THE EFFECTS OF CAFFEINE INGESTION ON MUSCLE CRAMP THRESHOLD FREQUENCY

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ABSTRACT

Context: The effects of caffeine on muscle cramp threshold frequency were investigated. Objective: To determine the effect of caffeine on muscle cramp threshold frequency. Design: Randomized Trial. Experimental Design Setting: Institutional Research Laboratory. Participants: 11 males, 6 females. Intervention: Each subject ingested different caffeine doses over three testing days. Main Outcome Measures: Muscle cramp threshold frequency, hemoglobin, hematocrit, plasma sodium concentration, plasma potassium concentration, plasma chloride concentration, and osmolality were recorded. Results: There was a significant difference between baseline and post ingestion threshold frequency as well as significant differences between 0 mg to 500 mg and 250 mg to 500 mg. Hemoglobin, plasma potassium concentration, and osmolality were significantly different from pre to post ingestion. Conclusions: There was a significant difference from pre to post muscle cramp threshold frequency. Hemoglobin, plasma potassium concentration, and osmolality were significantly different pre to post ingestion. The significance of these findings requires further study.

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INTRODUCTION

Caffeine is the most widely consumed substance for recreational and ergogenic purposes. Eighty-four to ninety-one percent of men and women between the ages of 18-35 consume, on average, 238 mg per day.[1] Caffeine is normally consumed in beverages such as coffee (71%), tea (16%), and soft drinks (12%).[1] Soda and coffee caffeine content varies as soda contains 20-55 mg of caffeine (12 oz.)[2] and coffee can contain up to 80-120 mg of caffeine (8 oz.).[2] Daily caffeine consumption varies, but has been reported to be as high as 600 mg per day.[1]

Caffeine ingestion causes several physiological effects such as stimulating the central and peripheral nervous systems. Caffeine has been shown to cause ergogenic effects by ingesting 2-3 mg/kg.[3] Consuming caffeine prior to exercise improves endurance in trained cyclists,[4] but appears to have negligible effects on anaerobic performance (e.g., strength and power).[5] While ingested at rest, urine production increases,[6] however when caffeine is ingested prior to exercise or during exercise, urine production is unaffected.[7,8,9] The neurological effects of caffeine is also well documented.[10,11,12] Caffeine ingestion stimulates the sympathetic nervous system,[13] which may increase resting metabolic rate and internal heat storage, leading to higher core body temperatures and sweat rates.[13] Moreover, caffeine ingestion increases spinal excitability (i.e. Hoffman reflex)[11] and motor unit self-sustaining firing rates.[12]

Exercise-associated muscle cramps (EAMC) are a common injury affecting recreational and competitive athletes.[14] EAMC are thought to be caused by dehydration/electrolyte imbalances[15] and changes in neuromuscular control.[16] The dehydration and electrolyte imbalance theory proposes EAMC occur when select motor nerves become hyperexcitable due to fluid and electrolyte losses incurred during exercise.[15] The neuromuscular control theory states that EAMC

occur because of fatigue-induced changes to muscle spindles and golgi tendon organs which alters alpha motor neuron activity.[16]

Since EAMC are unpredictable, scientists induce them in laboratory settings. Cramp threshold frequency (TF) is the minimal amount of electrical stimulation needed to elicit a muscle cramp.[17] Scientists have used TF as a quantitative indicator of cramp risk.[18] Individuals with a self-reported history of muscle cramping have a lower TF than those who self-report no history of cramping.[19] Therefore, cramp TF may be useful as a clinical tool to identify individuals at risk of cramping and develop treatment and prevention strategies.

Because caffeine may cause diuresis[13] and excitation of the peripheral nervous system,[10, 11, 12] ingesting it may increase cramp risk. No scientific data exist on the effect of caffeine on cramp TF. The purpose of this study is to determine if caffeine ingestion decreases cramp TF and increases the likelihood of EAMC occurring in physically active individuals. This study also determined if caffeine ingestion causes dehydration 1-hour post ingestion by looking at pre and post blood analysis of hemoglobin, hematocrit, plasma sodium concentration, plasma potassium concentration, plasma chloride concentration, and osmolality.

Research Question

1. Did ingesting either 0 mg (placebo), 250 mg, or 500 mg of caffeine decrease cramp TF?

Assumptions

1. An electrically induced muscle cramp (EIMC) of the flexor hallucis brevis is similar to all EIMCs induced through the body.
2. An EIMC of the flexor hallucis brevis is the same as an EAMC of the flexor hallucis brevis.

3. Caffeine consumed in a pill is the same as caffeine ingested in beverages (e.g. Red Bull, coffee).

Limitations

1. Individuals likely consumed caffeine in beverages (e.g. energy drinks, coffee) rather than in pills.
2. Caffeine ingestion was not specific to subject's body mass (mg vs. mg/kg).
3. The study looked at the effects of caffeine on cramp TF at rest and not during physical activity.
4. Subjects self-reported a history of not having any neurological, neuromuscular, cardiovascular, or thermoregulatory disorders especially cardiomyopathy, heart arrhythmia, heart murmur, high blood pressure, high cholesterol, and a history of heart disease in their immediate family.

Inclusion Criteria

1. Subjects self-reported not having any neurological, neuromuscular, cardiovascular, or thermoregulatory disorders especially cardiomyopathy, heart arrhythmia, heart murmur, high blood pressure, high cholesterol, and a history of heart disease in their immediate family.
2. Male and female subjects were between the ages of 18-35.
3. Subjects were excluded if they had injured or had surgery in their dominant lower limb within the last 12 months preceding data collection.
4. Subjects had self-reported a history of cramping within the 6 months preceding data collection.
5. Subjects were excluded if they smoke tobacco products.

6. Subjects were excluded if their body mass index is ≥30 kg/m^2.
7. Female subjects were excluded if they are currently taking oral contraceptives.
8. Female subjects were tested during the follicular phase of their menstrual cycle.
9. Subjects were excluded if they exercise vigorously (≥60% VO_2R)[22] for more than 5 days per week.
10. Subjects avoided caffeine ingestion three days prior to data collection.

Significance of Study

This study was significant as recent literature had looked at the effects of caffeine ingestion in physically active individuals competing in heat-stressed environments,[6,7,8] dehydration,[4] and normal physiological functions.[4,13] However, there is no current literature discussing the possibilities of increased risks of EAMC following caffeine ingestion.

Definition of Terms

Caffeine (1,3,7-trimethylxanthine): common ingredient found in tea, cocoa. Acts as an ergogenic aid.[21]

Body mass index (BMI): a number calculated from a person's weight and height indicating the percentage of body fat. BMI= body mass (lbs.) x 703/ height (in.).[2]

Hoffman (H-Reflex): a monosynaptic reflex consistently obtained in normal adults only by electrically stimulating the tibial nerve, generally in the popliteal fossa, while recording from the gastrocnemius-soleus muscle group.[10]

Hypohydration: the state of water output exceeding water intake, resulting in a body water deficit.[21]

Electrolyte: minerals in blood and other body fluids that carry an electric charge and affects water volume, blood pH, muscle function, and other bodily functions.[21]

Euhydration: the state of being hydrated. Urine specific gravity less than or equal to 1.012.[20]

Glomerular filtration rate: the rate at which renal function occurs to process water absorption and excretion.[21]

Golgi tendon organ: a proprioceptor located in muscle tendons that provide information about changes in muscle tension.[21]

Muscle spindle: a proprioceptor located within muscles that provide information about changes in muscle length.[21]

Sympathetic nervous system: originates in the thoracic regions of the spinal cord; opposes physiological effects of the parasympathetic system: reduces digestive secretions; speeds the heart; contracts blood vessels.[21]

LITERATURE REVIEW

The purpose of this study determined if caffeine ingestion decreased cramp threshold frequency and increased the likelihood of exercise associated muscle cramps occurring in athletes and physically active individuals. The following research question was addressed during this study: Did ingesting 0mg (placebo), 250mg or 500 mg of caffeine decrease cramp TF?

This literature review discussed the prevalence of caffeine usage and associated factors leading to muscle cramps. Specific emphasis was placed on the athletic population and those that live an active lifestyle. The following key areas regarding caffeine and muscle cramping were discussed: Caffeine, Physiological Effects of Caffeine, Caffeine and Aerobic Performance or Anaerobic Performance, Dehydration Theory, Muscular Fatigue Theory, Prevention of EAMC, and Caffeine and Muscular Functions relating to EAMC.

Caffeine

Caffeine is widely used and found in natural sources such as coffee beans, tea leaves, kola nuts, bissy nuts, cacao beans, guarana, and mate.[1] These natural sources of caffeine are added to foods, beverages, herbal supplements, and medications.[1] Besides food, beverages containing caffeine are considered the gold standard of caffeine consumption.[1] Soft drink consumption in 18-35 year olds increased 11 gal/year to 49 gal/year and coffee consumption has decreased from 16 gal/year to 9 gal/year since 2002.[1] This is due to an increase of specialty coffee, caffeinated water, and energy drink production.

Consumption is not only popular among adults (ages 18-65 years old), but children as well. Among children, ages 2-11 years old, data showed that 36 % (5,765/15,716) consume beverages or food containing caffeine.[1] Tea and soft drink consumption was higher (mean=4.8 mg/daily, 8.0 mg/daily) in children, ages 2-5, than flavored dairy products and sweetened grains

(mean=1.0mg/daily, 1.1 mg/daily).[1] Children, ages 6-11, consumed tea and soft drinks at higher rates (mean=6.1 mg/daily, 14.7 mg/daily) compared to flavored dairy products and sweetened grains (mean=1.7 mg/daily, 1.5 mg/daily).[1] Caffeine consumption is also popular among college age athletes (ages 18-22).[1] Athletes widely consume caffeine prior to competition in regards to increasing their focus and performance (i.e. endurance, aerobic capacity).[11] In a study showing caffeine ingestion prior to competition, 89 % of females (1,073/15,716) and 91% males (1,135/15,716) consumed either food or beverages containing caffeine.[1] Coffee ingestion within the 18-34 age group was popular (mean=111.5 mg/daily), but as age increases coffee consumption increases (ages 35-54; mean=260.9 mg/daily).[1]

Widely considered for its use for increasing focus and energy, caffeine is a popular ergogenic aid across the world.[23] Data shows that 82-95 % of adults in the United Kingdom and Denmark consume caffeine.[23] Caffeine consumption was reported in more than 40% British athletes in order to enhance performance.[4] Research collected on Ironman World Triathlon Championship participants showed that 89% of the athletes competing planned on using caffeine prior to competition.[23]

Energy drinks and supplements that contain caffeine is popular among college-aged individuals.[1] Energy drink production reaches annual global sales near several billion dollars.[24] Among the total sales, adolescents, young adults, and college-aged individuals are the top consumer.[24] American adolescents, young adults, and college-aged individuals account for 30% of the total population that consumes energy drinks.[23]

