BIOMARKERS, 1998, VOL. 3, NO. 4/5, 367–377

Effects of lifestyle and genetic polymorphisms on consumption of coffee or black tea and urinary caffeine levels

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Received 30 July 1997, revised form accepted 20 January 1998

To find the cause of individual differences in caffeine intake and its metabolism, we investigated the effects of lifestyle and genetic polymorphisms of caffeine metabolic enzymes on coffee or black tea and urinary caffeine levels among 259 male Japanese. It was seen that cigarette smokers drank more coffee or black tea than non-smokers ($p < 0{\cdot}001$). There was an inverse correlation between the amount of coffee or black tea consumed and age or the frequency of alcohol drinking ($p < 0{\cdot}05$). Genetic polymorphisms of *N*-acetyltransferase 2 (NAT2), cytochrome P450 (CYP)1A1 and 2E1 did not significantly affect the habit of drinking coffee or black tea. The frequency of allele 1, the NAT2 allele of rapid acetylators, increased according to coffee or black tea consumption ($0{\cdot}05 < p < 0{\cdot}1$). Among lifestyle factors, two factors, i.e. smoking and the amount of coffee or black tea consumed, were related to urinary caffeine levels ($p < 0{\cdot}05$). Geometric means of urinary caffeine levels were higher in the group who consumed higher amounts of coffee or black tea ($p < 0{\cdot}05$) and those of smokers were lower than non-smokers – approximately 70% of non-smokers ($p < 0{\cdot}05$). The genetic polymorphisms of NAT2, CYP1A1 and CYP2E1 were not significantly associated with the urinary caffeine levels according to each consumption level of coffee or black tea. This study suggests that smoking should be considered for the proper appreciation of individual differences in caffeine intake and urinary caffeine levels.

Keywords: caffeine, smoking, coffee, black tea, genetic polymorphisms.

Introduction

Caffeine is unconsciously ingested through the consumption of coffee, black tea, green tea, cola, etc., in daily life. Among these sources of caffeine intake, higher contents of caffeine are known to be in coffee or black tea than in the others (Resources Council, Science and Technology Agency, Japan 1995). As for coffee, it may possibly be involved in human bladder cancer (IARC 1991) but whether or not consumption of coffee is related to cancer is still open to question.

The metabolism of caffeine has been extensively studied (figure 1). Within 24 h, approximately 50–60% of administered caffeine is excreted as its metabolites (Carrillo and Benitez 1994). On the other hand, 0·5–3% of administered caffeine is excreted in unmetabolized form, i.e. as caffeine itself (Birkett and Miners 1991). Caffeine is mainly metabolized by polycyclic aromatic hydrocarbons (PAHs)-inducible cytochrome P450s (CYPs) (Berthou *et al.* 1991). PAHs in cigarettes induce CYP1A1, CYP1A2, etc., which are involved in caffeine demethylation, thus the enzyme induction by cigarette smoking is considered to accelerate caffeine

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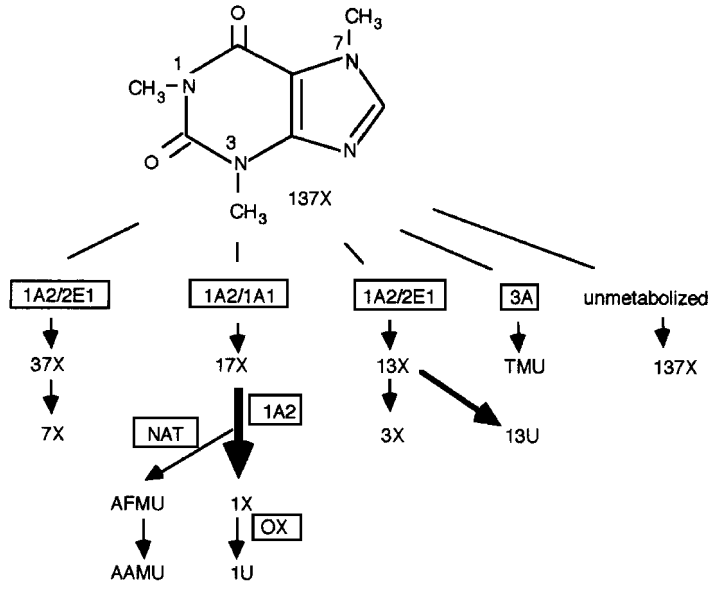

