Emulsification increases the acute ketogenic effect and bioavailability of medium-chain triglycerides in humans

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Funding: Financial support from Natural Sciences and Engineering Research Counsel of Canada (NSERC), Fonds de Recherche du Québec – Santé (FRQS) and Université de Sherbrooke


ABSTRACT

Background: Lower brain glucose uptake is commonly present before the onset of cognitive deterioration associated with aging and may increase the risk of Alzheimer’s disease. Ketones are the brain’s main alternative energy substrate to glucose. Medium-chain triglycerides (MCT) are rapidly β-oxidized and are ketogenic, but also have gastrointestinal side effects. We assessed whether MCT emulsification into a lactose-free skim milk matrix (MCT-E) would improve ketogenesis and/or reduce side-effects compared to the same oral dose of MCT consumed without emulsification (MCT-NE).

Hypotheses: In healthy adults, (i) MCT-E will induce higher ketonemia and have fewer side effects than MCT-NE. (ii) The effects of MCT-NE and MCT-E on ketogenesis and plasma medium chain fatty acids will be dose-dependent.

Methods: Using the same metabolic study day protocol, 10 healthy adults were each given three separate doses (10, 20 and 30 g) of MCT-NE or MCT-E with a standard breakfast compared to a no treatment Control test. Blood samples were taken every 30 min for 4 h to measure plasma ketones (β-hydroxybutyrate and acetoacetate), octanoate, decanoate and other metabolites. Participants completed a side-effects questionnaire at the end of each study day.

Results: Compared to the no treatment Control, MCT-NE increased ketogenesis by 2 fold with no significant difference between doses. MCT-E increased total plasma ketones by 2-4 fold, in a dose-dependent manner. Compared to MCT-NE, MCT-E increased plasma medium chain fatty acid bioavailability (F) by 2-3 fold, and decreased the number of side-effects by about 50%.
Conclusions: Emulsification increased the ketogenic effect and decreased side effects in a dose-dependent manner for single doses of MCT up to 30 g under matching conditions. Further investigation is needed to establish whether emulsification could sustain ketogenesis and minimize side effects and therefore be used as a treatment to change brain ketone availability over a prolonged period of time.

Key words: Medium-chain triglycerides, emulsification, ketogenesis
INTRODUCTION

The ketones, β-hydroxybutyrate (βOHB) and acetoacetate (AcAc), are the main alternative fuel to glucose for brain energy metabolism, and can meet at least two-thirds of the brain’s total energy requirement during a prolonged fast or while on a very low carbohydrate ketogenic diet (1-3). Whereas brain glucose uptake is not directly related to plasma glucose concentration (4), there is a direct relation between plasma ketones and brain ketone uptake over a wide range of plasma ketone concentrations (5-7).

Medium-chain triglycerides (MCT; 6-12 carbons) are well known to be ketogenic in humans (8-10). Their physico-chemical properties enable their rapid absorption from the gut through the portal vein to the liver, and rapid diffusion into hepatocytes (11). MCT are more rapidly β-oxidized than long-chain fatty acids (≥14 carbons) (12), which increases acetyl-CoA in the liver leading to ketogenesis and ketone release into the circulation (13). The inclusion of MCT as part of a ketogenic diet to treat intractable epilepsy in children demonstrates their superior ketogenic efficacy over long chain fats in humans (14, 15) and rats (16). However, MCT can be associated with gastro-intestinal side effects (9), which can decrease tolerability and the sustainability of their ketogenic effect.

In animal studies, emulsification improves enteral absorption and bioavailability of MCT (12, 17, 18), presumably because gastric and/or pancreatic lipases liberate the medium chain fatty acids (MCFA) more easily from smaller lipid droplets, thereby enhancing their absorption. In humans, emulsification increases absorption and metabolism of long chain fatty acids such as docosahexaenoic acid (19). Nevertheless,
little is known about how emulsification of MCT affects ketogenesis or their side effects in humans.

