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PHARMACOGENETICS

Induction of CYP1A2 by heavy coffee consumption in Serbs and Swedes

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Abstract
Objectives To investigate the influence of coffee consumption on CYP1A2 enzyme activity controlling for the effects of smoking and oral contraceptive (OC) use among Serbs and Swedes and to compare CYP1A2 activity between the two populations.
Methods Data on oral contraceptive use, habitual coffee consumption and smoking habits were obtained from 100 Serbian and 149 Swedish healthy volunteers using a detailed questionnaire. CYP1A2 activity was estimated by plasma paraxanthine/caffeine (17X/137X) ratio analysed by reversed-phase HPLC after oral administration of 100 mg caffeine.
Results Daily consumption of at least three cups of coffee significantly increased CYP1A2 enzyme activity in both Serbs (P=0.0002) and Swedes (P<0.0001). Among non-smokers and non-OC users, heavy coffee consumption significantly increased CYP1A2 activity in Serbs (mean difference 0.11; 95% CI of the mean difference 0.04, 0.18; P=0.003) and Swedes (mean difference 0.07; 95% CI of the mean difference 0.01, 0.12; P=0.02). Significantly higher 17X/137X ratio was detected in Serbian smokers compared to non-smokers. There was no significant gender difference in CYP1A2 activity in Serbs. Controlling for the effect of smoking, heavy coffee consumption habit and oral contraceptive use, significantly lower 17X/137X ratio was observed in Serbs than in Swedes (P=0.0003).
Conclusions Habitual heavy coffee consumption increases CYP1A2 activity. Polycyclic aromatic hydrocarbons formed during roasting of coffee beans might partly be responsible for this effect. The reason for the observed lower CYP1A2 activity in Serbs as compared to Swedes remains to be investigated.

Keywords CYP1A2 · Caffeine · Phenotype · Coffee · Ethnicity

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Introduction

Cytochrome P450 1A2 enzyme (CYP1A2) is involved in the metabolism of various endogenous substrates, such as melatonin and estrogenes [1], and in the activation of procarcinogens, such as heterocyclic amines, arylamines and aflatoxin B_1 [2]. More than 20 clinically used drugs, including propranolol, theophylline, clozapine and olanzapine are metabolized by CYP1A2 [3]. Caffeine, a frequent ingredient of many beverages, foods and medications, is almost entirely metabolised by CYP1A2 [4]. Therefore, caffeine clearance is considered to be the standard probe for the assessment of CYP1A2 activity in vivo [5].

Considerable inter-individual variations in CYP1A2 activity due to genetic and/or environmental factors have been reported [6, 7]. To date, more than 30 different alleles of the *CYP1A2* gene have been described (http://www.cypalleles.ki.se), but only a few of them are associated with altered enzyme activity [6, 8]. CYP1A2 is an inducible enzyme whose activity is modified by various factors. It has

been confirmed that cigarette smoking [9], intake of charcoal-grilled meat [7], omeprazole [10] and carbamazepine [11] induce CYP1A2. On the other hand, fluvoxamine [12] and oral contraceptives (OCs) [13] inhibit CYP1A2 enzyme activity.

Under the assumption that caffeine might induce its own metabolism, association between consumption of caffeine-containing foods or beverages and CYP1A2 activity has been investigated, but conclusions are inconsistent [14, 15]. Inter-ethnic differences in CYP1A2 activity have been confirmed by lower enzyme activity in black compared to white populations [16, 17]. Recently, we reported the finding of significantly lower CYP1A2 activity in Koreans compared to Swedes that could not be explained by the genetic polymorphism being investigated [13]. However, intra-ethnic variations have not been well explored. Gender difference in CYP1A2 activity has been reported [16], but numerous attempts to confirm higher enzyme activity in men than in women yielded conflicting results [13, 15].

In the present study, we examined the effect of habitual heavy coffee consumption on CYP1A2 enzyme activity, controlling for the induction effect of cigarette smoking and inhibition by oral contraceptives among healthy Serbian and Swedish volunteers. Moreover, variation in enzyme activity between the two populations, as well as the influence of smoking habit and gender in Serbs, was also investigated. The results indicate that CYP1A2 activity increases with heavy coffee consumption in both Serbian and Swedish subjects.

