A short-term intervention combining aerobic exercise with medium-chain triglyceride (MCT) is more ketogenic than either MCT or aerobic exercise alone: A comparison of normoglycemic and pre-diabetic older women.

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A short-term intervention combining aerobic exercise with medium-chain triglyceride (MCT) is more ketogenic than either MCT or aerobic exercise alone: A comparison of normoglycemic and pre-diabetic older women.

Camille Vandenberghe, Christian-Alexandre Castellano, Mathieu Maltais, Mélanie Fortier, Valérie St-Pierre, Isabelle J. Dionne, Stephen C. Cunnane

Author for correspondence:
Camille Vandenberghe
Research Center on Aging, 1036 Belvedere St. South, Sherbrooke, QC, CAN J1H 4C4 and Departments of Pharmacology and Physiology, Université de Sherbrooke, Sherbrooke, QC, CAN Tel: 1 819 780-2220, ext 45235; Camille.Vandenberghe@USherbrooke.ca

Authors:
Christian-Alexandre Castellano: Research Center on Aging, 1036 Belvedere St. South, Sherbrooke, QC, Canada J1H 4C4; Tel: 1 819 780-2220, ext 45623; Christian.Alexandre.Castellano@USherbrooke.ca

Mathieu Maltais: Research Center on Aging, 1036 Belvedere St. South, Sherbrooke, QC, Canada J1H 4C4 and Faculty of the Science of Physical Activity, Université de Sherbrooke, Sherbrooke, QC, Canada; Tel: 1 819 780-2220, ext 45310; Mathieu.Maltais@USherbrooke.ca

Mélanie Fortier: Research Center on Aging, 1036 Belvedere St. South, Sherbrooke, QC, Canada J1H 4C4; Tel: 1 819 780-2220, ext 45252; Mélanie.Fortier2@USherbrooke.ca
Valérie St-Pierre: Research Center on Aging, 1036 Belvedere St. South, Sherbrooke, QC, Canada J1H 4C4; Tel: 1 819 780-2220, ext 45286; Valérie.R.St-Pierre@USherbrooke.ca

Isabelle J. Dionne: Research Center on Aging, 1036 Belvedere St. South, Sherbrooke, QC, Canada J1H 4C4 and Faculty of the Science of Physical Activity, Université de Sherbrooke, Sherbrooke, QC, Canada; Tel: 1 819 780-2220, ext 45671; Isabelle.Dionne@USherbrooke.ca

Stephen C. Cunnane: Research Center on Aging, 1036 Belvedere St. South, Sherbrooke, QC, Canada J1H 4C4 and Departments of Pharmacology and Physiology and Department of Medicine, Université de Sherbrooke, Sherbrooke, QC, Canada; Tel: 1 819 780-2220, ext 45670; Stephen.Cunnane@usherbrooke.ca
ABSTRACT

Objectives: Determine whether – (1) a five-day aerobic exercise (AE) program combined with a medium-chain triglyceride (MCT) supplement would increase the plasma ketone response in older women more than either intervention alone, and (2) ketonemia after these combined or separate treatments was alike in normoglycemic (NG) versus pre-diabetic (PD) women.

Design: Older women (NG=10; PD=9) underwent a 4 h metabolic study after each of four different treatments: (i) no treatment control, (ii) five days of MCT alone (30 g/day), (iii) one session of 30 min of AE alone, and (iv) five days of MCT and AE combined (MCT+AE). Blood was sampled every 30 minutes over 4 h for analysis.

Results: In NG, MCT+AE induced the highest AUC for plasma ketones (835 ± 341 µmol h/L), values that were 69% higher than MCT alone (P<0.05). AUCs were not different between MCT alone and MCT+AE in PD, but both treatments induced a significantly higher AUC than the control or AE alone (P<0.05). Except for a trend towards a higher ketone AUC in NG vs. PD on AE alone (P=0.091), there was no significant difference between the ketone AUCs in PD and NG.

Conclusion: Combination of MCT+AE was more ketogenic in older women than MCT or AE alone. MCT+AE had a synergistic effect on ketonemia in NG but not in PD. Whether by improving insulin sensitivity with a longer term AE intervention can improve the ketogenic effect of MCT in PD and thereby increase brain ketone uptake in older people merits further investigation.

