Effects of A Ketone/Caffeine Supplement On Cycling and Cognitive Performance

THESIS

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Abstract

An increasing amount of experimental and empirical evidence indicates that low carbohydrate, high fat ketogenic diets may enhance various aspects of athletic and cognitive performance. A ketogenic diet induces a state of nutritional ketosis defined by an elevated ketone concentration within the blood range >0.5 to 5 mmol/L that over time results in a keto-adapted phenotype. Ketones contribute to many of the therapeutic and performance benefits associated with nutritional ketosis, and as a result, exogenous ketone supplements have become commercially available that are capable of inducing acute nutritional ketosis without restricting carbohydrate. The primary purpose of this study was to test the efficacy of a ketone-salt/caffeine supplement versus water on physical and cognitive performance in non-keto-adapted individuals. Twelve recreationally trained individuals (4 women; 8 men; mean age 22.9 ± 1.9 yr, body mass 79.3 ± 16.7 kg) participated in two experimental sessions in a randomized and balanced order. Subjects consumed either a ketone-salt/caffeine supplement or water (control condition) 15 min prior to performing a staged cycle ergometer time to exhaustion test followed immediately by a 30 sec Wingate test. Symbol digit modality tests were administered at baseline, immediately post-exercise and 30-/60-min post-exercise. Blood ketone concentrations were significantly increased peaking 15 min after ingestion by more than 2-fold and staying elevated throughout 60 min recovery. Compared to the water trial, ingestion of the ketone/caffeine supplement significantly increased time to
exhaustion (8.3%) and average power during the Wingate (4.3%). There were no significant differences between conditions in peak power output, cognitive performances, or blood glucose responses. These results indicate that ingestion of exogenous ketone-salts combined with caffeine is well tolerated and induces acute nutritional ketosis and is associated with improved high intensity exercise capacity in non-keto-adapted individuals.
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Chapter 1: Introduction

The metabolic pathways responsible for the synthesis, transport and utilization of ketones are highly conserved and have served an important evolutionary role for humans during prolonged periods of food deprivation. Today, this metabolic program is largely silenced owing to the abundance of carbohydrate-dense food present in the food supply of modern humans. During starvation or when dietary carbohydrate is restricted to less than ~40 grams/day, the liver accelerates production of ketones in order to maintain energy availability. Over the course of several days of a very low-carbohydrate ‘ketogenic’ diet, circulating ketone concentrations increase from <0.2 to 0.5-4.0 mmol/L, a range referred to as ‘nutritional ketosis’. After several weeks of chronic nutritional ketosis, the body undergoes a coordinated set of metabolic adaptations that ensures proper inter-organ fuel supply in the face of low carbohydrate availability. This process of ‘keto-adaptation’ has been shown to be an effective therapeutic approach in the management of insulin resistant conditions (e.g., metabolic syndrome and type-2 diabetes) (McKenzie et al., 2017) and neurological disorders (e.g., epilepsy) (Neal et al., 2008; R L Veech, Chance, Kashiwaya, Lardy, & Cahill Jr., 2001). More recently ketones have been shown to be potent signaling molecules positively affecting gene expression and cellular function (Shimazu, 2013). Another emerging application of keto-adaptation is with athletes and military personnel who often need to perform physically and
cognitively under stressful conditions. A growing number of athletes, especially ultra-endurance athletes, have switched to a very low-carbohydrate ketogenic diet. Some of these athletes have gone on to set personal records and even win races and set national records while following low carbohydrate/ketogenic diets (Volek et al., 2015).

As more scientific knowledge and empirical evidence becomes available supporting the benefits of ketosis from keto-adaptation, there has been motivation to develop exogenous ketone supplements. Several products are now commercially available. The goal of these supplements is to elevate blood concentrations of ketones to achieve a state of acute nutritional ketosis. Whether acute nutritional ketosis recapitulates the pleiotropic effects attributed to chronic nutritional ketosis/keto-adaptation remains unclear. The majority of ketone supplement research at this time has examined ketone esters proving their safety (Clarke et al., 2013) as well as their potential to improve physical performance (Cox et al., 2016). The benefits to performance may be attributed to ketones efficiency at producing ATP (Cahill & Veech, 2003; Kashiwaya, King, & Veech, 1997; Sato et al., 1995; Richard L. Veech, 2004). Outside of glucose, ketones are the primary energy substrate able to cross the blood brain barrier to fuel brain energy requirements and therefore may impact cognitive performance, especially when glucose availability is limited (Laffel, 1999; Owen et al., 1967).

Ketone-salts have not been studied in humans and thus need to be examined to demonstrate tolerance and efficacy. Manufacturers often combine ketones with caffeine owing to caffeine’s ability to mildly increase lipolysis, decreased perceived exertion, and in many cases increase exercise performance (McLellan, Caldwell, & Lieberman, 2016).
The purpose of this study was to examine the impact of a ketone-salt/caffeine supplement compared to water on ketosis, intense exercise and cognitive performance. We hypothesized that ingestion of a ketone/caffeine supplement would induce ketosis and enhance performance.
Chapter 2: Literature Review

Exogenous ketone supplementation was originally exclusively utilized in clinical settings as the only mode of delivery was via intravenous infusion (Dye & Chidsey, 1939). Recent advances have made it possible to orally consume ketones to induce acute levels of ketosis (via products such as Pruvit’s KETO//OS MAX CHARGED). Recent increase in ketogenic diet popularity, has fueled investment in research and development as companies have begun produce exogenous ketones supplements. Although many studies have been conducted related to ketogenic diets and keto-adaptation, research on the impact of exogenous ketone ingestion, acute ketosis, especially in regards to physical and cognitive performance, is lacking.

