Acute coffee ingestion with and without medium chain triglycerides decreases blood oxidative stress markers and increases ketone levels

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Acute coffee ingestion with and without medium chain triglycerides decreases blood oxidative stress markers and increases ketone levels

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Abstract

**Background:** Ingestion of ketone supplements, caffeine and medium chain triglycerides (MCTs) may all be effective strategies to increase blood levels of the ketone body beta-hydroxybutyrate (D-BHB). However, acute ingestion of a bolus of lipids may increase oxidative stress (OS). The purpose of the study was to investigate the impact of adding varying amounts of MCTs to coffee on blood levels of D-BHB and markers of OS. **Methods:** Ten college-aged men ingested coffee with 0g, 28g, and 42g of MCT in a randomized order. Blood samples were collected pre, as well as two and four hours postprandial and analyzed for D-BHB, total cholesterol (TC), high density lipoprotein cholesterol (HDL-c), glucose, triglycerides (TAG), insulin, as well as OS markers: advanced oxidation protein products (AOPP), glutathione (GSH), malondialdehyde (MDA), and hydrogen peroxide (H$_2$O$_2$). **Results:** All three treatments resulted in a significant increase in D-BHB, HDL-c, and TC, as well as a significant decrease in TAG, MDA, H$_2$O$_2$, and insulin. The 42g treatment was associated with significantly higher levels of AOPP and MDA. **Conclusions:** Acute ingestion of coffee results in favorable changes to markers of cardiometabolic health that were not impacted by the addition of 28g MCT. However, 42g MCT caused significantly greater OS.
**Introduction**

During periods of carbohydrate restriction or prolonged fasting ketone bodies such as beta hydroxybutyrate (BHB), acetoacetate, and acetone are produced by the liver to serve as an alternative fuel source (Robinson and Williamson, 1980). Hyperketonemia (>0.5 mM) has been proposed to have a therapeutic role in the attenuation of various disease states including hypoxia, neurodegeneration, chronic oxidative stress (OS), inflammation, and insulin resistance (Veech, 2004). In peripheral tissues, the ketone isoform D-BHB is released by the liver into circulation and oxidized by cardiac as well as skeletal muscle (Dedkova and Blatter 2014). A ketogenic diet is an effective tool to increase endogenous D-BHB production (Cavaleri and Bashar 2018) and has been shown to favorably alter the redox environment resulting in improved mitochondrial function, reduced inflammation and OS (Pinto et al. 2018). However, due to the impractical sustainability of the ketogenic diet, other means to acutely increase D-BHB levels may include the use of exogenous ketone salts or esters (Cavaleri and Bashar 2018; Evans and Egan 2018; Stubbs et al. 2017), caffeine (Vandenberghe et al. 2017), and medium chain triglycerides (MCT) (Vandenberghe et al. 2019).

Medium chain triglycerides taken as synthetically purified oils or coconut oil, are lipids that typically contain between 6 and 12 carbons. Unlike long chain fatty acids (i.e., > 14 carbons), MCT are metabolized directly by the liver and are an effective strategy for artificially elevating blood levels of D-BHB (0.5 – 1.0 mM) (Henderson et al. 2009). It is however important to note that acute ingestion of a bolus of lipids, is well established in the literature as a major cause of OS (Bloomer and Lee, 2013; Bloomer et al. 2013), potentially resulting in increased damage to cellular proteins, lipids, and DNA (Mikhed et al. 2015). Therefore,
interventions which offer a practical means for elevating D-BHB without the associated elevations in OS and inflammatory markers are needed.

Finally, caffeine offers an additional method that is both safe and practical for an individual to increase D-BHB levels via increased metabolic rate and free fatty acid availability (Vandenberghe et al. 2017). These findings have led to the commercial promotion of dietary supplements/products that claim to act as ketogenic beverages by adding lipids to caffeine-rich beverages such as coffee. However, it is not clear if the addition of MCT to a caffeine-rich beverage facilitates greater increases in D-BHB compared to caffeine alone. Further, coffee in particular has powerful antioxidative properties which may attenuate OS responses from acute ingestion of a bolus of lipids (Bloomer et al. 2013). Based on the aforementioned, the current research was conducted to address the following gaps existing in the literature: 1) the impact of adding MCT to coffee on blood D-BHB levels and markers of OS and 2) the optimal dose of MCT that is required to achieve this effect.