Physiological Effects of Caffeine

The main physiological effects of caffeine are diuresis, ergogenic aid, lipolysis, and secondary electrolyte excretion at rest. While there are physiological effects at rest, caffeine has

shown little to no physiological changes while exercising.[7] The main physiological action of caffeine at rest is it acts as a diuretic.[7] Diuresis is the act of increased fluid loss through increased urine output.[7] Athletes should refrain from caffeine during exercise due to the diuretic effect, especially in hot environments which can increase dehydration status through increased fluid loss.[7]

The process of diuresis influences the kidney's glomerular filtration rate.[7] Caffeine increases blood flow to the kidneys causing a greater glomerular filtration rate.[7] Glomerular filtration rate increases urine production.[8] Grandjean et al. observed no differences in urine volume over a 24-hour urine collection.[9] Even though participants consumed various doses of water, caffeinated and carbonated beverages there were no significant differences between the three groups (water, caffeinated beverages, carbonated beverages) and its effect on urine output.[9] However, Millard-Stafford et al. states that consuming a caffeinated sports drink while exercising in hot environments does not affect urine output.[9] Millard-Stafford et al. studied 16 cyclists completing submaximal cycling at 60-75% VO_2 max for 120 min.[9] Participants were given a placebo, carbohydrate-electrolyte, or caffeinated carbohydrate-electrolyte beverage on 3 different trials.[9] The caffeinated carbohydrate-electrolyte beverage maintained hydration as well as the carbohydrate-electrolyte beverage with no change in sweat and urine production.[9] Researchers have concluded that performing submaximal prolonged exercise show no effect on urine production with consumption of a caffeinated sports drink.[9]

The purpose of using caffeine as an ergogenic aid has shown improvements in endurance performance.[9] The use of caffeine in endurance activities such as cycling, running, and swimming has improved overall aerobic capacity times.[4] Endurance athletes are frequent users of caffeine pre-exercise and during exercise.[4] Caffeine stimulates lipolysis, the breakdown and

mobilization of free fatty acids (FFA).[10] FFA utilization delays usage of muscle glycogen stores.[10] Sparing muscle glycogen stores can increase performance by delaying the onset of fatigue during prolonged endurance activities.[10]

Electrolyte excretion at rest is present with caffeine ingestion even though limited research exsists.[7] The diuretic effects increase urine production and results in excretion of sodium, chloride, and potassium.[7] The electrolytes are excreted due to decreased reabsorption by the nephrons located within the kidneys.[7] Del Coso et al. observed no significant change in cyclists exercising in hot-dry environment.[7] Seven endurance trained cyclists performed submaximal cycling for 120 min. at 63 % of VO_2 max.[7] Each cyclist hydrated during exercise by completing each trail of either hydrating 97% of total sweat loss with water, an 6% carbohydrate-electrolyte solution, no replacement of total sweat loss, or combing the three treatments over different testing intervals with 6 mg/kg of body mass of caffeine.[7] The results showed increased urine excretion and sweat electrolyte loss, but not enough to cause dehydration.[7]

Caffeine Effects on Aerobic Performance and Anaerobic Performance

Caffeine ingestion acts as a stimulant of the central and peripheral nervous systems by increasing focus and attention by retaining natural energy stores (fatty free acid, carbohydrates, etc.[26] Though this is true, caffeine's effects on performance while exercising are limited. Limited scientific data have shown what occurs within the central and peripheral nervous systems with caffeine ingestion during exercise.[26] However, there is evidence that states improved performance during prolonged exercise.[26]

As previously noted, caffeine ingestion stimulates the lipolysis.[25] Lipolysis is the process of chemically breaking down lipids into FFA.[25] FFA can then be utilized as energy while at rest

or exercising.[25] During exercise, FFA is utilized towards energy expenditure and spare muscle glycogen.[25] When muscle glycogen stores are depleted, fatigue and exhaustion is evident in the participant. Decreased time to fatigue results in spare FFA and sparing of muscle glycogen stores.[25]

Caffeine's emphasis towards endurance performance proves its effectiveness as an ergogenic aid.[26] Lockwood et al. looked at this theory in 38 sedentary men. The men participating were randomly assigned to Group A (exercise and energy drink) or Group B (energy drink only).[26] Each participant was to drink one energy drink (12 fluid oz.) (Celsius, Celsius, Inc., Delray Beach, Florida) for 70 days.[26] Guidelines for the exercise protocol performed by Group A were established by the standards set by the American College of Sports Medicine (ACSM).[26] Each participant completed resistance training two days per week and aerobic training 3 days per week. Body fat percentage, cardiovascular function, and strength measures were assessed.[26]

The participants in Group A had significant improvements compared to Group B. Body fat percentage showed a significant difference ($p<0.05$) between Group A and Group B (-2.50 kg, -2.12%; +0.57 kg, +0.29% respectively).[26] Cardiovascular measures improved for Group B ($p=0.001$) while Group A improved significantly ($p\leq0.010$) from baseline.[26] Strength measures assessing 1-rep max in bench press and leg press improved significantly for both groups A (+11.64% and 8.14% respectively; $p<0.001$) and B (+9.54% and +10.72% respectively; $p=0.001$).[26]

Walsh and his colleagues further examined the point by investigating caffeine and endurance.[23] Though FFA concentration was not analyzed, the amount of work performed by the participants was assessed.[23] Work was assessed by the amount of time until exhaustion while

performing a VO_2 max test.[23] Fifteen subjects (9 men, 6 women) participated in a randomized study. Each participant completed a 5 min. warm-up followed by running on the treadmill at a self-selected speed. Every 2 minutes, the inclination increases 2% while maintaining the speed.[23] Caffeine ingestion at pre-exercise showed a significant difference in performance over time. Participants consuming the energy drink supplement were able to run 12.5% longer than participants that consumed the non-caffeinated beverage.[23] Visual analog scales (VAS) were established focusing on focus, energy, and fatigue.[23] Participants ingesting the energy supplement pre-exercise reported greater focus ($p=0.031$), energy ($p=0.016$), and less fatigue ($p=0.005$) than the non-caffeinated beverage.[23] Significant differences were also shown at 10 minutes during exercise with greater focus ($p=0.026$) and energy ($p=0.004$).[23] There were no significant differences between the control and treatment groups post exercise.[23]

Further studies have concluded caffeine ingestion prior to exercise can improve endurance.[27,28] Ivy et al. and Rutherford et al. observed cyclists over prolonged exercise bouts.[27-28] In both trials, cyclists that consumed caffeine (Red Bull) showed some to significant improvements ($p<.01$; $p>0.05$).[27-28] More importantly, both studies provided facts of FFA utilization over time.[27-28] Cyclists ingesting caffeine prior to exercise showed greater fatty acid oxidation, leading to increased performance.[27-28] Two studies looking at the effects mentioned above indicated that caffeine ingestion prior to exercise improves aerobic activity such as endurance.[1,13] However, caffeine ingestion prior to anaerobic conditioning shows minor to no effects on increasing strength and power.[5]

The effects of caffeine ingestion on anaerobic performance are still unclear. Studies showed that caffeine ingestion is indicative towards muscular endurance, but no correlation was made between caffeine ingestion and muscular force.[29] Caffeine dosages varied amongst studies

testing for anaerobic performance.[29] This research concludes that muscular endurance and focus improves, but no cause and effect relationship between caffeine ingestion and muscular measures (e.g. peak torque, strength, power, force).[24]

Two studies trying to prove a theorem behind caffeine ingestion and anaerobic performance involved 12 strength-trained males and 12 strength-trained males with 4 strength-trained females, respectively.[5,29] Both studies looked at caffeine ingestion and power measure output while performing the Wingate cycle test and bench press repetition test.[5,29] All participants either consumed commercially available energy drinks (Red Bull, Redline Extreme) or a placebo, 60 minutes and 10 minutes pre-exercise respectively.[5,29] After caffeine ingestion, Wingate cycle test and bench press repetition test were initiated. Significant evidence was found, but nothing to correlate caffeine ingestion to muscular power.[5,29] Red Bull ingestion showed an increase in the number of repetitions performed (34 ± 9; placebo: 32± 8).[5,29] There was no effect on post Red Bull ingestion on peak or average power (peak power: 701± 124 watts) placebo: 700± 132 W, average power: 479± 74 W; placebo: 471± 74 W).[5,29] Redline Extreme ingestion increased focus among those consuming the beverage (3.8± 0.5) compared to placebo (3.3± 0.7).[5,29] However, there were no improvements to anaerobic power measures.[5,29]

Many biomechanical functions have been tested to determine cause and effect of caffeine ingestion and anaerobic performance measures. Primary biomechanical functions scientists have looked at include catecholamine levels and potassium.[30] Catecholamine stimulates adrenaline secretion and caffeine consumption increases adrenaline.[30] Increased adrenaline levels increase glycolytic flux, which can lead to improved endurance.[30] Nonetheless, limited studies have shown no increase in glycolytic flux when adrenaline and catecholamine levels were increased.[30]

Potassium plays a role in the excitation-contraction coupling series in muscle.[30] Na^+/K^+ pumps are essential for depolarization of muscle cells.[30] During muscle contractions, K^+ diffuses into the extracellular space creating a more negative environment.[30] Caffeine has been known to stimulate K^+ transport in skeletal muscle by increasing Na^+/K^+ ATPase activity.[30] However, little research has been done to compare K^+ levels during anaerobe activity following caffeine ingestion.[30]

Two physiological effects of caffeine not proven while exercising anaerobically are adenosine antagonism and pain perception.[30] First, caffeine stimulates the central nervous system primarily through adenosine receptor antagonism.[30] Adenosine is regulated through adenine nucleotide breakdown, which increases during exercise.[30] Adenosine has been shown to reduce pain perception, induce sleep, reduce arousal, and act as a neuromodulator.[30] However, caffeine can inhibit the effects of adenosine.[30]

Adenosine receptors (A_1, A_{2a}, A_{2b}, A_3 respond to different stimuli.[30] Inhibitory effects primarily affect A_1 receptors and excitatory effects primarily affect A_2 receptors.[30] Caffeine can cross the blood-brain barrier through simple diffusion and acts as an adenosine inhibitor.[30] A_1 and A_{2a} receptors are primarily affected through caffeine ingestion as they contain more levels of adenosine compared to A_{2b} and A_3 receptors.[30] Thus leading to decreased pain perception while maintaining motor unit firing rates.[30] This is yet to be proven while exercising anaerobically.[30]

Second, pain perception. The logic behind pain perception is it would play a role in anaerobic performance.[30] Pain perception influences motor unit recruitment by decreasing firing rates.[30] Adenosine has been shown to cause muscle pain in healthy and unhealthy subjects.[30] Caffeine binds on to adenosine receptors and inhibits the acts of adenosine.[30] Through simple

logic though unproven, caffeine's inhibitory effects on adenosine can reduce pain perception and maintain good motor unit recruitment.[30]

Caffeine is an ingredient available in over-the-counter medication such as headache medication.[30] One of caffeine's main roles is inhibiting adenosine by binding on adenosine receptors.[30] This is a leading hypothesis in finding a correlation between caffeine and anaerobic performance.[30] Inhibition of pain signals as experienced in exercise and post-exercise, athletes and physically-active individuals should see improvements in performance, however adenosine pre and post caffeine ingestion levels and adenosine pre and post injury with caffeine ingestion has few to very minimal data sources to support this premise.[30]

