

**FATIGUE MECHANISMS IN SEDENTARY AND ENDURANCE
TRAINED ADULTS: EFFECTS OF NUTRITIONAL
COUNTERMEASURES**

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**FATIGUE MECHANISMS IN SEDENTARY AND ENDURANCE
TRAINED ADULTS: EFFECTS OF NUTRITIONAL
COUNTERMEASURES**

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LIST OF SYMBOLS AND ABBREVIATIONS

ACC	Accuracy
CAF	Caffeine
CHO	Carbohydrate
CNS	Central Nervous System
EE	Energy Expenditure
EET	Electrically Evoked Torque
EI	Energy Intake
FN	False Negatives
FP	False Positives
HR	Heart Rate
ITT	Interpolated Twitch Technique
kCal	Kilocalories (or Calories)
LT	Lactate Threshold
MVC	Maximal Voluntary Contraction
PLA	Placebo
PREC	Precision
TN	True Negatives
TP	True Positives
RER	Respiratory Exchange Ratio
RPE	Rating of Perceived Exertion
VO ₂ peak	Peak Oxygen Consumption
W	Watts

SUMMARY

Physical inactivity is a major risk factor for chronic disease; yet, despite this evidence, 70% of the U.S. population does not meet physical activity recommendations, with fatigue being a primary underlying reason. Surveys conducted within the U.S. workforce [1] indicate a 38% prevalence of fatigue, with 66% of these workers reporting lost productivity time, resulting in an overhead cost to employers of > \$100 billion annually. Fatigue is a complex phenomenon with multiple physiological and psychological origins but, ultimately, fatigue may be manifested by an increased perception of effort to complete a task or the inability to sustain a task, whether the task requires mental and/or physical effort. Mental fatigue, specifically, has been defined as a “psychobiological state caused by prolonged periods of demanding cognitive activity” and “characterized by subjective feelings of lack of energy” [2, 3]. Mental fatigue can be observed by the impairment of cognitive function, particularly tasks that require sustained attention or the ability to direct and focus cognitive ability over a period of time [2, 4, 5].

While fatigue is often considered to be a byproduct or result of a demanding effort, it can also be thought of as a perception or feeling that results in lack of motivation or energy to allocate effort or attention to continue a task or activity. Because reducing fatigue is essential for athletic performance, extensive research has been done in physically fit and highly trained populations to evaluate efficacy of ergogenic aids. Common nutritional supplements such as caffeine (CAF) and carbohydrate (CHO) are frequently taken prior to and during exercise and can delay fatigue, particularly in trained athletes; but, whether these same reductions in fatigue translate to sedentary individuals

is less clear. In addition to delaying fatigue in physical efforts, CAF and CHO may also provide benefit during mentally fatiguing cognitive tasks; presumably since both endurance exercise and cognitive tasks requiring sustaining attention over a period of time involve similar brain areas that are involved in higher order functions such as maintaining attention, processing sensory inputs, evaluating effort versus reward, and decision making. Although CAF and CHO may be ergogenic through some level of peripheral action, both are also able to act via the central nervous system (CNS) within the same brain areas involved in attention, sensory processing, motivation, reward, and decision making. There is evidence to support that CAF and CHO both increase exercise capacity; and when combined, CAF+CHO may provide additional benefits to physical and mental performance. However, nutritional strategies using these products to reduce physical or mental fatigue have not been systematically examined and compared in sedentary versus trained individuals.

While CHO ingestion rates of 30-6 g/h are typically recommended to improve endurance exercise for athletes, recent research also provides evidence that simply rinsing the mouth with a CHO solution can also improve physical performance and exercise capacity, even without ingestion. Since the majority of the population who does not engage in regular physical activity could benefit from these same nutritional countermeasures to fatigue, but possibly require different doses or recommendations from those given to athletes, more research is warranted for the general population. Commonly ingested CHO drinks (i.e. sports drinks, juices, and energy drinks) can be high in sugar and calories and may have health-related consequences (i.e. dental caries, obesity, insulin resistance, diabetes) [6-8]. Thus, the American Heart Association

recommends adults limit daily sugar intake to 30-45 g/d [9] and the American Dental Association also recommends “limiting between meal sipping and snacking on sugary beverages and foods”. For this reason, low-calorie, low-CHO drinks have become increasingly popular given the possible negative health effects of ingesting sugar-sweetened beverages, particularly under sedentary conditions. More recent research from exercise [10-14] and psychology [15-18] literature also provides evidence that the simple presence of CHO in the mouth can enhance performance even without ingestion or augmenting blood glucose. Signals from CHO-sensing receptors in the oral cavity, digestive tract, and liver and neural signaling from gut peptides to the brain are possible mechanisms underlying the ergogenic benefit of CHO “oral rinse” and low amounts of CHO ingestion [10, 11, 16, 19-23]. In addition, while CAF has benefits for exercise capacity, it may also have detrimental effects to glycemic regulation through transient insulin resistance when ingested in the sedentary state [24]. Thus, it is unclear whether the potential benefits of CAF outweigh metabolic disadvantages for a sedentary population who may be predisposed to diabetes or insulin resistance. The main goal of this research is to investigate the influence of nutritional aids: CAF, low-calorie CHO, and their combination CAF+CHO to delay physical and mental fatigue in healthy but sedentary men and women compared to endurance-trained counterparts.

The minimum amount of CHO that could elicit a benefit to attentional performance without eliciting a metabolic response when ingested remains unclear. Thus, our **first research aim** was to evaluate glycemic response after ingestion of CHO drinks ranging from 0 to 6% CHO; and, to determine effects of two methods of CHO administration (ingest and rinse) on sustained attention. We hypothesized that based on

evidence that “low calorie beverages” do not appear to alter blood glucose [25]: 1) ingesting a low-CHO (<1%) drink would not elicit a glycemic response different from an artificially-sweetened placebo and, 2) either ingesting low-CHO or rinsing an equivalent amount (grams) of CHO without ingestion would provide greater benefit to attenuate mental fatigue compared to rinsing with a CHO-free solution. Our **second aim** was to evaluate the efficacy of CAF, low-CHO, and the combination of CAF+CHO on exercise capacity in trained versus sedentary groups. Our hypothesis was that ingestion of CAF and CAF+CHO would improve exercise capacity by reducing factors related to fatigue independent of fitness level; and, that each nutritional component alone would improve measures of fatigue compared to a placebo (PLA). Since acute exercise also benefits cognition independent of CAF or CHO, our **third aim of research** was to determine: (1) the effect of moderate intensity exercise on sustained attention in comparison to a similar duration of seated rest; and 2) whether CAF provides additional benefit to sustained attention, perceived mental energy and mental fatigue when combined with exercise. We hypothesized that moderate intensity exercise would benefit sustained attention independent of fitness status; however, unlike trained individuals, sedentary subjects would experience impaired attention following exercise performed to fatigue. Furthermore, we also hypothesized that, compared to non-caffeinated placebo, caffeine would maintain sustained attention and, when combined with exercise, would maintain sustained attention and associated perceptual measures (mental energy and mental fatigue) independent of fitness level.

CHAPTER 1

INTRODUCTION

Overview

Physical inactivity and low cardiorespiratory fitness are known risk factors for chronic diseases such as cardiovascular disease, diabetes, hypertension, and obesity [26-30]; yet, 70% of the US population does not participate in regular physical activity (www.cdc.gov). The reasons for a sedentary lifestyle include the lack of motivation or energy to perform physical activity, pain, discomfort, and/or fatigue experienced either during or following activity [31, 32]. Fatigue is a complex phenomenon and its effects can be manifested in both physical and mental tasks. While physical fatigue is generally defined as a decreased capacity to exert muscle force or power during exercise, mental fatigue has been defined as a “psychobiological state caused by prolonged periods of demanding cognitive activity” and “characterized by subjective feelings of lack of energy” [2, 3, 33]. In habitually sedentary individuals, low exercise tolerance or motivation to be physically active may be related to perception or sensation of fatigue that arises from “central” signals in the brain. Not only does perceived fatigue affect physical behavior, but surveys conducted within the U.S. workforce [1] indicate a 38% prevalence of fatigue, with 66% of these workers reporting lost productivity time, resulting in an overhead cost to employers of > \$100 billion annually.

Central Fatigue Mechanisms

The mechanisms underlying fatigue can occur at multiple levels starting from the brain and spinal cord level in the central nervous system (CNS) to peripheral factors

originating from the working skeletal muscle and organs. Motor drive from the brain dictates voluntary physical activity and decreases in motor drive may be related to lower recruitment of motor units or decreased motor unit firing frequency [34]. *Central fatigue* is a general term that is often used to attempt to describe CNS-related impairments that reduce motor drive. However, some suggest central fatigue is not entirely a physical process; but, a “conscious perception of a sensation” that leads to decreased performance or discontinuation of activity [33, 35, 36], perhaps when perceived level of effort becomes greater than the perceived reward of the activity [2]. In this psychological sense, fatigue has been characterized as a subjective feeling of lack of energy or tiredness due to prolonged cognitive activity and/or depletion of energy [2]. But, in addition to fatigue being a byproduct or result of demanding work, it can also be thought of as an emotion that *influences* how individuals allocate mental resources or attend to a task or activity [2, 37, 38]. If an individual feels mentally fatigued, he or she may choose to disengage from a current task in favor of a distraction or other more “rewarding” behavior. Brain areas that are involved in effort versus reward analysis, decision making, motivation, and behavior monitoring include the orbitofrontal cortex, basolateral amygdala, insula, anterior cingulate cortex, nucleus accumbens, and prefrontal cortex, whether the task is physical and/or mental. Individuals are constantly presented with inputs and stimuli from the environment and/or other areas of the brain or periphery. During a prolonged physical or mental task that demands attention, individuals are constantly reevaluating motivation or dedication to the task and effort versus reward of continuing the task [2, 39].

Physical Fatigue

Motor drive from the brain dictates voluntary physical activity. During physical activity, onset of fatigue can occur without a decrement in task performance as additional motor units or muscles can be recruited to compensate for those fatiguing and this may be accompanied by an increased sense of effort to maintain the task. Individuals report an

increased rating of perceived exertion (RPE) [40] before fatigue is measurable by decreased force or power output (e.g. during a test of maximal voluntary contractile (MVC) force) [33, 41] or the inability to sustain a physical task. A higher RPE at a fixed submaximal workload may be an indicator of central fatigue, as is decreased time to voluntary fatigue [33] during an endurance task, particularly when metabolic fuel (i.e. glycogen status) is not limiting.

Motor drive and perceived effort are complex and influenced by multiple factors, including mental fatigue [3, 42], afferent feedback (metabolic, ionic, thermal, and mechanical) from the periphery [43], sensation of pain or discomfort [44], decreased motivation [33], hypoglycemia [45]; and, presence of adenosine [46, 47], an endogenous neuromodulator that inhibits dopamine transmission [48] and decreases the firing rate of central neurons (Figure 1). Supraspinal dopamine transmission is associated with increased arousal and motivation, spontaneous motor activity, and prolonged exercise time [41]; therefore, adenosine's inhibition of dopamine may directly impact central fatigue and related physical activity.

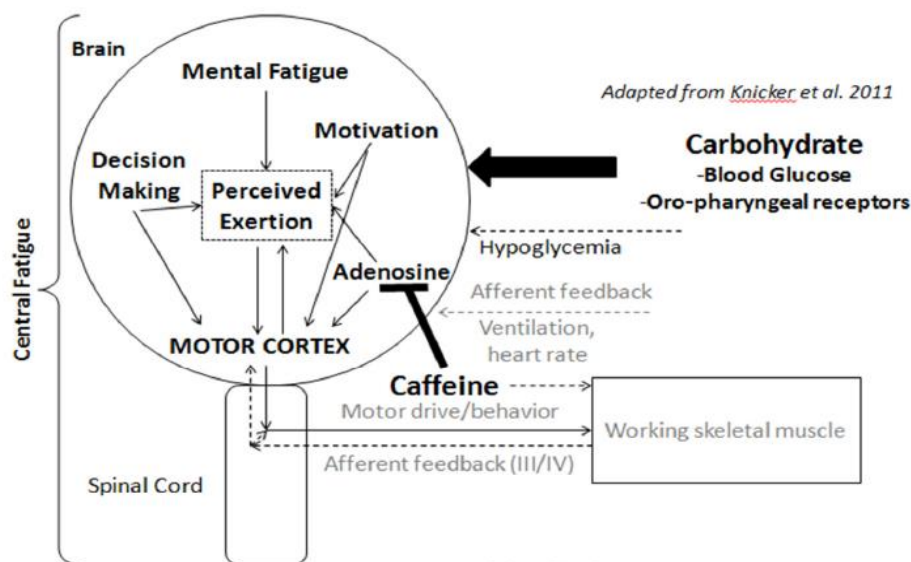


Figure 1. Mechanisms Underlying Central Fatigue

Mental Fatigue

Mental fatigue may impair exercise tolerance through neurocognitive mechanisms [3] involving the anterior cingulate cortex [49, 50] by influencing perception of effort and effort-related decision making [51, 52]. The anterior cingulate cortex is a primary component of the brain's attention network which also includes frontoparietal areas such as the prefrontal cortex, which are not only involved in decision-making and RPE, but also in maintaining attention relevant to behavioral goals and inhibition of irrelevant or distracting stimuli. When multiple sensory inputs are received (i.e. afferent feedback signals during exercise) or presence of carbohydrate in the mouth, the anterior cingulate cortex engages with the prefrontal cortex for goal-related conflict monitoring. The prefrontal cortex also has connectivity to emotional, perceptual, and motor areas of the brain. Mental fatigue can be experienced independent of exercise, but since the physiological and psychological mechanisms underlying mental fatigue also overlap with those involved in physical fatigue, mental fatigue is an important factor to consider when addressing the problem of physical fatigue.

The Relationship Between the Brain and Physical Activity

Just as the brain can influence physical activity and perception of fatigue, a single bout of exercise also has the acute effect to improve cognition and feelings of energy following exercise [53-56] by influencing neural activity in prefrontal areas [57], arousal, and allocation of mental resources [54, 58-61]. Sustained attention, or the ability to direct and focus cognitive ability over a period of time [2, 4, 5], is one aspect of cognition that is reflective of energy and fatigue processes [37]. Sustained attention and self-assessed perceived energy [56] are improved immediately following exercise [62]; however, the

optimal exercise intensity that elicits the greatest response is unclear. For example, high intensity activity may result in feelings of physical fatigue that may persist after exercise, masking potential reductions in mental fatigue or increases in positive affect that may otherwise be observed after low to moderate intensity exercise [56, 63, 64]. An individual's fitness status may also influence the emotional or psychological response to exercise of varying intensities [54]. The specific factors contributing to fatigue, perception of afferent signals, and the time course of onset of these factors may also be different based on fitness status or exercise experience and also influenced by nutrition intake.

Influence of Physical Fitness Level

Moderate-intensity exercise consistently improves both simple and complex cognitive tasks in trained individuals [59]; whereas, less physically fit individuals may experience greater anxiety during or after high intensity exercise [65, 66], contributing to lower task performance. An “inverted-U curve has been suggested to describe the relationship between exercise intensity and attentional processes; specifically, cognitive performance improves with moderate exercise intensities but deteriorates at high exercise intensity [67]. However, this type of relationship between exercise intensity and cognition is likely different based on an individual's level of fitness [67-69]. The basis to prescribe an exercise intensity that elicits similar efforts across groups with different fitness status is a design issue that has been primarily addressed by utilizing either relative oxygen uptake or maximum heart rate [67, 70]. However, this may not be the most appropriate design approach to examine the interaction between exercise intensity and fitness status on cognitive function. Sedentary participants typically accumulate more

lactic acid (i.e. reach lactate threshold) at a lower exercise intensity (based on HR or VO_{2max}) compared to higher fit participants [71]; thus, the use of lactate threshold to prescribe exercise intensities across groups is advantageous.

Ergogenic Aids and Fatigue Research Based on Fitness Level

Ergogenic aids such as caffeine (CAF) and carbohydrate (CHO) are commonly used by athletes and recreationally-active individuals to improve exercise performance and delay fatigue during prolonged exercise [72-77]. Studies investigating CAF and CHO effects on endurance capacity have been limited primarily to trained athletes and physically fit individuals [72, 73, 77-79] and the efficacy of some of these nutritional strategies for the general population of less fit individuals has been questioned [80, 81]. Few studies have investigated either CAF [82-85], CHO [14, 86-90], or the combination [91, 92] on endurance capacity in untrained but otherwise healthy adults. Moreover, the classification of “untrained” and “active” is not always clear within this small group of studies, with some defining untrained as $VO_{2max} < 50$ ml/kg-min [86-88, 91], others using $VO_{2max} < 45$ ml/kg-min or “performance level 1” based on established criteria [93], and yet others indicating untrained status but not reporting fitness level [82, 83, 85, 89, 92]. In the studies not reporting fitness level, the definition of physical activity level was also variable and ranged from participating in <60 min/wk [83] to 3 d/wk [92], to < 5 h/wk [84], or simply reporting “no regular PA” [82]. CAF and CHO and their combination have extensive research to support their use for athletic performance; and, both are frequently consumed by the general population. However, whether these nutrition aids can be effective for less active individuals, despite their known metabolic side effects under sedentary conditions [24], must be evaluated.

Caffeine

Caffeine is a well-documented ergogenic aid [72-74, 94-101] that reduces fatigue through a number of potential mechanisms: direct effect on muscle via increased mobilization of intracellular calcium and/or increased sensitivity of myofibrils to calcium [98, 101], increased motor unit recruitment in the CNS [100, 102], and/or increased lipolysis and fat oxidation with associated glycogen sparing [98, 103-105] although the latter has recently become less accepted [106]. CAF can also reduce symptoms of fatigue and increase perceived energy, [107-110] through psychological effects serving to enhance task persistence and determination [94, 111]. CAF acts as a stimulant potentiating sympathetic activity; but, also has a neuromodulatory function as a nonspecific adenosine receptor antagonist. In the brain, CAF increases transmission of dopamine in frontal and prefrontal areas [112, 113], contributing to increased arousal [113, 114]. In trained athletes, CAF intake between 3-6 mg/kg [74, 97] can improve exercise capacity and influence RPE and motor drive, perhaps allowing individuals to perform more physical work at the same RPE or perceive exercise as more tolerable, less painful, or fatiguing with CAF [72, 73, 83, 95, 109, 110, 115-118]. Based on exercise studies that have investigated CAF in healthy but untrained individuals, 3-6 mg/kg CAF ingested prior to exercise also appears to improve exercise capacity [82-84] by reducing perceived effort or allowing more work to be completed at a same relative effort.

Studies have also examined the effect of a moderate dose of CAF (e.g. ~1-2 cups of regular coffee) on mental fatigue [119, 120]. Caffeine improves higher order cognitive measures such as sustained attention [109, 110, 113, 121-124] with a concomitant increase in mental energy and alterations in neural activity in the anterior cingulate cortex

[112, 123] and additional brain areas involved in maintaining attention [125]. It has been suggested [113] that CAF may be more effective when arousal and attentional control of perceptual functions is low (i.e. fatigued state [126] or when CNS resources are compromised). Although CAF has clear benefits for exercise capacity and cognition, it may also have detrimental effects to insulin action and glycemic regulation when 3-5 mg/kg is ingested in the sedentary state [24], even though negative side effects (i.e. mental confusion or “intoxication”) are more often observed at higher doses ~9 mg/kg [99]. However, even a single dose of 3 mg/kg CAF is evidenced to increase feelings of tremor, compared to placebo [127]. Therefore, it is unclear whether the potential benefits of CAF outweigh metabolic disadvantages for a sedentary population who may be predisposed to diabetes or insulin resistance. Likewise, CAF in commonly ingested doses [24, 128] seems to be a worthwhile countermeasure to fatigue that should be further explored; but, possible metabolic consequences should be considered. Because there is large inter-individual variability in response to CAF and some individuals can subjectively perceive mood effects of as little as 10 mg [129], we elected to select volunteers that were regular consumers of low to moderate amounts of caffeine (~40 to 500 mg/d) [130, 131] but not caffeine naïve.

Carbohydrate

Carbohydrate enhances exercise capacity [77, 132] when 30-60 g/h CHO is ingested during prolonged exercise (> 1 h) [75, 76]. CHO delays fatigue by maintaining blood glucose and enhancing CHO oxidation, and may also improve exercise capacity at lower doses of <15 g/h [22, 133] in conditions when glycogen status is not limiting and blood glucose is well maintained (i.e. durations < 60 min) [76]. However, blood glucose

levels do not always predict exercise capacity in untrained individuals [86]. More recent evidence suggests CHO can also be ergogenic through CNS-mechanisms by acting as a signal or sensory input that can influence physical and mental task performance independent of glycemic fluctuation and insulin response [11, 134]. To test this hypothesis, studies have administered a bolus of CHO to be swirled in the mouth for a short duration (5-10 s), then expectorated rather than ingested immediately prior to a task. The benefit of a CHO mouth rinse was originally observed in exercise studies that also indicated infusion of glucose and bypassing oral ingestion did not improve performance [10], indicating the importance of sensory input. Since that original study, several other exercise studies and reviews have provided support that CHO can be ergogenic even when it is not ingested [11, 12, 14, 75, 76, 132, 135-140].

Non-exercise studies have also demonstrated a benefit derived from the simple presence of CHO (25 ml of 6.4 to 18% CHO) versus artificially-sweetened placebo in the mouth on mental task performance, independent of change in blood glucose [15, 17, 18, 141] when the protocol was performed after >3 h of fasting. It has been suggested [15] that while participants are able to maintain better self-control (including attention, impulsivity, cognitive processing) after a CHO mouth rinse, it cannot be ruled out that glucose metabolism may also influence task performance through central *and* peripheral mechanisms. Therefore, studies should consider including rinse and ingest conditions in parallel to test whether both methods result in similar effects on cognitive behavior [15]. CHO mouth rinse studies seem to agree that perception of CHO in the mouth may activate the brain's reward systems [10-14] and influence an individual's persistence during a task requiring goal-directed attention. During such a task, an individual must

continuously evaluate cost versus benefit of continuing to allocate effort and attention to a fatiguing task [142]. Aside from its ability to provide energy for metabolism, CHO appears to function a signal or input that influences decision making systems that ultimately determine allocation of neural resources and maintenance of effort during a task, whether the task is mental or physical [142] and whether the CHO is ingested or not [15, 16].

Although high rates of CHO ingestion during exercise are beneficial for athletic performance, others may find that ingesting typical CHO sports drinks and supplements counterproductive if they are exercising for weight loss. Sugarless caffeinated energy drinks have also been efficacious for submaximal exercise in non-athletes by improving “psychophysiological” responses [143, 144]. These energy drinks and sugar-free and low-CHO sports drinks are also heavily marketed to the general population; but, there is a relative lack of research on low-calorie drinks and their ergogenic and metabolic effects. It is possible that a low-CHO drink with CAF could mutually benefit sedentary as well as trained individuals, perhaps through CNS mechanisms similar to those triggered by CAF and CHO mouth rinse.

Caffeine with Carbohydrate

As evidenced by the similar brain networks that are affected by CAF and CHO, it is tempting to speculate that the two nutrition aids may have some additive or interactive effects when combined. In athletes, higher rates of CHO ingestion with CAF delays exercise-induced fatigue [78], and evidence suggests a benefit of CHO+CAF [145-147] on exercise performance, muscular fatigue and perceived exertion. Additionally, when used as an oral rinse without ingestion, CHO+CAF appears to improve sprint power

output to a greater degree than CHO alone [148] whereas rinse with CAF alone did not improve exercise performance or perceived exertion in a separate study [149]. When CAF (6 mg/kg) and a small amount of CHO (<1 g) were ingested together, untrained (<3 d physical activity per week) women were able to complete an “all out” cycling time trial faster with higher work output under CAF+CHO compared to CHO alone [92]. Using a similar treatment (5 mg/kg CAF with <1 g CHO), a treatment effect of CAF was observed in an endurance-trained group on endurance performance (by 1.6-2%) whereas sedentary experienced a non-significant 0.3-1% improvement, compared to CHO [91]. However, relative intensity between groups was not fixed, making it difficult to compare the treatment and exercise effect among groups of different fitness level, further suggesting the need for additional well-controlled studies comparing CAF and CAF+CHO in trained and sedentary populations.

Cognitive research also supports a benefit of co-ingestion of CHO with low to moderate doses of CAF (<50 to 200 mg). The combination of CAF+CHO reduces deficits in vigilance and attention; and, reduces subjective fatigue during high cognitive demand [109, 110, 150-156]. Therefore, beverages containing both CHO and CAF may have interactive effects on allocation of attentional resources that may influence cognitive behavior such as sustained attention [123, 152, 155], as well as exercise capacity.

Post-exercise Effects based on Fitness Level

The efficacy of exercise interventions for sedentary adults has been questioned [157] due to potential post-exercise compensatory behaviors that decrease spontaneous non-exercise physical activity [158-161]; and, although less likely, may increase energy intake [162, 163]. However, several factors can contribute to post-exercise compensation

such as lasting feelings of fatigue from the exercise itself [161], the exercise intensity [164, 165], and affective response to exercise [166]. While evidence suggests that spontaneous physical activity level is not different between trained and untrained [167] in a controlled environment, less is known about the difference in post-exercise compensation between trained versus untrained in free-living conditions. More recent findings [168] suggest that active and inactive individuals differ in free-living energy expenditure and compensate differently after a low-intensity bout of exercise. Limited available research [169] suggests that self-reported feelings of physical and mental energy are significant influencers of moderate and vigorous exercise behavior in a healthy young-adult sample of participants.

Because of the extended half-life of CAF (3-6 h), it is tempting to speculate that CAF, when ingested during exercise, may maintain perceived energy and result in a larger negative energy balance later in the day following exercise [159-161, 170]. Post-exercise sedentary time may not be affected by moderate CAF intake [171], but the assessment of long term physical activity is variable within the literature. Using indirect calorimetry to assess resting energy expenditure, Fernandez-Elias et al. [170] observed trained athletes ingesting 4.5 mg/kg CAF during 60 min of moderate intensity cycling had 15% higher post-exercise energy expenditure above placebo condition during the 3 hours following exercise. In addition, Schubert et al. [163] demonstrated higher energy expenditure as measured with indirect calorimetry during and following exercise (resting energy expenditure) and higher fat oxidation with CAF (taken as two doses of 3 mg/kg) compared to with placebo but no difference in post-exercise energy intake between CAF and placebo conditions. This dose of CAF resulted in the participants perceiving the bout

of exercise as less effortful and more enjoyable and resulted in a greater post-exercise energy deficit compared to when placebo was ingested. Another study [172] determined 3 mg/kg CAF in a low carbohydrate (2 g CHO) energy drink decreased perceived fatigue the following morning after an exercise bout compared to when exercise was performed with a placebo, providing further rationale for caffeine as a useful nutrition aid for developing liking and adherence to a regular physical activity program aimed at weight loss. The present research study is not only important for improving exercise capacity in trained and sedentary individuals; but also for improving mood and perceived energy that can subsequently improve work performance and energy expenditure during the rest of the day following exercise.

Assessment of Daily Energy Expenditure and Physical Activity

Rapid advancement in technology have resulted in several techniques to assess daily energy expenditure, physical activity, and sedentary time; and can be useful to answer the fundamental question of how a controlled bout of exercise in combination with a nutrition intervention affects post-exercise compensatory behavior (e.g. altered non-exercise physical activity or sedentary time) under free-living conditions [173]. Wearable accelerometers or activity monitors are able to track and capture movement and provide data regarding time spent in physical activity versus sedentary time, the intensity of activity, calculate number of steps taken, and estimate energy expenditure. There is considerable variability among activity monitors and their usefulness should be validated under controlled laboratory conditions against gold standard methods (e.g. indirect calorimetry or doubly labeled water) [173, 174] and detailed written daily activity logs. It is also recommended that, since activity patterns are highly variable in young, healthy

populations; that one week of activity recording is recommended to obtain an accurate measure of physical activity and sedentary time in free-living conditions [173].

Research Aims

Fatigue, both mental and physical, is a prevalent issue affecting not only productivity, but also the health of the US population. Similar areas of the brain are involved in attending to mental and physical tasks, decision making, evaluation of effort and reward, and task persistence whether the task is mental, physical, or involves elements of both. Substantial research has been done on methods to counteract fatigue for athletic performance but the physiological demands and make-up of athletes differ from non-athletes; as does the psychological and emotional response to exercise. The overall goal of this research was to identify potential strategies that may improve quality of life including perceived energy, cognition, mental fatigue, and physiological health for the majority of the population who does not typically engage in regular physical activity. By testing the following specific aims, we evaluated how nutrition aids known to influence the CNS, perception, and behavior could be used to improve an exercise bout and/or the response to an exercise bout; both for individuals who are trained athletes and those who are healthy but habitually inactive.

