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Caffeine intake increases plasma ketones: an acute metabolic study in humans

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ABSTRACT

Brain glucose uptake declines during aging and is significantly impaired in Alzheimer's disease. Ketones are the main alternative brain fuel to glucose so they represent a potential approach to compensate for the brain glucose reduction. Caffeine is of interest as a potential ketogenic agent owing to its actions on lipolysis/ lipid oxidation but whether it is ketogenic in humans is unknown. This study aimed to evaluate the acute ketogenic effect of two doses of caffeine in healthy adults (2.5; 5.0 mg/kg) during a 4-hour metabolic study period. Caffeine given at breakfast significantly stimulated ketone production in a dose-dependent manner (+88%; +116%) and also raised plasma free fatty acids. Whether caffeine has long-term ketogenic effects or could enhance the ketogenic effect of medium chain triglycerides remains to be determined.

Key words: Ketones; Ketonemia; Caffeine; Free fatty acids; Medium chain triglycerides; Lipolysis; Alzheimer's disease.

RÉSUMÉ

La consommation cérébrale de glucose diminue avec l'âge et, tout particulièrement, avec la maladie d'Alzheimer. L'élaboration de différentes stratégies nutritionnelles pour optimiser la production de cétones, le principal carburant alternatif cérébral, est nécessaire afin de soutenir les besoins énergétiques du cerveau vieillissant. La caféine est une molécule d'intérêt en raison de son action sur le métabolisme lipidique. L'effet aigu de différentes doses de caféine (2.5; 5.0 mg/kg) sur la production de cétones était évalué chez dix sujets. Nos résultats ont montré que la caféine ajoutée à un repas stimule significativement la cétonémie à des concentrations comparables à un jeûne de 12h et cette réponse est dose-dépendante (+88 à +116%). Ainsi, la prise de caféine combinée avec une source alimentaire cétogène comme les triglycérides à chaîne moyenne dans le but de maximiser la cétonémie constitue une piste prometteuse d'intervention en concomitance avec d'autres traitements thérapeutiques dans un contexte de maladies neurodégénératives.

INTRODUCTION

Caffeine upregulates metabolic rate (Miller et al. 1974), and stimulates energy expenditure. It is an adenosine receptor antagonist that increases sympathetic activity (Bellet et al. 1969) and inhibits cyclic nucleotide phosphodiesterase, which is responsible for catalyzing the conversion of cyclic adenosine monophosphate (cAMP) to AMP (Butcher et al. 1968; Quan et al. 2013). As a result, higher tissue concentrations of cAMP activate hormone-sensitive lipase and promote lipolysis (Acheson et al. 2004; Butcher et al. 1968). Free fatty acids (FFA) are the product of lipolysis and can be used as an immediate source of energy by many organs. They can also be converted by the liver into ketones (acetoacetate [AcAc], β -hydroxybutyrate [β -HB] and acetone). Most organs use glucose and FFA as energy substrates. However, the brain is unable to use FFA for energy, and requires ketones as the principal alternative fuel to glucose (Cunnane et al. 2016). Plasma ketones are highly positively correlated to their utilization by the brain (Cunnane et al. 2016; Mitchell et al. 1995) and can provide up to 70% of brain's total energy during period of hypoglycaemia as, for example, during fasting (Owen et al. 1967).

Brain glucose uptake is 10-15% lower during normal aging (Nugent et al. 2014), and can be up to 35% lower in certain brain regions in neurodegenerative diseases such as Alzheimer's disease (AD) (Castellano et al. 2015). Several studies suggest that brain glucose hypometabolism potentially contributes to the onset and/or progression of AD (Cunnane et al. 2016; Mosconi et al. 2005; Nugent et al. 2014; Reiman et al. 2004; Schöll et al. 2011). A ketogenic supplement could therefore potentially help support the brain's energy needs during aging. Hence, the primary aim of this study was to evaluate whether the lipolytic effect of caffeine acutely increases plasma ketones in healthy adults during a four-hour metabolic study period. The secondary aim was to confirm whether caffeine increases FFA as previously reported (Acheson et al. 1980; Acheson et al. 2004).

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 the 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 regular high consumption of caffeine (>300 mg/day), smoking, diabetes or glucose intolerance (fasting glucose >6.1 mmol/L and glycosylated hemoglobin >6.0%), untreated hypertension, dyslipidemia, abnormal renal, liver, heart or thyroid function. This project is registered on ClinicalTrials.gov (NCT 02694601).

