reduce biological variability.

6.2 Shortcomings

Although trained subjects were used for this study, they were not specifically trained in sprint cycling. The fatigue scale used for this study, although used by others studying the effects of caffeine and performance (Astorino et al, 2011.) has only been shown to be valid and reliable during rest and in older adults who are physically active or suffer from various disease states. The fatigue and pain scales were also administered following the active cool-down rather than immediately after the sprint which may have altered the results.

Future Research Direction

In future research for sprint exercise, the number of orientation trials each subject performs should be increased to limit possible pacing strategies during condition days. In addition, more research should be performed on caffeine's effects on potassium ion handling during sprint performance, rather than steady state exercise. A more sensitive measure for adenosine blocking and its effects on an individual's pain and fatigue would greatly benefit caffeine research. Further research needs to be conducted into whether increased catecholamine levels are due to direct stimulation of the central nervous system, alone, or if adenosine receptor blocking contributes to this increase in catecholamine levels. Finally, the pain perception scale (Cook et al, 1998) and visual analogue scales for fatigue used in the present study, combined with the timing that these

instruments were administered post-exercise (Underwood et al, 2006), may not have been sensitive enough to detect perceived changes in pain and fatigue with caffeine usage.

Conclusion

The purpose of this study was to investigate the effects of caffeine on pain and fatigue perception, plasma catecholamine concentrations and plasma potassium concentrations, to determine whether altered central nervous system perception and potassium ion handling were associated with an enhanced performance during a 1 km cycling time-trial. The results of this study suggest that caffeine ingestion at a dose of 5 mg·kg-1 body mass did not improve simulated 1 km cycling time-trial performance. Consumption of caffeine prior to a 1 km simulated cycling time-trial did, however, attenuate potassium levels in plasma prior to the sprint performance and increased post-exercise catecholamine and lactate levels over the placebo group. These metabolic changes were not reflected in an improved 1 km performance, possibly due to changes in pacing strategies implemented by the subjects and/or the fitness status and experience with 1 km sprinting. Despite the lack of statistical significance for 1 km performance times between the caffeine and placebo conditions, there did appear to be a person-by-treatment effect, with 8 out of 13 subjects actually improving their performance times. This information is important for sprint cyclists who may supplement with caffeine expecting performance enhancements. In conclusion, caffeine consumption prior to a 1 km simulated cycling time-trial expecting performance improvements is not warranted at the current time.

References

Anderson, M., Bruce, C., Fraser, S., Stepto, N., Klein, R., Hopkins, W., et al. (2000). Improved 2000-meter rowing performance in competitive oarswomen after caffeine ingestion. *International Journal of Sport Nutrition & Exercise Metabolism*, *10*(4), 464-475.

Anselme, F., Collomp, K., Mercier, B., Ahmaiedi, S., & Prefaut, C. (1992). Caffeine increases maximal anaerobic power and blood lactate concentration. *European Journal of Applied Physiology & Occupational Physiology*, *65*(2), 188-191.

Astorino, T. A., Terzi, M. N., Roberson, D. W., & Burnett, T. R. (2011). Effect of Caffeine Intake on Pain Perception During High-Intensity Exercise. *International Journal Of Sport Nutrition & Exercise Metabolism*, *21*(1), 27-32.

Bell, D. G., Jacobs, I. I., & Ellerington, K. K. (2001). Effect of caffeine and ephedrine ingestion on anaerobic exercise performance. *Medicine & Science in Sports & Exercise*, *33*(8), 1399-1403.

Berkowitz, B.A., Tarver, J.H., & Spector, S. (1970). Release of Norepinephrine in the Central Nervous System by Theophylline and Caffeine. *European Journal of Pharmacology, 10,* 64-71.

Bridge, C., & Jones, M. (2006). The effect of caffeine ingestion on 8 km run performance in a field setting. *Journal of Sports Sciences*, *24*(4), 433-439.

Bruce, C., Anderson, M., Fraser, S., Stepto, N., Klein, R., Hopkins, W., & Hawley, J.A. (2000). Enhancement of 2000-m rowing performance after caffeine ingestion. *Medicine & Science in Sports & Exercise*, *32*(11), 1958-1963.