In Chapter 2 we address research **Aim 1**: Determining potential “central” effects of two methods of CHO administration (ingest and rinse) on mental performance during a sedentary cognitive task requiring sustained attention. Based on evidence that “low calorie beverages” do not appear to alter blood glucose [25], the first part of a two-part study evaluated effects five drinks (0-6% CHO) on glycemic response. In the second part of the study, a CHO rinse of 25 ml 6% CHO solution was chosen as a positive control to

compare with low-CHO ingestion. This dose (1.5 g CHO) exposed to the oral cavity for 5 s is evidenced to improve physical capacity [14, 76, 135] and mental activity [17, 18], thus we expected it to improve sustained attention compared to a 0 g CHO control rinse. To compare an equivalent “dose” of low-CHO ingestion we administered 400 ml of 0.4% CHO to also provide 1.5 g CHO. While the oral receptors were not stimulated by the same concentration of CHO (6% versus 0.4%), the grams of CHO was standardized. Evidence suggests [16, 19-21, 23] that CHO receptors exist not only in the mouth but also in the pharynx, digestive tract, and liver. Thus, ingestion may also stimulate a CNS response through receptors beyond the oral cavity similar to that elicited by mouth rinsing a 6% CHO solution without ingestion. In the present study, we examined sustained attention after an overnight fast to determine if a low-CHO drink would attenuate mental fatigue compared to an artificially-sweetened placebo. We hypothesized:

1) Ingesting a low-CHO (<1%) drink would not elicit a glycemc response different from an artificially-sweetened placebo (Study A)

2) Either ingesting low-CHO or rinsing an equivalent amount (grams) of CHO without ingestion would provide greater benefit to attenuate mental fatigue compared to rinsing with a CHO-free solution (Study B).

In Chapter 3 we address research **Aim 2:** Examining the metabolic and endurance capacity effects of low-calorie (<1% CHO solution) CHO, CAF (3 mg/kg), and their combination (CAF+CHO) in endurance-trained athletes compared to healthy sedentary adults. If a low-CHO drink provides benefit compared to placebo, it is likely due to carbohydrate acting via a central mechanism [22] since the amount of CHO in the treatment is insufficient to contribute to blood glucose or exogenous CHO oxidation.

Based on evidence that CAF and CAF+CHO benefits endurance in trained [72-74, 78, 98, 146] and lower fit individuals [91, 92], we hypothesized:

- 1) *Ingesting CAF and CAF+CHO would improve exercise capacity by reducing factors related to fatigue independent of fitness level*
- 2) *Each nutritional component alone would improve measures of fatigue compared to a placebo.*

In Chapter 4 we address research **Aim 3**: Determining: (1) the effect of moderate intensity exercise on sustained attention compared to a similar duration of seated rest in endurance-trained versus sedentary adults; and 2) whether caffeine ingestion provides additional benefit to sustained attention, perceived mental energy and mental fatigue when combined with exercise. Since acute exercise and caffeine ingestion may each influence the CNS resulting in attenuated mental fatigue and improved attention; and, fitness status may influence the response to exercise of varying intensities, [54] we hypothesized:

- 1) *Moderate intensity exercise would benefit sustained attention independent of fitness status; however, unlike trained individuals, sedentary subjects would experience impaired attention following exercise performed to fatigue.*
- 2) *Compared to non-caffeinated placebo, caffeine would maintain sustained attention and, when combined with exercise, would maintain sustained attention and associated perceptual measures (mental energy and mental fatigue) independent of fitness level.*

CHAPTER 2

CARBOHYDRATE INGESTION BUT NOT MOUTH RINSE MAINTAINS SUSTAINED ATTENTION WHEN FASTED

Abstract

BACKGROUND: Carbohydrate (CHO) receptors in the mouth signal brain areas involved in cognitive tasks relying upon motivation and task persistence. However, the minimal CHO dose that improves mental activity is unclear. **PURPOSE:** To identify a CHO dose (via ingestion or oral rinse) that influences sustained attention without eliciting glycemic responses in a fasted state. **METHODS:** Five solutions (0-6% CHO) were ingested to evaluate glycemic response in six adults. Peak blood glucose for 6% and 1.5% CHO was greater ($p < 0.05$) than 0%, 0.4% CHO. Thus, 0.4% CHO was evaluated further. Following an overnight fast, ten healthy adults completed three trials in a crossover design: 1) 400 ml 0.4% CHO ingested (CHO-I/I), 2) 375 ml control (CON) 0% CHO ingested followed by 25 ml of 6% CHO rinse without ingestion (CON-I/CHO-R), and 3) CON ingest followed by CON rinse (CON-I/R). CHO-I/I and CON-I/CHO-R trials exposed 1.5 g CHO to the mouth for 5 s. Following the last 25 ml bolus, a 20 min Continuous Performance Task (CPT) was performed to assess accuracy (ACC) and precision (PREC). **RESULTS:** Ingestion of 1.5 g CHO (CHO-I/I) maintained ACC over 20 min CPT compared to significant decreases ($p < 0.05$) with CHO and CON rinses. CHO-I/I elicited a lower ($p < 0.05$) relative decrease in ACC ($-0.5 \pm 3.7\%$) and PREC ($-0.4 \pm 11.2\%$) compared to CON-I/R ($-7.5 \pm 7.3\%$, $-15.5 \pm 16.6\%$) and tended to be less ($p = 0.06$, $p = 0.08$) than CON-I/CHO-R ($-6.9 \pm 8.2\%$, $-16.1 \pm 22.9\%$). No differences in glycemic responses were observed. CHO mouth rinse did not differ from CON rinse. **CONCLUSIONS:** Compared to mouth rinsing CHO (1.5 g in 6% CHO), an isoenergetic low-CHO drink maintained sustained attention over a 20 min mentally fatiguing task and appears to be effective without eliciting a glycemic response after an overnight fast.

Introduction

Commonly ingested carbohydrate (CHO) drinks (i.e. sports drinks, juices, and energy drinks) contain sugar and calories (i.e. 6-12 g CHO or 24-48 kcal in 100 ml) and may have health-related consequences (i.e. dental caries, obesity, insulin resistance, diabetes) [6-8]. Thus, recommendations are to limit daily sugar intake to 30-45 g/d [9]. Given these concerns, low-CHO drinks have become increasingly popular particularly under sedentary conditions; however, research to support them is lacking. Aside from being an effective aid for physical performance, CHO may also benefit cognition, particularly during mentally fatiguing tasks [26, 37-39].

Carbohydrate is essential for brain function although performance of mental tasks may not be dependent on CHO metabolism or related to blood glucose levels [38, 175]. Moreover, prolonged mental work requiring sustaining attention over time does not consistently elicit glycemic changes [3]; and, systemic blood glucose levels typically measured are not reflective of cerebral blood glucose during mental tasks [16, 142, 176]. Yet, provision of glucose may improve mental performance [16] by eliciting a signal to the central nervous system (CNS). This input may influence decision making systems, allocation of neural resources, and greater motivation to sustain effort during a mental or physical task [16, 142, 177].

Evidence that CHO can act as an acute signal to influence task performance independent of glycemic fluctuation and insulin response [11, 134] is provided in studies by either ingestion [16] or administering a CHO bolus swirled in the mouth for a short duration (5-10 s), then expectorated rather than ingested immediately prior to a task. The simple presence of as little as 1.5 g CHO (25 ml of solution of 6% CHO) in the mouth

versus artificially-sweetened placebo benefits mental task performance [15, 17, 18, 141] when performed after >3 h of fasting [15]. Even though taste and ingestion of artificially-sweetened beverages may be pleasurable and stimulate brain reward centers, the same cognitive improvements are not observed as with CHO energy-containing drinks [16, 178]. Participants maintain better self-control (e.g. attention, impulsivity, cognitive processing) through a proposed mechanism triggered by stimulation of oral receptors by CHO [10, 11]. Brain imaging studies using fMRI [11, 179] suggest [38, 175, 180] sensory input from CHO in the mouth activates the brain's reward systems and influence persistence during performance tasks which require maintaining attention to a goal. Individuals must continuously evaluate cost versus benefit of continuing to allocate effort and attention to a fatiguing task [142].

While CHO mouth rinse triggers a CNS-response, CHO ingestion may also influence task performance through central *and* peripheral mechanisms. Therefore, studies should compare rinse and ingest conditions in parallel to partition the effects on cognitive behavior [15]. The dose and type of CHO ingested that may enhance cognition is unclear [16]. In animals, doses as low as 10 mg/kg glucose can improve memory; [181] whereas, in humans, the dose appears to be ~ 300 mg/kg (~20 g), and with greater effect during more difficult tasks or when attention is divided between multiple tasks [16, 182]. Further research on the dose-response relationship of glucose to behavior is warranted in humans with additional consideration to the method of administration (i.e. ingest versus rinse) [15, 16, 182]. The minimum amount of CHO ingested that elicits a cognitive benefit without a glycemic effect is unknown. The aim of the present study was to determine the “central” effects of two methods of CHO administration (ingest and mouth

rinse) on performance during a cognitive task requiring sustained attention. Based on evidence that “low calorie beverages” do not alter blood glucose [25], we hypothesized that: 1) ingesting a low-CHO (<1%) drink would not elicit a glycemic response different from artificially-sweetened placebo (Study A) but lower than a 6% CHO sports drink; and, 2) either ingesting low-CHO or rinsing an equivalent amount (grams) of CHO without ingestion would attenuate mental fatigue compared to rinsing with a CHO-free solution (Study B).

Methods

Participants

Six healthy males (n=3) and females (n=3) with mean (\pm SD) age of 25.2 ± 5.7 y and BMI 26.6 ± 3.7 kg/m² volunteered to participate in Study A. All participants completed a health-history screening questionnaire to ensure they met all inclusion criteria. Participants were excluded if: diabetic, pregnant, on a special diet or weight loss diet, lost or gained significant body weight in the last 3 months, sensitive to artificial sweeteners such as aspartame, saccharin, or sucralose; or, used prescription medications that affect appetite, mood, energy level or blood sugar. Participants provided written informed consent as approved by the University Institutional Review Board.

Study A

Research Design and Experimental Protocol

Each participant served as his or her own control performing all five tests. Treatment order began with an equal number of subjects completing the 0% and 6% CHO treatment in counterbalanced order. The next three trials were completed in order of

increasing CHO concentration for all subjects. Each visit was separated by a minimum of 3 d and scheduled at the same time of morning following an overnight fast. Before each visit, participants refrained from exercise for 24 h and caffeine for 12 h. Upon arrival to the lab, 24 h diet recall and history questionnaire were completed. After the first visit, the 24 h diet recall was copied and returned to the participant to use as a guide for replicating 24 h dietary intake before the subsequent visits.

Treatments

All treatments were mixed using varying proportions of fruit punch flavored Powerade ® (The Coca Cola Company, Atlanta, GA) and Powerade Zero ® (The Coca Cola Company, Atlanta, GA) to provide 400 ml of taste matched CHO drinks of the following concentrations: 0.4% CHO, 0.75% CHO, 1.5% CHO along with the 6% CHO drink (Powerade ®) and 0% CHO (Powerade Zero ®).

Blood Glucose

Participants remained seated for 10 min prior to obtaining a resting blood sample to confirm fasting blood glucose (<100 mg/dl). The sample was drawn by lancing a warmed fingertip into a heparinized capillary tube (Microvette® CB300, Sarstedt AG&Co., Numbrecht, Germany) for whole blood analysis of blood glucose (YSI Life Sciences, Inc. Yellow Springs, OH). Each participant then consumed 400 ml of the treatment beverage. Participants were asked to consume the entire beverage within a 5 min period. Blood samples were obtained at 7.5, 15, 30, 45, and 60 min after beverage ingestion was completed. Glucose area under the curve (AUC) was calculated using the linear trapezoidal rule between 0 and 60 min. Peak blood glucose was determined by the

highest glucose reading obtained after ingestion of each treatment, independent of time point.

Statistical Analyses

Data were reported as mean \pm standard deviation (SD) and analyzed using SPSS 17.0 (Chicago, IL). Two-way (treatment x time) repeated measures ANOVA (treatment as within-subjects factor with repeated measures over time) was used to examine differences in blood glucose between treatments. The Greenhouse-Geisser correction was used if the sphericity assumption of equal variances across groups was violated. If a significant F ratio was obtained, the Bonferroni post hoc test was used to detect significant differences in pairwise comparisons between time points or treatments. Within-factor (treatment) repeated measures ANOVA with Bonferroni post hoc was used to detect significant differences between treatments for peak blood glucose and glucose AUC.

Study B

Ten healthy males (n=4) and females (n=6) age 28.3 ± 6.7 y and BMI 24.5 ± 2.6 kg/m² volunteered to participate in Study B with three of the subjects also participating in Study A.

Research Design and Experimental Protocol

Three visits were each separated by a minimum of 3 d and scheduled at the same time of morning following an overnight fast. Pre-trial conditions and baseline measures were administered using methods from Study A (above). The schematic of the test protocol is illustrated in Figure 2 to examine CHO drink ingestion or mouth rinse on

glycemic response and sustained attention after an overnight fast. Following baseline measures of fasting blood glucose, each subject consumed an initial bolus (375 ml) of the treatment beverage administered as 1 x 25 ml sip every min over a 15 min period. Five min later, immediately prior to administering a 20 min cognitive task, each participant was given another 25 ml bolus of the treatment to swish around the mouth for 5 s before either swallowing or expectorating the entire contents back into the cup. After the 20 min cognitive task, participants had 30 min recovery before repeating the cognitive task for 5 min. Immediately prior to the final 5 min cognitive task, another 25 ml bolus was given for ingestion or rinse without ingestion. The rinse protocol was based on that previously described [17].

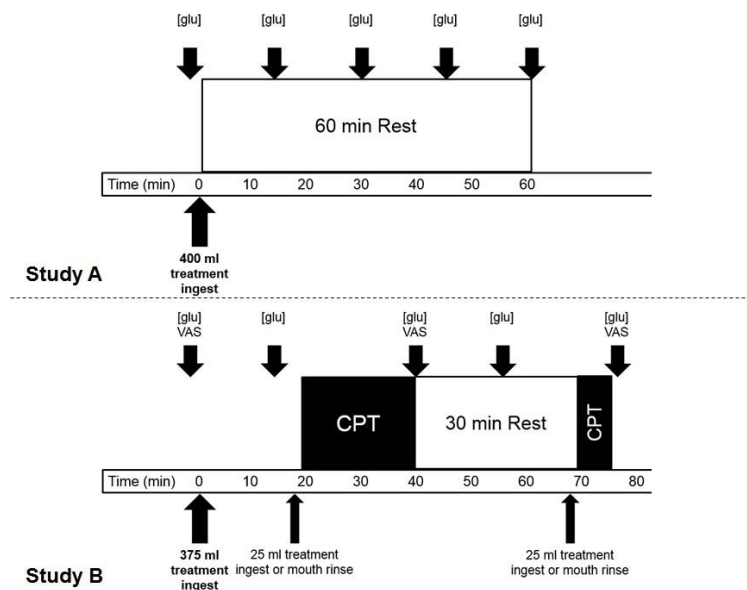


Figure 2. Top panel: Schematic of the test protocol for Study A: Glycemic response measured over 60 min following ingestion of 400 ml treatment (0 to 6% CHO). [glu] indicates timing of blood glucose assessment. Bottom panel: Test protocol for Study B: A Continuous Performance Test (CPT) assessed sustained attention and induced mental fatigue over 20 min. Timing of mental energy and fatigue visual analog scales (VAS) , blood glucose [glu] and treatment ingest or mouth rinse are noted.

Treatments

Details of the three treatments are given in Table 1. In the CHO ingestion condition (CHO-I/I), the initial bolus was 400 ml (15 x 25 ml boluses over 15 min plus 1 x 25 ml bolus ingested immediately prior to 20 min cognitive task) of low-CHO (0.4% CHO) beverage containing 1.5 g CHO manufactured and provided by Glaceau Vitaminwater® (Whitestone, NY). In the two rinse conditions, CHO and control (CON), 375 ml of artificially-sweetened (0 g CHO) CON prepared using water, lemon juice, and stevia was ingested (15 x 25 ml boluses over 15 min). Participants were not aware that only one of the treatments ingested was a placebo. We simply told participants that the two drinks (low-CHO and CON) had different amounts of CHO. In the CHO rinse condition (CON-I/CHO-R), 25 ml of 6% CHO (Powerade® providing 1.5 g CHO) was rinsed and expectorated immediately prior to the 20 min cognitive task. In the CON rinse condition (CON-I/R), 25 ml of CON (0 g CHO) was rinsed and expectorated.

Following 30 min recovery, another 25 ml treatment bolus was given. In CHO-I/I, 25 ml of 6% CHO (Powerade®) providing 1.5 g CHO was ingested. In CON-I/CHO-R, 25 ml of 6% CHO (Powerade®) providing 1.5 g CHO was rinsed. And, in CON-I/R, 25 ml of CON was rinsed.

Table 1 Treatment Administration in Research Aim 1, Study B (n=10)

Treatment	15 x 25 ml (from 0-15 min)	1 x 25 ml (at 20 min)	1 x 25 ml (at 70 min)
CHO-I/I	0.4% CHO ingest	0.4% CHO rinse+ingest	6% CHO rinse+ingest
CON-I/CHO-R	CON (0% CHO) ingest	6% CHO rinse only	6% CHO rinse only
CON-I/R	CON (0% CHO) ingest	CON rinse only	CON rinse only

Blood Glucose

Whole blood samples were analyzed in duplicate following the same procedures as Study A. In addition to the pre-treatment baseline sample, blood glucose was measured at 15 min (after 375 ml treatment), 40 min (after 20 min cognitive task), 55 min (during 30 min recovery), and 75 min (after 5 min cognitive task) of the protocol.

Continuous Performance Task (CPT)

We administered the Continuous Performance Task (CPT) [183, 184], a test that requires sustained attention, working memory, response inhibition, and error monitoring [185], and has been used in other studies to induce mental fatigue [3, 186]. The CPT is associated with significant activation in the anterior cingulate cortex [185], an area of the brain that is affected by mental fatigue [5] and responsive to CHO in the mouth [11]. Dependent variables obtained from the CPT were true positives (TP) or correct hits, true negatives (TN) or correct misses, false positives (FP) or hits on non-target letters, and false negatives (FN) or missed targets. Accuracy (ACC) was calculated as $=(TP+TN)/(TP+TN+FP+FN)$ and precision (PREC) = $TP/(TP+FP)$. Together, ACC and PREC were used as measures of sustained attention and decreases in ACC and PREC were considered indices of mental fatigue.

ACC and PREC were averaged over four 5 min time blocks starting at the beginning of the 20 min CPT (*0-5 min CPT*) through the last 5 min of 20 min CPT (*15-20 min CPT*). Results are presented as ACC and PREC scores over time, as well as the *relative change* in ACC and PREC compared to the starting *0-5 min* time block using the equation: $((\text{Post} - \text{Pre})/\text{Pre}) \times 100$ where “Pre” is the *0-5 min* time block and, “Post” is the later time point. ACC and PREC for the 5 min CPT following 30 min rest was

compared to the last 5 min of the 20 min CPT (*15-20 min CPT*) in order to determine recovery from mental fatigue.

Visual Analog Scales (VAS)

The State-Trait Energy and Fatigue (STEF) subjective survey instrument, a 10 cm VAS (Appendix C), was used to assess the intensity of “state of mental energy and fatigue” [110] at baseline when participants arrived to the lab and immediately after each CPT. Mental energy subscales included “energetic, vigorous, and full of pep” items and mental fatigue subscales included “fatigued, exhausted, and worn-out”. Low and high VAS anchors referred to the absence of a feeling or to the strongest feeling ever felt, respectively. Participants were asked to focus on feelings of “mental” energy and fatigue and to place a mark on each 10 cm line. Subscale items were summed and averaged to generate a numerical score for subjective mental energy and mental fatigue.

Statistical Analyses

Data are reported as mean \pm standard deviation (SD) and were analyzed using SPSS 17.0 (Chicago, IL). Two-way (treatment x time) repeated measures ANOVA (treatment as a within-subjects factor with repeated measures over time) was used to examine differences in blood glucose, ACC and PREC scores, relative change in ACC and PREC, mental energy, and mental fatigue between treatments. The Greenhouse-Geisser correction was used if the sphericity assumption of equal variances across groups was violated. If a significant F ratio was obtained, the Bonferroni post hoc test was used to detect significant differences in pairwise comparisons between time points or treatments. Pearson product moment correlation was used to detect significant relationships between changes in ACC and PREC with rating of mental energy and

fatigue; and, between blood glucose and ACC, PREC, mental energy, and mental fatigue. Statistical significance was set at an alpha level of $p < 0.05$.

Results

Study A

Blood Glucose

Post-hoc power analysis was calculated using SPSS based on treatment effect on peak blood glucose of $F(1,6) = 26.07$, $p = 0.002$, $\eta^2 = 0.84$, experiment-wise α level of 0.05. Observed power with sample size of $n=6$ was 0.99. There was no effect of treatment ($p = 0.07$) or treatment x time interaction ($p = 0.11$) for the five drinks. Mean blood glucose remained between 2.5 and 5.2 mmol/L (normal physiological resting range) [24] for all drinks except 6% CHO (Figure 3, top panel). There was no difference among drinks ($p = 0.06$) for glucose AUC, although 6% CHO tended ($p = 0.08$) to be higher compared to 0% CHO. Peak glucose with 6% CHO (5.8 ± 0.6 mmol/L or 37% increase) and 1.5% CHO (5.1 ± 0.3 mmol/L or 20% increase) were significantly higher ($p < 0.05$) than 0% CHO (4.5 ± 0.2 mmol/L, or 10% increase) and 0.4% CHO (4.6 ± 0.3 mmol/L, or 7% increase) (Figure 4). Peak glucose for 0.75% CHO (4.7 ± 0.3 mmol/L, or 9.7% increase) was not different from any of the other treatments. Based on no treatment difference in glycemic response compared to 0% CHO, the 0.4% CHO solution was selected to evaluate further in Study B.

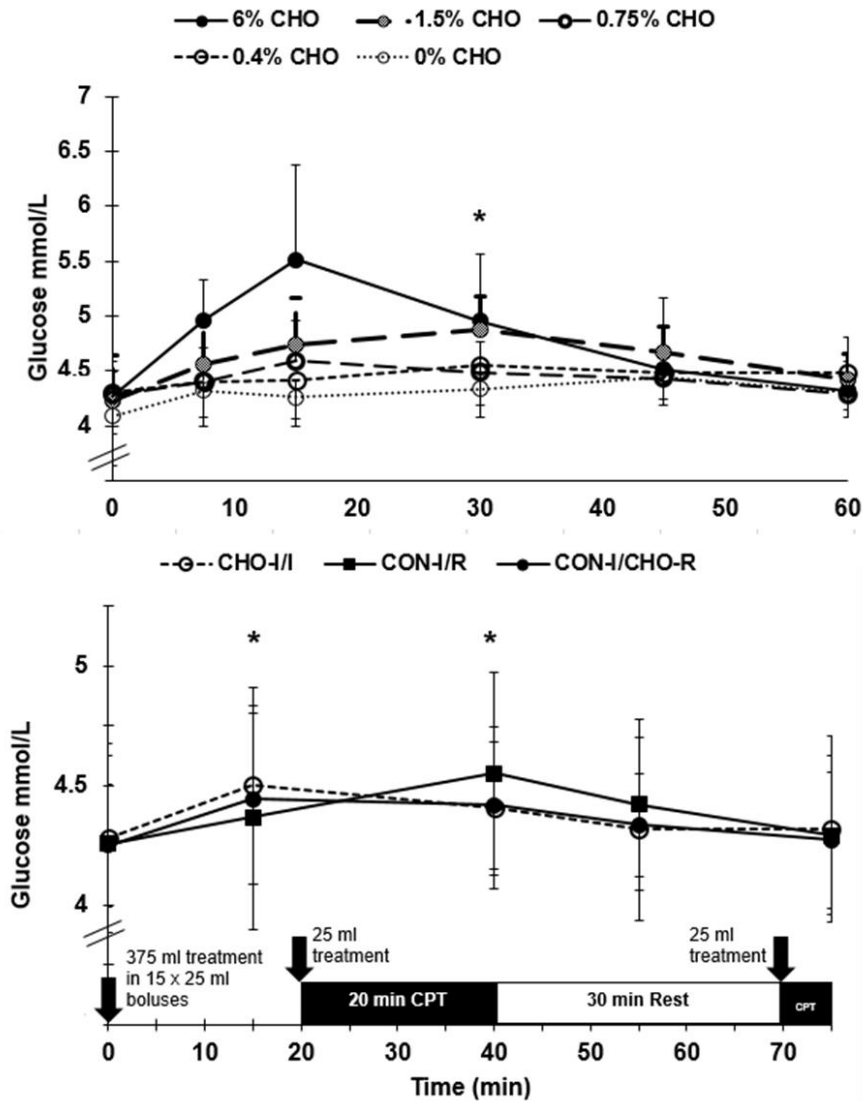


Figure 3: Top panel: Blood glucose after 400 ml ingestion of varying carbohydrate (CHO) solutions over 60 min (Study A). Low-CHO (0.4%) used in Study B indicated with same symbol --o-- * Higher ($p = 0.04$) at 30 min compared to pre-treatment baseline (0 min) across all treatments. Bottom panel: Glycemic response to 400 ml low-CHO (0.4%) ingestion (CHO-I/I), 375 ml control (CON) ingestion + 25 ml 6% CHO rinse (CON-I/CHO-R), and 375 ml CON ingestion + 25 ml CON rinse (CON-R/R) in Study B. * Higher ($p < 0.01$) glucose at 15 and 40 min compared to pre-treatment baseline for all treatments.

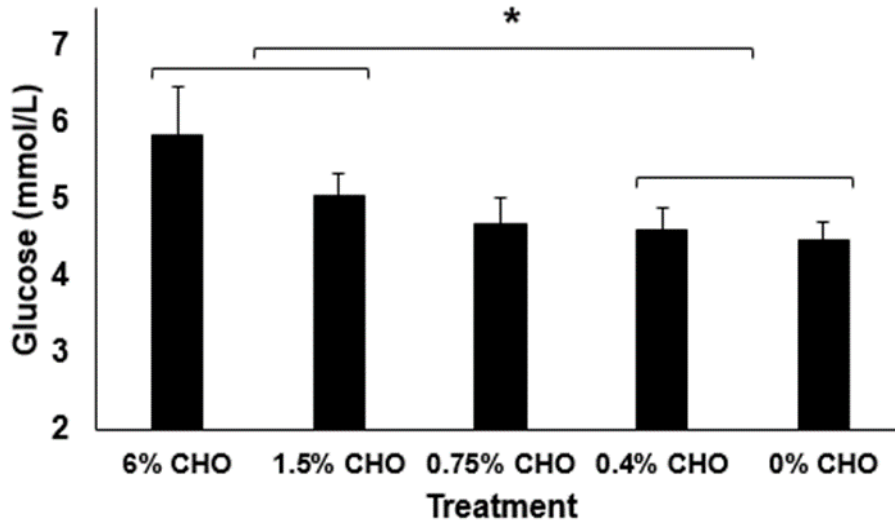


Figure 4: * Peak glucose concentration for 6% carbohydrate (CHO) and 1.5% CHO was higher ($p < 0.05$) compared to both placebo and 0.4% CHO. Peak glucose for 0.75% CHO was not different from any of the other treatments.

Study B

Carbohydrate Ingestion versus Rinse on Sustained Attention

Post-hoc power analysis was calculated using SPSS based on treatment x time effect on ACC of $F(4,36) = 3.14$, $p = 0.03$, $\eta^2 = 0.26$, experiment-wise α level of 0.05. The achieved power with our sample size of $n=10$ was 0.76.

As presented in Figure 5, there was no treatment effect on ACC or PREC scores over the 20 min CPT, but there was a significant treatment x time interaction for ACC ($p = 0.03$) and PREC ($p = 0.02$). ACC and PREC were not different between the ingestion or rinse treatments at the beginning of the 20 min CPT (0-5 min CPT). However, ACC scores significantly declined over 20 min with CON-I/R ($p = 0.03$) and CON-I/CHO-R ($p = 0.046$), but were maintained ($p = 1.0$) with CHO-I/I. PREC scores tended to decline

with CON-I/R ($p = 0.058$) and CON-I/CHO-R ($p = 0.05$) and were maintained with CHO-I/I ($p = 1.0$). There were no differences in ACC or PREC between the two rinse treatments over time. After 30 min recovery, ACC was significantly higher ($p < 0.05$) for all treatments and PREC was higher ($p < 0.05$) for the two rinse treatments compared to mental fatigue (end of the 20 min CPT).

There was a significant treatment effect for ACC ($p = 0.01$) and PREC ($p = 0.02$) based on the relative change in scores from beginning (*0-5 min*) to the end of the mentally fatiguing 20 min CPT (Figure 6). The relative decrease in ACC with CON-I/R ($-7.5 \pm 7.3\% \Delta$) was significantly greater ($p = 0.02$) compared to CHO-I/I ($-0.5 \pm 3.7\% \Delta$) but not different ($p = 1.0$) from CON-I/CHO-R ($-6.9 \pm 8.2\% \Delta$). Also, the decrease in ACC with CON-I/CHO-R tended to be greater ($p = 0.06$) compared to CHO-I/I. The relative decrease in PREC with CON-I/R ($-15.5 \pm 16.6\% \Delta$) was also greater ($p = 0.02$) compared to CHO-I/I ($-0.4 \pm 11.2\% \Delta$) but not different ($p = 1.0$) from CON-I/CHO-R ($-16.1 \pm 22.9\% \Delta$), which also tended to be different ($p = 0.08$) than CHO-I/I. The relative change in ACC and PREC did not become significant until the end of the CPT (reported above). When % change at the intermediate 5-min time blocks were examined (end of 10, 15, and 20 min), there was a treatment effect for ACC ($p = 0.04$) but not PREC ($p = 0.08$). Compared to *0-5 min*, ACC was maintained ($p > 0.05$) with CHO-I/I at 10 min ($-0.8 \pm 2.7\%$) and 15 min ($-2.0 \pm 5.4\%$) of the task. For the two rinse treatments, ACC decreased by $-4.4 \pm 6.7\%$ (at 10 min) and $-6.0 \pm 7.8\%$ (at 15 min) with CON-I/CHO-R, and by $-4.1 \pm 5.6\%$ (at 10 min) and $-5.5 \pm 5.9\%$ (at 15 min) with CON-I/R, but these changes were not significant ($p > 0.05$).