**Methods**

**Participants**

Participants were considered moderately active (150 minutes physical activity per week) as defined by the American College of Sports Medicine (ACSM), apparently healthy, (i.e., low risk according to ACSM criteria (ACSM's Guidelines for Exercise Testing and Prescription 2018), and with no known history of cardiometabolic disorders. Participants were free from prescription medication, tobacco use or any dietary supplements (e.g., vitamins, antioxidants) for the duration of the study. If they were initially taking a dietary supplement prior to the start of research, ingestion was suspended two weeks prior to the start of the study. Vegetarians were excluded and participants were asked to minimize coffee ingestion and red wine (<16 oz/day) for
the duration of the study. However, they did report being habitual coffee drinkers (i.e., 8-16 oz/day).

All participants completed a health history questionnaire and provided written informed consent. All procedures were reviewed and approved by the University’s Institutional Review Board. Prior to the first session, each participant had their height measured with a stadiometer (Detecto, Webb City, MO, USA) and body mass was recorded while wearing minimal clothing using a physician’s scale (Detecto, Webb City, MO, USA).

Overview of Experimental Testing

Participants reported to the Metabolic & Applied Physiology (San Marcos, TX, USA) laboratory for experimental testing a total of three times. The participants were randomly assigned (in a double blinded fashion) the order in which they ingested the following three treatments: 1) coffee with no added lipids (primarily in the form of MCT), 2) coffee with moderate dose of lipids, and 3) coffee with high dose of lipids. Upon the first session, participants were asked to complete a 24 hour dietary recall and were asked to match the diet as closely as possible prior to each subsequent testing session. Participants arrived to each testing session following $\geq 8$ hour fast and were asked to avoid strenuous exercise for 48 hours, and alcohol consumption and nicotine exposure for 24 hours. Participants were provided with 16 oz of freshly brewed coffee and allowed 15 min to finish the beverage during each testing session. Blood samples were collected at pre-, 120 min post-, and 240 min post-prandial. Note that timing started after beverage ingestion was completed. Participants remained in the building for the duration of the blood draws, unless for academic purposes (i.e. class attendance), in which participants were excused to attend and return immediately following. No additional food intake was allowed during this time. However, water was allowed ad libitum.
Coffee Ingestion

Each treatment included 16 oz of Folgers medium roast (Orrville, OH, USA) that was brewed each morning immediately prior to ingestion. Coffee was prepared using 16 oz of bottled water for each 50 grams (3.5 tbsp) of ground coffee. A stevia-based sweetener (In The Raw, Brooklyn NY, USA) was provided and the amount added was recorded and consistent for each participant throughout the study. Coffee was administered either with 0g lipids, ~28g lipids, or ~42g lipids. The lipids consisted of 75% MCT oil (Now Foods, Bloomingdale, IL, USA) and 25% coconut oil (“HEB Organics”, San Antonio, TX, USA). The choice to include 25% coconut oil was chosen based off pilot trials which produced this as generally the most tasteful preference. Prior to ingestion, the coffee was mixed in a blender (SharkNinja, Needham, MA, USA) 15 minutes after brewing with all additional ingredients (e.g. lipids, stevia) added to it. The drink was allowed to cool at -20 ºC to a drinkable temperature for 10 minutes prior to ingestion.

