Genetic determinants of blood pressure responses to caffeine drinking[1–3]

Giulia Renda, Marco Zimarino, Ivana Antonucci, Alfonso Tatasciore, Benedetta Ruggieri, Tonino Bucciarelli, Tina Prontera, Liborio Stuppia, and Raffaele De Caterina

ABSTRACT

Background: The widely observed between-subject variability in cardiovascular responses to coffee may have a genetic basis.

Objective: We evaluated acute blood pressure (BP) responses to caffeine and explored whether they are influenced by candidate gene variants affecting caffeine metabolism (for cytochrome P450 1A2), adenosine metabolism (for adenosine receptor and AMP deaminase), or catecholamine receptors.

Methods: We recruited 110 healthy male habitual moderate coffee drinkers who refrained from drinking coffee on the day preceding the study. Each subject underwent ambulatory BP monitoring at 6-min intervals for 2 h. Each participant was administered, in a double-blind design, 40 mL of either a decaffeinated coffee preparation plus 3 mg caffeine/kg (caf) or the corresponding vehicle (decaf). The protocol was repeated 24 h later with the alternative preparation. Blood samples were collected for genetic and plasma caffeine and catecholamine evaluations.

Results: Compared with decaf, caf was associated with a mean (± SD) significant increase in systolic BP of 4 ± 12 mm Hg and in diastolic BP of 3 ± 10 mm Hg ($P < 0.001$ for both). Plasma caffeine and adrenaline increased after caf, but not after decaf. Of 11 gene polymorphisms analyzed, a relation was observed between the *ADORA2A TT* variant and the change in SBP peak and between the *ADRA2B I* variant and the changes in both SBP mean and peak; mean peak change in SBP; these variants were associated with increased SBP responses to caf.

Conclusions: Variability in the acute BP response to coffee may be partly explained by genetic polymorphisms of the adenosine A2A receptors and α_2-adrenergic receptors. This trial is registered at clinicaltrials.gov as NCT01330680. *Am J Clin Nutr* 2012;95:241–8.

INTRODUCTION

The precise mechanisms underlying the actions of coffee on the cardiovascular system are incompletely understood. Many effects have been attributed to caffeine, although coffee is a mixture of hundreds of chemical substances, many of which, such as polyphenols, are pharmacologically active (1). The main mechanism of action of caffeine is to antagonize adenosine receptors (2); a secondary effect is the inhibition of phosphodiesterases (2), with the subsequent accumulation of cyclic AMP and an intensification of the effects of catecholamines (3). Such effects translate, in most people, in a psychoactive response (4) and in a complex cardiovascular response, mainly consisting of an increase in BP[4] (5).

It is also well known, however, that considerable variability exists in the cardiovascular and psychoactive responses to coffee drinking. In part, such variability is due to tolerance (6), but there is evidence that some may have a genetic basis (7). CYP1A2 accounts for ~95% of caffeine metabolism and has wide interindividual variability in activity (8). An A→C substitution at position 734 (*CYP1A2*1F*) decreases enzyme inducibility, which results in impaired caffeine metabolism (9) and has been associated with an increased risk of myocardial infarction (10). However, most pharmacologic effects of caffeine result from the antagonism of adenosine, mostly the A2A (ADORA2A), receptors (11). The 1976 C→T genetic variant of the ADORA2A gene has been shown to be related to susceptibility to anxiety (12) and sleep changes (7). Moreover, the 34 C→T genetic mutation of AMP deaminase, which catalyzes the deamination of AMP to inositol monophosphate and thus reduces enzyme activity (13), may increase adenosine availability for its receptors (14). Because circulating catecholamines may also be involved in cardiovascular responses to coffee (15), genetic

[1] From the Institute of Cardiology (GR, MZ, AT, BR, and RDC), the Department of Biomedical Sciences (TB), and the Department of Oral Sciences, Nano- and Biotechnologies (IA and LS)—Center of Excellence on Aging, "G. d'Annunzio" University, Chieti, Italy, and the "G. Monasterio" Foundation, Pisa, Italy (TP and RDC).

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[3] Address correspondence to R De Caterina, Institute of Cardiology—"G. d'Annunzio" University–Chieti, C/o Ospedale SS. Annunziata, Via dei Vestini, 66013 Chieti, Italy. E-mail: rdecater@unich.it.

[4] Abbreviations used: ADORA2A, A2A-adenosine receptor; ADRA1A, α_{1A}-adrenergic receptor; ADRA2B, α_{2B}-adrenergic receptor; ADRB1, β1-adrenergic receptor; ADRB2, β2-adrenergic receptor; ADRB3, β3-adrenergic receptor; BP, blood pressure; CYP1A2, cytochrome P450 1A2; DBP, diastolic blood pressure; ECG, electrocardiogram; HR, heart rate; *I/D*, insertion/deletion genotypes; LRT, likelihood ratio test; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SBP, systolic blood pressure; Δ, change.

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polymorphisms of adrenergic receptors may also contribute to the variability in cardiovascular responses to coffee. Polymorphism of ADRA1A and ADRA2B (16, 17) and of ADRB1, ADRB2, and ADRB3 (18–25) influence cardiovascular responses to catecholamines.

Against this background, we aimed at evaluating acute cardiovascular effects of coffee by exploring whether they are influenced by the genetic asset of caffeine or adenosine metabolism or catecholamine α- and β-receptors.

SUBJECTS AND METHODS

Subjects

We recruited 110 healthy men from among medical students of the "G. d'Annunzio University" of Chieti, Italy. The inclusion criteria were as follows: age 18–40 y, male sex (to avoid variation because of the female hormonal cycle), no known active ongoing disease (apparent good health), nonsmoking status (to avoid contributory effects of nicotine or other tobacco alkaloids to caffeine effects or tolerance), average coffee intake (1 to $\leq$3 cups/d of Italian espresso coffee; *see* caffeine content below).