Burke, L. (2008). Caffeine and sports performance. *Applied Physiology, Nutrition & Metabolism*, *33*(6), 1319-1334.

Cohen, J. (1977). F Tests on Means in the Analysis of Variance and Covariance. Statistical Power Analysis for the Behavioral Sciences: Revised Edition. (pp. 273-406). Orlando, Florida; Academic Press, Inc.

Collomp, K. K., Ahmaidi, S. S., Audran, M. M., Chanal, J. L., & Prefaut, C. H. (1991). Effects of caffeine ingestion on performance and anaerobic metabolism during the Wingate Test. *International Journal of Sports Medicine, 12*(5), 439-443.

Collomp, K., Ahmaidi, S., Chatard, J., Audran, M., & Prefaut, C. (1992). Benefits of caffeine ingestion on sprint performance in trained and untrained swimmers. *European Journal of Applied Physiology & Occupational Physiology*, *64*(4), 377-380.

Cook, D. B., O'Connor, P. J., Eubanks, S. A., Smith, J. C., & Lee, M. M. (1997). Naturally occurring muscle pain during exercise: assessment and experimental evidence. *Medicine & Science in Sports & Exercise*, *29*(8), 999-1012.

Cook, D.B., O'Connor, P.J., Oliver, S.E., & Lee, Y. (1998). Sex differences in naturally occurringl eg muscle pain and exertion during maximal cycle ergometry. *The International Journal of Neuroscience, 95,* 183–202.

Cox, G.R., Desbrow, B., Montgomery, P.G., Anderson, M.E., Bruce, C.R., Macrides, T.A., Martin, D.T., Moquine, A., Roberts, A., Hawley, J.A., & Burke, L.M. (2002). Effect of different protocols of caffeine intake on metabolism and endurance performance. *Journal of Applied Physiology*, *93*, 990-999.

Crowe, M. J., Leicht, A. S., & Spinks, W. L. (2006). Physiological and Cognitive Responses to Caffeine During Repeated, High-Intensity Exercise. *International Journal of Sport Nutrition & Exercise Metabolism*, *16*(5), 528-544.

Davis, J.M., Zhao, Z., Stock, H.S., Mehl, K.A., Buggy, J., & Hand, G.A. (2003). Central nervous system effects of caffeine and adenosine on fatigue. *American Journal of Physiology- Regulatory, Integrative and Comparative Physiology*, *284*(2), 399-404.

Davis, J., & Green, J. (2009). Caffeine and Anaerobic Performance. *Sports Medicine*, *39*(10), 813-832.

Doherty, M. M., Smith, P. M., Davison, R. R., & Hughes, M. G. (2002). Caffeine is ergogenic after supplementation of oral creatine monohydrate. *Medicine & Science in Sports & Exercise*, *34*(11), 1785-1792.

Doherty, M., Smith, P., Hughes, M., & Richard Davison, R. (2004). Caffeine lowers perceptual response and increases power output during high-intensity cycling. *Journal of Sports Sciences*, *22*(7), 637-643.

Doherty, M., & Smith, P. M. (2005). Effects of caffeine ingestion on rating of perceived exertion during and after exercise: a meta-analysis. *Scandinavian Journal of Medicine & Science in Sports*, *15*(2), 69-78.

Egerton, T., Brauer, S. G., & Cresswell, A. G. (2009). Fatigue After Physical Activity in Healthy and Balance-Impaired Elderly. *Journal of Aging & Physical Activity*, *17*(1), 89-105.

Elliott, A. D., & Grace, F. (2010). An examination of exercise mode on ventilatory patterns during incremental exercise. *European Journal of Applied Physiology*, *110*(3), 557-562.

Fletcher, D. K., & Bishop, N. C. (2012). Caffeine ingestion and antigen-stimulated human lymphocyte activation after prolonged cycling. *Scandinavian Journal Of Medicine & Science In Sports*, *22*(2), 249-258.

Fredholm, B.B., Battig, K., Holmen, J., Nehlig, A., & Zvartau, E.E. (1999). Actions of caffeine in the brain with special reference to factors that contribute to it's widespread use. *Pharmacological Review, 51*(1), 83-133.

