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The Effects of Acute Aerobic Exercise and Caffeine on Working Memory and Caffeine Withdrawal

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Abstract

Caffeine is one of the most widely used psychoactive substances worldwide. Although caffeine elicits cognitive benefits, there are concerns regarding caffeine's effects on certain health domains. Acute, aerobic exercise has been shown to improve cognition. The effects of aerobic exercise in comparison to caffeine on working memory (WM) in non-caffeine and caffeine consumers remains unknown. Furthermore, the effects of aerobic exercise in reducing caffeine withdrawal symptoms has yet to be examined. In Phase I, twenty-nine non-caffeine and thirty caffeine consumers completed a WM assessment (n-back task), followed by aerobic exercise and caffeine administration. In Phase II, twenty-five caffeine consumers underwent a WM assessment and reported caffeine withdrawal symptoms following a 12-hour deprivation period. Aerobic exercise and caffeine administration improved WM accuracy in both types of consumers and reduced caffeine withdrawal symptoms. WM performance was not reduced following caffeine deprivation, hence whether exercise and caffeine could restore WM was not tested.

Keywords

Caffeine

Aerobic Exercise

Acute Exercise

Working Memory

Cognition

Caffeine withdrawal

Summary for Lay Audience

Caffeine is found in a wide variety of beverages and foods including coffee, tea, soft-drinks, energy-drinks, chocolate, and medications. Many individuals consume caffeine daily to feel alert. Caffeine improves aspects of cognition, which refers to our ability to acquire and utilize information. Furthermore, caffeine improves feelings of energy and mood. However, caffeine consumption in certain individuals can have negative health effects such as increased anxiety and muscle tremors. Caffeine consumption has also been linked to some negative health effects for pregnant women and their fetuses, such as delayed growth. Another concern with caffeine consumption is withdrawal symptoms, which occur when a regular consumer does not consume caffeine. Withdrawal symptoms can include headaches, tiredness, decreased mood, irritability, and difficulty concentrating. Thus, it is important to determine if there is an alternative for caffeine that can improve cognition, energy, and mood, without the negative health effects. The primary aim of this project was to determine whether twenty minutes of brisk walking would be comparable to ingesting caffeine on a task that measures your ability briefly hold and update information in your mind. The secondary aim was to determine whether twenty minutes of brisk walking would be comparable to ingesting caffeine in reducing withdrawal symptoms after abstaining from caffeine for 12 hours. Our findings suggest brisk walking for 20 minutes can improve cognition and help reduce caffeine withdrawal symptoms. This research could have an impact on our understanding of the relationship between aerobic exercise and cognition, as well as how we can best use aerobic exercise to improve the overall health and well-being of individuals.

Acknowledgments

The verb “to acknowledge” is believed to come from a blend of the Middle and Old English words “aknow” which refers to admitting or showing one’s knowledge and “oncnawan” which refers to understanding or coming to recognize. Throughout my master’s I have come to recognize that this project would not have been possible without the support of many people. To my supervisor, Harry Prapavessis, thank you for your guidance and unparalleled support over the past two years. Your passion for research has fueled my desire to pursue further graduate studies and has made this experience one I will cherish for years to come. To my first undergraduate supervisor, Ayesha Khan, thank you for whetting my appetite for research and encouraging me to pursue this path. To everyone in the Exercise and Health Psychology Lab (past and present) thank you for all your support and laughs, this experience would not have been the same without all of you. To the girls of “write club” thank you for making thesis writing a more enjoyable experience. To my friends outside of research (Stephanie, Nina, Mary, Morgan, and Rochelle), thank you for providing sympathetic ears to the trials and tribulations of grad school and for always cheering me on. Finally, to my family, thank you for instilling the value of perseverance and for always encouraging me to pursue my dreams!

Table of Contents

List of Tables

List of Figures

List of Appendices

Chapter 1 : Introduction and Literature Review

Caffeine is one of the most widely used psychoactive substances worldwide (WHO, 2004). A comprehensive assessment of caffeine consumption from the National Health and Nutrition Examination Survey, found approximately 89% of adults in the United States (US) consume caffeine regularly (Fulgoni, Keast, & Lieberman, 2015). Caffeine is present in numerous products such as coffee, tea, soft-drinks, energy-drinks, chocolate, and medications. The cognitive and mood-enhancing benefits of caffeine have been cited as one of the primary motivators for its consumption (Temple, Dewey, & Briatico, 2010; Yeomans, 2010). Caffeine consumption has been specifically associated with increased energy, alertness, self-confidence, positive mood, and cognitive performance (Griffiths, Juliano, & Chausmer, 2003). However, for some individuals, caffeine consumption has been associated with negative effects such as anxiety and muscle tremors (Alsene et al., 2003; Bovim, Naess, Helle, & Sand, 1995; Childs et al., 2008). Caffeine has also been identified as a reinforcing and potentially addictive substance (Ferré, 2016; Hughes et al., 1993). Cessation of caffeine consumption often results in withdrawal symptoms such as: headache, fatigue, difficulty concentrating, and decreased contentedness (Juliano & Griffiths, 2004). Taken together, these findings indicate that although caffeine consumption elicits several benefits to cognition and mood, there are several concerns regarding caffeine's potential negative effects and withdrawal symptoms.

Pharmacokinetics

Caffeine (1,3,7-trimethylxanthine) is an alkaloid derived from the nuts, seeds, and leaves of numerous plant species (Graham, 1978). Once ingested orally, caffeine is rapidly absorbed through the small intestine, allowing entry into the bloodstream, and distribution to bodily tissues (Mumford et al., 1996). Caffeine reaches peak plasma level in approximately 30 to 60 minutes (Benowitz, 1990). Caffeine is primarily metabolized by the liver via the cytochrome P450 enzymes (CYP 1A2), with a half-life of approximately 4 to 6 hours (Benowitz, 1990; Lelo et al., 1986). Cigarette smoking and

exercise have been documented to significantly reduce caffeine's half-life, while acute alcohol consumption, oral contraceptive use, and pregnancy have been shown to significantly increase caffeine's half-life (Benowitz, 1990; Collomp et al., 1991; Knutti et al., 1982; Patwardhan et al., 1980).

Mechanisms of Action

Caffeine's primary mechanism of action occurs via antagonism of adenosine receptors in the central nervous system (CNS) (Fredholm et al., 1999). Adenosine is a neuromodulator primarily responsible for inhibitory effects in the CNS. The presence of caffeine in the synaptic clefts of CNS neurons results in the blockade of adenosine binding to adenosine receptors, ultimately promoting "wakefulness" and "alertness". Although caffeine acts as an antagonist at all four adenosine sub-receptors (A_1, A_{2A}, A_{2B}, A_3), its actions are primarily exerted through interactions at A_1 and A_{2A} sub-receptors (Fredholm et al., 1999). Adenosine receptor antagonism also stimulates release of neurotransmitters such as dopamine, norepinephrine, and acetylcholine (Carter et al., 1995; Fredholm & Jonzon, 1988; Hadfield & Milio, 1989). The release of the aforementioned neurotransmitters has been associated with enhanced motor activity, arousal, information processing, and attentional control (Acquas et al., 2002; Coull et al., 1995; Powell, Iuvone, & Holtzman, 2001).

Caffeine Sources and Intake

Caffeine is present in a growing number of foods, beverages, and supplements. Beyond traditional sources such as coffee, tea, and soft-drinks, caffeine is being added to candy, gum, and pre-workout supplements (Drewnowski & Rehm, 2016). In a nationally representative sample of US adults, coffee was found to be the most widely used source of caffeine (64%), followed by soft-drinks (18%), and tea (16%) (Fulgoni et al., 2015). In Canada, coffee is the second most consumed beverage by adults and accounts for approximately 80% of caffeine consumption, followed by tea (12%), and soft-drinks (6%) (Garriguet, 2008). The amount of caffeine in the above sources varies depending on the brand and preparation; however, reference values have been compiled by the United

States Department of Agriculture (USDA) and the Canadian Nutrient File (CNF; See Appendix 13). In Canada, the average daily caffeine intake for adults is approximately 2.4mg/kg of body weight (equivalent to approximately 173 mg/day for an individual weighing the Canadian average of 72.03 kg) (Chou, 1992; Statistics Canada, 2017).

Caffeine Consumption: Risks and Benefits

Caffeine consumption has been associated with risks and benefits to human health and well-being. Extensive systematic reviews examining caffeine's effects on human health suggested caffeine intake below 400 mg/day in healthy adults was not associated with adverse health effects (Nawrot et al., 2003; Wikoff et al., 2017). However, for a subset of individuals and for certain populations, caffeine consumption may result in negative health outcomes. For instance, one of the risks associated with caffeine consumption is increased anxiety/anxiety-related symptoms. Several studies have indicated a subset of individuals experience symptoms such as nervousness and restlessness after consuming caffeine (Alsene et al., 2003; Childs et al., 2008). One proposed hypothesis suggests possession of genetic variants of the *ADORA2A* and/or *CYP1A2* genes, which are associated with adenosine receptors and caffeine metabolism, may be associated with heightened sensitivity to caffeine (Alsene et al., 2003; Childs et al., 2008; Fulton et al., 2018). In a study investigating the effect of caffeine consumption in school-aged children, total weekly caffeine intake was a significant predictor of anxiety after controlling for covariates such as diet, demographics (e.g., sex, school), and lifestyle (e.g., sleep hours, exercise frequency), indicating caffeine may play a unique role in inducing anxiety-related symptoms in childhood (Richards & Smith, 2015). Furthermore, adults with pre-existing anxiety disorders have been documented to experience exacerbated anxiety symptoms post-caffeine consumption (Bruce et al., 1992; Nardi et al., 2009).

Another risk associated with caffeine consumption in certain individuals is increased muscle tremors. Bovim and colleagues (1995) detected reduced motor steadiness in healthy adults during neuropsychological testing post-caffeine consumption.

Similarly, in a study examining psychomotor tremors in both low ($\overline{x}$ =37.07 mg/day) and moderate ($\overline{x}$ = 316.2 mg/day) caffeine consumers, greater motor tremors were observed following caffeine consumption in both groups (Sands et al., 2015). A recent review of factors affecting tremors in surgeons found caffeine consumption negatively impacted surgical dexterity. The study authors encouraged reduction of caffeine consumption prior to conducting a surgical procedure to maintain dexterity (Fargen, Turner, & Spiotta, 2016).

Caffeine use has been associated with both dependence and withdrawal symptoms upon cessation (Hughes et al., 1991; Strain, Mumford, Silverman, & Griffiths, 1994). A small proportion of caffeine users (13%) display clinically significant levels of dependence consisting of "continued use despite psychological or physical harm, difficulty stopping caffeine use, and using more caffeine than intended" (Juliano & Griffiths, 2004; Meredith, Juliano, Hughes, & Griffiths, 2013). A larger proportion of caffeine users report experiencing a wide range of withdrawal symptoms at varying severities including: headache, fatigue, decreased contentedness, and decreased alertness (Juliano & Griffiths, 2004; See Section: Caffeine Withdrawal: Subjective and Cognitive Effects).

When considering subsets of the population vulnerable to the effects of caffeine, women planning to become pregnant or who are pregnant, have been identified as being at a greater risk of experiencing adverse health effects due to caffeine consumption. During pregnancy, the rate of caffeine clearance is significantly reduced, promoting caffeine accumulation in the body. The accumulated caffeine passes the placental barrier, potentially resulting in a disrupted neonatal environment (Knutti et al., 1982). Although numerous studies have investigated the effects of caffeine consumption on both maternal and fetal health, the results have been mixed. Several studies and reviews have found caffeine consumption was associated with negative health outcomes such as: delayed conception, increased risk of spontaneous abortion, preterm birth, low birth weight, and fetal growth restriction (Bech et al., 2005; Brent et al., 2011; Hahn et al., 2015; Maslova et al., 2010; Sengpiel et al., 2013). Thus, to mitigate potential harms to fetal development,

health agencies such as Health Canada recommend lower caffeine intake limits (i.e., <300mg/day) for reproductive-aged women than for the general population (i.e., <400mg/day; Nawrot et al., 2003).

Although specific risks are present with caffeine consumption for a proportion of individuals, caffeine is also associated with benefits to several health domains. Caffeine consumption has been associated with improved metabolic health outcomes (e.g., decreased Type II diabetes risk, increased insulin sensitivity, etc.), decreased risk of neurological disorders (e.g., Parkinson's disease), and enhancements to human behaviour (e.g., cognitive performance, athletic-related performance, and mood; For an in-depth review see: Nawrot et al., 2003; Pourshahidi et al., 2016). The remainder of this section will examine caffeine-induced benefits to cognition.

Caffeine and Cognition

Caffeine has been associated with improvements to multiple cognitive domains. For instance, caffeine is associated with improved information processing, attention, and specific types of memory (i.e., short-term, episodic, spatial). Caffeine administration has consistently elicited faster reaction times in both simple and choice reaction time tasks (Lieberman et al., 1987; Smit & Rogers, 2000). When examining attention, caffeine has marked effects on measures of sustained attention in both "normal" and "impaired" conditions, such as following sleep deprivation. Under "normal" conditions, caffeine administration (200 mg) improved accuracy on both auditory and visual vigilance tasks (Fagan, Swift, & Tiplady, 1988; Fine et al., 1994). Furthermore, caffeine (200 mg) improved the number of detected stimuli and reduced reaction times during a 45-minute visual vigilance task (Olson et al., 2010). Foxe and colleagues (2012) found that participants who completed the sustained attention to response task (SART) following caffeine consumption (50 mg) decreased omission errors (not responding to targets) by 50% compared to placebo.

Caffeine exerts similar effects on sustained attention under "impaired" conditions. In soldiers undergoing sleep deprivation (3 hours of total sleep), caffeine administration

resulted in similar performance in a vigilance task compared to non-sleep deprived controls (McLellan et al., 2005). Kamimori and colleagues (2005) tested participants who underwent a 29-hour wakeful period on the Psychomotor Vigilance Test (PVT). Individuals who were provided multiple doses of caffeine during the 29-hour wakeful period committed less attentional lapses and maintained baseline PVT performance throughout the entire testing period. Studies examining caffeine's effects on different types of memory have found variable results, with some studies detecting benefits and others finding null effects (Nehlig, 2010). The following section will focus on caffeine and working memory.