The exclusion criteria were as follows: treatment with any drug (to avoid any interference with the autonomic nervous system or with the effects of caffeine; this included therapy with sympathomimetic drugs, α- or β-adrenergic receptor blockers, theophylline, and any antihypertensive therapy), hypertension, and a BMI (in kg/m^2) $>$30 (obesity) or $<$18.5.

The volunteers were asked to completely refrain from consuming coffee, tea, cola drinks, energy drinks, other caffeine-containing drinks, chocolate, and any other food containing any of these substances on the day preceding the study. A written list of such drinks was provided to the candidates at the time of screening. Candidates were also screened for the absence of cardiovascular disease with the following examinations performed by a cardiologist: medical history, physical examination, basal ECG, ECG stress test, and 2-dimensional color-Doppler echocardiography.

Written informed consent was obtained from each volunteer. The protocol was approved by the Ethics Committee at the G. d'Annunzio University of Chieti, Italy. We originally recruited 127 healthy volunteers, 17 of whom did not complete the requirements on the second day of the trial, which resulted in a total of 110 subjects.

Study design

This was a prospective, double-blind, randomized study with crossover design. To this purpose, investigators administering the drinks to study subjects were kept unaware of which coffee preparations they were administering, with other investigators being in charge of the preparations of study materials. In the absence of reliable data on which to construct a sample-size hypothesis, we recruited a number of subjects similar to those from a previous study that reported on the association of the A2A receptor gene polymorphisms with caffeine-induced anxiety (12).

The study was run in the early afternoon of 2 consecutive days. All subjects were required to remain fasting for $\geq$2 h after a light lunch.

Basal phase (before the administration of coffee)

Study participants were equipped with an automated BP monitoring device (Spacelabs 90207; 26), which was set to measure BP and HR at 6-min intervals. They rested for 15 to 30 min before the first BP measurement, after which three 2-min interval measurements of BP and HR were recorded at baseline and the mean was considered the basal value. A first venous blood sample (basal) was collected to determine blood counts and differentials, for genetic analyses, and for basal measurements of plasma caffeine and catecholamines.

Coffee administration

Each subject received a 40-mL dose of a decaffeinated preparation to which caffeine was added at a dose of 3 mg/kg (caf; for comparison: 1 cup espresso has ~80 mg caffeine); the control consisted of the decaffeinated vehicle preparation without added caffeine (decaf). The 2 preparations were administered in a double-blind fashion in random order on the basis of a randomization list.

Analyses of cardiovascular responses to coffee administration

BP and HR measurements were repeated at 6-min intervals for 2 h. Two additional blood samples were taken: at 30 min for caffeine measurements and at 2 h after coffee administration for measurements of caffeine and catecholamines. The overall session duration on each of the 2 d of the study was ~2 h. The same session protocol was repeated 24 h later in each volunteer with administration of the alternative coffee preparation. The study design is shown in **Figure 1**. The protocol also included cognitive task measures, performed between 30 min and 2 h after caf or decaf administration. The results of the association of genetic variants with cognitive task measures will be the subject of an independent report.

Laboratory tests

Blood counts and differential were measured in EDTA-anticoagulated blood at the Central Hematology Laboratory of the Chieti University Hospital. Plasma caffeine concentrations were assayed in 3 heparin-treated blood samples taken before and 30 min and 2 h after coffee consumption. Caffeine was measured by inverse-phase HPLC (27). Plasma catecholamines, including adrenaline and noradrenaline, were measured in 2 heparin-treated blood samples obtained before and 2 h after coffee administration by HPLC (28).

Genetic analyses were performed with the use of a single EDTA-treated whole-blood sample. The gene variants analyzed, their known biological effects, corresponding proteins and effectors, and the primers and annealing temperatures used are reported elsewhere (*see* Supplemental Table 1 under "Supplemental data" in the online issue). Genomic DNA was extracted by using the High Polymerase Chain Reaction Template Preparation kit (Roche). PCR analyses for the amplification of the specific DNA sequences investigated were performed in a final reaction volume of 25 μL with primers and annealing temperatures as reported elsewhere (*see* Supplemental Table 1 under "Supplemental data" in the online issue). PCR conditions were as follows: denaturation at 94°C for 1 min, annealing at 58–63°C for 1 min, and extension at 72°C for 1 min (30 cycles). For the

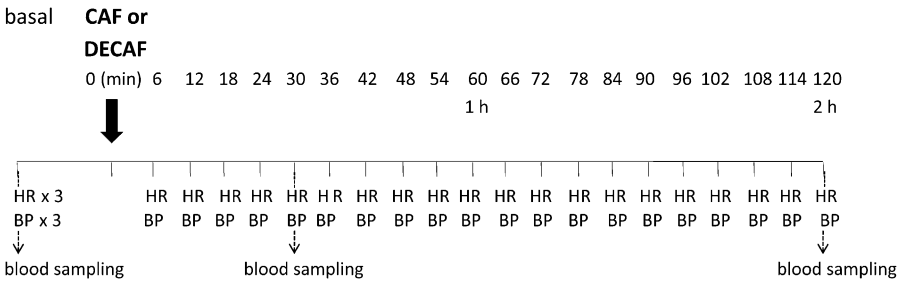

FIGURE 1. Illustration of the study design. CAF and DECAF were administered alternatively on the first or second day of the study. BP and HR were measured 3 times before (baseline) and then at 6-min intervals after coffee intake for 2 h. Blood samples were collected before (baseline) and 30 min and 2 h after coffee administration for genetic and plasma caffeine and catecholamine evaluations. BP, blood pressure; CAF, decaffeinated coffee preparation plus 3 mg caffeine/kg; DECAF, decaffeinated coffee without added caffeine. HR, heart rate.

study of the *ADRB1*, *ADRB2,* and *ADRB3* polymorphisms, gene amplification products were prepared and sequenced according to a method described previously (29). For the analysis of the *Arg347Cys* (*1475C→T*) variant in the A1A adrenergic receptor, a 502-bp fragment was genotyped by PCR-RFLP analysis of the PCR product, as previously described (30). The β_2-adrenergic receptor gene codon 164 polymorphisms were detected by using restriction enzyme digestion, as previously described (31). The CYP1A2(*−163A→C*), ADORA2A(*1976C→T*), and AMP deaminase 1 (*34C→T*) polymorphisms were detected by PCR-RFLP as previously described (32–34). The ADRA2B *I/D* genotypes were determined by separating PCR-amplified DNA fragments by 4% agarose electrophoresis (16). Identification of the *I* (long) and *D* (short) alleles was achieved by using a method described previously (16). All analyses were run blindly by investigators who were unaware of the treatment allocation of the study subjects.