Blood Sampling and Overview of Analysis

A total of 14 mL were collected in sealed, heparinized vacutainers immediately prior to ingestion, as well as two and four hours postprandial. These timepoints were chosen based on previous work showing changes in oxidative stress biomarkers in a four hour postprandial window (Bloomer et al. 2013). Immediately after sample collection, an initial aliquot of 700µL of whole blood was mixed with an equal volume of a 5% solution of 5-sulfosalicylic acid dehydrate (Sigma Aldrich, St Louis, MO, USA). These samples were then incubated for 10 min at 4ºC and centrifuged at 14,000rpm for 10 min. The supernatant was aliquoted and also stored at -80ºC and later assayed for total glutathione (GSH) concentrations according to assay instructions (Arbor Assays, Ann Arbor, MI, USA). Further, a 25µl sample of whole blood was
used to measure ketone levels using a portable analyzer (Abbott, Columbus, OH, USA).

Immediately after collection, whole blood samples were then centrifuged for 15 min at 2500 rpm at 4°C and plasma was aliquoted and stored at -80°C for subsequent analysis. Plasma samples were analyzed in duplicate for hydrogen peroxide (H$_2$O$_2$), insulin, triglycerides (TAG), glucose, total cholesterol (TC), HDL-c, advanced oxidation protein products (AOPP), malondialdehyde (MDA), and beta-hydroxybutyrate (D-BHB).

**Analysis of plasma D-BHB, HDL-c & TAG**

Plasma samples were thawed and analyzed for D-BHB, glucose, HDL-c and TAG levels using an enzyme immunoassay (EIA) according to assay instructions (Pointe Scientific, Canton, MI, USA). Absorbance was read using a spectrophotometer (Pointe 180 QT, Canton, MI, USA). The TAG assay uses the glycerol phosphate oxidase method (Fossati and Prencipe, 1982). The HDL-c assay involved initial separation of HDL-c where HDL-c precipitating reagent was added to plasma samples. For the HDL-c assay, samples were then centrifuged 2000 x g 10 min to allow for separation of HDL-c. The supernatant was aliquoted and added to a cholesterol reagent (Pointe Scientific, Canton, MI, USA) for subsequent determination of absorbance and analysis of HDL-c values.

**Analysis of insulin, H$_2$O$_2$, AOPP, MDA, total cholesterol & glucose**

Plasma concentrations of insulin were determined in using a high sensitivity assay (ALPCO, Salem, NH, USA) and an automated plate washer was used (Biotek, Winooski, VT, USA). Plasma levels of H$_2$O$_2$ were determined using the Amplex red assay by methods described by the manufacturer (Molecular Probes, Invitrogen Detection Technologies, Eugene, OR, USA). Plasma samples were assayed for AOPP using a commercially available kit (STA-318 OxiSelect AOPP; Cell Biolabs, Inc., San Diego, CA, USA) with methods previously described (Witko-
MDA was analyzed utilizing a commercial colorimetric kit (NWK-MDA01; Northwest Life Science Specialties, Vancouver, WA, USA) using similar methods as previously described (Jentzsch et al. 1996). Absorbance was determined using a Biotek Epoch II colorimetric reader (Winooski, VT, USA). Total cholesterol and glucose levels were analyzed using plasma samples on an Alfa Wasserman Vet Axcel chemistry analyzer (West Caldwell, NJ, USA). Reagents were supplied in manufacturer produced kits for cholesterol (S1010) and glucose (SA1014).

Statistical analysis

Statistical analyses were conducted using SAS v 9.4 (Cary, NC, USA). For each blood marker, a four-hour area under curve (AUC) was conducted as previously described (Pruessner et al. 2003). Subsequently, AUC values for each treatment were compared with one-way repeated measure ANOVAs. In addition, two way (treatment x time) repeated measure ANOVAS were conducted for each blood marker to compare changes across time, and between treatments. In the instance of a significant main effect, Fisher’s least significant difference post hoc test was conducted due to the number of comparisons (i.e., three treatments, three time points). Outliers were managed using the Robustreg procedure SAS v 9.4.