Muscle Cramps: Dehydration Theory

Exercise associated muscle cramps (EAMC) can affect healthy individuals that have no serious medical condition.[31] The underlying cause of EAMC is unknown; however, there are two theories that might help explain it: the dehydration theory and the muscular fatigue theory.[15,16,31] Clinical and perceptual diagnoses of EAMC through years have been related to dehydration and electrolyte imbalances.[15,16,31] Global corporations (e.g. Gatorade, Powerade) market products based on the premise of maintaining hydration through carbohydrate-electrolyte ingestion in hopes of decreasing chances of EAMC occurring. Proprietors in sport marketing focus on developing new beverages to maintain hydration and reduce cramp risks. For years athletic trainers and athletes have believed that EAMC occur due to electrolyte imbalances leading to clinical practices that include sport drink, pickle juice, and banana ingestion.[31]

According to the dehydration theory the human body does not store enough water during exercise.[31] Athletes and active individuals begin to lose water and do not consume enough water to restore water losses.[31] Water losses increase fluid and electrolyte depletion causing

desensitization of nerve terminals.[31] Sensitization of select nerve terminals increase mechanical pressure on motor nerve endings, resulting in an EAMC.[31]

Exercising in hot, humid conditions exacerbate these effects.[31] EAMC and dehydration theory data inflict cause and effect with increased fluid losses and prevalence of EAMC.[31] Research has reported that 95% (87/92) of cramping occurs in hot months.[31] Athletes participating in football were more at risk due to environmental conditions that could inquire a heat –related illness.[31] Case studies and observational studies involving athletes and workers developing EAMC were either competing or working in hot, humid environments.[31]

High sweat rates accompany the dehydration theory. Sodium and chloride are two main electrolytes lost in sweat.[15] A sodium and chloride deficit occurs when an individual partakes in prolonged exercise when sodium and chloride losses are greater than salt intake.[15] A 20-30% estimated sweat loss of the Na^+ pool has been seen with severe muscle cramping.[15]

Compensation occurs from increased plasma volume losses. Water from the interstitial space of the body shifts to the intravascular space.[15] As sweat rates continue, the interstitial fluid becomes more contracted.[15] As the interstitial compartment becomes more contracted, neuromuscular junctions can become hyperexcitable by mechanical deformation.[15] Increased levels of acetylcholine, electrolytes, and exercise-related metabolites could trigger nerve fibers to fire or cause an end-plate current and postsynaptic potential.[15] As water levels increase in the intravascular space, nerve terminals and post-synaptic membranes can be affected, prompting cramping in various muscle fibers and bundles.[15]

Athletes and active individuals can develop EAMC; however, hot, humid conditions is not the only climate prevalent in EAMC.[31] Marathon runners (18%, 15/82) still develop EAMC when competing in ambient, cool temperatures (10-12°C; 50-54°F).[31] The notion of developing

EAMC from hot, humid environments is limitless as athletes can develop EAMC in cool temperatures.[31]

Plasma and blood volume losses in those with EAMC and without EAMC had shown no significant difference.[31] A study looking at sweat rates, sodium, and fluid losses in athletes with EAMC and without EAMC showed no significant differences.[31] Concluding the same study, body weight losses between athletes with EAMC and without EAMC had shown significant difference.[31]

More significantly, carbohydrate-electrolyte ingestion shows no difference in fluid balance.[31] The dehydration theory focuses on increased fluid and electrolyte losses. Carbohydrate-electrolyte ingestion should restore fluid and electrolyte losses reproduced through exercise. However, when athletes consumed carbohydrate-electrolyte beverages, 69% (9/13) still developed EAMC post ingestion.[31] EAMC and the dehydration theory has its points, but no logical evidence to state that increased fluid and electrolyte losses cause EAMC.[15,16,31]

Muscle Cramps: Muscle Fatigue Theory

Muscle fatigue and EAMC is a well hypothesized and critical link for pathology of EAMC. Muscle fatigue during times of fluid and electrolyte imbalances result from prolonged exercise.[15,16,31] A study investigating 1383 marathon runners were asked about the prevalence of EAMC.[16] The majority of the runners (60%, 830/1383) stated they were experiencing muscle fatigue prior to episodes of EAMC.[16] Of the 1383 runners, 26 % (536/1383) stated they had a history of EAMC.[16] Altered muscular function leads to the next available hypothesis for EAMC.

EAMC during muscle fatigue are caused by an increased excitatory response in muscle spindles and a decrease inhibitory response in Golgi tendon organs (GTO).[15] This response leads to abnormal alpha (α) motor neuron control and activity.[15] The neural control of detecting

muscle tension by the GTO is disrupted and muscle contractions are unlimited.[15] The afferent activity of the muscle spindles enhances excitatory activity by causing involuntary muscle contractions.[15] The inhibitory effects of the GTO opposing an increase in excitatory activity by muscle spindles are limited.[15]

Already shortened muscles while exercising are more vulnerable to muscle cramps.[15] Neuromotor end-plate depolarization and GTO inhibitory activity are disrupted, especially in the shortened position.[15] Stretching can help relieve EAMC. Stretching causes tension in the muscle tendon activating the GTO, thus the inhibitory effects of the alpha motor neuron increases creating the balance between inhibitory and excitatory activity.[15,16,31]

A multi-analysis looking at EAMC and muscular fatigue was done in normal college students. One hundred fifteen college students performed maximal muscle contractions at rest and during exercise.[16] Eighteen percent (21/115) sustained muscle cramps prior to exercise and 26% (30/115) sustained muscle cramps after a 20-30 minute workout.[16] The muscle cramps endured in this study were tested by electromyography and were easily relieved with stretching.[16] The conclusions from this study showed that muscle cramps could be caused by increased and decreased activity originating in the central nervous system.[16]

Muscle fatigue has more direct cause and effect correlation with EAMC.[15,16,31] A study looked at 13 healthy men focused on EAMC and muscle fatigue. Each male participant completed an exercise protocol designed to fatigue the gastrocnemius.[16] Each participant ingested 1.0 to 1.5 L per hour of fluids containing carbohydrates and electrolytes prior to exercise.[16] After a mean of 15 minutes, the mean sweat rate was 2.0 L per hour and mean total fluid loss was 500 mL.[16] Significant episodes of EAMC were experienced by all participants, but sweat rate and sodium losses were not high enough to cause dehydration.[16]

Muscle fatigue and EAMC have been well documented in animal research.[16,31] Though animal research does not reflect on human purposes, it has been shown that EAMC can be caused by muscle fatigue. Muscle fatigue in animals was shown by disrupting the functional ability of peripheral muscle receptors.[16] Increased firing rate of the muscle spindle's type Ia and II afferent was present.[16] Also, a decrease in the type IIb afferent activity from the GTO was present.[16] Therefore, a combination of the increased muscle spindle activity and decreased GTO activity could be present during prolonged exercise.[16] Results from this would be sustained α-motor neuron activity and possible EAMC.[16]

EAMC and muscle fatigue has more premises behind the occurrence of muscle cramps.[15,16,31] Though these premises occur, scientific data while exercising have yet to be proven to correlate EAMC and muscle fatigue.[15,16,31] Muscle injury and muscle damage from exercise can result in a spasm causing involuntary muscle contractions.[16] Decreased signals from pain or pressure receptors could alter neuromuscular control through central nervous system output.[16] Muscle spindles and GTO would be the recipients of those altered neuromuscular changes.[16]

Not only has dehydration and prevalence of EAMC been studied by amount of electrolyte levels after exercise, but as well as total body weight loss and muscle re-education. Weight loss levels near 3 % hypohydration and near 5 % hypohydration have shown no effect on EAMC threshold frequency from pre to post threshold frequency.[32,33] Through these total body water losses, it is also concluded that electrolytes prevalent in sweat and perspiration lost during exercise has shown no effect on those with a self-reported history of EAMC during prolonged exercise.[34,35,36,37] Finally, a case study looked at the effect of muscle re-education by properly training and eliciting the correct firing sequence.[38] The patient suffered from bilateral episodes

of EAMC in his gastrocnemius during prolonged training for triatholons.[38] The patient underwent initial electrolyte replacement therapy via enhanced diet and continued to have bouts of EAMC. The patient began physical therapy focusing on strengthening his core, with emphasis on his glute and pelvic girdle muscles.[38] After three months of therapy, the patient began to train and never had one episode of EAMC occur while obtaining personal record times.[38]

Muscular fatigue and EAMC are more prevalent than EAMC and dehydration. EAMC has occurred in many athletes and workers, but the evidence comes from case studies, clinical observations, and a case-control study.[16,31] Studies focusing on EAMC show that while working in hot, humid environments, cramp risks increase.[16,31] The observations were not tested and scientific data are irrelevant for a majority of this theory.

Evidence supporting muscle fatigue theory was reported in humans inducing muscle cramps. Spinal reflex activity measuring type I, type IIa, and type IIb afferents in animals provides significant data.[16,31] Field studies measuring electromyography were recorded in humans during and after fatiguing exercise that induced muscle cramps.[16] While there are more things to be resolved, muscle fatigue and EAMC acts as the main physiological response of EAMC in comparison to dehydration and electrolyte imbalances.[15,16,31]

Prevention of EAMC

Treatment and prevention methods of EAMC contain aspects from both theories. Ingesting fluids high in electrolytes is a common practice for treatment of EAMC.[31] Many sport drinks do not contain the necessary amount of electrolytes in order to replace the electrolyte losses through sweat.[31] The National Athletic Trainers Association (NATA) recommends adding 0.3-0.7 g/L of sodium to your drink in order to reduce the occurrence of muscle cramps.[39] Further recommendations is adding one-quarter teaspoon of salt to 500 cc of water.[40]

Ingesting fluids for immediate treatment for cramps have been misconceived. Mustard, pickle juice, bananas, dextrose have been used for immediate treatment for EAMC.[40] However, ingesting these substances have not shown to alleviate EAMC.[21] It has been shown that fluids and electrolytes are not absorbed immediately.[31] Even hypotonic fluids have been shown to be absorbed into the circulatory system after 13 minutes.[31] Beyond increasing carbohydrate-electrolyte ingestion, stretching is a common mode of treating EAMC. Stretching is an effective remedy for treating EAMC and is one the more widely used practices in treating EAMC.[31,40]

Common practices are available to prevent occurrence of EAMC. Acclimation to environmental temperatures compliments the dehydration theory.[40] Ingesting at least one liter of fluids (e.g. water, sports drink) prior to competition prompts proper absorption of fluids and availability of electrolytes and nutrients for any chance of EAMC.[31] During competition, consumption of powder and gel supplements may boost depleted electrolytes stores and reduce the chance of EAMC.[40]