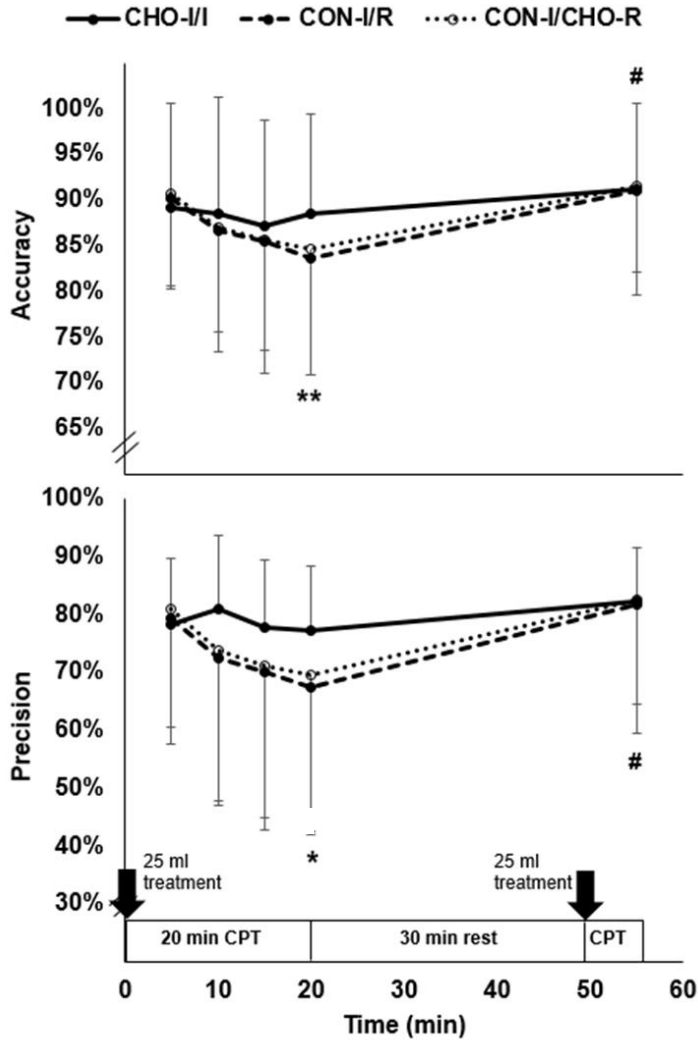


Figure 5. Mean (\pm SD) Accuracy (top panel) and Precision (bottom panel) during Continuous Performance Task (CPT) preceded by 375 ml ingestion and last 25 bolus of treatment. Top panel: ** significantly lower ($p < 0.05$) after 20 min CPT compared to 5 min for Control Rinse (CON-I/R) and Carbohydrate Rinse (CON-I/CHO-R). # significantly higher ($p < 0.05$) after 30 min rest for all treatments compared to after 20 min CPT. Bottom panel: # significantly higher ($p < 0.05$) after 30 min recovery for CON-I/CHO-R and CON-I/R compared to after 20 min CPT. Decline after 20 min CPT tended to be greater for CON-I/R ($p = 0.058$) and CON-I/CHO-R ($p = 0.05$).

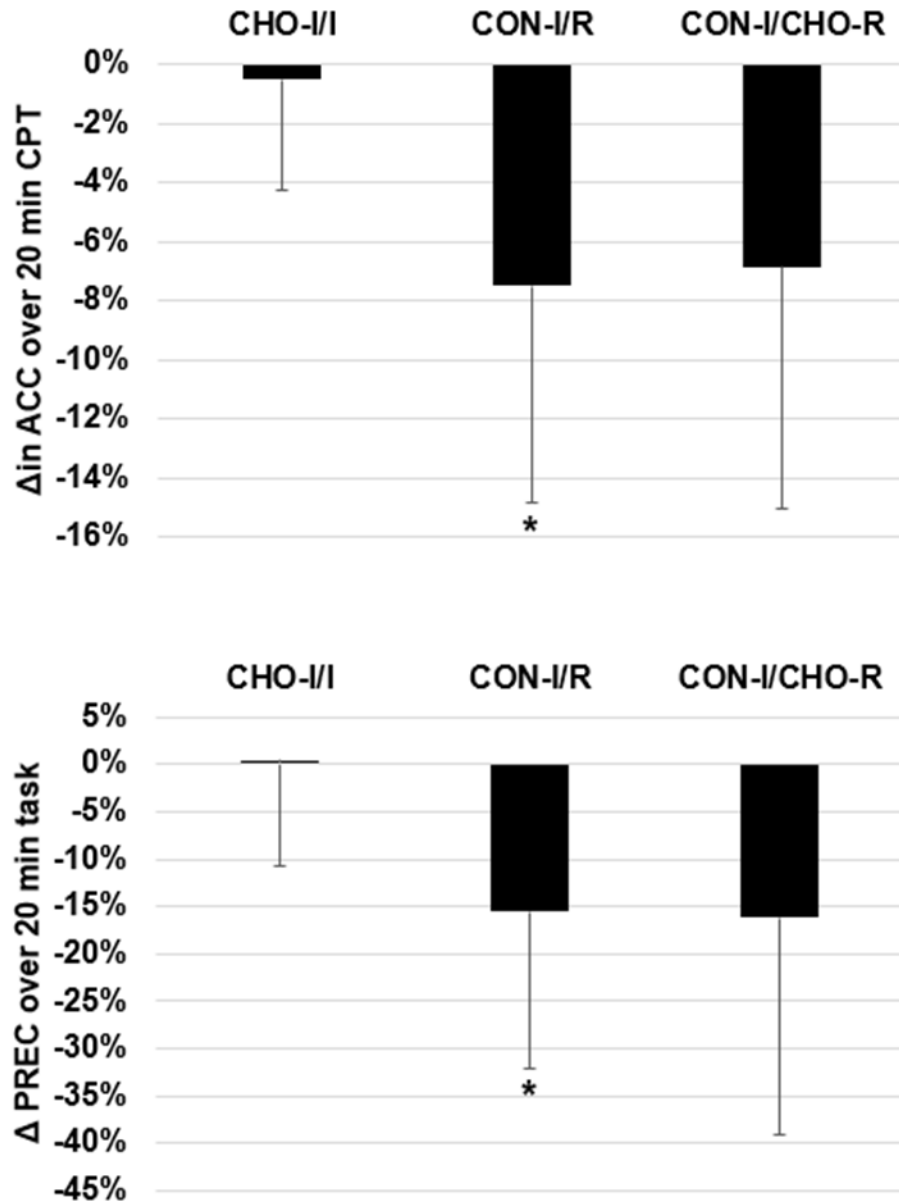


Figure 6: Top panel: Relative change in accuracy (ACC) at the point of mental fatigue

(end of 20 min Continuous Performance Task or CPT) compared to minutes 0-5. *

Significantly larger % decrease ($p < 0.05$) with control rinse (CON-I/R) compared to low-carbohydrate ingest (CHO-I/I). Bottom panel: Relative change in precision (PREC) at the

end of 20 min CPT compared to minutes 0-5. * Significantly larger % decrease ($p < 0.05$)

with CON-I/R compared to CHO-I/I.

Blood Glucose Response over Time

Blood glucose was not affected by treatment ($p = 0.88$) but did change over time ($p=0.002$). Compared to fasted baseline (4.3 ± 0.3 mmol/L), blood glucose was higher 15 min post-ingestion (4.4 ± 0.4 mmol/L, $p= 0.003$) and after the 20 min CPT (40 min time point) (4.5 ± 0.3 mmol/L, $p = 0.008$). While these differences are statistically significant, the ~5% relative change from baseline is practically not meaningful since all values remained within the normal physiological range for fasting blood glucose. No participants' glucose fell below 3.6 mmol/L at any time across treatments (2.5 mmol/L is considered hypoglycemic) (Figure 3, lower panel).

Perceived Mental Energy and Fatigue

There was no treatment effect ($p = 0.11$) or treatment x time interaction ($p = 0.34$) but perceived mental energy changed over time ($p = 0.001$). Compared to baseline (4.7 ± 1.1), mental energy decreased ($p = 0.02$) after 20 min CPT (3.8 ± 1.1) and increased ($p = 0.001$) back to baseline (5.1 ± 1.2) after 30 min recovery. A larger decline in ACC over the 20 min CPT was associated with a lower rating of mental energy ($r= -0.66$, $p = 0.04$) and the decline in PREC also was associated with lower post-test mental energy ($r= -0.77$, $p = 0.009$). However, blood glucose was not related to mental energy ($r=0.03$, $p=0.93$) after 20 min CPT.

There was no treatment effect ($p = 0.28$) or treatment x time interaction ($p = 0.43$), but perceived mental fatigue changed over time ($p < 0.001$). Compared to baseline (4.6 ± 1.3), mental fatigue increased ($p = 0.004$) after 20 min CPT (6.1 ± 0.7) and decreased ($p = 0.004$) back to baseline (4.8 ± 1.1) after 30 min recovery. Declines in ACC or PREC over the 20 min CPT were not significantly associated with post-test

rating of mental fatigue ($r=0.19$, $p=0.61$ and $r=0.26$, $p=0.47$, respectively). Blood glucose after 20 min CPT was also not significantly related to mental fatigue ($r=0.02$, $p=0.96$).

Discussion

The present study initially investigated glycemic response to carbohydrate solutions of $\leq 6\%$ CHO. Over 1 h, a 0.4% CHO solution did not induce a glycemic response different from 0% CHO (Powerade Zero ®) but 0.4% CHO had lower peak response compared to 1.5% and 6% CHO. Based on these findings; and, that peak glycemic response with 0.75% CHO was not different from 1.5% and 6% CHO, we selected a 0.4% CHO solution to evaluate whether ingestion of a low-CHO drink influenced sustained attention during a mentally fatiguing task. Since a small amount of CHO (1.5 g in 25 ml 6.4% CHO) may benefit mental activity when rinsed in the mouth prior to a cognitive task [15, 17, 18], we evaluated the efficacy of ingesting 1.5 g CHO compared to mouth rinsing 1.5 g CHO using a 6% CHO as our positive control [17, 18]. Although we expected ingestion and rinse of CHO to have equivalent benefit compared to rinsing 0% CHO control, our results suggest that low-CHO ingestion offers greater benefit to attenuating mental fatigue compared to rinsing CHO prior to a 20 min mental task when fasted.

Mental fatigue has been defined as a subjective feeling of tiredness or lacking energy due to prolonged cognitive activity and/or depletion of energy [2]. As a result of demanding work, mental fatigue can influence how individuals allocate mental resources or attend to a task or activity [2, 37, 38]. When mentally fatigued, an individual may disengage from a current task in favor of a distraction or other less effortful or more

“rewarding” behavior. Our results indicate that, in a fasted state, participants became mentally fatigued over a 20 min CPT when control fluid was ingested prior to mouth rinsing either 6% CHO or control. However, ingesting a small amount of CHO was able to attenuate the ~7-8% decline in accuracy and ~15% decline in precision that was observed with 6% CHO and control mouth rinse treatments over the 20 min task. While perceived mental fatigue increased and mental energy decreased following the 20 min task, neither rating was directly influenced by treatment. Only the degree to which sustained attention declined was related to the decrease in mental energy.

The presence of glucose or energy in the mouth is an example of an input or stimulus that can influence brain areas involved in reward, motivation, and decision making [2, 10, 11, 38, 187, 188] both with [13, 189] and without ingestion [11, 179]. Based on our findings, it appears that the mechanisms triggered by carbohydrate ingestion and absorption may be more important during a sustained mental task than just obtained by the mouth alone. Glucose ingestion can stimulate the CNS through the liver by sensing peripheral glucose and subsequently delivering neural responses via the Vagus nerve to areas of the brain involved in sensory processing and cognition [19]. Glucose sensing neurons that connect to the brain may sense peripheral glucose changes as small as 0.1 mmol/L [21] and facilitate dose-dependent alterations in cognition [16]. In addition to memory tasks, other cognitive tasks including those requiring attention also appear to be influenced by neural signaling via gut peptides and/or the Vagus nerve in response to CHO ingestion, independent of acute changes in blood glucose [20].

In contrast to our findings, Molden et al. observed a moderately strong effect ($d = 0.73$) [17] of a single 25 ml bolus of 6% CHO mouth rinse of 5 s on a subsequent Stroop

task. However, our 20 min CPT was likely longer in duration by at least 10 min compared to the Stroop task previously used, suggesting that CHO mouth rinse may have an acute benefit persisting for only a limited duration (< 20 min). Thus, we examined the intermediate 5 min time blocks during the 20 min CPT to determine whether CHO mouth rinse was effective earlier during the CPT (i.e. from 5-10 min or 10-15 min) compared to low-CHO ingestion and control mouth rinse. No treatments were different in the first 5 min of CPT. However, ACC and PREC, beginning with the 5-10 min block, appeared to steadily decline with both 6% CHO and control rinse treatments over the remainder of the task (from 10-15 min and 15-20 min), whereas low-CHO ingestion did not change over 20 min. Systematic review of studies from the exercise literature [135] indicates serial mouth rinsing (mouth rinse administered every 8-10 min) can be ergogenic during ~1 h exercise tasks [76]. Whether a CHO mouth rinse would have been as effective as low-CHO ingestion in sustaining attention during a 20 min task *if* additional rinses were provided throughout the task (i.e. every 5 min) is unclear; and, further investigation with prolonged attention tasks is warranted.

While cognitive psychology literature provides evidence that CHO mouth rinse benefits tasks involving self-regulation (e.g. engagement of attentional resources and inhibition of distracting stimuli), ingestion of an equivalent “dose” of CHO was not compared with the CHO rinse and the non-caloric artificially-sweetened control rinse [15, 17, 18]. The present study took into consideration inherent differences between ingesting and rinsing in order to compare equivalent oral exposure of CHO between methods, an important consideration when evaluating the role of oral CHO receptors in cognitive control. Unlike exercise studies that compared ingestion with rinse [139, 190], we

standardized the total fluid volume consumed (control solutions for 15 min prior) so that hydration status and volume load to the gut would be similar; and, we attempted to control the contact time of fluid in the mouth for ingestion vs. rinse. Ingesting a beverage typically involves immediately swallowing the fluid; whereas, CHO has longer contact time with oral receptors during a 5-10 s mouth rinse protocol [15, 16, 139, 182], potentially explaining differences in previous ingest versus rinse protocols. One reason ingestion was better than rinse in our findings may be that ingesting 1.5 g CHO in small boluses over 20 min while standardizing the amount of time CHO was in the mouth had a greater combined signal to the CNS from the mouth, pharynx, digestive tract, and liver [15, 19-21] compared to a single oral rinse of higher concentration (6% CHO providing 1.5 g CHO) exposed only to the mouth. We do not, however, have brain imaging data to support this suggestion. It is also possible that rinsing and expectorating immediately prior to the cognitive task was distracting. Unfortunately, we did not include a no-ingest, no-rinse control condition to determine the impact on mental fatigue for comparison [136]. Moreover, we would also expect that if rinsing and expectorating was a distraction, performance during the 0-5 min of the 20 min CPT would have been impaired with rinse versus ingestion. We did not, however, observe any difference between treatments during the first 5 min of the task.

Glycemic response was not influenced by low-CHO ingestion and the mental task did not elicit hypoglycemia for any subjects following an overnight fast. Glycemic status was also unrelated to perceived mental energy and fatigue; whereas, the decline in sustained attention during mouth rinse trials appeared related to lower perceived mental energy but not perceived mental fatigue. It has been suggested that the degree to which

perceived energy is altered modulates effects on perceived fatigue [56]; thus, it is possible that the decrease in mental energy was not severe enough to indicate a parallel significant increase in perceived mental fatigue.

Conclusion

Our findings indicate ingestion of a 0.4% CHO solution does not induce a glycemic response different from artificially-sweetened 0% CHO but elicits lower peak glucose compared to 6% and 1.5% CHO. Furthermore, the ingestion of 0.4% CHO (providing 1.5 g) attenuated mental fatigue in a 20 min cognitive task requiring sustained attention, compared to the ingestion of isovolumetric control solution followed by a rinsing either with 6% CHO (1.5 g) or control. Perceived mental energy and fatigue were not directly influenced by CHO but a larger decline in sustained attention was related to lower mental energy following the task. Contrary to previous studies, mouth rinsing 6% CHO did not maintain sustained attention better than 0% CHO rinse over the 20 min task. While evidence for an ergogenic effect of CHO mouth rinse appears strong in the exercise literature, more studies are needed to discern if benefits of oral exposure to CHO apply to sustained bouts of cognitive work for the general population.

CHAPTER 3

CAFFEINE BUT NOT LOW-CARBOHYDRATE INGESTION IMPROVES EXERCISE CAPACITY IN BOTH SEDENTARY ADULTS AND TRAINED ATHLETES

Abstract

BACKGROUND: Caffeine (CAF) and carbohydrate (CHO) delay fatigue during prolonged exercise but this is primarily documented in trained athletes. **PURPOSE:** To determine if exercise capacity is similarly improved in sedentary (SED) and endurance trained (ET) adults with CAF and/or CHO ingestion. **METHODS:** Using a double-blind crossover design, twelve ET and twelve SED completed four exercise trials consisting of 30 min cycling (MOD-EX) followed by cycling time to fatigue (TTF). After consuming a 43 g CHO nutrition bar, the following were ingested: CAF (3 mg/kg), low-CHO (0.4% solution, 2 g total CHO), CAF+CHO, and placebo (PLA). **RESULTS:** Rating of perceived exertion (RPE) did not differ between groups during MOD-EX and TTF. TTF was also similar (23.8 ± 3.1 in ET and 24.1 ± 2.6 min in SED). Low-CHO did not influence RPE or exercise capacity vs. PLA and CAF+CHO did not provide benefit compared to CAF. However, the two CAF treatments resulted in ~5% lower RPE ($p < 0.05$) and longer TTF (26.3 ± 10.4 min) compared to no-CAF (21.7 ± 9.9 min). Blood glucose and lactate were higher ($p < 0.05$) with CAF vs. no-CAF after MOD-EX and TTF. Compared to no-CAF, CAF increased oxidation of CHO but not fat. Following MOD-EX, CAF tended ($p = 0.055$) to maintain higher maximal voluntary isometric knee extensor strength compared to no-CAF (445 ± 198 N-m vs. 416 ± 195 N-m).

CONCLUSIONS: CAF reduced perceived effort and increased endurance capacity similarly for ET and SED; but, these effects were not improved by adding low-CHO. CAF mechanism of action appeared to be primarily through the central nervous system versus altering substrate oxidation in both ET and SED.

Introduction

Caffeine and carbohydrate are commonly used by athletes and recreationally-active individuals to improve performance and delay fatigue during prolonged exercise [72-74, 76, 77]. Fatigue is a complex phenomenon manifested by an increased perception of effort to complete a task or the inability to sustain a task. Specific factors contributing to fatigue may differ based on fitness status (sedentary compared to highly fit individuals) and influence the effectiveness of nutritional strategies which have primarily been tested on athletic populations.

Caffeine (CAF) is a well-documented ergogenic aid [72-74, 94-101] that may reduce fatigue through a number of potential mechanisms: direct effect on muscle via increased mobilization of intracellular calcium and/or increased sensitivity of myofibrils to calcium [98, 101], increased motor unit recruitment in the central nervous system (CNS) [100, 102], and/or increased lipolysis and fat oxidation with associated glycogen sparing [98, 103-105] although the latter has recently become less accepted [106]. CAF also has psychological effects serving to enhance task persistence and determination [94, 111, 143] and neuromodulatory effects in the brain [96, 100, 114]. Intake between 3-6 mg/kg CAF [74, 97] prior to exercise [72] can improve exercise capacity and influence perceived effort and motor drive, allowing individuals to perform more physical work at the same effort or perceive exercise as more tolerable, less painful, or fatiguing [72, 73, 83, 95, 115-118].

Carbohydrate (CHO) is another well-documented ergogenic aid used to enhance exercise capacity [77, 132]. Although ~30-60 g CHO/h ingested during prolonged exercise (> 1 h) may delay fatigue by maintaining blood glucose and enhancing CHO

oxidation [75, 76], it may also improve exercise capacity at lower doses <15 g/h [22, 133] when glycogen status is not limiting and blood glucose is well maintained [76], suggesting CHO also acts via a central mechanism. The central benefit of CHO for exercise capacity has been attributed to stimulation of CHO-sensitive receptors in the oral cavity. Neural signals are sent from CHO receptors to brain areas that modulate behavioral response to rewarding stimuli; possibly increasing motivation and allocation of neural resources to maintain and/or enhance performance during a physical effort [10, 11, 76, 139, 191].

Studies investigating CAF and CHO effects on endurance capacity have been limited primarily to trained athletes and recreationally-active individuals [72, 73, 77-79]. A meta-analysis of 20 studies suggests that individuals of higher fitness (VO_{2max}) may attain greater benefit from CAF during exercise [73] based on a slight but significant negative correlation between mean change in perceived effort during exercise and mean VO_{2max} . Yet, few studies have investigated CAF [82-85], CHO [14, 86-90], or the combination [91, 92] on endurance capacity in untrained but otherwise healthy adults.

Although there are many health benefits of exercise, sedentary individuals have low motivation or tolerance for fatigue during exercise [91, 92, 192]. It follows that CAF and/or CHO may allow these individuals to self-select higher exercise intensities and/or exercise for longer duration at the same perceived effort [132]. Since the mechanisms underlying fatigue and related metabolic processes during exercise are complex and differ based on fitness level; and, both CAF and CAF+CHO may also have negative metabolic side effects under sedentary conditions [24], further research is warranted to

better understand if CAF and CHO are similarly beneficial for a less active population to improve physical capacity or exercise tolerance.

Providing CHO calories during exercise would be counterproductive for sedentary individuals who exercise to lose weight; thus, if CHO works via the CNS, sedentary might benefit from a low-CHO (i.e. a low calorie “energy drink”) that may stimulate CHO-sensitive receptors to improve exercise capacity without added energy intake [12, 19, 21]. But, it is unclear whether low-CHO with or without CAF may reduce measures of physical fatigue in a practical scenario of a fed state. The purpose of this research was to determine if exercise capacity is similarly improved similarly in sedentary as in trained individuals using low-calorie (<1% CHO solution) low-CHO, CAF (3 mg/kg), and their combination (CAF+CHO). Based on evidence that CAF and CAF+CHO benefits endurance trained [72-74, 78, 98, 146] and individuals with variable physical activity [91, 92], our main hypothesis was that ingestion of CAF and CAF+CHO would improve exercise capacity by reducing factors related to fatigue independent of fitness level; and, that each nutritional component alone would improve measures of fatigue compared to a placebo (PLA). Our expectation that low-CHO would provide benefit compared to PLA is based on low-CHO acting via a central mechanism since the amount of CHO in the treatment is insufficient to contribute to blood glucose or exogenous CHO oxidation. Further, we expected that CAF+CHO would only be more beneficial than CAF alone if low-CHO provided benefit compared to PLA.

Methods

Participants

Twelve ET and 12 healthy SED males (n=10) and females (n=2) volunteered to participate in this study. Participants provided written informed consent prior to the study as approved by the Institutional Review Board at Georgia Tech. ET and SED individuals were matched pairwise by gender, age (within 3 yr), body mass, and BMI (within 2 kg/m²). The physical characteristics of subjects are presented in Table 2. ET were recruited from the local cycling and triathlon community and collegiate cycling and cross-country teams and SED were primarily recruited from the college campus. ET reported spending at least six hours per week performing physical training while SED did not spend more than 60 min per week in any planned exercise and no exercise above low intensity. Inclusion criteria for ET or SED were based on the $\text{VO}_{2 \text{ peak}}$ (< 50 ml/kg-min for SED men and < 40 ml/kg-min for SED women) *and* the amount of reported exercise per week in the initial screening questionnaire. Weekly non-exercise physical activity (i.e. walking or riding a bike to class or work) was also validated using the Core and Expanded Physical Activity STEPS version 2.0 Instrument [28]. The groups differed ($p < 0.05$) in body composition (% body fat), maximal aerobic capacity ($\text{VO}_{2 \text{ peak}}$), % $\text{VO}_{2 \text{ peak}}$ at lactate threshold (LT), and minutes of exercise per week; but, were similar in the amount of weekly non-exercise physical activity (Table 2).

All subjects completed a health-history screening questionnaire and a habitual caffeine intake questionnaire [146] to ensure they met all inclusion criteria. Exclusion criteria eliminated subjects who were either naïve to caffeine usage or on the “high” range [130, 131] (i.e., consuming > 500 mg/d approximately equivalent to more than two

premium coffees (~0.73 mg caffeine per ml) or energy drinks five or more times per week).

Table 2

Mean (\pm SD) physical characteristics of subjects.

	Endurance-trained (n=12)	Sedentary (n=12)
Age (yr)	27.7 \pm 5.5	26.8 \pm 7.0
Body mass (kg)	72.2 \pm 8.3	72.7 \pm 11.4
BMI (kg/m ²)	23.1 \pm 2.1	23.7 \pm 2.9
Body fat (%)	14.1 \pm 5.8	22.6 \pm 10.1*
VO _{2 peak} (ml/kg-min)	56.5 \pm 9.0	36.9 \pm 7.9*
Watts at VO _{2peak} (Wmax)	319 \pm 69	217 \pm 62*
Blood lactate at VO _{2peak} (mmol/L)	7.6 \pm 1.9	9.7 \pm 1.1*
% VO _{2 peak} at LT	75.7 \pm 10	67.5 \pm 9.1*
% Wmax at LT	69.4 \pm 18	63.5 \pm 4.5
RPE at LT	13.0 \pm 1.7	14.0 \pm 1.4
%HRmax at LT	82.3 \pm 7.4	83.7 \pm 8.2
Blood lactate at LT (mmol/L)	2.4 \pm 1.0	4.2 \pm 1.1*

* p<0.05 indicates significant difference versus endurance-trained subjects. LT indicates values at lactate threshold

Research Design

A double-blind, placebo-controlled, repeated measures crossover design was used and treatment order was assigned using a Latin Squares method. All participants served as their own control performing four trials (low-CHO, CAF, CAF+CHO, and PLA).

Preliminary Testing

Anthropometric measures were taken during the first laboratory visit: body mass using a digital scale (Pennsylvania 50, Lancaster, PA), height using a stadiometer (Seca, Hanover, MD), and body composition (% body fat) using the GE Lunar Prodigy Dual X-Ray Absorptiometry (DXA) scanner (GE Healthcare, Hatfield, United Kingdom). In a thermoneutral environment, participants completed a ramped exercise protocol to volitional fatigue on an electrically-braked Lode Excalibur Sport cycle ergometer (Lode, Groningen, The Netherlands) to determine VO_{2 peak}. Participants warmed up for 5 min between 50 to 100 watts (W) prior to the test, followed by 2 min stages with power

output increases of 25 to 50 W until volitional fatigue. Gas exchange data were obtained with the ParvoMedics True One 2400 metabolic cart (ParvoMedics, Sandy, UT). Heart rate (HR) and rating of perceived exertion (RPE) [40] were recorded every minute. We ensured a maximal effort was obtained in ET and SED based on similar respiratory exchange ratio (RER) (1.16 ± 0.05 and 1.15 ± 0.11) and RPE (18.3 ± 1.7 and 17.7 ± 2.4). Two min after the graded exercise test, blood lactate was measured using a 0.3 μ L blood sample obtained from the ear lobe (Lactate Pro LT-1710 analyzer, Arkray, Japan).

In order to assign comparable exercise workloads for ET and SED, each participant's LT was determined on the second visit, also using a ramped cycling protocol. Participants began cycling at 50 W and power output was increased by 25 to 50 W every three min [193] until reaching a RPE of 16-17 ("Hard to Very Hard"). Blood lactate was measured at baseline and 2 min into each stage using 0.3 μ L blood samples obtained from the ear lobe using the same hand held analyzer. Each individual's LT was determined using the DMax method [193, 194] and used to calculate moderate ($10\% < LT$) and vigorous ($5\% > LT$) workloads for the moderate exercise (MOD-EX) and time to fatigue (TTF) portions of the protocol, respectively.

After the LT test, participants cycled for 5 min at 25 to 50 W before being familiarized with the predetermined MOD-EX and TTF workloads for 10 min each. If RPE reached > 16 ("very hard") during MOD-EX familiarization and/or > 18 ("extremely hard") during TTF familiarization, or the subject felt uncomfortable with the workload, it was adjusted by 5% increments until the subject reported a RPE in the target range (below "somewhat hard") for MOD-EX and ("hard") for TTF. Workload adjustments were made for four ET and three SED participants. All pre-test instructions

(obtaining adequate sleep, hydration, and refraining from caffeine intake and physical activity prior to subsequent experimental sessions) were given to subjects at the conclusion of the session. Because ET participants were still engaging in regular endurance training programs during the weeks of the testing protocol, we requested they refrain from exceptionally strenuous training or racing in the three days leading up to the experimental sessions and that they treated each experimental exercise trial as they would a competitive event and arrive well rested.

Maximal Voluntary and Electrically Evoked Isometric Contractions

Isometric strength of the right knee-extensor muscles was measured during both maximal voluntary contractions (MVC) and electrically evoked contractions. These measurements were made using a modified leg-extension/curl machine (model NT-1220, Nautilus Fitness Products, Louisville, CO). The participant was seated in a semi-reclined position on the machine with hip- and knee-flexion angles at 80° and 70°, respectively [146]. The leg-extension arm was connected to a force transducer (model SBO-300-T, Transducer Techniques, Temecula, CA), enabling the determination of isometric torque production at the knee. The right ankle was secured to the leg extension arm and a seatbelt was used at the waist. Two 3"x4" adhesive electrodes (Dura-Stick® PLUS, Chattanooga Medical Supply, Chattanooga, TN) were placed on the skin over the distal vastus medialis and proximal vastus lateralis muscles. During the experimental trials, electrode placement was marked on the skin using a pen so positioning would be the same before and after exercise.

