Nutrition 29 (2013) 635–640

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

Stimulation of mild, sustained ketonemia by medium-chain triacylglycerols in healthy humans: Estimated potential contribution to brain energy metabolism

Alexandre Courchesne-Loyer M.Sc. [a,b], Mélanie Fortier M.Sc. [a], Jennifer Tremblay-Mercier M.Sc. [a], Raphaël Chouinard-Watkins M.Sc. [a,b], Maggie Roy M.Sc. [a,b], Scott Nugent B.Sc. [a,b], Christian-Alexandre Castellano Ph.D. [a], Stephen C. Cunnane Ph.D. [a,b,*]

[a] Research Center on Aging, Health and Social Services Center, University Institute of Geriatrics of Sherbrooke, Sherbrooke, Québec, Canada
[b] Departments of Medicine and Physiology and Biophysics, Université de Sherbrooke, Sherbrooke, Québec, Canada

ARTICLE INFO

Article history:
Received 4 May 2012
Accepted 14 September 2012

Keywords:
Ketone bodies
Medium-chain triacylglycerols
Metabolism
Supplementation
Brain

ABSTRACT

Objective: In humans consuming a normal diet, we investigated 1) the capacity of a medium-chain triacylglycerol (MCT) supplement to stimulate and sustain ketonemia, 2) ^{13}C-β-hydroxybutyrate and ^{13}C-trioctanoate metabolism, and 3) the theoretical contribution of the degree of ketonemia achieved to brain energy metabolism.
Methods: Eight healthy adults (26 ± 1 y old) were given an MCT supplement for 4 wk (4 times/d; total of 20 g/d for 1 wk followed by 30 g/d for 3 wk). Ketones, glucose, triacylglycerols, cholesterol, free fatty acids, and insulin were measured over 8 h during two separate metabolic study days before and after MCT supplementation. Using isotope ratio mass spectroscopy, ^{13}C-D-β-hydroxybutyrate and ^{13}C-trioctanoate β-oxidation to $^{13}CO_2$ was measured over 12 h on the pre- and post-MCT metabolic study days.
Results: On the post-MCT metabolic study day, plasma ketones (β-hydroxybutyrate plus acetoacetate) peaked at 476 μM, with a mean value throughout the study day of 290 μM. Post-MCT, ^{13}C-trioctanoate β-oxidation was significantly lower 1 to 8 h later but higher 10 to 12 h later. MCT supplementation did not significantly alter ^{13}C-D-β-hydroxybutyrate oxidation.
Conclusions: This MCT supplementation protocol was mildly and safely ketogenic and had no side effects in healthy humans on their regular diet. This degree of ketonemia is estimated to contribute up to 8% to 9% of brain energy metabolism.

Introduction

The human brain has an energy requirement disproportionate to its size; although it represents only ~2% of adult weight, it uses ~22% of the body's oxygen consumption, mostly for aerobic glucose oxidation [1]. In an ad libitum–fed, healthy adult, roughly 97% of the brain's requirement is met directly or indirectly by glucose, with the remaining 3% coming primarily from ketones (β-hydroxybutyrate [β-OHB], acetoacetate [AcAc], and acetone) [1,2]. Under ketogenic conditions, β-oxidation of fatty acids in the liver [3] and possibly in astrocytes [4] produces most, if not all, the ketones used by the brain. Under normal conditions, plasma ketone concentrations are relatively low (≤0.2 mM), but during ketogenic conditions, i.e., extended fasting or a very high-fat ketogenic diet, plasma ketones can increase to 5 mM, at which point they provide up to two-thirds of the brain's energy requirement [5]. Monocarboxylate transporter-1 in the blood–brain barrier is responsible for ketone transfer to the brain [6].

Medium-chain triacylglycerol (MCT) oils are composed of fatty acids of 8 to 12 carbons, mostly octanoate (8:0) and decanoate (10:0). MCTs are mainly absorbed as free fatty acids directly from the portal vein, thus reaching the liver faster than long-chain fatty acids (≥14 carbons), which are absorbed from the small intestine and lymphatic system. In the liver, MCT β-oxidation is rapid because it does not need to be activated by coenzyme A, making MCT a good ketogenic substrate [7]. Several studies have described the short-term efficacy of

This study was supported by the Natural Sciences and Engineering Research Council of Canada.
* Corresponding author. Tel.: +819-780-2220, ext.: 45670; fax: +819-829-7141.
E-mail address: Stephen.Cunnane@USherbrooke.ca (S. C. Cunnane).