Ganio, M., Klau, J., Case, D., Armstrong, L., & Maresh, C. (2009). Effect of Caffeine on Sport-Specific Endurance Performance: A Systematic Review. *Journal of Strength & Conditioning Research (Lippincott Williams & Wilkins)*, *23*(1), 315-324.

Glaister, M., Patterson, S. D., Foley, P., Pedlar, C. R., Pattison, J. R., & Mcinnes, G. (2012). Caffeine and Sprinting Performance: Dose Responses and Efficacy. *Journal of Strength & Conditioning Research, 26*(4), 1001-1005.

Gliottoni, R. C., & Motl, R. W. (2008). Effect of Caffeine on Leg-Muscle Pain During Intense Cycling Exercise: Possible Role of Anxiety Sensitivity. *International Journal of Sport Nutrition & Exercise Metabolism*, *18*(2), 103-115.

Goldstein, E., Ziegenfuss, T., Kalman, D., Kreider, R., Campbell, B., Wilborn, C., et al. (2010). International society of sports nutrition position stand: caffeine and performance. *Journal of the International Society of Sports Nutrition*, *7*1-15

Graham, T. (2001). Caffeine and exercise: metabolism, endurance and performance. *Sports Medicine*, *31*(11), 785-807.

Graham, T.E., Helge, J.W., MacLean, D.A., Kiens, B., & Richter, E.A. (2000). Caffeine ingestion does not alter carbohydrate or fat metabolism in human skeletal muscle during exercise. *Journal of Physiology*, *529*(3), 837-847.

Graham, T., Hibbert, E., & Sathasivam, P. (1998). Metabolic and exercise endurance effects of coffee and caffeine ingestion. *Journal of Applied Physiology*, *85*(3), 883-889.

Green, H. J. (1997). Mechanisms of muscle fatigue in intense exercise. *Journal Of Sports Sciences*, *15*(3), 247-256.

Greer, F., McLean, C., & Graham, T. (1998). Caffeine, performance, and metabolism during repeated Wingate exercise tests. *Journal of Applied Physiology*, *85*(4), 1502-1508.

Gutmann, I. & Wahlefeld, A.W. (1974). Lactate: Determination with lactate dehydrogenase and NAD. In H.U. Bergmeyer (Ed.), *Methods of Enzymatic*

Analysis (pp. 1464-1468). New York and London; Verlag Chemie, Weinheim/Academic Press, Inc.,

Hampson, D. B., St Clair Gibson, A. A., Lambert, M. I., & Noakes, T. D. (2001). The influence of sensory cues on the perception of exertion during exercise and central regulation of exercise performance. *Sports Medicine*, *31*(13), 935-952.

Hunter, A.M., Gibson, A.St.C., Collins, M., Lambert, M., & Noakes, T.D. (2002). Caffeine Ingestion Does Not Alter Performance During a 100-km Cycling Time-Trial Performance. *International Journal of Sport Nutrition and Exercise Metabolism*, *12*, 438-452.

Irwin, C., Desbrow, B., Ellis, A., O'Keeffe, B., Grant, G., & Leveritt, M. (2011). Caffeine withdrawal and high-intensity endurance cycling performance. *Journal of Sports Sciences*, *29*(5), 509-515.

Ivy, J., Kammer, L., Zhenping, D., Bei, W., Bernard, J., Yi-Hung, L., & Hwang, J. (2009). Improved Cycling Time-Trial Performance After Ingestion of a Caffeine Energy Drink. *International Journal of Sport Nutrition & Exercise Metabolism*, *19*(1), 61-78.

Jackman, M., Wendling, P., Friars, D., & Graham, T. (1996). Metabolic, catecholamine, and endurance responses to caffeine during intense exercise. *Journal of Applied Physiology*, *81*(4), 1658-1663.

Jacobson, T.A., Febbraio, M.A., Arkinstall, M.J., & Hawley, J.A. (2001). Effect of caffeine co-ingested with carbohydrate or fat on metabolism and performance in endurance-trained men. *Experimental Physiology, 86*, 137-144.