Statistical analysis

The results are expressed as means ± SDs for normally distributed continuous variables, as medians and ranges for non-normally distributed continuous variables, and as counts and percentages for discrete variables. The normality of the distribution was tested by using the one-sample Kolmogorov-Smirnov test. For non-normally distributed variables, a mathematical transformation was performed as appropriate.

A paired 2-sample *t* test was used to test for differences among BP and HR after the intake of coffee with and without caffeine and among catecholamine concentrations before and 2 h after consumption of caf or decaf. The interaction between time and treatment was then investigated with ANOVA for repeated measures. To test for differences among caffeine concentrations before and 30 min and 2 h after caf or decaf, we used 1-factor ANOVA. For post hoc multiple comparisons of continuous variables between groups, we set the threshold for significance at a preassigned probability value of 0.05/3 (Bonferroni's correction; 0.016).

The effect of each genetic variable on the BP (either mean or peak) response to the intake of coffee with and without caffeine was analyzed first with univariable linear regression analysis considering BP variables as continuous. Next, we applied a conditional logistic regression model using BP variables as discrete; the null hypothesis of no association was tested with the LRT. For each genetic variant, a departure from a per allele pattern of inheritance was assessed by comparing, with the use of an LRT, the model that does not assume any model for the effect (each genotype is allowed to have its own effect) and the model assuming a per allele model (the effect of the heterozygotes is half way between the effect of the wild-type and the mutant homozygotes). Evidence of a departure from a per allele model is given by a *P* value for the LRT being statistically significant. The effect of each genetic factor was expressed as the OR and its 95% CI for homozygotes of a specific gene variant to have BP changes above the median. ORs were computed from the corresponding estimated regression coefficients in the model and their SEs. A *P* value <0.05 was considered significant, unless otherwise indicated. All analyses were performed with SPSS release 15.0 software package (SPSS Inc).

RESULTS

Characteristics of the study subjects are reported in **Table 1**. Medical history, physical examination, basal ECG, ECG stress test, and 2-dimensional color-Doppler echocardiography excluded the presence of cardiovascular disease in the entire population.

Response in cardiovascular variables after caffeinated or decaffeinated coffee consumption

Baseline HR (mean of 3 automated measurements in each subject) was similar before caf or decaf administration: 81 ± 12 and 80 ± 12 bpm, respectively (NS). No significant differences were observed after administration of caf compared with decaf throughout the study (**Figure 2**A).

Baseline SBP and DBP (means of 3 automated measurements in each subject) were similar before caf or decaf administration (SBP: 132 ± 12 compared with 131 ± 11 mm Hg, NS; DBP: 79 ± 10 compared with 78 ± 9 mm Hg; NS).

Both SBP and DBP were significantly higher only 18 min after caf intake compared with decaf intake, and this difference persisted until the end of the study (2 h) (Figure 2, B and C). Mean SBPs after intake of the caf and decaf drinks were 133 ± 10 and 129 ± 10 mm Hg, respectively ($P < 0.001$); corresponding DBPs were 81 ± 8 and 78 ± 8 mm Hg ($P < 0.001$). The mean difference between caf and decaf was 4 ± 12 mm Hg for SBP and 3 ± 10 mm Hg for DBP (**Figure 3**). The wide SD for these measurements, with most subjects experiencing an increase in BP (but some experiencing a decrease in BP) compared with the decaf control, confirmed a wide intersubject variability in the BP responses to coffee.

TABLE 1
Characteristics of the study subjects[1]

Characteristic	Value (n = 110)
Age (y)	26 ± 4[2]
Age (y)	25 (20–38)[3]
Sex (% male)	100
Weight (kg)	75 ± 10
BMI (kg/m^2)	24 ± 3
RBC count (×10^6/μL)	5.18 ± 0.34
WBC count (×10^6/μL)	7.07 ± 1.66
Platelets (×10^3/μL)	224 ± 58
Basal SBP (mm Hg)	131 ± 11
Basal DBP (mm Hg)	78 ± 9
Basal HR (bpm)	80 ± 12

[1] bpm, beats per minute; DBP, diastolic blood pressure; HR, heart rate; RBC, red blood cells; SBP, systolic blood pressure; WBC, white blood cells.
[2] Mean ± SD (all such values).
[3] Median; range in parentheses.

SBP peak values, defined as the maximum SBP for each subject during the study period, were 147 ± 12 mm Hg after caf and 143 ± 12 mm Hg after decaf ($P < 0.001$). The corresponding peak values for DBP were 94 ± 10 mm Hg after caf and 93 ± 9 mm Hg after decaf (NS). The difference between caf and decaf was 4 ± 11 mm Hg for SBP peak and 1 ± 10 mm Hg for DBP peak.

Plasma caffeine

Plasma caffeine concentrations were similar before the administration of caf or decaf (0.6 ± 0.8 and 0.7 ± 0.8 μg/mL, respectively; NS). Measurements 30 min after caf drinking showed a significant increase in plasma caffeine concentrations (3.7 ± 1.5 μg/mL; $P < 0.001$ compared with baseline), which persisted at 2 h (4.1 ± 1.2 μg/mL; $P < 0.001$ compared with baseline, NS compared with 30 min) (**Figure 4**A), whereas no significant changes in plasma caffeine concentrations were observed after administration of decaf (0.8 ± 1.1 μg/mL at 30 min and 0.7 ± 0.9 μg/mL at 2 h; NS compared with baseline) (Figure 4B).