Performing exercises targeting the neuromuscular system compliments the muscular fatigue theory. Exercises activating the muscle spindles and GTO receptors can be implemented to reduce muscular fatigue.[31] Plyometric exercises implemented into an athlete's regimen elicit adaptations in muscle spindle and GTO receptor firing sequences controlling the neuromuscular system.[31] Endurance training also serves a purpose in neuromuscular control by increasing plasma volume and extracellular fluid during exercise.[31] Therefore, increasing endurance and plyometric training can delay neuromuscular fatigue.[31]

Caffeine and Muscular Functions relating to EAMC

Caffeine and prevalence of muscle cramps have not been shown to elicit neuromuscular changes after ingestion. However, a case study regarding abundant use of caffeine on a daily

basis eventually led to frequent cramping episodes.[41] The patient from the following case ingested caffeinated pills (Finimal) six to seven times a day, four cups of coffee, and two cups of tea daily.[41] Upon ingestion of beverages and pills, the patient smoked 10 cigarettes daily.[41]

The patient had recurrent muscle cramps at night. Reflecting on the patient's use of caffeine, scientists performed a double-blind study prescribing varying doses of caffeine to the patient. Through one week of testing, the patient reported back to the scientist. Patient's vulnerability of muscle cramping increased when the dosage exceeded 600 mg.[41] Below 600 mg of caffeine, the occurrence of cramping dispersed to no episodes of cramping.[41]

Caffeine and neuromuscular patterns provide a greater insight on possible events occurring between caffeine ingestion and EAMC. Scientists have found some key points on neuromuscular patterns following caffeine ingestion. Even though research around caffeine ingestion and EAMC are limited, caffeine ingestion and neuromuscular patterns explain some possible points of what could occur with caffeine and EAMC. One theory that can be made is the Hoffman Reflex or H-reflex.

The H-reflex is an electrically induced reflex that is similar to an mechanically induced spinal stretch reflex.[42] The difference between the H-reflex and a spinal stretch reflex is the H-reflex bypasses the muscle spindle allowing assessment of monosynaptic reflex activity occurring in the spinal cord.[42] The H-reflex is an estimate of alpha motor neuron excitability when presynaptic inhibition and intrinsic excitability of the alpha motor neurons are constant.[42] The H-reflex is a tool to assess the nervous system during application of many neurological conditions.[42]

The H-reflex has been known to decrease in amplitude post exercise.[10] Scientists have found that the H-reflex remains at the same amplitude, but the state anxiety after exercise

increases post caffeine ingestion.[10] State anxiety is described as the amount of tension within the muscle assessed by the H-reflex post exercise.[10] Motl and Dishman observed 16 participants cycling at 60% of VO_{2Peak}.[10] H-reflex and state anxiety were assessed before and one hour post caffeine ingestion as well as 10 minutes and 30 minutes post cycling. No changes in the amplitude of the H-reflex was shown, but state anxiety increased after caffeine consumption.[10]

The clinical application of the H-reflex can relate to sport-specific conditions. H-reflex measurements can assess the excitability actions occurring within the spinal cord. Assessing this by using the H-reflex can associate excitability levels and muscular fatigue. Walton, Kalmar, and Cafarelli[11] noted this action between caffeine and H-reflex. Seven subjects were either given caffeine (6 mg/kg) or placebo-filled capsules.[11] Assessments by stimulating the tibial nerve were made pre-ingestion and one hour post-ingestion. Excitatory function of the spinal cord increased 43±17% ($P<0.05$) showing increased excitability following caffeine ingestion.[11]

H-reflex and caffeine ingestion is documented, as well as caffeine ingestion and skeletal muscle function. Caffeine's effect on adenosine and the sarcoplasmic reticulum questions its role in human skeletal muscle. Caffeine inhibits the actions of adenosine increasing excitatory neurotransmitter release by lowering the threshold for neuronal activity.[11] Caffeine also increases calcium permeability within the sarcoplasmic reticulum allowing the contraction mechanism to be readily available.[43]

These observations are well documented and proven to occur in human skeletal muscle. Walton, Kalmar, and Cafarelli[12] observed this change in humans. Their main premise was observing self-sustained firing (SSF) in human motor units.[12] SSF is the continued firing of a motor neuron following a recruitment by a synaptic excitation during a constant, maybe decreasing, level of synaptic drive.[12]

Caffeine's inhibition effects on adenosine causes other neurologic processes.[12] Serotonin and noradrenaline increase following adenosine inhibition.[12] Serotonin and noradrenaline facilitate as neurotransmitters allowing plateau potentials in SSF to occur.[12] By ingesting caffeine, increases should be show during SSF function in motor units.[12]

Stimulation of the tibialis anterior motor units performed by Walton et al.[12] provided clarity in this premise. Two hundred-fourteen trials among seven male participants showed an increase in SSF ($P<0.05$).[12] The caffeine group had a significantly higher SSF (87.0±5.8%) than the placebo group (64.6±9.7%).[12] Although this showed the effects of caffeine on SSF, further research needs to be conducted to conclude this fact between SSF and caffeine ingestion.

Effects of caffeine ingestion on neuromuscular function have mixed results. While there is evidence to point to caffeine ingestion causing effects on neuromuscular function, Kalmar[44] shows that there is something greater to the concept of caffeine ingestion and neuromuscular function. Kalmar notes that the effects of caffeine has some general points on neurological modulation, but there is no empirical evidence stating the effects of caffeine on neuromuscular function.[44] The effects of caffeine at the spinal and supraspinal level have been noted in some studies, but no direct cause and effect relationship between caffeine and neuromuscular function exists at the time of this research, but limited research has been done since 2005.[44]

Research purposing a relationship between caffeine ingestion and neuromuscular function is slightly outdated (1999), but provides a good insight on what can occur. Kalmar and Cafarelli[45] induced electrical stimulation of the tibial nerve following caffeine ingestion (6 mg/kg).[45] Maximum voluntary contraction of the knee extensors and maximal H-reflexes of the soleus were assessed.[45] Maximum voluntary contraction increased 3.50±1.01% ($P<0.01$), but no change in H-reflex amplitude was shown.[45] Therefore, caffeine increased excitatory responses at

the supraspinal level.[45] The effect of the caffeine did not reach the spinal level, which could dismay the fact that the caffeine had any role at all in causing altering neuromuscular function.[45]

Summary

EAMC is prevalent among all populations, especially athletes and individuals living an active lifestyle. Many athletes and active individuals consume caffeine on a regular basis in efforts to increase mood, focus, alertness, and possibly performance. Caffeine is shown to increase endurance during prolonged exercise, however no conclusive evidence states a correlation between caffeine and improvements in anaerobic performance (e.g. strength, power, torque). Physiological aspects of caffeine ingestion at normal doses include diuresis, increased thermoregulatory function, and possibly altered neuromuscular alteration. Therefore, EAMC should be more prevalent and the likelihood should increase during exercise. Furthermore, more evidence of caffeine usage prior to exercising needs to be concluded.

METHODS

The purpose of this study determined if caffeine ingestion decreased cramp threshold frequency and increased the likelihood of exercise associated muscle cramps occurring in athletes and physically active individuals. The following research question was addressed during this study: Did ingesting 0 mg (placebo), 250 mg or 500 mg of caffeine decrease cramp TF?

Experimental Design

A 2x3 factorial crossover design guided data collection. The order of treatments were randomized and counterbalanced with a Latin square. The dependent variable was flexor hallucis brevis cramp threshold frequency (TF). Threshold frequency was measure in hertz. The independent variable was caffeine dosage (0 mg, 250 mg, and 500 mg). Caffeine consisted of a powder and was inserted into an ingestible capsule. Hematocrit, hemoglobin, plasma sodium concentration, plasma potassium concentration, plasma chloride concentration, and osmolality were identified and analyzed for any change in hydration status prior to and post-caffeine ingestion respectively.

Subjects

A convenience sample of 11 male and 6 female participants detecting a significant difference in caffeine ingestion and EAMC ($P<0.05$) with a self-reported history of muscle cramping within the last six months prior to data collection was recruited by word of mouth and social media advertisement. Exclusion criteria for subject's participation consisted of 1) injury or surgery to the dominant limb within the last 12 months; 2) any neurologic, cardiovascular, or neuromuscular condition/disease; or 3) not between the ages of 18-35. All subjects provided a written informed consent and the study was reviewed and approved by the North Dakota State University Institutional Review Board prior to data collection.

Procedures

Participants reported to the Research Lab (BBF 14) and were instructed to maintain their current diet, water intake, and refrain from strenuous exercise for the next 24 hours. A baseline TF was established with each subject on the first day. The participant was informed by reading and providing a written informed consent and fill out the health history questionnaire prior to baseline testing. Concluding the health questionnaire the subject was given a detailed brochure on what they can and can't consume for the next 72 hours. The brochure listed foods and beverages that contained caffeine or caffeine substitutes.

The participant laid supine on the table. Preparation of the participant's dominant lower limb occurred once their weight had been taken. Dominant lower limb selection was done by asking the participant if they were to kick a ball, what leg would they use to kick the ball. To determine the exact location of the flexor hallucis brevis, participant was asked to dorsiflex the 1st ray. The co-investigator marked the joint space in between the distal phalanx and metatarsal of the 1st ray, the joint space of the 1st metatarsal, and the 1st cuneiform. Hair was removed around the tibial tuberosity depending on the participant. Then the following areas were debrided using fine sandpaper: 1) tibial tuberosity, 2) medial plantar aspect of the mid 1st metatarsal, and 3) medial malleolous. Isopropyl alcohol was applied to the same areas following debridement of the fine sandpaper. The 1st metatarsal was dorsiflexed and 2- 8mm Ag-AgCl electrodes (Biopac Systems, Inc.) over the flexor hallucis brevis was applied. Inter-between electrodes was be 2 cm.

The co-investigator located the tibial pulse and applied a mark for tibial nerve. The co-investigator began the stimulation of the tibial nerve to find a strong, yet proper contraction. The flexor hallucis brevis contraction should not be felt in the heel by the participant. The co-investigator also noted the involuntary flexion of the big toe. A dispersive electrode was applied

to the lateral malleolous while the stimulating electrode was applied along the medial malleolous to stimulate the tibial nerve. While trying to identify the tibial nerve, the area was stimulated 2-4 times with a 1-ms electrical stimulus to determine the exact location of the tibial nerve. Once located, the stimulating electrode was re-applied with tape to secure the correct location and the dispersive electrode was secured with an elastic wrap.

The participant was advised of types of sensations and would receive electrical stimulation beginning at 80-V trains at 2 second bursts per minute. Each participant began with 4 Hz. Each participant increased the electrical stimulation they receive by 2 Hz from the previous stimulation. Over a 2 second period, the participant received 8 electrical bursts. If the participant did not cramp after the bout of stimulation, the participant rested for one minute. This process continued until a muscle cramp occurred.