Kalmar, J.M., & Cafarelli, E. (1999). Effects of caffeine on neuromuscular function. *Journal of Applied Physiology, 87 (2),* 801-808.

Kalmar, J. M. (2005). The Influence of Caffeine on Voluntary Muscle Activation. *Medicine & Science in Sports & Exercise*, *37*(12), 2113-2119.

Lee, K.A., Hicks, G., & Nino-Murcia, G. (1991). Validity and reliability of a scale to assess fatigue. *Psychiatry Research, 36*(3), 291–298.

LeMura, L. M., von Duvillard, S. P., & Stanek, F. F. (2001). Time course changes and physiological factors related to central and peripheral determinant of perceived exertion in highly trained adolescent alpine skiers. *Journal of Exercise Physiology Online*, *4*(4), 29-39.

Leung, A. S., Chan, C. H., Lee, A. S., & Lam, K. H. (2004). Visual Analogue Scale Correlates of Musculoskeletal Fatigue. *Perceptual & Motor Skills*, *99*(1), 235-246.

Lindinger, M. I., Graham, T. E., & Spriet, L. L. (1993). Caffeine attenuates the exercise-induced increase in plasma K+ in humans. *Journal of Applied Physiology*, *74*(3), 1149-1155.

Lynge, Hellsten, & Hellsten, Y. (2000). Distribution of adenosine A1, A2A and A2B receptors in human skeletal muscle. *Acta Physiologica Scandinavica*, *169*(4), 283-290.

Magkos, F., & Kavouras, S. (2005). Caffeine Use in Sports, Pharmacokinetics in Man, and Cellular Mechanisms of Action. *Critical Reviews in Food Science & Nutrition*, *45*(7/8), 535-562.

Medbø, J. I., Noddeland, H. H., & Hanem, S. S. (2009). Acid-Base Status of Arterial and Femoral-Venous Blood During and After Intense Cycle Exercise. *Acta Kinesiologiae Universitatis Tartuensis*, 14, 66-94.

Mohr, T., Van Soeren, M., Graham, T., & Kjaer, M. (1998). Caffeine ingestion and metabolic responses of tetraplegic humans during electrical cycling. *Journal of Applied Physiology*, *85*(3), 979-985.

Mohr, M., Nielsen, J., & Bangsbo, J. (2011). Caffeine intake improves intense intermittent exercise performance and reduces muscle interstitial potassium accumulation. *Journal of Applied Physiology*, *111*(5), 1372-1379.

Nielsen, O., & de Paoli, F. (2007). Regulation of Na+–K+ homeostasis and excitability in contracting muscles: implications for fatigue. *Applied Physiology, Nutrition & Metabolism*, *32*(5), 974-984.

Pasman, W. J., van Baak, M. A., Jeukendrup, A. E., & de Haan, A. A. (1995). The effect of different dosages of caffeine on endurance performance time. *International Journal of Sports Medicine*, *16*(4), 225-230.

Pickard-Holley, S. (1991). Fatigue in Cancer Patients; A descriptive study. *Cancer Nursing, 14*(1), 13-19.

Smith, A., Brice, C., Nash, J., Rich, N., & Nutt, D.J. (2003). Caffeine and Central Noradrenaline: Effects on Mood, Cognitive Performance, Eye Movements and Cardiovascular Function. *Journal of Psychopharmacology, 17*(3), 283-292.

Spriet, L.L., & Howlett, R.A. (2000). Caffeine. In R.J. Maughan (Ed.), Nutrition in Sport (7^{th} edition, 379-392). Blackwell Science Ltd.

Stuart, G. R., Hopkins, W. G., Cook, C., & Cairns, S. P. (2005). Multiple Effects of Caffeine on Simulated High-Intensity Team-Sport Performance. *Medicine & Science in Sports & Exercise*, *37*(11), 1998-2005.

Syrotuik, D. G., MacFadyen, K. L., Harber, V. J., & Bell, G. J. (2005). Effect of Elk Velvet Antler Supplementation on the Hormonal Response to Acute and Chronic Exercise in Male and Female Rowers. *International Journal Of Sport Nutrition & Exercise Metabolism*, *15*(4), 366-385.