Figure 1. The simplified metabolic pathway of caffeine: 137X, caffeine; 37X, theobromine; 17X, *para*xanthine; 13X, theophylline; TMU, 1,3,7-trimethyluric acid; 7X, 7-methylxanthine; 13U, 1,3-dimethyluric acid; AFMU, 5-acethylamino-6-formylamino-3-methyl-uracil; AAMU, 5-acethylamino-6-amino-3-methyluracil; 1U, 1-methyluric acid; OX, xanthine oxidase; 1A2, cytochrome P4501A2; 2E1, cytochrome P4502E1; 1A1, cytochrome P4501A1; 3A, cytochrome P4503A; NAT, N-acetyltransferase (Birkett and Miners 1991, Tassaneeyakul *et al.* 1992, Carrillo and Benitez 1994).

metabolism (Vistisen *et al.* 1992). Similarly to smoking, other lifestyle factors may affect caffeine intake and metabolism. In the present study, we investigated the effects of lifestyle factors on the consumption of coffee or black tea and urinary caffeine levels.

Among caffeine metabolic enzymes, CYP1A1, CYP2E1 and *N*-acetyltransferase2 (NAT2) are genetically polymorphic and it is supposed that their genetic polymorphisms are associated with their enzyme activities. In the case of CYP1A1, the point mutation from adenine (A) to thymine (T) in exon 7 of CYP1A1 leads to the substitution of isoleucine (Ile) for valine (Val). This mutation was reported to increase arylhydrocarbon hydroxylase activity and susceptibility to lung cancer (Kawajiri *et al.* 1993). However, there was a report that there were no differences in the two CYP1A1 variants in an *in vitro* kinetic study (Persson *et al.* 1997). *RsaI* genetic polymorphism of CYP2E1 was due to the transition of C (cytosine) to (thymine) on the 5′ flanking region of CYP2E1. Watanabe *et al.* (1994) reported that the expression of CYP2E1 mRNA in the *c1/c2* or *c2/c2* type of CYP2E1 was higher than that in the *c1/c1* type by RT-PCR using human peripheral lymphocyte. NAT2, which has been called polymorphic NAT, can be classified into three phenotypes – rapid, intermediate and slow acetylators – by combinations of NAT2 genotypes (Deguchi 1992). However, there are only a few papers on the relationship between genetic polymorphisms and caffeine intake or urinary caffeine levels (Tsukada *et al.* 1996). In this study, the effects of genetic polymorphisms of CYP1A1, CYP2E1 and NAT2 on the consumption of coffee or black tea and urinary caffeine levels were also investigated.

Subjects and methods

Subjects and questionnaire

The study subjects were 259 male workers (age range: 20–71years) who lived in the Kitakyushu region in southwest Japan. Every subject completed a questionnaire concerning consumption of tobacco, coffee, black tea , alcohol , etc.

Analysis of urinary caffeine and creatinine

Morning preprandial urine specimens were obtained from all subjects. Urinary caffeine was analysed by the ion pair reversed high performance liquid chromatography (HPLC) method, as described by Takeda *et al.* (1993), with a minor modification, i.e. 0·3ml of 3 N NaOH was added to 1 ml of urine sample. The mixture was extracted twice with 3 ml of CH2Cl2. After centrifugation (1500 rpm, 5 min), 1·5 ml of the CH_2Cl_2 layer was transferred to a 1·5 ml bottle and evaporated by nitrogen gas. The residues were dissolved by 1 ml of distilled water. Using the autosampler, 20 µl of the solution was injected into the HPLC. The conditions of HPLC (Hitachi-HPLC: L-4200 UV-VIS Detector, L-6210 Intelligent Pump, 6554-52 Column Oven, 655A-40 Autosampler, D-2500 Chromato-Integrator) were as follows : mobile phase, 20 mM of KH_2PO_4 (pH 4·7) in water containing 3 mM of sodium 1-decanesulphonate/CH3CN (85/15); temperature = 50 ºC, flow rate = 1·0 ml min^{-1}, column = TSK-GEL ODS-80^{TM}, detection = UV absorbance at 254 nm.