The primary objective of the present study was therefore to conduct a dose-response study to determine whether emulsification would improve the acute ketogenic effect in healthy adults of a single dose of MCT compared to the same dose of non-emulsified MCT oil. The two secondary objectives were to assess (i) the dose-response relationship between the change in plasma ketones and change in plasma MCFA after an oral dose of emulsified or non-emulsified MCT, and (ii) whether MCT emulsification would be associated with fewer gastro-intestinal side effects. A lactose-free skim milk matrix was used to emulsify the MCT to avoid lactose intolerance in susceptible individuals. The metabolic tests were done over 4 hours which is sufficient to observe significant increases in plasma ketones post-dose (7). A 4 hour period is also sufficient to compare the plasma ketone area-under-the-curve (AUC) of the various treatments (9), while limiting potential side effects to the shortest period possible.
METHODS

Ethical approval for this study was obtained from the Research Ethics Committee of the Centre Intégré Universitaire de Santé et de Services Sociaux de l'Estrie-Centre Hospitalier Universitaire de Sherbrooke, which oversees all human research done at the Research Center on Aging (Sherbrooke, QC, Canada). All participants provided informed consent prior to their inclusion to the study. This study is registered at ClinicalTrials.gov with identification number NCT02409927.

Participants

There were ten participants, aged 31 ± 3 y, six men and four women, all of whom were judged to be in good health after review of their medical histories and screening of a blood sample obtained after a 12 h overnight fast (Table 1). All participants were non-smokers, non-diabetic (fasting glucose <6.1 mM and glycosylated hemoglobin <6.0%), had normal renal function, serum electrolytes, liver function (normal aspartate aminotransferase and alanine aminotransferase), thyroid stimulating hormone, HDL and LDL cholesterol, triglycerides, and albumin, and no overt nutritional problems.

MCT emulsification

The composition of the MCT was 60% octanoate (8:0) and 40% decanoate (10:0) and it contained no protein or carbohydrates (Alpha Health Products, Burnaby, BC, Canada). The three separate doses of non-emulsified MCT (MCT-NE) were manually stirred into lactose-free skim milk to a final volume of 300 mL. The emulsified MCT (MCT-E) was the same MCT oil and at the same doses as for MCT-NE but was
emulsified into lactose-free skim milk (Natrel brand, St-Hubert, Quebec, Canada, lactose
free; 0 g lipids; 9 g carbohydrates; 12 g proteins per 250 mL) to a concentration of 10%
using a high-pressure homogenizer at 2000 psi (dairy products pilot plant, Institute of
Nutrition and Functional Foods, Université Laval, Quebec City, QC). The final mean
MCT particle diameter was ~0.7 μm and the emulsion was shown to be stable at room
temperature for at least 28 days.

Metabolic test protocol

There were seven test conditions: no treatment control (CTL) and three matching
doses each of MCT-E and MCT-NE (10, 20, and 30 g). Participants received the three
doses of MCT-NE or MCT-E in random order; each test was done in the morning with
test days separated by a minimum of 3 days. Participants were blinded to the form and
dose of MCT they would receive. Prior to each metabolic test, participants underwent a
12 h overnight fast. On the morning of the metabolic test, a venous forearm catheter was
installed for blood sampling and a baseline sample was taken to evaluate fasting ketones,
glucose, insulin, cholesterol, triglycerides, lactate, and free fatty acids. Following the
installation of the catheter and collection of the baseline blood sample, a standardized
breakfast consisting of two pieces of toast with jelly and 300 mL of test supplement was
served and consume within 15 min. For the MCT-E, the volume of pre-emulsified
product was proportional to the MCT dose (10 g = 100 ml; 20 g = 200 ml; 30 g = 300 ml)
with the final 300 ml volume of the 10 and 20 g doses being made up with skim milk.
After consuming the drink and breakfast, blood samples were collected every 30 min for
the next 4 h. Participants were asked to stay as relaxed as possible, to not engage in any
physical exertion (since it might stimulate ketogenesis), and to consume only water. At
the end of each metabolic test, participants completed a questionnaire on the side effects
they experienced.