Key words: Medium-chain triglycerides, aerobic exercise, ketones, free fatty acids and pre-diabetic
INTRODUCTION

Brain glucose uptake decreases during healthy aging (Nugent et al. 2014), a problem that worsens as Alzheimer’s disease (AD) develops (Lying-Tunell et al. 1981; Cunnane et al. 2011; Castellano et al. 2015b; Croteau et al. 2017). Similarly to AD, decreased insulin sensitivity occurs years before the onset of symptoms and diagnosis of diabetes (Dankner et al. 2009). Brain glucose hypometabolism and mild cognitive deficits have been identified in diseases characterized by disrupted peripheral energy utilisation such as polycystic ovary syndrome (Castellano et al. 2015a), diabetes or pre-diabetes (Baker et al. 2011; Burns et al. 2013; Roberts et al. 2014).

Normally, when blood glucose decreases for more than a couple of hours, ketonemia develops in response to decreased insulin and the brain will use ketones as an alternative endogenous fuel (Cahill 2006). Ketones can provide up to 80% of total brain energy requirements in obese patients undergoing a prolonged fast (Owen et al. 1967; Drenick et al. 1972). The entry of ketones into the brain is directly correlated with their plasma concentration (Cunnane et al. 2016). Unlike for glucose, brain ketone uptake is unaffected during cognitively healthy aging (Nugent et al. 2014; Croteau et al. 2017). However, conditions of insulin resistance and high blood insulin may impact negatively on availability and/or uptake of both the brain’s main fuels. Aerobic exercise (AE) mildly increases plasma ketones endogenously via the mobilisation of free fatty acids (FFA) from the adipose tissue in healthy adults (Koeslag et al. 1980; Koeslag 1982; Féry and Balasse 1983; Féry and Balasse 1986; Féry and Balasse 1988). AE also increase brain ketone uptake in AD (Castellano et al. 2017).

Dietary supplementation with medium-chain triglycerides (MCT) transiently and safely induces mild ketosis (Reger et al. 2004; Henderson 2008; Courchesne-Loyer et al. 2013). The 8 and 10 carbon fatty acids in MCT are absorbed via the portal vein to the liver where they are
rapidly β-oxidized to acetyl-CoA (Cunnane et al. 2016; Schönfeld and Wojtczak 2016). In contrast to long chain fatty acids that need to be transformed into acylcarnitines by carnitine palmityl transferase I before being β-oxidized in the mitochondrial matrix (Bach and Babayan 1982), medium-chain fatty acids do not require this shuttle system to entry the mitochondria (Schönfeld and Wojtczak 2016), so are β-oxidized more rapidly.

To our knowledge, whether combining AE and MCT together has a synergistic effect on short-term ketogenesis has not previously been reported. If so, this would facilitate strategies to provide more ketones to fuel the aging brain by at least partially bypassing the brain glucose uptake deficit. Our overall goal was therefore to assess a potential synergistic effect of a five-day intervention with AE and/or MCT on plasma ketones during a four-hour metabolic study (Courchesne-Loyer et al. 2013; Vandenberghe et al. 2017a). Hence, our primary objective was to determine whether a five-day AE program combined with an MCT supplement would increase plasma ketone response in older women more than either intervention alone. Given the dampening influence of insulin and insulin resistance on ketogenesis (Fukao et al. 2004; Veech 2004), our secondary aim was to assess whether plasma ketones increase similarly after these combined or separate treatments in normoglycemic (NG) versus pre-diabetic (PD) women.

