

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

Matthew J. McAllister, Hunter S. Waldman, Liliana I. Rentería, Andrew E. Gonzalez, Matthew B. Butawan, and Richard J. Bloomer

**Abstract:** 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. Ten college-aged men ingested coffee with 0, 28, and 42 g of MCT in a randomized order. Blood samples were collected pre- as well as 2 and 4 h postprandial and analyzed for D-BHB, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), glucose, triglycerides (TAG), insulin, and OS markers: advanced oxidation protein products (AOPP), glutathione (GSH), malondialdehyde (MDA), and hydrogen peroxide ( $H_2O_2$ ). All three treatments resulted in a significant increase in D-BHB, HDL-c, and TC as well as a significant decrease in TAG, MDA,  $H_2O_2$ , and insulin. The 42 g treatment was associated with significantly higher levels of AOPP and MDA. Acute ingestion of coffee results in favorable changes to markers of cardiometabolic health that were not impacted by the addition of 28 g of MCT. However, 42 g of MCT caused significantly greater OS.

**Key words:** caffeine, fat, beta-hydroxybutyrate, metabolism, ketosis.

**Résumé :** L'ingestion de suppléments cétoniques, de caféine et de triglycérides à chaîne moyenne (TCM) pourrait constituer autant de stratégies efficaces en vue d'augmenter les taux sanguins de bêta hydroxybutyrate (D-BHB), un corps cétonique. Cependant, l'ingestion aiguë d'un bolus de lipides pourrait entraîner une augmentation du stress oxydatif (SO). Cette étude portait sur l'incidence de l'ajout de diverses quantités de TCM au café sur les taux sanguins de D-BHB et de marqueurs du SO. Dix hommes d'âge universitaire ont ingéré du café avec 0, 28 et 42 g de TCM dans un ordre déterminé de façon aléatoire. Nous avons prélevé des échantillons sanguins avant, ainsi que 2 et 4 h après les repas, et mesuré les taux de D-BHB, de cholestérol total (CT), de cholestérol lié à des lipoprotéines de haute densité (HDL-c), de glucose, de triglycérides (TAG), d'insuline, ainsi que des marqueurs du SO : produits protéiques d'oxydation avancée (PPOA), glutathion (GSH), malondialdéhyde (MDA) et peroxyde d'hydrogène ( $H_2O_2$ ). Chacun des trois modes d'administration a entraîné des augmentations notables des taux de D-BHB, de HDL-c et de CT, ainsi qu'une hausse marquée des TAG, du MDA, de l' $H_2O_2$  et de l'insuline. L'administration de 42 g était associée à des taux nettement plus élevés de PPOA et de MDA. En conclusion, l'ingestion aiguë de café entraîne des variations favorables quant aux marqueurs de la santé cardiometabolique, lesquelles n'étaient pas perturbées par l'ajout de 28 g de TCM. Cependant, les 42 g de TCM ont entraîné un SO nettement plus important. [Traduit par la Rédaction]

**Mots-clés :** caféine, matières grasses, bêta hydroxybutyrate, métabolisme, cétose.

## 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 the circulation and oxidized by cardiac as well as skeletal muscle (Dedkova and Blatter 2014). A ketogenic diet is an effective tool to increase en-

dogenous 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 (Vandenbergh et al. 2017) and medium-chain triglycerides (MCT) (Vandenbergh et al. 2019).

MCT 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

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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 that 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 (FFA) availability (Vandenbergh 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 that 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 min physical activity per week) as defined by the American College of Sports Medicine (ACSM), apparently healthy (i.e., low risk according to ACSM criteria (Riebe et al. 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 2 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, Missouri, USA) and body mass was recorded while wearing minimal clothing using a physician's scale (Detecto).