Urinary creatinine was also analysed by the ion pair reversed HPLC method of Ogata and Taguchi (1986).

Determination of genotypes

Genomic DNA was isolated from the buffy coat fraction of peripheral blood (7 ml) samples of all subjects by a DNA extractor (Applied Biosystems Inc., Model 340A, Japan) after complete digestion with 100 µg ml^{-1} of proteinaseK (Wako Pure, Japan). The genetic polymorphism of CYP1A1 was analysed by designed restriction fragment length polymorphism (Oyama *et al.* 1995). Genotypes of CYP1A1 were classified under three types, i.e. Ile/Ile (the predominant homozygote: alleles of isoleucine), Ile/Val (the heterozygote: alleles of isoleucine and valine) and Val/Val (the rare homozygote: alleles of valine), according to the naming by Kawajiri *et al.* (1993).

The *RsaI* genetic polymorphism of CYP2E1 was determined by the method of Kawamoto *et al.* (1995) and classified into the predominant homozygote alleles(*c1/c1*), the heterozygote alleles (*c1/c2*) and the rare homozygote alleles (*c2/c2*).

Genotypes of NAT2 were determined by the method of Abe *et al.* (1993). The alleles of NAT2, which were reported to be of four types – allele 1, 2, 3 and 4 – were determined by the combination of digestion patterns by *TaqI* and *BamHI*. As the frequency of allele 4, which has a point mutation at Asp718 site, is approx. 1% in the Japanese population (Deguchi 1992), we did not examine allele 4. NAT2 phenotype was predicted from the combination of these NAT2 genotypes (Deguchi 1992, Abe *et al.* 1993), i.e. rapid acetylator (the homozygous genotype of an allele 1), intermediate acetylator (the heterozygous genotype of an alleles 1 and 2 or 3) and slow acetylator (the other genotypes).

Chemicals

Caffeine, Taq polymerase, 4dNTP, HincII, RsaI, TaqI, BamHI and other chemicals were purchased from Wako Pure Chem. Ind. Ltd (Osaka, Japan). Sodium 1-decanesulphonate was obtained from Aldrich Chem Co. (Milw, USA). All reagents were of analytical grade.

Statistical analysis

Lifestyle factors and genetic polymorphisms were quantified as follows: consumption of coffee or black tea (rare = 0; 1 cup per day = 1; 2 cups per day = 2; 3 cups per day or more = 3), frequency of alcohol drinking (rare = 0; 1–2 times per week = 1; 3–4 times per week = 2; 5 times per week or more = 3), smoking (non-smoker = 0; smoker = 1), the genotypes of NAT2 (slow acetylators = 1; intermediate acetylators = 2; rapid acetylators = 3), the genotypes of CYP1A1 (Ile/Ile = 1; Ile/Val = 2; Val/Val = 3) and the genotypes of CYP2E1 (*c1/c1* = 1; *c1/c2* = 2; *c2/c2* = 3). The correlations between coffee or black tea consumption and lifestyle or the genetic polymorphisms were studied by one-way analysis of variance (ANOVA) Mann-Whitney U test and Spearman Rank Correlation. The two-way ANOVA was used to compare urinary caffeine concentrations after classification of coffee or black tea consumption and smoking or genotypes. The statistical analyses were performed using the JMP^{R} program package (version 3, SAS Institute, USA). The criterion for significance was $p < 0{\cdot}05$.

Table 1. Effects of lifestyle on coffee or black tea consumption (*n*(%)).

Coffee/black tea Cups/day	Age Mean ± SD (Years)	Smoking Non-smokers	Smoking Smokers	Frequency of alcohol consumption Rare	1–2	3–4	≧5 times/week	Total
Rare	45±11	23 (63·9%)	13 (36·1)	4 (11·1%)	6 (16·7)	6 (16·7)	20 (55·5)	36 (100·0%)
1	33±13	35 (59·3)	24 (40·7)	8 (13·6)	20 (33·9)	12 (20·3)	19 (32·2)	59 (100·0)
2	33±11	53 (55·2)	43 (44·8)	35 (36·5)	18 (18·8)	20 (20·8)	23 (23·9)	96 (100·0)
≧3	32±11	27 (39·7)	41 (60·3)	25 (36·8)	17 (25·0)	10 (14·7)	16 (23·5)	68 (100·0)
Total	34±11	138 (53·2)	121 (46·7)	72 (27·8)	61 (23·6)	48 (18·5)	78 (30·1)	259 (100·0)
Statistical analysis	$p < 0{\cdot}001$[a]	$p < 000{\cdot}1$[b]		$p < 0{\cdot}05$[c]				