PARTICIPANTS AND METHODS

Participants

Ethical approval for this study was obtained from the Research Ethics Committee of the Integrated University Health and Social Services of Eastern Townships – Sherbrooke University Hospital Center, which oversees all human research done at the Research Center on Aging (Sherbrooke, QC, Canada). All participants provided written informed consent prior to beginning the study. They underwent a screening visit, including the analysis of a blood sample collected
after a 12 h overnight fast. Exclusion criteria included smoking, diabetes (fasting glucose >7.0 mmol/l and glycated hemoglobin >6.9%), unfit to practice exercise, untreated hypertension, dyslipidemia, and abnormal renal, liver, heart or thyroid function. PD participants needed to have a fasting plasma glucose of 6.0-6.9 mmol/l and/or a plasma glycated hemoglobin of 6.1-6.4% in order to be eligible to be included in the study (Golderngerg and Punthakee 2013). The homeostasis model assessment computational method was used to estimate insulin resistance (HOMA2-IR) from fasting plasma glucose and insulin (Levy et al. 1998). The Physical Activity Scale for the Elderly (PASE) questionnaire was used as an additional tool to characterise participants’ baseline physical activity. To be eligible, participants had to be doing structured physical exercise ≤ two times per week. Participants taking medications known to affect triglyceride or carbohydrate metabolism (i.e. diuretics, beta-blockers, steroids, insulin sensitizers) were excluded. Body composition and visceral adipose tissue area were measured by dual-energy x-ray absorptiometry (GE Healthcare LUNAR iDXA, Madison, WI, USA; (Hangartner et al. 2013). This project is registered on ClinicalTrials.gov (NCT 02678390).

**Experimental design**

Participants completed a 4 h metabolic study after each of the four following sequential experimental conditions: (i) no treatment control (CTL), (ii) five days of MCT supplementation alone (MCT), (iii) 30 min of aerobic exercise alone (AE), and (iv) five days of MCT supplementation combined with aerobic exercise (MCT+AE; Figure 1). Participants started with the CTL metabolic study during which they received only the lactose-free skim milk vehicle for the MCT drink. The CTL metabolic study served to set the baseline ketogenic response for the other three experimental conditions. Participants then took the MCT supplement daily for five
days; on the fifth day, they repeated the metabolic study, this time with a drink containing 15 g MCT at breakfast. During the second week of the study, participants repeated the metabolic study with vehicle only (such as CTL) but did 30 minutes of AE. This allowed assessment of the effect of a single acute session of AE on the ketone response during the 4 hour metabolic study. They then did five days of MCT supplementation simultaneously with five days of AE (30 min/day). On the fifth day of the combined intervention, they repeated the metabolic study with a drink containing 15 g MCT at breakfast but also followed by 30 min AE at 1.0 - 1.5 h. The 5 days period of MCT supplementation alone was separated from the MCT+AE period by a 1-3 week washout.

Metabolic studies

For each metabolic study, the participants arrived at 7:30 a.m. after a 12 h overnight fast and a minimum of 24 h without alcohol intake. A forearm venous catheter was installed and the baseline fasting blood sample withdrawn (Time 0 h). Participants then received a standardized breakfast comprised of two pieces of toast with raspberry jam and the MCT drink or the vehicle, which was 250 ml lactose-free skim milk. Water was available ad libitum throughout the study period. Blood samples were taken every 30 min for 4 h in EDTA collection tubes (BD Vacutainer, NJ, USA). Blood samples were centrifuged at 2846 g for 10 min at 4°C and plasma stored at -80°C until analyzed.

MCT supplement

The MCT used to make the emulsion was 55% tricaprylin oil (8-carbon MCT) and 35% tricaprin oil (10-carbon MCT; Captex 355, Abitec, Columbus, OH, USA). The emulsion was manufactured under aseptic conditions at Université Laval Laboratory of Food Technologies.
(Québec, QC, CAN) using our proprietary technology. The emulsion was prepared using 120 g MCT per liter of lactose-free skim milk (12%) and provided to participants in 250 ml bottles. When taking the supplement, participants consumed 15 g of MCT at breakfast (125 ml of the drink) and 15 g again at supper (total MCT 30 g/d; a previously used dose (Courchesne-Loyer et al. 2013) for five consecutive days. Compliance was measured by bottle count.