### Overview of experimental testing

Participants reported to the Metabolic and Applied Physiology (San Marcos, Texas, 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 h 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 a  $\geq 8$  h fast and were asked to avoid strenuous exercise for 48 h and alcohol consumption and nicotine exposure for 24 h. 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 postprandial. 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 (Orville, Ohio, USA) that was brewed each morning immediately prior to ingestion. Coffee was prepared using 16 oz of bottled water for each 50 g (3.5 tbsp) of ground coffee. A stevia-based sweetener (In The Raw, Brooklyn, New York, USA) was provided and the amount added was recorded and consistent for each participant throughout the study. Coffee was administered either with 0,  $\sim 28$ , or  $\sim 42$  g of lipids. The lipids consisted of 75% MCT oil (Now Foods, Bloomington, Illinois, USA) and 25% coconut oil ("HEB Organics", San Antonio, Texas, USA). The choice to include 25% coconut oil was chosen based on pilot trials that produced this as generally the most tasteful preference. Prior to ingestion, the coffee was mixed in a blender (SharkNinja, Needham, Massachusetts, USA) 15 min after brewing with all additional ingredients (e.g., lipids, stevia) added to it. The drink was allowed to cool at  $-20^{\circ}\text{C}$  to a drinkable temperature for 10 min prior to ingestion.

### Blood sampling and overview of analysis

A total of 14 mL was collected in sealed, heparinized vacutainers immediately prior to ingestion as well as 2 and 4 h postprandial. These timepoints were chosen based on previous work showing changes in OS biomarkers in a 4 h postprandial window (Bloomer et al. 2013). Immediately after sample collection, an initial aliquot of 700  $\mu\text{L}$  of whole blood was mixed with an equal volume of a 5% solution of 5-sulfosalicylic acid dehydrate (Sigma-Aldrich, St. Louis, Missouri, USA). These samples were then incubated for 10 min at  $4^{\circ}\text{C}$  and centrifuged at 10 000g for 10 min. The supernatant was aliquoted and also stored at  $-80^{\circ}\text{C}$  and later assayed for total glutathione (GSH) concentrations according to assay instructions (Arbor Assays, Ann Arbor, Michigan, USA). Further, a 25  $\mu\text{L}$  sample of whole blood was used to measure ketone levels using a portable analyzer (Abbott, Columbus, Ohio, USA). Immediately after collection, whole blood samples were then centrifuged for 15 min at 1100g at  $4^{\circ}\text{C}$  and plasma was aliquoted and stored at  $-80^{\circ}\text{C}$  for subsequent analysis. Plasma samples were analyzed in duplicate for hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), insulin, triglycerides (TAG), glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), advanced oxidation protein products (AOPP), malondialdehyde (MDA), and D-BHB.

### Analysis of plasma D-BHB, HDL-c, and 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, Michigan, USA). Absorbance was read using a spectrophotometer (Pointe 180 QT, Canton, Michigan, 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 2000g for 10 min to allow for separation of HDL-c. The supernatant was aliquoted and added to a cholesterol reagent (Pointe Scientific) for subsequent determination of absorbance and analysis of HDL-c values.

### Analysis of insulin, $\text{H}_2\text{O}_2$ , AOPP, MDA, TC, and glucose

Plasma concentrations of insulin were determined in using a high-sensitivity assay (ALPCO, Salem, New Hampshire, USA) and an automated plate washer was used (Biotek, Winooski, Vermont, USA). Plasma levels of  $\text{H}_2\text{O}_2$  were determined using the Amplex red assay by methods described by the manufacturer (Molecular Probes, Invitrogen Detection Technologies, Eugene, Oregon, USA). Plasma samples were assayed for AOPP using a commercially available kit (STA-318 OxiSelect AOPP; Cell Biolabs, Inc., San Diego,

**Table 1.** Mean AUC values between treatments.

	AUC (4 h)		
	0 g of MCT	28 g of MCT	42 g of MCT
D-BHB (mM)	1.3±1.0	1.2±0.8	1.6±1.2
D-BHB (EIA) (mM)	1.5±0.9	2.0±1.8	1.9±1.1
H <sub>2</sub> O <sub>2</sub> (μM)	56.0±24.0	44.7±15.4	61.5±29.6
Insulin (μIU/mL)	21.3±10.8	23.8±9.8	18.7±7.4
TAG (mg/dL)	352.3±236.8	229.2±110.2	322.1±188.3
GSH (μM)	4399.7±453.6	4632.5±1014.4	4638.4±697.8
AOPP (μM)*	107.1±53.6	79.0±54.2	134.9±49.9
Glucose (mg/dL)	410.3±20.4	398.8±23.0	398.4±21.6
MDA (μM)*	4.1±1.5	3.5±0.9	4.7±1.5
TC (mg/dL)	777.1±91.0	776.7±125	814.8±66.3
HDL-c (mg/dL)*	225.6±61.8	253.7±68.5	263.3±78.3