n, number of subjects; SD, standard deviation. [a]Comparison between age and amounts of coffee or black tea consumed (by one way ANOVA). [b]Comparison of coffee or black tea consumption between smokers and nonsmokers (by Mann-Whitney U test). [c]Comparison of coffee or black tea consumption between alcohol consumption frequencies (by Spearman Rank Correlation).

Results

Effects of lifestyle on coffee or black tea consumption

Table 1 shows the average age and the lifestyle of subject groups divided by coffee or black tea consumption. Approximately 14% of the subjects rarely consumed coffee or black tea; 23%, 1 cup per day; 37%, 2 cups; 26%, 3 cups or more. Age and the frequency of alcohol drinking were inversely correlated to the amount of coffee or black tea consumed ($p < 0{\cdot}001$ and $p < 0{\cdot}01$, respectively). Smokers drank more coffee or black tea than non-smokers ($p < 0{\cdot}001$).

Effects of genetic polymorphisms on coffee or black tea consumption

The frequency of each genotype of CYP1A1, CYP2E1 and NAT2 was studied in the subjects divided according to coffee or black tea consumption (table 2). After being quantified as described in 'Statistical analysis', the relationship between coffee or black tea consumption and the genetic polymorphisms was studied. Although there was a trend of changes in coffee or black tea consumption according to NAT2 allele frequency ($0{\cdot}05 < p < 0{\cdot}1$), no significant correlations were observed between genotypes or allele frequency and amounts of coffee or black tea consumed.

Distribution of urinary caffeine

Urinary caffeine was detected in specimens from 97% of the subjects. The range of urinary caffeine levels was 0·10–40·12 mg l^{-1} (median, 0·83 mg l^{-1}). As the distribution of urinary caffeine levels was almost logarithmically normal regardless of creatinine modification, the concentrations of urinary caffeine were calculated after geometric transformation.

Effects of lifestyle on urinary caffeine levels

Table 3 shows the correlation among lifestyle and urinary caffeine levels. The amount of coffee or black tea consumed was significantly associated with urinary caffeine levels, regardless of creatinine modification. Thus, u[illegible]

Table 2. Effects of genetic polymorphisms on coffee or black tea consumption (*n*(%)).

Coffee or black tea (cups/day)	NAT2					CYP1A1					CYP2E1					
	Genotypes[a]			Gene frequency		Genotypes			Gene frequency		Genotypes			Gene frequency		
	Slow	Interme	Rapid	Allele1	Others	Ile/Ile	Ile/Val	Val/Val	Ile	Val	*c1*/*c1*	*c1*/*c2*	*c2*/*c2*	*c1*	*c2*	Total
Rare	2 (5·6%)	17 (47·2)	17 (47·2)	0·71	0·29	23 (63·9%)	9 (25·0)	4 (11·1)	0·76	0·24	20 (55·6%)	16 (44·4)	0 (0·0)	0·79	0·21	36 (100·0%)
1	12 (20·3)	20 (33·9)	27 (45·8)	0·63	0·37	35 (59·3)	18 (30·5)	6 (10·2)	0·75	0·25	35 (59·3)	23 (38·9)	1 (1·7)	0·78	0·22	59 (100·0)
2	13 (13·5)	36 (37·5)	47 (48·9)	0·64	0·36	61 (63·5)	29 (30·2)	6 (6·3)	0·79	0·21	59 (61·5)	32 (33·3)	5 (5·2)	0·81	0·19	96 (100·0)
≧3	6 (8·8)	21 (30·9)	41 (60·3)	0·76	0·24	46 (67·7)	19 (27·9)	3 (4·4)	0·82	0·18	43 (63·2)	24 (35·3)	1 (1·5)	0·79	0·21	68 (100·0)
Total	33 (12·7)	94 (36·3)	132 (50·9)	0·68	0·32	165 (63·7)	75 (28·9)	19 (7·3)	0·78	0·22	157(60·6)	95 (36·7)	7 (2·7)	0·78	0·22	259 (100·0)
Statistical analysis[b]	$p = 0·18$[c]			$p = 0·08$[d]			$p = 0·48$[e]		$p = 0·38$[f]			$p = 0·88$[g]		$p = 0·93$[h]		