MVC strength of right knee-extensor muscles was determined each morning following blood sampling and prior to treatment ingestion to obtain a baseline measure

for each testing day. MVC strength was also measured within 5 min after MOD-EX and TTF. Participants performed five 3-s MVC trials at 1-min intervals and verbal encouragement was given to ensure maximal efforts. The highest and lowest peak-torque values were discarded and the middle three were averaged and used in the subsequent analyses. Electrically evoked strength of the knee-extensors was also determined using the interpolated twitch (ITT) protocol and equipment previously described [195] and used to calculate percent voluntary muscle activation at baseline and after MOD-EX during each trial. The strength of the electrical stimulation was first determined using a current optimization protocol [146] previously described. The current setting was adjusted to the minimum needed to elicit peak strength during the baseline measure of each testing day. At 2.5 s into each 3-s MVC, the knee extensors were stimulated using a computer-controlled Digitimer DS7AH stimulator (Digitimer, Hertfordshire, England). The stimulation was brief (10 msec) and peak force was recorded both prior to and during the stimulation. Any additional torque generated from this stimulus was considered to be ITT torque. At 2 and 4 s after the end of the MVC (i.e., while the muscles are relaxed), the quadriceps were stimulated again and the peak forces (electrically evoked torque or EET) recorded and averaged together. Percent voluntary activation is calculated using the following equation: % Voluntary Activation = $(1 - (\text{ITT torque} / \text{EET})) \times 100$

Because it may be difficult for some individuals to truly maximally contract and tolerate the electrical stimulus, the MVC, EET, and voluntary activation measures require adequate familiarization and “learning” prior to the experimental trials. Two sessions of the current optimization protocol and five subsequent MVC contractions with electrical

stimulus were completed at the conclusion of the preliminary testing days in order to provide each participant with adequate familiarization of the MVC+ITT procedure.

Treatment Ingestion

Fruit punch solutions were provided in de-identified containers by Glaceau Vitaminwater® with either CAF (0.34 mg/ml), low-CHO (3.6 g/L), CAF+CHO, or PLA to maintain the double blind design for participants and investigators. Fluid volumes were administered based on individual's body mass to provide 3 mg/kg CAF and <2 g CHO, resulting in 464 ± 85 ml of fluid. The main treatment bolus (all but 100 ml) was given 40 min prior to exercise with 75 ml given during MOD-EX and 25 ml given at the start of TTF. These small amounts of fluid given during exercise were provided to ensure a final dose of 3 mg/kg CAF dose prior to the test of exercise capacity (TTF) in the CAF+CHO and CAF trials and to provide periodic acute stimuli of the treatment similar to mouth rinse protocols [12]. However, we did not standardize the time of oral exposure with any of the boluses and participants simply ingested the boluses without any other “swishing” instructions. Because our research question was pertinent to the practical scenario of the “fed state”, an energy bar (PowerBar® Harvest Energy, PowerBar USA, Florham Park, NJ) was given to participants for breakfast along with the treatment beverage. The bar provided containing 250 kcal, 5 g fat, 43 g carbohydrate, 9 g protein, and no caffeine. Thus, the total CHO dose when combined with the initial bolus of treatment beverages was 43 g in CAF and PLA trials and 44.7 ± 0.3 g in CAF+CHO and low-CHO trials.

Experimental Protocol

The next four visits were each separated by a minimum of 7 d and scheduled at the same time of morning following an overnight fast. Before each visit, participants

refrained from exercise for 24 h and caffeine for 12 h, confirmed with a brief 24 h history questionnaire at the beginning of each trial. The schematic of the test protocol is illustrated in Figure 7. Upon arrival to the lab, participants also completed a 24 h diet recall. After the first visit, the 24 h diet recall was copied and returned to the participant to use as a guide for replicating 24 h dietary intake before the subsequent visits. A blood sample was drawn from a lanced fingertip into a heparinized capillary tube (Microvette® CB300, Sarstedt AG&Co., Numbrecht, Germany) for whole blood analysis of blood glucose and lactate (YSI Life Sciences, Inc. Yellow Springs, OH). Blood lactate during exercise was thereafter performed with this analyzer instead of the hand-held model used for LT determination. Baseline measures of maximal voluntary and electrically evoked contractile properties of the right quadriceps muscle were also obtained.

In order to give sufficient time for CAF to reach peak concentration [196], the exercise protocol began 40 min after treatment ingestion. During MOD-EX, participants warmed up at 50 W (sedentary) or 100-150 W (trained) for 5 min, then cycled continuously for 25 min at the individual's predetermined moderate workload. VO_2 and respiratory-exchange ratio were measured during the last 3 min of each 10-min interval. Total-body CHO and fat oxidation were calculated from VO_2 and the RER. RPE and HR were assessed every 5 min during cycling and blood glucose and lactate were measured at 15 and 30 min using whole blood samples drawn from the ear lobe. Immediately after cycling, maximal voluntary and electrically evoked contractile properties were measured (within 5 min of cessation). After a 10 min break, participants began TTF at the predetermined workload. The point of fatigue was defined as the participant voluntarily deciding to stop exercise due to feelings of fatigue and/or the inability to maintain a

minimum cadence of 40 rpm. No information on time elapsed, heart rate or external motivation was given to the participants. HR and RPE were measured every min during the trial and blood glucose and lactate were measured immediately after the point of fatigue. Within 5 min after cessation of the fatigue trial, maximal voluntary strength assessment was repeated.

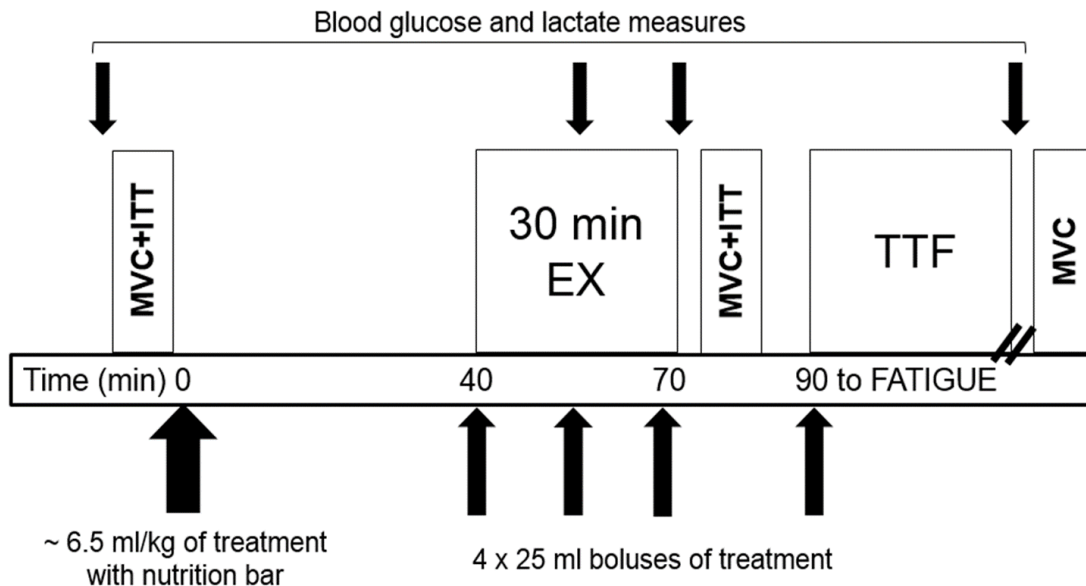


Figure 7. Schematic of the test protocol for the four drink trials (one initial bolus and four small boluses during exercise) consisting of moderate-intensity cycling (EX) followed by cycling to volitional fatigue (TTF) with timing of Maximum Voluntary Contractions (MVC) with and without Interpolated Twitch (ITT).

Statistical Analyses

Data are reported as mean \pm standard deviation (SD) and were analyzed using SPSS 17.0 (Chicago, IL). Three-way (group x treatment x time) mixed model ANOVA (group as the between-subject factor and treatment as the within-subject factor with repeated measures over time) was used to examine differences in RPE and physiological variables. A one-way (within factor treatment) ANOVA was also used to examine

differences in time to fatigue and overall RPE during MOD-EX and TTF in all subjects. The Greenhouse-Geisser correction was used to account for the sphericity assumption of unequal variances across groups. If a significant F ratio was obtained, Bonferroni post hoc test was used to detect significant differences in pairwise comparisons. Two-way (i.e. treatment x time) repeated measures ANOVA with Bonferroni post hoc pairwise comparisons at each time point also used to examine differences in measures, when significant interactions were present. Since TTF was variable, we also normalized each TTF session based on percent of time complete in 10% increments in order to compare RPE and HR at the same relative point in time over the course of the trial (from 10% to 100% of time complete). A paired t-test was used to examine difference in total g CHO and fat oxidized during MOD-EX between CAF and non-CAF treatments. Pearson product moment correlations were used to examine if habitual caffeine intake was associated with TTF. Effect sizes (ES) given are based on Cohen's d. Statistical significance was set at an alpha level of $p < 0.05$. Sample size was based on a priori power analysis completed with G*Power 3.1 using a repeated measures ANOVA with a between-factor of group and repeated factor of treatment. Four levels were assumed for the repeated factor. CAF has a moderate ES of 0.5-0.7 compared to PLA on endurance capacity and RPE based on two meta-analyses [72, 73]. Based on an expected moderate ES of 0.7 for the treatment effect of CAF and CAF+CHO, a total sample size of 24 yielded statistical power of 0.8 ($\rho = 0.5$, alpha level = 0.05, two groups, four repeated measures/nutritional treatments).

Results

Exercise Workloads

Exercise intensities were based on workloads (Watts) that elicited 90% of LT (MOD) and 105% LT (TTF). When comparing ET and SED, %Wmax (Figure 8), RPE, and %HRmax were not different at LT; however, the percentage of VO₂peak and blood lactate at LT was different ($p < 0.05$) between groups (Table 2 and Figure 8). ET cycled at a higher VO₂ uptake during MOD-EX ($p < 0.001$) (36.2 ± 4.8 ml/kg-min) compared to SED (25.7 ± 4.8 ml/kg-min).

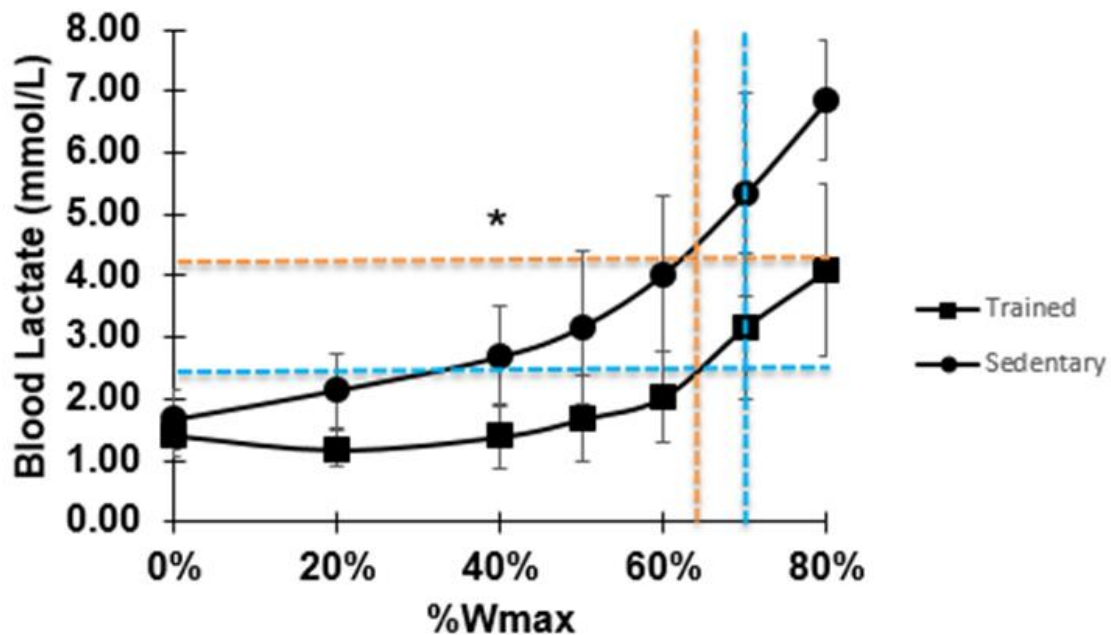


Figure 8. Mean (\pm SD) blood lactate response during lactate threshold (LT) test for Trained and Sedentary groups relative to percentage of Watt maximum (Wmax). Horizontal blue and orange dashed lines indicate higher (* $p < 0.05$) mean blood lactate at LT for trained and sedentary, respectively. ($p < 0.05$) Vertical blue and orange dashed lines indicate similar ($p > 0.05$) mean %Wmax at LT for trained and sedentary, respectively.

Low-CHO Treatment

Compared to PLA, low-CHO did not result in any significant differences in RPE, TTF (Table 4), or muscular strength. In addition, low-CHO did not affect HR, blood glucose, blood lactate, or substrate utilization (Table 3). The addition of low-CHO to CAF also did not elicit significant differences compared to CAF alone. Thus, the results are presented as comparisons between CAF treatments (CAF alone and CAF+CHO) and no-CAF treatments (low-CHO and PLA) unless otherwise specified.

RPE

RPE averaged over 30 min MOD-EX was similar ($p = 0.50$) in SED (12.4 ± 1.2) and ET (12.0 ± 0.9) and increased significantly ($p < 0.001$) between 10 min (11.7 ± 1.1) and 25 min (13.1 ± 1.2). For all subjects, CAF resulted in ~5% lower ($p = 0.005$) overall RPE (11.9 ± 1.0) compared to no-CAF (12.5 ± 1.2) (Figure 9).

RPE averaged over the TTF trials was similar ($p = 0.41$) in SED (16.0 ± 1.0) and ET (16.4 ± 1.5). For all subjects, CAF increased ($p = 0.003$) the time until rating a 17 or “very hard” (14.8 ± 7.3 min) compared to no-CAF (11.2 ± 5.5 min). When the TTF trial lengths were normalized from 0 to 100% of trial completed, there was no main effect of CAF ($p = 0.30$) but there was a three-way interaction between group x treatment x time ($p < 0.001$). Compared to no-CAF, CAF resulted in lower ($p < 0.05$) RPE early in the trial (during the first 20% of total cycling time) for ET, but this effect was not observed in SED (Figure 9).

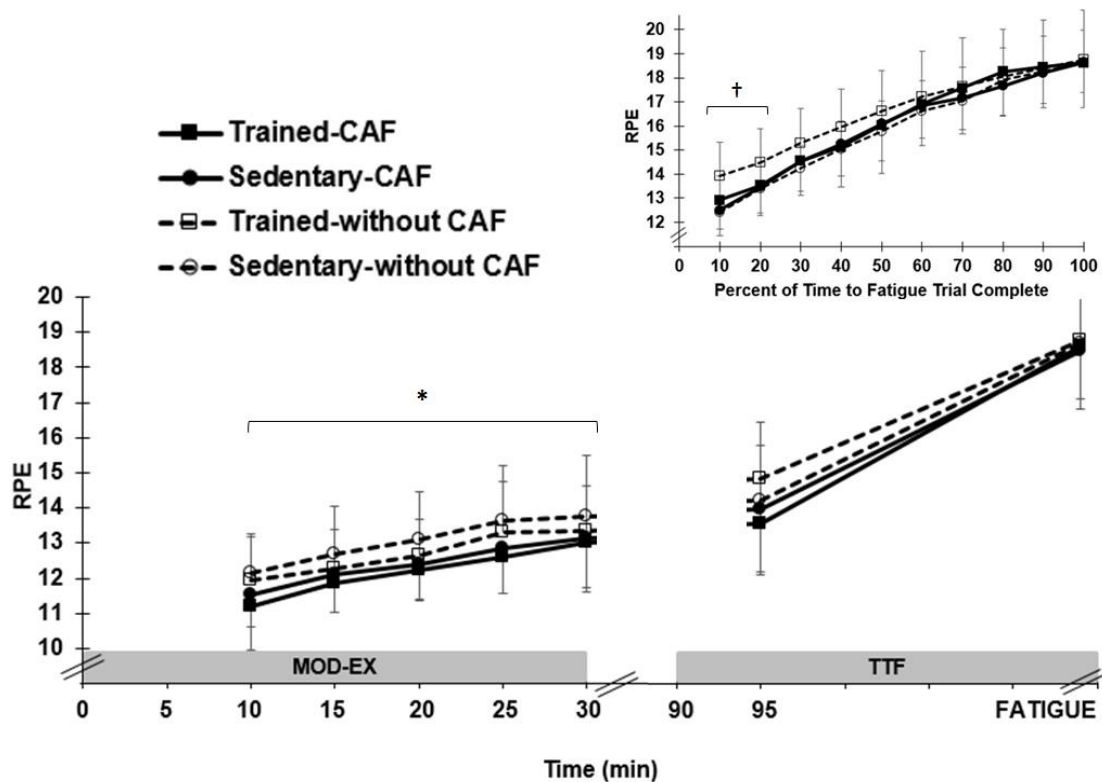


Figure 9. Mean (\pm SD) rating of perceived exertion (RPE) by group (trained and sedentary) and caffeinated (CAF) vs. un-caffeinated treatment over time. * Significant ($p < 0.01$) overall lower RPE with CAF compared to without CAF for both groups. Upper right panel: RPE during time to fatigue (TTF) normalized by percentage of trial completed. † Significantly higher ($p < 0.05$) RPE in trained subjects without CAF for first 20% of TTF.

Heart Rate

HR expressed as the percentage of each individual's maximum (%HRmax) was similar between ET and SED during MOD-EX ($p = 0.33$) and TTF ($p = 0.63$). There was a treatment effect of CAF ($p = 0.01$) and a significant group \times treatment interaction ($p = 0.02$) during MOD-EX. CAF resulted in higher %HRmax compared to no-CAF ($81.1 \pm 5.3\%$ vs. $78.9 \pm 6.2\%$, $p = 0.001$) in ET, whereas no difference was observed in SED

(82.9 ± 7.7 vs. $82.8 \pm 8.3\%$, $p = 0.86$). During TTF, there was a significant treatment effect of CAF ($p < 0.001$) but no group x treatment interaction ($p = 0.51$). %HRmax averaged over the entire TTF trial was higher with CAF ($89.2 \pm 4.8\%$) vs. no-CAF ($86.5 \pm 5.0\%$), although this data is difficult to directly compare since the TTF protocol elicited different durations (see Results section below *Time to Fatigue*).

Substrate Utilization

Substrate utilization and energy expenditure (EE) measures for all four treatments are indicated in Table 3 for trained ($n=11$) and sedentary ($n=12$) groups. RER was significantly lower ($p = 0.04$) in ET (0.94 ± 0.03) compared to SED (0.98 ± 0.04) and decreased ($p < 0.001$) over 30 min MOD-EX. CAF tended ($p = 0.19$) to result in higher average RER during MOD-EX (0.961 ± 0.05) compared to no-CAF (0.955 ± 0.05) for all subjects. The percent of kcal derived from CHO during 30 min MOD-EX also tended to be higher ($p = 0.07$) for SED ($88.5 \pm 12.5\%$) compared to ET ($79.8 \pm 10.9\%$) but was not affected by CAF ($p = 0.13$).

CHO oxidation rate during 30 min MOD-EX was not different ($p = 0.09$) between ET (2.34 ± 0.43 g/min) and SED (2.02 ± 0.49 g/min). For all subjects, CAF resulted in higher ($p = 0.006$) CHO oxidation rate (2.23 ± 0.3 g/min) vs. no-CAF (2.13 ± 0.5 g/min). Total CHO oxidized over 30 min MOD-EX was also higher ($p=0.006$) with CAF (66.7 ± 13.7 g) vs. no-CAF (63.9 ± 12.9 g).

Fat oxidation rate during 30 min MOD-EX was higher ($p = 0.03$) in ET (0.27 ± 0.15 g/min) compared to SED (0.13 ± 0.16 g/min); as was total fat oxidized in 30 min

(Table 3). CAF did not affect fat oxidation rate ($p = 0.65$) or total fat oxidized over 30 min ($p = 0.65$).

Total EE during 30 min MOD-EX was higher ($p = 0.009$) in ET (354 ± 64 kcal) compared to SED (276 ± 70 kcal). CAF resulted in higher ($p = 0.02$) EE (319 ± 64 kcal) vs. no-CAF (312 ± 65 kcal) for all subjects with no group x treatment interaction ($p = 0.91$).

Table 3

Mean \pm SD EE (kcal) during 30 min MOD-EX (10% below LT)

	CAF+CHO	CAF	PLA	Low-CHO	All Treatments
Trained (n=11)	357 \pm 60	358 \pm 58	355 \pm 59	347 \pm 69	354 \pm 64*
Sedentary (n=12)	279 \pm 69	281 \pm 69	274 \pm 66	272 \pm 71	276 \pm 70
All Participants	316 \pm 75	318 \pm 74†	312 \pm 74	308 \pm 78	

* $p < 0.05$ higher than sedentary group

† $p < 0.05$ higher than CHO for all participants

Mean \pm SD respiratory exchange ratio (RER) during 30 min MOD-EX

	CAF+CHO	CAF	PLA	Low-CHO	All Treatments
Trained (n=11)	0.947 \pm 0.033	0.944 \pm 0.043	0.942 \pm 0.033	0.927 \pm 0.033	0.940 \pm 0.03*
Sedentary (n=12)	0.969 \pm 0.045	0.983 \pm 0.048	0.967 \pm 0.059	0.984 \pm 0.031	0.976 \pm 0.04
All Participants	0.954 \pm 0.044	0.959 \pm 0.054	0.953 \pm 0.049	0.950 \pm 0.044	

* $p < 0.05$ lower than sedentary group

Mean \pm SD carbohydrate oxidation (total grams) during 30 min MOD-EX

	CAF+CHO	CAF	PLA	Low-CHO	All Treatments
Trained (n=11)	71.8 \pm 9.7	72.4 \pm 17.1	70.9 \pm 11.8	65.4 \pm 14.8	70.1 \pm 13.1
Sedentary (n=12)	60.0 \pm 13.8	62.7 \pm 16.4	57.6 \pm 13.8	61.6 \pm 14.8	60.5 \pm 14.0
All Participants	65.7 \pm 13.2	67.4 \pm 17.1	63.9 \pm 14.3	63.4 \pm 14.6	

Mean \pm SD fat oxidation (total grams) during 30 min MOD-EX

	CAF+CHO	CAF	PLA	Low-CHO	All Treatments
Trained (n=11)	7.7 \pm 5.1	7.6 \pm 6.7	7.9 \pm 5.3	9.5 \pm 5.2	8.2 \pm 4.6*
Sedentary (n=12)	4.3 \pm 5.2	3.3 \pm 4.8	4.8 \pm 5.1	2.9 \pm 2.7	3.8 \pm 4.2
All Participants	6.0 \pm 5.2	5.5 \pm 5.8	6.4 \pm 5.2	6.2 \pm 4.1	

* $p < 0.05$ higher than sedentary group

Blood Glucose

Blood glucose was not different ($p = 0.70$) between ET and SED, but there was a significant group \times time ($p = 0.006$) interaction (Figure 10). Fasting blood glucose was similar between ET (4.1 ± 0.2 mmol/L) and SED (4.3 ± 0.3 mmol/L) but ET had lower ($p = 0.02$) blood glucose (3.1 ± 0.6 mmol/L) after 15 min MOD-EX (55 min post-treatment and energy bar) compared to SED (3.4 ± 0.7 mmol/L). However, glucose after 30 min MOD-EX (70 min post-treatment and energy bar) was again similar between groups and remained similar after TTF. There was a main effect of CAF ($p = 0.02$) and treatment \times time interaction ($p = 0.04$) for blood glucose. CAF resulted in higher glucose compared to no-CAF after 30 min MOD-EX ($p = 0.04$) and after TTF ($p = 0.02$) (Figure 10).

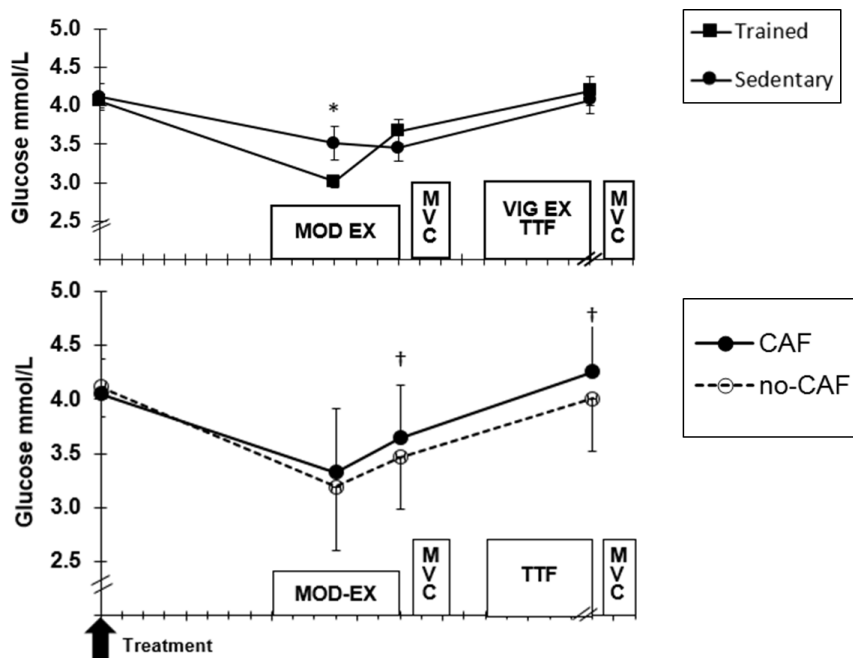


Figure 10. Top panel: Mean (\pm SD) glucose at baseline, moderate exercise (EX) and after time to fatigue (TTF) across treatments for trained and sedentary groups. * Higher ($p < .05$) for sedentary at 15 min of EX. Bottom panel: Treatment effect for all subjects. † higher ($p < 0.05$) for CAF compared to no-CAF.

Blood Lactate

Blood lactate during exercise was higher ($p = 0.001$) in SED compared to ET and there was also a significant ($p < 0.001$) group x time interaction (Figure 11). Resting blood lactate was similar ($p = 0.46$) between groups but ~ 1 mmol/L higher in SED compared to ET throughout MOD-EX ($p \leq 0.001$) and after TTF (3.5 ± 0.3 mmol/L vs. 2.2 ± 0.3 mmol/L, $p = 0.008$). For all subjects, CAF resulted in higher ($p < 0.001$) overall blood lactate during exercise (2.5 ± 0.9 mmol/L) vs. no-CAF (2.2 ± 0.9 mmol/L) and a significant treatment x time interaction ($p = 0.007$). Blood lactate was not different at pre-treatment baseline or after 15 min MOD-EX; however, lactate was higher with CAF vs. no-CAF ($p=0.001$) after 30 min MOD-EX (Figure 11) and remained higher after TTF ($p=0.004$), although subjects cycled longer to fatigue with CAF versus without CAF.

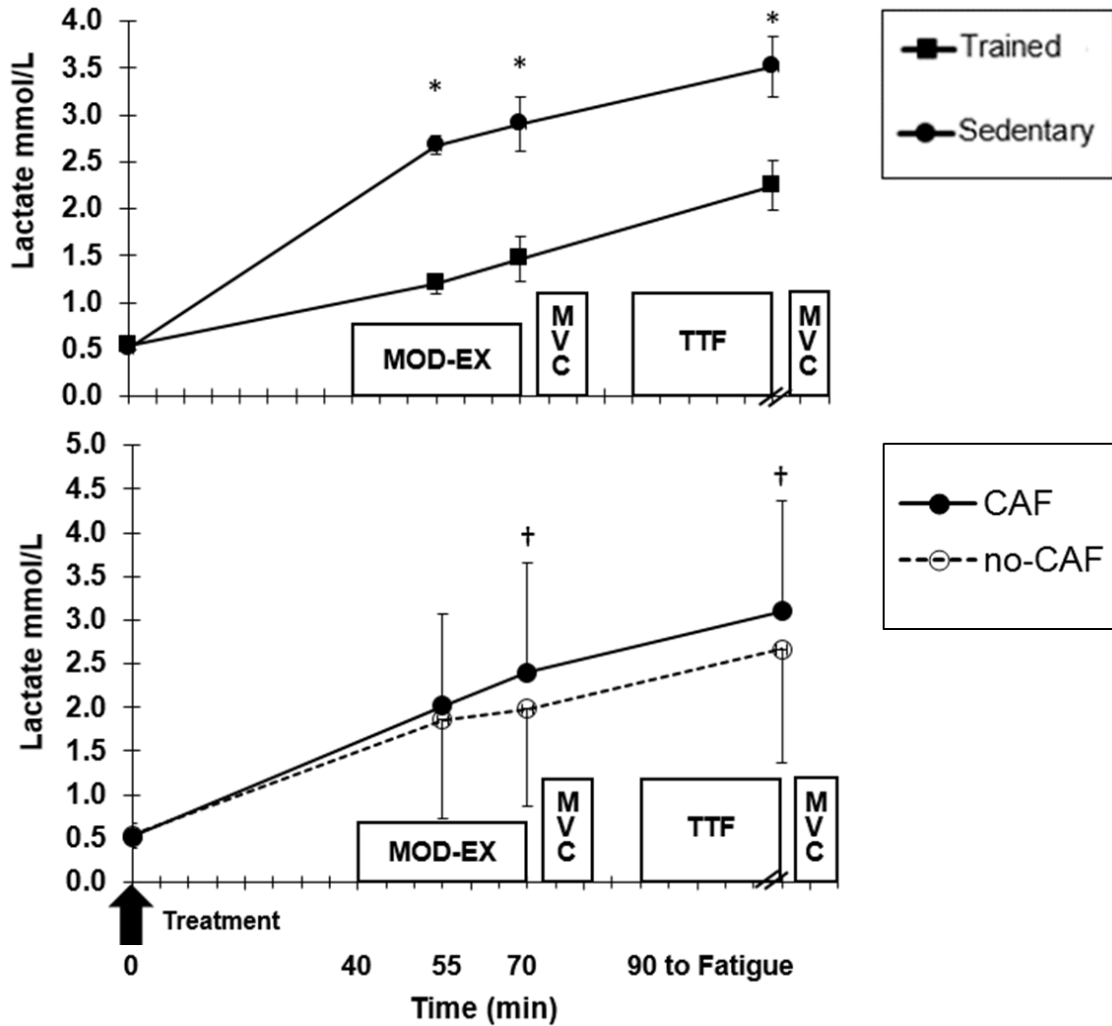


Figure 11. Mean (\pm SD) lactate at baseline, during moderate exercise (EX) and after time to fatigue (TTF). Top panel: Trained and sedentary values for all treatments combined. * Higher ($p < 0.01$) for sedentary vs. trained. Bottom panel: Treatment effect for all subjects combined. † higher ($p < 0.01$) for CAF compared to no-CAF.

