

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 and lipid oxidation but whether it is ketogenic in humans is unknown. This study aimed to evaluate the acute ketogenic effect of 2 doses of caffeine (2.5; 5.0 mg/kg) in 10 healthy adults. 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 du glucose diminue avec l'âge et est nettement compromise dans 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 la lipolyse et l'oxydation 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. Ajoutée à un repas, la caféine stimule significativement la cétonémie selon une relation dose-dépendante (+88 à +116 %). Celle-ci permet aussi d'augmenter efficacement le taux d'acides gras libres plasmatiques de manière proportionnelle à la dose. Ainsi, la prise de caféine combinée avec une source alimentaire cétogène comme les triglycérides à chaîne moyennes dans le but de maximiser la cétonémie constitue une piste prometteuse d'intervention en concomitance avec d'autres traitements thérapeutiques.

Mots-clés : cétones, cétonémie, caféine, acides gras libres, triglycérides à chaîne moyenne, lipolyse, maladie d'Alzheimer.

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 the brain's total energy during period of hypoglycemia 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 (Castellano et al. 2015). Several studies suggest that brain glucose hypometabolism potentially contributes to the onset and (or) progression of Alzheimer's disease (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 4 h metabolic study period. The secondary aim was to confirm whether caffeine increases FFA as previously reported (Acheson et al. 1980, 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, Quebec, Canada). All participants provided written informed consent prior to beginning the study. They underwent a screening visit, including the analysis of a blood

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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; and abnormal renal, liver, heart, or thyroid function. This project is registered on ClinicalTrials.gov (NCT 02694601).

Experimental design

The protocol involved 3 randomized 4 h metabolic study days: a baseline metabolic day (CTL) and 2 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 0800 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 the 4 h period. After the catheter was installed and the first blood sample was taken, participants received a standard breakfast of 2 pieces of toast with raspberry jam, a piece of cheese, applesauce, and 100 mL of juice. The breakfast contained 85 g of carbohydrate, 9.5 g of fat, and 14 g of protein. Commercially available caffeine tablets (200 mg extra-strength Life Brand, Ontario, Canada) were hand crushed to powder and 2 doses were provided (2.5 mg/kg and 5.0 mg/kg) on separate test days. The low dose corresponds to 1.5 cups 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 2846g 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 Kit from Neogen (Wisconsin, USA), according to the manufacturers' protocol with the following modifications. Caffeine (Sigma-Aldrich, St. Louis, Missouri, USA) was diluted with the Neogen kit buffer at multiple dilutions ending with the standard curve dilutions from 0 to 25 ng/mL. Plasma samples were then diluted with Neogen kit 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., Massachusetts, USA) at 690 nm.

Metabolites

Plasma glucose, lactate, triglycerides, total cholesterol (Siemens Medical Solutions USA, Inc., Deerfield, Illinois, USA), and FFA (Randox Laboratories Ltd., West Virginia, USA) were measured using commercial kits on a clinical biochemistry analyzer (Dimension Xpand Plus, Siemens Healthcare Diagnosis Inc., Deerfield, Illinois, 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).

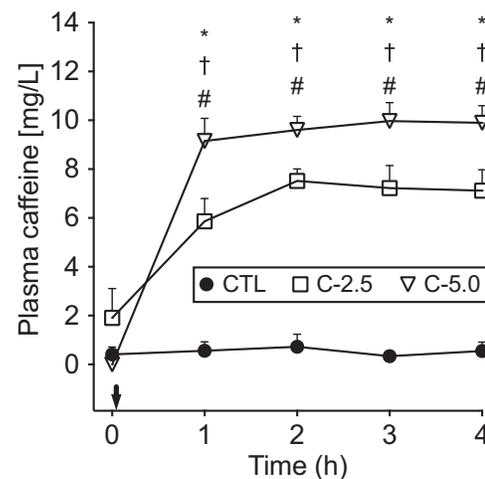
Statistical analysis

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 to account for variability at the beginning of the study day. For post-caffeine ketone and FFA analysis, the area under the curve was calculated from 2 to 4 h 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, Illinois, USA). Comparison of the 3 test conditions was done using the Friedman test, and the effect

Table 1. Baseline demographic and biochemical parameters of the participants (2 men and 8 women).