Caffeine and Working Memory

Working memory (WM) has been conceptualized as a system that provides storage and manipulation of information necessary for cognitive tasks (Baddeley, 1992). Previous studies show mixed effects of caffeine administration on WM. Addicott and Laurienti (2009) found administering 250 mg of caffeine to regular caffeine consumers (2-5 cups of coffee/day) following either 30 hours of caffeine abstinence or normal caffeine use resulted in improved accuracy (% correct responses) on the n-back task (continuous performance task assessing WM capacity) in both the abstained and normal state. Haskell and colleagues (2005) examined the effect of either 75mg or 150mg of caffeine in both caffeine ($\overline{x}$ = 217mg/day) and non-caffeine consumers ($\overline{x}$ = 20mg/day). Caffeine administration (150mg) significantly improved reaction time on the numeric WM task in both types of consumers (Haskell et al., 2005). A randomized, double-blind investigation of the effects of energy drink ingredients on cognitive performance examined the effects of 200 mg of caffeine on verbal (letter-stimuli), object (shape-stimuli), and spatial (shape-stimuli in differing locations) WM tasks. Caffeine reduced reaction time on the most difficult load of the verbal WM task and increased sensitivity (an accuracy index composed of hit rate and false alarm rate) across all loads of the spatial and object WM tasks (Giles et al., 2012).

Personality type has been found to moderate the effects of caffeine on WM. A randomized, double-blind, placebo-controlled study examining caffeine, WM, and personality type (introvert, extrovert) found 200 mg of caffeine improved performance (% correct responses) on the 3-back load in extraverts (Smillie & Gokçen, 2010). Furthermore, a study examining the effect of caffeine and personality type on several components of WM detected similar results at a lower caffeine dose (65mg). Caffeine interacted with extraversion, improving two components of WM (articulatory loop and central executive), while also improving simple reaction time and speed of information encoding across both personality types, suggesting a unique interaction between extraversion, caffeine, and WM (Smith et al., 2013). A number of studies have not detected a caffeine-induced improvement to WM (Childs & De Wit, 2006; Koppelstaetter et al., 2008; Smith, 1999; Warburton, 1995). Warburton (1995) found no change to WM following caffeine ingestion, but did cite high WM performance in the placebo group, allowing a small margin for caffeine-driven improvement. Smith (1999) did not find overall improvement on the WM tasks (serial recall task, running memory task, and spatial memory task) in the caffeine condition, but did detect improved encoding of new information in a masked categorical search task, indicating perhaps the tasks chosen to assess WM were not sensitive enough to detect subtle caffeine-driven changes. Although, Koppelstaetter and colleagues (2008) did not detect accuracy or reaction time differences between caffeine and placebo administration on the n-back task, the highest load assessed in their paradigm was 2-back and previous studies examining the effect of substances such as nicotine on cognition have indicated the 3-back load is the most sensitive to the drug effect (Loughead et al., 2009). Koppelstaetter et al (2008) did however determine via functional magnetic resonance imaging (fMRI) that caffeine modulated neuronal activity in frontal brain regions associated with executive and attentional functions during the WM task. In concert, these findings suggest caffeine administration influences WM processes.

Caffeine Withdrawal: Subjective and Cognitive Effects

Caffeine has been identified as a reinforcing and addictive substance in murine models and humans (Griffiths & Woodson, 1988; Hughes et al., 1991). Early research on human caffeine withdrawal determined caffeine consumers (3-7 coffee cups/day) who underwent double-blind interleaved periods of caffeinated (100mg) and decaffeinated coffee consumption displayed withdrawal symptoms, particularly headache, on decaffeinated days. Furthermore, the presentation of headache predicted self-administration of caffeinated coffee (Hughes et al., 1991). Several studies have replicated the presence of withdrawal symptoms following caffeine deprivation with larger sample sizes (Silverman et al., 1992), as well as characterized the doses at which withdrawal symptoms occur (Evans & Griffiths, 1999). Caffeine doses as low as 100 mg per day have been shown to produce withdrawal symptoms upon cessation (Juliano & Griffiths, 2004).

Juliano and Griffiths (2004) conducted an extensive review of human caffeine withdrawal studies with the objective of characterizing and empirically validating reported symptoms. Withdrawal symptoms met validity criteria if there was "statistical demonstration of the symptom in six or more studies that include two or more double-blind studies that used methodologies in which the conclusion of caffeine withdrawal effects was not confounded by direct effects of caffeine" (Juliano & Griffiths, 2004). The following ten caffeine withdrawal symptoms met full validity criteria: headache, fatigue, decreased energy/activeness, decreased alertness, drowsiness, decreased contentedness, depressed mood, difficulty concentrating, irritability, and foggy/not clear headed. Caffeine withdrawal symptoms occur 12 to 24 hours after abstinence and can persist for several days at varying intensities (Griffiths & Woodson, 1988). Administration of caffeine post-deprivation has been shown to reduce withdrawal symptom presence and severity (Addicott & Laurienti, 2009). Although expectancy effects have been raised as a potential confound in relation to caffeine withdrawal, a recent balanced-placebo study examining caffeine dose (caffeinated versus decaffeinated) and expectancy (told caffeinated or told decaffeinated) detected no expectancy effects on withdrawal

symptoms or cognitive performance, suggesting a pharmacological basis for caffeine withdrawal (Juliano, Kardel, Harrell, Muench, & Edwards, 2019).

Alongside the subjective effects of caffeine withdrawal, negative effects on cognitive performance have been detected. Lane and Phillips-Bute (1998) found overnight caffeine abstinence in regular caffeine consumers (2-10 coffee cups/day) slowed reaction times and reduced accuracy on a vigilance task. James (1998) replicated these findings in regular caffeine consumers (3-5 caffeine beverages/day) over a longer deprivation period (24-hour) on a character recognition task, which assesses information transfer and short-term memory. Similarly, Yeomans and colleagues (2002) found a 24-hour caffeine abstinence period resulted in slower reaction times and increased errors on the Rapid Visual Information Processing (RVIP) task. However, studies have suggested the effects of caffeine withdrawal may be reversed. Yeomans et al., (2002) determined administering 1 mg/kg of caffeine after an overnight abstinence period restored RVIP task performance (i.e., decreased reaction times and increased response accuracy). An investigation of caffeine deprivation on cognitive performance, as measured by a choice-reaction and n-back task, determined 30 hours of deprivation in regular caffeine consumers (2-5 coffee cups/day) reduced performance on choice reaction time (Addicott & Laurienti, 2009). Furthermore, administration of 250 mg of caffeine post deprivation reduced reaction time on the choice-reaction task and improved accuracy on the 1-back load of the n-back task (Addicott & Laurienti, 2009). These findings suggest caffeine deprivation results in withdrawal symptoms and reduces performance on a subset of cognitive tasks, which are both restored by caffeine administration.

Caffeine Withdrawal Reversal

James (1998) first posited the concept of withdrawal reversal, suggesting caffeine has limited direct effects, but rather operates by "reversing" withdrawal effects. In 2005, James and Rogers outlined that several laboratory studies required an overnight abstinence period before conducting cognitive and subjective assessments, inducing a caffeine-withdrawn state. Thus, the administration of caffeine in these studies may have

reduced the negative effects of withdrawal, such as tiredness, rather than represented a caffeine-driven benefit. To address this concern, several studies have incorporated low to non-caffeine consumer groups, ad-libitum caffeine consumption, and long-term withdrawal periods to delineate whether caffeine induces direct effects (Addicott & Laurienti, 2009; James & Rogers, 2005; Warburton, Bersellini, & Sweeney, 2001). Heterogeneous results have been reported, with some studies finding direct effects of caffeine (Addicott & Laurienti, 2009; Childs & deWit, 2006; Haskell et al., 2005 Smith, Christopher, & Sutherland, 2013), while others finding evidence supporting caffeine withdrawal reversal (James, 1998; James, Gregg, Kane & Harte, 2005).

Alternative Modalities to Enhance Cognition: Acute Exercise

Given the aforementioned concerns associated with caffeine consumption, examining alternative modalities to improve cognitive performance is critical. Acute exercise (single bout) has been suggested as a potential intervention to improve cognitive performance. Previous studies have shown reliable improvements in cognition following acute exercise (Chang, Labban, Gapin, & Etnier, 2012; Lambourne & Tomporowski, 2010; Tomporowski, 2003). In a meta-analysis conducted by Chang et al. (2012), the authors found acute exercise (aerobic, anaerobic, resistance, and combination) had a small (Hedge's g = 0.097), but positive effect on cognition. Furthermore, these positive cognitive effects were found during exercise, immediately following exercise, and after a delay (Chang et al., 2012). Regarding the assessment of cognitive performance, tasks gauging executive functions such as the Stroop Task, were more sensitive to the effects of acute exercise in comparison to other cognitive task types (Chang et al., 2012).

In addition to the above findings, Chang and colleagues (2012) examined potential moderators of the acute exercise and cognition relationship including: timing of cognitive assessment, exercise duration, and exercise intensity. When examining timing of cognitive assessments, testing immediately following exercise resulted in the largest effect (Cohen's d = 0.108), followed by testing after a delay (d = 0.103), and testing during exercise (d = 0.101). When collapsing testing immediately following with delayed

testing, 11-20 minutes of exercise produced the greatest effect (d = 0.262). Exercise intensity had differential effects depending on the timing of cognitive testing. Positive effects on cognition were only observed for very light (d = 0.152), light (d = 0.169), and moderate intensity exercise (d = 0.120) when cognitive testing occurred immediately after exercise. When cognitive testing occurred after a delay, positive effects on cognition were found at every intensity except very light (d = -0.133). Chang et al.'s (2012) findings suggest acute exercise lasting 11-20 minutes, at an intensity ranging from light to moderate, may produce the greatest post-exercise cognitive benefit.

Further studies examining the relationship between exercise intensity and cognitive performance have suggested moderate intensity may confer the greatest post-exercise cognitive benefit, particularly in executive functioning (EF) tasks (McMorris, Sproule, Turner, & Hale, 2011; McMorris & Hale, 2012). McMorris and colleagues (2011) conducted a meta-analysis of studies utilizing acute, moderate intensity exercise to enhance EF as assessed by several different tasks (e.g., Flanker Task, Switching Visual Attention Task, Stroop Task, etc.). Acute, moderate intensity exercise had a strong, beneficial effect on reaction times in EF tasks (Hedges' g = -1.41), but a small, negative effect on accuracy (Hedge's g = 0.40). In a subsequent investigation of the differential effects of exercise intensities on cognition speed and accuracy, McMorris and Hale (2012) detected a small but positive effect size on overall cognitive performance (Hedge's g = 0.14). The two studies together suggest the increased arousal elicited by moderate intensity may result in faster information processing speed. Regarding accuracy, the small effect may be due to the cognitive assessments lacking the appropriate sensitivity to detect subtle exercise-induced changes to accuracy (McMorris & Hale, 2012).

Concerns have been raised regarding whether cardiorespiratory fitness influences the relationship between acute exercise and cognitive performance. Chang et al. (2014) addressed these concerns in an investigation of cardiorespiratory fitness, acute exercise, and executive functioning. Healthy college-aged adults completed a maximal graded treadmill test to assess cardiorespiratory fitness and were subsequently categorized into

low, moderate, and high fitness groups. Individuals in all fitness groups were assessed on the Stroop Task, which measures executive functioning, pre- and post- completion of 20 minutes of cycling (65% of participant $VO_{2\ max}$). Participants performed better on the Stroop Task post-exercise, irrespective of cardiorespiratory fitness. However, on the incongruent condition of the Stroop Task, moderate fitness individuals exhibited the fastest reaction times, while high fitness individuals exhibited the slowest reaction times, indicating cardiorespiratory fitness may affect specific domains of information processing, but not overall performance.

Beyond cognitive functioning, acute exercise has also been shown to provide other psychological benefits such as improved mood, feelings of energy, and well-being (Maraki et al., 2005; Loy et al., 2003; Bartholomew, Morrison, & Ciccolo, 2005). Alongside psychological benefits, acute exercise also confers physical health benefits such as improved cardiovascular health, skeletal muscle, and immune function (Rosenwinkel, Bloomfield, & Arwady, 2001 ; Schenk & Horowitz, 2007 ; Rowbottom & Green, 2000). Thus, acute exercise is a promising alternative for caffeine in that it has been documented to improve cognitive, psychological, and physical health.

Exercise and Cognition: Mechanisms

Several neurobiological mechanisms have been proposed to underlie the observed exercise-induced benefits to cognition. The following mechanisms will be reviewed below: (i) neurogenesis and angiogenesis, (ii) increased neurochemical release, and (iii) changes to cerebral blood flow and neurotransmitter release. Neurogenesis and angiogenesis refer to the production of new neurons and blood vessels, respectively. Non-human animal studies have indicated aerobic exercise training resulted in neurogenesis, specifically in the hippocampus, a brain region associated primarily with memory (Creer, Romberg, Saksida, van Praag & Bussey, 2010; van Praag, Christie, & Gage, 1999). In addition, following aerobic exercise training, angiogenesis was detected in brain regions nearby the hippocampus. Some studies have found increased neurogenesis was associated with improvements in some but not all cognitive tasks (Clark, Brzezinska, Thomas,

Ryzhenko, Toshkov, & Rhodes, 2008). Taken together, these findings suggest although there are reliable aerobic exercise-induced structural changes to the brain, the dose of exercise required and the manner in which the changes promote cognitive function remain unclear.

Regarding neurochemical changes, two neural growth factors have been identified as being heavily involved in the exercise-induced benefits to cognitive performance. Brain-derived neurotrophic factor (BDNF) is a neurotrophin, a protein involved in the development, function, and survival of neurons (Barde, 1994). BDNF has been identified as a key component in the neurochemical cascades associated with neuroplasticity (Huang et al., 2006). Neuroplasticity facilitates learning through modifying neural connections (Hennigan, O'Callaghan, & Kelly, 2007). Non-human animal studies have identified that a single bout of exercise increased BDNF levels in the brain (Rasmussen et al., 2009). Furthermore, a systematic review of acute exercise studies in humans determined that 69% of studies examining acute, aerobic exercise in healthy individuals resulted in a "mostly transient increase in serum or plasma BDNF concentration" (Knaepen, Goekint, Heyman, & Meeusen, 2010). The transient increases in BDNF post-exercise may thus promote neuroplasticity in regions contributing to cognition.

