A short-term intervention combining aerobic exercise (AE) with medium-chain triglycerides (MCT) on ketonemia: a comparison of normoglycemic and prediabetic older women

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Participants

This study was conducted with the informed written consent of all the participants and was approved by the appropriate ethics committees (Health and Social Services Center – Sherbrooke University Geriatrics Institute and the Centre hospitalier universitaire de Sherbrooke). Participants 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. Prediabetic (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 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).

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). 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

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. 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).

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