A muscle cramp must meet the following criteria: 1) involuntary contraction of the flexor hallucis brevis immediately after electrical stimulation resulting in great toe flexion and 2) the participant states they are having a cramp. Cramp TF was recorded at baseline and after each caffeine ingestion trial. Each participant was asked to perform this task over three testing days with 48 hours in between each testing session. Each day included dosages of caffeine (0 mg, 250 mg, 500 mg) administered in random order and was ingested one hour prior to the stimulation bouts. On each testing day, the participant was asked to empty their bladder and provide the co-investigator a collected urine sample. Subjects with a urine specific gravity of 1.020 or less can be determined as euhydrated and begin the equilibration stage. If the urine sample was greater than 1.020, the participants would ingest 3 mL/kg of water and rest for 30 minutes to allow water absorption. Urine specific gravity was re-assessed until the participants had reached a euhydrated state.

Following urine analysis, the subject laid supine to begin the equilibration stage for 30 minutes. This would allow the body's internal fluid compartments to balance and provide a proper blood sample. After 30 minutes, a venous catheter was inserted in the subject's forearm vein and a 5 mL sample was drawn. The subject picked a number out of a hat which included either a 1, 2, 3, 4, 5, or 6. The number correlates with the sequence relating to the dosage order balanced via Latin Square and ingested by subject over the three testing days.

Once the first blood draw had been performed and the subject's dose sequence established, the subject was given their first dose. The subject would either ingest 0 mg, 250 mg, or 500 mg of caffeine depending on their selected dose sequence. Caffeine doses were blinded for all participants and ingested one hour prior to the stimulation bouts. After 1 hour, a second blood sample was drawn and a urine sample collected if subject needed to empty their bladder. The subject then began the stimulation bout. The results were recorded following the subject's maximal cramp threshold frequency. The subject did this over a course of three days and was compensated following their last testing day.

Instrumentation

Muscle Stimulation

An 8 mm Ag-AgCl shielded active electrode and an 8 cm^2 dispersive electrode were applied to the foot of each participant.[46] The muscle action potential of the flexor hallucis brevis was collected using the MP150 analog-to-digital data acquisition system with Acq*Knowledge* software (version 3.7.3; Biopac Systems Inc.).[46] Signals were amplified using the TEL 100C (Biopac Systems, Inc.) with a gain set to 5000 from disposable, long-term recording electrodes with a center-to-center interelectrode distance of 2 cm.[46] The EMG signals were sampled at 2000 Hz.[1] The total EMG recording consisted of 1 second of resting activity, 2 seconds of

stimulation, and 12 seconds of post stimulus activity (cramping).[46] The train (bouts of stimulation) of the electrical stimuli were delivered to the tibial nerve by a Grass S88 stimulator and SIU5 Stimulation Isolation Unit (Astro-Med, Inc., West Warwick, RI).[1]

Urine Analysis

Urine specific gravity was measured with a handheld refractometer (SUR-Ne; Atago USA Inc., Bellevue, WA). A specific gravity less than or equal to 1.020 indicated subject's state of euhydration.[20] If specific gravity was greater than 1.020, the subject would continue to hydrate on testing day until their specific gravity was less than or equal to 1.020.

Blood Analysis

Hematocrit, hemoglobin, plasma sodium concentration, plasma potassium concentration, plasma chloride concentration, and osmolality were measured by conducting venipuncture of subject's antecubital region. The subject's antecubital region was sterilized with alcohol and one, single-use, 20-gauge venous catheter (BD, Sandy, UT) was inserted into a superficial forearm vein. The catheter was attached to a 3-way stopcock (Tyco Healthcare Group LP, Mansfield, VA) with extension tubing (B. Braun Medical Inc., Bethlehem, PA). A 5 mL blood sample was drawn. Four mL of whole blood was drawn into a 6 mL lithium heparin vacutainer (BD, Franklin Lakes, NJ) and chilled in a crushed-ice bath until the last blood sample was drawn. To measure hematocrit, about 1 mL from the 5 mL sample of whole blood was drawn into heperanized microcapillary tubes and centrifuged at 3000 rpm for 5 minutes (IEC Micro-MB; IEC, Needham Heights, MA). The final hematocrit was read using a microcapillary reader (IEC 2201; Damon/IEC, Needham Heights, MA). Hemoglobin was measured by mixing 20 µL of whole blood with 5 mL of cyanmethemoglobin reagent. The sample was read with a standard

spectrophotometer at 540 nm (iMark; Biord, Hercules, CA). Hemoglobin and hematocrit were sampled and measured for each time for reliable measures.

The remaining 4 mL of whole blood was centrifuged at 3000 rpm for 15 minutes at 3°C (5804; Eppendorf North America Inc., New York, NY) and plasma was drawn from the red blood cells. Plasma osmolality was determined with freezing point depression osmometry (3D3; Advanced Instruments Inc., Norwood, MA). Plasma electrolyte concentrations were measured two times using an ion-sensitive electrode system (NOVA 16; NOVA Biomedical, Waltham, MA).

Statistical Analysis

Means and standard deviations were calculated and used for statistical analysis with the factors being time and dose. A two-way analysis of variance (ANOVA) was used to determine the effect of caffeine dosages on cramp threshold frequency, pre and post hemoglobin, hematocrit, plasma sodium concentration, plasma potassium concentration, plasma chloride concentration, and osmolality. If significant F-values were noted through two-way analysis of variance, Tukey-Kramer post hoc tests and one-way analysis of variance (ANOVA) for each dose were used to identify significant differences between baseline muscle cramp TF and post caffeine ingestion muscle cramp TF as well as pre and post hemoglobin, hematocrit, plasma sodium concentration, plasma potassium concentration, plasma chloride concentration, and osmolality. The α-level was set at 0.05 (SPSS: 20th edition; Pearson Education Inc., Upper Saddle River, NJ).

MANUSCRIPT. THE EFFECTS OF CAFFEINE INGESTION ON MUSCLE CRAMP THRESHOLD FREQUENCY

Introduction

Caffeine is the most widely consumed substance for recreational and ergogenic purposes. Eighty-four to 91 percent of men and women between the ages of 18-35 consume, on average, 238 mg of caffeine per day.[1] Caffeine is normally consumed in beverages such as coffee (71%), tea (16%), and soft drinks (12%).[1] The caffeine in soda and coffee varies as soda contains 20-55 mg of caffeine (12 oz.)[2] and coffee contains up to 80-120 mg of caffeine (8 oz.).[2] Daily caffeine consumption varies, but it has been reported to be as high as 600 mg per day in adults ages 18-35.[1]

Caffeine ingestion causes several physiological effects such as stimulating the central and peripheral nervous systems. Caffeine has been shown to cause ergogenic effects ingesting 2-3 mg/kg.[3] Consuming caffeine prior to exercise improved endurance in trained cyclists,[4] but it appeared to have negligible effects on anaerobic performance (e.g., strength and power).[5] When ingested at rest, urine production increased;[6] however, when caffeine was ingested prior to exercise or during exercise, urine production was unaffected.[7,8,9] The neurological effects of caffeine are also well documented.[10,11,12] Caffeine ingestion stimulates the sympathetic nervous system,[13] which may increase resting metabolic rate and internal heat storage, leading to higher core body temperatures and sweat rates.[13] Moreover, caffeine ingestion increases spinal excitability (i.e. Hoffman reflex)[11] and motor unit self-sustaining firing rates.[12]

Exercise associated muscle cramps (EAMC) are a common injury affecting recreational and competitive athletes.[14] EAMC are thought to be caused by dehydration/electrolyte imbalances[15] and changes in neuromuscular control.[16] The dehydration and electrolyte imbalance

theory proposes EAMC occur when select motor nerves become hyperexcitable due to fluid and electrolyte losses incurred during exercise.[15] The neuromuscular control theory states that EAMC occur because of fatigue induced changes to muscle spindles and golgi tendon organs which alters alpha motor neuron activity.[16]

Since EAMC are unpredictable, researchers induce them in laboratory settings. Cramp threshold frequency (TF) is the minimal amount of electrical stimulation needed to elicit a muscle cramp[17] and is used as a quantitative indicator of cramp risk.[18] Individuals with a self-reported history of muscle cramping have a lower TF than those who self-report no history of cramping.[19] Therefore, cramp TF may be useful as a clinical tool to identify individuals at risk of cramping and develop treatment and prevention strategies. Because caffeine may cause diuresis[13] and excitation of the peripheral nervous system,[10, 11, 12] ingesting it may increase cramp risk. No scientific data exist on the effect of caffeine on cramp TF.

The purpose of this study determined if caffeine ingestion decreased cramp threshold frequency and increased the likelihood of exercise associated muscle cramps occurring in physically active individuals. This study also determined if caffeine ingestion causes dehydration 1-hour post ingestion by looking at pre and post blood analysis of hemoglobin, hematocrit, plasma sodium concentration, plasma potassium concentration, plasma chloride concentration, and osmolality. The following research question was addressed: Did ingesting either 0 mg (placebo), 250 mg, or 500 mg of caffeine decrease cramp TF?

Methods

Participants

A convenience sample of 11 male and 6 female healthy, physically active participants (females; age: 22.16 ± 2.32 years, height: 166.37 ± 9.32 cm, weight: 64.86 ± 15.82 kg, males;

age: 22.72 ± 4.05 years, height: 178.49 ± 9.30 cm, weight: 84.95 ± 12.67 kg) with a self-reported history of muscle cramping within the last six months prior to data collection was recruited by word of mouth and social media advertisement. Exclusion criteria for subject's participation consisted of 1) injury or surgery to the dominant limb within the last 12 months; 2) any neurologic, cardiovascular, or neuromuscular condition/disease; or 3) not between the ages of 18-35. All subjects signed an informed consent and our study was approved by the Institutional Review Board prior to data collection.

Instruments

Muscle Stimulation

An 8 mm Ag-AgCl shielded active electrode and an 8 cm^2 dispersive electrode were applied to the dominant foot of each participant.[20] The muscle action potential of the flexor hallucis brevis was collected using the MP150 analog-to-digital data acquisition system with Acq*Knowledge* software (version 3.7.3; Biopac Systems Inc.).[20] Signals were amplified using the TEL 100C (Biopac Systems, Inc.) with a gain set to 5000 from disposable, long-term recording electrodes with a center-to-center interelectrode distance of 2 cm.[20] The EMG signals were sampled at 2000 Hz.[1] The total EMG recording consisted of 1 second of resting activity, 2 seconds of stimulation, and 12 seconds of post stimulus activity (cramping).[20] The train (bouts of stimulation) of the electrical stimuli was delivered to the tibial nerve by a Grass S88 stimulator and SIU5 Stimulation Isolation Unit (Astro-Med, Inc., West Warwick, RI).[1]

Urine Analysis

Urine specific gravity was measured with a handheld refractometer (SUR-Ne; Atago USA Inc., Bellevue, WA). A specific gravity less than or equal to 1.020 indicated the subject's

state of euhydration. If specific gravity was greater than 1.020, the subject would continue to hydrate on testing day until their specific gravity was less than or equal to 1.020.