The second neurotrophic factor that has been documented to play a role in the exercise-cognition relationship is insulin-like growth factor 1 (IGF-1; Voss, Nagamatsu, Liu-Ambrose, & Kramer, 2011). In non-human animals, aerobic exercise has resulted in elevated IGF-1 production (Trejo, Carro, & Torres-Aleman, 2001). One study found IGF-1 and BDNF work in tandem to promote neurogenesis and angiogenesis, particularly in the hippocampus (Lopez-Lopez, LeRoith, & Torres-Aleman, & 2004; Trejo, Carro, & Torres-Aleman, 2001). Increases to both neurotrophic factors have been linked to increased neuroplasticity and other brain network-related changes, however the manner in which these changes impact cognition warrant further investigation.

The aforementioned mechanisms underlying changes to brain structure typically operate over time. Thus, mechanisms which operate transiently, such as changes to

cerebral blood flow (CBF; Vissing, Andersen, & Diemer, 1996) and increased neurotransmitter release (Dishman, 1997; Wang et al., 2000) are more likely to underlie the cognitive changes following acute exercise. Several non-human animal studies have indicated acute exercise-induced changes to CBF (Delp et al., 2001; Vissing, Andersen, & Diemer, 1996). Human studies have detected changes to the oxygenation of CBF with concomitant improvements to cognitive performance following acute exercise (Bediz et al., 2016; Yanagisawa et al., 2010). Regarding neurotransmitter release, non-human animal studies have consistently shown changes to the release of acetylcholine, dopamine, epinephrine, and norepinephrine following acute exercise (Kashihara et al., 2009; Poehlman et al., 1992; Soya et al., 2007). Several human studies have also detected changes to release of neurotransmitters, namely dopamine and norepinephrine, during and following acute exercise (McMorris et al., 2008; Wang et al., 2000). Although several neurotransmitters have been associated with the facilitation of cognitive processes (Blokland, 1995), the nature of the relationships between acute exercise-induced neurotransmitter release and cognition remains to be elucidated.

Acute Exercise: Restoring Cognitive Performance and Reducing Withdrawal Symptoms during Cessation of other Substances

As mentioned previously caffeine deprivation often results in negative effects on cognitive performance (Addicott & Laurienti, 2009; James, 1998; Phillips-Bute, 1998; Yeomans et al., 2002 and withdrawal symptoms (Juliano & Griffiths, 2004). With respect to cognitive performance, there is no literature to support the tenet that cognitive deficits seen through caffeine deprivation can be restored following an acute bout of exercise. One indirect non-inferiority study found light-to-moderate intensity exercise pragmatically increased cognition to a similar level as nicotine in a non-deprived smoking model (Fagan, Guirguis, Smith, Sui, Rollo, and Prapavessis, unpublished). The authors concluded that exercise is a healthier alternative to nicotine for cognitive enhancement and may weaken the maintenance of tobacco use for cognitive enhancement.

Cessation of other substances such as nicotine, alcohol, opioids, and benzodiazepines have also been associated with withdrawal symptoms (WHO, 2018). Although withdrawal management is often pharmacological in nature, in the context of smoking cessation (Haasova, Warren, Ussher, Van Rensburg, Faulkner, & Cropley, 2013; Roberts, Maddison, Simpson, Bullen, & Prapavessis, 2012) and recently alcohol cessation (Stoutenberg, Rethorst, Lawson, & Read, 2016), acute exercise has been successfully employed as an intervention to reduce the intensity and frequency of withdrawal symptoms and cravings. In two comprehensive systematic and meta-analysis reviews, Roberts et al., (2012) using aggregate data and Haasova et al., (2013) using individual participant data found weighted mean differences in both "desire to smoke" [-1.90 and-2.04 points, respectively] and 'strength of desire to smoke" [-2.41 and -1.91 points, respectively] that favored the acute exercise condition over the control condition following a temporary period of abstinence. The effect sizes found in these studies ranged from d = .4 to 1.9, which are considered moderate-to-large in size (Cohen, 1988). Furthermore, craving reduction effects lasted up to 30 minutes post-exercise (Ussher, Cropley, Playle, Mohidin, & West, 2009). Unfortunately, the mechanisms through which exercise exerts its craving effect are not well understood. Potential mechanisms of action that have received some support include affect and mood (De Jesus & Prapavessis, 2018), shifts in attention (Janse Van Rensburg et al., 2012), and cortisol secretion (Roberts et al., 2015).

Regarding tobacco withdrawal symptoms, light and moderate intensity exercise significantly reduced symptoms, while vigorous exercise increased symptoms (Roberts et al., 2012). Withdrawal symptoms positively affected by acute exercise included stress, difficulty in concentration, tension, restlessness, depression, and irritability (Roberts et al., 2012). Although the mechanisms underlying exercise-induced reductions to withdrawal symptoms remain unclear, several biological and cognitive mechanisms have been proposed. Changes in beta-endorphins, opioids, and cortisol have been identified as factors potentially mediating the exercise-induced reductions in tobacco withdrawal symptom reductions (Scerbo et al., 2007). Additionally, heart rate variability (HRV) has

been identified as a psychophysiological marker that changes following smoking cessation and exercise, highlighting its potential role in elucidating exercise-driven withdrawal symptom reduction (Stein et al., 1996; Sandercock, Bromley, & Brodie, 2005). Cognitive changes such as shifts in the allocation of cognitive resources, such as attention, may also be involved in reducing specific withdrawal symptoms (Ekkekakis & Acevedo, 2006). Several of the withdrawal symptoms reported in the tobacco cessation context overlap with caffeine cessation (Irons et al., 2016). The shared symptomatology lends to assessing the utility of acute exercise in reducing caffeine withdrawal symptoms during caffeine deprivation.

Summary

There is robust evidence that caffeine leads to improvement in cognitive performance. Furthermore, when considering the health-concerns associated with caffeine consumption for specific individuals and subsets of the population, examining alternative modalities to improve cognitive performance is warranted. An acute bout of exercise has also shown to enhance cognitive performance, while providing additional health benefits. To date, the effects of acute exercise in comparison to caffeine on cognitive performance in both non-caffeine and caffeine users remain unknown. Furthermore, cognitive deficits and withdrawal symptoms accompany periods of caffeine deprivation among caffeine users. It also remains unknown whether acute exercise can reverse these cognitive deficits and withdrawal symptoms to the same extent as caffeine.

Objectives

The objectives of the present study are as follows:

Phase I

i) To determine the effects of an acute bout of moderate intensity aerobic exercise and caffeine administration on working memory (WM) in both non-caffeine and caffeine consumers

Phase II

i) To determine whether a 12-hour caffeine deprivation period in caffeine consumers increases caffeine withdrawal symptoms and reduces WM performance.

ii) To determine whether an acute bout of moderate intensity aerobic exercise and caffeine administration can reduce caffeine withdrawal symptoms and restore WM performance.

Hypotheses

The hypotheses of the present study are as follows:

Phase I

i) In comparison to baseline WM performance, aerobic exercise and caffeine administration will improve WM comparably in both non-caffeine and caffeine consumers.

Phase II

ii) A 12-hour caffeine deprivation period in caffeine consumers will increase caffeine withdrawal symptoms and reduce WM performance.

iii) Aerobic exercise or caffeine administration will reduce caffeine withdrawal symptoms and restore WM performance comparably.

Chapter 2 : Methods

Participants

The inclusion criteria consisted of: (1) aged 18-64 years, (2) ability to read and write in English, and (3) consumption of less than 30 milligrams of caffeine per day (non-caffeine consumer) or consumption of greater than or equal to 150 milligrams of caffeine per day (caffeine consumer). The exclusion criteria consisted of: (1) contraindications to exercise (as assessed by the Physical Activity Readiness Questionnaire), (2) self-reported cognitive difficulties, (3) self-reported taking of medication for depression or anxiety, and (4) pregnancy. In (data analyses (See: Table 1). In Phase II, twenty-five participants (caffeine consumers) completed study procedures.

	Caffeine Consumers *(n = 30)*	Non-Caffeine Consumers *(n = 29)*
Age	24.1 (4.8)	24.8 (3.4)
Sex (% males)	43.3%	51.7%
Weight (kg)	72.7 (15.1)	70.1(12.2)
Education (%)		
Undergraduate	50.0%	13.33%
Graduate	43.3%	86.67%
Employed	6.67%	0%
Caffeine Intake (mg)		
Weekly	2110.2(1194.8)	74.7 (64.4)
Daily	301.5 (170.7)	10.7 (9.8)
Time of Last Caffeine Consumption (h)	10.33 (9.31)	
Years of Caffeine Consumption	6.7 (4.1)	
Preferred Type of Caffeine Administration	Coffee	
Physical Activity (minutes of MVPA/week)	1213 (752.8)	1324.19 (1044.296)

Table 1. Demographics: Means and Standard Deviations (SD)

Study Design

Phase I

Phase I utilized a randomized counterbalanced crossover design such that each participant was randomly assigned treatment order (i.e., caffeine administration followed by exercise or exercise followed by caffeine administration) but completed both treatments irrespective of being non-caffeine and caffeine consumers. Treatments were conducted on separate days

Phase II

Phase II involved only caffeine consumers and utilized a randomized design such that each participant was randomly assigned to receiving either caffeine administration or exercise following a 12-hour caffeine deprivation period. Randomization was completed using a computer-generated numbers table.

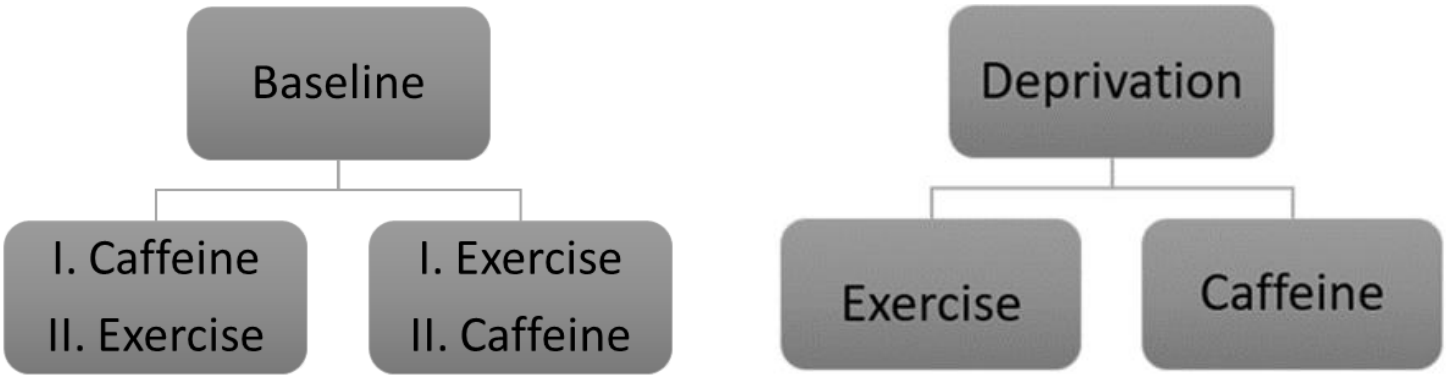

Figure 1. Study Design (Phase I on the left, Phase II on the right)

Primary Outcome Measure: Working Memory

Working memory (WM) was assessed through the n-back task. The n-back task has been widely used in the cognition literature to gauge WM, as it requires both short-term recognition of and operation on stimuli (Baddeley, 1992; Conway, Kane, Bunting, Hambrick, Wilhelm, & Engle, 2005). The n-back task consists of a series of stimuli that are presented rapidly on a screen, with the participant deciding whether the target stimuli matches the stimuli 'n' items back (Jonides, Schumacher, Smith, Lauber, Awh, Minoshima, & Koeppe, 1997).

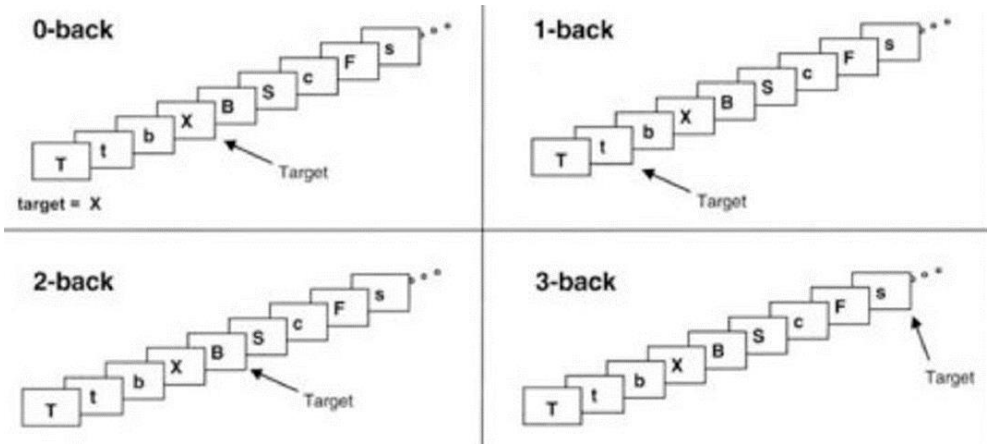

Figure 2. n-back task (Jonides et al., 1997)

The n-back task was run on Inquisit (version 4.0.8.0; Millisecond Software, 2008). Instructions for the task are presented on the screen and a practice phase precedes the evaluation. The participant must score a minimum of 75% of the trials correctly during the practice phase to proceed to the evaluation. The 75% accuracy threshold was deemed appropriate for mitigating the learning effect on the n-back task in a previous study examining WM in smokers and non-smokers (Fagan, Guirguis, Smith, Sui, Rollo, and Prapavessis, unpublished).