Characteristics	Mean \pm SEM
Age (years)	33 \pm 19
Mass (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

Fig. 1. Plasma caffeine 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. Values are presented as mean \pm SEM ($n = 10$ /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.



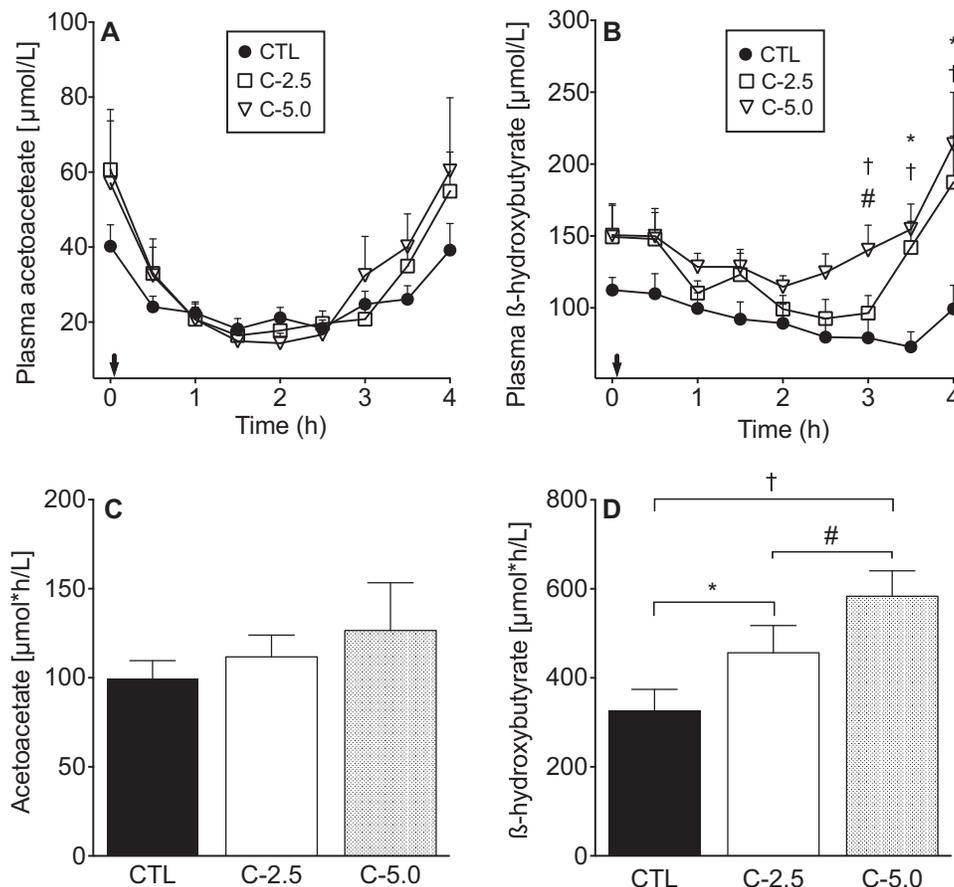
of caffeine supplementation was determined in each group using a Wilcoxon's signed rank test. Differences were considered statistically significant at $p < 0.05$. Data were graphed using Prism version 6.0 (GraphPad Software Inc., San Diego, California, USA).