2.1 Safety

As of now exogenous ketones can be orally consumed by being bound in one of two forms; esters and salts, both of which have shown to be successful at elevating blood ketone levels to achieve acute ketosis (Clarke et al., 2013; Johnson & Walton, 1972). The safety of ketone ester and salt exogenous supplementation has been demonstrated with minimal side-effects (Clarke et al., 2013; Hashim & Vanitallie, 2014; Shivva et al., 2016; Richard L. Veech, 2004). It is important to note that some individuals may experience mild adverse symptoms including; flatulence, nausea, diarrhea, constipation,
vomiting, abdominal distension and abdominal pain. It’s unclear if these adverse feelings were due to the ketones themselves or if it was the overall quantity of fluid being consumed in the study (Clarke et al., 2013).

The ketone levels achieved via supplementation in various studies have ranged from <0.5 mmol/L at baseline to 3.5 mmol/L at some of the higher levels of intake by those who are not keto-adapted (keto-naïve) (Clarke et al., 2013; Cox et al., 2016). This is of utmost importance as ketone levels typically are <0.5 mmol/L if keto-naïve, 0.5-4 mmol/L if following a well formulated ketogenic diet and between 5-8 mmol/L after prolonged starvation (Cahill, 2006; Owen, Felig, Morgan, Wahren, & Cahill, 1969). All of these ketone levels are physiologically safe and it’s not until levels reach upwards of near 25 mmol/L in diabetic ketoacidosis (Richard L. Veech, 2004) where ketones can become potentially harmful. The concept of safely achieving acute ketosis without having to adhere to a ketogenic diet is one that the athletic world has found intriguing. Ketone supplements may be a way to help those who are keto-naïve achieve some of the unique benefits seen within keto-adapted athletes. While those who are already keto-adapted may see the ketone supplements augment the benefits that they are already experiencing.

### 2.2 Energy Production

Beta-hydroxybutyrate is able to be oxidized and provide energy by conversion to acetyl-CoA and entering the TCA cycle for ATP production (Laffel, 1999). This process tends to be more efficient than glycolysis in part due to the ability of ketones to produce
more energy per unit of oxygen (Cahill & Veech, 2003; Kashiwaya et al., 1997; Sato et al., 1995; Richard L. Veech, 2004). Ketolysis also has the potential to generate energy quicker than glycolysis due to fewer steps needing to occur for production of acetyl-CoA to enter the TCA cycle (Gropper & Smith, 2013). With the ability to be utilized quickly and efficiently, it seems logical to believe that beta-hydroxybutyrate supplementation could potentially improve anaerobic performance. Endurance athletes have already demonstrated the potential that a ketogenic diet can have at aiding performance to at least match what can be done on a higher carbohydrate diet (Volek et al., 2015). The impact of ketogenic diets on anaerobic performances has been looked at less and it’s less clear as to what type of overall impact it could have. With high intensity exercise and the rapid need for energy it is unclear if the body is able to produce enough ketones to sustain that energy demand as compared to a higher carbohydrate diet but perhaps supplementing exogenous ketones could augment overall energy production as ketones utilize different transporters (monocarboxylate transporters – MCT) (Evans, Cogan, & Egan, 2011) than glucose (glucose transporters – GLUT) (Bryant, Govers, & James, 2002).

**Ketone Utilization**

There does appear to be a difference in the body’s ability to utilize ketones for energy depending on what levels the concentration of ketones are raised to. When ketone levels are elevated to 1-2 mmol/L, the body is able to take up and utilize them up to five-times faster at the onset of exercise (Balasse, Fery, & Neef, 1978; Fery & Balasse, 1988; Féry & Balasse, 1986). If ketone levels are too elevated, the clearance rate of them may
not be significantly impacted by the initial onset of exercise (Fery & Balasse, 1988). There appears to be an inverse relationship between blood ketone levels and the rate at which the body is able to utilize and clear them. Higher starting ketone levels causes slower uptake and the levels to remain elevated longer whereas as the lower ketone levels become or start at, they will be utilized quicker and return back to baseline levels sooner (Clarke et al., 2013; Fery & Balasse, 1988; Féry & Balasse, 1986). This leaves the question of whether exogenous ketones could potentially provide a source of energy for the final push at the end of a race or game if the supplementation induces high enough ketone levels to suppress their utilization rates until later on in exercise. Supplementation could help to ensure that ketones are available at this time of need when intensity is at its highest and endogenous ketone production is inhibited due to blood flow being directed to the muscles and away from the liver where ketones are endogenously produced (Rowell, Blackmon, & Bruce, 1964). Conversely, if ketone levels remain relatively low to be utilized faster and earlier on in exercise, it could preserve glucose stores from being tapped into until later on during exercise. This theoretically would result in improved endurance to exercise longer and/or increased power production (Cox et al., 2016). Thus, without exogenous ketones the body would be relying more heavily on its glucose and glycogen stores leaving an increased susceptibility for fatigue during both endurance and power tests. Despite these tests being exact opposites it seems possible for ketones to potentially help one or the other.