Experimental design

The protocol involved three randomized four-hour metabolic study days: a baseline metabolic day (CTL) and two days each with a different dose of caffeine (2.5 mg/kg [C-2.5] and 5.0 mg/kg [C-5.0]). On each metabolic study day, the participants arrived at 8:00 a.m. after 12 h of fasting and 24 h without caffeine intake. At the time of signing the consent form, participants were aware of the 12 h fast and to abstain from consuming caffeine. They also received a reminder call 24 h before the metabolic study day. A forearm venous catheter was installed and blood samples were taken every 30 min during 4 hours. After installing the catheter and the first blood sample, participants received a standard breakfast comprised of two pieces of toast with raspberry jam, a piece of cheese, applesauce and 100 ml of juice. The breakfast contained 85 grams of carbohydrate, 9.5 g of fat and 14 g of protein. Commercially available caffeine tablets (200 mg extra-strength Life BrandTM, ON, Canada) were hand crushed to powder and two doses were provided (2.5 mg/kg and 5.0 mg/kg) on separate test days. The low dose corresponding to 1½ cup of coffee and the high dose to 3 cups of regular coffee, the highest quantity recommended by Health Canada. The caffeine dose to be given was mixed in 104 ml of applesauce and consumed during breakfast. No caffeine was added to the breakfast for CTL. Water was available *ad libitum* throughout the study day. Blood samples were centrifuged at 3500 rpm for 10 min at 4°C and plasma was stored at -80°C until further analysis.

Plasma analyses

Caffeine

Plasma caffeine was measured using a complete ELISA Kits from Neogen (WI, USA), according to the manufacturers' protocol with the following modifications. Caffeine (Sigma-Aldrich®, St-Louis, Mo, USA) was diluted with the Neogen kit buffer (EIA) at multiple dilutions ending with the standard curve dilutions from 0 to 25 ng/ml. Plasma samples were then diluted with EIA buffer at a 1:50 000 dilution. Both standards and samples were run in duplicate. The absorbance was then measured with a plate reader (VICTOR, Perkin Elmer Inc, MA, USA) at 690 nm.

Metabolites

Plasma glucose, lactate, triglycerides, total cholesterol (Siemens Medical Solutions USA, Inc., Deerfield, IL, USA) and free fatty acids (Randox Laboratories Ltd, West Virginia, USA) were measured using commercial kits on a clinical biochemistry analyzer (Dimension Xpand Plus, Siemens Healthcare Diagnosis Inc., Deerfield, IL, USA) as previously described (Courchesne-Loyer et al. 2013). Plasma β -HB and AcAc were evaluated by an automated colorimetric assay as previously described (Courchesne-Loyer et al. 2013).

Statistical analysis

All results are given as mean $\pm$ SEM. Ten participants were sufficient to meet the statistical power ($\beta=0.80$) needed to observe a significant difference in plasma FFA with the caffeine supplementation (Acheson et al. 1980). For lactate, metabolic study day values were normalized to baseline in order to account for variability at the beginning of the study day. For post-caffeine ketone and FFA analysis, the area under the curve (AUC) was calculated from 2 to 4-hour post-dose because that was when maximal plasma caffeine was achieved. All statistical analyses were carried out using SPSS 23.0 software (SPSS Inc., Chicago, IL, USA). Comparison of the three test conditions was done using the Friedman test, and the effect of caffeine supplementation was determined in each group using a Wilcoxon's signed rank test.

Differences were considered statistically significant at $p \leq 0.05$. Data were graphed using Prism version 6.0 (GraphPad Software Inc., San Diego, CA, USA).

RESULTS

Two men and eight women completed all three test conditions (Table 1). Participants were 33 ± 19 years of age and had a body mass index of 24 ± 8 ($n=10$). The participant's baseline biochemical parameters corresponded to normal references values from the Sherbrooke University Hospital Center (Sherbrooke, Qc). No significant side effects were reported following caffeine intake. Baseline plasma caffeine values did not significantly differ from zero on any of the three study days (Fig.1). There was no difference in plasma glucose, triglycerides, or cholesterol response across the three metabolic days (*data not shown*). Plasma lactate differed across the three metabolic days ($p=0.045$), but after normalizing the data to baseline, these differences disappeared ($p=0.607$).

A dose-response was observed for plasma caffeine across the three metabolic days ($p<0.05$; Fig. 1). Plasma caffeine significantly increased during the first hour post-dose ($p<0.05$). C-2.5 increased plasma caffeine to a maximum of 7.5 ± 1.5 mg/L at 2 h and C-5.0 increased plasma caffeine to a maximum of 10.0 ± 2.3 mg/L at 3 h ($p<0.05$). No difference in plasma AcAc levels was observed across the three test days ($p=0.497$; Fig 2A, 2C). However, after normalizing the data to baseline, there was a significant group difference between baseline and the two doses of caffeine at 3.5 h, at which time AcAc was significantly increased ($p<0.05$; *data not shown*). A group difference was observed for the β -HB response from 2 to 4 h post-dose ($p<0.05$; Fig. 2B and 2D). Caffeine increased plasma β -HB by 88% and 116% in a dose-dependent manner ($p<0.05$). No significant difference in plasma FFA was observed during 0-2 h post-dose (Fig. 3A). Globally, FFA decreased from 711 ± 398 μ M to 91 ± 42 μ M during this period (Fig. 3A). Between 2 – 4 h after the breakfast, a dose-related increase of FFA was observed with the two doses of caffeine ($p<0.005$; Fig. 3B). C-2.5 raised plasma FFA concentrations to 548 ± 276 μ M after 4 h whereas C-5.0 raised plasma FFA to 695 ± 433 μ M.