Plasma catecholamines

Plasma adrenaline concentrations were similar before the administration of caf or decaf (32 ± 23 and 35 ± 25 pg/mL, respectively; NS). At 2 h after caf consumption, a significant increase in plasma adrenaline concentration was observed (45 ± 31 pg/mL; $P < 0.001$ compared with baseline) (Figure 4C), whereas no significant changes in plasma adrenaline concentrations were observed 2 h after decaf (31 ± 19 pg/mL; NS compared with baseline) (Figure 4D). No significant differences were observed in plasma noradrenaline concentrations before the administration of caf or decaf (261 ± 115 and 264 ± 113 pg/mL, respectively; NS), nor at 2 h (259 ± 151 and 258 ± 132 pg/mL, respectively; NS).

Effect of genetic polymorphisms on BP response to coffee

Overall frequencies of gene variants studied in our population are shown in **Table 2**. The genotype distributions among study subjects did not deviate from Hardy-Weinberg equilibrium according to a Pearson chi-square test with 1 df.

To test the hypothesis of a genetic basis for the observed variability in BP responses to coffee, we first considered pressure outcomes as continuous variables and we analyzed data by univariable linear regression.

To test for any difference in caffeine metabolism, the *CYP1A2* genotype was first considered. No difference in plasma caffeine concentrations across such genotypes was observed: carriers of the "slow metabolism" *CYP1A2*1F* allele showed a difference (2 h – baseline) after coffee intake similar to that of homozygotes for the "rapid metabolism" *CYP1A2*1A allele* (3.6 ± 0.7 and 3.3 ± 0.8 μg/mL, respectively; NS). No relation was observed between the *CYP1A2* genotype and BP variables chosen for the analysis (**Table 3**).

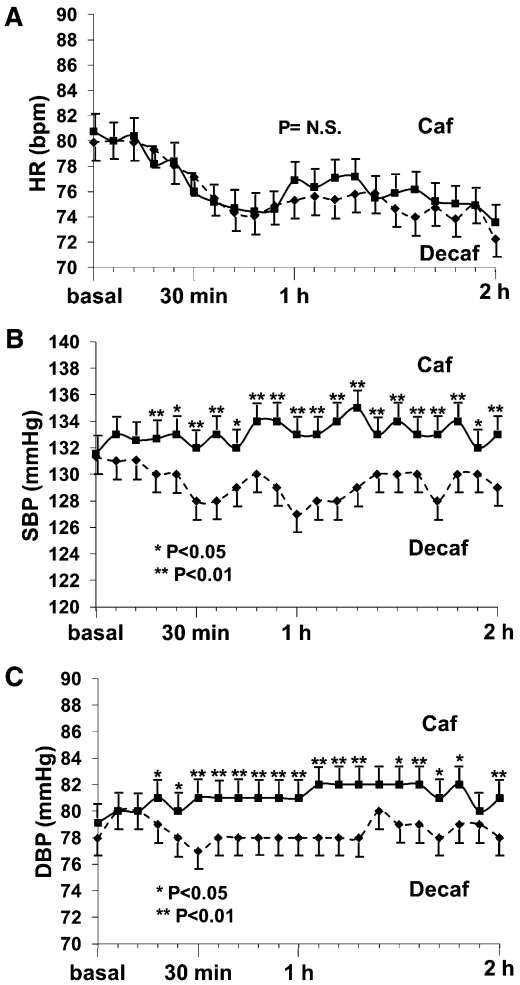

FIGURE 2. Mean (±SEM) trends in cardiovascular variables after consumption of caf (n = 110) or decaf (n = 110). A: Trend in HR at baseline and at 6-min intervals until 2 h after coffee consumption. No statistically significant difference was observed between the caf and decaf conditions at any time point. There was no significant time-by-treatment interaction for HR after caf and decaf (P = NS). B: Trend of SBP at baseline and at 6-min intervals until 2 h after coffee consumption. There was a significant time-by-treatment interaction for SBP after caf and decaf (P <0.01 for both). C: Trend of DBP at baseline and at 6-min intervals until 2 h after coffee consumption. There was no significant time-by-treatment interaction for DBP after caf and decaf (P = NS). Data were analyzed with a paired 2-sample t test. Repeated-measures ANOVA was used to test for time-by-treatment interactions. Differences between caf and decaf are shown at the different time points. caf, decaffeinated coffee preparation plus 3 mg caffeine/kg; decaf, decaffeinated coffee without added caffeine; DBP, diastolic blood pressure; HR, heart rate; SBP, systolic blood pressure.

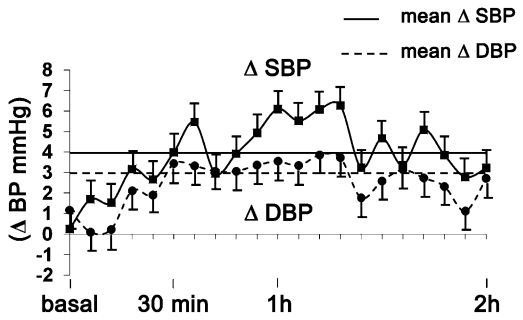

FIGURE 3. Mean (± SEM) changes in SBP and DBP between after consumption of caf (n = 110) or decaf (n = 110) throughout the study (beginning at baseline and then at 6-min intervals thereafter until 2 h). There was a significant time-by-treatment interaction for ΔSBP ($P < 0.01$) but not for ΔDBP (P = NS). The horizontal lines represent the mean values of the differences for SBP (continuous) and DBP (dotted). Data were analyzed with a paired 2-sample t test. Repeated-measures ANOVA was used to test for time-by-treatment interactions. BP, blood pressure; caf, decaffeinated coffee preparation plus 3 mg caffeine/kg; decaf, decaffeinated coffee without added caffeine; DBP, diastolic blood pressure; SBP, systolic blood pressure; Δ, change.