It is unclear how much skeletal muscle is able to utilize ketones for energy as previous studies have found it to be relatively minimal when in a glycogen depleted state
or during chronic nutritional ketosis (Fery & Balasse, 1983; Phinney, Bistrian, Evans, Gervino, & Blackburn, 1983). Under standard physiological conditions and following a higher carbohydrate diet, acute nutritional ketosis appears to allow for significant ketone utilization (Cox et al., 2016). During exercise itself, the body’s ability to take up and utilize ketones may depend on the type of work being done as the concentration of MCTs are different on different muscle fibers. There appears to be greater MCT concentration on type-1 slow-twitch muscle fibers, which may be one variable as to why ketogenic diets are generally touted for benefiting endurance sports (Bonen, 2001). The extra MCTs on the type-1 fibers theoretically might enhance their ability to take up and utilize ketones more readily than type-2 fast-twitch muscle fibers despite type-2 fibers having a greater concentration of mitochondria (Winder, Holloszy, & Baldwin, 1974). Ketones could continue to provide a significant overall contribution to energy production but attribute to a smaller percentage of it as the intensity of exercise increases (Cox et al., 2016). Regardless of muscles ability to utilize ketones, the brain is able to run off of ketones (discussed more in depth in the cognitive function section) and preserve glucose and glycogen for muscles to utilize.

In a research setting, the intensity at which someone is working at can be measured by using a metabolic cart and looking at the respiratory exchange ratio (RER). RER is the proportion of how much carbon dioxide is produced, relative to how much oxygen is consumed and is used to help gauge the intensity at which someone is exercising. Additionally RER can be used to give an idea as to what types of sources the body is getting its energy from (Frayn, 1983). An RER value of 0.7 indicates the body is
predominately-utilizing fat for energy, which typically occurs during low intensity, whereas an RER value of 1.0 indicates carbohydrates are predominately being used, typically occurring during high intensity. Consumption of exogenous ketones would be believed to cause some difficulty in interpreting this as beta-hydroxybutyrate when utilized produces a respiratory quotient (not exactly the same as RER but generally similar) value of 0.89 (Frayn, 1983). This could potentially inflate the portrayed intensity at which the participant is working and thus insinuate that the participant may be utilizing carbohydrates more, even though in reality they could be using more ketones. The opposite could occur too so that at a higher intensity, which would typically induce an RER of greater than or equal to 1.0, utilization of the ketones could bring the RER down. Due to the variability of individual RER values and the many factors that can impact it (Goedecke et al., 2000) the study was designed to eliminate some of these factors by having the participants consume the same food the day before each testing visit, come in fasted and have them all be at least recreationally trained.

*Trained vs. Untrained*

The more trained an individual is, the more able they theoretically can make use of ketones for energy. Exercising pushes the body to increase mitochondria production and enhances metabolic efficiency by allowing it to utilize fat for energy production than someone untrained doing the same work (Burgomaster et al., 2008; Holloszy & Coyle, 1984). These findings have helped confirm that training impacts fat utilization but ketone utilization specifically hasn’t yet been studied as intensively to look at the different
utilization between trained and untrained individuals. Indirectly looking at this, it appears that trained individuals are less likely to exhibit episodes of post-exercise ketosis (Ohmori, Kawai, & Yamashita, 1990; Rennie, Jennett, & Johnson, 1974; Svensson, Albert, Cardel, Salatino, & Handschin, 2016; Winder et al., 1974). These numbers can be impacted by how long post-exercise the study continued monitoring the participants with individualized responses (Koeslag, Noakes, & Sloan, 1980). It is unable to be determined what the exact cause of post-exercise ketosis is. The body’s ability to utilize the ketones being produced or the actual rate at which ketones are produced at the end of exercise are two critical variables impacting the state of post-exercise ketosis.

**Cognitive Function**

Most textbooks state the central nervous system requires a minimum amount of carbohydrates so that it can properly function due to the copious supply of glucose. Unfortunately those tests possess inaccuracy due to human ability to utilize ketones for its brain energy needs even when no carbohydrates are available (Cahill & Owen, 1968; Laffel, 1999; Owen et al., 1967). The production of ketones is able to make up for the decreased glucose availability and keep the brain functioning properly during apparent carbohydrate/energy crisis.

When exercising the body is put into a similar glucose/glycogen depleted state due to the body’s increased energy needs. As the duration of exercise increases, the brain is able to detect the gradual decline in glucose levels and serves as a major contributor to the body’s perception of fatigue (Noakes, 2011). Carbohydrates have been the popular
recommendation for many years now for athletes to consume during exercise to help alleviate fatigue from setting in by replenishing those glucose levels (Coyle et al., 1983). With ketones’ ability to cross the blood brain barrier it isn’t unreasonable to believe that exogenous ketones could provide similar benefits in delaying central fatigue to allow for increased physical performance even when blood glucose becomes low (Dalsgaard & Secher, 2007).

Cognitive abilities are also impaired when blood glucose levels become depleted which can be deleterious in a multitude of applications: sports, military, school or activities of daily living (Warren & Frier, 2006). When in a situation of hypoglycemia where clear thinking is required, ketones may serve as an alternative energy source to combat cognitive decline (Cahill & Owen, 1968; Laffel, 1999; Owen et al., 1967). As mentioned earlier, if ketone levels reach around 2 mM or higher, utilization during exercise could be preserved early in the activity and used more at the end (Clarke et al., 2013; Fery & Balasse, 1988; Féry & Balasse, 1986). If ketone levels fail to reach highly ketotic levels (<1.0mmol/L) they may be oxidized earlier in the exercise portion and improve physical performance but the cognitive test scores may not be changed (Balasse et al., 1978; Fery & Balasse, 1988; Féry & Balasse, 1986).