Results

A total of 11 men were recruited to participate in the study. Due to gastrointestinal discomfort, one participant withdrew from the study, therefore a total of ten men (age 23.7 ± 2.8 years, height 175.1 ±7.0 cm, mass 80.6 ±8.5 kg) completed experimental sessions. One participant requested to avoid ingestion of the high dose of MCT due to gastrointestinal distress, therefore that participant was removed from the double blinded design and only completed two of the three treatments.
**Beta Hydroxybutyrate**

With respect to AUC values for whole blood levels of D-BHB as measured by the ketone strip, there was no difference between treatments \((F = 1.37, p = 0.297)\). Mean AUC values for D-BHB are shown in Table 1. In terms of mean D-BHB values, there was no treatment x time interaction \((F = 0.54, p = 0.704)\) and no main effect for treatment \((F = 1.63, p = 0.206)\). However, there was a main effect for time \((F = 11.74, p < 0.001)\). Post hoc analysis showed a significant increase in D-BHB levels from baseline at two \((p < 0.001)\) and four hours \((p = 0.001)\) post prandial, with no significant difference between D-BHB levels at two and four hours. Mean D-BHB levels between treatments and across time are shown in Figure 1.

With respect to AUC values for plasma levels of D-BHB as measured by EIA, there was no difference between treatments \((F = 1.14, p = 0.347)\). Mean AUC values for insulin are shown in Table 1. There was also no significant treatment x time interaction \((F = 1.00, p = 0.411)\) and no main effect for treatment \((F = 1.00, p = 0.371)\). However, there was a main effect for time \((F = 5.24, p = 0.0075)\). Post hoc analysis showed a significant increase from baseline to two \((p = 0.001)\) hours postprandial. There was no difference in D-BHB levels at two and four hours post prandial \((p = 0.20)\). Mean D-BHB levels between treatments and across time are shown in Figure 1.

**Insulin**

In terms of AUC values for insulin, there was no difference between treatments \((F = 0.69, p = 0.519)\). Mean AUC values for insulin are shown in Table 1. There was no treatment x time interaction \((F = 0.72, p = 0.581)\) and no main effect for session \((F = 0.82, p = 0.443)\). However, there was a main effect for time \((F = 3.27, p = 0.045)\). Post hoc analysis showed significantly lower insulin values at four hours compared to two hours post prandial \((p = 0.013)\), with no
other differences between time points. Insulin levels for both treatments across time are shown in Figure 1.

**Glucose**

No difference was noted between treatments for mean AUC values for glucose (F = 1.36, p = 0.28). Mean AUC values for glucose are shown in Table 1. There was no treatment x time interaction (F = 0.61, p = 0.065) and no main effect for time (F = 1.04, p = 0.35). There was a main effect for treatment (F = 4.12, p = 0.02). Significantly higher glucose levels were associated with the 0g treatment compared to 28g (p = 0.01) and 42g (p = 0.01). However, it is important to note that none of the treatments had a significant effect on plasma glucose levels. Mean glucose levels between treatments and across time are shown in Figure 1.

**Triglycerides**

There was no difference between treatments in relation to AUC values for TAG (F = 0.76, p = 0.485). Mean AUC values for TAG are shown in Table 1. No significant treatment x time interaction was noted (F = 0.28, p = 0.888). No significant treatment effect was noted (F = 2.30, p = 0.108). However, there was a significant main effect for time (F = 9.05, p = 0.0003). Post hoc analysis showed a significant decrease in TAG levels from pre to two (p = 0.028) and four (p < 0.001) hours post prandial. Mean TAG levels for both treatments across time are shown in Figure 2.

**Total Cholesterol**

There was no difference between treatments regarding mean AUC values for TC (F = 0.85, p = 0.44). Mean AUC values for TC are shown in Table 1. There was no significant treatment x time interaction (F = 0.22, p = 0.92). However, there was a main effect for treatment (F = 3.15, p = 0.04) and time (F = 4.88, p = 0.01). Post hoc analysis demonstrated a significant
increase from pre-ingestion to two (p = 0.01) and four hours (p = 0.005) post ingestion. Further, TC was significantly higher with 42g of MCT compared to 0g (p = 0.02) and 28g (p = 0.03). Mean TC values between treatments and across time are shown in Figure 2.