**Note:** Data are represented as mean ± SD. D-BHB, beta-hydroxybutyrate; AOPP, advanced oxidation protein products; EIA, enzyme immunoassay analysis with plasma. An asterisk indicates a significant main effect for treatment ( $p < 0.05$ ) with significantly greater values with the 42 g MCT treatment compared to 0 and 28 g. Due to missing blood samples, the number of observations ( $n$ ) varies: GSH: 0 g of MCT ( $n = 9$ ), 28 g of MCT ( $n = 9$ ), and 42 g of MCT ( $n = 9$ ); H<sub>2</sub>O<sub>2</sub>: 0 g of MCT ( $n = 8$ ), 28 g of MCT ( $n = 9$ ), and 42 g of MCT ( $n = 8$ ); glucose: 0 g of MCT ( $n = 9$ ), 28 g of MCT ( $n = 9$ ), and 42 g of MCT ( $n = 9$ ); HDL-c: 0 g of MCT ( $n = 9$ ), 28 g of MCT ( $n = 9$ ), and 42 g of MCT ( $n = 9$ ); AOPP: 0 g of MCT ( $n = 8$ ), 28 g of MCT ( $n = 9$ ), and 42 g of MCT ( $n = 9$ ); BHB-D strip: 0 g of MCT ( $n = 9$ ), 28 g of MCT ( $n = 4$ ), and 42 g of MCT ( $n = 9$ ); BHB-D EIA: 0 g of MCT ( $n = 9$ ), 28 g of MCT ( $n = 8$ ), and 42 g of MCT ( $n = 9$ ); insulin: 0 g of MCT ( $n = 8$ ), 28 g of MCT ( $n = 8$ ), and 42 g of MCT ( $n = 7$ ).

California, USA) with methods previously described (Witko-Sarsat et al. 1996). MDA was analyzed utilizing a commercial colorimetric kit (NWK-MDA01; Northwest Life Science Specialties, Vancouver, Washington, USA) using similar methods as previously described (Jentzsch et al. 1996). Absorbance was determined using a Biotek Epoch II colorimetric reader. TC and glucose levels were analyzed using plasma samples on an Alfa Wasserman Vet Axcel chemistry analyzer (West Caldwell, New Jersey, 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, North Carolina, USA). For each blood marker, a 4 h 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 × 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 10 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. Data are shown as mean ± SD unless otherwise specified.

#### D-BHB

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 × 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 2 ( $p < 0.001$ ) and 4 h ( $p = 0.001$ ) postprandial, with no significant difference between D-BHB levels at 2 and 4 h. Mean D-BHB levels between treatments and across time are shown in Fig. 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 D-BHB are shown in Table 1. There was also no significant treatment × 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 2 h ( $p = 0.001$ ) postprandial. There was no difference in D-BHB levels at 2 and 4 h postprandial ( $p = 0.20$ ). Mean D-BHB levels between treatments and across time are shown in Fig. 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 × 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 4 h compared to 2 h postprandial ( $p = 0.013$ ), with no other differences between time points. Insulin levels for both treatments across time are shown in Fig. 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 × 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 0 g treatment compared to 28 g ( $p = 0.01$ ) and 42 g ( $p = 0.01$ ). 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 Fig. 1.

#### TAG

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 × 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 2 h ( $p = 0.028$ ) and 4 h ( $p < 0.001$ ) postprandial. Mean TAG levels for both treatments across time are shown in Fig. 2.