Syrotuik, D.G., & Bell, G.J. (2004). Acute creatine monohydrate supplementation: A descriptive physiological profile of responders vs. nonresponders. *Journal of Strength and Conditioning Research, 18*(3), 610-617.

Tarnopolsky, M. (2008). Effect of caffeine on the neuromuscular system - potential as an ergogenic aid. *Applied Physiology, Nutrition & Metabolism*, *33*(6), 1284-1289.

Tim Hortons (2012). Tim Hortons Caffeine Content. Retrieved October 15, 2012 from. www.timhortons.com/ca/pdf/CaffeinceContentEN.pdf

Underwood, J., Clark, P.C., Blanton, S., Aycock, D.M., & Wolf, S.L. (2006). Pain, Fatigue, and Intensity of Practice in People With Stroke Who Are Receiving Constraint-Induced Movement Therapy. *Physical Therapy*, *86*(9), 1241-1250.

Wiles, J., Bird, S., Hopkins, J., & Riley, M. (1992). Effect of caffeinated coffee on running speed, respiratory factors, blood lactate and perceived exertion during 1 500-M treadmill running. *British Journal of Sports Medicine*, *26*(2), 116-120.

Wiles, J., Coleman, D., Tegerdine, M., & Swaine, I. (2006). The effects of caffeine ingestion on performance time, speed and power during a laboratory-based 1 km cycling time-trial. *Journal of Sports Sciences*, *24*(11), 1165-1171.

Williams, J., Signorile, J., Barnes, W., & Henrich, T. (1988). Caffeine, maximal power output and fatigue. *British Journal of Sports Medicine*, *22*(4), 132-134.

Woolf, K., Bidwell, W., & Carlson, A. (2009). Effect of Caffeine as an ergogenic aid during anaerobic exercise performance in a caffeine naïve collegiate football players. *Journal of Strength & Conditioning Research (Lippincott Williams & Wilkins)*, *23*(5), 1363-1369.

Yeomans, M. R., Ripley, T., Davies, L. H., Rusted, M., & Rogers, P. J. (2002). Effects of caffeine on performance and mood depend on the level of caffeine abstinence. *Psychopharmacology*, *164*(3), 241.

Zajac, A. A., Jarzabek, R. R., & Waskiewicz, Z. Z. (1999). The diagnostic value of the 10- and 30-second Wingate Test for competitive athletes. *Journal Of Strength & Conditioning Research*, *13*(1), 16-19.

Zouhal, H., Jacob, C., Delamarche, P., & Ariette, G. (2008). Catecholamines and the Effects of Exercise, Training and Gender. *Sports Medicine*, *38*(5), 401-423.

Appendix A. Experimental Design

Bloods	Sample		Sample		Sample		Sample	Sample	Sample	Sample
Supplement		C(P)								
Exercise	BP		BP	Warm-Up			Cool-Down	Pain + Fatigue Scales		
Time	-80	-75	-20	-15	-5	Test	0	5	10	15

Sample= 10 ml blood sample
P= Placebo
C= Caffeine Supplementation
BP= Blood Pressure

Appendix B. Scale of Perceived Pain

0	No Pain at all
0.5	Very faint pain (just noticeable)
1	Weak Pain
2	Mild Pain
3	Moderate Pain
4	Somewhat strong Pain
5	Strong Pain
6	
7	Very Strong Pain
8	
9	
10	Extremely intense Pain (almost unbearable)

Appendix C. Fatigue Visual Analogue Scale

Overall I feel:

Not at all fatigued Extremely fatigued

_____

My legs feel:

Not at all fatigued Extremely fatigued

_____

Appendix D. Participant letter

The effects of caffeine supplementation on fatigue and pain perception and plasma potassium concentrations during a 1 km cycling time trial.

Principal Investigator(s):

Dean Cordingley, Graduate Student, Faculty of P.E. and Rec. cordingl@ualberta.ca.

Co-Investigator(s):

Dan Syrotuik, Ph.D., Faculty of P.E. and Rec. dan.syrotuik@ualberta.ca.

