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Ketogenic response to cotreatment with bezafibrate and medium chain triacylglycerols in healthy humans

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ABSTRACT

Objectives: The aim of this study was to compare the ketogenic effect of the peroxisome proliferator-activated receptor-α stimulator, bezafibrate (BEZA), alone or in combination with medium-chain triacylglycerols (MCTs) in healthy adults.
Methods: Eighteen healthy adults completed the study: 10 were given a therapeutic dose of BEZA (400 mg/d) for 8 wk followed by a further 4 wk of BEZA (400 mg/d) plus MCT (60 g/d). Eight other participants were given MCT alone (60 g/d) for 4 wk. All participants underwent identical metabolic study days: (a) pretreatment (the control), and after (b) BEZA combined with MCT (BEZA+MCT) or (c) an equal dose of MCT only. On the metabolic study days, a standard breakfast and lunch were given and blood samples were taken hourly to measure plasma ketones, glucose, and fatty acids.
Results: The combination of BEZA+MCT increased ketones twofold during the metabolic study day. The addition of BEZA increased early ketogenic efficiency of MCT by 2.5-fold but did not result in higher peak or mean concentration of ketones during the metabolic study day. No other differences were seen in plasma metabolites or insulin during metabolic study days. On the final metabolic study day, MCT or BEZA+MCT had different effects on the plasma acetoacetate-to-β-hydroxybutyrate ratio compared with control.
Conclusions: BEZA mildly potentiated the ketogenic action of MCT but did not increase peak plasma ketone concentration or overall ketone production during the metabolic study day.

Introduction

Normally about 97% of the adult brain's energy substrate is glucose, with the remaining 3% being provided by the ketones β-hydroxybutyrate (β-OHB), and acetoacetate (AcAc) [1,2]. When circulating glucose and insulin levels are low, ketones are produced from the β-oxidation of fatty acids (FAs), mainly in the liver [3]. During prolonged fasting or when on a very high-fat, low-carbohydrate diet, plasma ketone levels can rise to ≥5 mM, at which concentration they provide ≤70% of total brain energy [2,4].

Medium-chain triacylglycerols (MCTs) contain FAs of 8 to 12 carbons and are good ketogenic substrates. Following absorption mainly via the portal vein, MCTs entering the liver bypass the rate-limiting enzyme, acyl-carnitine translocase, and enter mitochondria directly for β-oxidation. These two characteristics make MCT better ketogenic substrates compared with long-chain FAs (≥14 carbons), which are mainly absorbed as triacylglycerols (TGs) via chylomicrons and the lymphatic system, and reach the liver and peripheral tissues after transport through the general circulation [5,6]. MCTs and ketogenic diets have been shown to improve cognitive function on a short-term basis in older adults

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with mild cognitive impairment or Alzheimer's disease (AD) [7–9], possibly because of their ability to increase plasma ketones in all healthy adults including older people [10,11].

In addition to providing more ketogenic substrate such as dietary MCT, a second strategy to stimulate ketogenesis is by increasing hepatic FA β-oxidation. Previous research has shown that bezafibrate (BEZA), a second-generation fibrate, modestly increases postprandial plasma ketone response in humans [12]. Fibrates are mostly prescribed to treat hypertriacylglycerolemia in humans. They activate peroxisome proliferator-activated receptor (PPAR)α, although some studies suggest they may also have some interaction with PPARγ and PPARδ [13]. Through the activation of PPARα, fibrates increase expression of hydroxymethylglutaryl coenzyme-A (HMG-CoA) synthase and lyase, both key enzymes in ketogenesis [14].

Several studies have demonstrated that brain glucose metabolism is impaired by 10% to 15% in cognitively healthy older individuals and by 20% to 25% in patients with AD [15–18]. In individuals genetically susceptible to AD, brain glucose hypometabolism may occur before the onset of clinical symptoms of cognitive decline [19]. Because brain ketone uptake seems not to be affected by AD [17,20,21], we hypothesized that providing alternative substrates such as ketones could increase energy availability for glucose-deficient brain regions. The aim of the present study, therefore, was to determine whether BEZA could increase the ketogenic effect of MCT and if this effect could lead to a more sustained state of mild ketonemia than MCT alone in healthy humans on a typical Western diet.

Methods

Ethical approval for this study was obtained from the Research Ethics Committee of the Health and Social Services Center—Sherbrooke University Geriatrics Institute, which oversees all human research done at the Research Center on Aging (Sherbrooke, Quebec, Canada).

Participants

Twenty participants started the project, 10 men and 10 women. Two women dropped out of the BEZA+MCT group because of gastrointestinal side effects after the initiation of MCT supplementation. Ten participants, aged 49 ± 5 y, in the BEZA+MCT group and eight participants, ages 26 ± 1 y, in the MCT group completed the study. All were judged to be in good health after review of their medical histories and blood screening performed after a 12-h overnight fast. All participants were non-smokers and non-diabetic (fasting glucose <6.1 mmol/L and glycosylated hemoglobin <6%); had normal renal function, serum electrolytes, liver function (normal aspartate aminotransferase [AST] and alanine aminotransferase [ALT]), thyroid-stimulating hormone, high- and low-density lipoprotein (HDL and LDL) cholesterol, TGs, and albumin; had no overt nutritional problems; had no medical history of serious disease; and were not taking any medication or dietary supplements. Lifestyle was assessed with the aid of a self-administered questionnaire during the preselection visit.