#### TC

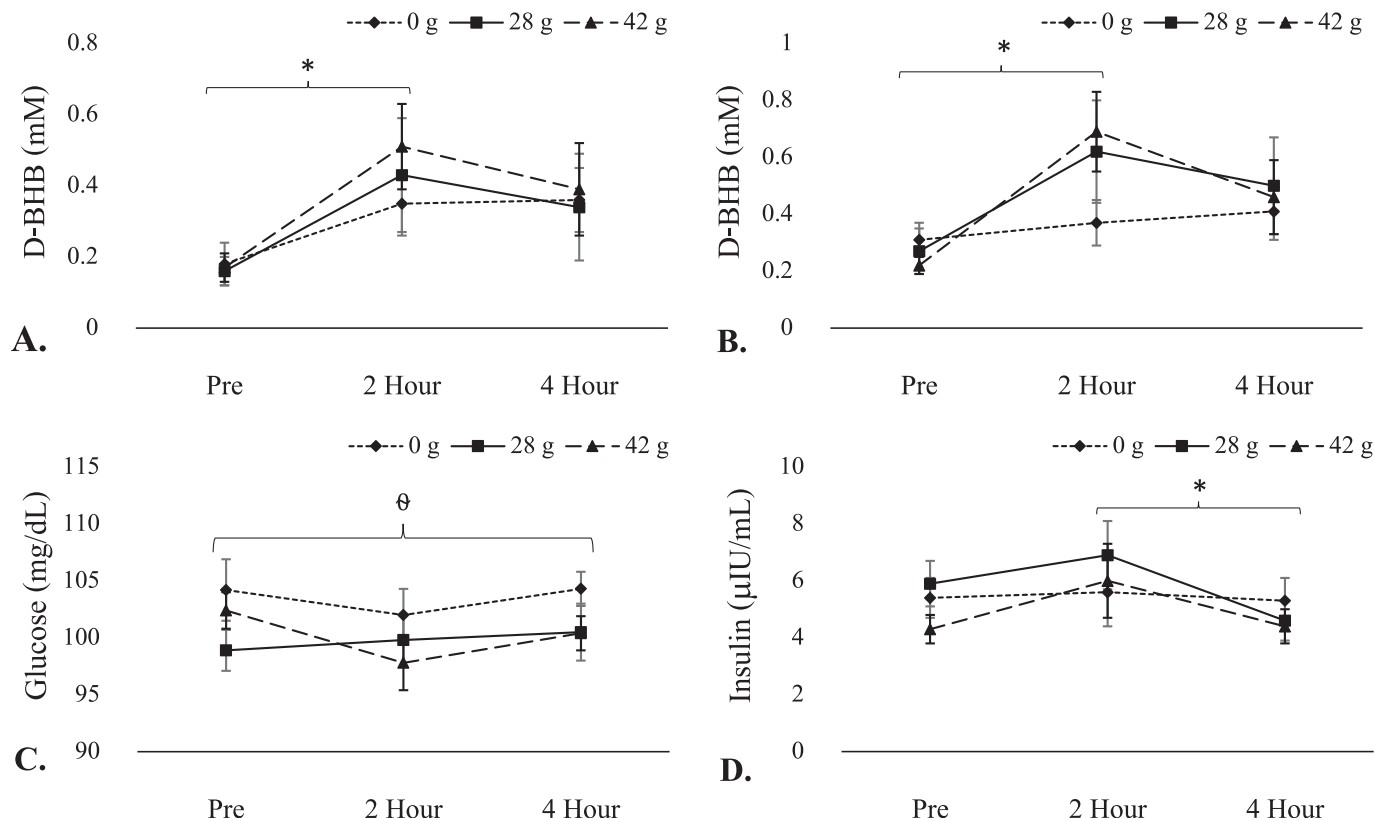
There was no difference between treatments regarding mean AUC values for TC ( $F = 0.85$ ,  $p = 0.44$ ). Mean AUC values for TC are shown in Table 1. There was no significant treatment × time interaction ( $F = 0.22$ ,  $p = 0.92$ ). However, there was a main effect for treatment ( $F = 3.15$ ,  $p = 0.04$ ) and time ( $F = 4.88$ ,  $p = 0.01$ ). Post hoc analysis demonstrated a significant increase from preingestion to 2 h ( $p = 0.01$ ) and 4 h ( $p = 0.005$ ) postingestion. Further, TC was significantly higher with 42 g of MCT compared to 0 g ( $p = 0.02$ ) and 28 g ( $p = 0.03$ ). Mean TC values between treatments and across time are shown in Fig. 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 42 g of MCT treatment compared to both 0 g of MCT ( $p = 0.004$ ) and 28 g