Concerning all the other polymorphisms, a relation between *ADORA2A TT* and peak ΔSBP (r = 0.214, P = 0.024) was observed (Table 3); moreover a relation between the *ADRA2B I* variant and mean ΔSBP mean (r = 0.226, P = 0.017) and peak (r = 0.225, P = 0.018) was found (Table 3). No differences in caffeine and plasma adrenaline concentrations (2 h – basal) were observed after coffee intake between subjects homozygous or heterozygous for the genetic variants of *ADORA2A*C→T or *ADRA2B I/D* (NS; data not shown).

Then, the study population was divided into groups: above or below the median values of the distributions for ΔSBP and ΔDBP mean and above or below the median values of the distributions for ΔSBP and ΔDBP peak. Medians and ranges of these BP variables are reported in **Table 4**. We then assessed the number of subjects with SBP and DBP values above or below the median according to the gene variants studied. No relation was observed between the *CYP1A2* genotype and BP variables according to this statistical method (**Figure 5**).

We observed a significantly higher proportion of subjects with a ΔSBP mean above the median in homozygotes for the *ADRA2B I* variant than in the other genotypes at the same locus (OR: 3.7; 95% CI: 1.4, 9.5; P = 0.006).

The other polymorphisms did not show any significant relation with ΔSBP mean (Figure 5). No relation was observed between ΔSBP peak and any polymorphism by this analysis (*see* Supplemental Figure 1 under "Supplemental data" in the online

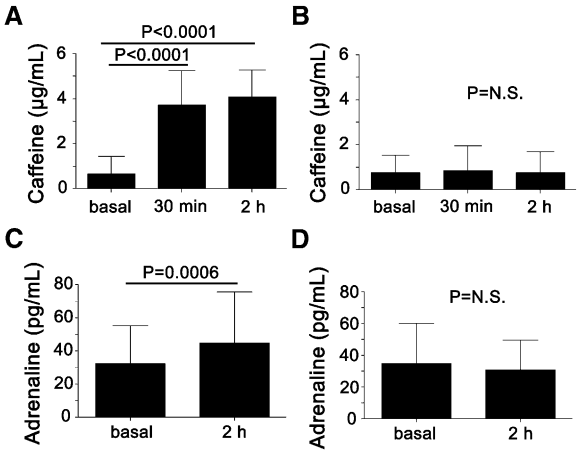

FIGURE 4. Mean (±SD) plasma caffeine and adrenaline concentrations before and after consumption of caf (n = 110) or decaf (n = 110). A: Plasma caffeine concentrations at baseline and 30 min and 2 h after caf intake. B: Plasma caffeine concentrations at baseline and 30 min and 2 h after decaf intake. C: Plasma adrenaline concentrations at baseline and 2 h after caf intake. D: Plasma adrenaline concentrations at baseline and 2 h after decaf intake. Plasma caffeine data were analyzed by one-factor ANOVA with Bonferroni's correction. Plasma adrenaline data were analyzed with a paired 2-sample t test; caf, decaffeinated coffee preparation plus 3 mg caffeine/kg; decaf, decaffeinated coffee without added caffeine.

TABLE 2
Genotype distributions in the study population[1]

Variant	Genotype frequency
	n (%)
CYP1A2-734A→C	
AA	37 (34)
AC	71 (64)
CC	2 (2)
ADORA2A-1976C→T	
CC	48 (44)
CT	34 (31)
TT	28 (25)
AMPD1-C34T	
CC	50 (46)
CT	60 (54)
TT	0 (0)
ADRA1A-Arg347Cys	
ArgArg	21 (19)
ArgCys	56 (51)
CysCys	33 (30)
ADRA2B-Ins+910Del	
II	45 (41)
ID	57 (52)
DD	8 (7)
ADRB1-Arg389Gly	
ArgArg	55 (50)
ArgGly	36 (33)
GlyGly	19 (17)
ADRB1-Ser49Gly	
SerSer	88 (80)
SerGly	18 (16)
GlyGly	4 (4)
ADRB2-Arg16Gly	
ArgArg	17 (15)
ArgGly	48 (44)
GlyGly	45 (41)
ADRB2-Glu27Gln	
GluGlu	24 (22)
GluGln	44 (40)
GlnGln	42 (38)
ADRB2-Thr16Ile	
ThrThr	104 (94)
ThrIle	6 (6)
IleIle	0 (0)
ADRB3-Trp64Arg	
TrpTrp	103 (94)
TrpArg	7 (6)
ArgArg	0 (0)

[1] ADORA2A, A2A-adenosine receptor; AMPD, AMP deaminase; ADRA1A, α_{1A}-adrenergic receptor; ADRA2B, α_{2B}-adrenergic receptor; ADRB1, β_1-adrenergic receptor; ADRB2, β_2-adrenergic receptor; ADRB3, β_3-adrenergic receptor; CYP1A2, cytochrome P450 1A2.

TABLE 3
Genetic variables related to differences in ΔSBP and ΔDBP mean and peak between caf and decaf[1]