Materials and methods

Study subjects One hundred unrelated healthy Serbian volunteers, 55 men and 45 women, with a median age of 27 years (range 18–46 years) and a median weight of 74 kg (range 43–120 kg) participated in the present study. Among them, 16 men and 12 women were smokers (smoking between two and forty cigarettes per day), and 16 men and 20 women were heavy coffee consumers (with regular daily intake of three or more cups of coffee). None of the subjects had chronic or acute use of any medications and none of the female participants was pregnant or breast-feeding or used oral contraceptives. The Swedish subjects participating in the present study also participated in a previous study that investigated the effect of genetic polymorphism and smoking habit on CYP1A2 enzyme activity [13].

After being acquainted with the study, study participants signed written informed consent and answered a detailed questionnaire about their habitual coffee consumption, smoking habit and oral contraceptive use. Subjects abstained from taking coffee, tea, chocolate and any other caffeine-containing food or beverage for at least 24 h prior to and throughout the study period. They received a 100 mg oral dose of caffeine (Koffein Recip; Recip AB, Årsta, Sweden) and 4.0 h later a 10-ml venous blood sample was collected into EDTA-containing Vacutainer tubes (Sarstedt, Nümbrecht, Germany), centrifuged and plasma separated. Recruitment of the subjects and collection of samples were conducted at the Medical Faculty, University of Kragujevac, and at the Clinical Centre in Kragujevac, Serbia. All samples were frozen at −80°C, packed on dry ice and sent to Karolinska University Hospital, Huddinge, Sweden, for analysis. The study was approved by the ethics committees at the Medical Faculty, University of Kragujevac, Serbia, and at Karolinska Institutet, Sweden.

Caffeine phenotyping The molar concentrations of caffeine (1,3,7-trimethylxanthine or 137X) and its metabolite paraxanthine (1,7-dimethylxanthine or 17X) in plasma samples were determined by reversed-phase HPLC with UV detection, according to Ghotbi at al. [13]. In vivo CYP1A2 enzyme activity was estimated by the 17X/137X ratio. At 16.3 μM for caffeine and 10.3 μM for paraxanthine, within-day and between-day coefficients of variations were less than 10 and 5% respectively.

Statistical analysis Statistical analyses were performed with Statistica, version 7.1 (StatSoft Inc, Tulsa, OK, USA). The 17X/137X ratio was log-transformed before statistical analyses. For all statistical procedures, $P<0.05$ was considered as significant. Consistency of log-transformed data with the normal distribution was assessed by Kolmogorov-Smirnov test. Student's *t*-test or Mann-Whitney tests for independent groups were used to determine the influence of different factors on 17X/137X ratio, as well as to compare Serbs with Swedes in terms of CYP1A2 activity. One-way ANOVA was used to test the effect of the number of cigarettes smoked per day on the level of CYP1A2 induction.

Results

Activity of CYP1A2 in Serbs was estimated by plasma 17X/137X ratio in 100 healthy volunteers after administration of 100 mg caffeine (137X) as a single oral dose. A seven-fold variation in the 17X/137X ratio was observed (range 0.16–1.24), and the log-transformed values showed a relatively normal distribution (Fig. 1). To examine the influence of heavy coffee consumption on enzyme activity among Swedes, we used the 17X/137X ratio for 149 healthy Swedish volunteers [13], after excluding oral contraceptive users. None of the Serbs were oral contraceptive users. To compare CYP1A2 activity between the Serbian and Swedish populations, smokers and heavy coffee consumers were excluded as well.

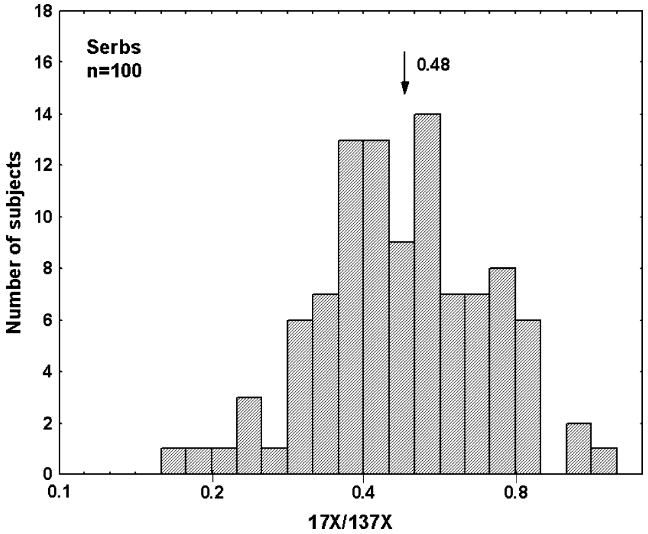

Fig. 1 Frequency distribution of log-transformed 17X/137X ratio in Serbs. The *arrow* indicates the median value

In the Serbian population, smokers had significantly higher enzyme activity compared to non-smokers ($P<0.0001$; 95% CI for the mean difference 0.15, 0.27). The difference remained significant controlling for the effect of heavy coffee consumption habit ($P<0.0001$; 95% CI for the mean difference 0.13, 0.33). The number of cigarettes smoked per day showed a significant effect on the level of CYP1A2 induction in all Serbs ($P<0.0001$) as well as in non-heavy coffee consumers ($P=0.0002$).