0899-9007/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nut.2012.09.009

MCTs for ketogenesis and for supporting normal cognitive function in the elderly and during experimentally induced hypoglycemia [8–12]. Long-term supplementation with MCTs may potentiate the ketogenic response to a single dose of MCT [13].

In the elderly, 10% to 15% lower brain glucose uptake has been widely reported, a value that increases to 20% to 25% in Alzheimer's disease [14–17]. Several studies have suggested that the uptake and/or use of glucose by the brain can deteriorate long before the normal threshold of the clinical symptoms of cognitive decline, i.e., in young adult carriers of apolipoprotein E4, an important genetic risk factor for Alzheimer's disease, and in those with a maternal history of Alzheimer's disease [17]. In the absence of any effective strategy to prevent or reverse the aging-related decline in brain glucose uptake, mildly stimulating ketogenesis may be a way to bypass the significant deficit in brain glucose uptake in persons with or at risk of developing aging-related cognitive decline [17–19]. The high-fat ketogenic diet has shown efficacy in the treatment of epilepsy [20,21]. In addition to trials of ketogenic supplements in Alzheimer's disease [9,13], this approach is being assessed for the treatment of other neurologic disorders, including Parkinson's disease, brain tumors, and stroke [18,22,23].

The three aims of the present study were to 1) determine whether 4 wk of supplementation with MCTs could induce a relatively sustained yet safe level of ketonemia in healthy adults consuming their usual diets, 2) evaluate labeled ketone and octanoate oxidation before and after MCT supplementation, and 3) estimate a plasma ketone level theoretically capable of replacing the apparent brain fuel deficit caused by lower brain glucose uptake in Alzheimer's disease.

Materials and 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, QC, Canada).

Participants

There were eight participants, 26 ± 1 y old, judged to be in good health after a review of their medical histories and blood screening performed after a 12-h overnight fast (Table 1). All participants were non-smokers, non-diabetic (fasting glucose <6.1 mmol/L and glycosylated hemoglobin <6.0%), had normal renal function (normal serum electrolytes), liver function (normal aspartate aminotransferase and alanine aminotransferase), thyroid function (normal thyroid stimulating hormone), and no overt nutritional problems (normal high-density and low-density lipoprotein cholesterol, triacylglycerols, and albumin).

MCT supplementation

The MCT supplement consisted of a commercially available emulsion made from MCTs and water (50:50, vol:vol; Liquigen, SHS International, Inc. [Nutricia Ltd.], Liverpool, UK). The fatty acid composition of the MCT supplement was 80% octanoic acid, 15% decanoic acid, and 5% lauric acid (12:0). The supplement contained no protein or carbohydrates. An artificial strawberry flavor and aspartame were added to the supplement before consumption. To acclimatize to the MCT supplement, during the first week of the study participants consumed 5 g of MCT three times per day during meals and once before going to sleep (total MCT 20 g/d). From weeks 2 to 4 of supplementation, each MCT dose was increased to 7.5 g, for a total MCT of 30 g/d.

Experimental design

The protocol involved 4 metabolic study days in total, 2 before MCT supplementation (pre-MCT) and 2 after MCT supplementation (post-MCT). On the first pre-MCT metabolic study day, the β-oxidation of β-OHB was evaluated with a 50-mg oral dose of ^{13}C-β-OHB ([2,4-^{13}C]-β-OHB; Cambridge Isotope Laboratories, Andover, MA, USA). On the second pre-MCT metabolic study day, fasting plasma metabolites and body composition were evaluated in addition to MCT β-oxidation (50 mg orally of [U-^{13}C]-trioctanoate; Cambridge Isotope Laboratories). The protocol on the metabolic study days post-MCT was the same as those pre-MCT.