	ΔSBP mean		ΔDBP mean		ΔSBP peak		ΔDBP peak	
	r	*P*	*R*	*P*	*r*	*P*	*r*	*P*
*CYP1A2*1F*	0.048	0.62	0.050	0.60	0.016	0.87	0.032	0.74
*CYP1A2*1A*	0.069	0.47	0.176	0.65	0.035	0.71	0.114	0.23
ADORA2A-CC	0.002	0.99	0.038	0.69	0.114	0.23	0.163	0.089
ADORA2A-TT	0.150	0.11	0.087	0.36	0.214	0.024	0.031	0.75
AMPD1-CC	0.033	0.73	0.025	0.80	0.030	0.75	0.003	0.97
ADRA1A-347ArgArg	0.062	0.52	0.122	0.20	0.051	0.59	0.044	0.65
ADRA1A-347CysCys	0.061	0.53	0.032	0.74	0.057	0.55	0.024	0.80
ADRA2B-II	0.226	0.017	0.100	0.30	0.225	0.018	0.031	0.75
ADRA2B-DD	0.093	0.34	0.103	0.29	0.073	0.45	0.173	0.071
ADRB1-49SerSer	0.099	0.30	0.011	0.91	0.015	0.88	0.076	0.43
ADRB1-49GlyGly	0.011	0.91	0.016	0.87	0.033	0.73	0.064	0.51
ADRB1-389ArgArg	0.003	0.97	0.043	0.65	0.034	0.73	0.170	0.077
ADRB1-389GlyGly	0.095	0.32	0.084	0.39	0.055	0.57	0.069	0.47
ADRB2-16GlyGly	0.111	0.25	0.036	0.71	0.004	0.97	0.105	0.27
ADRB2-16ArgArg	0.019	0.84	0.016	0.87	0.059	0.54	0.005	0.96
ADRB2-27GluGlu	0.039	0.68	0.046	0.64	0.003	0.97	0.011	0.91
ADRB2-27GlnGln	0.080	0.41	0.061	0.52	0.120	0.21	0.010	0.92
ADRB2-164ThrThr	0.036	0.70	0.110	0.25	0.020	0.84	0.035	0.72
ADRB3-64TrpTrp	0.084	0.38	0.139	0.15	0.138	0.15	0.013	0.89

[1] The data were analyzed with linear regression analysis. *P* values < 0.05 are statistically significant. ΔSBP, change between caf and decaf in systolic blood pressure; ΔDBP, change between caf and decaf in diastolic blood pressure. caf, decaffeinated coffee preparation plus 3 mg caffeine/kg; decaf, decaffeinated coffee without added caffeine.

issue). In addition, no relation was observed between diastolic BP variables and the various polymorphisms (*see* Supplemental Figures 2 and 3 under "Supplemental data" in the online issue).

DISCUSSION

The effects of coffee on the cardiovascular system are still debated. Evidence that caffeine is an antagonist of adenosine receptors (35) suggests the hypothesis that coffee induces vasoconstriction and elevates SBP and DBP acutely (36, 37), but the chronic effects of coffee consumption on BP and cardiovascular disease remain controversial (15, 36, 38–43). Various confounding factors may have influenced the results of the different studies, including differences in the study design, populations examined (age, sex, usual frequency of coffee drinking, and smoking), types of coffee blend used, and types of preparations used. Variability in the physiologic responses to coffee is well documented, with subjects totally indifferent to even a large amount of coffee ingestion, and subjects deriving profound cardiovascular and neuropsychological consequences to coffee drinking (44–46). We hypothesized that part of this variability may be explained by genetic factors.

With the purpose of limiting some of the variables involved, we adjusted caffeine intake for body weight, chose only nonsmoking male subjects, and specifically restricted our study to subjects with moderate habitual average coffee intakes to limit the effects of tolerance. The use of decaffeinated coffee as control and of decaffeinated coffee with added caffeine as the test drink allowed us to isolate the effects of caffeine. The double-blind, randomized, prospective, controlled, crossover design—with each subject serving as his own control—virtually eliminated the influence of systematic bias. In the absence of specific data, we estimated the needed sample size on the basis of previous neuropsychological literature. Although the sample size may be considered small for genetic studies, it would mostly increase type II error, precluding the possibility of drawing conclusions from negative findings, but should have less effect on the possibility of biological inference from statistically significant data.

In agreement with some previous observations, we found an increase in SBP and DBP after coffee drinking, which was associated with an increase in plasma caffeine and adrenaline concentrations. These results suggest that caffeine may determine an acute BP increase through adrenaline incretion. Of note, and in agreement with previous studies, BP responses were variable between subjects, with most subjects experiencing an increase in BP, others showing no change, and some even showing BP reductions.

We analyzed some genetic polymorphisms selected as candidates to influence cardiovascular responses to coffee. We found no association between expected changes in caffeine metabolism exerted by *CYP1A2* gene variants and plasma caffeine concentrations or BP variables during the 2 h of the study. However, it is possible that the 2-h time frame of our study was too short to

TABLE 4
Medians and ranges for ΔSBP and ΔDBP mean and peak between caf and decaf[1]

	ΔSBP mean	ΔDBP mean	ΔSBP peak	ΔDBP peak
	mm Hg	*mm Hg*	*mm Hg*	*mm Hg*
Median	2.96	2.49	4.5	1.5
Range	−14.2–20.4	−13.6–21.8	−31–29	−27–27

[1] ΔSBP, change between caf and decaf in systolic blood pressure; ΔDBP, change between caf and decaf in diastolic blood pressure. caf, decaffeinated coffee preparation plus 3 mg caffeine/kg; decaf, decaffeinated coffee without added caffeine.

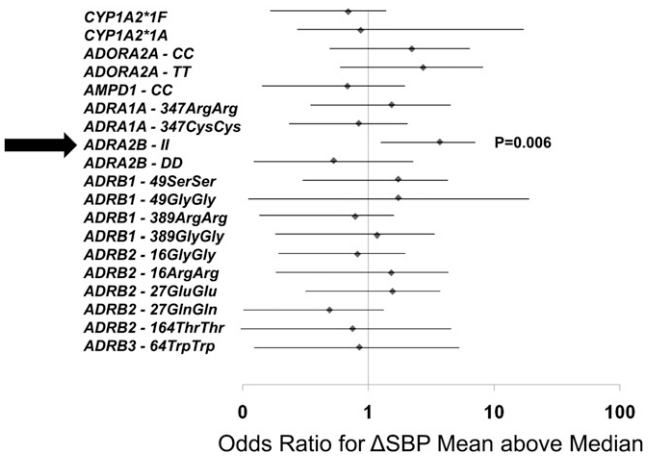

FIGURE 5. Forest plot of the effect of genetic variants on the risk of having an above-the-median *Δ*SBP mean between after consumption of caf (n = 110) or decaf (n = 110). The genetic variants evaluated are listed on the left and are grouped according to the gene where they are located. The plot shows, for the homozygotes of each single genetic variant, the specific effect on BP [expressed as ORs and 95% CIs (horizontal lines)] against both the alternative genotypes at the same locus. The homozygotes not shown (*AMPD1-TT*, *ADRB2-164IleIle*, *ADRB3-64ArgArg*) were not present in our population. The data were analyzed by a conditional logistic regression model by using BP as the discrete variable; the null hypothesis of no association was tested with the likelihood ratio test. The vertical line represents no effect. The overlap of the CI with this line indicates that the effect size does not deviate significantly from no effect. BP, blood pressure; caf, decaffeinated coffee preparation plus 3 mg caffeine/kg; decaf, decaffeinated coffee without added caffeine; *Δ*SBP, change in systolic blood pressure.