**HDL-c**

There was a significant main effect for treatment in terms of mean AUC values for HDL-c (F = 5.75, p = 0.014). Post hoc analysis showed significantly greater AUC values associated with the 42g MCT treatment compared to both 0g (p = 0.004) and 28g MCT (p = 0.037). AUC data for HDL-c are shown in Table 1. There was no significant treatment x time interaction (F = 0.20, p = 0.938). However, there was a main effect for treatment (F = 11.95, p < 0.001), and time (F = 18.44, p < 0.001). Post hoc analysis showed significantly greater HDL-c values for the 42g MCT treatment compared to 0g MCT (p < 0.001) and 28g MCT (p = 0.003). In addition, HDL-c levels were significantly increased at two and four hours post prandial compared to pre ingestion levels (p < 0.001). Data demonstrating changes across time and between treatments for HDL-c are shown in Figure 2.

**Oxidative Stress Markers**

There was no difference between treatments in terms of mean AUC values for GSH (F = 0.51, p = 0.613). AUC values for GSH are shown in Table 1. No significant treatment x time interaction was noted for mean GSH levels (F = 1.18, p = 0.328). There was also no main effect for treatment (F = 0.83, p = 0.440), or time (F = 0.95, p = 0.393). Mean GSH values between treatments and across time are shown in Figure 3.

There was no treatment effect for AUC values for plasma H$_2$O$_2$ (F = 2.09, p = 0.163). Mean AUC values for H$_2$O$_2$ are shown in Table 1. No significant treatment x time interaction for H$_2$O$_2$ was noted (F = 0.38, p = 0.821). There was no main effect for treatment (F = 2.13, p =
0.12). However, there was a main effect for time (F = 3.82, p = 0.027). Post hoc analysis indicated significantly lower H$_2$O$_2$ levels at two (p = 0.040) and four (p = 0.010) hours post prandial. No difference was noted between two and four hours post prandial (p = 0.605). Changes in H$_2$O$_2$ levels over time are shown in Figure 3.

In terms of mean AUC values for AOPP, there was a significant main effect for treatments (F = 4.04, p = 0.04) with significantly higher AOPP values associated with ingestion of 42g of MCT compared to 28g (p = 0.01). Mean AUC values for AOPP are shown in Table 1. Further, there was a significant treatment x time interaction (F = 3.17, p = 0.02) as well as a main effect for treatment (F = 11.27, p < 0.001) and time (F = 5.84, p = 0.005). Post hoc analysis revealed significantly higher AOPP values at two hours post ingestion associated with the 42g treatment compared to the 28g treatment (p < 0.001). In addition, AOPP values were significantly higher at four hours post ingestion with the 42g treatment compared to 28g (p = 0.01). Finally, only ingestion of 42g of MCT resulted in a significant rise in AOPP values from pre to two hours post ingestion (p < 0.001). Mean AOPP values are shown in Figure 3.

There was no difference between treatments regarding mean AUC values for MDA (F = 2.08, p = 0.15). Mean AUC values for MDA are shown in Table 1. In addition, no treatment x time interaction was noted (F = 0.53, p = 0.715). There was a main effect for treatment (F = 3.72, p = 0.029 and time (F = 7.49, p = 0.001). Post hoc analysis revealed a significant decrease in MDA from pre to two (p = 0.022) and from pre to four hours post ingestion (p < 0.001). Mean MDA values were significantly higher following ingestion of 42g MCT compared to 28g MCT (p = 0.008). Changes in MDA values across time and between treatments are shown in Figure 3.

**Discussion**
The main findings from this investigation are: 1) coffee ingestion resulted in significant decreases in blood levels of triglycerides, H_2O_2 and MDA as well as increases in D-BHB, HDL-c and TC, 2) the addition of MCT to coffee does not significantly elevate D-BHB levels compared to the ingestion of coffee alone, and 3) the addition of a high lipid dose, 42g of MCT, to coffee caused OS shown by significantly greater AOPP levels at two hours post prandial compared to both 0g and 28g treatments. These findings are in alignment with previous studies that show antioxidative, lipolytic properties of coffee ingestion (Endesfelder et al. 2019; Endesfelder et al. 2017) and that a large bolus of lipids causes OS (Bloomer and Lee 2013; Bloomer et al. 2013).