MCFA analysis

Plasma samples were prepared for MCFA (C8:0 [octanoic or caprylic acid] and
C10:0 [decanoic or capric acid]) analysis with modification of a previously reported
method (20). Plasma samples (25 µL) were spiked with isotopic standards (10 µL each of
1,2,3,4-13C4 octanoic acid and methyl-D3 decanoic acid). After mixing 5 µL of 9 M KOH
with a 25 µL sample of plasma, the tubes were place in a water bath at 60°C for 0.5 h.
After adjusting the pH by adding 20 µL of 2.25 M HCl, 450 µL of acetonitrile was added
and the samples centrifuged at 16,400 g for 0.5 h. Finally, 80 µL of the supernatant was
added to 120 µL of 5 mM ammonium bicarbonate and stored at 4°C until analysis.
MCFA analysis was performed by ultra-high performance liquid chromatography
(Nexera X2, Shimadzu) coupled to tandem mass spectrometry (API-3000, ABSciex). The
chromatography was carried out using an Acquity UPLC HSS T3 1.8 µm column fitted
with a BEH C18 1.7 µm VanGuard pre-column both of which were maintained in a
heating compartment at 30°C (Waters, Milford, MA, USA). The MCFAs were eluted by a
binary gradient starting at 75% solvent A and 25% solvent B and increasing linearly to
100% solvent B in 5 min, at a flow rate of 0.5 mL/min. Solvent A was a aqueous solution
of ammonium bicarbonate (5 mM) adjusted to pH 6 by the addition of formic acid, and
solvent B was 90% acetonitrile in water. The gradient was held at 100% solvent B for 4
min before equilibrating for another 4 min at initial conditions. A 10 µL injection volume
was employed. Under these conditions, C8:0 and C10:0 MCFAs eluted at approximately 2.5 and 3.3 min, respectively. The natural and isotopic derivatives of MCFAs were detected by tandem mass spectroscopy in negative mode using multiple reaction monitoring with both quadruples (Q1 and Q3) set to the corresponding molecular ions: natural octanoic acid (m/z 143); isotopic octanoic acid (m/z 147); natural decanoic acid (m/z 171); isotopic decanoic acid (m/z 174). The source temperature was 500°C and the collision energy was set to -15 eV on the instrument. The concentration of MCFA in the plasma samples was determined from the ratio of natural compound to isotopic standard added prior to sample preparation. An isotopically-labeled internal standard was added before sample preparation to take into account of any changes in extraction efficiency and sensitivity of detection by LC-MS/MS. Calibrations were performed for each assay to ensure the precision of the method and the LC-MS/MS response was linear over the entire range of concentrations of MCFAs measured in plasma samples. The linearity was verified after every batch of 50-100 samples, and the slope remained fairly constant with a batch to batch variation of about 20%.

Analyses

Plasma ketone concentrations were evaluated by automated colorimetric assay as previously described (10, 21-23). Calibrations and quality controls were performed for each assay (coefficient of variation between tests was 5 ± 1% based on n = 360 measurements). Plasma insulin was analyzed by enzyme-linked immunosorbent assay (Alpco Diagnostics Ltd., Salem, NH, USA) with a microplate reader (Victor multi-label...
plate reader 2030; Perkin Elmer, MA, USA). Plasma glucose, cholesterol, triglycerides (Siemens Medical Solutions USA, Inc., Deerfield, IL, USA), and free fatty acids (Wako Diagnostics, Richmond, VA, USA) were measured using commercially available kits.

MCFA bioavailability

Two parameters were determined for all the test conditions: AUC (0-4 h post-dose; Prism 6.0, GraphPad, La Jolla, CA, USA), and relative bioavailability ($F\%$) of the MCFA, defined as $\frac{AUC_{(MCT-E)}}{AUC_{(MCT-NE)}} \times 100$.

Statistical analysis

All statistical analyses were performed using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA). Due to small sample size ($n < 30$) and the non-normality of the distribution as calculated by the Fisher test, a non-parametric test (Friedman test) with an $\alpha$ set at 0.05 was employed to determine whether there were statistically significant differences between the test substances at each hour of the metabolic test. If a difference was observed on the Friedman test at a given time point, a post-hoc Wilcoxon rank-sum test was employed to determine the level of significance ($\alpha = 0.05$). Correlations were assessed using the Spearman correlation coefficient. The comparisons then underwent a $P \leq 0.05$ false discovery rate (FDR) correction (24).

RESULTS

Metabolic parameters
Ten participants completed all seven tests with no dropouts. Data from men and women were pooled since previous studies showed that there was no gender difference in short-term ketogenesis after a dose of MCT given to healthy adults (9, 10). All participants had plasma metabolite values in the normal range at baseline (Table 1). Changes in plasma cholesterol, triglycerides, free fatty acids, glucose, and insulin responses did not differ significantly between any of the seven metabolic tests (data not shown).