evaluate caffeine metabolism, because differences between "slow" and "rapid" metabolizers could well manifest at later time points. We found an association between the *TT* variant of the *ADORA2A* polymorphism and SBP peak after coffee consumption. This may suggest that an acute increase in SBP after caffeine consumption may be affected by genetic variants of adenosine receptors. Caffeine is an antagonist of adenosine receptors, which normally mediate vasodilation. However, whereas the *ADORA2A* polymorphism was reported to modulate some psychological response to caffeine, and individuals with the *ADORA2A TT* variant had greater anxiety after acute caffeine administration than did other groups (12), there is no clear evidence of an association between this polymorphism and BP response to caffeine, although this could not be excluded.

Of the various polymorphisms of adrenergic receptors investigated, we found that the *ADRA2B I/D* polymorphism contributed to some of the variability in the acute BP responses to coffee. The presence of 2 copies of the *I* allele of the *ADRA2B I/D* polymorphism confers susceptibility for higher BP responses.

The *ADRA2B I→D* polymorphism involves the deletion of 3 glutamic acid residues from a repeat element in the third intracellular loop of the α_2-receptor protein, which results in a loss of agonist-promoted desensitization (47); this should mean that carriers of the *D* variant have α_{2B}-receptors that are more sensitive to agonists and promote vasoconstriction. However, *ADRA2B I* was associated with higher BP in Chinese men (48). This finding agrees with our results, which indicate that homozygotes for the *I* allelic variant have a higher BP response to caffeine, probably mediated by adrenaline. On the other hand, our carriers of the *D* allele seemed to be more protected against an increase in BP.

Study limitations

The main limitation of our study was the sample size, which was considered small for a genetic study and limited the possibility of interpreting the data obtained and drawing conclusions from negative findings. However, the sample size—the largest used thus far in a study exploring physiologic effects of caffeine—was of the same order of magnitude of previously published studies that investigated the neuropsychological effects of coffee. Another weak point was that our data cannot be extended to the entire population because only healthy nonsmoking young men were included. The caffeine concentration attained in the subjects, although realistic, may reflect only extremes of habitual coffee consumption; therefore, we cannot draw inferences on the effects of lower doses. Finally, the clinical implications of the study are indirect and are based on the assumption that an increase in BP related to coffee drinking and caffeine may translate to an increased cardiovascular disease risk. Such inferences are provisional because *1*) we did not directly evaluate clinical endpoints and *2*) absolute differences in BP were small and mostly in the range of normal values. In principle, however, continuous exposure to even small changes in BP may adversely affect cardiovascular outcomes.

In conclusion, this was the first study in the literature to report an associations between gene variants in the adenosine and α_2-adrenergic receptors and BP responses to caffeine. Nutrigenetic interactions may explain part of the variability in the cardiovascular effects of coffee drinking. In principle, the exposure to higher BP values as the result of such nutrigenetic interactions in some genetically predisposed individuals may expose such individuals to a higher coffee-related cardiovascular disease risk. This hypothesis should be tested in large population-based studies.

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The authors' responsibilities were as follows—GR: conducted the research (experiments and data collection) and wrote the manuscript; MZ: conducted the research (experiments and data collection) and performed the statistical analysis; IA: conducted the research (genetic analyses); AT and BR: conducted the research (experiments and data collection); TB: conducted the research (caffeine analyses); TP: conducted the research (catecholamine analyses); LS: conducted the research (genetic analyses) and contributed to the data interpretation; and RDC: designed the research (project conception, development of overall research plan, and study oversight), wrote the manuscript, and takes primary responsibility for the final content. The Institute for Scientific Information on Coffee reviewed the protocol at the time of the grant application and suggested some protocol improvements. The Italian Istituto Nazionale Ricerche Cardiovascolari had no role in the study design. ISIC was informed of the study results at the time of manuscript submission. Neither entity had any role in the analysis or discussion of the results. None of the authors declared any conflicts of interest.

REFERENCES

1. Ferruzzi MG. The influence of beverage composition on delivery of phenolic compounds from coffee and tea. Physiol Behav 2010;100:33–41.
2. Ribeiro JA, Sebastiao AM. Caffeine and adenosine. J Alzheimers Dis 2010;20(suppl 1):S3–15.
3. Robertson D, Froelich J, Carr K, Watson J, Hollifield J, Shand D, Oates J. Effects of caffeine on plasma renin activity, catecholamines and blood pressure. N Engl J Med 1978;298:181–6.
4. Lieberman HR. The effects of ginseng, ephedrine, and caffeine on cognitive performance, mood and energy. Nutr Rev 2001;59:91–102.