[a]Slow, slow acetylators ; Interme, intermediate acetylators ; Rapid, rapid acetylators. [b]Comparison of coffee or black tea consumption between genotypes (by Mann-Whitney U test) or gene (allele) frequencies (by Spearman Rank Correlation). [c]Between slow or intermediate acetylators and rapid acetylators ($p = 0·35$). [d]Between frequencies of allele1 and others ($p = 0·08$). [e]Between Ile/Ile and Ile/ Val or Val/Val type ($p = 0·81$). [f]Between frequencies of allele Ile and Val ($p = 0·57$). [g]Between *c1*/*c1* and *c1*/*c2* or *c2*/*c2* type ($p = 0·88$). [h]Between frequencies of allele *c1* and *c2* ($p = 0·93$).

Table 3. Matrix of correlation coefficients among lifestyle and log of urinary caffeine levels.

	Age	Frequency of alcohol	Smoking	Coffee/black tea	Urinary caffeine (mg/l)	Urinary caffeine (mg/g creatinine)
Age	1·000	*0·101*	*–0·106*	*–0·143**	*–0·046*	*0·055*
Frequency of alcohol	0·107	1·000	*0·134**	*–0·131**	*0·017*	*0·058*
Smoking	–0·109	0·099	1·000	0·235***	*–0·201***	*–0·179***
Coffee/black tea	–0·257***	–0·132*	0·250***	1·000	*0·154**	*0·143**
Urinary caffeine (mg/l)	–0·051	–0·037	–0·166**	0·134*	1·000	0·822***
Urinary caffeine (mg/g creatinine)	0·047	0·034	–0·149*	0·129*	0·915***	1·000

Plain, simple correlations. Italic, partial correlations. *$p < 0·05$, **$p < 0·01$ and ***$p < 0·001$, by Pearson product-moment correlations.

reflected caffeine intake from coffee or black tea. In addition, smoking was inversely related to urinary caffeine levels.

Considering the consumption of coffee or black tea, which was found to be related to urinary caffeine levels, we studied in detail the distribution of urinary caffeine levels due to smoking (table 4). Geometric means of urinary caffeine levels were significantly higher in the group who consumed higher amounts of coffee or black tea and those of smokers were lower than non-smokers – approximately 70% of non-smokers – in the overall consumption levels of coffee or black tea. When urinary caffeine was corrected with creatinine, these trends became weak.

Effects of genetic polymorphisms on urinary caffeine levels

As cigarette smoking and coffee or black tea consumption were related to the urinary caffeine levels, the effects of genetic polymorphisms on urinary caffeine levels were studied after controlling the amounts of coffee or black tea consumed with separating non-smokers and smokers.

Compared with the rapid acetylators, the slow acetylators were very rare, thus, they were combined with intermediate acetylators. It was seen that the geometric means of urinary caffeine levels among rapid acetylators were higher than those among slow or intermediate acetylators in almost all classified consumption levels of coffee or black tea, regardless of smoking habit (figure 2). However, this trend was not significant (non-smokers, $p = 0·22$; smokers, $p = 0·57$).

As the Val/Val type of CYP1A1 was very rare, compared with the Ile/Ile type, the Val/Val type was combined with the Ile/Val type against the Il/Ile type. As a result (figure 3), urinary caffeine levels in the Ile/Ile type were higher than those in the Ile/Val or Val/Val type among non-smokers at all classified consumption levels of coffee or black tea ($p = 0·10$), while this trend was not shown among smokers ($p = 0·78$).