**Fig. 1.** (A) Changes in beta-hydroxybutyrate (D-BHB) values across time and between treatments as measured in whole blood. Data are shown as mean  $\pm$  SE. Note the number of observations ( $n$ ) may vary due to missing or inadequate samples: 0 g of MCT ( $n = 9-10$ ), 28 g of MCT ( $n = 4-5$ ), and 42 g of MCT ( $n = 9$ ). (B) Changes in D-BHB values across time and between treatments via EIA (enzyme immunoassay analysis): 0 g of MCT ( $n = 9-10$ ), 28 g of MCT ( $n = 9-10$ ), and 42 g of MCT ( $n = 9$ ). (C) Changes in glucose levels across time and between treatments: 0 g of MCT ( $n = 9-10$ ), 28 g of MCT ( $n = 9-10$ ), and 42 g of MCT ( $n = 9$ ). (D) Changes in insulin levels across time and between treatments. An asterisk indicates a significant ( $p < 0.05$ ) main effect for time;  $\theta$  indicates a significant main effect for treatment with higher values in the 0 g treatment compared to 28 and 42 g of MCT: 0 g of MCT ( $n = 8-9$ ), 28 g of MCT ( $n = 8-9$ ), and 42 g of MCT ( $n = 7-8$ ).



of MCT ( $p = 0.037$ ). AUC data for HDL-c are shown in Table 1. There was no significant treatment  $\times$  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 42 g of MCT treatment compared to 0 g of MCT ( $p < 0.001$ ) and 28 g of MCT ( $p = 0.003$ ). In addition, HDL-c levels were significantly increased at 2 and 4 h postprandial compared to preingestion levels ( $p < 0.001$ ). Data demonstrating changes across time and between treatments for HDL-c are shown in Fig. 2.

### OS 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  $\times$  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 Fig. 3.

There was no treatment effect for AUC values for plasma  $H_2O_2$  ( $F = 2.09$ ,  $p = 0.163$ ). Mean AUC values for  $H_2O_2$  are shown in Table 1. No significant treatment  $\times$  time interaction for  $H_2O_2$  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  $H_2O_2$  levels at 2 h ( $p = 0.040$ ) and 4 h ( $p = 0.010$ ) postprandial. No difference was noted between 2 and 4 h postprandial ( $p = 0.605$ ). Changes in  $H_2O_2$  levels over time are shown in Fig. 3.