**Aerobic exercise**

During the AE phase of the study, participants exercised for 30 minutes in the morning for five consecutive days, a simple, short protocol feasible for older women. Exercise sessions were supervised by a kinesiologist and were performed on a treadmill or on a stationary bicycle at 55-75% of heart rate reserve, which was calculated as maximum heart rate - resting heart rate using the Karvonen equation (Karvonen and Vuorimaa 1988). Once the participant selected one of the two exercise methods (treadmill or stationary bicycle), it was kept throughout the 5 days of AE. Theoretical maximum heart rate was estimated as 206.9-(0.67 × age) (Tanaka et al. 2001) and the resting heart rate was measured before exercise. During the aerobic exercise sessions, heart rate was monitored with a Polar FT2 watch and a T31 heart rate sensor strap (Polar Electro, Kempele, Finland). A physician was on hand in case of a medical emergency.

**Plasma metabolite analyses**

Plasma acetoacetate (AcAc) and β-hydroxybutyrate (BHB) were measured by an automated colorimetric assay as previously described (Courchesne-Loyer et al. 2013). Briefly, for AcAc, 25 µL of plasma was mixed with 330 µL of fresh reagent (Tris buffer, pH 7.0, 100 mmol/L, 20 mmol/L sodium oxamate; 0.15 mmol/L NADH and 1U/mL β-hydroxybutyrate dehydrogenase [BHBDH]). For BHB, the reagent was Tris buffer (pH 9.0; 20 mM sodium
oxamate, 1 mmol/L NAD, and 1U/mL BHBDH). Tris, oxamic acid, DL-BHB sodium salt, Li-AcAc standard, and NAD were purchased from Sigma (St. Louis, MO, USA), NADH, from Roche (Mannheim, Germany), and BHBDH from Toyobo (Osaka, Japan). The change in absorbance at 340 nm between 15 and 120 s after the addition of the reagent was measured on an automated clinical chemistry analyzer (Dimension Xpand Plus; Siemens, Deerfield, IL, USA). Plasma glucose, lactate, triglycerides (Siemens Medical Solutions USA, Inc., Deerfield, IL, USA) and FFA (Randox Laboratories Limited, West Virginia, USA) were analysed using commercial kits. 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). Glycated hemoglobin was measured by HPLC-723G7, a fully automated high performance liquid chromatography instrument-reagent system (Tosoh Bioscience, King of Prussia, PA, USA).

Statistical analysis

All results are given as the mean ± SEM. The sample size calculation was based on a previous study in which 9 participants were sufficient to measure a significant difference (β = 0.80) in plasma ketones after consuming 20 g of MCT during a study with the same 4-hour metabolic study period (Vandenberghe et al. 2017b). We recruited n=10/group for the present study in case of a dropout during the two weeks. To analyze the acute effect of AE on plasma metabolites during the metabolic study, the % change was calculated during the 30 min AE session (1.0 vs. 1.5 h time point) and again during the 30 min immediately after the AE session (post-AE; 1.5 vs. 2.0 h time points).

When plasma ketones are present, this refers to the total of AcAc and BHB combined. For all metabolites, the area-under-the-curve (AUC) was calculated according the trapezoid method.
from 0-4 h during the metabolic study (Gagnon and Peterson 1998). The Shapiro-Wilk test
demonstrated that the plasma metabolite data were not normally distributed, so the results of the
four conditions were compared using Friedman’s test, and the effect of the treatments was
determined in each group using Wilcoxon’s signed rank test. All statistical analyses were carried
out using SPSS 23.0 software (SPSS Inc., Chicago, IL, USA). NG and PD were treated as
independent groups and compared using Mann-Whitney’s test. Differences were considered
statistically significant at \( P \leq 0.05 \). Graphs were prepared using Prism version 6.0 (GraphPad
Software Inc., San Diego, CA, USA).

RESULTS

Ten NG and nine PD women completed all four treatments of the study (Table 1).
Baseline anthropometry and plasma metabolites corresponded to references values from the
Sherbrooke University Hospital Center (Sherbrooke, QC). During AE, average exercise intensity
for NG and PD was 65 ± 6% and 68 ± 2% of heart rate reserve, respectively (\( P = 0.25 \)). Average
heart rate was 122 ± 12 (NG) and 127 ± 8 (PD) beats/min (\( P = 0.37 \)). HOMA-IR was 1.24 ± 0.17
and 1.25 ± 0.61 for NG and PD respectively (\( P = 0.22 \)). No gastrointestinal side-effects of the
MCT supplement were reported.