DISCUSSION

This short-term study showed that caffeine intake can stimulate ketogenesis by increasing β -HB concentrations by 88-116% with a maximum within four hours post-dose. A dose-response was observed for plasma β -HB (Fig. 2D) but not for AcAc (Fig. 2C), which could be explained by the larger inter-group variation in AcAc. The increase in plasma ketones obtained with these doses of caffeine could at least transiently contribute to 5-6% of brain energy needs (Cunnane et al. 2016).

The increased plasma FFA after caffeine seen in the present study confirms prior results (Acheson et al. 1980; Acheson et al. 2004; Bellet et al. 1968; Bellet et al. 1969). Caffeine competes for the adenosine receptor, inhibits phosphodiesterase activity and increases plasma FFA. FFA entering the liver are beta-oxidized and converted to ketones due to condensation of pairs of acetyl-CoA units as their availability exceeds their utilization by the tricarboxylic acid cycle (Wang et al. 2014).

The increase in blood ketones shown here was equivalent to that observed after an overnight fast. Another way of increasing blood ketones is to provide a source of medium-chain triglyceride (MCT) (Courchesne-Loyer et al. 2013). Caffeine combined with an MCT supplement could potentially prolong mild ketonemia. Such products are already available on the market although no reports are available on the ketogenic effect of the combination of these products.

One limitation of this study design is that the metabolic study period was only 4 hours. However, this was sufficient to observe an effect on plasma ketones and FFA within the period during which peak plasma caffeine was observed. The half-life of caffeine is 4.5 hours, which suggests that its peak metabolic effect would take place over 2-3 hours. Furthermore, the effect of each caffeine dose was only assessed once, so a longer term study would be useful.

In conclusion, by enhancing lipolysis and increasing blood FFA levels, which in turn provide substrates for ketogenesis, caffeine at doses of 2.5 and 5.0 mg/kg stimulated safe and mild ketonemia in healthy adults to a ketone level twice that seen after an overnight fast. Several studies suggest that regular caffeine

consumption may be linked to the decreased risk of developing late-life cognitive decline (Panza et al. 2015). Further studies are needed to evaluate caffeine's long term effect on ketonemia and its impact on brain function during aging.

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148 Table 1. Baseline demographic and biochemical parameters of the participants
 149 (Mean $\pm$ SEM)

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Characteristics			
Age (y)	33	$\pm$	19
Men/Women	2/8		
Weight (kg)	65	$\pm$	14
Height (cm)	163	$\pm$	16
Body mass index (kg/m ²)	24	$\pm$	8
Glucose (mmol/L)	4.2	$\pm$	0.4
Lactate (mmol/L)	1.86	$\pm$	1.0
Glycated hemoglobin (%)	5.3	$\pm$	0.3
Total cholesterol (mmol/L)	4.3	$\pm$	0.8
Triacylglycerol (μ mol/L)	749	$\pm$	282
Free fatty acids (μ mol/L)	711	$\pm$	392
Ketones (μ mol/L)	175	$\pm$	65

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Figure 1. Plasma caffeine concentrations during the control (CTL) metabolic study day (●), after receiving a 2.5 mg/kg (C-2.5) (□) or 5.0 mg/kg dose of caffeine (C-5.0) (▽). Arrow indicates breakfast. Values are presented as mean ± SEM ($n = 10/\text{point}$); * $p < 0.05$ CTL vs C-2.5, † $p < 0.05$ CTL vs C-5.0, # $p < 0.05$ C-2.5 vs C-5.0.

Figure 2. Plasma acetoacetate [A] and β-hydroxybutyrate [B] concentrations during the control (CTL) metabolic study day (●), and after receiving a 2.5 mg/kg (C-2.5) (□) or 5.0 mg/kg dose of caffeine (C-5.0) (▽). Arrow indicates breakfast. The area under the curve was measured from 2 to 4-hour post-dose for acetoacetate [C] and β-hydroxybutyrate [D]. Values are presented as mean ± SEM ($n = 10/\text{point}$); * $p < 0.05$ CTL vs C-2.5, † $p < 0.05$ CTL vs C-5.0, # $p < 0.05$ C-2.5 vs C-5.0.

Figure 3. Plasma free fatty acids (FFA) concentrations [A] during the control (CTL) metabolic study day obtained before (●), after receiving a 2.5 mg/kg dose (C-2.5) (□) or 5.0 mg/kg dose of caffeine (C-5.0) (▽). Arrow indicates breakfast. The area under the curve [B] was measured from 2 to 4-hour post-dose. Values are presented as mean ± SEM ($n = 10/\text{point}$); * $p < 0.05$ CTL vs C-2.5, † $p < 0.05$ CTL vs C-5.0, # $p < 0.05$ C-2.5 vs C-5.0.