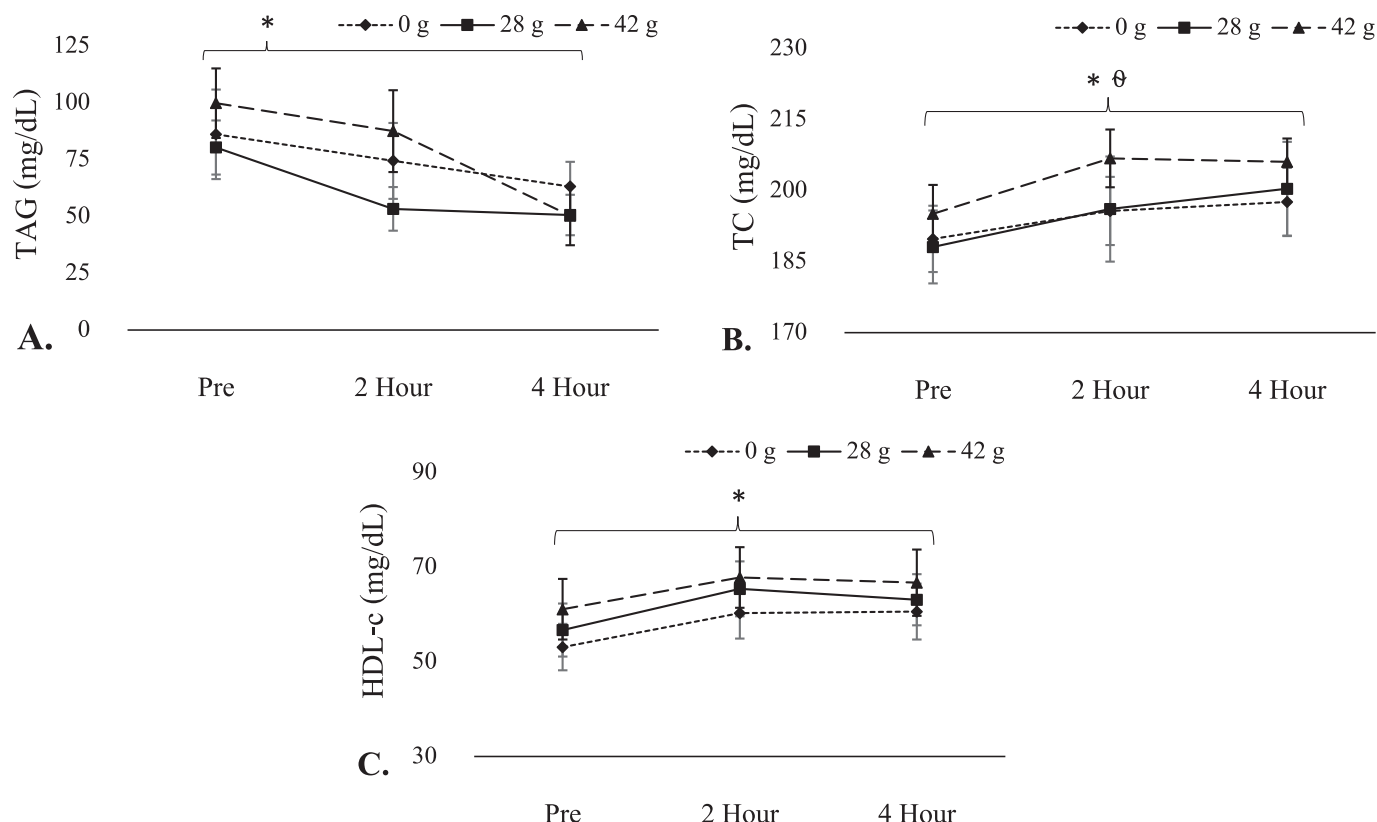
In terms of mean AUC values for AOPP, there was a significant main effect for treatments ( $F = 4.04$ ,  $p = 0.04$ ) with significantly higher AOPP values associated with ingestion of 42 g of MCT compared to 28 g ( $p = 0.01$ ). Mean AUC values for AOPP are shown in Table 1. Further, there was a significant treatment  $\times$  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 2 h postingestion associated with the 42 g treatment compared to the 28 g treatment ( $p < 0.001$ ). In addition, AOPP values were significantly higher at 4 h postingestion with the 42 g treatment compared to 28 g ( $p = 0.01$ ). Finally, only ingestion of 42 g of MCT resulted in a significant rise in AOPP values from pre- to 2 h postingestion ( $p < 0.001$ ). Mean AOPP values are shown in Fig. 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  $\times$  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 2 h ( $p = 0.022$ ) and from pre- to 4 h postingestion ( $p < 0.001$ ). Mean MDA values were significantly higher following ingestion of 42 g of MCT compared to 28 g of MCT ( $p = 0.008$ ). Changes in MDA values across time and between treatments are shown in Fig. 3.