The AUC for the plasma glucose response was significantly higher in PD compared to
NG during the CTL, MCT and MCT+AE but not AE treatments (\( P < 0.05 \); Table 2). In NG, the
MCT supplement decreased the AUC for the plasma lactate response compared to CTL, whereas
in the PD group, AE alone increased the AUC for the plasma lactate response compared to CTL
(\( P < 0.05 \); Table 2). There was no difference in the AUC of the plasma FFA, triglyceride or insulin
response across the four different treatments or between ND and PD (all \( P > 0.05 \); Table 2).
Plasma ketones during the metabolic studies

During the CTL metabolic study, basal plasma ketones varied between 60-190 µmol/L in both the NG and PD groups (Figure 2A). Compared to the CTL metabolic study, MCT alone significantly elevated plasma ketones in NG for 1.5 hour post-breakfast and 3 hour in PD ($P<0.01$; Figure 2C) with peak ketones in NG and PD of $436 \pm 180$ and $479 \pm 266$ µmol/L, respectively ($P=0.93$). After of one session of AE alone, plasma ketones were significantly higher at 2 h in the NG compared to PD ($P<0.05$; Figure 2B). The MCT+AE treatment significantly elevated plasma ketones compared to CTL during most time point ($P<0.05$; Figure 2D). NG plasma ketones peaked at $434 \pm 173$ µmol/L (2 h) whereas PD peaked at $559 \pm 269$ µmol/L at 1 h ($P>0.05$).

In NG, MCT+AE induced the highest AUC for plasma ketones ($835 \pm 341$ µmol h/L), values which were 69%, 6 fold and 17 fold more elevated than for MCT alone, AE alone or CTL, respectively (all $P<0.05$; Figure 3). The plasma ketone AUC in PD was not different between the MCT alone and MCT+AE, and both were about 40 fold higher than the CTL or AE alone (all $P<0.05$). Except for a trend towards a higher ketone AUC in NG vs. PD on the AE alone ($P=0.091$), there was no significant difference between ($P>0.30$) the ketone AUCs in PD and NG under these experimental conditions.

Plasma glucose

The plasma glucose response was significantly higher in PD vs. NG at most time points during the CTL metabolic study ($P<0.05$; Figure 4A). On the MCT treatment alone, plasma glucose peaked at 1.5 h in NG ($6.7 \pm 1.0$ mmol/L) and at 2 h in PD ($7.6 \pm 1.2$ mmol/L (Figure 4C). During the metabolic study with the AE treatment, plasma glucose was lowest at the end of the 30 min AE session in both groups, and the overall glucose response was higher in PD.
At the end of the MCT+AE period, plasma glucose peaked at 2 h for the NG and at 2.5 h for the PD (both ~ 7.4 mmol/L; Figure 4D).

**Plasma insulin**

During the CTL metabolic study, plasma insulin peaked at 1 h at 496 ± 260 pmol/L (NG) and at 438 ± 177 pmol/L (PD) with no significant difference in the peaks between the two groups (Figure 5A). During the AE treatment alone, plasma insulin concentration was lower at 2 h in PD than ND (Figure 5B; *P*=0.01). At the end of the MCT or MCT+AE periods, plasma insulin response was significantly lower in both the NG and PD groups compared to CTL (Figures 5C, 5D; *P*<0.05).

**Metabolite changes during AE and immediately post-AE**

During the 30 min session of AE, plasma ketones and insulin % changes tended to increase in NG compared to PD (*P*<0.02), whereas glucose, lactate and FFA remained unchanged when compared between groups (*P*>0.05; Figure 6; upper). In the 30 min post-AE, the % increased in glucose, insulin, ketone and FFA (but not lactate) was significantly higher in NG compared to PD (*P*<0.03).