Blood Analysis

Hematocrit, hemoglobin, plasma sodium concentration, plasma potassium concentration, plasma chloride concentration, and osmolality were measured by conducting venipuncture of subject's antecubital region. The subject's antecubital region was sterilized with alcohol and one, single-use, 20-gauge venous catheter (BD, Sandy, UT) was inserted into a superficial forearm vein. The catheter was attached to a 3-way stopcock (Tyco Healthcare Group LP, Mansfield, VA) with an extension tubing (B. Braun Medical Inc., Bethlehem, PA). A 5 mL blood sample was drawn. Four mL of whole blood was drawn into a 6 mL lithium heparin vacutainer (BD, Franklin Lakes, NJ) and chilled in a crushed ice bath. To measure hematocrit, approximately 1 mL from the 5 mL sample of whole blood was drawn into heparinized microcapillary tubes and centrifuged at 3000 rpm for 5 minutes (IEC Micro-MB; IEC, Needham Heights, MA). The final hematocrit was read using a microcapillary reader (IEC 2201; Damon/IEC, Needham Heights, MA). Hemoglobin was measured by mixing 20 µL of whole blood with 5 mL of cyanmethemoglobin reagent. The sample was read with a standard spectrophotometer at 540 nm (iMark; Biorad, Hercules, CA). Hemoglobin and hematocrit was sampled and measured twice each time for reliability.

The remaining 4 mL of whole blood was centrifuged at 3000 rpm for 15 minutes at 3°C (5804; Eppendorf North America Inc., New York, NY) and plasma was drawn from the separated red blood cells. Plasma osmolality was determined with freezing point depression osmometry (3D3; Advanced Instruments Inc., Norwood, MA). Plasma electrolyte

concentrations were measured two times for each sample using an ion-sensitive electrode system (NOVA 16; NOVA Biomedical, Waltham, MA).

Procedures

Participants reported to the Research Lab and were instructed to maintain their current diet, water intake, and refrain from strenuous exercise for the next 24 hours. A baseline TF was established with each subject on the first day. The participants were informed of the testing procedures and signed an informed consent and filled out the health history questionnaire. Next the subject was given a detailed brochure on what they could and couldn't consume for the next 72 hours. The brochure listed foods and beverages that contained caffeine and caffeine substitutes.

To determine baseline muscle cramp TF the participant laid supine on the table. Preparation of the participant's dominant lower limb occurred once their height and weight had been taken. To determine the exact location of the flexor hallucis brevis on the subjects dominate leg, participants were asked to dorsiflex the 1st ray. The joint space between the distal phalanx and metatarsal of the 1st ray, the joint space of the 1st metatarsal, and the 1st cuneiform were marked for electrode placement. If necessary, hair was removed around the tibial tuberosity and the following areas were debrided using fine sandpaper: 1) tibial tuberosity, 2) medial plantar aspect of the mid 1st metatarsal, and 3) medial malleolous. Isopropyl alcohol was applied to the same areas following debridement of the fine sandpaper. The 1st metatarsal was dorsiflexed and 2- 8mm Ag-AgCl electrodes (Biopac Systems, Inc.) were applied over the flexor hallucis brevis. Inter-between distance for the electrodes was 2 cm.

The tibial pulse was located and marked to identify the tibial nerve. Stimulation bursts to the tibial nerve were used to identify a strong, yet proper contraction of the flexor hallucis brevis.

While trying to identify the tibial nerve, the area was stimulated 2 to 4 times with a 1-ms electrical stimulus to determine the exact location of the tibial nerve. A dispersive electrode was applied to the lateral malleolous while the stimulating electrode was applied along the medial malleolous to stimulate the tibial nerve. Once located, the stimulating electrode was re-applied with tape to secure the correct location and the dispersive electrode was secured with an elastic wrap.

The participants were advised of the various types of sensations and received electrical stimulation beginning at 80-V trains at 2 second bursts per minute. Each participant began with 4 Hz. The participant's amount of electrical stimulation increased by 2 Hz with each unsuccessful bout that provided no sign of a muscle cramp. Over a two-second period, the participant received eight electrical bursts. If the participant did not cramp after the bout of stimulation, the participant rested one minute. The process continued until the muscle cramp occurred.

A muscle cramp met the following criteria: 1) involuntary contraction of the flexor hallucis brevis immediately after electrical stimulation resulting in great toe flexion and 2) the participant stated they had a cramp. Cramp TF was recorded at baseline (day 1) and after each caffeine ingestion trial (days 2, 3, 4). On the first caffeine ingestion trial, the participant picked a number out of a hat which included 1, 2, 3, 4, 5, or 6. The number correlated with a sequence relating to the dosage order balanced via Latin Square and ingested by the participant over the three testing days. Each participant ingested 0 mg (placebo), 250 mg, and 500 mg of caffeine and performed this process over the three caffeine ingestion days with 48 hours between each session. Caffeine doses were blinded for all participants and ingested one hour prior to the stimulation bouts.

On each testing day, the participant was asked to empty their bladder and provide a urine sample. Participants with a urine specific gravity of 1.020 or less were determined as euhydrated and began the equilibration stage. If the urine sample was greater than 1.020, the participants ingested 3 mL/kg of water and rested for 30 minutes to allow water absorption. Urine specific gravity was re-assessed until the participants reached a euhydrated state. Table 1 shows specifications on each subject's urine specific gravity for each testing days.

Table 1. Urine Specific Gravity Specifications Prior to Intervention.

Urine Specific Gravity (Subjects)	0 mg Caffeine (n=17)	250 mg Caffeine (n=17)	500 mg Caffeine (n=17)
1	1.008	1.009	1.009
2	1.010	1.010	1.012
3	1.012	1.012	1.010
4	1.009	1.013	1.011
5	1.005	1.020	1.011
6	1.019	1.007	1.017
7	1.015	1.020	1.016
8	1.020	1.005	1.020
9	1.017	1.020	1.015
10	1.018	1.020	1.017
11	1.012	1.019	1.020
12	1.014	1.020	1.020
13	1.020	1.017	1.011
14	1.011	1.018	1.018
15	1.019	1.020	1.020
16	1.020	1.013	1.020
17	1.010	1.009	1.011
	*1.014 ± .004	*1.014 ± .005	*1.015 ± .004

*****Values are Means ± SD

Following urine analysis, the participants laid supine and began the equilibration stage for 30 minutes. This allowed the body's internal fluid compartments to balance and provide a proper blood sample. After 30 minutes, a venous catheter was inserted in the subject's forearm vein and 5 mL of blood was drawn.

Once the first blood draw had been performed and the participant's dose sequence established, the participant was given his/her first dose. The participant either ingested 0 mg, 250 mg, or 500 mg of caffeine depending on their selected dose sequence. The participant ingested the dosage immediately and continued to lay supine for one hour. After one hour, a second blood sample was drawn followed by the muscle stimulation bout. The results were recorded following the participant's maximal cramp threshold frequency. The participant repeated this procedure over the course of three testing days and was compensated on their last testing day.

Statistical Analysis

Means and standard deviations were calculated and used for statistical analysis with the factors being time and dose. A two-way analysis of variance (ANOVA) was used to determine the effect of caffeine dosages on cramp threshold frequency, pre and post hemoglobin, hematocrit, plasma sodium concentration, plasma potassium concentration, plasma chloride concentration, and osmolality. If significant F-values were noted through two-way analysis of variance, Tukey-Kramer post hoc tests and one-way analysis of variance for each dose were used to identify significant differences between baseline muscle cramp TF and post caffeine ingestion muscle cramp TF as well as pre and post hemoglobin, hematocrit, plasma sodium concentration, plasma potassium concentration, plasma chloride concentration, and osmolality. The α-level was set at 0.05 (SPSS: 20th edition; Pearson Education Inc., Upper Saddle River, NJ).

Results

Ingesting 0 mg, 250 mg or 500 mg of caffeine increased muscle cramp threshold frequency. Means and standard deviations for all dependent variables are found in Table 2; results of ANOVA's for caffeine dose are found in Table 3. For muscle cramp threshold

frequency, a significant time by dose interaction was found. Follow-up analysis showed that, at each dosage level, post-test threshold frequency was significantly higher than baseline. In addition, when post-test cramp threshold was compared across the three dosages, there was a significant difference from 0 mg to 500 mg and 250 mg to 500 mg. There was no significant difference found between 0 mg and 250 mg with post-test cramp threshold frequency (see Table 4).

For hemoglobin, plasma potassium concentration, and osmolality, a significant main effect was also found for time. Hemoglobin, plasma sodium concentration, and osmolality showed significant increases from pre to post caffeine ingestion for all three doses, with the exception of hemoglobin decreasing from pre to post caffeine ingestion at 0 mg. No effect for dose or for the interaction of dose and time was found. Finally, no significant effects of any type were found for hematocrit, plasma chloride concentration, or plasma sodium concentration.

Table 2. Mean and Standard Deviations of Dependent Variables.

Pre Ingestion	0 mg	250 mg	500 mg
Hb (g/dl)	16.14 ± .67	16.11 ± .74	16.14 ± .56
Hct (%)	43.04 ± 1.26	42.98 ± 1.75	43.42 ± 1.86
[Na] (mmol/l)	138.56 ± 3.29	139.85 ± 2.80	139.09 ± 1.82
[K] (mmol/l)	3.68 ± .29	3.7 ± .40	3.64 ± .30
[Cl] (mmol/l)	104.97 ± 2.28	105.21 ± 2.64	105.82 ± 1.89
OsM (mOsm/kg H_2O)	286.35 ± 5.98	288.82 ± 2.29	288 ± 2.55
Baseline Muscle CTF (Hz)	15.65 ± 4.19		
Post Ingestion			
Muscle CTF (Hz)	16 ± 4.84	17.41 ± 6.11	19.41 ± 6.43
Hb (g/dl)	16.26 ± .56	16.35 ± .59	16.38 ± .63
Hct (%)	43.21 ± 1.40	43.45 ± 1.43	44.04 ± 1.58
[Na] (mmol/l)	138.59 ± 2.31	139.53 ± 2.76	139.09 ± 1.96
[K] (mmol/l)	3.76 ± .21	3.78 ± .36	3.78 ± .27
[Cl] (mmol/l)	105.65 ± 2.12	105.35 ± 2.52	106.09 ± 1.91
OsM (mOsm/kg H_2O)	288.41 ± 2.09	289.35 ± 3.08	289 ± 3.52

*Values are Means ± SD (n=17)

*CTF, cramp threshold frequency; Hb, hemoglobin; Hct, hematocrit, [Na], plasma sodium concentration; [K], plasma potassium concentration; [Cl], plasma chloride concentration; OsM, osmolality

Table 3. ANOVA Dependent Variable Results.