Time to Fatigue

TTF was similar ($p = 0.94$) in ET (23.8 ± 8.1 min) and SED (24.1 ± 11.3 min). TTF data for all four treatments are presented in Table 4. There was a main treatment effect ($p = 0.004$) but no group \times treatment interaction. TTF was significantly longer ($p =$

0.03) with CAF+CHO (26.7 ± 12.6 min) compared to low-CHO (21.2 ± 10.3 min) (Table 4) but not different from any other treatment. When treatments were averaged to examine the low-CHO effect, TTF was not different (24.0 ± 10.7 min) from no-CHO (24.0 ± 9.4 min) (ES = -0.003). When treatments were averaged to examine CAF effect, TTF was longer ($p = 0.003$) (26.3 ± 10.4 min) compared to no-CAF (21.7 ± 9.9 min) (ES = 0.45, $p < 0.01$). TTF was not correlated with participants' habitual caffeine intake during either the CAF trials ($r = -0.18$, $p = 0.394$) or no-CAF trials ($r = 0.038$, $p = 0.861$).

Table 4

Mean (\pm SD) minutes to volitional fatigue during vigorous exercise (5% above LT).

Treatment	Endurance-trained	Sedentary	All Participants
Caffeine (CAF)+Low-carbohydrate (CHO)	26.5 ± 9.6 (d = 0.4)	26.9 ± 15.6 (d = 0.38)	26.7 ± 12.6 (d = 0.39)
Caffeine (CAF)	26.0 ± 10.7 (d = 0.32)	25.6 ± 9.2 (d = 0.37)	25.8 ± 9.8 (d = 0.36)
Placebo (PLA)	22.8 ± 9.0	21.5 ± 12.8	22.1 ± 10.8
Low-CHO	19.8 ± 10.3 (d = -0.31)	22.5 ± 10.4 (d = 0.09)	$21.2 \pm 10.2^*$ (d = -0.1)
All treatments	23.8 ± 3.1	24.1 ± 2.6	24.0 ± 2.6

* $p < 0.05$ indicates significantly lower compared to CAF+CHO.

Effect size (Cohen's d) is treatment compared to PLA for all participants.

MVC Strength

Relative change in MVC strength between pre- and post-exercise measures was not different between groups but the average loss in MVC following 30 min MOD-EX tended to be higher ($p = 0.09$) in SED (-5.9%) vs. ET (-4.6%). There was no effect of CAF vs. no CAF ($p = 0.44$), nor group x treatment ($p = 0.33$) or treatment x time interactions ($p = 0.74$) for the relative change in MVC strength; though, CAF tended to maintain post-TTF MVC strength compared to baseline better than no-CAF for ET, but not SED (Table 5).

Table 5

 Mean (\pm SD) Relative Change (%) in MVC Strength from baseline

Treatment	Baseline to post MOD-EX			Baseline to post TTF		
	Trained	Sedentary	All Participants	Trained	Sedentary	All Participants
CAF	-1.0 \pm 8.7	-8.7 \pm 12.2	-5.0 \pm 11.1	-1.2 \pm 15.6	-10.6 \pm 10.6	-5.9 \pm 13.9
No-CAF	-1.8 \pm 18.1	-9.9 \pm 7.9	-5.9 \pm 14.3	-7.8 \pm 10.2	-8.7 \pm 9.9	-8.2 \pm 9.8

When absolute MVC strength was compared between CAF and no-CAF over time, there was no significant effect of CAF although CAF tended ($p = 0.055$) to result in higher overall MVC strength compared to no-CAF (447.6 ± 191.2 N-m vs. 427.03 ± 191.2 N-m). There was a main effect of time ($p = 0.002$) but no treatment x time interaction ($p = 0.33$). Compared to baseline (454.6 ± 189.2 N-m), MVC strength declined ($p = 0.008$) after 30 min EX (430.4 ± 195.4 N-m) or $\sim -5\%$ relative decrease for all subjects (Figure 12). MVC strength did not decline between post MOD-EX and after TTF ($p = 1.0$), but MVC after TTF (427.1 ± 192.7 N-m) remained lower than baseline ($p = 0.007$) for all subjects.

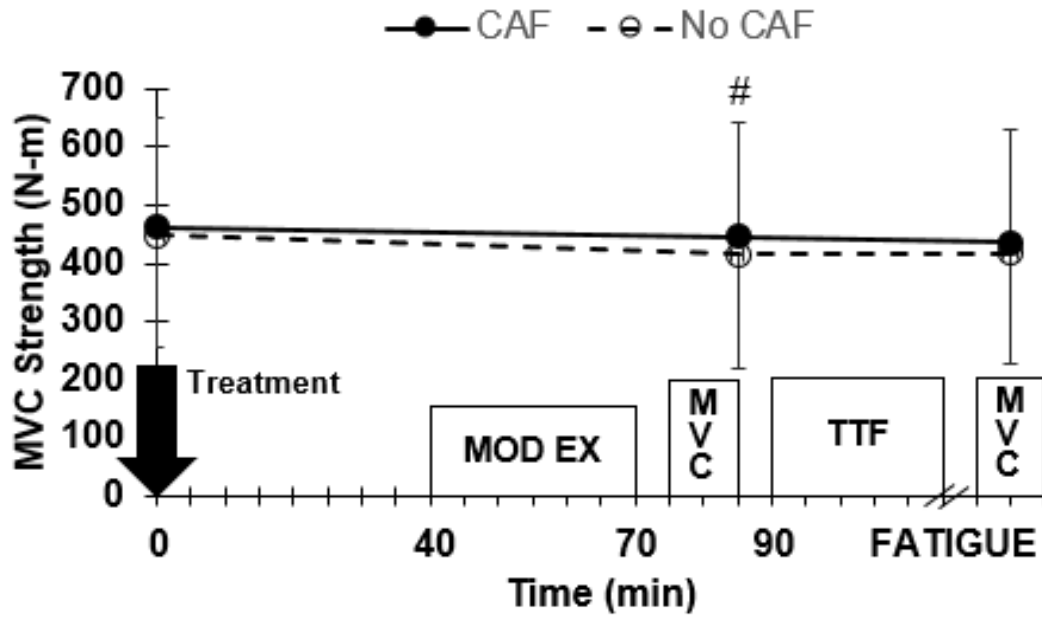


Figure 12. Mean (\pm SD) maximum voluntary contractile (MVC) isometric strength at baseline, after moderate exercise (EX) and after time to fatigue (TTF). # lower ($p < 0.01$) MVC strength following EX compared to baseline for both caffeinated (CAF) and non-caffeinated (No CAF) treatments.

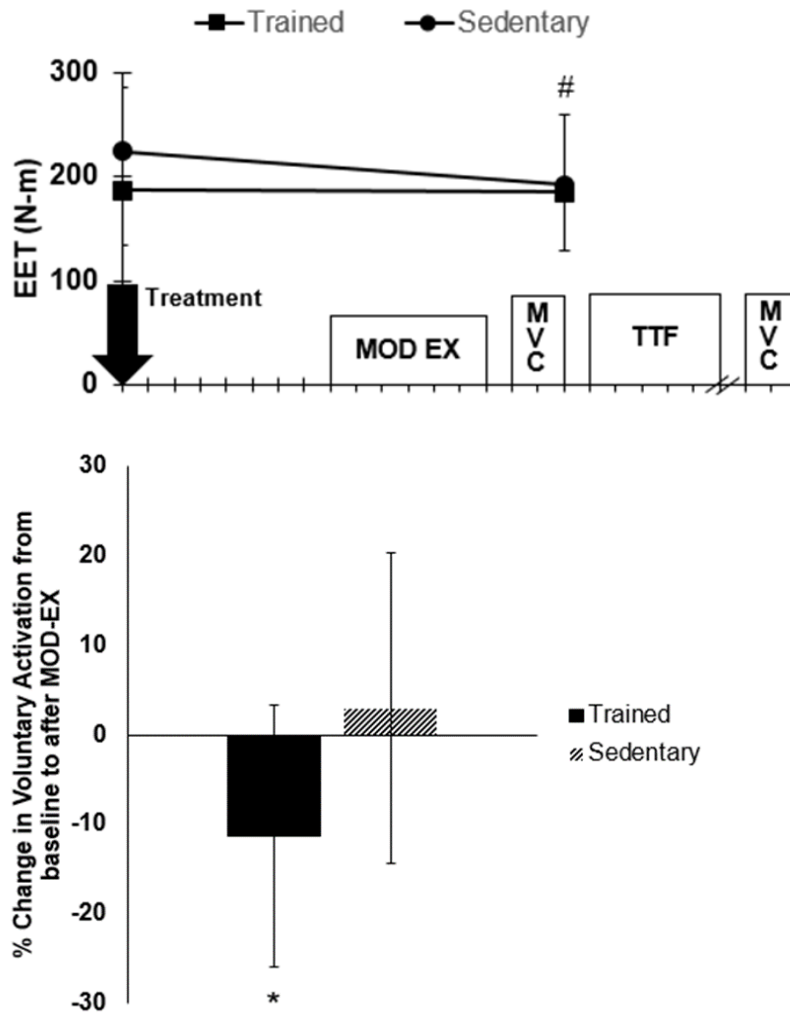


Figure 13. Top panel: Mean (\pm SD) electrically evoked torque (EET) during the maximal voluntary contraction (MVC) with interpolated twitch completed at baseline and after moderate exercise (MOD EX). # lower ($p < 0.001$) EET following MOD EX compared to baseline for sedentary participants only. Bottom panel: Mean (\pm SD) relative change in voluntary activation between baseline and after MOD EX. * larger ($p < 0.05$) change in trained compared to sedentary.

Electrically Evoked Torque (EET)

While EET over time was similar ($p = 0.36$) in ET (185.7 ± 58.4 N-m) and SED (208.3 ± 58.4 N-m), there was a significant group x time ($p = 0.001$) interaction. In SED, EET declined approximately 14% following MOD-EX compared to baseline (192.2 ± 61.3 N-m vs. 224.3 ± 57.0 N-m, $p < 0.001$) but no change was observed in ET (Figure 13). There was no treatment effect ($p = 0.61$) on EET (197.7 ± 60.6 N-m vs. 196.3 ± 56.9 N-m with CAF and no-CAF, respectively) nor treatment x time ($p = 0.24$) or group x treatment ($p = 0.72$) interactions.

Changes in Voluntary Activation

Overall, voluntary activation of the knee extensors was not different ($p = 0.54$) between groups but there was a significant group x time interaction ($p = 0.009$). Voluntary activation declined $11.3 \pm 14.6\%$ ($p = 0.006$) from baseline following MOD-EX in ET but did not change in SED ($2.9 \pm 17.4\%$, $p = 0.3$); and, the magnitude of change in voluntary activation was significantly larger ($p = 0.04$) for ET compared to SED (Figure 13). CAF did not influence the change in voluntary activation between baseline and after MOD-EX ($-0.5 \pm 20.7\%$) compared to no-CAF ($-4.9 \pm 20.0\%$) and there was no treatment x time ($p = 0.94$) or group x treatment ($p = 0.74$) interaction.

Discussion

Low physical activity and low physical fitness are known health risks [30, 197]. Therefore, our aim was to examine if nutritional countermeasures to fatigue (i.e., CAF and CHO) would improve exercise capacity in sedentary individuals through similar mechanisms as trained athletes [72, 73, 77, 78, 83, 94, 97, 143]. We observed a moderate

dose of CAF (3 mg/kg) lowered perceived effort during moderate intensity exercise and increased exercise capacity in both trained and sedentary individuals. Compared to non-caffeinated treatments, CAF increased CHO oxidation, blood glucose and lactate for all subjects; but, only increased heart rate in trained during moderate intensity exercise. Furthermore, ingestion of low-CHO did not improve exercise capacity or reduce perception of effort in either group. Our findings collectively suggest CAF improves exercise tolerance in sedentary individuals, appearing to act primarily through central versus peripheral mechanisms similar to endurance-trained.

While CAF has purported peripheral mechanisms of action (metabolic, neuromuscular) at physiological doses, the primary mechanism appears to be adenosine antagonism in the CNS [100, 114]. Our findings are consistent with a meta-analysis [73] indicating CAF reduces perceived effort in trained individuals; and, that CAF facilitates greater work output in sedentary individuals without an increase in RPE [83] compared to placebo. Though the exact mechanism by which CAF can influence perception of effort during exercise is still unknown, it is likely that a combination of the following mechanisms are involved [73]: reducing sensation of pain through peripheral and/or CNS adenosine receptors in the nociceptive system [73, 117, 198], decreasing sensation of force during muscular contraction [118], and improving mood state in response to exercise [91, 94]. In the present study, CAF reduced RPE during moderate intensity exercise compared to no-CAF by ~5% for individuals of high and low fitness. And, consistent with previous findings [73, 82, 92], CAF effects on RPE were more evident in steady state exercise below LT. CAF did, however, increase the time both SED and ET participants were able to tolerate exercise above LT before rating the effort as “very

hard”. In agreement with others [72, 74, 146] demonstrating an ergogenic benefit of a moderate dose of caffeine for trained athletes, our results indicate ~21% longer exercise capacity with CAF compared to no-CAF (ES = 0.45) for both trained and sedentary. Further, the effect of CAF on endurance capacity was “moderate” in trained (ES=0.54) compared to “small to moderate” in sedentary (ES=0.38), consistent with the reported effect in physically fit individuals [72].

Although studies on sedentary or untrained populations are more limited, evidence [82, 83, 92] suggests 3-6 mg/kg CAF improves exercise capacity during exercise below LT, but not all studies have observed a benefit. However, the classification of “untrained” and “active” is not always clear within this small group of studies. Some studies defined untrained as $VO_{2max} < 50$ ml/kg-min [86-88, 91] while others used $VO_{2max} < 45$ ml/kg-min or indicated untrained status with variable physical activity, but did not report fitness level [82-85, 89, 92]. Additionally, CAF may not benefit sedentary individuals if the exercise intensity is too high (e.g., 80% VO_{2peak}) [84] or be effective during a “time trial” effort due to less experience with pacing than trained athletes. For example, caffeine did not reduce RPE in sedentary females [85] during steady-state cycling or performance during a subsequent 10 min time trial compared to placebo. Findings from the present study observed sedentary individuals improved exercise capacity performed to volitional fatigue at an intensity near LT with CAF. Specifically, CAF allowed sedentary individuals to complete moderate exercise with less perceived effort and improved tolerance to sustain exercise which could potentially influence energy expenditure during activity and facilitate gains in fitness over time.

In studies that reported CAF benefits in less fit subjects [82, 83], no effects on substrate oxidation [83], blood lactate or free fatty acids [82] were observed. The present study, however, observed higher lactate with CAF vs. no-CAF after 30 min of moderate exercise in all subjects, but this did not affect their ability to exercise longer before reaching volitional fatigue with CAF. While CAF resulted in a small but significant increase in CHO oxidation during MOD-EX (~3 g CHO in 30 min) compared to no-CAF, it did not alter the relative contribution of CHO to total EE during MOD-EX in either group. Our findings are in partial agreement with others [199] indicating increased exogenous CHO oxidation during 2 h cycling exercise after ingestion of 5 mg/kg-h CAF with glucose, although this was attributed to increased intestinal absorption of glucose with CAF even though blood glucose concentration was not increased. While our results indicate ET had higher overall fat oxidation compared to SED, we did not observe a CAF effect on fat oxidation in either group, consistent with Ivy et al. [116]; although others [103, 104, 200] observed increased lipolysis with CAF in endurance trained individuals. Chronic endurance training results in increased capacity for fat oxidation through mechanisms such as higher hormone sensitive lipase induced lipolysis, increased mitochondria and capability for beta-oxidation, and greater inhibition of glycolytic enzymes [201]. Thus, it stands to reason that sedentary individuals would have less capacity to enhance fat oxidation even with CAF. It has also been suggested [98] that trained individuals may have increased adenosine receptor sensitivity in adipose tissue compared to untrained or higher muscle sensitivity to CAF, which could imply that sedentary individuals may require a higher CAF dose (>3 mg/kg) to increase fat

metabolism [82, 115]. Regarding a metabolic basis for CAF effects, although enhanced fat metabolism is unlikely, we cannot rule out impact on CHO metabolism.

CAF ingestion resulted in higher lactate for all subjects after 30 min of MOD-EX and TTF, compared to no-CAF. These results are consistent with previous work indicating, compared to placebo, 4-5 mg/kg CAF increases blood lactate in both physically fit and unfit adults [103, 104, 202]. While we did not measure catecholamine levels, Collomp et al. [103] demonstrated a strong correlation between epinephrine and lactate, suggesting CAF increases glycolytic processes via increased epinephrine and/or increased lactate release from the muscle [203]. Increased epinephrine could also explain our findings that CAF increased HR during MOD-EX in the trained group.

Independent of CAF ingestion, blood glucose was higher in sedentary compared to trained after 15 min moderate exercise, likely as a result of ingesting ~40 g CHO prior to exercise and/or a higher rate of glucose uptake by skeletal muscle by trained during exercise [204] compared to sedentary. However, by the end of both 30 min MOD-EX and TTF, blood glucose was similar in sedentary and trained. A limitation of the present study is that glycemic response to the pre-exercise “feeding” and treatment beverage was not serially measured prior to exercise, and we are uncertain of the impact that the pre-exercise feeding with or without 3 mg/kg CAF had on insulin sensitivity in each group. Previous research indicates CAF (3-5 mg/kg) ingested 1 h prior to an oral glucose tolerance test (75 g glucose ingested under sedentary conditions) can result in ~26% increase in insulin response [24] concomitant with decreased insulin sensitivity in young healthy subjects. The reported metabolic impairments resulting from CAF ingestion are likely due to a combination of CAF effects on glucose delivery, membrane signaling, and

intracellular signaling and processing [24]. These insulin-dependent processes are more relevant and affected by CAF in the sedentary state because muscular contraction during exercise triggers GLUT4 translocation to skeletal cell membranes for insulin-independent glucose uptake.

Despite the growing popularity of caffeinated low-calorie “energy drinks”, only a few studies have compared the efficacy of CAF with low-CHO to placebo [91, 92, 172] to understand if the ergogenic benefits are attributed to CAF. Previously, we found that low-CHO ingestion attenuated mental fatigue compared to a CHO mouth rinse in a fasted state (unpublished doctoral dissertation). In the present study, a small amount (<2 g CHO in a 0.4% CHO solution) was ingested along with a ~40 g CHO energy bar prior to exercise; and, 100 ml of the 0.4% CHO solution was ingested in small increments during exercise. We did not observe any effect of low-CHO on exercise capacity (TTF) compared to PLA (ES = -0.09). When 3 mg/kg CAF was added to low-CHO, there was also no effect on TTF compared to CAF alone (ES = 0.08). Thus, it appears that ergogenic effects of the treatments in the present study can primarily be attributed to a moderate CAF dose but not ingestion of low-CHO in the fed state.

MVC strength and electrically evoked strength of the right knee extensors were measured before and after exercise in order to determine whether CAF might influence mechanisms underlying physical fatigue differently in trained compared to sedentary.. MVC strength decreased in all subjects between baseline and after exercise but the average loss tended to be higher in sedentary vs. trained. After moderate exercise, electrically evoked strength only decreased in sedentary, whereas voluntary activation only decreased in trained. These findings suggest peripheral fatigue in the knee extensors

may have been higher in sedentary, whereas the decline in MVC strength in trained appeared due to lower central drive. However, since we did not make all measures after volitional fatigue, these explanations are speculative. CAF tended to maintain MVC strength following TTF better for trained than for sedentary. The lack of CAF effect on MVC strength and electrically evoked measures in the present study may be attributed to the lower dose of CAF [146, 205] and/or that our participants were not in similar states of fatigue as in previous studies [146, 205]. Our muscular strength data were also highly variable, due to the inclusion of both men and women of mixed fitness levels; and, our trained participants were not all cyclists (included runners and swimmers); whereas MVC strength maintenance with CAF [146] tested highly trained cyclists.

Conclusion

A moderate dose of caffeine (3 mg/kg) reduced perceived effort during moderate intensity exercise and improved exercise capacity and tolerance (longer duration of cycling prior to rating “very hard” effort) for sedentary and trained individuals. However, low-carbohydrate did not provide benefit to exercise capacity in either group. The metabolic effects of caffeine combined with a pre-exercise energy bar did not increase fat utilization during exercise; but appears to impact carbohydrate oxidation, blood lactate and glycemic responses. Caffeine tended to maintain maximum voluntary isometric strength after exercise but did not appear to affect neuromuscular activation or electrically evoked properties. Thus, caffeine appeared to act centrally (perception of effort) versus peripherally (direct skeletal muscle activation). Although caffeine appears efficacious, additional research is warranted to understand the optimal dose of pre-

exercise caffeine (with and without carbohydrate) for habitually sedentary individuals to improve exercise tolerance without excess caloric intake or adverse metabolic consequences.

CHAPTER 4

EXERCISE AND CAFFEINE IMPROVE SUSTAINED ATTENTION FOLLOWING FATIGUE INDEPENDENT OF FITNESS STATUS

Abstract

Background: Exercise improves cognition, but whether fitness status and caffeine modulate this effect remains unclear. Purpose: To determine if sustained attention is improved following exercise with and without caffeine in endurance-trained versus sedentary adults. Methods: A Continuous Performance Task (CPT) induced mental fatigue over 20 min and assessed accuracy and precision. Following the 20 min CPT, trained and sedentary participants completed 30-min rest (REST) or moderate-intensity cycling (EX) below lactate threshold. EX trials were completed with placebo and caffeine (3 mg/kg) and followed by cycling to volitional fatigue (FATIGUE). Results: EX improved ($p < 0.05$) accuracy and precision after 20 min CPT (by $9.0 \pm 6.3\%$, $22.5 \pm 16.6\%$) compared to REST ($3.7 \pm 8.0\%$, $6.9 \pm 22.3\%$) and was not different between groups. Accuracy and precision declined ($p < 0.05$) with placebo during CPT but were maintained with caffeine resulting in higher accuracy and precision following EX (by $1.9 \pm 4.1\%$, $5.2 \pm 12.7\%$) and FATIGUE (by $2.8 \pm 6.5\%$, $7.8 \pm 19.6\%$) versus placebo. Mental energy declined after CPT with placebo but not caffeine; but compared to EX, FATIGUE resulted in lower mental energy/ greater mental fatigue for both treatments. Conclusions: EX improved sustained attention following mental fatigue independent of fitness status; and, when coupled with caffeine, provided greater benefit to accuracy, precision, and mental energy. Although caffeine's beneficial effect on sustained attention persisted after FATIGUE, it did not prevent increased perceived mental fatigue or decreased mental energy.

Introduction

Fatigue may be manifested by an increased perception of effort to complete a task or the inability to sustain a task. Mental fatigue has previously been defined as a “psychobiological state caused by prolonged periods of demanding cognitive activity” and “characterized by subjective feelings of lack of energy”. [2, 3] Surveys conducted within the U.S. workforce [1] indicate a 38% prevalence of fatigue, with 66% of these workers reporting lost productivity time, resulting in an overhead cost to employers of > \$100 billion annually. Mental fatigue can be observed by the impairment of cognitive function, particularly tasks that require sustained attention or the ability to direct and focus cognitive ability over a period of time. [2, 4, 5]

The acute effect of exercise on cognition and perceived energy appears beneficial [55, 206, 207] due to increased neural activity, [57] arousal, and allocation of neural resources [54, 58-60]. Sustained attention, one aspect of cognition that reflects energy and fatigue processes, [37] is improved immediately following exercise [62] along with self-assessed feelings of energy. [206] However, the optimal exercise intensity that elicits greater perceived energy and sustained attention is unclear. High intensity activity may result in feelings of physical fatigue that persist after exercise, masking potential reductions in mental fatigue or increases in positive affect that have been observed after low-moderate intensity exercise. [206, 208]

Fitness status may also influence the emotional or psychological response to exercise of varying intensities. [54] Twenty to 60 min of moderate-intensity exercise near lactate threshold consistently improves simple and complex cognitive tasks in trained individuals; [59] whereas, less fit individuals may experience greater anxiety during or

after high intensity exercise, contributing to lower task performance. [65, 66] Training status may influence brain hemodynamics and neural activity associated with impairment in certain cognitive tasks in untrained (vs. trained) individuals participating in high intensity exercise [209]. However, the basis to prescribe an exercise intensity that elicits similar efforts across groups of individuals with different fitness status is a design issue that has been primarily addressed by utilizing either relative oxygen uptake or maximum heart rate [67].

In addition to exercise, caffeine may also benefit cognition and perception [107-110] through psychological effects serving to enhance task persistence and determination [94, 111] and physiological effects in the central nervous system (CNS). Caffeine acts as a stimulant potentiating sympathetic activity, may reduce mental fatigue, [30, 33] and improves higher order cognitive measures such as sustained attention [109, 110, 113, 123] with a concomitant increase in perceived mental energy.

Therefore, acute exercise and caffeine ingestion may each influence the CNS resulting in attenuated mental fatigue and improved attention. The interactions of these and other modulating factors (i.e., fitness status, exercise intensity, and physical fatigue) on mental fatigue, mental energy, and attention remain to be clarified. Thus, our purpose was to determine: (1) the effect of moderate intensity exercise on sustained attention, using individual lactate threshold as the basis for exercise prescription, in comparison to a similar duration of seated rest in endurance-trained versus sedentary adults; and 2) whether caffeine ingestion provides additional benefit to sustained attention, perceived mental energy and mental fatigue when combined with exercise. We hypothesized that moderate intensity exercise would benefit sustained attention independent of fitness

status; however, unlike trained individuals, sedentary subjects would experience impaired attention following exercise performed to fatigue. Furthermore, we also hypothesized that, compared to non-caffeinated placebo, caffeine would maintain sustained attention and, when combined with exercise, would maintain sustained attention and associated perceptual measures (mental energy and mental fatigue) for all subjects.

Methods

Participants

Twelve endurance-trained males (n=10) and females (n=2) and an equal number of healthy sedentary males and females volunteered for this study. Participants provided written informed consent as approved by the University Institutional Review Board. Physical characteristics of subjects are presented in Table 6. Endurance-trained participants were recruited from the local endurance sports community and reported training \geq six hours per week. Sedentary participants were recruited from the college campus and spent \leq 60 min per week in low intensity activity (e.g., riding a bike to class) and did not participate in any regular exercise. Exercise training history via a screening questionnaire and $VO_{2\text{ peak}}$ criteria (< 50 ml/kg-min for sedentary men, < 40 ml/kg-min for sedentary women) stratified participants into groups. One sedentary male was slightly above this cut point for $VO_{2\text{ peak}}$ but his activity status was verified as meeting sedentary criteria.

Typical weekly physical activity was also validated using the Core and Expanded Physical Activity STEPS version 2.0 Instrument. [210] Trained and sedentary individuals were matched pairwise by gender, age (within 3 yr), and BMI (within 2 kg/m²) and, thus, groups differed ($p < 0.05$) only in body composition (% body fat), maximal aerobic

capacity ($\text{VO}_{2 \text{ peak}}$), % $\text{VO}_{2 \text{ peak}}$ elicited at lactate threshold and minutes of exercise per week. Groups were similar in non-exercise physical activity (i.e. transport activities such as walking).

All subjects completed a health-history screening questionnaire and typical caffeine usage questionnaire for a 7-day period [56] to ensure they met all inclusion criteria. Exclusion criteria eliminated subjects who were either naïve to caffeine or on the “high” range [131] of caffeine use (i.e., consuming > 500 mg /day). The trained group tended to have higher reported habitual caffeine intake than sedentary subjects, but the groups were not significantly different (Table 6) and all participants were considered low-to-moderate caffeine users. These classifications also appear to be physiologically valid based on brain activation [57], not just population normative values.

Table 6

Physical characteristics (mean \pm SD) of subjects.

