Original Article

Is there a sex difference in adult salivary clearance of caffeine (1,3,7-trimethylpurine-2,6-dione)?

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ABSTRACT

Background: The salivary caffeine clearance is a non-invasive, safe, saliva-based method for assessing hepatic function and diagnosing chronic liver disease. The elimination of caffeine from the body follows first-order kinetics and principally involves catabolism by hepatic CYP1A2, with a half-life usually between three and 7 h. It is known that this process is affected by age and smoking tobacco. It has been suggested that sex might also be important, but there is scant evidence for this. The aim of this study was to assess whether there is a sex difference in salivary caffeine clearance in adults.

Methods: A cohort of 213 adults was studied. They were all non-smokers and none suffered from chronic liver disease. They consisted of 67 men (mean age 40.0 years) and 146 women (mean age 44.7 years). Following a period of dietary caffeine abstinence lasting at least 24 h, each subject ingested a single oral dose in the morning of caffeine, at a dose of 3 mg per kg body mass. Salivary samples were collected at 2 h and 14 h post-caffeine ingestion and were spectrophotometrically assayed for their caffeine concentrations.

Results: The two groups were matched for age. The mean (standard error) salivary caffeine clearance in the male subjects was 1.51 (0.10) mL min⁻¹ kg⁻¹, while that in the female subjects was 1.60 (0.07) mL min⁻¹ kg⁻¹ ($p = 0.495$).

Conclusion: This relatively large study provides no evidence of a sex difference in salivary caffeine clearance.

1. Introduction

Caffeine (1,3,7-trimethylpurine-2,6-dione) is the natural alkaloid most widely ingested by humans.¹ While murine caffeine pharmacokinetics are non-linear, in adult humans the elimination of caffeine from the body follows first-order kinetics and principally involves catabolism by hepatic CYP1A2 (with the rs762551 polymorphism of *CYP1A2* decreasing enzyme inducibility), with a half-life usually between three and 7 h^{1,2,3}. The main catabolic pathways in adult humans are illustrated in Fig. 1; caffeine is mainly catabolised by cytochrome P450, with around 90 per cent of these reactions involving CYP1A2.^{4,5,6}

In humans, the caffeine clearance from saliva has been shown to act as a non-invasive liver function test.⁷ Importantly, Tripathi and colleagues have recently shown that salivary caffeine clearance can be used for diagnosing chronic liver disease and assessing residual hepatic function in this condition.⁸

The activity of CYP1A2, and therefore the salivary caffeine

clearance rate, is affected by factors such as age, drinking coffee and smoking tobacco.^{2,5,9,10} It has not been established, however, whether the sex of an adult has a significant effect. Tantcheva-Poór and colleagues reported that, on average, the CYP1A2 activity of healthy adult females studied by their group was lower than that of healthy men by a factor of 0.9.¹¹ On the other hand, a significant effect of sex has not been reported by other groups. Indeed, following their study of the influence of sex on CYP1A2 activity, Kashuba and colleagues concluded that: 'Stratification by ... sex need not be performed for pharmacokinetic or clinical investigations of substrates for CYP1A2 ... in which the subjects are adults.'¹²

Clinically, it is clearly important to ascertain whether or not sex has a significant effect on the rate of salivary caffeine clearance. The aim of this study was to assess whether or not such a sex difference exists.

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Fig. 1. The main catabolic pathways of caffeine in adult humans.

2. Materials and method

2.1. Subjects

An audit was carried out of 213 patients, mean (standard error) age 43.2 (1.11) years, who were non-smokers and who were attending an environmental health clinic. Sixty-seven of the patients were male

(mean age 40.0 (2.2) years) and the remaining 146 were female (mean age 44.7 (1.3) years). None of the patients suffered from chronic liver disease. A detailed nutritional history was taken from each subject. The audit was carried out with research ethics committee approval and was carried out in accordance with the Declaration of Helsinki. All subjects gave full informed consent to undergo assessment of the salivary caffeine clearance.

2.2. Samples

Following a period of dietary caffeine abstinence lasting at least 24 h, each subject ingested a single oral dose in the morning of caffeine, at a dose of 3 mg per kg body mass. Salivary samples were collected at 2 h and 14 h post-caffeine ingestion and were spectrophotometrically assayed for their caffeine concentrations by the Medical Research Laboratory of MetaMetrix Inc. (Norcross, Georgia, USA).

2.3. Statistical analysis

The data were analysed using R version 3.6.1 and RStudio version 1.2.1335 running on an x86_64-w64-mingw32/x64 (64-bit) platform.^{13,14} Group comparisons were carried out using the Welch *t*-test, which is more conservative than an independent samples *t*-test and which does not assume equal variances. Boxplots were created using ggplot2.¹⁵

3. Results

The two groups did not differ significantly in respect of age ($t = 1.89$, $df = 111.29$, NS). The mean salivary caffeine clearance in the male subjects was 1.51 (0.10) mL min⁻¹ kg⁻¹, while that in the female subjects was 1.60 (0.07) mL min⁻¹ kg⁻¹ ($t = 0.68$, $df = 131.68$, NS). Boxplots showing these results are shown in Fig. 2.

4. Discussion

This relatively large study has found no evidence that salivary caffeine clearance rates are lower in adult females compared with adult men, as reported in one previous study.¹¹ Indeed, the present study, in

Fig. 2. Boxplots showing the salivary caffeine clearance rates in the male and female subjects. F, female; M, male.

which the age and smoking status of the two groups were carefully matched, showed a non-significantly higher clearance rate in the adult female group compared with the male group. This is a reassuring finding for clinicians who wish to make use of the non-invasive salivary caffeine clearance assay to help assess hepatic function and to diagnose chronic liver disease in both men and women.

The finding that there does not appear to be a sex-related difference in the catabolism of caffeine has other important potential implications given that this purine alkaloid is widely ingested in humans, is a prescription drug and has a wide range of actions. We shall briefly consider each of these important aspects in turn.

First, as mentioned earlier, caffeine is the most widely ingested natural alkaloid in humans.¹ Beverages which are relatively rich in caffeine include coffee, tea, coca cola and energy drinks, while dark chocolate is also generally a rich source.^{16,17} Some students use caffeine pills for neuroenhancement.¹⁸ Athletes sometimes also use caffeine in an attempt to enhance sports performance; even low doses of less than 3 mg/kg body mass may be ergogenic.¹⁹

Second, caffeine is an ingredient in some prescription drugs. For example, in some countries a preparation combining ergotamine, cyclizine and caffeine may be prescribed for the treatment of acute migraine and for migraine variants which are unresponsive to analgesics.²⁰ Indeed, caffeine appears in the World Health Organisation's 2019 list of essential medicines, albeit for the treatment of neonates.

Third, caffeine intake is associated with a wide variety of effects, for example in relation to alertness, learning, memory, sleep regulation and locomotor activity.²¹ Its actions appear to be dose-dependent, with caffeine concentrations of 1–10 μ M being associated with antagonism of adenosine receptor signalling; higher concentrations of 0.5–10 mM are associated with inhibition of phosphodiesterases, thereby inhibiting the breakdown of the second messenger cAMP into AMP; while caffeine concentrations in the range of 0.5–30 mM appear to sensitise ryanodine receptors, which are present in the endoplasmic reticulum and sarcoplasmic reticulum of skeletal and cardiac muscle, by lowering the associated calcium ion channel-opening threshold.^{21,22,23,24}

5. Conclusion

Based on our findings, we agree with Kashuba and colleagues that there is no need to stratify by sex when considering the salivary caffeine clearance in adult humans.¹²

Declaration of competing interest

None to declare.

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