Plasma MCFA

At the pre-MCT baseline (time 0 h), plasma C8:0 and C10:0 were 4.2 ± 0.7 µM and 9.3 ± 1.7 µM, respectively. Throughout the CTL test, plasma C8:0 and C10:0 averaged 2.8 ± 0.7 and 11.6 ± 1.7 µM, respectively, values that did not differ significantly from baseline. The MCT-NE induced a gradual increase in plasma C8:0 that started at 3 h with the 10 g dose and at 30 min with both the 20 and 30 g doses (Figure 1A, B, C). The highest plasma C8:0 concentration attained on all three doses of MCT-NE was at 4 h with a dose-response effect between the 10 and 20 g doses (45.7 ± 9.0 µM vs. 92.2 ± 19.4 µM; Figure 1A, B), but no significant difference between the 20 and 30 g doses (92.2 ± 19.4 µM vs. 99.8 ± 35.6 µM; P = 0.33; Figure 1B, C). All three doses of the MCT-E significantly increased plasma C8:0 starting at 30 min post-dose. A significant dose-response relationship was observed with MCT-E in which the maximum plasma C8:0 attained increased from 47.8 ± 5.5 µM to 108.8 ± 13.2 µM to 144.8 ± 29. µM for the 10, 20, and 30 g doses, respectively (P < 0.001; Figure 1A, B, C).
The 10 g dose of MCT-NE did not significantly increase plasma C10:0 (Figure 3D), but the 20 and 30 g doses did increase plasma C10:0 at 4 h to 59.8 ± 15.7 µM and 63.3 ± 12.2 µM, respectively (Figure 1E, F). MCT-E significantly increased plasma C10:0 starting at 2.5, 2, and 1.5 h for the 10, 20, and 30 g doses, respectively. A significant dose-response relationship for plasma C10:0 was observed after the 10, 20, and 30 g doses of MCT-E, with highest concentrations of 68.0 ± 18.0 µM, 11.0 ± 18.6 µM and 139.4 ± 34.3 µM, respectively (P = 0.003; Figure 1D, E, F).

The increase in plasma C8:0 from baseline was significantly correlated with the increase in plasma ketones on the 10, 20, and 30 g doses of MCT-NE (Figure 2A; r= +0.84, P=0.005; r= +0.86, P=0.003; r= +0.98, P<0.001, respectively). The increase in plasma C8:0 from baseline was not significantly correlated to the increase in plasma ketones after the 10 g dose of MCT-E (Figure 2A; r= -0.11, P=0.78), but the correlation was significant after the 20 and 30 g doses of MCT-E (Figure 2A; r= +0.72, P=0.03; r= +0.80, P=0.009, respectively). The increase in plasma C10:0 from baseline was significantly correlated with the increase in plasma ketones on the 10, 20, and 30 g doses of MCT-NE (Figure 2B; r= +0.93, P<0.001; r= +0.92, P<0.001; r= +0.89, P=0.001, respectively). The correlation between the increase in plasma C10:0 from baseline and the increase in plasma ketones was not significant for any of the three doses of MCT-E (Figure 2B; r= -0.46, P=0.22; r= -0.15, P=0.70; r= -0.04, P=0.93, respectively).

The relative bioavailability (F; %) of C8:0 from the MCT-E/MCT-NE value was 137%, 113%, and 177% for the 10, 20, and 30 g doses, respectively. For C10:0, F was 196%, 236%, and 298% for the 10, 20, and 30 g doses, respectively, in favour of MCT-E.
For C8:0 and C10:0 combined, the F was 188%, 185%, and 304% for the 10, 20, and 30 g doses, respectively, in favour of MCT-E.