It is well established that coffee has antioxidative properties (Martini et al., 2016). Therefore, the finding that markers of OS measured by H_2O_2 and MDA decreased in all three treatments following acute ingestion are likely due to polyphenols and melanoidins (Borrelli et al. 2002; Delgado-Andrade et al. 2005; Sanchez-Gonzalez et al. 2005). It is important to note however, that while the antioxidative properties of coffee ingestion have been extensively reported (Martini et al. 2016), data showing metabolic effects of acute coffee ingestion are scarce. Therefore, the current study is unique in showing one dose of caffeine rich coffee is effective at reducing markers of OS, facilitating hyperketonemia, and increasing HDL-c. However, previous work has shown that ingestion of a lipid and carbohydrate rich meal may overwhelm such benefits of acute coffee ingestion (Bloomer et al. 2013).

Previous work has shown coffee ingestion results in increases in GSH levels (Martini et al. 2016) which may be responsible for reduced OS; however, the present results suggest this effect is not achieved in response to acute ingestion as whole blood GSH was not changed. As mentioned by Martini et al., (Martini et al. 2016) it is possible that coffee ingestion may alter other antioxidant enzymes that were not measured such as superoxide dismutase, catalase,
glutathione peroxidase. While the lack of data regarding these enzymes could be viewed as a limitation to the study, it is not clear if coffee ingestion has a strong effect on these enzymes. It should also be noted that the current study is limited to a small sample size (n=10) therefore caution is warranted when considering the potential application of these findings to various populations. In addition, the study is also limited by not further examining changes in biomarkers beyond a four hour postprandial window. Finally, this study is also limited by not including an MCT alone treatment. However, it is important to note that the antioxidative responses noted, were likely due to the ingestion of coffee as opposed to MCT.

In terms of the impact of coffee ingestion on blood lipids, previous work has shown coffee ingestion increases TC without increasing HDL-c (Cai et al. 2012; Onuegbu and Agbedana 2001). The current findings are in agreement since acute ingestion resulted in increased TC. However, those changes may in fact be due to an increase in HDL-c. Since LDL-c, was not measured, this is only speculation and should be viewed as a limitation. While LDL-c can be predicted using the Fridewald equation however, this equation may overestimate LDL-c in individuals with low TAG and high TC levels (Ahmadi et al. 2008). Many of the reports on coffee ingestion and blood lipids findings typically come from analysis of associations with daily coffee intake (Cai et al. 2012; Condon et al. 2018; Kuang et al. 2018). As pointed out by Kokjohn et al., (Kokjohn et al. 1993) these results may be impacted by a number of confounding variables. Zargar et al., (Zargar et al. 2013) investigated metabolic effects of acute ingestion of ingestion of café latte and found no change in total cholesterol, LDL-c, or non HDL-c. Similarly, TAG levels decreased significantly, and HDL-c increased significantly which was also supported by the current findings. However, blood samples were collected up to 30 minutes after ingestion (Zargar et al. 2013) which differs from the current study, as samples were collected at two and
four hours post ingestion. As mentioned by Zargar et al., (Zargar et al. 2013), a decrease in blood TAG levels following acute ingestion may be due to increased fasting duration. However, it is important to note that TAG levels decreased in all conditions in the current study, despite the ingestion of a moderate and high doses of MCT’s. Given these findings, it is likely that the caffeine content of coffee triggered lipolysis (Acheson et al. 2004) which is responsible for the decreases in blood TAG. Caffeine content in coffee can vary significantly but it is expected that the amount of caffeine ingested was between approximately 100-200mg (Desbrow et al. 2012).