**Caffeine**

Even though the main ingredient within the product being tested is ketones, the other ingredients cannot be ignored especially when one such ingredient is caffeine which has been studied intensively for its impact of physical and cognitive performance as well
as perceived exertion (McLellan, Caldwell, & Lieberman, 2016). The benefits of caffeine on physical performance have been shown in a wide variety of sports/exercises (Burke, 2009). However, the benefits have not been universally shown (Attison & Nnes, 2012; Burke, 2009; McLellan et al., 2016). There is still a wide array of variables working together that impact the efficacy of caffeine such as unique individual responses, different exercise protocols, dosage and timing of the caffeine intake. It is currently recommended to consume 3-5 mg/kg of caffeine an hour before exercise to allow for the caffeine to be fully absorbed and reach peak concentration to achieve maximum efficacy.

Pruvit’s KETO//OS MAX CHARGED contains 120mg of caffeine. This amount would be <2 mg/kg for the participants in the current study. Based on the current study design and participants exercise initiation 15 minutes post-consumption, the small amount of caffeine consumed wouldn’t have time to achieve peak concentration and thus could be expected to have a minimal impact on cycling performance. There is a chance however the caffeine consumptions could impact the cognitive tests administered immediately after exercise and during the recovery period after more time has elapsed (McLellan et al., 2016).

As the interest in ketones ergogenic possibilities burgeons, this study will help provide more insight into the use of exogenous ketone-salts. Exogenous ketones are beginning to become commercially available and these findings will help justify whether or not they are worth the financial expense for people looking to receive the desired ergogenic effect.
Therefore, the purpose of this study was to help determine the effects that consumption of an exogenous ketone/caffeine supplement would have on cycling and cognitive performance. We hypothesized that supplementation would provide ketones as an additional energy source to improve cycling time to exhaustion, anaerobic power and post-exercise cognitive abilities compared to the projected decline seen when water is consumed in place of the supplement.
Chapter 3: Methods

Experimental Approach

The current study utilized a crossover control trial design comparing ketone supplementation versus control (water). Subjects visited the lab on 4 occasions to complete the consent process, maximal aerobic testing and familiarization session, and two experimental exercise sessions: one involving ingestion of a ketone/caffeine supplement and the other a water control condition. The two experimental sessions were performed in a randomized and balanced order. Each exercise test day lasted approximately 2 hours with a 2-7 day rest period in between testing based upon the subject’s availability to return and allowing time to recover from the physical exertion of the test. Overall the study took 1-2 weeks for each participant. The specific test days are listed below:

1. Screening/Complete consent form
2. Baseline testing – Vo2Max Cycle Test; cognitive test
3. Cycling test & cognitive testing with ketone supplement
4. Cycling test & cognitive testing with control (water)
Subjects

12 recreationally trained participants (4 female, 8 male: mean ± SD age, 23.1 ± 1.9 years; weight, 78.9 ± 16.2 kilograms; height, 176.6 ± 14.0 cm; VO\textsubscript{2}max 41.2 ± 5.2 ml/kg/min) participated in this study (Table 1). To ensure eligibility, participants completed a medical history questionnaire and physical activity questionnaire before signing an informed consent document approved by the institutional review board. The inclusion criteria for this study required participants to be; between the ages of 18-50, recreationally trained as deemed by meeting ACSM recommendations of at least 150 minutes of moderate activity per week and habitually consuming a traditional mixed diet determined by food frequency questionnaire administered during the consent meeting. Participants were excluded if the administered questionnaires detected; metabolic or endocrine dysfunction, cardiovascular or respiratory diseases, gastrointestinal disorders, regularly smoking, consuming alcoholic beverages in excess of three drinks per day, epilepsy, chronic headaches or if pregnant. If taking participants were asked to discontinue if able any use of probiotics, antibiotics, antifungals and any supplements known to impact exercise performance, antioxidant or inflammatory status.
**Supplement**

The supplement used in this study consisted of 12 grams of beta-hydroxybutyrate in the salt form (sodium, magnesium, and calcium) and 120 mg caffeine (KETO//OS MAX CHARGED, Pruvit). One 18.4 g individual serving supplement packet was mixed with 16 oz of water prior to ingestion. During the control condition, subjects consumed an equal volume of water only. Other ingredients are listed below on the nutrition facts panel.

![Supplement nutrition label](image)

**Figure 2: Supplement nutrition label**

**Testing Preparation**

Prior to testing, participants completed a 24 hr food record. Participants were asked to replicate the same eating pattern before the second day of testing. Subjects avoided any food and caloric beverages within 8 hours of their scheduled testing time to ensure they arrive at the lab in a postabsorptive state. The day immediately prior to testing the participants were specifically advised to avoid any alcohol, caffeine and
voluntary physical activity. Participants were advised to maintain their current activity level throughout the duration of the study with the exception being the day before testing in which the participants were advised to avoid exercising.

**Baseline Testing**

Participants arrived at the lab in the morning having fasted for at least eight hours prior to arrival and without participating in any voluntary exercise the day before. They immediately were asked to provide a urine sample to determine specific gravity (Reichert TS 400 clinical refractometer) to ensure acceptable hydration was met (all subjects had a USG <1.025). Female participants completed a urine pregnancy test (Sure-Vue urine hCG strips). Height and weight were assessed (seca 763, Deutschland) before being familiarized with the symbol digit modality test (SDMT), which was used to measure cognitive function. SDMT is a 90 second test in which there is a key with nine unique shapes each representing a specific number 1-9. The participant was given a series of those shapes and asked to write the corresponding number for each one in order to complete as many as they can as accurately as they can.