**DISCUSSION**

The aim of this study was to determine whether a short, moderate intensity AE program combined with a MCT supplement would increase plasma ketones in older women more than either intervention alone. Our results confirm previous reports that, separately, MCT supplementation (Henderson 2008; Courchesne-Loyer et al. 2013) or moderate AE (Balasse et al. 1978; Koeslag et al. 1980; Koeslag 1982; Féry and Balasse 1983; Féry and Balasse 1986; Féry
and Balasse1988) increase plasma ketones in older NG women (Figure 2). We extend those results and show that the combination of MCT+AE was more ketogenic in older NG women than MCT or AE alone. Specifically, in NG, plasma ketone AUC nearly doubled during the 4-h metabolic study after MCT+AE compared to MCT alone and increased by six-fold compared to AE alone. However, in PD, MCT+AE and MCT alone treatments didn’t have the same impact: compared to AE alone, MCT alone or MCT+AE increased the plasma ketone AUC significantly more in PD than in NG (Figure 3). A 15-gram dose of MCT is not directly comparable to a 30 min period of AE; nevertheless, the AE added to MCT was 69% more ketogenic than MCT alone in the NG group.

Since brain ketone uptake is proportional to plasma ketones (Cunnane et al. 2016), the implication of the present results is that enhanced plasma ketone response induced by a combination of AE and MCT has the potential to improve ketone supply to the brain in older women more than either alone. However, utilization of ketones by skeletal muscle can increase by as much as five-fold during AE (Passmore and Johnson 1958; Balasse et al. 1978; Féry and Balasse 1983; Féry and Balasse 1986; Evans et al. 2016), so the increase in plasma ketones is by no means limited to supplying more fuel to the brain. In order to optimize the effect of MCT+AE on plasma ketone availability specifically for the brain, it would be worth assessing whether the AE should be performed first, with the MCT supplement taken a short time later.

During the metabolic study, the dose of MCT significantly reduced the post-prandial glucose response (Figure 4), an effect that would be consistent with MCT slowing down the rate of absorption of glucose from the breakfast (Cunningham and Read 1989). As expected, the five days of MCT supplementation alone and combined with AE also delayed peak plasma glucose and insulin compared to the CTL in both groups (Figure 4-5 C; D). The 4 h glycemic response was flattened by consuming MCT, thereby inhibiting significant post-prandial excursions in
plasma glucose. Exogenous ketones (BHB infusion, dietary ketone monoester supplement) have also been recently shown to significantly lower plasma glucose in healthy young individuals (Mikkelsen et al. 2015; Stubbs et al. 2017; Myette-Côté et al. 2018). Combining the acute glucose-lowering effect of ketogenic supplements with the improved blood glucose control due to AE (Colberg et al. 2010) could be a useful strategy to improve glucose management in type 2 diabetic patients.

The second objective of this study was to assess whether the plasma ketone response was normal in PD. In fact, during the four-hour metabolic study, PD had a similar ketone plasma response to MCT supplement in comparison with NG (Figure 3), so a mildly hyperglycemic or insulin resistant state may not affect ketone production from MCT in older women. However, the synergistic effect of AE with MCT on plasma ketone AUC seen in NG (Figure 3) was not observed in PD in whom the post-prandial glucose response improved but did not normalize after AE. The capacity of skeletal muscle to switch from lipid to carbohydrate oxidation during insulin stimulation is impaired in pre-diabetics individuals (Kelley and Mandarino 2000; Færch and Vaag 2011). Exercise permits better control of compromised fuel oxidation owing to insulin-independent glucose uptake by skeletal muscle (Martin et al. 1995; Colberg et al. 2010). Previous data suggest that the rate of carbohydrate utilization by skeletal muscle during exercise is increased in hyperglycemic patients and is sustained by the mass effect of glucose plasma availability (Martin et al. 1995). Mild hyperglycemia in PD (Figure 4) would diminish lipid oxidation (Kelley and Mandarino 2000). In fact, the entry of FFA into skeletal muscle requires carnitine before the FFA are β-oxidized and converted to ketones in mitochondria (Koeslag 1982). Muscle free carnitine availability is reduced during conditions of high glycolytic flux such as elevated carbohydrate availability thus inhibiting long-chain fatty acids oxidation via transporter carnitine palmityl transferase I (Stephens 2018). Hence, compared to NG, skeletal
muscle energy metabolism during AE in PD probably depends more on carbohydrate breakdown as the primary substrate, thus decreasing FFA mobilization and ketogenesis (Jeukendrup et al. 1998). This interpretation is consistent with the differences observed in the immediate post-AE period during which ketones, glucose, FFA and insulin all rebounded more in NG than in PD (Figure 6).