Increases in lipolysis and free fatty acid mobilization may also be responsible for increases in blood D-BHB levels. To illustrate, a recent study (Vandenberghe et al. 2019) reported aerobic exercise facilitates greater increases in D-BHB from MCT ingestion which is likely due to increased FFA availability which is driven by lipolysis. It is therefore important to note that previous findings showing an inverse relation between D-BHB levels and FFA are coming from studies investigating the impact of exogenous ingestion or infusion of ketones (Pinckaers et al. 2017; Stubbs et al. 2017). This study is different in that exogenous ketones were not provided—rather, endogenous ketone production increased which was not facilitated by the ingestion of MCT, but likely facilitated by increased lipolysis. While FFA’s were not measured, it is assumed that the decrease in TAG is due to increased lipolysis and blood FFA levels induced by caffeine ingestion (Acheson et al. 2004). In fact, many of these noted outcomes, in addition to the antioxidative activity may also be attributed to caffeine (independent of coffee) (Metro et al. 2017). Caffeine has been shown to have antioxidative (Endesfelder et al. 2017) and lipolytic (Endesfelder et al. 2019) properties.

In summary, the current study is, to our knowledge the first to report metabolic effects of acute coffee ingestion (with and without varying amounts of added MCT). The addition of MCT
to coffee did not impact blood D-BHB levels, as an increase in ketone levels were found in all conditions. Coffee ingestion resulted in reductions of blood markers of OS and increases in HDL-c. However, the 42g dose of lipids causes greater levels of AOPP post prandial. Future studies should investigate the effect of acute coffee ingestion among populations that are susceptible to OS, such as overweight individuals or those exposed to high amounts of occupational stress (i.e., firefighters, military personnel, etc.).

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References


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Table Captions

Table 1. Mean AUC values between treatments. Data are represented as mean ± SD. D-BHB = beta hydroxybutyrate. AOPP = advanced oxidation protein products. EIA = enzyme immunoassay analysis with plasma. * indicates a significant main effect for treatment (p<0.05) with significantly greater values with 42g MCT treatment compared to 0g and 28g. Due to missing blood samples, the number of observations (n) varies and is reported: GSH: 0 g MCT (n = 9); 28 g MCT (n = 9); 42 g MCT (n = 9); H2O2: 0 g MCT (n = 8); 28 g MCT (n = 9) 42 g MCT (n = 8); Glucose: 0 g MCT (n = 9); 28 g MCT (n = 9) 42 g MCT (n = 9); HDL-C: 0 g MCT (n = 9); 28 g MCT (n = 9) 42 g MCT (n = 9); BHB-D Strip: 0 g MCT (n = 9); 28 g MCT (n = 4) 42 g MCT (n = 9); BHB-D EIA: 0 g MCT (n = 9); 28 g MCT (n = 9) 42 g MCT (n = 9); Insulin: 0 g MCT (n = 8); 28 g MCT (n = 9) 42 g MCT (n = 7)

Figure Captions

Figure 1. A – Changes in D-BHB (beta hydroxybutyrate) values across time and between treatments as measured in whole blood. Note the number of observations (n) may vary due to missing or inadequate samples: (0 g MCT (n = 9-10); 28 g MCT (n = 4-5); 42 g MCT (n = 9)) B – Changes in D-BHB values across time and between treatments via EIA (enzyme immunoassay analysis). (0 g MCT (n = 9-10); 28 g MCT (n = 9-10); 42 g MCT (n = 9)) ( C – Changes in glucose levels across time and between treatments. (0 g MCT (n= 9-10); 28 g MCT (n = 9-10); 42 g MCT (n = 9)) D – Changes in insulin levels across time and between treatments. * indicates a significant (p<0.05) main effect for time. θ indicates a significant main effect for treatment with higher values in the 0g treatment compared to 28 and 42g MCT. (0 g MCT (n = 8-9); 28 g MCT (n = 8-9); 42 g MCT (n = 8-9))
**Figure 2.** A – Changes in triglyceride (TAG) levels across time and between treatments. Note the number of observations (n) may vary due to missing or inadequate samples: (0 g MCT (n = 8-10); 28 g MCT (n = 9-10); 42 g MCT (n = 8-9)) B – Changes in total cholesterol (TC) levels between treatments and across time. (0 g MCT (n = 9-10); 28 g MCT (n = 9-10); 42 g MCT (n = 9)) C – Changes in HDL-c levels across time and between treatments. (0 g MCT (n = 9-10); 28 g MCT (n = 9); 42 g MCT (n = 9))* indicates a significant (p<0.05) main effect for time. ø indicates a significant main effect for treatment with significantly higher values in the 42g treatment compared to both 0g and 28g MCT. Note: after outlier detection, two data points removed from TAG data: treatment 28g, pre ingestion as well as treatment 42g four hours postprandial.