The n-back task utilized in this study employed letter stimuli. Each letter stimulus was presented upon the computer screen for 500 milliseconds (ms), followed by a 2000 ms interstimulus (blank screen). The number of stimuli presented changed depending on the working memory load. For example: 0-back = 48 letters, 1-back = 51 letters, 2-back = 51 letters, and 3-back = 54 letters. The individuals would complete each load (0-back, 1-back, 2-back, and 3-back) three times in a randomized order. A correct response would be when the participant pressed the letter 'A' on the keyboard which would indicate the letter in the sequence is the same as the target letter 'n' items back. In the 0-back, the target letter precedes the assessment for that block, for example, the program states "the target is W", hence every time a 'W' appears on the screen the individual should press the 'A' key. In the 1-back, a correct response would be if the letter and the consecutive letter are the same, for example, 'F', 'interstimulus', 'F'. In the 2-back, a correct response would be if a letter matched a previous letter that appeared 2 back in the sequence, for example 'T', 'interstimulus', 'X' 'interstimulus', 'T'. In the 3-back, a correct response would be if a letter matched a previous letter that appeared 3 back in the sequence, for example 'M', 'interstimulus', 'P', 'interstimulus', 'T' 'interstimulus', 'M'. Reaction time (ms) and accuracy (percentage of errors) were tabulated for each load. Previous research has identified the 3-back load as being the most sensitive to drug administration (Loughead, Wileyto, Valdez, Sanborn, Tang, Strasser, Ruparel, Ray, Gur &, Lerman, 2009). Furthermore, the 3-back load has also been shown to be sensitive to acute exercise (Tomporowski et al., 2003; Fagan, Guirguis, Smith, Sui, Rollo, and Prapavessis, unpublished).

Other Measures

Demographics

The following information was collected: age, sex, weight (kg), and education level.

Caffeine and drug consumption history

Acute and chronic caffeine history (i.e., approximate time of last caffeine consumption, amount of years regularly consuming caffeine, preferred type of caffeine administration) was assessed. Consumption of drugs (i.e., smoking, alcohol) in the past 18 hours was assessed. No participants reported drug consumption 18 hours prior to the experiment. No participants were smokers.

Physical activity

The Physical Activity Readiness Questionnaire (PAR-Q; Thomas, Reading, & Shephard, 1992) was utilized to assess ability to participate in physical activity safely. The PAR-Q is appropriate to administer to individuals aged 15-69 years (Thomas, Reading, & Shephard, 1992). The PAR-Q has seven items, each with only two possible response options: yes or no. If a participant indicated yes to any of the seven items they were deemed not able to participate in physical activity and were thus excluded from the study (See Appendix 3).

The Short Questionnaire to Assess Health-enhancing Physical Activity (SQUASH; Wendel-Vos, Schuit, Saris, & Kromhout, 2003) was administered to assess the frequency, duration, and perceived effort of physical activity during an average week in four domains: commuting (e.g. walking to school), leisure time (e.g. sports), household (e.g. washing dishes), and work/school (e.g. walking and standing between working at a desk) (Wendel-Vos et al., 2003). Frequency and duration are fillable options, such that the participant is able to indicate the number of days per week, as well as the amount of hours and minutes they partake in each activity, while perceived effort has three possible options: slow/light, moderate, and fast/intense. An assessment of the test-retest reliability of the SQUASH in an adult Dutch population determined acceptable reliability (Spearman's correlation = 0.58) (Wendel-Vos et al., 2003). An investigation of the validity of the SQUASH via doubly labelled water, determined the SQUASH is a valid self-report tool for assessing physical activity energy expenditure (Campbell, Gaston, Gray, Rush, Maddison, & Prapavessis, 2016; See Appendix 12).

Caffeine Consumption

The Caffeine Consumption Questionnaire Revised (CCQ-R; Irons, Bassett, Prendergast, Landrum, & Heinz, 2016) was administered to assess the consumption of caffeine-containing products (i.e. beverages, foods, and drugs) during an average week. The CCQ provides images of caffeine containing products to aid in the estimation of the serving size of products consumed. The modified CCQ has been shown to have acceptable reliability (Pearson product moment correlation, r = 0.77). An investigation of the validity of the CCQ in gauging weekly caffeine consumption determined the CCQ has acceptable criterion validity (>85% inter-rater agreement) (Irons et al., 2016; See Appendix 10). CCQ responses were converted to caffeine intake in milligrams/week using the reference values in Harland (2000; See Appendix 8).

Caffeine Withdrawal

The Caffeine Withdrawal Symptom Questionnaire (CWSQ; Juliano, Huntley, Harrell, & Westerman, 2012) was utilized to assess the type and severity of caffeine withdrawal symptoms experienced by the caffeine-consumers. The CWSQ uses twenty-three items which focus on seven symptom clusters: (1) fatigue/drowsiness, (2) low alertness/difficulty concentrating, (3) mood disturbances, (4) low sociability/motivation to work, (5) nausea/upset stomach, (6) flu-like feelings, and (7) headache. The CWSQ also includes nine additional items for consideration, four of which have not yet been empirically validated. Severity of each symptom is assessed on a five-point scale ranging from 0 (*not at all*) to 4 *(extremely*). A higher score reflects greater number of symptoms and symptom severity. The CWSQ remains in the initial stages of validation and further studies are warranted to assess its reliability (Juliano et al., 2012; See Appendix 11).

Intervention

Aerobic Exercise

The exercise intervention consisted of a single bout of moderate intensity aerobic exercise completed on a Woodway PPS treadmill (Woodway, Waukesh, WI). The intervention consisted of a 2.5 minute warm-up walk, 15 minutes walking at a moderate

intensity, and a 2.5 minute cool-down walk. Moderate intensity exercise was defined as 40 to 60% of Heart Rate Reserve (HRR; Karvonen, Kentala, & Mustala, 1957; ACSM, 2013). HRR was calculated using the formula ($HR_{maximum}$= 220-age) – (HR_{rest}). HR_{rest} was taken in a seated position prior to exercise with a heart rate monitor. HR during exercise was also taken with a heart rate monitor. The researcher controlled the speed and incline of the treadmill to ensure the participant exercised within their moderate intensity HRR range.

Caffeine Administration

The caffeine administration intervention consisted of oral ingestion of powdered caffeine. Each participant ingested 1.2mg/kg (body weight) of powdered caffeine (Sigma–Aldrich Foundation, St Louis, MO) dissolved in 100mL of water (Heatherly, Hayward, Seers, & Rogers, 2005). The participant then waited in a seated position for 20 minutes to permit caffeine absorption (Mumford, Benowitz, Evans, Kaminski, Preston, Sanneurd, Silverman, & Griffiths, 1996).

Procedure

The conduct of the study adhered to the Declaration of Helsinki (World Medical Association, 2013) and the Handbook for Good Clinical Research Practice (WHO, 2005). Ethical approval was granted from the Western University's Research Ethics Board (#110797) (See Appendix 1). All participants read the Letter of Information, had his/her questions pertaining to the study answered, and signed a Consent Form prior to study participation.

Participants were recruited from Western University via online advertisements email, and word-of-mouth. Participants were initially screened for eligibility via email or an in-person meeting. Screening questions pertained to age (i.e. between 18 and 64), ability to read and write in English, self-reported caffeine consumption (<30mg/day or ≥150mg/day), contraindications to exercise (i.e. a condition preventing the ability to walk

on a treadmill for twenty minutes at a moderate intensity), self-reported cognitive difficulties, self-reported taking of medication for depression or anxiety, and pregnancy.

For those eligible, a first session was scheduled at the Exercise and Health Psychology Lab (EHPL, www.ehpl.uwo.ca) at Western University. The first session began with administration of the PAR-Q. If a participant indicated yes to any of the seven items on the PAR-Q, they were deemed not able to participate in physical activity and were thus excluded from the study. Upon completion of the PAR-Q, participants were given the demographic questionnaire, caffeine and drug history questionnaire, SQUASH, CCQ-R, and CWSQ (caffeine consumers only) to complete.

A non-caffeine consumer was defined as an individual who consumes less than 30 mg of caffeine/day (Kennedy & Haskell, 2011). A caffeine consumer was defined as an individual who consumes equal to or greater than 150 milligrams of caffeine a day, which approximately equates to the amount of caffeine in a cup of brewed coffee (Harland, 2000). Non-caffeine consumers completed two one-hour sessions on two separate days (one exercise session and one caffeine administration session). Caffeine consumers completed three sessions on three separate days (one exercise session, one caffeine administration session, and one caffeine deprivation session). The order of sessions (i.e. caffeine administration followed by exercise or exercise followed by caffeine administration) was randomized. Participants were scheduled at approximately the same time to mitigate diurnal effects.

For both non-caffeine and caffeine consumers, blood pressure (BP) was taken in a seated position with an electronic sphygmomanometer (MPOW). Resting heart rate (HR) was taken in a seated position with a heart rate monitor (Polar RS100). Weight was measured using the Health-O-Meter Professional weight scale (Health-O-Meter 500 KL, Boca Ration, FL) to the nearest 0.1kg. Participants then completed the baseline n-back task (lasting approximately 10 to 15 minutes) on a portable computer in isolation. Upon completion of the baseline n-back task, participants completed either the aerobic exercise

session or the caffeine administration session. HR and BP were again taken at the end of each treatment session followed by the n-back task.

Participants returned on the second day and followed the same protocol to complete the treatment session they did not undergo on day one. Caffeine consumers underwent one additional session, which required a 12-hour caffeine deprivation period prior to arrival on the third day. Participants were told the researcher would be biologically confirming caffeine abstinence through a saliva swab, when in fact no salivary caffeine assays were conducted. This was simply a strategy to increase caffeine deprivation compliance (Rogers et al., 2003). Participants' BP and HR were taken in a seated position upon arrival. They then completed the CWSQ and the n-back task to assess caffeine-deprived performance. Upon completion of the n-back task, participants were randomized into receiving either the exercise session or caffeine administration session. At the end of either session, the CWSQ and n-back were administered again. At the end of the experimental protocols, participants' email addresses were entered into a draw to win a twenty-five-dollar gift card.

Sample Size Analysis

Phase I

Giles and colleagues (2012) detected a change in WM accuracy (composite score of hit rate and false alarm rate) between placebo and caffeine administration (Cohen's d = 0.418). Fagan, Guirguis, Smith, Sui, Rollo, and Prapavessis, unpublished detected a change in WM accuracy (% errors) between baseline and aerobic exercise (Cohen's d = 0.511). Based on the above findings, to be adequately powered to detect differences from baseline, caffeine, and aerobic exercise, a conservative approach of using a small-to-moderate effect size f = 0.20, power = 0.80, and alpha = 0.05, generated a sample size of 28 individuals (Cohen 1969; Cohen, 1988; Faul, Erdfelder, Lang, & Buchner, 2007).

Phase II

In developing the Caffeine Withdrawal Symptom Questionnaire (CWSQ), Juliano and colleagues (2012) detected a 2.69-point reduction in withdrawal symptoms (Cohen's $d = 0.866$) when caffeine was administered following a 16-hour caffeine deprivation period. Based on the above findings, to be adequately powered to detect the effects of caffeine administration following an overnight deprivation period, an approach of using the effect size of $d = 0.866$, power = 0.80, and alpha = 0.05, generated a sample size of 13 individuals (Cohen 1969; Cohen, 1988; Faul, Erdfelder, Lang, & Buchner, 2007).

Primary and secondary outcome analyses

Phase I

Repeated measures ANOVAs were conducted across baseline, caffeine, and exercise for both accuracy (% errors) and RT (ms) for non-caffeine and caffeine consumers on the n-back task. Analyses focused on the 3-back (primary outcome) and 2-back (secondary outcome) loads specifically. Means, standard deviations, and 95% confidence intervals associated with both non-caffeine and caffeine-consumers at all n-back loads are presented in Table 2.

Phase II

Repeated measures ANOVAs were conducted across non-deprived, caffeine-deprived, and post-caffeine withdrawal symptom scores as well as non-deprived, caffeine-deprived and post-exercise withdrawal symptom scores (primary outcome). Repeated measures ANOVAs were also conducted across non-deprived, caffeine-deprived and post-caffeine accuracy (% errors) and RT (ms) on the n-back task, as well as non-deprived, caffeine-deprived, and post-exercise accuracy (% errors) and RT (ms) on the n-back task (secondary outcome). Analyses focused on the 3-back and 2-back loads specifically.

For both phases, following significant repeated measures ANOVAs, Bonferroni-corrected post-hoc t-tests were conducted. The level of significance was accepted at $p < .05$ for all tests. Effect sizes (Cohen's d, $\eta 2$) accompany all reported findings. All bars

in figures represent standard deviation (SD). Data were analyzed using IBM SPSS Statistics (Version 23).

Chapter 3 : Results

Treatment of Data

Missing data. One non-caffeine participant had no data recorded during the post-exercise session and thus was not included in the analyses. Two BP measures from one participant were not recorded due to equipment malfunction and thus were omitted from the BP dataset and fidelity check.

Outliers. n-Back trials were excluded if trial RT <150ms (Miller & Low, 2000) and if the trial was identified as an outlier (>1.5 times the interquartile range above the upper quartile and below the lower quartile) via boxplots. Less than 3% of total trials were excluded from the n-Back data set.

Manipulation check (MC). Paired sample t-tests were conducted to determine whether a time effect (participants performing better on second assessment compared to first assessment irrespective of treatment) was present. Factorial repeated measures ANOVAs (2 treatment: caffeine, exercise by 2 treatment order: caffeine first, exercise first) were also conducted to determine whether treatment by order effects were present.

Non-caffeine consumers MC. All paired sample t-tests were non-significant [3-back accuracy, $t(28) = 1.231$, $p = 0.229$, $d = 0.190$. 3-back RT, $t(28) = -1.218$, $p = 0.233$, $d = 0.235$. 2-back accuracy, $t(28) = 1.231$, $p = 0.228$, $d = 0.313$. 2-back RT, $t(28) = -0.800$, $p = 0.430$, $d = 0.141$], indicating no time effects were present. Three-back, factorial repeated measures ANOVAs found no significant interaction effect for accuracy, $F(1,12) = 2.292$, $p = 0.156$, $\eta^2 = 0.160$ but a significant interaction effect for RT, $F(1,12) = 5.866$, $p = 0.032$, $\eta^2 = 0.328$. For the 2-back, factorial repeated measures ANOVAs found no significant interaction effects for accuracy, $F(1,12) = 0.359$, $p = 0.560$, $\eta^2 = 0.029$ or RT, $F(1,12) = 0.519$, $p = 0.485$, $\eta^2 = 0.041$. Taken together, these data show that no treatment by order effect was present except for RT on the 3-back.