This short-term intervention cannot necessarily be extrapolated to predict the effect of MCT supplementation and AE on long-term ketonemia. Previous studies have reported a sustained ketogenic effect of 4-12 wk of MCT supplementation in humans (Henderson 2008; Courchesne-Loyer et al. 2013). Currently there is no evidence that the ketogenic response to MCT and/or AE differs with the duration of the intervention but this should be validated. We also did not measure brain ketone or glucose uptake so we have no direct measure of whether the changes in plasma ketones reflect brain availability, although we would expect this to be the case from our previous work (Castellano et al. 2017).

We conclude that a combination of MCT with AE has a synergistic effect on ketonemia in NG older women. The ketonemia in older NG and PD women did not differ after MCT but did after AE or MCT+AE. MCT and AE merit further investigation as components of a multi-component strategy to increase availability of fuel to the aging brain.

COMPETING INTERESTS

Stephen C Cunnane has done consulting for or received honoraria for conference travel from Bulletproof, Keto-Products, Accera, Nisshin Oillio and Pruvit. Abitec Corp provided the MCT for this project. Nestlé has provided funding for some MCT research by Stephen C Cunnane’s group. The other authors have no conflict of interest.
FUNDING

MCT were provided by Abitec Corporation, Columbus, USA. Financial support was from NSERC.

AUTHORS’ CONTRIBUTIONS

Stephen C Cunnane, Isabelle J Dionne, Christian-Alexandre Castellano and Camille Vandenberghe designed the study. Camille Vandenberghe, Mathieu Maltais, Mélanie Fortier and Valérie St-Pierre conducted the study. Camille Vandenberghe, Isabelle J Dionne and Stephen C Cunnane analyzed and interpreted the data. All the authors contributed to the final article.

ACKNOWLEDGMENTS

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REFERENCES


Table 1

Baseline demographic and biochemical parameters of the participants

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Values are mean ± SEM.

Ketones= acetoacetate and β-hydroxybutyrate

ns = non significant
Table 2

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<td>PD 11.0 ± 2.8†</td>
<td>11.6 ± 2.8†</td>
<td>10.4 ± 3.0</td>
<td>10.3 ± 1.8†</td>
</tr>
<tr>
<td><strong>Insulin (nmol h/L)</strong></td>
<td>NG 0.9 ± 0.5</td>
<td>0.8 ± 0.3</td>
<td>0.8 ± 0.2</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>PD 0.8 ± 0.3</td>
<td>0.8 ± 0.3</td>
<td>0.7 ± 0.3</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td><strong>Lactate (mmol h/L)</strong></td>
<td>NG 6.2 ± 1.2</td>
<td>4.8 ± 1.3*</td>
<td>6.7 ± 1.5</td>
<td>5.9 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>PD 4.9 ± 0.9†</td>
<td>4.0 ± 1.0</td>
<td>7.5 ± 2.0*</td>
<td>5.0 ± 1.2</td>
</tr>
<tr>
<td><strong>Free fatty acids (mmol h/L)</strong></td>
<td>NG 1.3 ± 0.3</td>
<td>1.3 ± 0.2</td>
<td>1.3 ± 0.2</td>
<td>1.4 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>PD 1.3 ± 0.6</td>
<td>1.5 ± 0.4</td>
<td>1.4 ± 0.3</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td><strong>Triglycerides (mmol h/L)</strong></td>
<td>NG 3.6 ± 0.6</td>
<td>3.9 ± 0.4</td>
<td>4.0 ± 0.4</td>
<td>3.9 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>PD 3.8 ± 0.3</td>
<td>3.5 ± 0.5</td>
<td>3.7 ± 0.7</td>
<td>3.5 ± 0.8</td>
</tr>
</tbody>
</table>

Area under the curve of the plasma metabolites

Values are mean ± SEM;

CTL: Control; MCT: Medium chain triglycerides; AE: Aerobic exercise;

NG: Normoglycemic; PD: Prediabetic.