Participants were then familiarized with the equipment and procedures before completing a maximal oxygen consumption (VO$_2$max) test on a stationary bicycle (Lode Corival bicycle egometer, Groningen, Netherlands) using indirect calorimetry (Parvo Medics TrueOne 2400 Metabolic Measurement System; Sandy, Utah). Participants were fitted with headgear and a mouthpiece attached to a hose (HANS RUDOLPH 2700 Series non-rebreathing; Shawnee, Kansas) linked with the two-way air chamber (Parvo Medics
TrueOne 2400 Metabolic Measurement System; Sandy, Utah) with a nose-clip to ensure all air is collected from the mouth and not escaping out the nose. The protocol began with a 4 min warm-up period with zero resistance to allow adjustment of equipment for comfort and proper function while cycling. The staged protocol began at 20W of resistance and increased 20W every 60 sec. The test was completed when the participants voluntarily stopped pedaling, occurring within 18 min.

Measurements of VO\textsubscript{2} and VCO\textsubscript{2} via breath-by-breath gas exchange was recorded every 15 sec. These values were used to determine oxygen uptake, carbon expiration and respiratory exchange ratio (RER) to calculate carbohydrate and fat oxidation rates. Max VO\textsubscript{2} and RER were determined by the highest 15 sec interval value reported. After testing, the participant was given a food log to record what they ate during the 24 hr prior to the upcoming test day and a date for the first test session was scheduled within 7 days and after at least 48 hours to allow for adequate recovery.

**Full Test Day**

Subjects arrived to the testing center fasted with their dietary log completed. All participant’s hydration status was measured via urine and female participants completed a urine pregnancy test. A finger stick was conducted with a lancet to obtain capillary concentrations of beta-hydroxybutyrate and glucose at baseline (FreeStyle Optium beta-Ketone Blood beta-Ketone Test Strips, Abbott Nutrition, Columbus, Ohio) and glucose (ReliOn ULTIMA Blood Glucose Test Strips, Abbott Nutrition, Columbus, Ohio) using a handheld meter (Precision Xtra Blood Glucose and Ketone Monitoring System, Abbott
Nutrition, Columbus, Ohio). Upon completion, a baseline SDMT test was conducted prior to consuming the supplement or water, which was ingested within 5 min. Another finger stick was taken 10 and 15 min after beverage consumption. Subjects were fitted with the mouthpiece attached to the metabolic cart. The bike seat was positioned to be exactly the same as it was for the participant at baseline. An aerobic graded exercise protocol to exhaustion was then performed. Testing began with a 4 min warm-up period to allow for participants to get comfortable and ensure all equipment was properly positioned and functioning. Once started, each stage lasted 5 min starting at 65% of their determined VO$_2$max. Each stage increased by 5% of their VO$_2$max until 90% was reached where the resistance was maintained until exhaustion. At the end of each stage a rate of perceived exertion was collected using the 6-20 BORG RPE scale. One finger stick was taken at the end of the third stage to determine changes in ketones and glucose at the predicted mid-point. Exhaustion was defined when the revolutions per minute (RPM) on the bike became less than 60. The first time the participant fell below 60 RPM, a verbal warning was given and the participant had 5 sec to get the RPMs above 60 at which point testing resumed. If the participant was unable to get the RPMs back up within 5 sec or the second time the RPMs fall below 60, the test was ended. After exhaustion was achieved, the mouthpiece and headgear were removed and the participant was immediately moved to a second bike (Monark Ergomedic 894E bicycle ergometer, Vansbro, Sweden) to complete a 30 sec anaerobic Wingate test. The bike seat was adjusted prior to testing having begun and set to same measurements for both test days. Once completed the participant immediately completed another SDMT test followed by a
finger stick and completed a gastrointestinal distress questionnaire that involved a 0-10 point scale. The participant was then seated for 60 min to recover. Finger sticks were taken every 10 min throughout the recovery with a third and fourth SDMT completed at 30- and 60-min recovery. Upon completion of test session one, participants were given a copy of their completed food log to replicate the day before their scheduled second test day in which the same protocol was followed with the alternative beverage being consumed.

**Figure 3: Test day timeline**

**Statistics**

Dependent t-tests were used to examine differences between ketone/caffeine supplement and water conditions for physical and cognitive performance results. For ketones and glucose, a two-way analysis of variance with condition (ketone/caffeine vs
water) and time (11 time points during the testing session) as within factors were used.

Fisher’s least significant difference post hoc was used to examine comparisons between
conditions when significant main or interaction effects were present. The alpha level for
significance was set at $p \leq 0.05$. 
Chapter 4: Results

GI Distress

The supplement was well tolerated. Self-perceived ratings of GI distress immediately post exercise were not significantly different between conditions. There were three instances of vomiting that occurred, all of which were at the end of exercise during the water condition. In all cases, subjects had completed the exercise trial and all additional testing (cognitive tests, blood draws) were completed as scheduled.

Ketone and Glucose Levels

Capillary blood ketone concentrations at baseline (pre-ingestion) were similar, but all post-supplement consumption values were significantly higher than the water condition \( (p<0.05; \text{Figure 1}) \). Only one out of the twelve participants did not experience an increase in ketones from the supplement. Peak ketone levels typically occurred 15 min after ingestion and were more than two-fold higher after supplement ingestion. Ketone levels post-supplementation were significantly higher compared to baseline and also compared to the water condition at every time point. Under the water condition, ketone levels decreased after the onset of exercise and were significantly lower than baseline from the mid-point of the TTE through IP-40 except for IP-20 \( (p=0.07) \).
Glucose concentrations peaked immediately post-exercise, gradually returning to baseline levels over the course of the recovery time, but there were no significant differences between conditions (Figure 2). Glucose concentrations were significantly lower than baseline at the mid-point of TTE and significantly higher than baseline immediately, 10 and 20 min post-exercise.