Ketogenic response

The CTL test did not induce ketogenesis over 4 h; indeed, plasma ketones decreased after the meal and did not return to baseline during the next 4 h (Figure 3). The MCT-NE induced a ketogenic response starting 2.5 h after the 10 g dose and 3 h after the 20 or 30 g doses (Figure 3). The MCT-E induced a modest but rapid transient ketogenic effect at the 10 g dose, which was more pronounced and sustained at the 20 and 30 g doses (Figure 3). At the 20 g dose, there was a higher and more sustained ketogenic effect of MCT-E that peaked at +355 ± 56 μM after 30 min compared to MCT-NE for which ketones reached maximum concentration at +213 ± 51 μM after 4 h (Figure 1B). The 30 g dose of MCT-E induced the largest ketogenic response, peaking at 1 h post-dose at +617 ± 139 μM (Figure 3C). Ketosis on 30 g MCT-E was also more sustained and did not go under +190 μM during the 4 h metabolic study period. The ketogenic response to the 30 g dose of MCT-NE was slower than for MCT-E, and reached the highest concentration at 4 h at +378 ± 73 μM (Figure 3C). There were no significant differences in ketone AUC at the 10 g dose of either treatment compared to CTL (ketone AUC was -171 ± 88 μM·h/L for CTL vs. 78 ± 70 μM·h/L for MCT-NE and -135 ± 129 μM·h/L for MCT-E; Figure 4). An incremental ketogenic dose-effect was seen with MCT-NE with the AUC increasing from 78 ± 70 to 147 ± 94 and 311 ± 97 μM·h/L for the 10, 20, and 30 g doses, respectively (P = 0.01; Figure 4). There was also an incremental dose-effect relationship
with the 4 h AUC for the MCT-E increasing from -135 ± 129 to 560 ± 95 to 1320 ± 336 μM·h/L for the 10, 20, and 30 g doses, respectively (∼0.001; Figure 4).

Side effects

With the MCT-NE, the most common SE reported were abdominal discomfort and diarrhea, both of which represented 42% of the total number of reported side effects and were reported on all test days except the CTL. Normally the abdominal discomfort disappeared 30-60 min post-dose. The number and severity of reported side effects was dose-dependent with the MCT-NE; 2 side effects were noted with the 10 g dose whereas 11 were reported with the 30 g dose. With the MCT-E, the most common side effect reported was abdominal discomfort (53% of all side effects; reported on all 6 tests; Table 2). As with MCT-NE, abdominal discomfort decreased after 30-60 min after taking the MCT-E. There was no difference in total number of side effects reported for the 10 and 20 g doses of MCT-E. With the 30 g dose, almost twice as many side effects were reported for MCT-NE as for MCT-E (11 vs. 6); the main difference being the absence of diarrhea with MCT-E.

DISCUSSION

Our main observation is that a stable emulsion of MCT in lactose-free skim milk improves the ketogenic effect while reducing diarrhea, the side effect most commonly associated with MCT. Previous studies from our group have shown a potent and rapid effect of non-emulsified MCT on ketogenesis in humans (9), but this is the first time we
report the dose-response effect of emulsification on ketogenesis as well as the side effects after a single dose of MCT.

Emulsification has previously been shown to improve long chain fatty acid absorption and metabolism (18, 19), but we are aware of no studies that have reported whether it affects the ketogenicity or side effects of MCT (11). The significantly higher increase in plasma ketones for the three doses of MCT-E implies faster absorption of MCT after emulsification. It may be that preformed MCT-rich micelles are more easily hydrolysed by gastric and/or pancreatic lipase prior to absorption via the portal vein (17, 18), thereby increasing the ketogenic effect and reducing the amount of unabsorbed MCT reaching the large gut after the MCT-E versus MCT-NE. Our present results are in line with those previously reported in piglets in which a twofold increase in plasma C8:0 was noted following emulsification of a triglyceride of C8:0 and where the peak C8:0 concentration was achieved at 1 h (18). However, the effect of non-emulsified C8:0 in piglets differed from our results where the plasma ketone concentration did not start to increase before three hours post-dose. Previous studies have shown that substrate availability is probably a limiting factor in stimulating ketogenesis in adults (25), suggesting that the efficacy of absorption and bioavailability of MCT are probably directly linked to their subsequent ketogenic effect.

To the best of our knowledge, since the study by Freund et al. in 1966 (8), this is the first study to report the dose-response relationship of an oral dose of MCT on plasma ketones and MCFA and the first study to look at the effect of MCT formulation on ketogenesis in humans. The results demonstrate a direct relationship between the oral dose of MCT given (up to 30 g), and the bioavailability of MCFA in the plasma of adults,
a relationship that also correlates directly with the change in plasma ketones. A previous review looking into this dose-response relationship came to the same conclusion (7). The strong positive correlation between plasma ketones and plasma C8:0 after MCT-E was not observed with C10:0, implying that the increase in plasma ketones was more a function of C8:0 than C10:0. The 4 h metabolic tests did not, however, permit us to follow plasma C10:0 to its peak, so it was not possible to assess the full extent of the impact of C8:0 and C10:0 on plasma ketones beyond 4 h post-dose. Recent work from our group showed that C8:0 increases ketones significantly more in humans than C10:0 over the course of an 8 h study day. The same results were observed in neuronal cell culture where C8:0 but not C10:0 stimulated the production of ketone from astrocytes (26).