* Different from CTL; † Pre-diabetic different than normoglycemic; P<0.05
FIGURE LEGENDS

Figure 1. Outline of the experimental design. CTL: no treatment; MCT: medium-chain triglyceride; AE: aerobic exercise.

Figure 2. Plasma total ketones (β-hydroxybutyrate and acetoacetate combined) in normoglycemic (black symbols) and pre-diabetic (white symbols) older women during the metabolic studies: [A] control (CTL [no treatment]), [B] single 30 min session of aerobic exercise during the metabolic study (gray bar; AE) [C] five consecutive days of medium-chain triglyceride supplementation alone (MCT), and [D] five days consecutive days of MCT supplementation combined with 30 min aerobic exercise daily (MCT+AE; gray bar for AE). Arrow indicates when breakfast with MCT drink (C, D) or vehicle (A, B) was consumed. Values are the mean ± SEM for n= 10 normoglycemic and n=9 pre-diabetic. * Different from CTL, † Pre-diabetic different than normoglycemic; P< 0.05.

Figure 3. Four hour areas-under-the-curve (AUC) for plasma total ketones (acetoacetate and β-hydroxybutyrate combined) in normoglycemic (■) and pre-diabetic (□) older women during the metabolic studies: control (CTL; no treatment), five days of medium-chain triglyceride supplementation alone (MCT), single 30 min session of aerobic exercise (AE), or five days of MCT supplementation combined with 30 min aerobic exercise daily (MCT+AE). AUCs were defined as ‘net AUC’, which is the difference between the area of the peak above the baseline minus the area of peak below the baseline. Bars are the mean ± SEM for n= 10 normoglycemic and n=9 pre-diabetic. Means without a common letter differ significantly (A<B<C<D [normoglycemic], a<b [pre-diabetic]), P<0.05.

Figure 4. Plasma glucose in normoglycemic (black symbols) and pre-diabetic (white symbols) older women during the metabolic studies: [A] control (CTL [no treatment]), [B] single 30 min
session of aerobic exercise (gray bar; AE) [C] five days of medium-chain triglyceride supplementation alone (MCT) and [D] five days of a combination of MCT supplementation combined with 30 min aerobic exercise daily (MCT+AE; gray bar for AE). Arrow indicates when breakfast with MCT (C, D) or vehicle (A, B) was consumed. Values are presented as mean ± SEM for n= 10 normoglycemic and n=9 pre-diabetic. * Different from CTL, † Pre-diabetic different than normoglycemic; P< 0.05.

Figure 5. Plasma insulin in normoglycemic (black symbols) and pre-diabetic (white symbols) older women during the metabolic studies: [A] control (CTL [no treatment]), [B] single 30 min session of aerobic exercise (gray bar; AE) [C] five days of medium-chain triglyceride supplementation alone (MCT) and [D] five days of a combination of MCT supplementation combined with 30 min aerobic exercise daily (MCT+AE; gray bar for AE). Arrow indicates when breakfast with MCT (C, D) or vehicle (A, B) was consumed. Values are presented as mean ± SEM for n= 10 normoglycemic and n=9 pre-diabetic. * Different from CTL, † Pre-diabetic different than normoglycemic; P< 0.05.

Figure 6. Change in total ketones (β-hydroxybutyrate and acetoacetate), lactate, glucose, insulin and free fatty acids (FFA) during the 30 min aerobic exercise session (DURING AE; upper) or during the 30 min immediately after the exercise (POST-AE; lower). Data are expressed as % change in normoglycemic (■) and pre-diabetic (□) older women. Values are presented as mean ± SEM for n= 10 normoglycemic and n=9 pre-diabetic. † Pre-diabetic different than normoglycemic; P< 0.05.
DURING AE

% change

$P = 0.072$

POST-AE

% change

$P = 0.072$

Ketones  Lactate  Glucose  Insulin  FFA

††

†

††

††