**Time To Exhaustion**

Total time to exhaustion was significantly increased by 93 sec (8.3%) after consumption of the ketone supplement (20:53 ± 1:17 vs 19:17 ± 1:15 min:sec) (Table 2). Ten of 12 participants increased TTE and the two who showed decreased performance were less than 5% difference between trials (Figure 3). There were no significant correlations between ketones and TTE. Peak VO2 levels achieved during TTE showed a trend of being higher in the supplement group (p<0.08).

**Wingate Power**

Peak power achieved during the Wingate was not significantly different between the ketone/caffeine supplement and water trial (605 ± 66 W and 609 ± 65 W, respectively) (Table 3). Average power over 30 sec was significantly higher after the ketone/caffeine supplement than water (432 ± 45 and 414 ± 42 W respectively).

**Cognitive Testing**
There was no significant difference in test scores between conditions. There was an average increase of three correct answers immediately post-test compared to baseline in the ketone/caffeine supplement condition whereas there was a decrease of one correct answer during the water condition.

Rate of Perceived Exertion

The average RPE scores from stages 1-4 were significantly lower in the ketone/caffeine supplement condition (p<0.05; Figure 4). Four individuals were able to complete one additional stage under the ketone/caffeine supplement condition. Stages 5 and 6 were not used in the analysis due to too few participants completing those stages. Stage 3 was the only stage there was a significant difference between conditions (p<0.02).

Respiratory Exchange Ration

There were no significant differences in RER between conditions except at stage 3 RER was significantly lower for the ketone/caffeine supplement condition (p<0.01) (Table 4).
Chapter 5: Discussion

This study demonstrated that compared to a control condition when subjects consumed only water, acute ingestion of a ketone/caffeine supplement resulted in a mild ketosis, improved TTE and subsequent average power output in a fatigued state in healthy adults. Despite a greater physical output, the ketone/caffeine supplement was associated with decreased perceived exertion. The ketone/caffeine supplement was well tolerated by all participants. These results support the ergogenic potential of a combined ketone/caffeine supplement for enhancing exercise performance in non keto-adapted recreationally-active adults.

Ketone/caffeine supplementation successfully elevated ketone concentrations in every participant, but the effect was moderate (~0.3 mmol/L increase from baseline) and lower than reported in previous studies (Clarke et al., 2013; Cox et al., 2016). Four of the participants did not achieve ketone concentrations ≥0.5 mmol/L, a level generally not achieved in the fasted state for non-keto-adapted individuals and the lower end of the range of nutritional ketosis (Hyde, Miller, & Volek 2016). All subjects were provided a standard dose of ketones containing 12 g of BOHB. Whether a higher dose would result in increased ketone concentrations, and whether higher ketones would translate into greater improvements in exercise capacity remains unclear, but the variable response in ketosis to supplementation suggests that dosage requirements may need to be
personalized, perhaps based on body mass or body volume, to achieve a consistent ketone concentration. It is also possible that achieving a particular ketone concentration is not important, since there were no significant associations between magnitude of ketosis and exercise performance.

In both conditions glucose was typically less than 90 mg/dL at baseline, gradually declined throughout the TTE protocol, and then peaked above baseline immediately post anaerobic Wingate testing. This pattern was expected as glucose typically declines during submaximal exercise, and then increases during supramaximal exercise due to the hormonal response that drives augmented hepatic gluconeogenesis (Wasserman, 1995). Ketosis did not alter the glycemic response, indicating a net increase in availability of circulating energy substrates. This is contradictory to what may have been expected as previous research has found acute ketosis to inhibit hepatic glucose output and result in decreased glucose levels (Mikkelsen, Seifert, Secher, Grondal, & Van Hall, 2015). The increased glucose response attributed to high-intensity exercise may have overshadowed the more subtle effect of ketones on hepatic glucose output. There may be a dose response effect suggesting the levels of ketosis achieved in this study weren’t sufficient to induce the effects observed by Mikkelsen et al at concentrations of 1.7 mmol/L (Mikkelsen et al., 2015).

Cycling performance was improved after acute ingestion of the ketone/caffeine supplement as evidenced by an enhanced to cycle longer at 90% of VO2max and to achieve a higher average 30 sec power output in a fatigued stated. Similar improvements were reported after ketone supplementation in a 30 min timed trial performance test conducted after an hour of steady state workload at 75% Wmax on a stationary bike (Cox
et al., 2016). Although these results were obtained on a cycle ergometer in a laboratory setting, the ergogenic effect may translate to sporting events where enhanced endurance and power at the end of events is relevant. This ergogenic effect may be attributed to enhanced efficiency of ketones at producing ATP per unit of oxygen (Cahill & Veech, 2003; Kashiwaya et al., 1997; Sato et al., 1995; Richard L. Veech, 2004). Increased production of ATP with similar oxygen intakes would translate into improved performance since the ketone/caffeine supplement was associated with greater VO2. Although not measured directly in this study, the ketone supplement may have also improved performance via enhanced buffering as ketone esters have been shown to decrease accumulation of lactate (Cox et al., 2016) which may delay the onset of fatigue (Hogan, Gladden, Kurdak, & Poole, 1995; Mainwood & Renaud, 1985). Most studies refuting the ability of skeletal muscle to utilize ketones are done on participants while in a relatively glycogen depleted stated and oxidizing free fatty acids predominately such as in chronic nutritional ketosis (Fery & Balasse, 1983; Phinney et al., 1983). When the body is in a unique state of ketosis whilst maintaining glycogen stores, ketone utilization by muscle doesn’t appear to be hindered mirroring what was shown in this study (Cox et al., 2016).