	Trained (n= 12)	Sedentary (n= 12)
Age (yr)	27.7 \pm 5.5	26.8 \pm 7.0
BMI (kg/m^2)	23.1 \pm 2.1	23.7 \pm 2.9
Body mass (kg)	72.2 \pm 8.3	72.7 \pm 11.4
Body fat (%)	14.1 \pm 5.8	22.6 \pm 0.1*
$\text{VO}_{2 \text{ peak}}$ ($\text{ml}/\text{kg}\cdot\text{min}$)	55.6 \pm 8.5	38.2 \pm 9.1*
Peak heart rate (bt/min)	181 \pm 10	185 \pm 12
Peak power output (PPO) (Watts)	319 \pm 69	217 \pm 62*
% $\text{VO}_{2 \text{ peak}}$ at LT	75.7 \pm 10	67.5 \pm 9.1*
Average reported exercise (min/wk)	753 \pm 364	37 \pm 56*
Average reported transport time (min/wk)	130 \pm 136	108 \pm 106
Average daily caffeine intake (mg/d)	215.7 \pm 155.6	110.8 \pm 98.1
Minimum-maximum caffeine intake (mg/d)	31.1 – 450.1	31 – 291.3
% subjects with caffeine intake < 218 mg (treatment)	50	83.3

* $p < 0.05$ indicates difference versus trained subjects.

Research Design

Each subject served as his or her own control performing four prolonged exercise trials consisting of a 20 min mental task followed by 30 min moderate exercise (EX) and subsequent exercise bout to FATIGUE. Two of the trials were with caffeine and two with placebo. A “placebo effect” may factor into treatment studies, particularly for individuals who have expectations that caffeine is beneficial. Thus, in order to address placebo effects and minimize potential day-to-day variation in mental fatigue or learning effects, we utilized a double-blind, crossover design with treatment order assigned using a Latin Squares method. Mean scores were averaged across two caffeine+exercise and two placebo+exercise trials to reduce variability and bias from either subject or investigator.

In order to compare passive “recovery” following a 20 min mental task, a subgroup of subjects (n=20) performed a 5th control REST trial with placebo. This control trial (Figure 14 top line) was identical to the first portion of the exercise trials through 30 min of moderate exercise with placebo; but, instead subjects sat quietly (performing no mental work) for 30 min. The control trial was not “blinded” or fully counterbalanced among the four exercise trials.

Preliminary Testing and Familiarization Protocol

On the first laboratory visit, body mass and height were measured and body composition (% body fat) estimated using the GE Lunar Prodigy Dual X-Ray Absorptiometry (DXA) scanner (GE Healthcare, Hatfield, United Kingdom). Then, participants completed a ramped exercise protocol to volitional fatigue on an electrically-braked Lode Excalibur Sport cycle ergometer (Lode, Groningen, The Netherlands) to assess VO_2 peak. Participants warmed up for 5 min between 50 to 100 W, followed by

workload increases of 25 to 50 W every 2 min until volitional fatigue. Gas exchange data were obtained using the ParvoMedics True One 2400 metabolic cart (ParvoMedics, Sandy, UT) and rating of perceived exertion (RPE) [40] recorded every min. Subjects met criteria for a maximal test based on similar peak respiratory exchange ratio (1.16 ± 0.05 , 1.15 ± 0.11) and RPE (18.3 ± 1.7 , 17.7 ± 2.4) for trained and sedentary, respectively. After a cool down, participants were familiarized with the cognitive task.

In order to assign comparable exercise workloads for sedentary and trained individuals, each participant's lactate threshold was determined on the second visit, using a ramped cycling protocol. Participants began cycling at 50 W and workload increased by 25 to 50 W every three min [193] until reaching a RPE of 16-17 ("Hard to Very Hard"). Blood lactate was measured at baseline and following each stage using 0.3 μ L blood samples obtained from the ear lobe (Lactate Pro LT-1710 analyzer, Arkray, Japan). Each individual's lactate threshold (LT) was determined using the DMax method [193, 194] to calculate a moderate (10%<LT) and vigorous (5%>LT) intensity workload for the 30-min moderate EX and time to FATIGUE portions of the protocol, respectively.

After the lactate threshold test, participants cooled down before being familiarized with the predetermined moderate and vigorous workloads for 10 min. If, during familiarization, RPE reached > 16 ("very hard") during moderate and/or > 18 ("extremely hard") during vigorous, or the subject felt uncomfortable with the workload, it was adjusted by 5% until the subject reported a RPE in the target range (below "somewhat hard") for moderate and ("hard") for vigorous. Workload adjustments were made for four trained and three sedentary participants. All pre-test instructions (obtaining adequate sleep, hydration, and refraining from caffeine intake and exercise prior to

subsequent experimental sessions) were given to subjects. Trained participants continued to engage in their typical endurance training over the course of the study; however, we requested they refrain from exceptionally strenuous training or racing in the three days prior to experimental sessions, similar to pre-race preparation.

Experimental Protocol

The next five visits (four exercise, one resting control) were each separated by a minimum of 7 d and scheduled at the same time of the morning following an overnight fast. Before each visit, participants refrained from exercise for 24 h and caffeine for 12 h. Upon arrival to the lab, a 24 h diet recall, 24 h history questionnaire, and visual analog scales (VAS) for rating perceived mental energy and fatigue were completed. Reported sleep prior to each trial was similar between trained and sedentary (7.0 ± 0.6 h and 6.9 ± 0.6 h) and prior to caffeine (6.8 ± 0.6 h) and placebo (7.0 ± 0.7 h) trials. To simulate the practical scenario of the “fed state”, a nutritional bar (PowerBar® Harvest Energy, PowerBar USA, Florham Park, NJ) was given along with treatment beverages. The bar contained 250 kcal, 5 g fat, 42-43 g carbohydrate, 9 g protein, no caffeine, and given in complete packaging so participants could view the nutrition facts label. After the first visit, the 24 h diet recall was copied and returned to the participant to use as a guide for replicating 24 h dietary intake before the subsequent visits.

The schematic of the test protocol is illustrated in Figure 14. In the control trial (Figure 14 top line) comparing 30 min REST vs. EX, placebo was given 20 min prior to a Continuous Performance Task (CPT). Following a 20 min CPT, participants completed 30 min of quiet REST or EX before repeating a 5 min CPT. EX consisted of 30 min moderate intensity cycling ($10\% < LT$) designed to elicit RPEs below 13 or “somewhat

hard” which was accomplished (12.4 ± 1.2 and 12.0 ± 0.9 for sedentary and trained, respectively).

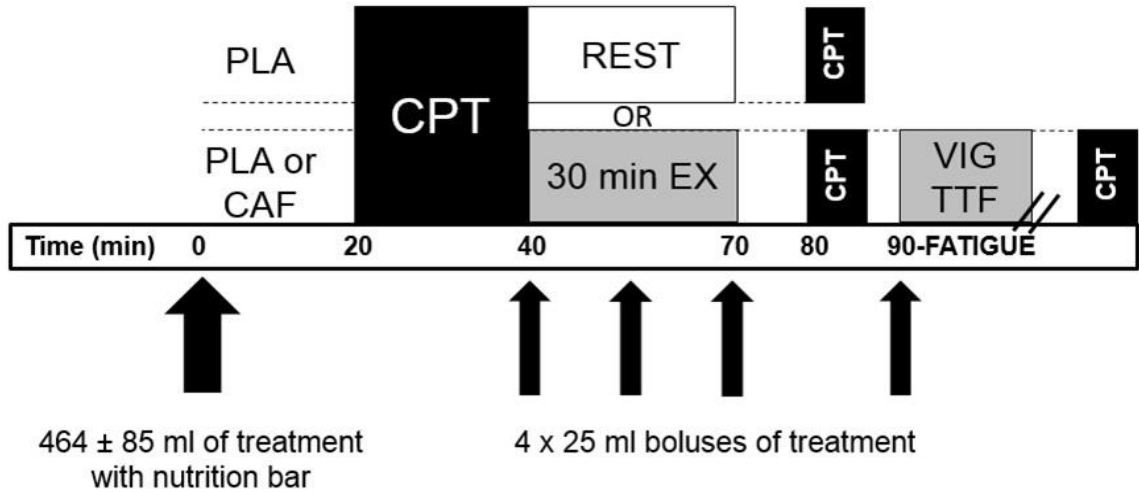


Figure 14. Schematic of the Test protocol. Top row indicates protocol for control REST condition (n=20) compared to 30 min moderate cycling condition (EX) following placebo (PLA) ingestion only. Lower row indicates full test protocol (n=24) comparing caffeine (CAF) versus non-caffeinated PLA treatment prior to EX and vigorous cycling to FATIGUE (VIG TTF). Periodic assessments of sustained attention using the Continuous Performance Test (CPT) are indicated. Bold arrows indicate timing of treatment. Reprinted with permission from *Fatigue: Biomedicine, Health, and Behavior*. Copyright 2015, IACFS/ME.

In the full exercise experimental protocol (Figure 14 lower line), two caffeine+exercise and two placebo+exercise trials were completed. In order to give sufficient time for caffeine absorption [58, 59], participants waited 20 min following treatment ingestion prior to completing the initial CPT. Following the 20 min CPT, 30 min EX was completed and followed by a 5 min CPT. Then, a subsequent cycling task at

an intensity 5% >LT to volitional FATIGUE was completed, eliciting an overall RPE of ~ 16 or “hard” for both groups. The point of FATIGUE was defined as voluntarily termination of exercise at participants’ request and/or the inability to maintain a minimum cadence of 40 rpm. Cycling time to FATIGUE was typically < 30 min for trained (23.8 ± 8.1 min) and sedentary (24.1 ± 11.3 min) subjects, but mean workload was lower by ~80 W in sedentary. Following FATIGUE, participants repeated a final 5 min CPT.

Treatment Ingestion

Fruit punch solutions were manufactured and provided (Glaxo Vitaminwater®, Whitestone, NY) in de-identified containers with either 0.34 g/L caffeine or placebo to maintain blinding of the investigators. Fluid volumes were administered based on individual’s body mass to provide ~3 mg/kg caffeine or equivalent placebo (464 ± 85 ml prior to moderate exercise or rest). This amount of caffeine (~218 mg) is similar to the average reported intake of adults in the U.S. [34]. A dose of 3 mg/kg of caffeine has been observed to be efficacious for mental tasks [30] and ingestion was timed in order to potentially observe caffeine effects towards the latter half of the 20 min CPT (30-40 min after ingestion). [58, 59] During moderate EX, three 25 ml boluses were given (25.5 mg caffeine in caffeine+exercise) and another 25 ml bolus to minimize “dry mouth”, offset sweat loss and ensure a 3 mg/kg caffeine dose prior to starting the ride to FATIGUE.

State-Trait Energy and Fatigue (STEF) Scale

The STEF subjective survey instrument, a 10 cm VAS, assessed the intensity of “state of mental energy and fatigue” [110] when participants arrived to the lab prior to

treatments and immediately after each CPT. Mental energy subscales included “energetic, vigorous, and full of pep” items and mental fatigue subscales included “fatigued, exhausted, and worn-out”. Low and high VAS anchors referred to the absence of a feeling or to the strongest feeling ever felt, respectively. Subscale items were summed and averaged to generate a numerical score for mental energy and mental fatigue.

Continuous Performance Task (CPT)

We administered a version of the CPT programmed and presented with MATLAB® v2011 (MathWorks, Natick, MA, USA) [183, 184]. This task requires sustained attention, working memory, response inhibition, and error monitoring, [185] and has been used in other motor-related and exercise studies to induce mental fatigue. [3, 186] During the task, a series of letters were visually presented in the middle of a computer screen for 0.6 s and participants were instructed to click a computer mouse upon seeing the target letter ‘T’. Participants were seated at a standard computer desk with the desktop monitor a fixed length from the chair and consistent lighting for all trials. The task and order of letter presentation was fixed and identical across all trials. The remaining letters of the alphabet served as non-target probes. Attention was required to click on target letters (true positives) while inhibiting response to non-targets. Letters were 1.5 inches in height and appeared in white capital font on a black background at a rate of 1.67 Hz or 100 letters/min. Each 5 min epoch consisted of 500 letters, 127 target letter ‘T’s of which 59 were preceded by a bait letter ‘S’, and 373 non-targets of which 14 were preceded by the bait letter ‘S’. Signal detection sensitivity was 0.81, indicating participants were sensitive to the difference between targets and non-targets.

Dependent variables obtained from the task were true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN). Response accuracy was calculated as $= (TP+TN)/(TP+TN+FP+FN)$ and precision $= TP/(TP+FP)$. Together, accuracy and precision were used as measures of sustained attention. Reduced inhibition and increased FP would be reflected in lower precision. Although we did not specifically program for reaction time, it is indirectly assessed in this task. Impaired reaction time would result in FN and possibly additional FP, thus lowering accuracy and/or precision. Accuracy and precision were averaged over 5 min blocks but comparisons were ultimately based on: 1) first 5 min of 20 min CPT (*Baseline-5 min CPT*), 2) last 5 min of 20 min CPT (*Post-20 min CPT*); and 3) the 5 min CPT after REST or EX; or, after FATIGUE. The relative change over two time points was calculated for accuracy and precision using the equation: $((\text{Post} - \text{Pre})/\text{Pre}) \times 100$ where “Pre” is the earlier time point; and, “Post” is the later time point.

Statistical Analyses

Data are reported as mean \pm standard deviation (SD) and were analyzed using SPSS 17.0 (Chicago, IL). Three-way (group x condition x time) mixed model ANOVA (trained versus sedentary group as the between-subject factor and REST versus EX condition as the within-subject factor with repeated measures over time) was used to examine differences in accuracy, precision, perceived mental energy and fatigue in control REST versus a single placebo+exercise trial. Three-way (group x treatment x time) mixed model ANOVA (caffeine versus placebo treatment as the within-subject factor with repeated measures over time) was used to examine differences in accuracy, precision, perceived mental energy and fatigue in caffeine+exercise versus

placebo+exercise trials. The Greenhouse-Geisser correction was used to account for the sphericity assumption of unequal variances across groups.

If a significant F ratio was obtained, the Bonferroni post hoc test was used to detect significant differences in pairwise comparisons. Two-way (between factor condition or treatment; within factor time) repeated measures ANOVA with Bonferroni post hoc pairwise comparisons at each time point and between time points were used to examine differences in mental energy, mental fatigue, and, when significant interactions were present, for accuracy and precision. Pearson product moment correlations were used to examine if habitual caffeine intake was associated with dependent variables. Statistical significance was set at an alpha level of $p < 0.05$.

Results

Effect of 30 min Moderate Exercise (EX) versus REST Control on Sustained Attention

Based on mixed model ANOVA (between factor group; within factors condition and time), changes in accuracy and precision in the EX and REST control trial were not significantly different between sedentary ($n=10$) and trained ($n=10$) groups ($p = 0.65$ and $p = 0.67$, respectively). Thus, condition (EX, REST) and time effects were analyzed with subjects combined using a two-way repeated measures ANOVA.

There was a significant main effect for time ($p < 0.001$) and a significant condition x time interaction for accuracy ($p = 0.02$). Accuracy declined during the initial 20 min CPT to a similar degree in both conditions. Accuracy declined ($p < 0.001$) from *Baseline-5 min CPT* to *Post-20 min CPT* prior to 30 min REST ($93.7 \pm 6.7\%$ to $89.6 \pm$

8.4%, or a relative Δ score of $-4.5 \pm 4.6\%$) and prior to EX ($91.2 \pm 8.4\%$ to $86.5 \pm 8.4\%$, or $-5.0 \pm 4.7\%$). Compared to *Post-20 min CPT*, the improvement in accuracy was higher ($p = 0.03$) after EX by $9.0 \pm 6.3\%$ compared to $3.7 \pm 8.0\%$ after REST (Figure 15, top panel), although absolute accuracy score was not different ($p = 0.41$) between conditions. EX and REST both restored accuracy to *Baseline-5 min CPT* although relative change in accuracy after EX tended ($p = 0.08$) to be above *Baseline-5 min CPT* (Δ of $3.4 \pm 6.7\%$) compared to after REST ($-1.1 \pm 7.9\%$).

There was also a significant main effect for time on precision ($p < 0.001$) and a significant condition x time interaction ($p = 0.02$). Unlike accuracy, precision only declined significantly (Δ of $-10.9 \pm 11.1\%$, $p < 0.001$) during the initial 20 min CPT (*Baseline-5 min CPT* to *Post-20 min CPT*) in the trial preceding the EX condition but not prior to REST ($-4.1 \pm 17.8\%$, $p = 0.08$). Compared to *Post-20 min CPT*, the improvement in precision was higher ($p = 0.03$) after EX (Δ of $22.5 \pm 16.6\%$) compared to REST ($6.9 \pm 22.3\%$) (Figure 15, bottom panel). EX and REST both restored precision to their respective *Baseline-5 min CPT* levels although precision after EX tended ($p=0.14$) to be higher (Δ of $8.7 \pm 18.9\%$) compared to after REST ($1.1 \pm 20.9\%$).

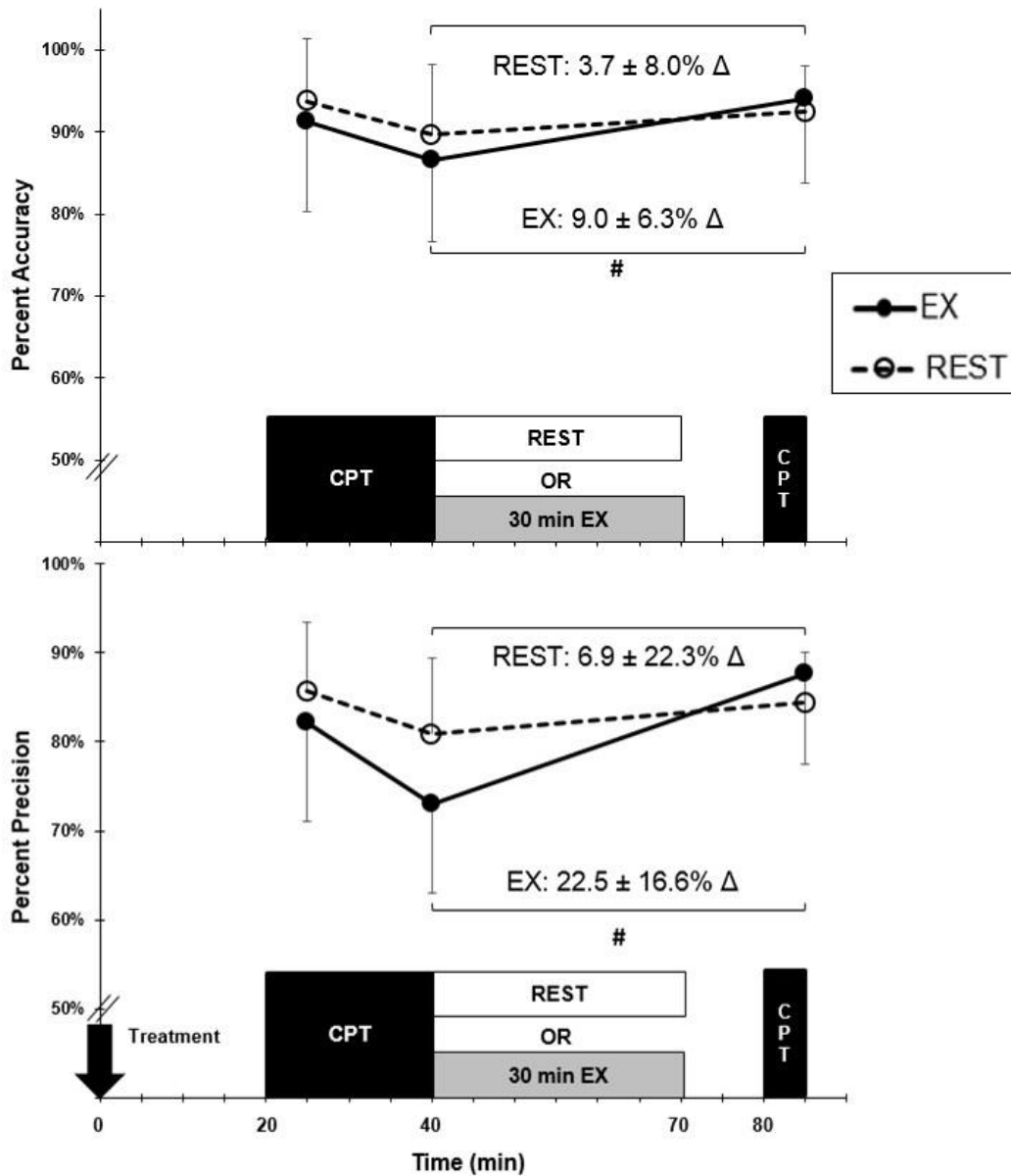


Figure 15. Mean (\pm SD) accuracy (ACC) (top panel) and precision (PREC) (bottom panel) from baseline, following 20min CPT compared to 30 min moderate intensity exercise (EX) versus REST. # indicates significantly larger ($p < 0.05$) relative change after EX versus REST compared to the end of 20 min CPT.

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Effect of Caffeine Combined with EX and FATIGUE on Sustained Attention

Changes in accuracy and precision in response to caffeine and placebo treatments were not significantly different between sedentary (n=12) and trained (n=12) ($p = 0.30$ and $p = 0.31$ in accuracy and precision, respectively). Thus, treatment and time effects were analyzed with subjects combined to compare caffeine vs. placebo on sustained attention before and after exercise (Figure 16).

There was a main treatment effect with caffeine eliciting higher overall accuracy ($p = 0.002$) and precision ($p = 0.002$) compared to placebo. There was also a treatment x time interaction for accuracy ($p < 0.001$) and precision ($p < 0.001$). No differences were present at *Baseline-5 min CPT*. Figure 16 reflects a progressive decline from *Baseline-5 min CPT* to *Post-20 min CPT* with placebo, but caffeine blunted this decline. Compared to *Post-20 min CPT*, caffeine maintained accuracy ($92.3 \pm 6.9\%$ to $91.2 \pm 5.9\%$, $p = 1.0$ or Δ of $1.0 \pm 4.6\%$) and precision ($84.3 \pm 13.7\%$ to $82.2 \pm 11.5\%$, $p = 1.0$ or Δ of $-0.9 \pm 14.4\%$) relative to *Baseline-5 min CPT*; whereas with placebo, accuracy and precision declined by $-5.1 \pm 5.8\%$ ($91.3 \pm 7.9\%$ to $86.4 \pm 8.2\%$, $p < 0.001$) and $-9.6 \pm 18.5\%$ ($82.3 \pm 15.9\%$ to $72.8 \pm 16.0\%$, $p < 0.001$), respectively.

Thereafter, accuracy remained $1.9 \pm 4.1\%$ higher with caffeine vs. placebo after EX ($95.5 \pm 4.0\%$ vs. $93.9 \pm 6.1\%$, $p = 0.02$) and $2.8 \pm 6.5\%$ higher after FATIGUE ($96.1 \pm 2.9\%$ vs. $93.8 \pm 6.0\%$, $p = 0.04$). Precision also remained $5.2 \pm 12.7\%$ higher with caffeine vs. placebo after EX ($90.5 \pm 7.8\%$ vs. $87.3 \pm 11.9\%$, $p = 0.03$) and $7.8 \pm 19.6\%$ higher after FATIGUE ($91.8 \pm 5.7\%$ vs. $87.1 \pm 12.0\%$, $p = 0.04$). Significant caffeine effects ($p < 0.002$) were also observed for all components of accuracy and precision (TP, TN, FP,

FN) in all subjects; but, there was no time effect or treatment x time interaction.

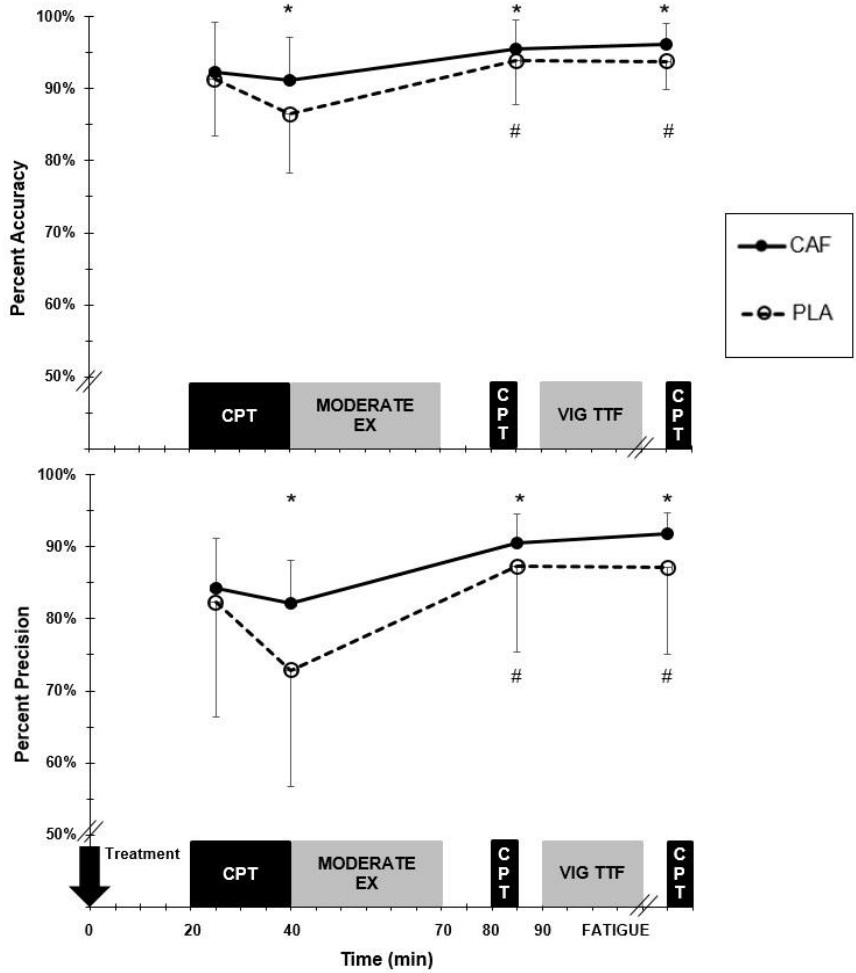


Figure 16: Caffeine (CAF) attenuated the decline in mean (\pm SD) accuracy (top panel) and precision (bottom panel) compared to placebo (PLA) and remained higher throughout exercise for all subjects. * Higher ($p < 0.05$) with CAF compared to PLA. # Significant ($p < 0.01$) time effect with both CAF and PLA where ACC and PREC were higher after moderate exercise (EX) and vigorous cycling to FATIGUE (VIG TTF) compared to pre-exercise (Baseline and Post 20 min CPT).

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There was also a main effect of time for accuracy ($p < 0.001$) and precision ($p < 0.001$). For both caffeine and placebo, accuracy ($p \leq 0.005$) and precision ($p \leq 0.006$) were higher after both EX and FATIGUE compared to both pre-exercise time points.

The accuracy and precision in responses following the “bait” letters (14.6% of all letters) were also examined. There was a main effect for caffeine ($p = 0.003$) eliciting higher accuracy ($88.6 \pm 8.3\%$) compared to placebo ($84.2 \pm 12.2\%$); however, precision remained high and was not different ($p = 0.27$) between caffeine ($99.1 \pm 1.0\%$) and placebo ($98.9 \pm 1.0\%$).

Effect of Fitness Status, Caffeine, and Exercise on Perceived Mental Energy

Rating of mental energy over time was not different between trained and sedentary groups ($p = 0.09$), but there was a significant group x time interaction ($p = 0.04$) (Figure 17 top panel) due to a higher Pre-treatment mental energy rating for sedentary ($p = 0.01$) compared to trained. This group difference remained consistent at Post-20 min CPT ($p = 0.04$) independent of treatment. Due to the significant group x time interaction, one-way repeated measures ANOVA (within factor time) was conducted for each group to examine change in mental energy over time. For trained individuals, significant time effect ($p = 0.03$) indicated mental energy was significantly higher ($p \leq 0.01$) after EX compared to both pre-exercise time points and FATIGUE; but, mental energy at FATIGUE was not different compared to pre-exercise time points. For sedentary, significant time effect ($p < 0.001$) indicated mental energy did not change after EX compared to *Pre-treatment* and *Post-20 min CPT*; but, mental energy was significantly lower ($p < 0.05$) at FATIGUE compared to all previous time points (Figure 17 top panel).