Caffeine consumers MC. All paired sample t-tests were non-significant [3-back accuracy, $t(29) = 1.039$, $p = 0.307$, $d = 0.213$. 3-back RT, $t(29) = 0.686$, $p = 0.498$, d

=0.165. 2-back accuracy, t(29) = 0.743, p = 0.464, d =0.187. 2-back RT, t(29), =0.556, p =0.582, d=0.140], indicating no time effects were present. Three-back, factorial repeated measures ANOVAs revealed a significant interaction effect for accuracy, F(1,14) =4.807, p = 0.046, η^2 = 0.256. No significant interaction effect was found for RT, F(1,14) = 0.288, p =0.600, η^2 = 0.020. For the 2-back, no interaction effect was found for accuracy, F(1,14) =0.244, p = 0.629, η^2 = 0.017 or RT, F(1,14) =0.142, p =0.712, η^2 = 0.010. These data, taken together, suggest there was no treatment by order effect present except for accuracy on the 3-back.

Fidelity check. A repeated measures ANOVA, followed by post-hoc t-tests were conducted across pre-exercise, during, and post-exercise treatment for heart rate (HR) combining both caffeine and non-caffeine consumers. There was a significant effect for HR [F(2,116) = 754.442, p < 0.000, η^2 = 0.929]. Paired sample post-hoc t-tests uncovered significant increases between pre-exercise and during exercise: t(59)=33.97, p <0.000, d=5.480, and significant decreases during exercise and post-exercise t(59)=30.260, p <0.000, d=4.846. A paired sample t-test was conducted between HR prior to caffeine administration and HR post caffeine administration (20 minutes) combining both caffeine and non-caffeine consumers. There was a significant decrease in HR [t(58) = 5.117, p < 0.000, d =0.584]. Descriptive HR data can be seen in Table 2 and Table 3.

Paired sample t-tests were conducted between systolic blood pressure (SBP) pre-exercise and post-exercise, as well as pre-caffeine and post-caffeine. There was no significant difference in SBP following exercise: [t(57) =0.240, p = 0.811, d =0.048]. There was a significant difference in SBP following caffeine: [t(57) =-2.925, p = 0.005, d =-0.546]. Paired sample t-tests were also conducted between diastolic blood pressure (DBP) pre-exercise and post-exercise as well as pre-caffeine and post-caffeine administration. There was no significant difference in DBP following exercise: [t(57) =0.527, p = 0.600, d = 0.118]. There was no significant difference in DBP following caffeine: [t(57) =0.125, p = 0.125, d = -0.283]. Descriptive BP data can be seen in Table 4 and Table 5.

	Non-Caffeine Consumers	Caffeine Consumers	Both Consumers
Pre-Exercise	71.76 (10.82)	74.36 (11.51)	73.15 (11.01)
Exercise	121.52 (5.69)	120.18 (5.40)	121 (5.57)
Post Exercise	74.51 (13.10)	76.96 (10.72)	75.88 (11.93)

Table 2. Exercise Treatment HR, Values are means and (SD), HR (beats/min)

	Non-Caffeine Consumers	Caffeine Consumers	Both Consumers
Pre-Caffeine	69.03 (9.03)	69.96 (10.2)	69.64 (9.45)
Post-Caffeine	64.45 (9.37)	63.61 (10.5)	64.05 (9.71)

Table 3. Caffeine Treatment HR, Values are means and (SD), HR (beats/min)

	Non-Caffeine Consumers	Caffeine Consumers	Both Consumers
Pre-Exercise SBP	120.43 (12.24)	115.11 (11.63)	117.86 (12.15)
Post-Exercise SBP	119.20 (11.33)	115.68 (14.89)	117.5 (13.17)
Pre-Exercise DBP	75.93 (9.89)	71.11 (10.57)	73.59 (10.42)
Post-Exercise DBP	72.93 (9.75)	72.43 (11.92)	72.69 (10.76)

Table 4. Exercise Treatment BP, Values are means and (SD), SBP and DBP (mmHg)

	Non-Caffeine Consumers	Caffeine Consumers	Both Consumers
Pre-Caffeine SBP	116.29 (11.52)	117.93 (12.24)	117.14 (12.36)

Post-Caffeine SBP	119.11 (12.25)	123.33 (13.19)	121.29 (12.36)
Pre-Caffeine DBP	68.03 (7.60)	74.07 (11.97)	71.16(10.46)
Post-Caffeine DBP	71.96 (9.63)	74.57 (11.85)	73.31 (10.82)

Table 5. Caffeine Treatment BP, Values are means and (SD), SBP and DBP (mmHg)

Group equivalency (Phase II only). Independent t-tests revealed no significant treatment group differences (between participants randomized to caffeine and participants randomized to exercise) for age: t(23)=1.231, p = 0.231, d = 0.490, weight: t(23) = 0.086, p= 0.932 , d = 0.034, years of caffeine consumption: t(23) =1.105 , p =0.281, d =0.437, daily caffeine consumption (mg): t(23) = 0.257, p=0.799, d =0.103, non-deprived caffeine withdrawal scores: t(23): -0.121, p= 0.905, d = 0.048, and MVPA per week (minutes): t(23)= -0.208, p=0.837, d=0.084. Chi-square tests revealed no significant group differences for sex χ^2 (1, n = 25) = 1.066, p = 0.302, Phi = -0.206 and education χ^2 (1, n = 25) = 0.051, p = 0.821, Phi =0.045.

Phase I

Primary Outcome (3-back)

Non-caffeine consumers. A repeated measures ANOVA for 3-back accuracy was statistically significant: F(2,56)=3.315, p=0.044, η^2 =0.106 (see Figure 3). Paired sample post-hoc t-tests uncovered non-significant differences between baseline and the caffeine condition: t(28)=2.60, p=0.052, d =0.345, baseline and the exercise condition: t(28) =2.30, p=0.107, d =0.313, and caffeine and exercise condition t(28) =0.25, p=1.000, d =0.0148. A repeated measures ANOVA for 3-back RT was not statistically significant: F(2,56) =1.233, p =0.299, η^2 =0.042 (see Figure 4).

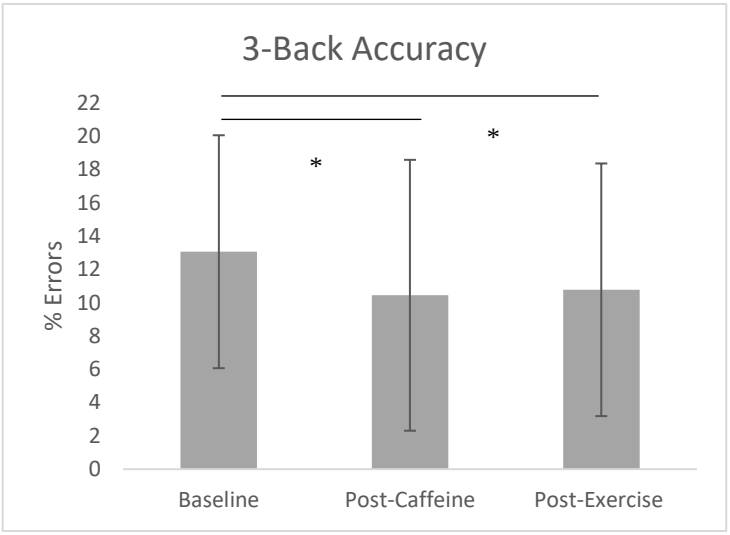

Figure 3. Changes to accuracy following treatments. Values are means ± SD. *p <0.05

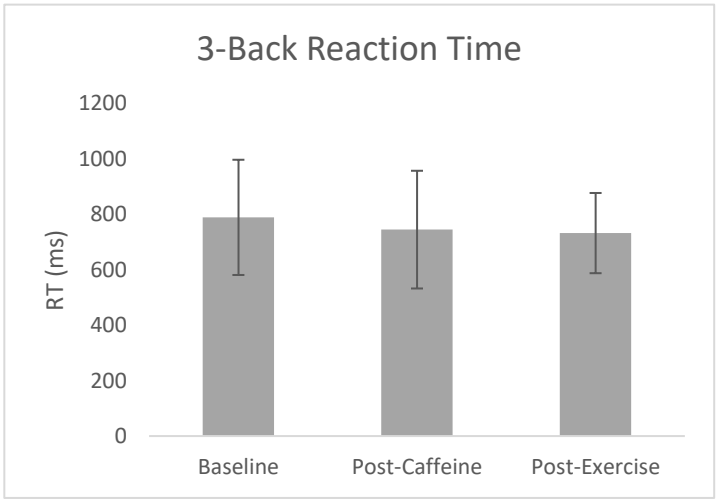

Figure 4. Changes to RT following treatments. Values are means ± SD. *p <0.05

Caffeine consumers. A repeated measures ANOVA for 3-back accuracy was statistically significant: F(2,58)=6.479, p=0.003, η^2 =0.183 (see Figure 5). Paired sample post-hoc t-tests uncovered significant differences between baseline and the caffeine condition: t(29) =2.818, p=0.027, d =0.512, and baseline and the exercise condition: t(29) =3.454, p=0.006, d =0.599. No significant difference was found between the caffeine and exercise condition t(29) =0.667, p=1.000, d = 0.112. A repeated measures ANOVA for 3-back RT was not statistically significant: F(2,58) =1.157, p =0.321, η^2 =0.038 (see Figure 6).

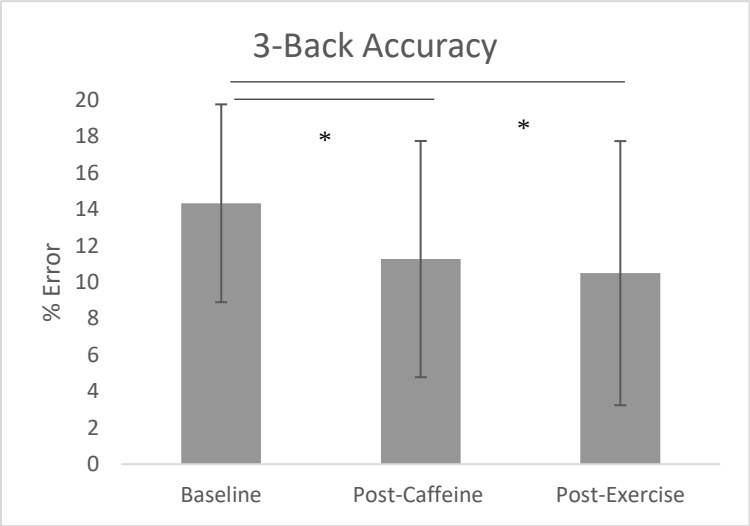

Figure 5. Changes to accuracy following treatments. Values are means ± SD. *p <0.05

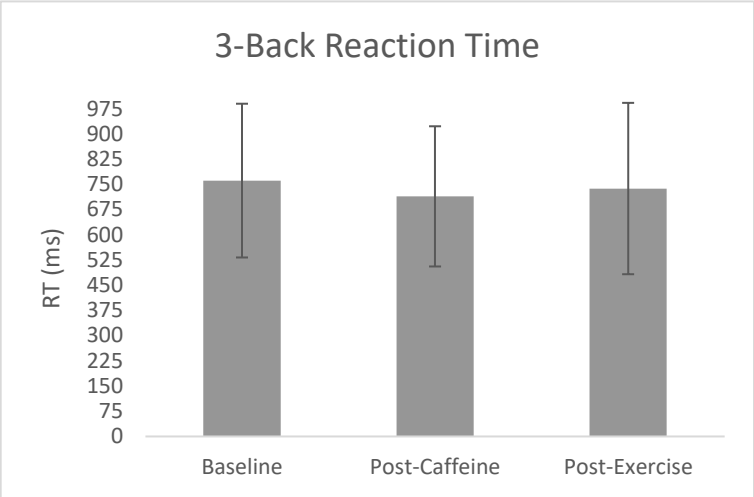

Figure 6. Changes to RT following treatments. Values are means ± SD. *p <0.05

Secondary Outcome (2-back)

Non-caffeine consumers. A repeated measures ANOVA for 2-back accuracy was not statistically significant F(2,56) =2.644, p=0.080, η^2 =0.086. A repeated measures ANOVA for 2-back RT was statistically significant F(2,56) =4.595, p =0.014, η^2 =0.141. Paired sample post-hoc tests uncovered significant differences between baseline and the caffeine condition only: t(28)=2.786, p=0.028, d =0.527.

Caffeine consumers. A repeated measures ANOVA for 2-back accuracy was statistically significant F(2,58) =9.179, p=0.000, η^2 =0.240. Paired sample post-hoc t-tests uncovered significant differences between baseline and the caffeine condition only: t(29) =3.90, p=0.002, d=0.679. A repeated measures ANOVA for 2-back RT was not statistically significant F(2,58) =2.239, p =0.116, η^2 =0.072.