Comparison of CYP1A2 enzyme activity between heavy coffee consumers and non-heavy coffee consumers in all Serbs and Swedes regardless of smoking habit as well as for the groups of smokers and non-smokers is shown in Table 1. Significantly higher CYP1A2 enzyme activity was observed in heavy coffee consumers compared to non-heavy coffee consumers in all Serbs ($P=0.0002$; 95% CI for the mean difference 0.08, 0.21) and Swedes ($P<0.0001$; 95% CI for the mean difference 0.06, 0.16).

Since smoking induces enzyme activity, comparisons of 17X/137X ratio were made separately within the groups of smokers and non-smokers. Among non-smokers, significantly higher 17X/137X ratio was observed in heavy coffee consumers compared to non-heavy coffee consumers in both Serbs ($P=0.003$; 95% CI for the mean difference 0.04, 0.18) and Swedes ($P=0.02$; 95% CI for the mean difference 0.01, 0.12). Among Swedish smokers, CYP1A2 activity was significantly higher in heavy coffee consumers compared to non-heavy coffee consumers ($P<0.0001$; 95% CI for the mean difference 0.13, 0.33). However, no significant difference was observed among Serbian smokers ($n=28$, $\alpha=0.05$, 5.6% power).

There were no differences in 17X/137X ratio between men and women in the Serbian population. Comparing the groups of non-smokers who were non-heavy coffee consumers, Serbs had significantly lower 17X/137X ratio compared to Swedes ($P=0.0003$; 95% CI for the mean difference −0.14, −0.04).

Discussion

The main findings of the present study are that heavy coffee consumption increases CYP1A2 enzyme activity and Serbs display lower enzyme activity compared to Swedes. We investigated the influence of habitual heavy coffee consumption on CYP1A2 enzyme activity and examined intra-ethnic variation in enzyme activity controlling for the confounding factors that alter enzyme activity, namely cigarette smoking and oral contraceptive use. Possible competitive inhibition was avoided by abstaining from intake of any caffeine-containing food, beverage and medication for at least 24 h before and during the study. Comparison of the enzyme activity between Serbs and Swedes was done using similar protocols with caffeine as a phenotyping probe.

The result demonstrated that daily coffee consumption of at least three cups of coffee increased CYP1A2 enzyme activity in all Serbs and Swedes. It is well documented that cigarette smoking increases and use of oral contraceptive decreases CYP1A2 activity [5, 11, 13]. We confirmed in the Serbian population previous findings of dose-dependent

Table 1 Comparisons of mean 17X/137X ratios between non-heavy and heavy coffee consumers in Serbs and Swedes based on smoking habit using *t*-test for two independent groups

		Non-heavy coffee consumers		Heavy coffee consumers		*P* (95% CI of the mean difference)
		n	Mean ± SD	*n*	Mean ± SD	
Serbs ($n=100$)	All	64	0.42±0.17	36	0.59±0.13	0.0002 (0.08, 0.21)
	Non-smokers	54	0.39±0.14	18	0.50±0.11	0.003 (0.04, 0.18)
	Smokers	10	0.66±0.17	18	0.68±0.11	0.77 (−0.09, 0.12)
Swedes ($n=149$)	All	91	0.48±0.14	58	0.62±0.17	<0.0001 (0.06, 0.16)
	Non-smokers	72	0.48±0.13	42	0.55±0.16	0.02 (0.01, 0.12)
	Smokers	19	0.50±0.16	16	0.85±0.14	<0.0001 (0.13, 0.33)

induction of CYP1A2 by cigarette smoking [15]. If there is an effect of coffee consumption on enzyme activity, it would be observed in the group of non-smokers who are non-OC users. OC users were not included in this study and we investigated the influence of coffee consumption and gender differences on CYP1A2 enzyme activity controlling for the effect of cigarette smoking. Among non-smokers, heavy coffee consumers displayed higher enzyme activity compared to non-heavy coffee consumers in both Serbs and Swedes. Similar results were obtained among Swedish smokers, but we were not able to show the same effect in Serbian smokers due to the low number of subjects in this group.