In the case of CYP2E1, the *c2/c2* type was very rare, compared with the *c1/c1* type of CYP2E1. Thus, we separated the types into the *c1/c1* type and the *c1/c2* or *c2/c2* type. There were no significant differences in urinary caffeine levels between the *c1/c1* type and the *c1/c2* or *c2/c2* type (non-smokers, $p = 0·34$; smokers, $p = 0·32$). As a conclusion, genetic polymorphisms of NAT2, CYP1A1 and CYP2E1 were not significantly associated with the urinary caffeine levels according to each consumption level of coffee or black tea.

Table 4. Changes in urinary caffeine concentrations by smoking and coffee or black tea consumption.

Consumption of coffee or black tea (cups/day)	Uncorrected urinary caffeine (mg/ l)								Creatinine-corrected urinary caffeine (mg/g creatinine)					
	Non-smoker			Smoker			Total		Non-smoker		Smoker		Total	
	N	G.M.	G.S.D.	N	G.M.	G.S.D.	G.M.	G.S.D.	G.M.	G.S.D.	G.M.	G.S.D.	G.M.	G.S.D.
Rare	23	0·515	2·156	13	0·173	1·977	0·361	2·741	0·390	2·223	0·282	1·762	0·346	2·309
1	35	1·128	1·561	24	0·492	1·904	0·824	2·670	0·599	2·074	0·423	1·959	0·536	3·589
2	53	0·979	2·284	43	0·745	2·362	0·831	2·589	0·641	2·488	0·472	2·344	0·565	2·951
≧3	27	1·258	2·931	41	1·002	2·491	1·012	2·581	0·896	3·122	0·708	3·837	0·764	3·019
Total	138	0·862	3·311	121	0·626	3·981	0·734	3·819	0·629	4·036	0·465	3·754	0·558	3·899
Statistical analysis[a]			$p < 0·001$[b]				$p < 0·001$[c]			$p < 0·05$[b]			$p < 0·05$[c]	

N, number of subjects; G.M., geometric mean of urinary caffeine concentrations; G.S.D., geometric standard deviation. [a]By two way ANOVA against smoking and coffee or black tea consumption. [b]Comparison of urinary caffeine levels between non-smokers and smokers after controling coffee or black tea consumption. [c]Comparison of urinary caffeine levels between amounts of coffee or black tea consumed after controling smoking

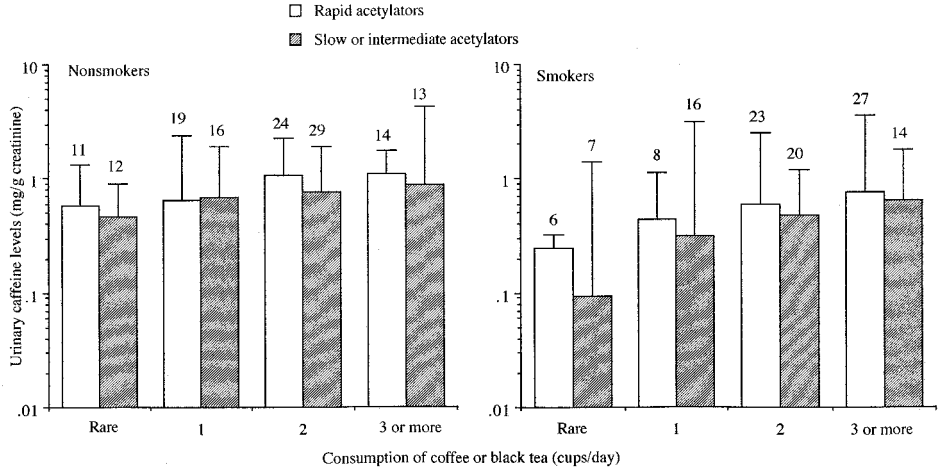

Figure 2. Effects of NAT2 genetic polymorphisms on urinary caffeine levels by coffee or black tea consumption. Data show GM and GSD of urinary caffeine levels: arabic figures, number in each group.