Means, standard deviations, and 95% confidence intervals for n-Back task

Trial	Caffeine Consumers		
	M	SD	*95% CI*
Baseline			
3-back Error %	14.33	5.43	[12.30, 16.36]
3-back RT	761.12	228.71	[675.72, 846.52]
2-back Error %	8.26	7.28	[5.54, 10.98]
2-back RT	665.80	174.99	[600.45, 731.14]
1-back Error %	5.53	7.69	[2.66, 8.41]
1-back RT	608.62	128.98	[560.45, 656.78]
0-back Error %	4.13	7.34	[1.39, 6.88]
0-back RT	496.29	96.54	[460.24, 532.33]
Caffeine			
3-back Error %	11.26	6.49	[8.84, 13.69]
3-back RT	714.19	208.50	[636.34, 792.05]
2-back Error %	4.37	3.87	[2.93, 5.82]
2-back RT	615.66	166.00	[553.67, 677.65]
1-back Error %	2.23	4.28	[0.65, 3.85]
1-back RT	554.52	132.0	[505.23, 603.81]

0-back Error %	1.50	4.28	[0.00, 0.31]
0-back RT	487.97	85.96	[455.87, 520.06]
Exercise			
3-back Error %	10.49	7.25	[7.78, 13.20]
3-back RT	737.50	254.86	[640.71, 834.29]
2-back Error %	5.88	4.97	[4.02, 7.74]
2-back RT	641.85	219.53	[559.87, 723.82]
1-back Error %	5.60	1.81	[-0.12, 1.23]
1-back RT	543.03	140.22	[490.67, 595.39]
0-back Error %	0.694	2.34	[0.18, 1.57]
0-back RT	488.9	78.63	[459.53, 518.26]

Means, standard deviations, and 95% confidence intervals for n-Back task

Trial	Non-Caffeine Consumers		
	M	SD	*95% CI*
Baseline			
3-back Error %	13.06	7.00	[10.40, 15.72]
3-back RT	789.20	207.90	[710.12, 868.28]

2-back Error %	7.23	10.60	[3.20, 11.26]
2-back RT	667.62	189.00	[595.72, 739.52]
1-back Error %	2.93	9.096	[-0.53, 6.389]
1-back RT	551.38	162.42	[489.60, 613.16]
0-back Error %	0.87	1.55	[0.28, 1.46]
0-back RT	473.88	59.49	[451.25, 496.51]
Caffeine			
3-back Error %	10.44	8.13	[7.35, 13.54]
3-back RT	744.93	212.36	[664.15, 825.70]
2-back Error %	4.35	9.69	[0.664, 8.03]
2-back RT	577.34	151.62	[519.66, 635.01]
1-back Error %	3.54	8.83	[0.183, 6.90]
1-back RT	511.56	94.87	[475.47, 547.65]
0-back Error %	1.19	2.88	[0.09, 2.29]
0-back RT	458.82	70.98	[431.82, 485.82]
Exercise			
3-back Error %	10.77	7.59	[7.89, 13.60]
3-back RT	732.44	144.71	[677.40, 787.48]

2-back Error %	5.47	8.69	[2.17, 8.78]
2-back RT	609.61	145.28	[554.35, 664.87]
1-back Error %	2.00	8.20	[-1.00, 6.00]
1-back RT	526.70	127.88	[478.05, 575.33]
0-back Error %	2.00	3.60	[0.00, 3.00]
0-back RT	478.34	95.88	[441.87, 514.81]

Table 6. Means, SDs, 95% CI for Non-Caffeine and Caffeine Consumers for all n-back loads

Phase II

Primary Outcome (Caffeine Withdrawal Symptoms)

A repeated measures ANOVA conducted between non-deprived CWSQ, deprived CWSQ, and post-caffeine CWSQ scores was statistically significant: $F(2,24)=11.058$, $p = 0.001$, $\eta^2 = 0.501$ (seed Figure 7). Paired sample post-hoc t-tests uncovered significant differences between baseline and the deprived condition, $t(11)= -3.856$, $p = 0.008$, $d=1.35$, as well as between the deprived condition and post-caffeine administration: $t(11)= -3.392$, $p = 0.018$, $d=1.15$.

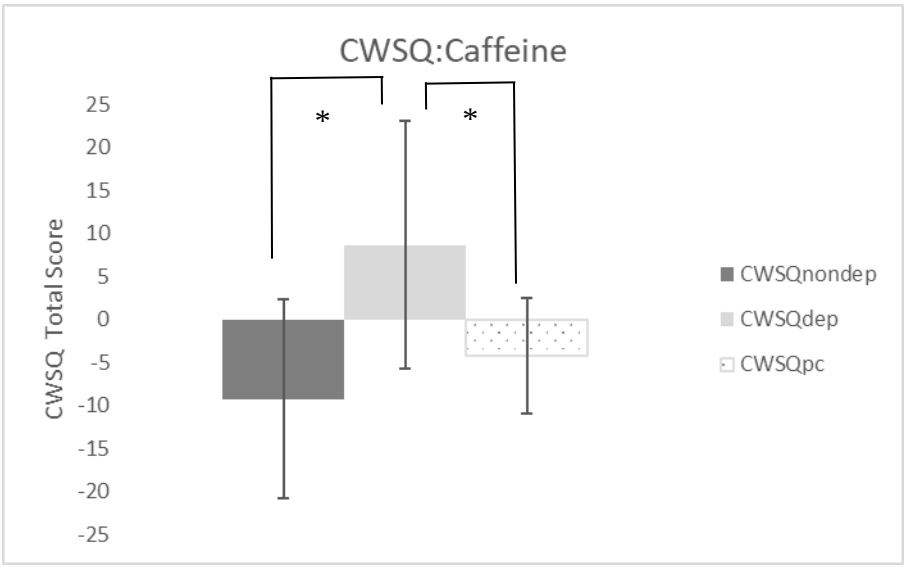

Figure 7. Changes to caffeine withdrawal symptoms from the non-deprived state , following 12-hour deprivation, and post caffeine administration. Values are means ± SD. *p <0.05

A repeated measures ANOVA conducted between non-deprived CWSQ, deprived CWSQ, and post-exercise CWSQ scores was also statistically significant: F(2,24)=5.786, p =0.009 η^2 = 0.325 (see Figure 8). Paired sample post-hoc t-tests uncovered a significant difference between baseline and the deprived condition, t(12) = -2.861, p=0.043, d=1.095, but a non-significant difference between the deprived condition and post exercise t(12)= -1.338, p = 0.617, d = 0.730.

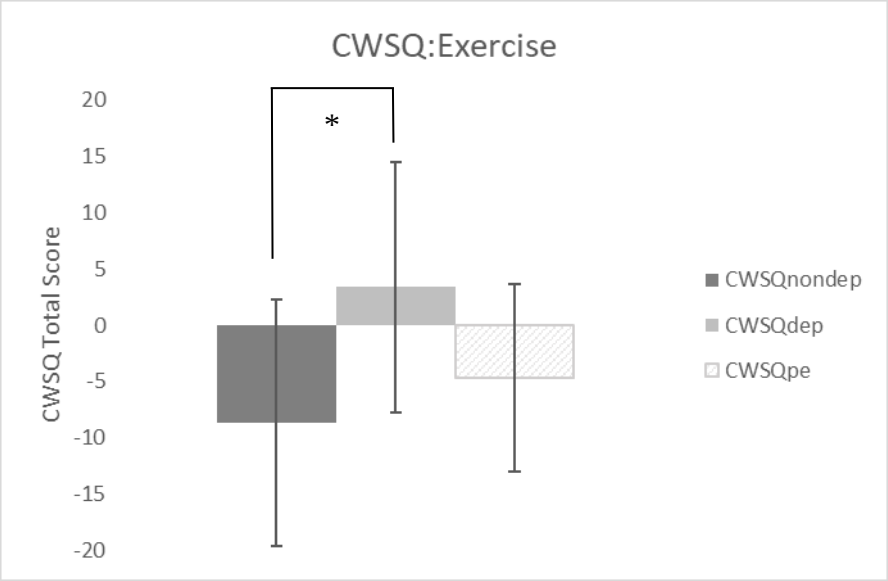

Figure 8. Changes to caffeine withdrawal symptoms from the non-deprived state , following 12-hour deprivation, and post exercise administration. Values are means ± SD. *p <0.05

Secondary Outcome (3-back)

A repeated measures ANOVA for 3-back accuracy between non-deprived, deprived, and post-caffeine was not statistically significant: F(2,22) =0.651, p = 0.531, η^2 = 0.056 (See Figure 9). A repeated measures ANOVA for 3-back RT between non-deprived, deprived, and post-caffeine was not statistically significant: F(2,22) =0.684, p = 0.515, η^2 = 0.059 (See Figure 10). A repeated measures ANOVA for 3-back accuracy between non-deprived, deprived, and post-exercise was not statistically significant: F(2,24) = 1.801, p = 0.187, η^2 = 0.131 (See Figure 11). A repeated measures ANOVA for 3-back RT between non-deprived, deprived, and post-exercise was not statistically significant: F(2,24) =0.486, p = 0.621, η^2 = 0.039 (See Figure 12).

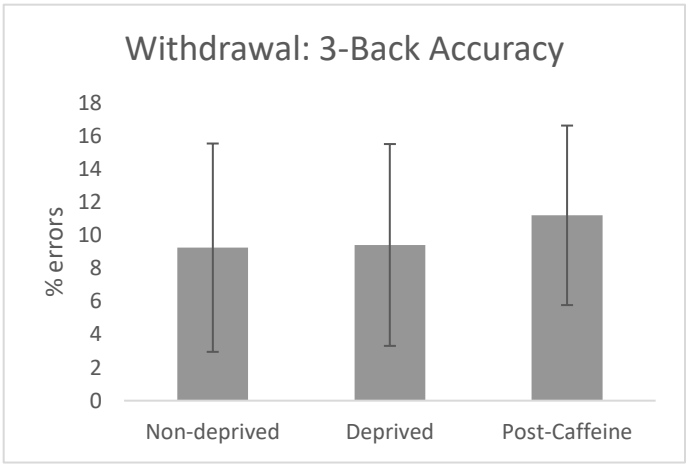

Figure 9. Accuracy comparison between non-deprived state, following 12-hour deprivation, and post-caffeine administration. Values are means ± SD. *p <0.05

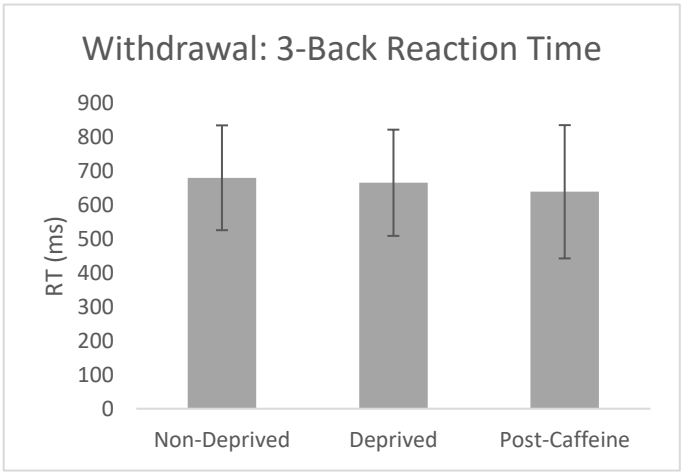

Figure 10. RT comparison between non-deprived state, following 12-hour deprivation, and post-caffeine administration. Values are means ± SD. *p <0.05

Figure 11. Accuracy comparison between non-deprived state, following 12-hour deprivation, and post-exercise administration. Values are means ± SD. *p <0.05

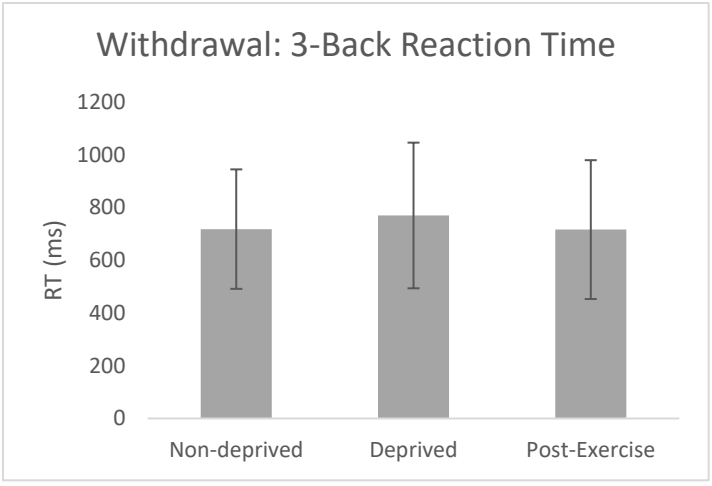

Figure 12. RT comparison between non-deprived state, following 12-hour deprivation, and post-exercise administration. Values are means ± SD. *p <0.05

Secondary Outcome (2-back)

A repeated measures ANOVA for 2-back accuracy between non-deprived, deprived, and post-caffeine was not statistically significant: $F(2,22) = 1.086$, $p = 0.355$, $\eta^2 = 0.090$. A repeated measures ANOVA for 2-back RT between non-deprived, deprived, and post-caffeine was not statistically significant: $F(2,22) = 1.467$, $p = 0.252$, $\eta^2 = 0.118$. A repeated measures ANOVA for 2-back accuracy between non-deprived, deprived, and post-exercise was not statistically significant: $F(2,24) = 0.549$, $p = 0.584$, $\eta^2 = 0.044$. A repeated measures ANOVA for 2-back RT between non-deprived, deprived, and post-exercise was not statistically significant: $F(2,24) = 1.442$, $p = 0.256$, $\eta^2 = 0.107$.

Relations between caffeine-deprived WM performance and caffeine withdrawal symptoms.

Bivariate correlations were conducted between deprived WM performance (3-back) and deprived withdrawal symptoms (CWSQ) scores. A Pearson correlation between deprived 3-back WM accuracy and deprived withdrawal symptoms in the post-caffeine group was not statistically significant: $r(10) = 0.209$, $p = 0.514$. A Pearson correlation between deprived 3-back WM accuracy and deprived withdrawal symptoms in the post-exercise group was not statistically significant: $r(11) = 0.321$, $p = 0.284$.

Chapter 4 : Discussion

The present investigation sought to determine the effects of acute, aerobic exercise in comparison to caffeine administration on working memory (WM) in non-caffeine and caffeine consumers. Additionally, the investigation sought to examine the utility of acute, aerobic exercise in reducing WM deficits and subjective withdrawal symptoms induced by caffeine deprivation. To begin, I will discuss the Phase I findings, followed by the Phase II findings.

Phase I

WM performance: Accuracy

In comparison to baseline WM accuracy, caffeine administration and acute, aerobic exercise improved WM accuracy in non-caffeine (3-back load only) and caffeine consumers (2- and 3-back loads). In non-caffeine consumers, WM accuracy improved following both caffeine administration and acute, aerobic exercise. In line with our hypothesis, caffeine administration and aerobic exercise conferred comparable improvements to accuracy (absolute percent difference: 2.62%, 2.29% and relative percent difference: 20.1%, 17.5% respectively). Caffeine administration conferring a slightly greater accuracy benefit may be due in part to the novelty of caffeine as a substance for non-caffeine consumers, as prior research has suggested non-caffeine consumers display heightened physiological and psychological responses to caffeine (Kennedy & Haskell, 2011). Furthermore, the non-caffeine consumers in this study reported high physical activity participation (1324 minutes of MVPA/week), suggesting tolerance of a single-bout of aerobic exercise with little fatigue and discomfort (Chiu & Barnes, 2003). Previous studies have identified that exercise tolerance is implicated in exercise-cognition investigations as individuals who do not regularly exercise are more likely to experience fatigue, which has been associated with impaired cognitive performance (Brown & Bray, 2015). It is also important to note that our findings contribute to the body of literature (Haskell et al., 2005; Childs & deWit, 2006; Addicott & Laurienti, 2009) supporting the notion that caffeine provides net benefits to cognition

and does not rely completely on the reversal of withdrawal symptoms, as non-caffeine consumers would not be expected to experience caffeine withdrawal.