Bezafibrate treatment and MCT supplementation

Participants in the BEZA+MCT group received BEZA (Bezalip SR; Actavis, Parsippany, NJ, USA) as a single 400-mg pill once daily for 12 wk, beginning the day after the control (CTL) metabolic study day. This was the same dose as is prescribed for hypertriacylglycerolmia. Liver enzymes (AST, ALT) were tested every 4 wk as a measure of liver injury. The MCT supplement consisted of a commercially available MCT oil (Alpha Health Products, Burnaby, BC, Canada), the FA composition of which was 60% octanoic acid (8:0) and 40% decanoic acid (10:0). The MCT oil contained no protein or carbohydrates. The MCT supplementation started 8 wk after the initiation of the BEZA. To acclimatize to the MCT supplement during the first week of supplementation, participants consumed 10 g of MCT four times per day during meals and once before going to sleep (total of 40 g MCT/d). During weeks 2 to 4 of supplementation, the MCT dose at each meal and in the evening was increased to 15 g, or a total of 60 g MCT/d. The MCT-alone group received only the 4-wk MCT oil treatment at the same dose schedule as the BEZA+MCT group. A 60 g/d dose of MCT was the highest well-tolerated dose as previously determined in our laboratory.

Metabolic study days

Participants in both groups underwent testing on two identical metabolic study days: before the introduction of BEZA or MCT (CTL), and after 4 wk on either supplementation with MCT oil alone (MCT) or the combination (BEZA+MCT). Potential side effects of BEZA were assessed by a questionnaire and weekly blood samples to measure liver enzymes (AST, ALT). Before each metabolic study day, participants fasted overnight for 12 h. On arrival at the lab, a forearm catheter was installed for blood sampling to evaluate baseline ketone, glucose, insulin, cholesterol, TGs, lactate and free fatty acids (FFAs). Blood samples were taken at 15-min intervals for the first hour and hourly thereafter for the next 7 h. After installing the catheter, a standard breakfast comprised of two pieces of toast with peanut butter and jelly and 200 mL orange juice was served. Four hours after the start of each metabolic study day, a lunch consisting of store-bought lasagna, tomato juice, sugar-free applesauce, and a granola bar was consumed. A ketogenic challenge (15 g dose of MCT) was given with both the breakfast and the lunch on the final metabolic study day.

Analyses

Plasma glucose, cholesterol, TGs, lactate (Siemens Medical Solutions USA, Inc., Deerfield, IL, USA), and FFAs (Wako Diagnostics, Richmond, VA, USA) were measured by commercially available kits. Ketone concentrations were evaluated by automated colorimetric assay as previously described [11,12,22,23]. Briefly, for AcAc, 25 μL of plasma was mixed with 330 μL of fresh reagent (Tris-buffer, pH 7.0, 100 mmol/L; sodium oxamate 20 mmol/L; NADH 0.15 mmol/L; β-hydroxybutyrate dehydrogenase [β-OHBDH]; 1 U/mL). For β-OHB, the reagent was Tris-buffer (pH 9.0; sodium oxamate 20 mmol/L; NAD 1 mmol/L; BHBDH 1 U/mL). Tris, oxamic acid, DL-β-OHB 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 15 to 120 sec after the addition of the reagent was measured on an automated clinical chemistry analyzer (Dimension Xpand Plus; Siemens). The assay was calibrated with freshly diluted standards from frozen aliquots of a 10 mmol/L standard of Li-AcAc or DL-β-OHB sodium salt, which is stable at −20°C for 2 and 6 mo, respectively. Calibrations and quality controls were performed for each assay to ensure the precision of the kits. Interassay coefficient of variation was 5% ± 1% (n = 360 measurements). Intraassay coefficient of variation was 4% ± 2% (n = 80 measurements). Plasma insulin was analyzed by enzyme-linked immunosorbent assay (Alpco Diagnostics Ltd., Salem, NH, USA) with a microplate reader (Victor multilabel plate reader 2030; Perkin Elmer: MA, USA).

Statistical analysis

All results are given as the mean ± SEM, unless otherwise stated, and differences were considered statistically significant at $P \leq 0.05$. Eight participants per group were sufficient to meet the statistical power ($\beta = 0.80$) needed to achieve a significant difference in plasma ketones during the metabolic study day. All statistical analyses were performed using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA). Due to sample size <30 per group, a nonparametric statistical test (one-way Kruskal-Wallis test) was employed to determine the differences in plasma data between the three groups at each hour of the metabolic study day. If a significant variability was observed at a given time point, a post hoc test (Wilcoxon rank-sum test) was employed for metabolic study day data pre- and post-MCT supplementation. The area under the curve (AUC) from 0 to 4 h, 4 to 8 h, and 0 to 8 h was determined using Prism version 6.0 (GraphPad Software Inc., San Diego, CA, USA).

Results

No modification in liver enzymes was observed in any participant on BEZA during the study. Other than age (26 ± 1 versus 49 ± 17; $P \leq 0.01$), there was no difference between the men and women so their data were combined for the analyses (Table 1). Plasma cholesterol, FFAs, TGs, glucose, insulin, and body composition did not change throughout the study (Table 2). A postprandial increase in plasma glucose and insulin was observed on the metabolic study days (data not shown). Lower fasting plasma lactate was noted in the MCT group (1 ± 0.1 mM [MCT] versus 1.8 ± 1 mM [BEZA+MCT]; $P = 0.043$ versus 1.7 ± 0.7 mM [CTL]; $P = 0.016$; Table 2). BEZA had no significant effect on any metabolic parameters measured over 8 wk (data not shown).