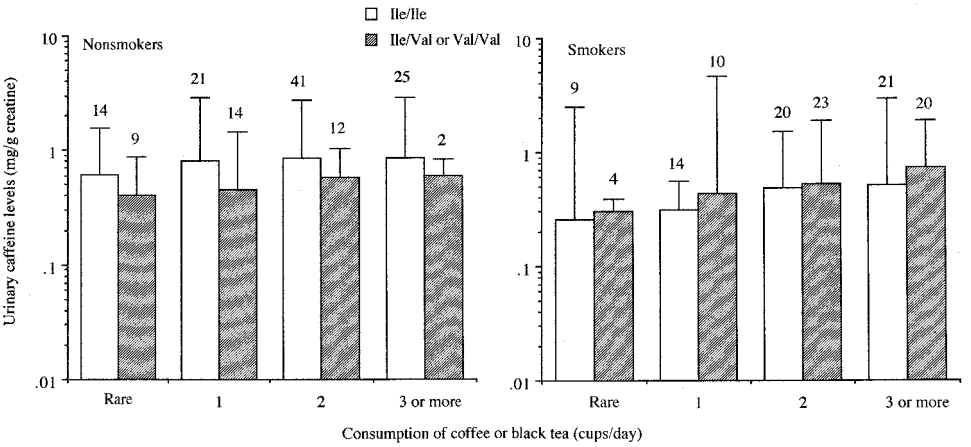

Figure 3. Effects of CYP1A1 genetic polymorphisms on urinary caffeine levels by coffee or black tea consumption. Data show GM and GSD of urinary caffeine levels: arabic figures, number in each group.

Discussion

We found that smokers drank more coffee or black tea than non-smokers and showed lower urinary caffeine levels than non-smokers. The increased consumption of coffee or black tea among smokers may be related to the accelerated metabolism of caffeine by smoking. Among the lifestyle factors, smoking was positively related to age and alcohol drinking, however coffee or black tea consumption was inversely related to age and alcohol drinking (table 3). In addition, age and alcohol drinking were not associated with urinary caffeine levels, so the reason for their effects on coffee or black tea consumption may not be due to merely caffeine metabolism. Therefore, the effects of lifestyle on caffeine intake will be due to other factors, e.g. social, cultural or behavioural differences, as well as caffeine metabolism.

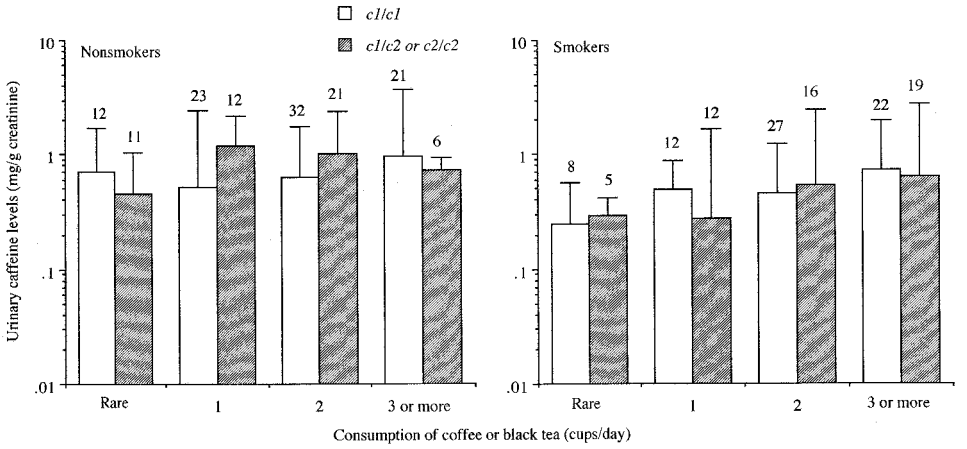

Figure 4. Effects of CYP2E1 genetic polymorphisms on urinary caffeine levels by coffee or black tea consumption. Data show GM and GSD of urinary caffeine levels: arabic figures, number in each group.

Genetic polymorphisms of NAT2, CYP1A1 and CYP2E1 were determined in the study subjects. The observed genotype distribution of each gene followed Hardy–Weinberg's law. The frequency of each allele of NAT2, CYP1A1 and CYP2E1 is in accordance with that of the previous studies dealing with the normal Japanese population (Shibuta *et al.* 1994, Watanabe *et al.* 1994, Oyama *et al.* 1995). Thus, the present subjects are considered to reflect the normal Japanese population.