In caffeine consumers, aerobic exercise improved accuracy to a greater extent (absolute percent difference: 3.84%, relative percent difference: 26.8%) than caffeine administration (absolute percent difference: 3.07%, relative percent difference: 21.4%). Aerobic exercise conferring a greater benefit to WM accuracy than caffeine may be due in part to caffeine tolerance (Evans & Griffiths, 1991). The caffeine dose administered (1.2mg/kg) equates to less than the mean daily caffeine consumption reported by the caffeine group (301.5mg/day), suggesting these consumers have likely developed some level of tolerance to the caffeine-driven cognitive effects. Similarly, to the non-caffeine consumers, caffeine consumers also reported regular participation in physical activity (1213 minutes of MVPA/week) supporting the notion that a single-bout of aerobic exercise was tolerated comfortably by this group. It is important to address that a treatment by order effect was detected for accuracy on the 3-back load in caffeine consumers, suggesting receiving caffeine on the the first day may have resulted in improved performance on the second day following acute, aerobic exercise, although treatment order was counterbalanced. A carry-over effect may have been present and thus utilizing a wash-out period greater than 24-hours may be required in future investigations.

Overall, our findings that WM accuracy improvements were detected in both groups (non-caffeine and caffeine consumers) at only the 2- and 3-back WM loads is in line with previous work which stated higher WM loads are most sensitive to drug and behavioural intervention effects (Loughead et al., 2009). Furthermore, our work substantiates prior findings that acute caffeine administration (Addicott & Laurienti, 2009) and acute exercise improve WM accuracy (Tomporowski et al., 2003).

WM performance: RT

Caffeine administration improved WM RT in comparison to baseline only in non-caffeine consumers on the 2-back load. Aerobic exercise resulted in no improvement to

WM speed in non-caffeine and caffeine consumers at the 2- and 3-back loads. These findings differ from those reported by Haskell et al., (2005) and McMorris et al., (2011). Diverging results could be due to the wide range in administered caffeine doses (Kaplan et al., 1997), type of cognitive task administered, and exercise intensity (Smit & Rogers, 2000; McMorris et al., 2011). Prior work by our group also detected no changes to RT on the n-back task following acute, aerobic exercise at a moderate intensity (Fagan et al., unpublished). It is important to note when examining the WM speed and accuracy findings in concert, improved WM was not due to a speed-accuracy trade-off (Reed, 1973). In other words, individuals were not committing less errors on the n-back task at a cost to response speed. Prior work has suggested caffeine may improve accuracy in cognitive tasks via increased alertness (Giesbrecht, Rycroft, Rowson, & DeBruin, 2010) and modulation of neuronal activity in regions associated with attention (Koppelstaetter et al., 2008). When considering acute, aerobic exercise it has been proposed that exercise selectively affects the activation and allocation of attentional resources (Sanders , 1983; Tomporowski et al., 2003).

Phase II

Caffeine Withdrawal Symptoms

A twelve-hour caffeine deprivation period increased subjective caffeine withdrawal symptoms (14.88-point increase on CWSQ from non-deprived state), which was in line with our hypothesis and prior work examining caffeine withdrawal (Juliano & Griffiths, 2004). Moreover, caffeine administration and aerobic exercise reduced caffeine withdrawal symptoms (12.91-point reduction, 8.07 point-reduction, respectively). Our results are in line with previous work suggesting caffeine re-administration reduces caffeine withdrawal symptoms (Addicott & Laurienti, 2009). Furthermore, our study suggests acute aerobic exercise demonstrates utility in reducing caffeine withdrawal symptoms, which is a novel finding, as well as provides further evidence that a single-bout of aerobic exercise improves “alertness”, “feelings of energy”, and mood (Maraki et al., 2005; Loy et al., 2013). In addition, our findings are consistent with work conducted

in the exercise and tobacco withdrawal literature, which determined acute, aerobic exercise successfully reduced withdrawal symptoms such as stress, difficulty concentrating, tension, restlessness, depression, and irritability (Roberts et al., 2012).

When conceptualizing the caffeine deprived phase of this investigation, the notion of non-inferiority was explored. Non-inferiority trials assess whether a novel intervention is not unacceptably lesser than a standard of care in clinical research (Rehal et al., 2016). Non-inferiority trials promote the comparison of advantages that a novel therapy may have over a standard therapy, such as fewer side effects or lower costs (Bouman et al., 2015). Caffeine use has been associated with withdrawal symptoms upon cessation in certain individuals and thus "caffeine-related disorders" have been introduced into the Diagnostic and Statistical Manual (DSM-5; Hughes et al., 1991; Strain, Mumford, Silverman, & Griffiths, 1994; Juliano & Griffiths, 2004; Addicott, 2014). Although caffeine-related disorders have been added to the DSM-5, diagnostic criteria for caffeine-use disorder have not been solidified due to uncertainties regarding caffeine's abuse potential and clinically relevant symptomology (APA, 2013). The lack of quantifiable diagnostic criteria during the time of this investigation barred calculating an appropriate non-inferiority margin for caffeine withdrawal symptoms to subsequently compare caffeine administration to acute, aerobic exercise for caffeine withdrawal relief.

WM performance

In contrast to the caffeine withdrawal symptoms, a 12-hour caffeine deprivation period did not reduce WM performance in caffeine consumers. No significant changes to WM accuracy or speed were detected between the non-caffeine deprived and caffeine-deprived conditions. These findings were not in line with our hypothesis or with work conducted by Yeomans et al., 2002. Differing results may be due to the duration of caffeine-deprivation utilized in our paradigm. Some studies have employed a 24-hour caffeine deprivation period which may have resulted in greater caffeine withdrawal severity and in turn greater cognitive deficits (Yeomans et al., 2002; Giles et al., 2012). Furthermore, when considering the cognitive tasks that were administered in the

investigations of caffeine withdrawal that detected a caffeine-deprivation induced cognitive deficit, a variety of cognitive tasks were used (e.g., Rapid Visual Information Processing task, Attention Network Test) and thus perhaps, the n-back alone may not have been the most sensitive to detect subtle WM deficits (Heatherely et al., 2004). Alternatively, the caffeine consumers in our study completed several iterations of the n-back task, thus the practice effect may have bolstered WM performance in the caffeine-deprived trials.

Addicott and Laurienti (2009) have also posited participants may exert more effort during the caffeine-deprived state to compensate for "withdrawal-related fatigue". Given that WM performance did not suffer following the 12-hour deprivation period, improvement to WM via caffeine administration or acute, aerobic exercise was unlikely. However, it is important to note that WM performance remained stable following both treatments. Previous literature has suggested that caffeine withdrawal effects worsen with time and withdrawal related fatigue could result in deteriorating performance on cognitive tasks (Juliano & Griffiths, 2004; Rogers et al., 2005). Thus, since we detected no change to WM performance, the caffeine administration and acute, aerobic exercise treatments my have buffered the caffeine-deprivation effects.

When examining the WM performance and caffeine withdrawal symptoms in concert, 12-hours of caffeine deprivation did not affect WM performance and caffeine withdrawal symptoms in the same manner. Twelve-hours of caffeine deprivation resulted in no significant decrements to WM performance as assessed by the n-back task, however, caffeine withdrawal symptoms significantly increased. Bivariate correlations between deprived WM accuracy (3-back load) and deprived CWSQ scores were weakly, positively correlated, suggesting that caffeine-deprivation may operate on cognition and caffeine withdrawal symptoms via distinct mechanisms, however, further investigations are needed to disentangle the effects of caffeine-deprivation.

Strengths, Limitations, Implications, and Future Directions

The present investigation had numerous strengths. The recruitment of a non-caffeine consumer group allowed our study to address methodological concerns highlighted in previous studies, as we could further explore whether caffeine-driven enhancements to cognition represent direct caffeine effects or the reversal of caffeine withdrawal effects. Furthermore, the use of both cognitive (n-back task) and self-report (CWSQ) measures following 12-hour caffeine deprivation enabled comparison of objective and perceived effects of caffeine withdrawal. When considering study design, the use of a within-subject counterbalanced design in Phase I provided advantages in terms of reducing variability associated with individual differences, as subjects act as their own control, as well as minimization of order effects via counterbalancing. Another strength included administration of the caffeine and aerobic exercise treatments on separate days, as this minimized carry-over effects and fatigue experienced when undergoing cognitive testing. The use of a between-subjects randomized design in Phase II provided advantages in reducing the number of times the n-back was conducted. Finally, the caffeine dosing utilized in our investigation accounted for participant body weight, while also being within doses typically consumed in real-world contexts.

Despite the aforementioned strengths, there are limitations to be acknowledged. One limitation is the practice effect associated with the n-back as well as other cognitive tasks, which refers to participants improving on the task as a result of repetition of the task. Future investigations should examine cognitive tasks that are more robust to the practice effect. Another limitation includes the lack of comparison to a placebo, which in this investigation was done to reduce the amount of times participants completed the n-back task. In the future, a between-groups design could be employed with one group receiving a placebo condition. Additionally, we detected a treatment by order effect in caffeine consumers regarding accuracy, which suggests carry-over between treatments may have been present. Thus, employing a longer wash-out period between treatments (i.e., > 24 hours) in future investigations may minimize contamination. Finally, the participants in the present investigation were young, physically active, and highly

educated. Future research should examine the effects of caffeine and acute aerobic exercise on WM across various ages, physical activity and education levels.

Determining the duration of the post-caffeine and post-exercise cognitive benefit, as well as investigating the potential effects of different exercise modalities on cognitive performance remain areas warranting further investigation. Additionally, examining the role of biological variables such as caffeine metabolism via genes such as *CYP1A2* could further clarify interactions between metabolism and caffeine-driven changes to cognitive performance. Finally, exploring the effects of sleep in tandem with caffeine administration and acute, aerobic exercise on cognitive functioning is another potential avenue of investigation. Through investigations of this nature, the utility of acute, aerobic exercise in lieu of caffeine consumption to optimize cognitive performance would be further clarified with the end-goal of guiding health-related interventions for both general and special populations.

Conclusion

Findings from the present study suggest caffeine administration and acute, aerobic exercise improve WM accuracy in both non-caffeine and caffeine consumers. Furthermore, caffeine administration and acute, aerobic exercise reduce caffeine withdrawal symptoms induced by a 12-hour caffeine deprivation period. WM is not reduced during caffeine deprivation, hence whether exercise and caffeine can restore WM remains unknown. Further research is required to elucidate the mechanisms in which acute, aerobic exercise exerts its effects on cognition and withdrawal symptoms.

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Appendices

Western Research

Date: 15 March 2018

To: Prof. Harry Prapavessis

Project ID: 110797

Study Title: The Acute Effects of Moderate Intensity Exercise and Caffeine Ingestion on Cognition in Non-Caffeine Consumers and Caffeine Consumers

Application Type: HSREB Initial Application

Review Type: Full Board

Meeting Date / Full Board Reporting Date: 19/Dec/2017

Date Approval Issued: 15/Mar/2018

REB Approval Expiry Date: 15/Mar/2019

Dear Prof. Harry Prapavessis

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above mentioned study as described in the WREM application form, as of the HSREB Initial Approval Date noted above. This research study is to be conducted by the investigator noted above. All other required institutional approvals must also be obtained prior to the conduct of the study.

Documents Approved:

Document Name	Document Type	Document Date	Document Version
caffeine_ad_2_26_2018	Recruitment Materials	26/Feb/2018	1
CWSQ_VD	Paper Survey	31/Dec/2017	2
debriefing_letter_VD_CLEAN	Debriefing Letter	31/Dec/2017	2
Letter of Information caffeine_clean_2_26_2018_CLEAN	Written Consent/Assent	26/Feb/2018	1
Questionnaires caffeine_VD	Paper Survey	31/Dec/2017	2
Supp_CCQ-R_VD	Paper Survey	31/Dec/2017	2

Documents Acknowledged:

Document Name	Document Type	Document Date	Document Version
overview of study caffiene cognition	Flow Diagram	22/Nov/2017	1
references for caffiene ethicsUPDATED	References	17/Nov/2017	1

No deviations from, or changes to, the protocol or WREM application should be initiated without prior written approval of an appropriate amendment from Western HSREB , except when necessary to eliminate immediate hazard(s) to study participants or when the change(s) involves only administrative or logistical aspects of the trial.

REB members involved in the research project do not participate in the review, discussion or decision.

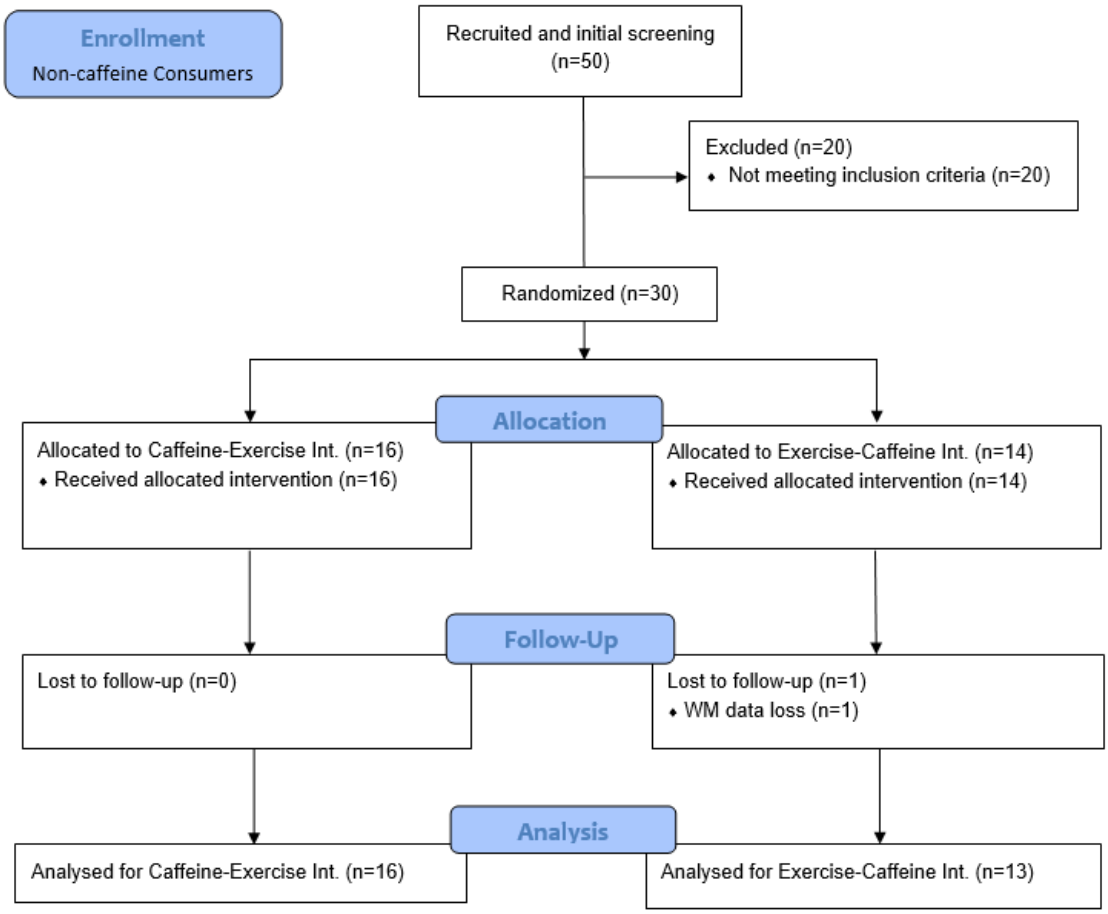
The Western University HSREB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