Results

Two men and eight women completed all 3 test conditions (Table 1). Participants were 33 \pm 19 years of age and had a body mass index of 24 \pm 8 ($n = 10$). The participant's baseline biochemical parameters corresponded to normal reference values from the Sherbrooke University Hospital Center (Sherbrooke, Quebec). No significant side effects were reported following caffeine intake. Baseline plasma caffeine values did not significantly differ from zero on any of the 3 study days (Fig. 1). There was no difference in plasma glucose, triglycerides, or cholesterol response across the 3 metabolic days (data not shown). Plasma lactate differed across the 3 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 3 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 \pm 1.5 mg/L at 2 h and C-5.0 increased plasma caffeine to a maximum of 10.0 \pm 2.3 mg/L at 3 h ($p < 0.05$). No difference in plasma AcAc levels was observed across

Fig. 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 h post-dose for acetoacetate (C) and β -hydroxybutyrate (D). Values are presented as mean \pm 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.



the 3 test days ($p = 0.497$; Figs. 2A and 2C). However, after normalizing the data to baseline, there was a significant group difference between baseline and the 2 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 ($p < 0.05$; Figs. 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 $711 \pm 398 \mu\text{mol/L}$ to $91 \pm 42 \mu\text{mol/L}$ during this period (Fig. 3A). Between 2 and 4 h after the breakfast, a dose-related increase of FFA was observed with the 2 doses of caffeine ($p < 0.005$; Fig. 3B). C-2.5 raised plasma FFA concentrations to $548 \pm 276 \mu\text{mol/L}$ after 4 h whereas C-5.0 raised plasma FFA to $695 \pm 433 \mu\text{mol/L}$.

Discussion

This short-term study showed that caffeine intake can stimulate ketogenesis by increasing β -HB concentrations by 88%–116% with a maximum within 4 h 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, 2004; Bellet et al. 1968, 1969). Caffeine competes for the adenosine receptor, inhib-

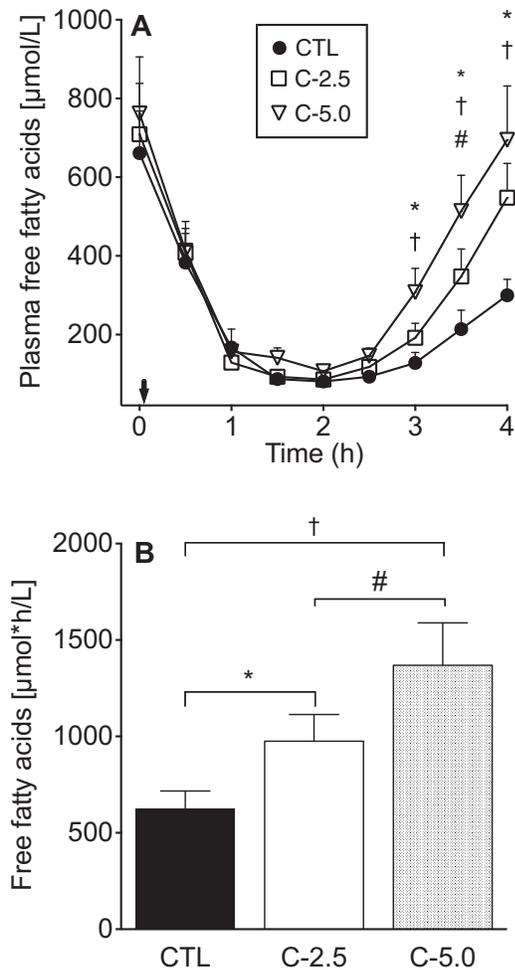
its phosphodiesterase activity, and increases plasma FFA. FFA entering the liver are beta-oxidized and converted to ketones due to condensation of pairs of acetyl-CoA units as their availability exceeds their utilization by the tricarboxylic acid cycle (Wang et al. 2014).

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 (Courchesne-Loyer et al. 2013). Caffeine combined with a medium-chain triglyceride 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 h. 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 h, which suggests that its peak metabolic effect would take place over 2–3 h. 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

Fig. 3. Plasma free fatty acid concentrations (A) during the control (CTL) metabolic study day (●), and 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 h post-dose. Values are presented as mean ± SEM (*n* = 10/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.



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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