On each metabolic study day, the participants arrived at 08:00 after having fasted overnight for 12 h. The 50-mg dose of ^{13}C-β-OHB or ^{13}C-trioctanoate was then aliquoted onto a piece of toast as part of a standard breakfast comprised of two pieces of toast with peanut butter and/or jelly and 200 mL of orange juice. Breath samples were collected every 30 min from 0 to 6 h and every hour from 6 to 12 h after the dose. After an overnight fast, on the morning of the second study day (pre- and post-MCT), an intravenous forearm catheter was installed and blood samples were taken to evaluate baseline ketone, glucose, insulin, cholesterol, triacylglycerol, and free fatty acid levels. Blood samples were taken at 15-min intervals for 1 h after the breakfast and hourly thereafter for another 7 h. Four hours after the start of each metabolic study day, a lunch consisting of lasagna, tomato juice, and apple sauce was consumed. A ketogenic challenge (7.5-g dose of MCTs) was given with the breakfast and with the lunch on the 2 post-MCT metabolic study days. Body composition was measured by dual-energy x-ray absorptiometry (GE Prodigy Lunar, Madison, WI, USA) pre- and post-MCT.

Analyses

Plasma glucose, cholesterol, triacylglycerols, lactate (Siemens Medical Solutions USA, Inc., Deerfield, IL, USA), and free fatty acids (Wako Diagnostics, Richmond, VA, USA) were measured by commercially available kits. Ketone concentrations were evaluated by an automated colorimetric assay as previously described [11,24–26]. 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; reduced nicotinamide adenine dinucleotide 0.15 mmol/L; β-OHB dehydrogenase 1U/mL). For β-OHB, the reagent was Tris buffer (pH 9.0; sodium oxamate 20 mmol/L, nicotinamide adenine dinucleotide 1 mmol/L, β-OHB dehydrogenase 1 U/mL). The Tris, oxamic acid, DL-β-OHB sodium salt, Li-AcAc standard, and nicotinamide adenine dinucleotide were purchased from Sigma (St. Louis, MO, USA), reduced nicotinamide adenine dinucleotide from Roche (Mannheim, Germany), and β-OHB dehydrogenase from Toyobo (Osaka, Japan). The change in absorbance at 340 nmol/L 15 to 120 s after the addition of the reagent was measured on an automated clinical chemistry analyzer (Dimension Xpand Plus; Siemens Medical Solutions USA). 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 or 6 mo, respectively. Calibrations and quality controls were performed for each assay to ensure the precision of the kits (coefficient of variation between tests 5 ± 1%). Plasma insulin was analyzed by an enzyme-linked immunosorbent assay (Alpco Diagnostics Ltd., Salem, NH, USA) with a microplate reader (Victor Multilabel Plate Reader 2030; Perkin-Elmer, Waltham, MA, USA).

Table 1
Baseline demographic and biochemical parameters of the participants

		Reference values*
Age (y)	26 ± 1	—
Men/women	6/2	—
Weight (kg)	73 ± 4	—
Height (cm)	177 ± 2	—
Body mass index (kg/m^2)	23 ± 1	—
Plasma measurements		
Albumin (g/L)	45.8 ± 0.4	35–52
Aspartate aminotransferase (IU/L)	20 ± 2	0–32
Alanine aminotransferase (IU/L)	19 ± 3	0–37
Thyroid-stimulating hormone (mIU/L)	1.90 ± 0.37	0.35–3.50
LDL cholesterol (mmol/L)	2.26 ± 0.27	<5.00
HDL cholesterol (mmol/L)	1.47 ± 0.13	≥1.30
Total cholesterol (mmol/L)	4.2 ± 0.3	3.0–5.2
Triacylglycerols (mmol/L)	1.0 ± 0.2	≤1.7
Glycated hemoglobin (%)	5.1 ± 0.1	4.0–6.0
Creatinine (μmol/L)	74 ± 3	46–92

HDL, high-density lipoprotein; LDL, low-density lipoprotein
Values are presented as mean ± SEM (n = 8).
* Reference values are from the Centre Hospitalier Universitaire de Sherbrooke.