There was no significant difference in the total number of reported side effects between MCT-E and MCT-NE. However, there was more diarrhea on the highest dose of MCT-NE compared to MCT-E ($P<0.001$). In contrast, nausea of short-duration ($\leq 30$ min) was more common on MCT-E than on the MCT-NE. In our 1-6 month feeding studies with the same MCT emulsion, we observe better tolerability when the participants start at a lower dose (typically 5 g in the morning and again in the evening) and gradually titrate towards 15 g/dose twice a day over at least one week.

The present study has some limitations. In particular, the single dose design does not provide information about the effect of MCT on ketogenesis and side effects in the long term. Nevertheless, there is presently no evidence that the ketogenic response to MCT differs after a long term intervention vs. a single dose (9, 27). Our 4 h metabolic study day model was therefore appropriate to evaluate the acute dose-response effects of
MCT-E or MCT-NE without side effects from repeated doses potentially causing lower compliance. The differences in AUCs reported from this 4 h metabolic day may however not reflect differences in the AUC that would be observed during a longer metabolic study day.

Several studies have shown 10-15% lower brain glucose uptake in the elderly, a deficit that increases to 20-25% in Alzheimer’s disease (28-31). Since this deterioration of brain glucose uptake is commonly present before the clinical (cognitive) onset of Alzheimer’s disease, it was postulated that deteriorating brain energy metabolism could be an important step in the evolution of the disease (7, 32, 33). However, brain ketone uptake is not affected in Alzheimer’s disease (34, 35). When given as a single dose (27, 36) or as a daily supplement for 3 months (27), MCT have been shown to improve cognition in mild-moderate Alzheimer’s disease, so it seems plausible that the beneficial cognitive effect of MCT occurs through the stimulation of ketogenesis (27, 36). Hence, optimizing the ketogenic effect and tolerability of MCT could potentially be beneficial in Alzheimer’s disease (36, 37).

CONCLUSION

Emulsification of MCT into a lactose-free skim milk matrix improved their ketogenic effect by up to four fold over 4 h, and also decreased diarrhea, a key side effect observed particularly at the 30 g dose of MCT. The effect on tolerability and compliance of emulsifying MCT therefore needs testing in longer term studies.

ACKNOWLEDGEMENT
Invaluable help was provided by our research nurse Christine Brodeur-Dubreuil with participant screening, blood sampling, and care of the participants.

Statement of authors’ contributions to manuscript

A.C.L., M.F., C.A.C, J.R.W. and S.C.C. designed research, A.C.L., C.M.L, V.S.P., and C.V. conducted research, A.C.L and C.M.L analysed data, and A.C.L, C.M.L, V.S.P., J.R.W. and S.C.C wrote the paper. All authors read and approved the final manuscript.
REFERENCES


Figure legends

**Figure 1.** Change in plasma C8:0 (left) and C10:0 (right) normalized to time (T) 0 during the metabolic test days on the no treatment Control (CTL) (□), non-emulsified (MCT-NE; ▲) or emulsified (MCT-E; ○) medium chain triglyceride at the 10 g (A, D), 20 g (B, E) and 30 g (C, F) doses. ▲: MCT consumed. a: MCT-E vs. MCT-NE, b: MCT-E vs. CTL, c: MCT-NE vs. CTL; all $P<0.05$.