It is known that some substrates of inducible enzymes can act as inducers [18], and animal studies have indicated that caffeine induces its own metabolism [19]. This suggested inducing effect of caffeine was investigated in humans as well, by considering regular consumption of caffeine-containing food or beverages in connection with CYP1A2 activity [14, 15, 20–22], but results were inconsistent. Kalow et al. [14], using urinary (AAMU+1X+1U)/17U ratio as an index of CYP1A2 activity, did not find any association with habitual coffee consumption. However, other studies described, but did not fully explain, an inducing effect of daily consumption of caffeine-containing food or beverages on CYP1A2 activity, using caffeine breath test [20], urinary (17X+17U)/137X ratio [21, 22] or saliva 17X/137X ratio as an index [15].

Induction of the CYP1A subfamily is mediated by the aryl hydrocarbon (Ah) receptor to which the inducer binds [18, 19]. Polycyclic aromatic hydrocarbons (PAHs), present in cigarette smoke and charcoal-grilled meat, enhance transcription of the *CYP1A2* gene by the same mechanism [18]. However, caffeine has very low affinity for the Ah receptor [19]. Tantcheva-Poor et al. [15] reported CYP1A2 induction to be more dependent on regular coffee consumption alone than on a combined estimate of the total daily caffeine intake, suggesting that an inducing effect might be due to other constituents present in coffee and not to caffeine itself.

In the present study, we have shown significantly higher CYP1A2 enzyme activity in heavy coffee consumers in two separate populations, using plasma 17X/137X ratio. Different species of coffee and different brewing methods are used in Serbia and Sweden, and the possibility of different caffeine levels in the coffee consumed by Serbs and Swedes can not be excluded [23]. On the other hand, all types of coffee beans have to be roasted before preparation and the temperature during roasting reaches approximately 220°C [24]. Incomplete combustion processes of organic material at high temperatures produce PAHs [25], and the concentration of PAHs from roasted coffee beans could reach up to 96 ng of PAHs per liter of coffee brew samples [26]. Apparently, there is evidence that regular coffee consumption does induce CYP1A2 activity, and we presume polycyclic aromatic hydrocarbons to be at least partly responsible for this effect, as already suggested [15].

Previous investigations of gender affecting CYP1A2 activity yielded contradictory results. Some investigators reported no difference in enzyme activity between men and women [6, 13, 22], while others, without taking into account possible confounding factors, described lower CYP1A2 indices in female populations [5, 14, 16]. Tantcheva-Poor et al. [15], considering the influence of known inducers/inhibitors, reported a small effect of gender on CYP1A2 activity. However, in our study, after excluding oral contraceptive use, cigarette smoking and coffee consumption as confounders, no significant difference in CYP1A2 activity between men and women was shown in the Serbian population.

Ethnicity is an important demographic variable that contributes to interindividual variability in drug disposition [27], and previous studies have confirmed its influence on CYP1A2 when comparing black with white populations [16, 17], or Koreans with Swedes [13]. CYP1A2 enzyme activity displayed normal distribution in Serbs, as observed in other populations [13]. Our study showed that between Serbs and Swedes a significant difference in CYP1A2 activity also exists, although both populations are of Caucasian origin. Within the white population, a higher CYP1A2 activity has been reported in Germany than in Bulgaria and Slovakia [15], but comparison was based on country of residence and not on ethnicity of participants. The effects of ethnicity on drug metabolism can be determined by both genetic and environmental factors, such as nutrition or lifestyle; alteration of CYP1A2 activity has been already associated with several *CYP1A2* alleles [6, 8], as well as with some other determinants [7]. Bearing this in mind, additional investigations are needed to explain the difference discovered.

In conclusion, our findings reveal heavy coffee consumption as an inducer of CYP1A2; we believe that polycyclic aromatic hydrocarbons, present in roasted coffee beans, are at least partly responsible for this effect. Cigarette smoking is confirmed to have significant influence on CYP1A2 activity. There was no significant difference in CYP1A2 activity between men and women. Serbs were shown to have significantly lower CYP1A2 activity than Swedes, which remains to be further explored.

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Conflict of interest statement Authors have identified no conflict of interest in relation to this manuscript.

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