Do you understand that you have been asked to be in a research study?	Yes	No
Have you read and received a copy of the attached Information Sheet	Yes	No
Do you understand the benefits and risks involved in taking part in this research study?	Yes	No
Have you had an opportunity to ask questions and discuss this study?	Yes	No
Do you understand that you are free to refuse to participate, or to withdraw from the study at any time, without consequence, and that your information will be withdrawn at your request?	Yes	No
Has the issue of confidentiality been explained to you? Do you understand who will have access to your information?	Yes	No

This study was explained to me by: _____

I agree to take part in this study:

_____	_____	_____
Signature of Research Participant	Date	Witness
_____		_____
Printed Name		Printed Name

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

_____	_____
Signature of Investigator or Designee	Date

Participant Information

The effects of caffeine supplementation on fatigue and pain perception and plasma potassium concentrations during a 1 km cycling time trial.

Principal Investigator: Dean Cordingley, Graduate Student, Faculty of P.E. & Rec.
cordingl@ualberta.ca.
Co-Investigator: Dan Syrotuik, Ph.D., Faculty of P.E. & Rec.
dan.syrotuik@ualberta.ca.

Dear Participant.

I (Dean Cordingley) am a graduate student in the Faculty of Physical Education and Recreation under the supervision of Dr. Dan Syrotuik. I am conducting a study that is looking at the effects of caffeine on a variety of things that can be measured in your blood and the time it takes to complete a 1 km cycling time trial. Caffeine is a "pick me up" and is found in certain foods (chocolate, sports gels, power bars, etc.), drinks (coffee, tea, chocolate milk, energy drinks, etc.) and can also be bought at health food stores. The purpose of this study will be to determine the effects of eating caffeine when combined with cycle exercise.

To be in our study, you must be a healthy male who participates in cycling with a sprint component (such as track cycling and mountain biking), free of any medical condition or food allergy since these may affect our results, and not taking any other nutrition aids such as creatine. You will be asked to attend a meeting in the exercise lab (directions will be provided) during which all the methods will be explained to you and allow us to answer any questions you have related to the study. You will be asked to complete a record of what you eat at home and fill it out on your own time over three days and we will ask you to not consume any alcohol during these days. When done we will ask that you email, or drop this form off at the lab. We will analyze and return this record to you and ask you to eat the same foods for the 2 days before each of the testing trials.

At the first meeting, we will measure your height and weight, record your age and ask you to do a fitness test. The fitness test will be done on a stationary bike while you are breathing in a mouthpiece designed to collect all the air you breathe out. This test will measure your highest oxygen consumption (VO2 max) during exercise. This test gets harder until you indicate you cannot go on. Exercise intensity is light at the beginning of the test and becomes more difficult every minute. The test lasts for about 12 to 15 minutes, with 5 to 10 minutes of warm-up and cool-down exercise before and after. Heart rate is also measured with a heart rate monitor.

Then each of the 2 testing trials will be in an order that may or may not be the same for everyone. The 2 days before each trial we will ask you to eat the same meals as on your diet record form. Then we will ask that you do not eat after 10:00 pm the night before each trial, but you can drink as much water as you require. The next day, we ask that you come to the lab between 7:00 and 9:00 am to begin each session. We ask that

you do not exercise (ride your bike to the lab for example) before you come as we need to take a resting blood sample on an empty stomach. We will weigh you again and a small blood sample (~10ml) will be taken from an arm vein with a needle. For all the other blood samples, a nurse will put a small sterile tube into a blood vessel in your forearm with a needle. This is similar to what is used if you have had an "IV" in a hospital or if you have donated blood at Blood Services. We will take blood samples before and after the cycling test for a total of 7 samples. This is around 70 ml's of blood for each trial but does not present any risk to your health at all. In addition we will measure the air you breathe during the testing trials using the same equipment as for your fitness test. We will also put a clip on your finger to measure the oxygen levels in your blood during all the exercise tests. Two additional measurements will be blood free fatty acid and glucose which do not require any more blood to be taken.

You will do each of the 2 testing trials as shown below. You will get at least 7 days off in between the bouts. The exercise will involve 1 bout of exercise, an all out 1 km sprint on the cycle. To stay hydrated, you will be allowed to drink as much water as you like during the exercise session. A pain scale and visual analogue scale will also be used to measure how much pain and tired you are from the exercise.