**Figure 2.** Correlation between difference in plasma medium-chain fatty acids (MCFA; C8:0, (A); C10:0, (B)) and difference in plasma ketones following non-emulsified medium chain triglyceride (MCT-NE) (dotted lines) at 10 g (●; C8:0: $r=0.84$, $P=0.0048$; C10:0: $r=0.93$, $P=0.0002$), 20 g (■; C8:0: $r=0.86$, $P=0.0029$; C10:0: $r=0.92$, $P=0.0004$) and 30 g (▲; C8:0: $r=0.98$, $P<0.0001$; C10:0: $r=0.89$, $P=0.0012$), or emulsified medium chain triglyceride (MCT-E) (solid lines) intake at 10 g (○; C8:0: $r=-0.11$, $P=0.7768$; C10:0: $r=-0.46$, $P=0.2174$), 20 g (□; C8:0: $r=0.72$, $P=0.0291$; C10:0: $r=-0.15$, $P=0.7005$) and 30 g (△; C8:0: $r=0.80$, $P=0.0091$; C10:0: $r=-0.04$, $P=0.9274$).

**Figure 3.** Change in plasma ketones normalized to time (T) 0 during the Control (CTL) (□), or metabolic tests with 10 g (A), 20 g (B) and 30 g (C) doses of non-emulsified (MCT-NE; ▲) or emulsified (MCT-E; ○) medium chain triglyceride. ▲ - MCT consumed. a: MCT-E vs. MCT-NE, b: MCT-E vs. CTL, c: MCT-NE vs. CTL; all $P<0.05$. 

In Figure 1, the graphs show changes in plasma C8:0 and C10:0 concentrations normalized to time (T) 0 on metabolic test days for different dietary conditions. CTL refers to the Control group, non-emulsified MCT-NE, and emulsified MCT-E. Each condition is represented by a different symbol (□, ▲, ○) for visual distinction.

In Figure 2, the correlation between plasma medium-chain fatty acids and ketones is illustrated for both non-emulsified (MCT-NE) and emulsified (MCT-E) conditions at 10, 20, and 30 g doses. The data points are marked by different symbols (●, ■, ▲) for each condition, and the Pearson correlation coefficients ($r$) and significance levels ($P$) are provided for each comparison.

In Figure 3, the change in plasma ketones is shown for the Control (CTL) and different dietary treatments with 10, 20, and 30 g of either non-emulsified (MCT-NE) or emulsified (MCT-E) medium chain triglycerides. The significance levels are indicated with asterisks for each comparison.
Figure 4. Normalized area under the curve for plasma ketones (acetoacetate + β-hydroxybutyrate) during the 4 h metabolic test for the no treatment control (Control; gray bar), non-emulsified (MCT-NE) (black bars) or emulsified MCT-E (white bars) medium chain triglyceride. Data are means ± SEM (n = 10 for each group). *P < 0.05, **P < 0.01.
Table 1
Baseline demographic and plasma parameters of the participants.

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<table>
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<tr>
<td>Age (y)</td>
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<td>Plasma measurements</td>
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<td>Acetoacetate (µM)</td>
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<td>β-Hydroxybutyrate (µM)</td>
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<td>Insulin (IU/L)</td>
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<td>Octanoate (µg/mL)</td>
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<tr>
<td>Decanoate (µg/mL)</td>
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\(^1\) Mean ± SEM (n = 10)
Table 2

Self-reported side effects over 4 h after a single dose of non-emulsified (MCT-NE) or emulsified (MCT-E) medium-chain triglycerides.

<table>
<thead>
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<th>Single dose</th>
<th>MCT-NE (n=10)</th>
<th>MCT-E (n=10)</th>
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<td></td>
<td>10 g</td>
<td>20 g</td>
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<tr>
<td>Abdominal discomfort</td>
<td>1 (M)</td>
<td>3 (S)</td>
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<tr>
<td>Decreased appetite</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gastric reflux</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nausea</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1 (M)</td>
<td>2 (M)</td>
</tr>
<tr>
<td>Headache</td>
<td>-</td>
<td>1 (M)</td>
</tr>
<tr>
<td># of participants reporting SE</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Total number of SEs reported</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>

Severity scale (0-10):
- Light (L): 0-3
- Moderate (M): 4-7
- Severe (S): 8-10

* Severity level was based on the highest score reported
Nutrition. DOI: 10.3945/cdn.117.000851

Δ ketones (μM)

Δ MCFA (μM)
Change in plasma ketones normalized to T0 (μM)

A

B

C

Time (h)

0 1 2 3 4

10 g

20 g

30 g

Nutrition. DOI: 10.3945/cdn.117.000851

on August 14, 2017 - First published online on June 21, 2017 in Current Developments in Nutrition.