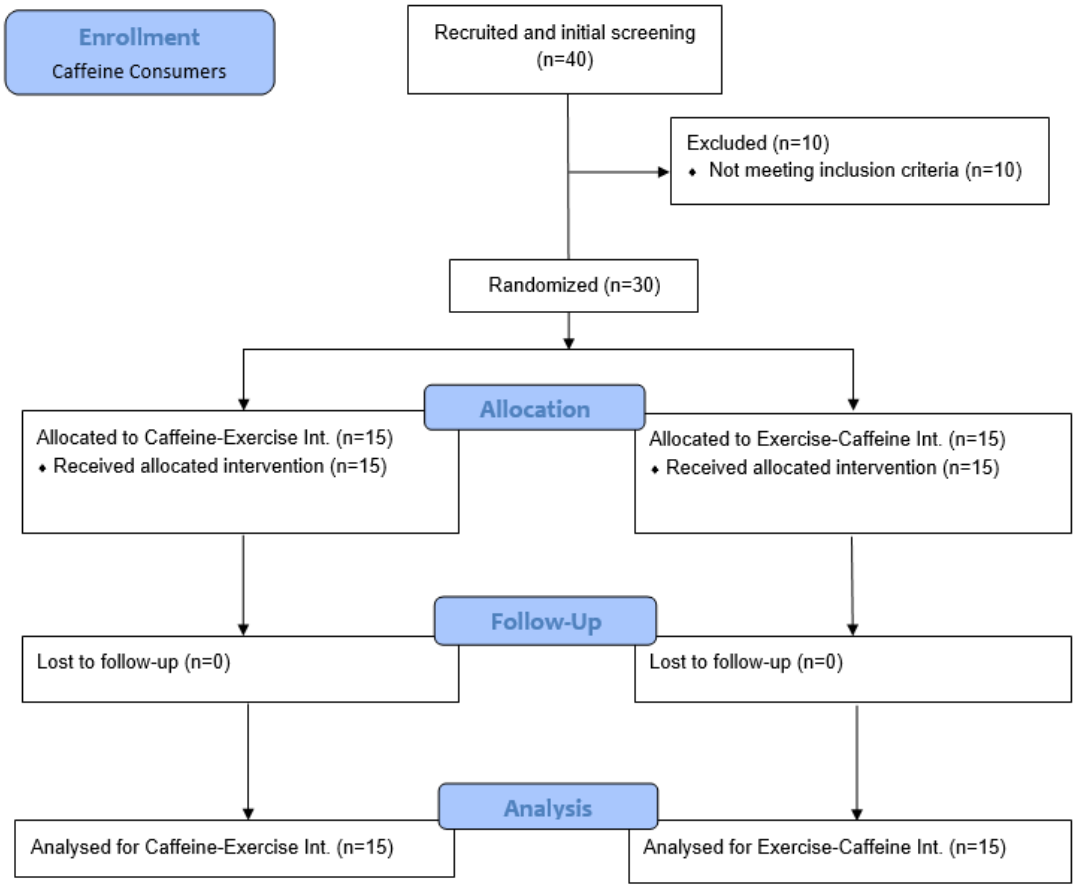
Sincerely,

Patricia Sargeant, Ethics Officer (ext. 85990) on behalf of Dr. Marcelo Kremenchutzky, HSREB Vice-Chair

Appendix 1. Approval notice from Western Research Ethics



Appendix 2. Participant Flow Diagram (Non-Caffeine Consumers)



Appendix 3. Participant Flow Diagram (Caffeine Consumers)

Physical Activity Readiness Questionnaire (PARQ)

1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?

a. [ ]Yes b. [ ]No

2. Do you feel pain in your chest when you do physical activity?

a. [ ]Yes b. [ ]No

3. In the past month, have you had chest pain when you were not doing physical activity?

a. [ ]Yes b. [ ]No

4. Do you lose your balance because of dizziness or do you ever lose consciousness?

a. [ ]Yes b. [ ]No

5. Do you have a bone or joint problem that could be made worse by a change in your physical activity?

a. [ ]Yes b. [ ]No

6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart?

a. [ ]Yes b. [ ]No

7. Do you know of any other reason why you should not do physical activity?

a. [ ]Yes b. [ ]No

Appendix 4. Physical Activity Readiness Questionnaire

Caffeine Consumption Questionnaire-R

Please answer the following questions as accurately as you can. **Indicate how many servings per week you normally consume of each item. Use the pictures to help guide your responses.**

Do you drink coffee at least once a week?

- ○ Yes
- ○ No

Please indicate how many servings of coffee you consume, on average, each week.

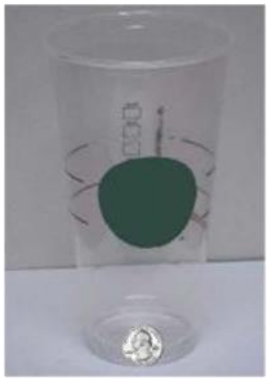
			
8 oz. coffee (short)	12 oz. coffee (tall)	16 oz. coffee (grande)	20 oz. coffee (venti)

Please indicate how many servings of **decaffeinated** coffee you consume, on average, each week.

			
8 oz. decaffeinated coffee (short)	12 oz. decaffeinated coffee (tall)	16 oz. decaffeinated coffee (grande)	20 oz. decaffeinated coffee (venti)

Appendix 5. Caffeine Consumption Questionnaire

Please indicate how many servings of **iced** coffee you consume, on average, each week.

			
12 oz. iced coffee (tall)	16 oz. iced coffee (grande)	24 oz. iced coffee (venti)	31 oz. iced coffee (trenta)

Soda

Do you drink soda at least once a week?

- ○ Yes
- ○ No

Please indicate how many servings of **soda** and **diet soda** you consume, on average, each week. Some sodas do not contain caffeine. Examples include: Sprite, 7-Up, Orange soda, and Root Beer.

			
12 oz. soda	16.9 oz. soda	20 oz. soda	32 oz. soda

Energy Drinks

Do you drink energy drinks at least once a week?

- ○ Yes
- ○ No

Please indicate how many servings of energy drinks you consume, on average, each week.

			
2 oz. Energy Shot	8.4 oz. energy drink	12 oz. energy drink	16 oz. energy drink

Teas

Do you drink tea at least once a week?

- ○ Yes
- ○ No

Please indicate how many servings of tea you consume, on average, each week.

			
8 oz. tea	12 oz. tea (tall)	16 oz. tea (grande)	24 oz. tea (venti)

Chocolate Beverages

Do you drink chocolate beverages at least once a week?

- ○ Yes
- ○ No

Please indicate how many servings of hot chocolate you consume, on average, each week.

			
8 oz. hot chocolate (short)	12 oz. hot chocolate (tall)	16 oz. hot chocolate (grande)	20 oz. hot chocolate (venti)

Please indicate how many servings of chocolate milk you consume, on average, each week.

			
8 oz. chocolate milk (short)	12 oz. chocolate milk (tall)	16 oz. chocolate milk (grande)	20 oz. chocolate milk (venti)

Food

Do you consume any food that contains caffeine (food including chocolate or coffee are prime examples)?

- ○ Yes
- ○ No

Please indicate how many chocolate bars (**purely chocolate**) you consume, on average, each week.

	
Chocolate Bars (1.55 oz.)	Mini Chocolate Bars

Please indicate how many candy bars (snickers, twix, butterfinger, etc.) you consume, on average, each week.

	
Candy Bars (full size)	Mini Candy Bar

Food containing **chocolate** (4 oz. servings)

	Please indicate how many servings of the following foodstuffs (4 oz.) you consume, on average, each week. servings
Yogurt	
Ice cream	
Baked goods	

Food containing coffee (4 oz. servings)

	Please indicate how many servings of the following foodstuffs (4 oz.) you consume, on average, each week. servings
Yogurt	
Ice cream	
Candy	
Baked goods	

Mint or Gum containing caffeine (Jolt gum, Alert Energy gum, Foosh mints, Hero mints, etc.)

	Please indicate how many pieces of the following foodstuffs you consume, on average, each week. pieces
Mint	
Gum	

Drugs

Do you consume any of the following over-the-counter caffeinated drugs?

	Please enter how many days each week you consume the drug(s), the serving size of each dose, and the number of times you consume the drug(s) each day.		
	days	serving size	amount
Vivarian			
NoDoz			
Excedrin			
Vanquish			
Anacin			
Dristan			
Xendrine			
Trimspa			
Other			

CWSQ

Below is a list of feelings/experiences people have. Circle the number that best describes how you are feeling/what you are experiencing **RIGHT NOW**.

	Not at all	A little	Moderately	Quite a bit	Extremely
1. Drowsy/sleepy	0	1	2	3	4
2. Self-confidence	0	1	2	3	4
3. Yawning	0	1	2	3	4
4. Alert	0	1	2	3	4
5. Tired/Fatigued	0	1	2	3	4
6. Content	0	1	2	3	4
7. Difficulty Concentrating	0	1	2	3	4
8. Irritable	0	1	2	3	4
9. Heavy feelings in arms and legs	0	1	2	3	4
10. Depressed Mood	0	1	2	3	4
11. Grouchy	0	1	2	3	4
12. Urge to do work related activity	0	1	2	3	4
13. Flu-like feelings	0	1	2	3	4
14. Headache	0	1	2	3	4
15. Talkative	0	1	2	3	4
16. Sluggish	0	1	2	3	4
17. Upset stomach	0	1	2	3	4
18. Clearheaded	0	1	2	3	4
19. Desire to socialize	0	1	2	3	4
20. Energetic	0	1	2	3	4
21. Nausea/vomiting	0	1	2	3	4
22. Muscle pain/stiffness/aches	0	1	2	3	4
23. Discouraged	0	1	2	3	4
Additional items for consideration:					
Queasy	0	1	2	3	4
Nauseous	0	1	2	3	4
Vomiting	0	1	2	3	4
Headachy	0	1	2	3	4
*Anxious	0	1	2	3	4
*Nervous	0	1	2	3	4
*Jittery	0	1	2	3	4
*Craving for caffeine	0	1	2	3	4
*Craving for coffee	0	1	2	3	4

* These symptoms have not been empirically validated as caffeine withdrawal symptoms

Appendix 6. Caffeine Withdrawal Symptom Questionnaire

Appendix A: ***The short questionnaire to assess health enhancing physical activity (SQUASH)***

Think about an average week in the past months. Please indicate **how many days per week** you performed the following activities, how much time **on average** you were engaged in this, and (if applicable) how strenuous this activity was for you?

COMMUTING ACTIVITIES (round trip)	**days per *week***	**average time per *day***	**Effort (circle please)**
Walking to/from work or school	days	hour minutes	slow/moderate/fast
Bicycling to/from work or school	days	hour minutes	slow/moderate/fast
Not applicable			

LEISURE TIME ACTIVITIES	**days per *week***	**average time per *day***	**Effort (circle please)**
Walking	days	hour minutes	slow/moderate /fast
Bicycling	days	hour minutes	slow/moderate /fast
Gardening	days	hour minutes	light/moderate /intense
Odd jobs	days	hour minutes	light/moderate /intense
Sports (please write down yourself) *e.g., tennis, fitness, skating, swimming, dancing*			
1.	days	hour minutes	light/moderate /intense
2.	days	hour minutes	light/moderate /intense
3.	days	hour minutes	light/moderate /intense
4.	days	hour minutes	light/moderate /intense

HOUSEHOLD ACTIVITIES	**days per *week***	**average time per day**
Light household work (cooking, washing dishes, ironing, child care)	days days	hour minutes hour minutes
Intense household work (scrubbing floor, walking with heavy shopping bags)		

ACTIVITY AT WORK AND SCHOOL	**average time per *week***
Light work	hour minutes
(sitting/standing with some walking, e.g., a desk job)	hour minutes
Intense work (regularly lifting heavy objects at work)	
Not applicable	

Appendix 7. Short Questionnaire to Assess Health Enhancing Physical Activity

Product	Serving Size (unless otherwise stated)		Milligrams of Caffeine (approximate values)
	oz	**ml**	
Coffee			
Brewed	8	237(1cup)	135
Roasted and ground, percolated	8	237	118
Roasted and ground, filter drip	8	237	179
Roasted and ground, decaffeinated	8	237	3
Instant	8	237	76 - 106
Instant decaffeinated	8	237	5

Product	Serving Size (unless otherwise stated)		Milligrams of Caffeine (approximate values)
	oz	**ml**	
Tea			

Appendix 8. Caffeine Content (Harland, 2000)

Average blend	8	237	43
Green	8	237	30
Instant	8	237	15
leaf or bag	8	237	50
Decaffeinated tea	8	237	0

Product	**Serving Size (unless otherwise stated)**		**Milligrams of Caffeine (approximate values)**
	oz	**ml**	
Cola Beverages			
Cola beverage, regular	12	355(1 can)	36 - 46
Cola beverage, diet	12	355	39 - 50

	oz	**ml**	
Cocoa Products			
Chocolate milk	8	237	8

1 envelope hot-cocoa mix	8	237	5
Candy, milk chocolate	1	28g	7
Candy, sweet chocolate	1	28g	19
Baking chocolate, unsweetened	1	28g	25 - 58
Chocolate cake	2.8	80g	36
Chocolate brownies	1.5	42g	10
Chocolate mousse	3.2	90g	15
Chocolate pudding	5.1	145g	9

Curriculum Vitae

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