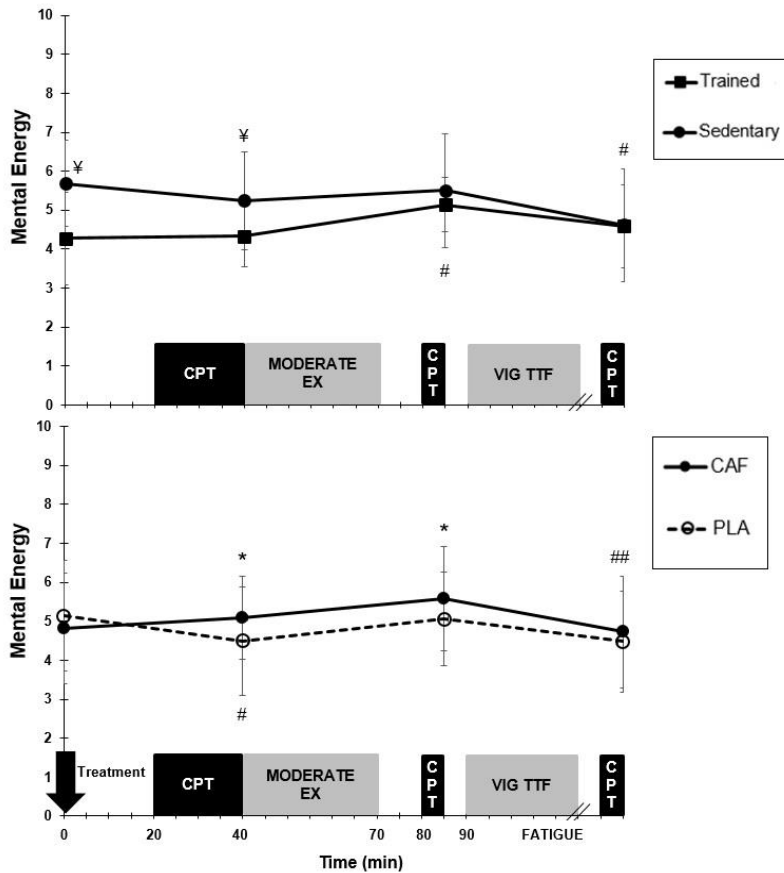


Figure 17. Mean (\pm SD) Visual Analog Scale ratings for mental energy by fitness group (top panel) and nutritional aids (bottom panel). Top panel: ¥ Higher ($p < 0.05$) mental energy for sedentary compared to trained at Baseline and after 20 min CPT. # significant time effect ($p \leq 0.01$) in trained after moderate exercise (EX), compared to all other time points; and, lower ($p < 0.05$) mental energy in sedentary after vigorous cycling to FATIGUE (VIG TTF) compared to all other time points. Bottom panel: * Higher ($p < 0.05$) mental energy with caffeine (CAF) compared to PLA. # Significant ($p < 0.05$) time effect from Baseline to Post 20 min CPT with placebo (PLA) but not caffeine (CAF). ## Significant ($p < 0.05$) time effect from EX to FATIGUE with PLA and CAF.

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There was no main effect of treatment although caffeine tended ($p = 0.06$) to result in higher mental energy compared to placebo overall. There was, however, a significant treatment x time interaction ($p = 0.004$). Caffeine mitigated the decrease in mental energy from *Pre-treatment* to *Post-20 min CPT* that occurred with placebo (Figure 17 bottom panel). However, mental energy decreased with both caffeine ($p = 0.02$) and placebo ($p = 0.007$) between EX and FATIGUE.

Effect of Fitness Status, Caffeine, and Exercise on Perceived Mental Fatigue

Ratings of mental fatigue over time were similar between trained and sedentary ($p = 0.57$), but there was a significant group x time interaction ($p = 0.003$) (Figure 18 top panel). Trained participants rated similar mental fatigue across time (*Pre-treatment*, *Post-20 min CPT*, after EX, and FATIGUE); however, sedentary rated significantly higher mental fatigue after FATIGUE compared to all previous time points ($p < 0.01$).

Because there was no between-group difference in rating of mental fatigue, treatment and time effects were analyzed with subjects combined to compare the effect of caffeine vs. placebo. There was no treatment effect ($p = 0.90$) or treatment x time interaction ($p = 0.06$), but there was a time effect ($p < 0.001$). Mental fatigue did not differ between *Pre-treatment*, *Post-20 min CPT*, and after EX. However, after FATIGUE, mental fatigue was rated significantly higher ($p < 0.05$) compared to all previous time points with both caffeine and placebo (Figure 18 bottom panel).

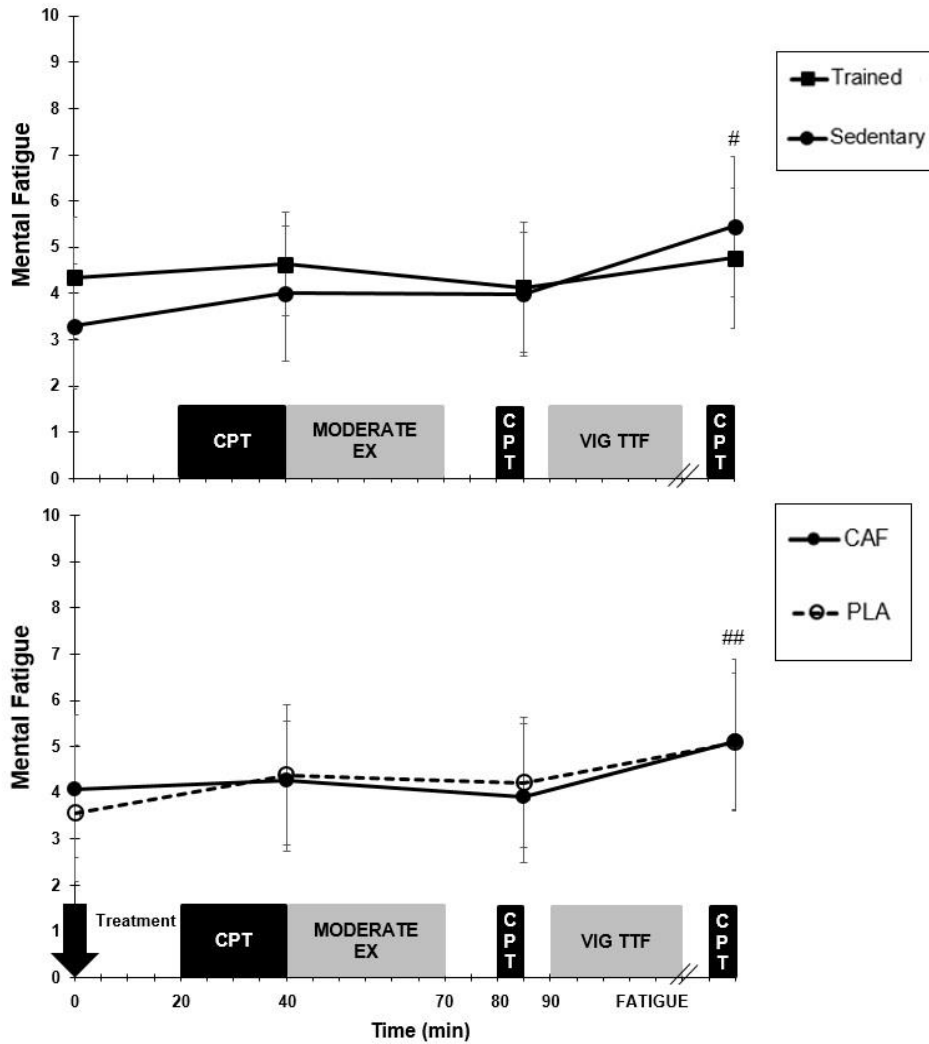


Figure 18. Mean (\pm SD) Visual Analog Scale ratings for mental fatigue by fitness group (top panel) and nutritional aids (bottom panel). Top panel: # Higher ($p < 0.01$) mental fatigue for sedentary participants only compared to other time points. Bottom panel: # Higher ($p < 0.05$) mental fatigue at physical FATIGUE compared to Baseline and after moderate exercise (EX), with both CAF and PLA.

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Potential Influence of Habitual Caffeine Intake

Habitual caffeine intake was not significantly related to accuracy ($r = 0.02$, $p = 0.94$) or precision ($r = 0.01$, $p = 0.97$) at *Baseline-5 min CPT* or *Post-20min CPT* ($r = 0.27$, $p = 0.21$; $r = 0.28$, $p = 0.19$) when consuming placebo. Thus, individuals with higher habitual caffeine intake did not exhibit lower accuracy or precision when deprived of caffeine.

Habitual caffeine intake was also not related to *Pre-treatment* mental fatigue ($r = 0.16$, $p = 0.47$), but the correlation with *Pre-treatment* mental energy approached significance ($r = -0.40$, $p = 0.05$). The relationship between habitual caffeine intake and mental energy *Post-20 min CPT* (with placebo) was not significant ($r = -0.34$, $p = 0.10$), nor with mental fatigue at *Post-20 min CPT* ($r = 0.02$, $p = 0.95$).

Discussion

Acute exercise increases neural activity in brain areas related to executive control and improves attention, particularly when the cognitive task is given after a slight delay (~10 min) following exercise. [53, 57, 67, 211, 212] However, the interplay between the optimal exercise intensity and related physiological responses (e.g. heart rate, brain neurotrophins, and dopamine response) [40] that elicit a cognitive benefit based upon the fitness level of the individual is less clear. Our results highlight that 30 min of moderate intensity exercise below lactate threshold restores sustained attention after a mentally fatiguing task faster than 30 min of rest for individuals with either high or low fitness levels. Moreover, sustained attention following vigorous exercise above lactate threshold to the point of physical fatigue remained higher compared to pre-exercise and also did not differ with fitness level. These findings contrast others [67, 68] reporting high intensity

exercise impairs cognitive function in lower fit individuals, and may be due to our exercise prescription using a similar relative intensity based on lactate threshold and/or the administration of our cognitive task 10 min after exercise rather than during exercise [20, 40, 41]. Furthermore, compared to placebo, caffeine attenuated the decline in accuracy and precision observed during a mentally fatiguing task and this benefit persisted when coupled with exercise even to the point of physical fatigue, again, independent of fitness status. However, caffeine could not preserve perceived mental fatigue/energy at the point of physical fatigue.

Acute Exercise and Cognition

Acute exercise has a small ($d = 0.16 - 0.20$) but significant benefit to improve cognition. [53, 54, 211, 213] However, there are a variety of moderating factors [58, 60, 213] which influence the magnitude of this effect such as mode of exercise (i.e. smaller effect in running vs. cycling), the type of cognitive task (i.e. smaller effect on processing tasks vs. memory tasks), duration of exercise, [60] and timing of the task following exercise. In addition, research designs that lack a control rest condition [54] result in an overestimation of the exercise effect. [54, 214] The present study suggests that, compared to rest, 30 min of moderate intensity exercise has a moderate ($d = 0.73$, $p = 0.01$) effect on accuracy during a task requiring sustained attention. This effect has been attributed to exercise-induced arousal, allocation and activation of processing resources in prefrontal brain areas following moderate intensity exercise, whereas decreased brain activation is observed following rest. [61] The duration of this benefit to restore sustained attention following mental fatigue, however, is unclear since only one post-exercise assessment was performed. The larger effect size in the present study could be due to a more

homogeneous basis of setting the moderate exercise intensity, the nature of the cognitive task (sustained attention), and/or the optimal duration of the exercise bout recently observed to be between 10-45 min. [60]

Other factors contributing to the magnitude of the acute exercise effect on cognition include exercise intensity and differences in participants' fitness status. [54, 59] An "inverted-U curve" has been suggested to describe the relationship between exercise intensity and cognition; specifically, cognitive performance improves with moderate intensities but deteriorates at high exercise intensity. [215] The aerobic vs. anaerobic contribution to energy at the different workloads may differ between groups when workloads are based on %HR or %VO_{2max} (rather than lactate threshold), likely influencing factors related to fatigue, perception, arousal, and task performance. [54] Therefore, to better understand the interaction between exercise intensity and fitness status on cognitive function, workloads need to be comparable across groups. Since sedentary participants typically reach lactate threshold at a lower exercise intensity (based on HR or VO_{2max}) compared to higher fit participants, [71] the use of lactate threshold to prescribe exercise intensities across groups is advantageous.

In the present study, we did not observe an "inverted-U" type of phenomenon in either group after basing exercise intensity on a fixed percentage of the lactate threshold. Our findings are in contrast to evidence suggesting that, compared to athletes, untrained individuals experience cognitive decrements, at higher exercise intensity (>~ 60% HR_{max}). [67, 68] A limitation in these studies is that fitness characteristics (i.e. VO_{2max} and lactate threshold) are often not measured, [67] making it unclear if the required perceived efforts between the groups were truly "equivalent". However, since our

participants did not perform the cognitive task *during* exercise, this may have masked potential impairments in sedentary since positive effects are more likely similar across fitness levels when cognitive performance is assessed following exercise [40].

Individuals of lower fitness may require more cognitive resources than physically fit individuals to sustain exercise, leaving fewer neural resources available for the less fit to perform cognitive tasks during high intensity exercise [40]. Yet, we did not observe this when perceived efforts were comparable across high and low fit groups.

Perceived Energy and Fatigue Related to Fitness Status

Although the present study observed no impact of fitness status on cognitive performance, the groups differed in their ratings of perceived energy and fatigue in response to exercise. However, trained subjects rated lower mental energy compared to sedentary prior to ingesting treatments or engaging in any tasks. We did not anticipate this difference since chronic aerobic training improves mood and feelings of mental energy and fatigue. [216] This between-group difference is a limitation in evaluating fitness status as a factor that influences perceptions related to mental tasks followed by varying intensities of exercise.

After exercise to physical fatigue, a change in perceived mental fatigue was more pronounced in sedentary compared to trained. This appears consistent with investigations indicating lower-fit individuals may require greater mental resources or increased effort to complete mental tasks due to lasting effects of a fatiguing physical task. [59, 208, 217-219] Other evidence also suggests emotional response to exercise of moderate versus high intensity may differ based on training status, possibly affecting cognitive resource allocation, motivation, and effort to maintain engagement in a task during exercise [59,

208, 217]. It is possible that time to volitional fatigue protocols without a definite endpoint may elicit greater perceived mental fatigue and less perceived energy following exercise compared to fixed-length exercise stimuli. The timing of assessments in our design (after the CPT, two of which were immediately preceded by exercise) limits our ability to determine specific effects of exercise versus specific demands of the cognitive task on perceived mental energy and fatigue. More investigation is needed to understand the optimal exercise intensity that maintains perceived mental energy for sedentary, especially given the recent popularity of high-intensity exercise programs. On the other hand, vigorous exercise, with advantages such as greater energy expenditure and fitness benefits, does not appear to sacrifice cognitive performance in less fit individuals.

Caffeine Effects on Sustained Attention and Perceived Energy and Fatigue

Similar to other studies, [109, 110, 121, 124] we observed moderate caffeine (equivalent to 1-2 cups of coffee) attenuated mental fatigue during a task requiring sustained attention. This was not unexpected since Schmitt et al. [122] suggest caffeine may be more beneficial to complex, higher order cognitive tasks versus simple processing tasks by improving concentration. Although exercise restored sustained attention after mental fatigue, caffeine provided additional benefit to sustained attention when coupled with moderate and vigorous intensity exercise, possibly due to its CNS actions which appear analogous to exercise effects [12, 28]. Our findings are in agreement with previous work indicating 100-150 mg of caffeine improves attention in endurance-trained athletes following fatiguing cycling exercise [220] and extends previous investigations by observing the same effect in those who do not perform regular physical activity.

Although caffeine was able to maintain cognitive performance after physical fatigue, it was not able to mitigate the drop in perceived energy or increase in perceived fatigue. While 6 mg/kg caffeine has been suggested to improve subjective affect dimensions (i.e., increase feelings of pleasure during moderate intensity exercise), no difference was noted in affective state following exercise. [94] In partial agreement with these results, 3 mg/kg caffeine in the present study improved perceived mental energy following moderate exercise, but not after physical fatigue. A recent systematic review also indicates an acute bout of moderate exercise has a similar effect on increasing perceived mental energy as the ingestion of ~64 mg caffeine (~1 mg/kg for reference in our study) [6]. This suggests that affective state following exercise may be more dependent on exercise duration and/or intensity rather than the dose of caffeine ingested.

Habitual caffeine intake tended to be higher (by ~ 100 mg/d, equivalent to one cup of regular coffee) in trained compared to sedentary participants, possibly contributing to lower pre-treatment mental energy in trained. However, all participants were classified as low-to-moderate caffeine users [57]. Although mental energy appears sensitive to caffeine dosages between 100-200 mg, [23] it is not clear our trained participants experienced a greater caffeine “withdrawal” effect, since lower mental energy with placebo was no longer significantly correlated to higher habitual caffeine intake following the 20 min cognitive task.

Our study was not designed to differentiate whether caffeine [28] and exercise [9-13, 55] exert distinct and/or synergistic effects. Future studies should include an additional control rest condition with caffeine to understand if caffeine alone restores sustained attention compared to the acute benefit of exercise. Although moderate exercise

increases allocation of resources related to complex cognitive tasks, prolonged or exhaustive exercise may decrease this response. [54, 65, 66] Caffeine may be more effective when attentional control of perceptual functions is low (i.e. fatigued state or when CNS resources are compromised), [113] explaining our findings that, after physical fatigue, caffeine maintained attention despite lower perceived energy and higher perceived fatigue. Practically, caffeine appears to be an effective countermeasure to fatigue when subsequent mentally and physically fatiguing tasks must be completed intermittently throughout a typical day.

Conclusion

The present study indicates moderate intensity exercise improves recovery of sustained attention following mental fatigue in both trained and sedentary individuals. Furthermore, sustained attention was not impaired in either group after exercise to the point of physical fatigue, despite greater perceived mental fatigue. Caffeine acted as a mental ergogenic aid by blunting the decline in sustained attention during a fatiguing cognitive task which persisted after both moderate and vigorous exercise for all individuals. Caffeine, however, could not maintain perceived mental energy or mitigate mental fatigue at the point of physical fatigue. It is also clear sustained attention is improved by acute exercise independent of fitness levels. However, more research is warranted to understand the optimal exercise prescription (e.g., intensity) for individuals (sedentary and trained) to maintain mental energy following exercise in order to perform subsequent cognitive tasks and physical activity.

CHAPTER 5

SUMMARY AND FUTURE DIRECTION

Integration of Dissertation Findings

Strategies to reduce perception of fatigue and increase daily activity and perceived energy are relevant to improve health outcomes, work performance, and quality of life. Extensive research on nutritional aids to reduce fatigue and improve exercise performance has been performed using physically fit subjects but more research is needed to understand how it may or may not apply to the general population. Two extensively researched ergogenic aids for counteracting physical fatigue, caffeine and carbohydrate, are widely consumed in the U.S., by a population that is largely (~70%) sedentary. Typical caffeine intake by adults in the U.S. averages ~300 mg/d with ~150-200 g/d from caffeine-containing energy supplements (e.g. energy drinks and shots) [128, 221]. Over the years, there have been mixed perceptions on the positive and negative health effects of caffeine. However, more recently, the US Department of Agriculture and Department of Health and Human Services has indicated that caffeine in moderate amounts <400 mg/d is not associated with health risks [222]. According to the Dietary Reference Intake in the US, ~75% of adults consume the recommended amount of carbohydrate in a day (~45-65% total daily calorie intake) [222]. However, recommendations indicate sugar consumption should be limited to 10% of total energy intake and Americans consume 6.5% of daily energy intake from sugar sweetened beverages alone [222].

Although carbohydrate is an important dietary nutrient, excess consumption of sugar sweetened beverages has been associated with overweight and obesity in

observational epidemiological studies and limited interventions [7, 222-225]. Recent evidence [226] also suggests that adding ~ 60 mg caffeine to sugar sweetened beverages increased their consumption by ~ 6% over 28 days compared to non-caffeinated sweetened beverages. Because of the potential metabolic consequences (e.g. transient insulin resistance, hyperinsulinemia, and reduced glycemic control) of ingesting caffeinated, sweetened beverages in a sedentary state [24], more research is needed to understand the relative efficacy of caffeinated and/or carbohydrate beverages to promote mental and physical energy and cognitive/physical work performance for those who are habitually sedentary or physically active.

We evaluated strategies to impact mental and physical fatigue in both trained and sedentary subjects. The effects of caffeine and carbohydrate treatment and moderating factor of fitness level on the dependent variables indicative of mental and physical fatigue are summarized in Table 7 along with estimated effect. Our findings revealed that, following an overnight fast, ingestion of a sweetened low carbohydrate drink (0.4% CHO) attenuated the decline in sustained attention over a 20 min cognitive task without impacting glycemic response. This suggests mental fatigue can be influenced by ingesting a very small amount of carbohydrate when breakfast cannot be consumed, possibly through stimulation of oral receptors and other CHO sensing receptors distal to the mouth. Based upon the potential benefit of low-CHO on mental fatigue, we evaluated the same low-CHO treatment to determine its efficacy for counteracting mental and physical fatigue in a fed state. In this scenario, while caffeine was beneficial to mental fatigue, low-CHO did not attenuate mental fatigue compared to placebo or provide benefit when added to caffeine versus caffeine alone. Similarly, low-CHO had no

apparent impact on physical fatigue whereas a moderate dose of caffeine reduced physical fatigue for both habitually trained and sedentary individuals.

Table 7. Summary Effects of Nutritional Strategies on Fatigue and the Impact of Fitness Level

	Low-CHO (0.4%) ingest vs. CHO rinse Fasted State	Low-CHO (0.4%) vs. Placebo Fed State	Caffeine (3 mg/kg) vs. No Caffeine Fed state	Fitness Interaction
Mental Fatigue				
Sustained Attention	+ (ES=0.27)	∅ (ES=0.02)	+ (ES=0.37)	No
Perceived Energy	∅ (ES=0.2)	∅ (ES=0.01)	+ (ES=0.18)	Lower in sedentary after TTF
Perceived Fatigue	∅ (ES=0.13)	∅ (ES=0.02)	∅ (ES=0.11)	Higher in sedentary after TTF
Physical Fatigue				
RPE	--	∅ (ES=0.03)	+ (ES=0.31) During MOD EX	Lower in trained 1 st 20% TTF No effect during TTF in sedentary
Exercise Capacity (Time to Fatigue)	--	∅ (ES=0.02)	+ (ES=0.34)	No
MVC of Knee Extensors	--	--	∅ (ES=0.16)	No
EET of Knee Extensors	--	--	∅ (ES=0.01)	Lower for sedentary after MOD EX
Voluntary Activation of Knee Extensors	--	--	∅ (ES=0.05)	Lower for trained after MOD EX
<p>CHO = carbohydrate; TTF = Time to Fatigue Task (5% > lactate threshold), MOD EX = 30 min moderate intensity cycling (10% < lactate threshold), MVC = Isometric maximal voluntary contractile strength; EET = Electrically evoked torque; ES = estimated effect (partial η^2) where 0.01 = small effect, 0.06 = moderate, and 0.14 = large effect</p> <p>-- = not applicable based on test protocol ∅ = no significant effect of treatment + = significant benefit of treatment (p < 0.05)</p> <p>Fitness Interaction: “No” = non-significant between-group effect or group x treatment interaction. Significant (p < 0.05) group x treatment interactions are indicated.</p>				

Our research findings indicate that moderate intake of caffeine (equivalent to ~2 cups of coffee) appears to maintain mental performance and perceived energy when demanding mental and physical tasks must be completed throughout a typical day. Moreover, this benefit is attained by both physically fit and less fit individuals. However, moderate exercise alone (with non-caffeinated placebo) resulted in ~5% larger restoration in accuracy and ~13% larger improvement in precision after a 20 min mental task, compared to an equivalent amount of rest. We did not, however, partition the effect of caffeine alone compared to exercise in recovery from mental fatigue, which remains to be investigated. Caffeine provided an acute small benefit in attenuating mental fatigue and maintaining perceived mental energy during the 20 min task; and, subsequently when combined with moderate exercise, resulting in a relative improvement of ~4% accuracy and ~7% precision, compared to baseline. These results suggest that 30 min of moderate intensity exercise below lactate threshold can restore prior mental fatigue; but, the addition of caffeine ingestion prior to exercise may provide a sustained benefit to subsequent attention tasks. Because similar brain areas are involved in sustaining attention during cognitive tasks and in sustaining physical effort during submaximal exercise tests, it is not surprising that caffeine also improved exercise capacity by reducing perceived effort at intensity below lactate threshold, possibly influencing the ability to tolerate subsequent fatiguing exercise. The present study provides indirect support that caffeine's ergogenic effect is primarily due to central mechanisms influencing perception of effort versus influencing metabolism during exercise, and this appears to be true for both trained and habitually sedentary individuals.

Although trained and sedentary responded similarly to caffeine (i.e. improved sustained attention and improved exercise capacity), fitness level was a moderating factor in *perceived* rating of mental energy and mental fatigue after the cognitive tasks. Even though performance in the sustained attention task and cycling tasks were similar between groups independent of the nutrition treatment, a reduction in mental energy and corresponding increase in perceived mental fatigue was more pronounced in sedentary following physical fatigue compared to trained individuals. Since cognitive task performance was maintained but perceptual measures were still impacted in sedentary after fatiguing exercise, it is unclear whether physical fatigue might impair subsequent mental “fatigue” as defined by impaired performance in more difficult or longer duration tasks in sedentary individuals.

Future Directions

More research should be done on both sedentary and trained populations with additional exercise interventions combined with nutritional strategies, particularly given the popularity of high intensity interval training. An optimal exercise prescription resulting in sustained positive feelings of energy and mood could increase the likelihood of adherence to an exercise routine. Strategies for weight loss are not limited to exercise, but involve altering energy balance by increasing energy expenditure relative to energy intake. However, individuals who engage in physical activity may compensate for the additional energy expenditure by decreasing subsequent physical activity and/or increasing subsequent energy intake due to physiological or behavioral cues triggered by exercise [192], although not all studies support this [158, 162, 227, 228].

Physical Activity in Free-living Conditions

Some [192] have suggested the lack of weight loss response to an exercise intervention could be due to volitional post-exercise compensation such as decreased non-exercise physical activity and/or increased sedentary time. Because the half-life of caffeine is 3-6 h following ingestion; we expected that following the caffeinated trials, participants would feel less fatigued and/or perceive more energy, allowing them to be more physically active or less sedentary for the remainder of the day [159-161, 170]. In order to test this hypothesis we obtained accelerometer data (Fitbit One™, Fitbit Inc., San Francisco, CA) from seven trained and nine sedentary participants for the remainder of the day until bedtime following each of the four experimental trials. We secured the activity monitor on the waist band prior to participants leaving the laboratory and instructed participants to wear the monitor on the hip for all activities except showering or swimming. We asked that they removed the monitor when they went to sleep for the night and they returned the device to our laboratory the following morning. Five trained and three sedentary participants were not able to provide complete data from four trials since two of the participants lost the monitor and the remaining participants forgot to wear the monitor for the entire day. We did not obtain self-reported activity logs to compare with the data downloaded from the Fitbit One™, a recommended consideration for future research. Because rapid advancement in technologies for accelerometers and activity monitors have resulted in many different choices in products, there is considerable variability among activity monitors. Not all activity monitors have been validated for use in free-living conditions. At the time of this research study, there was preliminary evidence that the Fitbit One™ was able to accurately quantify number of

steps [229]. However, recent evidence indicates ~10.4% error when estimating energy expenditure with the Fitbit One™ under free-living conditions, compared to using indirect calorimetry [230].

Similar to others suggesting that post-exercise sedentary time may not be affected by moderate caffeine intake [171]; the present study did not detect a difference in post-exercise sedentary time following trials with caffeine versus without caffeine. However, our participants tended ($p = 0.06$) to sit ~30 min less per day after ingesting caffeine during the morning experiment compared to the no-caffeine trials (Figure 19). Interestingly, there was no difference in how much time trained (561 ± 70 min) and sedentary (529 ± 70 min) spent sedentary throughout the day following the trials, independent of the treatment intervention. This is an important finding supporting previous evidence [231] that endurance athletes may also be at health risk from prolonged sitting outside their training sessions; and, that nutrition interventions involving caffeine or other aids that may increase perceived energy are equally important for increasing post-exercise activities in regular exercisers as well as habitually sedentary individuals.

Although we did not obtain subjective ratings of perceived energy and fatigue after participants left the laboratory, another study [172] determined a low-carbohydrate energy drink with caffeine decreased perceived fatigue the following morning after an exercise bout, compared to when exercise was performed with a placebo. While the caffeine (3 mg/kg or ~210 mg) and carbohydrate (2 g) content of their treatment was similar to that provided in our study, their energy drink also contained taurine, which may have contributed to perceptual measures [232]. Findings from the present study provide

support for previous literature [233] suggesting caffeine (with or without a small amount of carbohydrate) may increase enjoyment of physical activity, potentially facilitating long-term adherence to a regular physical activity program designed for weight loss [163].

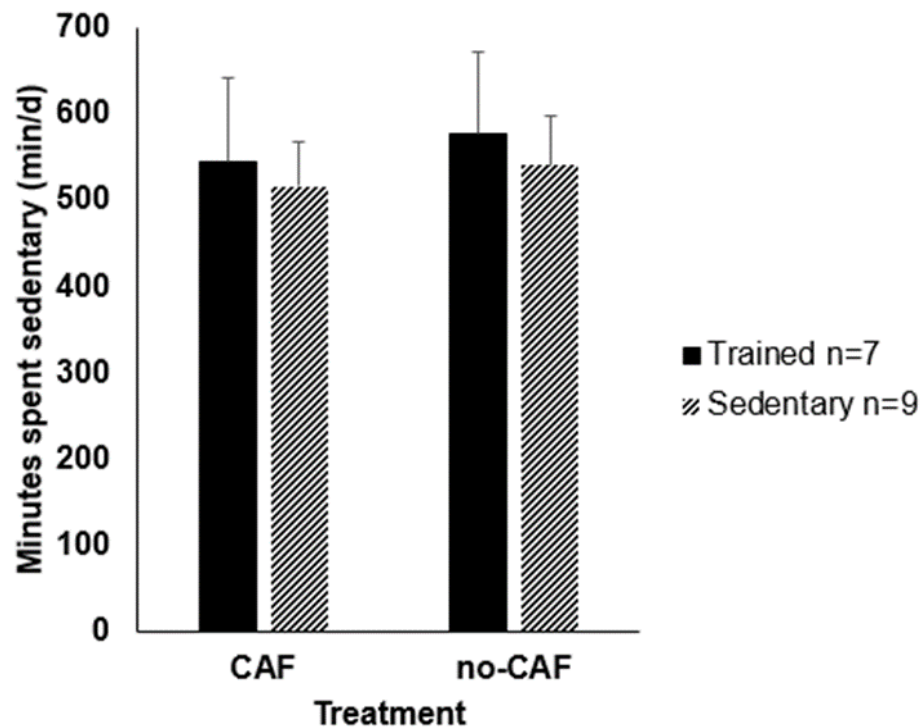


Figure 19. Mean (\pm SD) Sedentary time (min) following exercise trials with caffeine (CAF) and without (no-CAF) by group. No significant difference in time spent sedentary between Trained and Sedentary. CAF tended to result in less post-exercise sedentary time compared to no-CAF ($p = 0.06$).