### Discussion

The main findings from this investigation are that (1) coffee ingestion resulted in significant decreases in blood levels of TAG,

**Fig. 2.** (A) Changes in triglyceride (TAG) levels across time and between treatments. Data are shown as mean  $\pm$  SE. Note the number of observations ( $n$ ) may vary due to missing or inadequate samples: 0 g of MCT ( $n = 8-10$ ), 28 g of MCT ( $n = 9-10$ ), and 42 g of MCT ( $n = 8-9$ ). (B) Changes in total cholesterol (TC) levels between treatments and across time: 0 g of MCT ( $n = 9-10$ ), 28 g of MCT ( $n = 9-10$ ), and 42 g of MCT ( $n = 9$ ). (C) Changes in high-density lipoprotein cholesterol (HDL-c) levels across time and between treatments: 0 g of MCT ( $n = 9-10$ ), 28 g of MCT ( $n = 9-10$ ), and 42 g of MCT ( $n = 9$ ). An asterisk indicates a significant ( $p < 0.05$ ) main effect for time;  $\theta$  indicates a significant main effect for treatment with significantly higher values in the 42 g treatment compared to both 0 and 28 g of MCT. Note that after outlier detection, two data points were removed from the TAG data: treatment 28 g preingestion as well as treatment 42 g 4 h postprandial.



$H_2O_2$ , 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, 42 g of MCT, to coffee caused OS shown by significantly greater AOPP levels at 2 h postprandial compared to both 0 and 28 g 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).

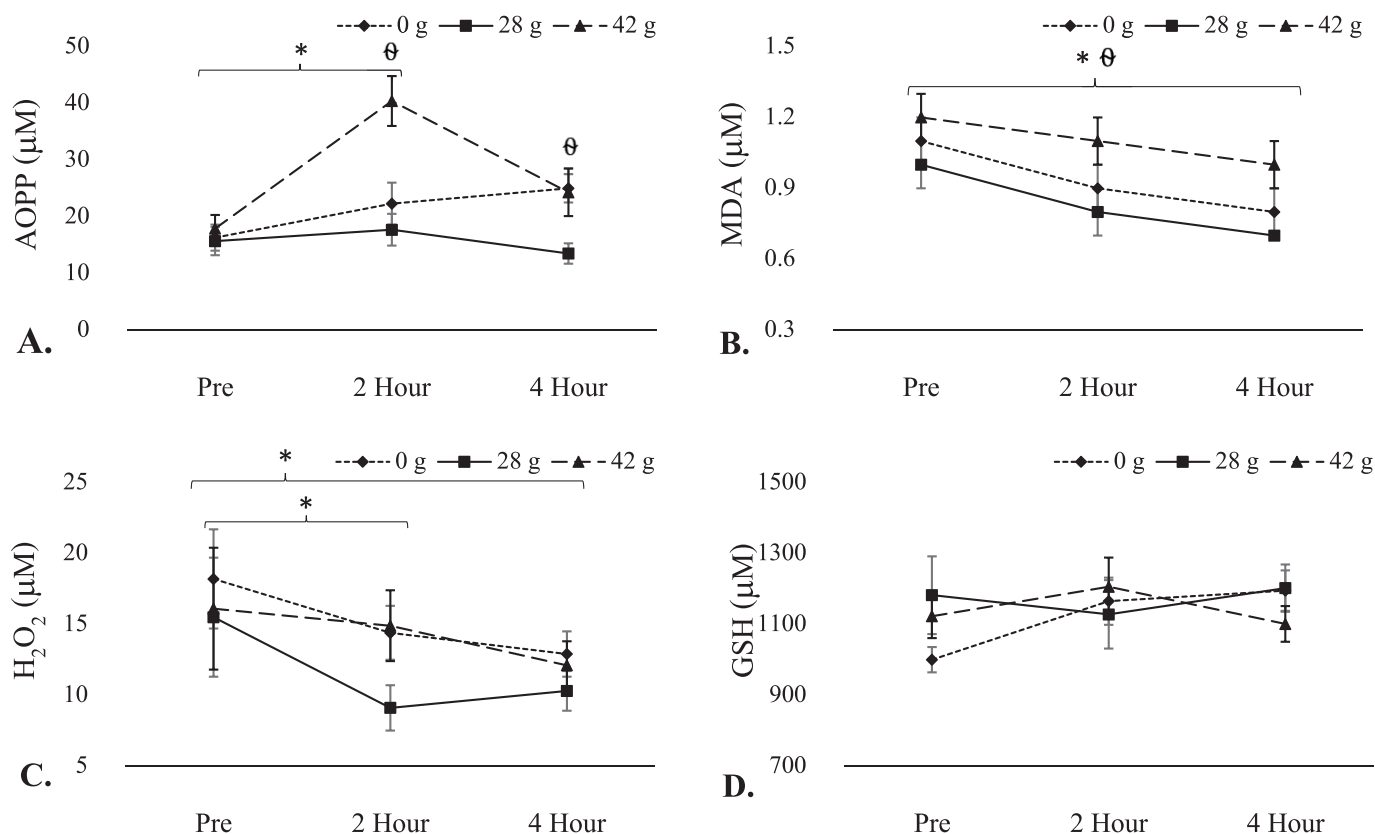
It is well established that coffee has antioxidative properties (Martini et al. 2016). Therefore, the finding that markers of OS measured by  $H_2O_2$  and MDA decreased in all three treatments following acute ingestion are likely due to polyphenols and melanoidins (Borrelli et al. 2002; Delgado-Andrade et al. 2005; Sánchez-González 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 that 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 that coffee ingestion results in increases in GSH levels (Martini et al. 2016), which may be responsible for reduced OS; however, the present results suggest that this