**Figure 3.** A – Changes in advanced oxidation protein products (AOPP) across time and between treatments. Note the number of observations (n) may vary due to missing or inadequate samples: (0 g MCT (n = 5-10); 28 g MCT (n = 8-10); 42 g MCT (n = 6-9)) B – Changes in malondialdehyde (MDA) levels across time and between treatments. (0 g MCT (n = 8-10); 28 g MCT (n = 9-10); 42 g MCT (n = 8-9)) C – Changes in hydrogen peroxide H₂O₂ levels across time and between treatments. (0 g MCT (n = 8-10); 28 g MCT (n = 9-10); 42 g MCT (n = 9)) D – Changes in whole blood glutathione levels across time and between treatments. (0 g MCT (n = 9-10); 28 g MCT (n = 9-10); 42 g MCT (n = 9)) *indicates a significant (p<0.05) main effect for time. ø denotes a significant main effect for treatment with higher values in the 42g MCT treatment compared to 0 and 28g MCT. However, note that regarding mean MDA, 42g MCT was only significantly higher than 28g MCT dose. Note eight data points removed from AOPP data following outlier detection. Two were removed from 28g treatment: one pre and one two hours post prandial; three data points (pre blood samples) from the 0g treatment; three pre
samples from the 28g treatment. Regarding MDA, a total of three data points were removed following outlier detection including one pre sample from the 0g treatment, one pre sample from the 42g treatment, and one two hours postprandial sample from the 42g treatment.
<table>
<thead>
<tr>
<th></th>
<th>0 g MCT</th>
<th>28 g MCT</th>
<th>42 g MCT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AUC (4hr)</strong></td>
<td></td>
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<td></td>
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<tr>
<td>D-BHB (mM)</td>
<td>1.3 ±1.0</td>
<td>1.2 ±0.8</td>
<td>1.6 ±1.2</td>
</tr>
<tr>
<td>D-BHB (EIA) (mM)</td>
<td>1.5 ±0.9</td>
<td>2.0 ±1.8</td>
<td>1.9 ±1.1</td>
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<tr>
<td>Hydrogen Peroxide (μmol·L⁻¹)</td>
<td>56.0 ±24.0</td>
<td>44.7 ±15.4</td>
<td>61.5 ±29.6</td>
</tr>
<tr>
<td>Insulin (μIU/mL)</td>
<td>21.3 ±10.8</td>
<td>23.8 ±9.8</td>
<td>18.7 ±7.4</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>352.3 ±236.8</td>
<td>229.2 ±110.2</td>
<td>322.1 ±188.3</td>
</tr>
<tr>
<td>Glutathione (μM)</td>
<td>4399.7 ±453.6</td>
<td>4632.5 ±1014.4</td>
<td>4638.4 ±697.8</td>
</tr>
<tr>
<td>*AOPP (μM)</td>
<td>107.1 ±53.6</td>
<td>79.0 ±54.2</td>
<td>134.9 ±49.9</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>410.3 ±20.4</td>
<td>398.8 ±23.0</td>
<td>398.4 ±21.6</td>
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<tr>
<td>*Malondialdehyde (μM)</td>
<td>4.1 ±1.5</td>
<td>3.5 ±0.9</td>
<td>4.7 ±1.5</td>
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<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>777.1 ±91.0</td>
<td>776.7 ±125</td>
<td>814.8 ±66.3</td>
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<tr>
<td>*HDL-c (mg/dL)</td>
<td>225.6 ±61.8</td>
<td>253.7 ±68.5</td>
<td>263.3 ±78.3</td>
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