Post-exercise Energy Intake

Behavioral compensation is not only related to less physical activity or more sedentary time following exercise, but it can also be related to increased energy intake [192] due to an automatic (appetite-driven) or volitional (behavioral-driven) response.

Either type of compensatory response could affect energy balance and limit weight loss. There is considerable variability in the relationship between exercise participation and weight loss, and activity status (i.e. regular exerciser versus habitually sedentary) is an important moderator of this relationship [168, 234]. While some evidence [235] suggests that short-term (e.g. within a single day) appetite control is stronger in active compared to sedentary adults, others [168] suggest that a longer time frame of assessment is necessary to capture delayed compensatory responses to bouts of exercise which appears to differ in active versus sedentary adults [168].

All participants (n=24) kept a detailed dietary record of all food and beverages consumed during the day following each of the four experimental trials. Intake started with the first meal or beverage following the trial and was recorded until bedtime that night. Self-reported intake was analyzed with NDSR (University of Minnesota Nutrition Data System for Research, Minneapolis, MN) and values obtained for content of the first eating episode following each experimental trial and for total intake over the course of the day.

Energy intake during the first eating episode following the trials was similar in trained (530 ± 248 kcal) and sedentary (614 ± 248 kcal). Although we did not measure energy expenditure during the vigorous bout of exercise, all participants exercised for a similar time until volitional fatigue. We were, however, able to estimate energy expenditure during the 30 min bout of moderate intensity exercise using oxygen uptake and RER; and, thus calculate relative energy balance between energy expended during moderate exercise and energy intake during the first post-exercise meal. Statistical analyses indicate that acute energy balance was similar between groups, although

sedentary tended to have higher energy intake during the first meal relative to energy expenditure by ~250 kcal compared to trained (Figure 20). There was a significant effect of CAF ($p = 0.046$) resulting in less relative energy intake compared to energy expenditure compared to no-CAF (Figure 20). Since CAF also allowed all participants to cycle for a longer duration during vigorous exercise, it is difficult to know if the greater time spent in vigorous activity blunted appetite or caffeine per se. Thus, the actual effect of CAF intake on acute energy balance may act in multiple ways to be determined.

For the remainder of the day, however, energy balance became more similar in trained (1664 ± 388 kcal) and sedentary (1670 ± 388 kcal), suggesting trained compensated with more energy intake later in the day versus in the first eating episode. There was no longer a treatment effect of CAF on whole-day energy balance, although energy balance tended to be ~120 kcal lower after CAF trials versus no-CAF trials, across all subjects, likely due to less post-exercise carbohydrate intake after CAF compared to no-CAF.

We were also interested in post-exercise intake of macronutrients, particularly for carbohydrate and fat in the two groups following exercise with and without CAF. Total carbohydrate and fat intake following the trials was not different between trained and sedentary but CAF intake resulted in lower ($p = 0.01$) post-exercise carbohydrate intake by ~30 g compared to after no-CAF throughout the day, in all subjects, even though subjects expended more energy during the CAF trials (longer TTF compared to no-CAF). However, we cannot be certain that this would be a direct effect of CAF ingestion or an effect of exercise mediated by CAF intake. Fat intake was not influenced by CAF treatment. These findings are in agreement with previous evidence [163] that post-

exercise energy intake was not affected by 6 mg/kg CAF compared to placebo. That study also determined that CAF increased energy expenditure during and following exercise; thus resulting in greater post-exercise energy deficit with CAF versus placebo. Consistent with previous evidence [163], our results suggest that a moderate amount (3 mg/kg) of CAF ingestion prior to and/or during exercise may contribute to a small but greater energy deficit in the hours following exercise as a result of both less energy intake *and* less sedentary time. Future studies should investigate this phenomenon with better methods of activity and energy intake monitoring over a longer period of time (≥ 1 day).

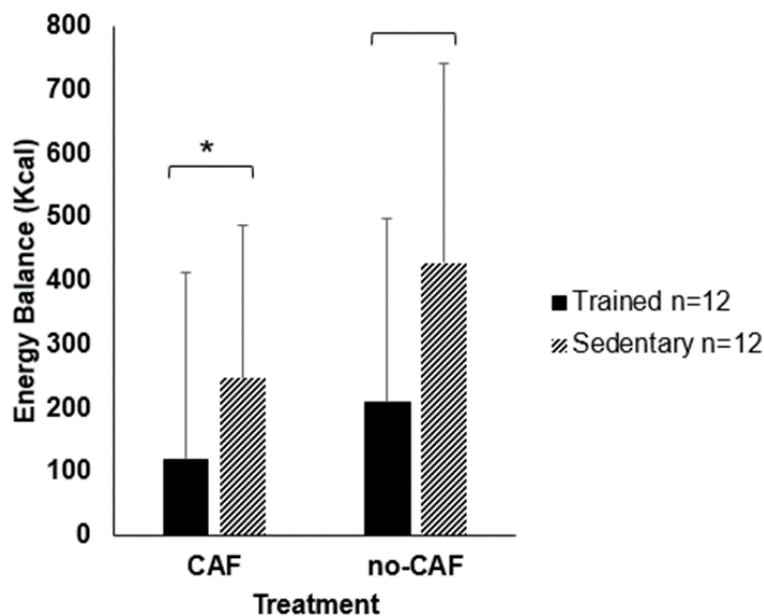


Figure 20. Mean (\pm SD) Energy balance (kcal) following exercise trials with caffeine (CAF) and without (no-CAF) by group. Energy balance = Kcal expended during 30 min moderate intensity exercise – Kcal ingested during first meal following exercise. No significant difference in energy balance between Trained and Sedentary but CAF resulted in lower energy intake relative to energy expenditure in all subjects * $p < 0.05$.

Metabolic Considerations

While CAF has well documented benefits for mental and physical performance, mood, and perceived energy, it can also have negative consequences to glucose tolerance in a sedentary state [24]. Our findings indicated that sedentary had higher blood glucose compared to trained after 15 min of moderate exercise (or 55 min post treatment ingestion); and, that 30 min of moderate exercise was sufficient to return the sedentary group blood glucose back to levels similar to trained. The treatment and ~40 g CHO energy bar given 40 min prior to starting exercise is common for a pre-exercise feeding but approximately half the CHO load given during a standard oral glucose tolerance test (OGTT). We did not, however, conduct an OGTT or measure post-treatment glycemic response as we did with the carbohydrate-only drinks under fasted, sedentary conditions. The endurance-trained group may have improved insulin sensitivity compared to sedentary but this is highly speculative since we also did not measure insulin response. Our groups were matched in body mass index, fasting glucose, and self-reported non-exercise physical activity (i.e. walking) but differed in percent body fat, aerobic fitness, and habitual exercise quantity. Thus, even in a young, healthy but sedentary population, glycemic control in response to a morning meal with carbohydrate may be compromised compared to response in individuals who engage in regular exercise [236], whether caffeine is ingested [24] or not.

Thus, future work should measure fasted blood glucose, post-prandial glycemic response and excursions, insulin response, and insulin sensitivity along with responses to varying concentrations of low-calorie drinks ingested with and without caffeine. Continuous monitoring over extended periods (> 60 min) would be of interest to better

understand the metabolic impact of ergogenic aids and exercise interventions in groups of different fitness levels; particularly since, apart from their regular training sessions, athletic individuals appear to be just as inactive as habitually sedentary individuals.

Conclusion

The present series of research studies examined fatigue, both mental and physical, which is a prevalent issue affecting the health and productivity of the US population.

Nutritional strategies known to be beneficial for athletes appear to act similarly for sedentary individuals. However, caffeine and carbohydrate, particularly in the form of sugar sweetened beverages such as energy drinks, are often criticized or result in potential metabolic consequences such as impaired insulin sensitivity and glycemic control under sedentary conditions. These data suggest that caffeine coupled with a meal and/or low-sugar beverage do not elicit acute adverse effects for sedentary or trained individuals when combined with exercise. Although oral rinse of carbohydrate solutions may be efficacious for exercise performance, we did not find that a single oral rinse attenuated mental fatigue. Instead, ingesting a low-carbohydrate drink was ergogenic during a mental task when in a fasted, sedentary state, but not after a small carbohydrate feeding. A thirty minute bout of moderate intensity exercise was also advantageous for sustained attention and in the recovery from mental fatigue compared to an equivalent amount of rest. A moderate dose of caffeine attenuated mental and physical fatigue and reduced perception of effort during a submaximal physical task for both trained and habitually sedentary individuals. However, the addition of low-carbohydrate to caffeine did not further improve caffeine's effects. Before nutritional countermeasures to mental

or physical fatigue can be recommended for public health, the distinct effects of the treatment versus the exercise itself are equally important aspects not rigorously examined in the present study. Future investigations are needed to determine the impact of acute exercise combined with and without nutrition strategies on volitional activity and dietary patterns not only immediately post-exercise, but also throughout the remainder of the day and longer, based on individuals' fitness status.

APPENDIX A

ADDITIONAL FIGURES

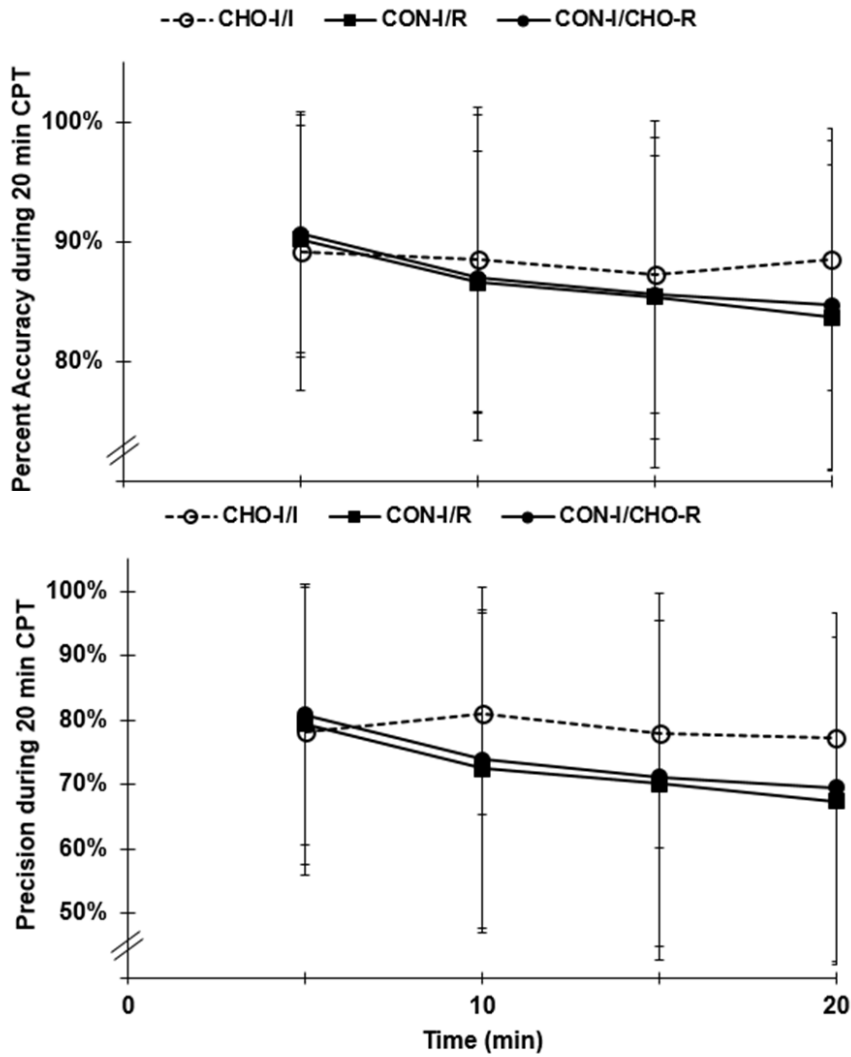


Figure A-1. Mean (\pm SD) Accuracy (top panel) and Precision (bottom panel) during 20 min Continuous Performance Task (CPT) over time in 5 min epochs by treatment.

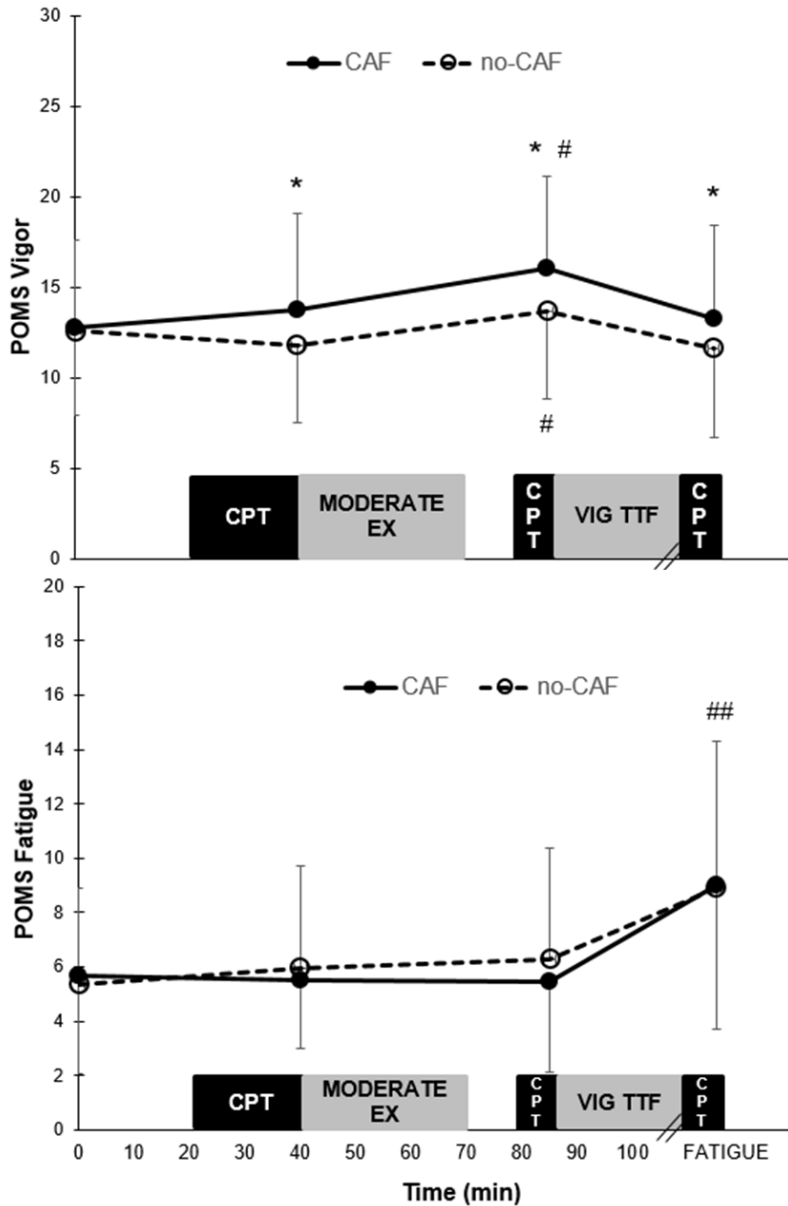


Figure A-2. Mean (\pm SD) rating of Vigor (top panel) and Fatigue (bottom panel) using Profile of Mood States (POMS) questionnaire with caffeine (CAF) and no-CAF treatments. Top panel: # Higher ($p < 0.01$) vigor with CAF compared to all other time points and higher ($p < 0.05$) with no-CAF compared to 40 min and after FATIGUE. * Higher ($p < 0.05$) with CAF vs. no-CAF. Bottom panel: # Higher ($p < 0.05$) compared to all other time points for both CAF and no-CAF.

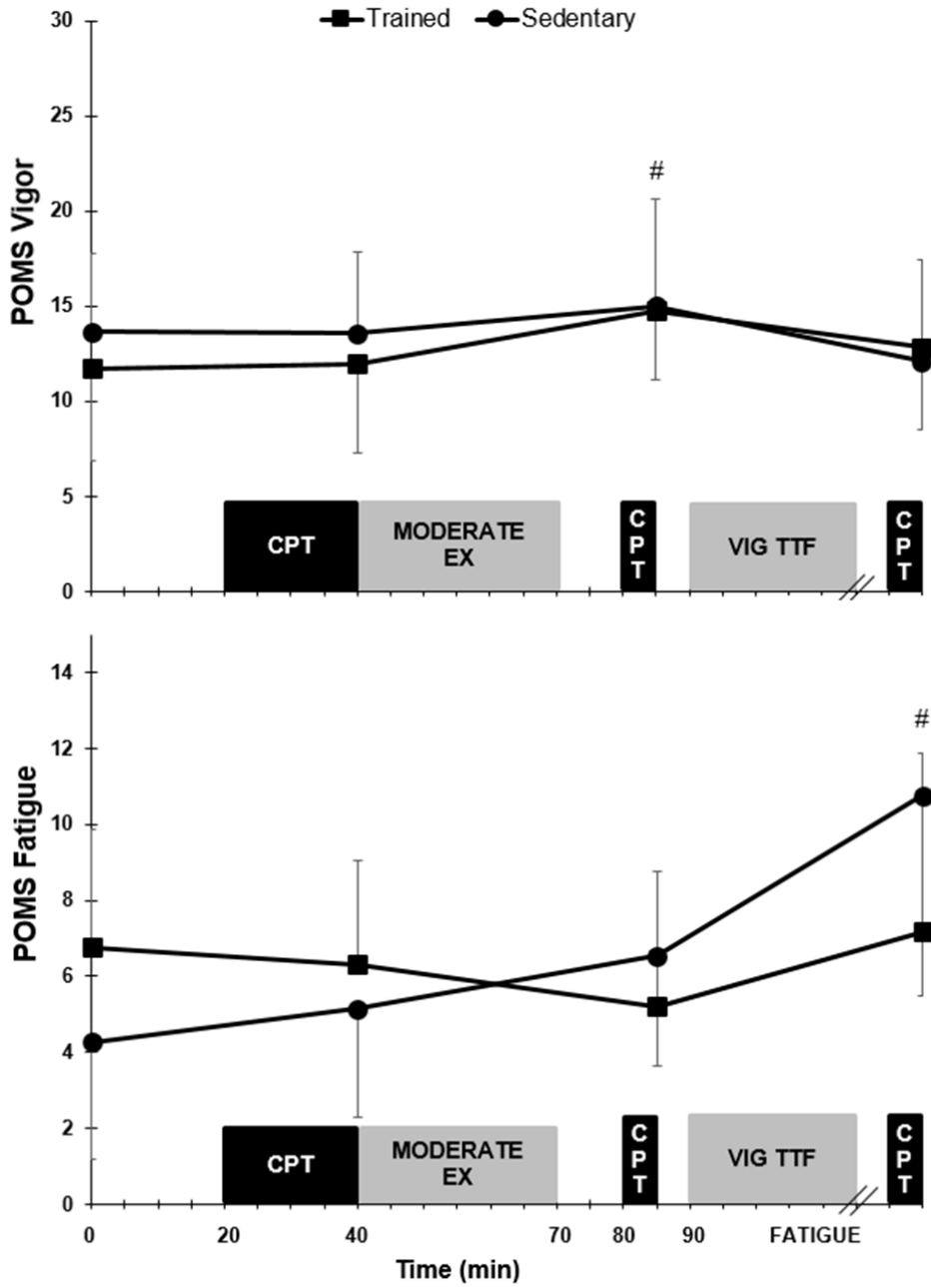


Figure A-3. Mean (\pm SD) rating of Vigor (top panel) and Fatigue (bottom panel) using Profile of Mood States (POMS) questionnaire for Trained and Sedentary. Top panel: # Higher ($p < 0.05$) vigor compared to all other time points for both groups. Bottom panel: # Higher ($p < 0.01$) compared to all other time points for Sedentary.

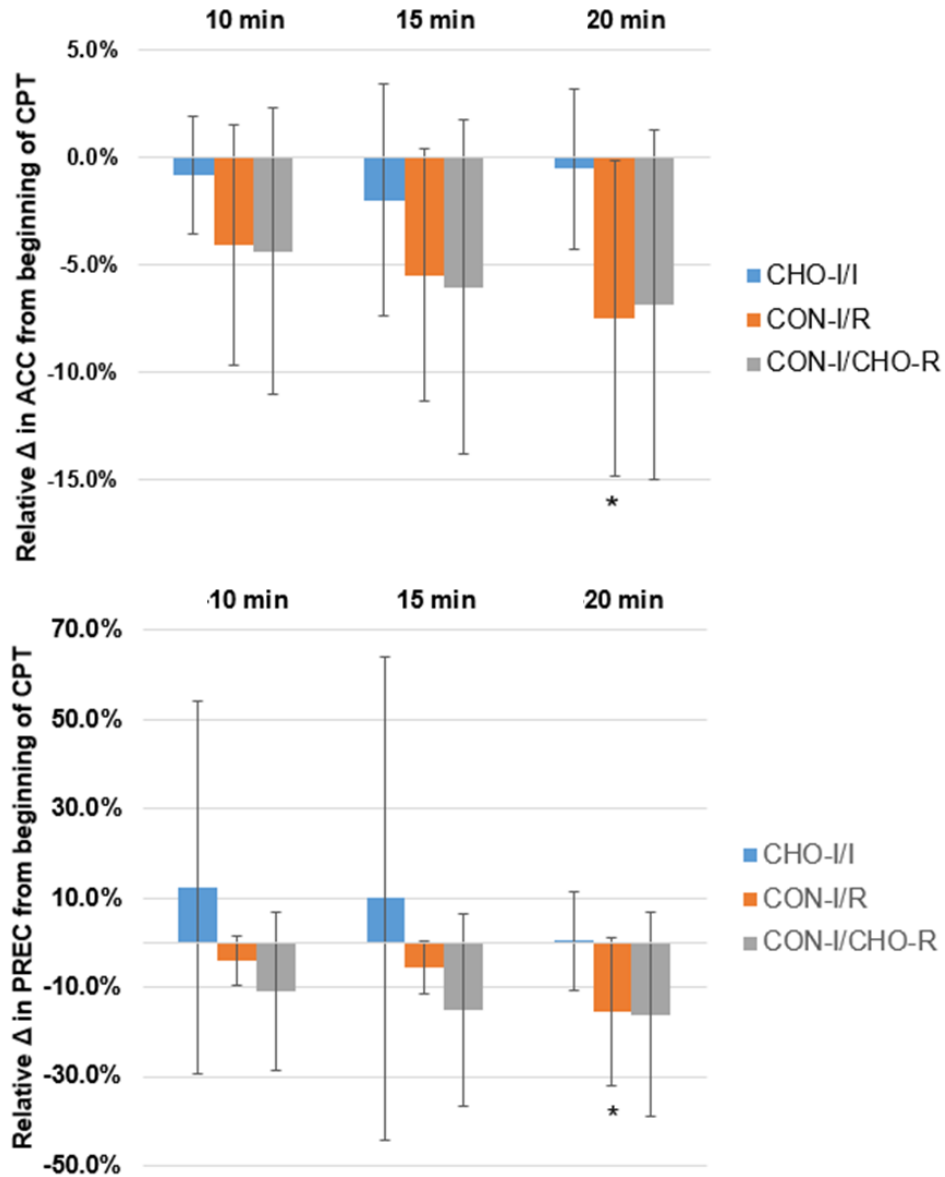


Figure A-4: Top panel: Relative change in accuracy (ACC) at intermediate points during 20 min Continuous Performance Task (CPT), compared to beginning of the test (0-5 min). * significant decrease ($p < 0.05$) in ACC at 20 min with control rinse (CON-I/R) compared to low-carbohydrate ingestion (CHO-I/I). Bottom panel: Relative change in precision (PREC) at intermediate points during 20 min CPT, compared to beginning of the test (0-5 min). * significant decrease ($p < 0.05$) in PREC at 20 min with control rinse CON-I/R compared to low-carbohydrate ingestion CHO-I/I.

APPENDIX B
SURVEY INSTRUMENTS AND QUESTIONNAIRES

How do you feel right now with regard to your capacity to perform your typical MENTAL ACTIVITIES.

I feel I have no energy Strongest feelings of energy ever felt

I feel no fatigue Strongest feelings of fatigue ever felt

I feel I have no vigor Strongest feelings of vigor ever felt

I feel no exhaustion Strongest feelings of exhaustion ever felt

I feel I have no pep Strongest feelings of pep ever felt

I have no feelings of being worn out Strongest feelings of being worn out ever

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24-Hour History

Date: _____ Time: _____

1. How much sleep did you get last night? (please circle one)

0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 10 (hours)

2. How long has it been since your last meal or snack? (please circle one)

0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5
9 9.5 10 11 11.5 12 12.5 13 13.5 14 14.5 15 15.5 (hours)

3. When did you last have:

- A cup of coffee or tea
- Soft drink / Soda/ Energy drink (please specify type)
- Cigarettes
- Drugs (including aspirin)
- Alcohol
- Vitamins or Supplements (please specify type)

4. How many servings of caffeinated beverages have you consumed in the last 24 hours? _____ cans _____ cups _____ other

5. What sort of exercise did you perform this past week; and for how long?

Saturday: _____ for _____ minutes

Sunday: _____ for _____ minutes

Monday: _____ for _____ minutes

Tuesday: _____ for _____ minutes

Wednesday: _____ for _____ minutes

Thursday: _____ for _____ minutes

Friday: _____ for _____ minutes

6. How much of this exercise would you consider to be moderate to hard work or intensity? _____ hours or _____ minutes

7. What sort of physical activity have you performed in the past 24 hours; and for how long?

TYPICAL WEEKLY CAFFEINE CONSUMPTION PATTERN

Instructions: Indicate how many of the following beverages or products you consumed last week or in a typical week. The data you provide will be used to help us analyze your response to the drinks. Thank you for your help and time.

Beverage	Days per week	# 8 oz cups per day	Brand/Size	Type	Prep Method
Coffee prepared at home	5	3	Peet's Coffee	Whole Bean	French Press
Coffee ordered out	5		Starbucks	Starbucks	Starbucks
Tea					
Cocoa/Chocolate					
Milk					
Coffee ice cream or yogurt					
Soda					
Energy Drinks					
Other products consumed					
Chocolate (# grams)					
Tylenol (# pills)					
Excedrin (# pills)					
M&M's (# pills)					
Other types of pain relievers containing caffeine					
Cold & allergy medications containing caffeine					

	Qty per day	Days per week	Brand/Type
Vivarin (# pills)			
NoDor (# pills)			
Other types of caffeine pills			
Diuretics containing caffeine (e.g., Aquas-Ban)			

Please indicate any other products you consumed that may have had caffeine in them (e.g., herbal supplements):

First Initial: Last Initial: Age:

Gender: Female Male

If you are female, please answer the following questions:

Are you pregnant or think you could be pregnant?

Yes No

Are you taking or have taken oral contraceptive pills in the last 8 weeks?

Yes No

Do you have regular menstrual periods (approximately every 20-40 days)?

Yes No

Weight: Height:

Amount of Hours in a Week Spent Exercising:

0-10 min 30 min-2 hours 2-5 hours 6-10 hours >10 hours

Please explain the type(s) of exercise you perform and how often:

Are you diabetic (Type I or II)? Yes No

Are you on a special diet or weight loss diet or have you lost or gained significant body weight in the last 3 months? *

Yes No

If you answered yes to the previous question, please explain:

Do you smoke? * Yes No Previous Smoker

Do you have or have you ever been diagnosed with a sleep, eating, or mood disorder? *

Yes No

If you answered yes to the previous question, please explain:

Are you taking any prescription stimulants or medications that affect your appetite, mood, energy level, or blood sugar? * Yes No

If you answered yes to the previous question, please explain and list medications:

Are you sensitive to artificial sweeteners such as aspartame, saccharin, or sucralose? *

Yes No

Do you often take in a very large amount of caffeine from drinks (>2.16 ounces cups of premium coffee or energy drinks per day) or pills (>1 caffeine pill per day)? *

Yes No

Physical Activity Questionnaire

1. Does your work involve vigorous-intensity activity that causes large increases in breathing or heart rate like *(carrying or lifting heavy loads, digging or construction work)* for at least 10 minutes continuously? (Circle one)

YES NO

IF YES, In a typical week, on how many days do you do vigorous-intensity activities as part of your work? (Circle one)

1 2 3 4 5 6 7

How much time do you spend doing vigorous-intensity activities at work on a typical day? _____ hours _____ minutes

2. Does your work involve moderate-intensity activity that causes small increases in breathing or heart rate such as brisk walking *(or carrying light loads)* for at least 10 minutes continuously? (Circle one)

YES NO

IF YES, In a typical week, on how many days do you do vigorous-intensity activities as part of your work? (Circle one)

1 2 3 4 5 6 7

How much time do you spend doing vigorous-intensity activities at work on a typical day? _____ hours _____ minutes

3. Do you walk or use a bicycle *(pedal cycle)* for at least 10 minutes continuously to get to and from places? (Circle one)

YES NO

IF YES, In a typical week, on how many days do you walk or bicycle for at least 10 minutes continuously to get to and from places? (Circle one)

1 2 3 4 5 6 7

How much time do you spend walking or bicycling for travel on a typical day?
_____ hours _____ minutes

Modified from WHO STEPS instrument www.who.int/chp/steps (World Health Organization 2010)

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