RER was significantly higher for the ketone supplementation condition. A higher RER is indicative of an increased utilization of glucose as an energy substrate (Goedecke et al., 2000). The trend for both conditions was a gradual increase as time spent cycling increased and intensity increased at each stage (Lj & Pl, 2001; Romijn et al., 1993). The fact that RER was higher at each stage in the ketone supplementation condition leads one to believe that the supplement may cause an increase in glucose utilization and decreased
fat utilization (Robinson & Williamson, 1980). In support of this idea, Cox et al found that acute nutritional ketosis suppressed fat oxidation indicated by a suppressed increases in both free fatty acids and glycerol (Cox et al., 2016). Surprisingly though, the same study found ketones to inhibit glycolysis indicating glucose utilization was also decreased but it may be impacted less than fat utilization (Cox et al., 2016). The stage with the most significant difference between conditions was seen at stage three when participants were cycling at 75% of their VO_{2}\text{max}. At this intensity, Cox et al saw a relatively similar contribution of ketones towards energy output between exercise at 40% and 75% VO_{2}\text{max} but a significantly higher absolute utilization of ketones was observed at the higher intensity (Cox et al., 2016). It leads to the question of whether ketone utilization itself is contributing to the increased RER value, which would be worth looking into more extensively in future studies.

Increased RER at stage three may also be connected with the significantly decreased RPE at stage three as well. If ketones are able to provide energy in a more efficient manner, perception of effort and fatigue would be expected to be lower despite the same work being done. Possible decreased lactate build up from the ketones (Cox et al., 2016), and the caffeine within the supplement may also be responsible for the impact on RPE (Costill, Dalsky & Fink, 1977; Hogan et al., 1995; Mainwood & Renaud, 1985; McLellan, Caldwell, & Lieberman, 2016). The decreased perceived effort may have allowed participants to complete one additional stage compared to the water condition.

As for the cognitive test scores, no significant effects of supplementation were detected. It was hypothesized that the combined effects of ketones and caffeine would increase cognitive performance after exercise. It was believed the ketones could provide
an alternative energy substrate for the brain, especially when glucose availability may be limiting due to competition between active skeletal muscle uptake and transport across the blood brain barrier. Better fuel flow to the brain was hypothesized to translate into improved cognitive functioning. The moderate level of ketosis reached (less than 1 mmol/L) and the possibility some of the ketones were taken up by skeletal muscle, may explain the lack of an effect on cognition. Caffeine was originally believed to help with cognitive function based on studies showing improved attention and memory (McLellan et al., 2016) but the dose may have been to low and/or caffeine may not be beneficial for the specific type of cognitive test administered in this study as results tended to be highly variable (Nehlig, 2010).

Caffeine has been studied extensively for its impact on exercise performance. Most benefits have been shown using higher dosages than what was provided in this supplement (Graham & Spriet, 1995; McLellan et al., 2016). When looking at graded exercise protocols similar to what was done in this study, it appears caffeine ingestion does not provide a significant ergogenic effect especially compared to other performance protocols (Dodd, Brooks, Powers, & Tulley, 1991; Doherty & Smith, 2004). For the sprint at the end of the protocol, caffeine has been shown to be ergogenic (Doherty & Smith, 2004) even in situations such as this when glucose/glycogen stores are depleted (Silva-Cavalcante et al., 2013). The reason for improved performance may stem from caffeine’s ability to decrease RPE (Costill, Dalsky & Fink, 1977; Doherty & Smith, 2005) and increase lipolysis (Harpaz, Tamir, Weinstein, & Weinstein, 2016) for energy helping to preserve glucose/glycogen. In regards to its impact on cognition the research is a bit mixed, especially when looking at the impact on short-term memory specifically
such as what was used with the SDMT test in this study (Nehlig, 2010). Overall the impact of caffeine is highly variable dependent upon the dosage and what tests are being conducted. Small caffeine dosages, such as the 120mg used in this study, have not been studied as intensively and the results on those are more variable making it difficult to discern the efficacy it may have as an ergogenic aid (Spriet, 2014). Despite this one cannot ignore the possible effect caffeine may have had. Testing this ketone supplement with caffeine is very beneficial as the majority of commercially available supplements being used as a pre-workout contain caffeine in them, which provides real world applicability to this study.

No adverse events were observed from consumption of the ketone/caffeine supplement. The supplement was well tolerated except for one participant was not fond of the flavor. Due to three participants vomiting under the water condition, it raises the question of whether the ketone supplement may have a soothing effect on one’s GI. This is an improvement compared to previous studies that showed problems with nausea, bloating and vomiting from ketone supplementation (Clarke et al., 2013). Part of those adverse effects may be attributed to the sheer quantity of fluid the participants had to consume (over 3 liters of supplement per day) compared to the mere 16 ounces provided in this study. Two of the participants vomited during the first day of testing and one participant vomited during the second day. Due to it occurring on both days it appears more likely that the soothing effect is more likely attributed to supplementation than adaptation to the exercise. It’s unclear what specific ingredient may be attributing to this soothing effect. Caffeine is typically believed to cause a decrease in blood pH level causing increased acidity and GI discomfort presenting itself as nausea and vomiting.
This may be more attributed to caffeine containing beverages such as coffee and the contents of coffee actually causing the negative effects (Tunnicliffe, Erdman, Reimer, Lun, & Shearer, 2008). Caffeine by itself hasn’t been shown to elicit an impact on GI distress. Indirectly though caffeine has been shown to increase fat oxidation (Harpaz et al., 2016). Increased fat oxidation would result in less glycolysis and thus less acid buildup causing GI distress. Further investigation looking into the ability of ketone-salts to augment fat oxidation would be useful to see if caffeine and ketone-salts are working synergistically to reduce GI distress.