effect is not achieved in response to acute ingestion, as whole blood GSH was not changed. As mentioned by Martini et al. (2016), it is possible that coffee ingestion may alter other antioxidant enzymes that were not measured such as superoxide dismutase, catalase, and 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 4 h 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 that 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 low-density lipoprotein cholesterol (LDL-c) was not measured, this is only speculation and should be viewed as a limitation. While LDL-c can be predicted using the Friedewald 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

**Fig. 3.** (A) Changes in advanced oxidation protein products (AOPP) across time and between treatments. Data are shown as mean  $\pm$  SE. Note the number of observations ( $n$ ) may vary due to missing or inadequate samples: 0 g of MCT ( $n = 5-10$ ), 28 g of MCT ( $n = 8-10$ ), and 42 g of MCT ( $n = 6-9$ ). (B) Changes in malondialdehyde (MDA) levels across time and between treatments: 0 g of MCT ( $n = 8-10$ ), 28 g of MCT ( $n = 9-10$ ), and 42 g of MCT ( $n = 8-9$ ). (C) Changes in hydrogen peroxide ( $H_2O_2$ ) levels across time and between treatments: 0 g of MCT ( $n = 8-10$ ), 28 g of MCT ( $n = 9-10$ ), and 42 g of MCT ( $n = 9$ ). (D) Changes in whole blood glutathione (GSH) levels across time and between treatments: 0 g of MCT ( $n = 9-10$ ), 28 g of MCT ( $n = 9-10$ ), and 42 g of MCT ( $n = 9$ ). An asterisk indicates a significant ( $p < 0.05$ ) main effect for time;  $\theta$  denotes a significant main effect for treatment with higher values in the 42 g of MCT treatment compared to 0 and 28 g of MCT. However, note that regarding mean MDA, 42 g of MCT was only significantly higher than 28 g MCT dose. Note eight data points were removed from the AOPP data following outlier detection. Two were removed from the 28 g treatment: one pre- and one 2 h postprandial. Three data points (preblood samples) were removed from the 0 g treatment and three presamples from the 28 g treatment. Regarding MDA, a total of three data points were removed following outlier detection including one presample from the 0 g treatment, one presample from the 42 g treatment, and one 2 h postprandial sample from the 42 g treatment.



daily coffee intake (Cai et al. 2012; Condon et al. 2018; Kuang et al. 2018). As pointed out by Kokjohn et al. (1993), these results may be impacted by a number of confounding variables. Zargar et al. (2013) investigated metabolic effects of acute 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 min after ingestion (Zargar et al. 2013), which differs from the current study, as samples were collected at 2 and 4 h post-ingestion. As mentioned by 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 MCTs. 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 and 200 mg (Desbrow et al. 2012).

Increases in lipolysis and FFA mobilization may also be responsible for increases in blood D-BHB levels. To illustrate, a recent study (Vandenbergh et al. 2019) reported that 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 FFAs 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 was found in all conditions. Coffee ingestion resulted in reductions of blood markers of OS and increases in HDL-c. However, the

42 g dose of lipids causes greater levels of AOPP postprandial. 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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