Variables	Caffeine Dose	Pre to Post Ingestion	Dose from Pre to Post Ingestion
Muscle CTF	$F_{2,15}$=.016	$F_{1,16}$=.068	$F_{2,15}$=.016
Hb	$F_{2,15}$=.906	$F_{1,16}$=.001	$F_{2,15}$=.692
Hct	$F_{2,15}$=.246	$F_{1,16}$=.091	$F_{2,15}$=.610
[Na]	$F_{2,15}$=.415	$F_{1,16}$=.349	$F_{2,15}$=.100
[K]	$F_{2,15}$=.904	$F_{1,16}$=.007	$F_{2,15}$=.417
[Cl]	$F_{2,15}$=.196	$F_{1,16}$=.113	$F_{2,15}$=.426
OsM	$F_{2,15}$=.247	$F_{1,16}$=.044	$F_{2,15}$=.682

*α value=.05

*CTF, cramp threshold frequency; Hb, hemoglobin; Hct, hematocrit, [Na], plasma sodium concentration; [K], plasma potassium concentration; [Cl], plasma chloride concentration; OsM, osmolality

Table 4. Caffeine Dose and Post Muscle Cramp Threshold Frequency Comparison.

Paired Sample Comparisons of Caffeine Dosages		95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
Pair 1	0 mg – 250 mg	-3.55538	.73185	-1.396	16	.182
Pair 2	250 mg – 500 mg	-3.88912	-.11088	-2.244	16	.039
Pair 3	500 mg – 0 mg	1.23754	5.58599	3.327	16	.004

Discussion

There is no current literature regarding the effects of caffeine on muscle cramp threshold frequency or the effects of caffeine on muscle cramping. Our study looked at the effect of caffeine ingestion and dosage on cramp threshold frequency. We found a significant increase for muscle cramp threshold frequency for each dose as well from pre to post ingestion. Hemoglobin, plasma potassium concentration, and osmolality showed significant differences from pre to post ingestion, but no significant effects for caffeine dose. These differences may be due to any change in body position. If the participant moved during the hour following caffeine ingestion, fluid compartments would rapidly shift from the intracellular space to the extracellular space resulting in significant increases in hemoglobin, plasma potassium, and osmolality. We measured hemoglobin, hematocrit, plasma sodium concentration, plasma potassium concentration, plasma chloride concentration, and osmolality as they are precursors to an individual's hydration status. Any change would indicate either dehydration or euhydration status of the individual.

Muscle cramping revolves around two theories: the dehydration theory and the muscle fatigue theory. According to the dehydration theory the human body does not store enough water during exercise.[21] Water losses increase fluid and electrolyte depletion causing desensitization of nerve terminals.[21] Desensitization of select nerve terminals increase

mechanical pressure on motor nerve endings, resulting in exercise associated muscle cramps (EAMC).[21] Our study looked at participants who were euhydrated (≤ 1.020) prior to ingesting caffeine on each testing day. Testing for euhydration controlled the outcome of any event relating to the dehydration theory and limiting any change in muscle cramp threshold frequency due to dehydration.

The muscle fatigue theory states that muscle fatigue is caused by an increased excitatory response in muscle spindles and a decreased inhibitory response in Golgi tendon organs (GTO).[15] This response leads to abnormal alpha (α) motor neuron control and activity.[15] The neural control of detecting muscle tension by the GTO is disrupted and muscle contractions are unlimited.[15] The afferent activity of the muscle spindles enhances excitatory activity by causing involuntary muscle contractions.[15] The inhibitory effects of the GTO opposing an increase in excitatory activity by muscle spindles are limited.[15]

Even though our study did not assess urine analysis post caffeine ingestion, our study assumes that dehydration does not have a significant effect on the participant's muscle cramp threshold frequency. From pre ingestion to post ingestion of caffeine, muscle cramp threshold frequency should decrease from baseline threshold frequency to post caffeine ingestion threshold frequency. The results from our study showed significant differences for dose pre ingestion to post ingestion, with cramp threshold frequency increasing over baseline for all three doses.

The relationship between caffeine ingestion and increased cramp threshold frequency maybe related to caffeine's effect on the functional capacity of the muscle. The effect of caffeine on adenosine and the sarcoplasmic reticulum dictates the role of neurotransmitters in normal skeletal muscle. Caffeine inhibits the actions of adenosine increasing excitatory neurotransmitter release by lowering the threshold for neuronal activity.[11] Also observed in

skeletal muscle relating to caffeine ingestion is self-sustained firing (SSF) in human motor units.[12] SSF is the continued firing of a motor neuron following a recruitment by a synaptic excitation during a constant, maybe decreasing, level of synaptic drive.[12] However, this does not explain the increase in muscle cramp threshold frequency in our study.

There are three premises describing how cramp threshold frequency increased following caffeine ingestion in our study. The first premise is pain perception was altered following caffeine ingestion. One study showed plasma b-endorphin concentrations nearly doubled during a two-hour cycling session when consuming caffeine with no significant increase in participants selected for the placebo group.[22] Exercise associated muscle cramps and electrically induced muscle cramps can be painful and cause discomfort for the individual. As caffeine dosage increased, so did the cramp threshold frequency. On many occasions, participants in this study stated they were not feeling a muscle cramp, but showed involuntary flexion of the great toe. They finally realized they were indeed having a muscle cramp when their great toe was stretched allowing the great toe to relax.

The second premise is the effect of caffeine on adenosine. Caffeine resembles adenosine and binds onto adenosine receptors. Adenosine inhibits stimulating events provided through the autonomic nervous system, primarily the sympathetic nervous system. Once caffeine binds to adenosine receptors, adenosine can no longer bind to these receptors allowing the individual's overall perception increase such as mood, alertness, focus, and energy levels. As caffeine dosage increases, the perception of muscle tension increases due to the flight or fight response. Through this reaction, the muscle is already available causing the effects of the caffeine to override the electrical stimulation.

The final premise is related to the individuals that participated in the study. Each individual was advised to refrain from moving and allow the muscle cramp to occur. A normal physical reaction during a muscle cramp event is to relieve the cramp by stretching the great toe. Many individuals tried to follow the recommendation, but had to receive an extra bout of stimulation if they did indeed try to alleviate their muscle cramp.

The clinical relevance of this study supports the view that caffeine did not contribute to causing cramps in active individuals. In the meantime, healthcare professionals should continue to advise physically active individuals to hydrate with uncaffeinated beverages following rehydration periods. Future research is needed in this area to conclude the effects of caffeine on muscle cramp threshold frequency.

Conclusion

There was a statistically significant difference from pre to post muscle cramp threshold frequency when subjects ingested caffeine doses of 0mg (placebo), 250mg, and 500mg. Hemoglobin, plasma potassium concentration, and osmolality were statistically significantly different from pre to post ingestion. Even though cramp thresholds were not lowered by the caffeine ingestion, healthcare professionals should continue to monitor patient's caffeine intake and modify with more suitable hydration sources. Along with hydration maintenance, further research needs to be done with caffeine and its effect on muscle cramp threshold frequency. Looking at the effects of caffeine on various neurotransmitters, caffeine's effect on cramp threshold frequency following physical activity, different modes of caffeine and the effect it has on cramp threshold frequency is recommended for further research. Additionally, further research investigating pre cramp threshold frequency test prior to ingesting caffeine along with performing a post cramp threshold frequency test is needed.

References

1. Frary C, Johnson R, Wang M. Food sources and intakes of caffeine in the diets of persons in the United States. *J Am Diet Assoc.* 2005;105:110-113.

2. Center for Science in the Public Interest: Caffeine Contents of Foods and Drugs. Available at: http://www.cspinet.org/new/cafchart.htm.

3. Jenkins N, Trilk J, Singhal A, O'Connor P, Cureton K. Ergogenic effects of low doses of caffeine on cycling performance. *Int J Sport Nutr Exerc.* 2008;18:328-342.

4. Armstrong L, Pumeratz A, Kellogg M, et al. Fluid, electrolyte, and renal indices of hydration during 11 days of controlled caffeine consumption. *Int J Sport Nutr Exerc.* 2005;15:252-265.

5. Hoffman JR, Kang J, Ratamess NA, Hoffman MW, Tranchina CP, and Faigenbaum AD. Examination of a pre-exercise, high energy supplement on exercise performance. *J Int Soc Sports Nutr.* 2009;6:2-8.

6. Fiala K, Casa D, Roti M. Rehydration with a caffeinated beverage during the nonexercise periods of 3 consecutive days of 2-a-day practices. *Int J Sport Nutr Exerc.* 2004;14:419-429.

7. Del Coso J, Estevez E, Mora-Rogriguez R. Caffeine during exercise in the heat: thermoregulation and fluid-electrolyte balance. *Med Sci Sport Exerc.* 2009;41:164-173.

8. Dias J, Roti M, Armstrong L, et al. Rehydration after exercise dehydration in heat: effects of caffeine intake. *J Sport Rehabil.* 2005;14:294-300.

9. Millard-Stafford M, Cureton K, Wingo J, Trilk J, Warren G, Buyck M. Hydration during exercise in warm, humid conditions: effect of a caffeinated sports drink. *Int J Sport Nutr Exerc.* 2007;17:163-177.

10. Motl R, Dishman R. Effects of acute exercise on the soleus H-reflex and self-reported anxiety after caffeine ingestion. *Phys Behav*. 2004;80:577-585.

11. Walton C, Kalmar J, Cafarelli E. Caffeine increases spinal excitability in humans. *Muscle Nerve*. 2003;28:359-364.

12. Walton C, Kalmar J, Cafarelli. Effects of caffeine on self-sustained firing in human motor units. *J Physiol*. 2002;545:671-679.

13. Armstrong L, Casa D, Maresh C, Ganio M. Caffeine, fluid-electrolyte balance, temperatureregulation, and exercise-heat tolerance. *Exerc Sport Sci Rev*. 2007;35:135-140.

14. Manjra S, Schwellnus M, Noakes T. Risk factors for exercise associated muscle cramping (EAMC) in marathon runners. *Med Sci Sports Exerc*. 1996;28:S167.

15. Bergeron M. Muscle cramps during exercise-is it fatigue or electrolyte deficit? *Curr Sports Med*. 2008;7:S50-S55.

16. Schwellnus M. Cause of exercise associated muscle cramps (EAMC)-altered neuromuscular control, dehydration or electrolyte depletion? *Br J Sports Med*. 2008;10:1-20.

17. Miller K, Knight K, Wilding S, Stone M. Duration of electrically induced muscle cramp increased by increasing stimulation frequency. *J Sport Rehabil*. 2012;12:182-185.

18. Serrao M, Nielsen L, Ge H, Pierelli F, Sandrini G, Farina D. Experimental muscle pain decreases the frequency threshold of electrically elicited muscle cramps. *Exp Brain Res*. 2007;182:301-308.

19. Miller K, Knight K. Electrical stimulation cramp threshold frequency correlates well with the occurrence of skeletal muscle cramps. *Muscle Nerve*. 2009;39:364-368.

20. Miller K., Knight K. Intial electrical stimulation frequency and cramp threshold frequency and force. *J Athl Train.* 2012; 47(5):1-5.

21. Miller K., Stone M., Huxel K., Edwards J. Exercise-associated muscle cramps: causes, treatment, and prevention. *Sports Health.* 2010; 2(4): 279-283.

22. Tarnopolsky M. Effect of caffeine on the neuromuscular system-potential as an ergogenic aid. *Appl Physiol Nutr Metab.* 2008; 33:1284-1289.