The other main ingredients in this supplement (l-taurine and l-leucine) have not been linked to impacting GI in neither a positive nor negative manner. When assessing the impact of the minerals it appears that calcium may actually induce potential adverse GI events (Lewis, Zhu, & Prince, 2012). As for magnesium and sodium, it appears they have a negligible impact on GI distress. Exogenous ketones impact is mixed and it may depend on the supplemented form and overall dosage. Ketone-salts seem to be well tolerated (Nair, Welle, Halliday, & Campbell, 1988; Plecko et al., 2002) yet ketone esters had more adverse affects associated with them but it’s tough to distinguish if it’s the quantity of ketones or volume of liquid consumed causing GI distress (Clarke et al., 2013). One potential reason for the tolerability of ketone-salts is their ability to blunt decreases in blood pH values typically seen during exercise similar to bicarbonate (Nair et al., 1988). Hydrogen production is the main contributor to the acidity experienced in exercise, which occurs at a higher rate during more intense exercise when lactate is produced (Messonnier, Kristensen, Juel, & Denis, 2007). This hydrogen build up and acidity causes vomiting as a way for the body to excrete excess acidity to return back to a
more basic pH level. Ketone-salts ability to buffer this acid build up could attribute to the improved GI responses post supplementation.

**Conclusion**

In conclusion, ingestion of a ketone/caffeine supplement was well tolerated, successfully elevated ketone concentrations and improved cycling performance while decreasing perceived exertion, but had no impact on cognitive function. The supplement used contained a combination of ketones and caffeine, and thus we cannot determine if the ergogenic effects were a result of one or a combination of these nutrients. These effects were observed in a group of healthy active adults who had not previously been keto-adapted. The results indicate that the combination of exogenous ketones with caffeine has ergogenic effects on physical performance and should be studied further to determine optimal dosages and underlying mechanisms for the enhanced performance.
References


Clarke, K., Tchabanenko, K., Pawlosky, R., Carter, E., King, M. T., Musa-Veloso, K., …


Johnson, R., & Walton, J. (1972). The effect of exercise upon acetoacetate metabolism in
athletes and non-athletes. *Experimental Physiology*, 57(1), 73–79.


Figure 4: Capillary blood beta-hydroxybutyrate responses

Ketone levels were assessed via blood from individual finger sticks at 11 different time points. (*) Indicates significantly different (p<0.05) from corresponding time point for water. (#) Indicates significantly different (p<0.05) than baseline value for respective condition.
Figure 5: Capillary blood glucose responses
Glucose levels were assessed via blood from individual finger sticks at 11 different time points. No statistically significant difference was observed at any point between conditions. (#) Indicates significantly different (p ≤ 0.05) than baseline value for both conditions.
Figure 6: Individual responses in time to exhaustion

The time cycled post-ketone/caffeine supplementation was significantly higher (p<0.01) determined via two-tailed t-test.
The BORG rating of perceived exertion (6-20) was assessed at the end of each stage cycled. Mean RPE was significantly lower in the ketone/caffeine supplement condition (p<0.05) at stage 3 (*; p<0.05) tested via two-tailed t-test.
# Appendix B: Tables

<table>
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<tr>
<th></th>
<th>Sex</th>
<th>Age</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>VO$_{2}$max (ml/kg/min)</th>
<th>Caffeinated Beverages / wk</th>
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</table>

|                | Mean ± SD | 79.3 ± 16.7 | 176.6 ± 13.0 | 41.2 ± 5.12 | 6.4 ± 9.4* |

**Table 1: Participant Characteristics**

* One participant consumes 35 caffeinated beverages per week. Excluding this outlier the mean/wk equates to 3.8 ± 2.6.
<table>
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<tr>
<th>Participant</th>
<th>Peak Ketone (mM)</th>
<th>TTE (min:sec)</th>
<th>Peak VO2 (ml/kg/min)</th>
<th>% TTE Change</th>
<th>% VO2 Change</th>
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<td>1</td>
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<td>27.8%</td>
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<td>3.0%</td>
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<td>0.0%</td>
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Mean: 0.57 ± 0.05 | 20:53 ± 1:17* | 40.2 ± 1.7 | 19:17 ± 1:15* | 38.8 ± 1.7 | 8.7 ± 2.8* | 1.5 ± 0.8

Table 2: Peak beta-hydroxybutyrate levels and TTE and VO2 responses

Data (mean ± standard error; n = 12) for TTE was statistically different (*) (p<0.01) determined via two-tailed t-test. A trend for higher VO2 after ketone/caffeine supplement consumption (p=0.08) was determined via two-tailed t-test.
<table>
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<th>Water</th>
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| Mean         | 605 ± 66          | 432 ± 45* | 609 ± 65          | 414 ± 42* | 0.3 ± 4.5 | 4.0 ± 1.8* |

**Table 3: Wingate power output responses**

Wingate testing occurred immediately after TTE testing and data (mean ± standard error) showed significant (*) improvement in average power within the ketone supplement group (p<0.05) via two-tailed t-test. No significant difference found in overall peak power.
Table 4: Respiratory exchange ratio responses

RER was calculated every 15 sec during TTE testing and averaging those values at each stage found significantly higher (*) RER in the ketone supplement.