NAT2 is involved in metabolism of 17X to AFMU (figure 1). Slow acetylators excrete lower AFMU levels than rapid acetylators (Kalow 1985). The effects of NAT2 genetic polymorphism on coffee or black tea consumption are not clear. Vineis (1993) suggested that, for some reason, e.g. the neurological effect of caffeine metabolites, slow acetylators tended to drink more coffee than rapid acetylators. In contrast, Tsukada *et al.* (1996) reported that among 30 Japanese subjects rapid acetylators preferred to drink coffee and took more caffeine than slow acetylators. Classifying consumption levels of coffee or black tea into 'under three cups per day' and 'three cups or more per day' , we found the consumers of 'three cups or more of coffee or black tea per day' made up 31 % (41 subjects) of rapid acetylators (132 subjects), while 21 % (27 subjects) were slow or intermediate acetylators (127 subjects). Though it is not significant (p = 0·07), there is a tendency that rapid acetylators drink more coffee or black tea. In addition, the frequency of allele 1, the allele of rapid acetylators, increased according to coffee or black tea consumption (table 2). On this point, the result of the present study supports not the previous suggestion (Vineis 1993) but the report of Tsukada *et al.* (1995). If the higher coffee consumption of rapid acetylators was associated with the accelerated caffeine metabolism among rapid acetylators, urinary caffeine levels among rapid acetylators would be lower than those among slow or intermediate acetylators. However, in the present study, geometric means of urinary caffeine levels among rapid acetylators were not lower than those among slow or intermediate acetylators (figure 2). NAT2 genetic polymorphism did not have an influence on urinary caffeine levels in this study. NAT is mainly involved in the latter stage of caffeine metabolism (figure 1). Thus, the fact that NAT does not

directly work on caffeine may be associated with the non-significant correlation between NAT2 genetic polymorphism and urinary caffeine levels. Thus, it is rash to conclude a relationship between NAT2 genetic polymorphism and coffee or black tea consumption.

CYP1A1 is virtually absent unless induced by inducers, e.g. smoking, air pollutant, black tea, etc. (Gonzalez and Gelboin 1992). Thus, non-smokers' CYP1A1 induction is dependent not on smoking but on the other inducers, e.g. coffee or tea. CYP1A1 activity was reported to be lower in the Ile/Ile type than the Ile/Val or Val/Val type (Kawajiri *et al.* 1993). As CYP1A1 is involved in caffeine metabolism (Tassaneeyakul *et al.* 1992), the unmetabolized form of caffeine, i.e. urinary caffeine, will be higher in the Ile/Ile type than the Ile/Val or Val/Val type. Among non-smokers in the present study, this bias was found in each consumption level of coffee or black tea (figure 3). To confirm the above correlation, an enlarged scale of studies is necessary in the near future. In addition, Persson *et al.* (1997) reported that there were no differences in substrate affinity or V_{max} for the two CYP1A1 variants. Thus, the effects of CYP1A1 genetic polymorphism on its enzyme activity should also be studied further.

CYP2E1 is involved in caffeine metabolism and its *RsaI* genetic polymorphism is considered to affect CYP2E1 mRNA expression (Watanabe *et al.* 1994). We studied the relationship between the CYP2E1 genetic polymorphism and coffee or black tea consumption or urinary caffeine levels. However, there were no significant correlations between them. As CYP2E1 contributed weakly to caffeine metabolism (Tassaneeyakul *et al.* 1994), CYP2E1 genetic polymorphism may not affect caffeine intake or urinary caffeine levels.

In conclusion, we studied the effects of lifestyle and genetic polymorphisms of NAT2, CYP1A1 and CYP2E1 on coffee or black tea consumption and urinary caffeine levels among 259 healthy Japanese male subjects. From our results, smoking is considered to increase coffee or black tea consumption and to decrease urinary caffeine levels. The genetic polymorphisms did not affect the amount of coffee or black tea consumed or the urinary caffeine levels.

Acknowledgements

This study was partially supported by the Grant-in-Aid for Scientific Research (C) of the Ministry of Education, Science and Culture, Japan and a grant for post-graduate students in the University of Occupational and Environmental Health, Japan. We wish to thank Professors Y. Kodama and K. Arashidani and Drs N. Kunugita, F. Kayama, M.Yoshikawa and S. Matsuda of the University of Occupational and Environmental Health, Japan, for their kind advice.

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