REFERENCES

1. Frary C, Johnson R, Wang M. Food sources and intakes of caffeine in the diets of persons in the United States. *J Am Diet Assoc.* 2005;105:110-113.

2. Center for Science in the Public Interest: Caffeine Contents of Foods and Drugs. Available at: http://www.cspinet.org/new/cafchart.htm.

3. Jenkins N, Trilk J, Singhal A, O'Connor P, Cureton K. Ergogenic effects of low doses of caffeine on cycling performance. *Int J Sport Nutr Exerc.* 2008;18:328-342.

4. Armstrong L, Pumeratz A, Kellogg M, et al. Fluid, electrolyte, and renal indices of hydration during 11 days of controlled caffeine consumption. *Int J Sport Nutr Exerc.* 2005;15:252-265.

5. Hoffman JR, Kang J, Ratamess NA, Hoffman MW, Tranchina CP, and Faigenbaum AD. Examination of a pre-exercise, high energy supplement on exercise performance. *J Int Soc Sports Nutr.* 2009;6:2-8.

6. Fiala K, Casa D, Roti M. Rehydration with a caffeinated beverage during the nonexercise periods of 3 consecutive days of 2-a-day practices. *Int J Sport Nutr Exerc.* 2004;14:419-429.

7. Del Coso J, Estevez E, Mora-Rogriguez R. Caffeine during exercise in the heat: thermoregulation and fluid-electrolyte balance. *Med Sci Sport Exerc.* 2009;41:164-173.

8. Dias J, Roti M, Armstrong L, et al. Rehydration after exercise dehydration in heat: effects of caffeine intake. *J Sport Rehabil.* 2005;14:294-300.

9. Millard-Stafford M, Cureton K, Wingo J, Trilk J, Warren G, Buyck M. Hydration during exercise in warm, humid conditions: effect of a caffeinated sports drink. *Int J Sport Nutr Exerc.* 2007;17:163-177.

10. Motl R, Dishman R. Effects of acute exercise on the soleus H-reflex and self-reported anxiety after caffeine ingestion. *Phys Behav*. 2004;80:577-585.

11. Walton C, Kalmar J, Cafarelli E. Caffeine increases spinal excitability in humans. *Muscle Nerve*. 2003;28:359-364.

12. Walton C, Kalmar J, Cafarelli. Effects of caffeine on self-sustained firing in human motor units. *J Physiol*. 2002;545:671-679.

13. Armstrong L, Casa D, Maresh C, Ganio M. Caffeine, fluid-electrolyte balance, temperatureregulation, and exercise-heat tolerance. *Exerc Sport Sci Rev*. 2007;35:135-140.

14. Manjra S, Schwellnus M, Noakes T. Risk factors for exercise associated muscle cramping (EAMC) in marathon runners. *Med Sci Sports Exerc*. 1996;28:S167.

15. Bergeron M. Muscle cramps during exercise-is it fatigue or electrolyte deficit? *Curr Sports Med*. 2008;7:S50-S55.

16. Schwellnus M. Cause of exercise associated muscle cramps (EAMC)-altered neuromuscular control, dehydration or electrolyte depletion? *Br J Sports Med*. 2008;10:1-20.

17. Miller K, Knight K, Wilding S, Stone M. Duration of electrically induced muscle cramp increased by increasing stimulation frequency. *J Sport Rehabil*. 2012;12:182-185.

18. Serrao M, Nielsen L, Ge H, Pierelli F, Sandrini G, Farina D. Experimental muscle pain decreases the frequency threshold of electrically elicited muscle cramps. *Exp Brain Res*. 2007;182:301-308.

19. Miller K, Knight K. Electrical stimulation cramp threshold frequency correlates well with the occurrence of skeletal muscle cramps. *Muscle Nerve*. 2009;39:364-368.

20. Casa D, Armstrong L, Hillman S, Montain S, Reiff R, Rich B, Roberts W, Stone J. National Athletic Trainers' Association Position Statement: Fluid replacement for athletes. *J Athl Train.* 2000;35:212-224.

21. Medical Dictionary. *Free Dictionary.* Available at http://medical dictionary.thefreedictionary.com/. Accessed May 9, 2013.

22. (2009). ACSM's guidelines for exercise testing and prescription. (8th ed.). Baltimore, MD: Lippincott Williams & Wilkins.

23. Walsh AL, Gonzalez AM, Ratamess NA, Kang J, and Hoffman JR. Improved time to exhaustion following ingestion of the energy drink Amino Impact. *J Int Soc Sports Nutr.* 2010;7:14.

24. Ragsdale FR, Gronli TD, Batool N, Haight N, Mehaffey A, McMahon EC, Nalli TW, Mannello CM, Sell CJ, McCann PJ, Kastello GM, Hooks T, and Wilson T. Effect of Red Bull energy drink on cardiovascular and renal function. *Amino Acids.* 2010; 38: 1193–1200.

25. Cohen B, Nelson A, Prevost M, Thompson G, Marx B, Morris G. Effects of caffeine ingestion on endurance racing in heat and humidity. *Eur J Appl Physiol O.* 1996; 73 (3/4):358-363.

26. Lockwood CM, Moon JR, Smith AE, Tobkin SE, Kendall KL, Graef JL, Cramer JT, and Stout JR. Low-calorie energy drink improves physiological response to exercise in previously sedentary men: A placebo-controlled efficacy and safety study. *J Strength Cond Res.* 2010; 24: 2227–2238.

27. Ivy JL, Kammer L, Ding Z, Wang B, Bernard JR, Liao YH, and Hwang J. Improved cycling time-trial performance after ingestion of a caffeine energy drink. *Int J Sport Nutr Exe.* 2009 19: 61–78.

28. Rutherford JA, Spriet LL, and Stellingwerff T. The effect of acute taurine ingestion on endurance performance and metabolism in well-trained cyclists. *Int J Sport Nutr Exe.* 2010; 20: 322–329.

29. Forbes SC, Candow DG, Little JP, Magnus C, and Chilibeck PD. Effect of Red Bull energy drink on repeated Wingate cycle performance and bench-press muscle endurance. *Int J Sport Nutr Exerc Metab.* 2007*;* 17:433–444.

30. Davis JK and Green JM. Caffeine and anaerobic performance: ergogenic value and mechanisms of action. *Sports Med.* 2009; 39:813–832.

31. Miller K., Stone M., Huxel K., Edwards J. Exercise-associated muscle cramps: causes, treatment, and prevention. *Sports Health.* 2010; 2(4): 279-283.

32. Braulick K, Miller K, Albrecht J, et al. *Br J Sports Med.* Published Online First: 5 December 2012 doi:10.1136/bjsports-2012=091501.

33. Miller K, Knight K, Mack G, et al. Three percent hypohydration does not affect the threshold frequency of electrically-induced muscle cramp. *Med Sci Sports Exerc.* 2010;42:2056-2063.

34. Schwellnus M, Nicol J, Laubscher R, et al. Serum electrolyte concentrations and hydration status are not associated with exercise associated muscle cramping (EAMC) in distance runners. *Br J Sports Med.* 2004;38:488-492.

35. Stofan J, Zachwieja J, Horswill C, et al. Sweat and sodium losses in NCAA football players: a precursor to heat cramps? *Int J Sport Nutr Exerc Metab.* 2005;15:641-652.

36. Bergeron M. Heat cramps: fluid and electrolyte challenges during tennis in the heat. *J Sci Med Sport.* 2003;6:19-27.

37. Jung A, Bishop P, Al Nawwas A, et al. Influence on hydration and electrolyte supplementation on incidence and time to onset of exercise-associated muscle cramps. *J Athl Train.* 2005;40:71-75.

38. Wagner T, Behnia N, Ancheta W, et al. Strengthening and neuromuscular reeducation of the gluteus maximus in a triathlete with exercise associated cramping of the hamstrings. *J Orthop Sports Phys Ther.* 2010;40:112-119.

39. Binkley H, Beckett J, Casa D, Kleiner D, Plummer P. National Athletic Trainers' Association postion statement: exertional heat illness. *J Athl Train.* 2002;37:329-343.

40. Poynter D, Thornton H. Heat cramps: treatment and prevention. *Athletic Training and Sport Health Care.* 2012;2:205-207.

41. Caffeine and muscle cramps: a stimulating connection. *The American Journal of Medicine.* 2007;120:e1-e2.

42. Palmeri R, Ingersoll C, Hoffman M. The Hoffman Reflex: methodologic considerations and applications for use in sports medicine and athletic training research. *J Athl Train.* 2004;39(3):268-277.

43. Lopes J, Aubier J, Jardim J, Aranda J, Macklem P. Effect of caffeine on skeletal muscle function before and after fatigue. *J Appl Physiol.* 1983;54(5):1303-1305.

44. Kalmar J. The influence of caffeine on voluntary muscle activation. *Med Sci Sports Exerc.* 2005;37(12):2113-2119.

45. Kalmar J, Cafarelli E. Effects of caffeine on neuromuscular function. *J Appl Physiol.* 1999;87(2):801-808.

46. Miller K., Knight K. Intial electrical stimulation frequency and cramp threshold frequency and force. *J Athl Train.* 2012; 47(5):1-5.

47. Tarnopolsky M. Effect of caffeine on the neuromuscular system-potential as an ergogenic aid. *Appl Physiol Nutr Metab.* 2008; 33:1284-1289.

APPENDIX. IRB APPROVAL

NDSU NORTH DAKOTA STATE UNIVERSITY

November 18, 2013

Pamela Hansen
Department of Health, Nutrition & Exercise Sciences
BBFH

IRB Approval of Protocol **#HE14087**, **"The Effects of Caffeine Ingestion on Muscle Crampe Threshold Frequency"**
Co-investigator(s) and research team: **Max Pagel**

Approval period: 11/18/13 to 11/17/14 **Continuing Review Report Due: 10/1/14**

Research site(s): **NDSU** Funding agency: **n/a**
Review Type: Full Board, meeting date – 11/8/13
Risk Level: A minor increase over minimal risk
IRB approval is based on original submission, with revised: protocol materials (received 11/14/13).

Additional approval is required:

- prior to implementation of any proposed changes to the protocol (*Protocol Amendment Request Form*).
- for continuation of the project beyond the approval period (*Continuing Review/Completion Report Form).* A reminder is typically sent two months prior to the expiration date; timely submission of the report is your responsibility. To avoid a lapse in approval, suspension of recruitment, and/or data collection, a report must be received, and the protocol reviewed and approved prior to the expiration date.

A report is required for:

- any research-related injuries, adverse events, or other unanticipated problems involving risks to participants or others within 72 hours of known occurrence (*Report of Unanticipated Problem or Serious Adverse Event Form*).
- any significant new findings that may affect risks to participants.
- closure of the project (*Continuing Review/Completion Report Form*).

Research records are subject to random or directed audits at any time to verify compliance with IRB regulations and NDSU policies.

Thank you for cooperating with NDSU IRB procedures, and best wishes for a successful study.

Sincerely,

Kristy Shirley, CIP
Research Compliance Administrator

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