> ***Caffeine + Exercise***, we will give you as much water as you like and 5 mg/kg of body weight of caffeine mixed in Crystal Light and you will complete the exercise bout.
> ***Placebo + Exercise,*** we will give you as much water as you like as well as Crystal Light and you will complete the exercise bout.

Note that we will provide you with a schedule of all your visits to the lab and these will be as flexible as possible to suit your personal lives.

Risks: There is a small risk of infection at the site of the blood sample if not properly cared for. However, sterile procedures, cleanliness and use of a band-aid greatly minimize this risk. A registered nurse using safe procedures will conduct the blood sample procedures.

Qualified people under the supervision of Dr. Dan Syrotuik will run the testing. Staff is trained to handle particular risks and certifications can be produced upon request. The researchers will continuously watch for unpleasant symptoms and will stop any test if at any time they are concerned about your safety. You can also stop any test at any time. All of the exercise tests may cause you some discomfort because they will be exercising to near exhaustion. Injuries that may result include muscle pulls, strains, and cramps, but these will be minimized by having the athlete perform a proper warm-up and stretching before the exercise tests. Please inform the testers of any of the above-mentioned symptoms experienced during or after the tests.

Benefits: The major benefit of your participation in this study will be to help the researchers understand the nature of caffeine when combined with a 1 km cycling time trial on certain responses in the body. As a participant you will be provided with a written report card of your personal results if you want. If you are interested in the future

research outcomes of this study, you may contact one of the researchers for this information as well.

Total Time Commitment:

First meeting, VO2max	fitness test	~1 hours
Filling out the diet record at home		~30 minutes (total time)
2-experimental trials		**~2×2 hours ~4 hours**
Total testing time =		***~5.5 hours***

Testing time does not include travel to and from the lab.

Confidentiality: To ensure secrecy and anonymity, personal information will be coded and stored in a file cabinet in a locked office to which only the investigators have access. There will be no way to identify individuals in results that may be published in any report or article. Normally, information is retained for a period of 5 years post publication, after which it may be destroyed.

Freedom to withdraw: For the purpose of the study you are required to participate in all the procedures but you can withdraw at any time without consequence by simply informing one of the testers verbally, phone call or email. If you decline to continue or withdraw from the study, all information will be removed from the study upon your request. Contacting either Dean Cordingley or Dan Syrotuik at anytime during the study can do this.

Additional contacts: If you have concerns about the study and wish to speak with someone who is not involved with this study, please call the University of Alberta Research Ethics Office at (780) 492-2615

Thank you,

Dean Cordingley	**Dan Syrotuik, Ph.D.**

Appendix E. Poster

Volunteers Needed for a Study Examining a Nutritional Supplement and Anaerobic Exercise

Researchers at the Exercise Physiology Lab, U. of A. are interested in the effects of consuming caffeine on how various blood components and mental perception are affected by a 1 km cycling sprint.

We are looking for healthy, male cyclists
(18-39 yrs old) who:

1. Have competed in any cycling discipline for at least 1 year.

Tests include: a maximal oxygen consumption test, a dietary analysis, a non-invasive pain scale, fatigue scale and blood samples will be taken by a registered nurse.
Volunteers will receive free information about their individual maximal oxygen consumption, and dietary status.
Testing will begin in February, 2012 and continue until enough participants are recruited.

Contact
Mr. Dean Cordingley, M.Sc. Graduate Student at
cordingl@ualberta.ca
or leave a phone message with Dr. Dan Syrotuik, Professor
@ (780) 492-6583.

Appendix F. Sample size calculations

$$\text{Effect size} = \frac{M_{(control)} - M_{(caffeine)}}{SD_{(control)}}$$

$$0.82 = \frac{73.3-71.1}{2.7}$$

Wiles, J., Coleman, D., Tegerdine, M., & Swaine, I. (2006). The effects of caffeine ingestion on performance time, speed and power during a laboratory-based 1 km cycling time-trial. *Journal of Sports Sciences*, *24*(11), 1165-1171.