5. Riksen NP, Rongen GA, Smits P. Acute and long-term cardiovascular effects of coffee: implications for coronary heart disease. Pharmacol Ther 2009;121:185–91.
6. Colton T, Gosselin R, Smith R. The tolerance of coffee drinkers to caffeine. Clin Pharmacol Ther 1968;9:31–9.
7. Yang A, Palmer AA, de Wit H. Genetics of caffeine consumption and responses to caffeine. Psychopharmacology (Berl) 2010;211:245–57.
8. Rasmussen BB, Brix TH, Kyvik KO, Brosen K. The interindividual differences in the 3-demthylation of caffeine alias CYP1A2 is determined by both genetic and environmental factors. Pharmacogenetics 2002;12:473–8.
9. Sachse C, Brockmoller J, Bauer S, Roots I. Functional significance of a C→A polymorphism in intron 1 of the cytochrome P450 CYP1A2 gene tested with caffeine. Br J Clin Pharmacol 1999;47:445–9.
10. Cornelis MC, El-Sohemy A, Kabagambe EK, Campos H. Coffee, CYP1A2 genotype, and risk of myocardial infarction. JAMA 2006; 295:1135–41.
11. Ongini E, Fredholm BB. Pharmacology of adenosine A2A receptors. Trends Pharmacol Sci 1996;17:364–72.
12. Alsene K, Deckert J, Sand P, de Wit H. Association between A2a receptor gene polymorphisms and caffeine-induced anxiety. Neuropsychopharmacology 2003;28:1694–702.
13. Morisaki T, Gross M, Morisaki H, Pongratz D, Zollner N, Holmes EW. Molecular basis of AMP deaminase deficiency in skeletal muscle. Proc Natl Acad Sci USA 1992;89:6457–61.
14. Feldman AM, Wagner DR, McNamara DM. AMPD1 gene mutation in congestive heart failure: new insights into the pathobiology of disease progression. Circulation 1999;99:1397–9.
15. Happonen P, Voutilainen S, Salonen JT. Coffee drinking is dose-dependently related to the risk of acute coronary events in middle-aged men. J Nutr 2004;134:2381–6.
16. Snapir A, Heinonen P, Tuomainen TP, Alhopuro P, Karvonen MK, Lakka TA, Nyyssonen K, Salonen R, Kauhanen J, Valkonen VP, et al. An insertion/deletion polymorphism in the alpha2B-adrenergic receptor gene is a novel genetic risk factor for acute coronary events. J Am Coll Cardiol 2001;37:1516–22.
17. Xie HG, Kim RB, Stein CM, Gainer JV, Brown NJ, Wood AJ. Alpha1A-adrenergic receptor polymorphism: association with ethnicity but not essential hypertension. Pharmacogenetics 1999;9:651–6.
18. Bengtsson K, Melander O, Orho-Melander M, Lindblad U, Ranstam J, Rastam L, Groop L. Polymorphism in the beta(1)-adrenergic receptor gene and hypertension. Circulation 2001;104:187–90.
19. Brodde OE, Buscher R, Tellkamp R, Radke J, Dhein S, Insel PA. Blunted cardiac responses to receptor activation in subjects with Thr164Ile beta(2)-adrenoceptors. Circulation 2001;103:1048–50.
20. Liggett SB, Wagoner LE, Craft LL, Hornung RW, Hoit BD, McIntosh TC. The Ile164*2-adrenergic receptor polymorphism adversely affets the outcome of congestive heart failure. J Clin Invest 1998;102:1534–9.
21. Maqbool A, Hall AS, Ball SG, Balmforth AJ. Common polymorphisms of beta1-adrenoceptor: identification and rapid screening assay. Lancet 1999;353:897.
22. Ranade K, Shue WH, Hung YJ, Hsuing CA, Chiang FT, Pesich R, Hebert J, Olivier M, Chen YD, Pratt R, et al. The glycine allele of a glycine/arginine polymorphism in the beta2-adrenergic receptor gene is associated with essential hypertension in a population of Chinese origin. Am J Hypertens 2001;14:1196–200.
23. Ringel J, Kreutz R, Distler A, Sharma AM. The Trp64Arg polymorphism of the beta3-adrenergic receptor gene is associated with hypertension in men with type 2 diabetes mellitus. Am J Hypertens 2000;13:1027–31.
24. White HL, Maqbool A, McMahon AD, Yates L, Ball SG, Hall AS, Balmforth AJ. An evaluation of the beta-1 adrenergic receptor Arg389Gly polymorphism in individuals at risk of coronary events. A WOSCOPS substudy. Eur Heart J 2002;23:1087–92.
25. Kelsey RM, Alpert BS, Dahmer MK, Krushkal J, Quasney MW. Beta-adrenergic receptor gene polymorphisms and cardiovascular reactivity to stress in Black adolescents and young adults. Psychophysiology 2010;47:863–73.
26. Baumgart P, Kamp J. Accuracy of the SpaceLabs Medical 90217 ambulatory blood pressure monitor. Blood Press Monit 1998;3:303–7.
27. Schreiber-Deturmeny E, Bruguerolle B. Simultaneous high-performance liquid chromatographic determination of caffeine and theophylline for routine drug monitoring in human plasma. J Chromatogr B Biomed Appl 1996;677:305–12.
28. Goldstein DS, Stull R, Markey SP, Marks ES, Keiser HR. Dihydrocaffeic acid: a common contaminant in the liquid chromatographic-electrochemical measurement of plasma catecholamines in man. J Chromatogr 1984;311:148–53.
29. Yuan M, Ohishi M, Ito N, Sugimoto K, Takagi T, Terai M, Katsuya T, Rakugi H, Wu Z, Ogihara T. Genetic influences of beta-adrenoceptor polymorphisms on arterial functional changes and cardiac remodeling in hypertensive patients. Hypertens Res 2006;29:875–81.
30. Mochtar CA, Laan W, Van Houwelingen KP, Franke B, De La Rosette JJ, Schalken JA, Kiemeney LA. Polymorphisms in the alpha1A-adrenoceptor gene do not modify the short- and long-term efficacy of alpha1-adrenoceptor antagonists in the treatment of benign prostatic hyperplasia. BJU Int 2006;97:852–5.
31. de Groote P, Lamblin N, Helbecque N, Mouquet F, McFadden E, Hermant X, Amouyel P, Dallongeville J, Bauters C. The impact of beta-adrenoreceptor gene polymorphisms on survival in patients with congestive heart failure. Eur J Heart Fail 2005;7:966–73.
32. Andreassi MG, Botto N, Laghi-Pasini F, Manfredi S, Ghelarducci B, Farneti A, Solinas M, Biagini A, Picano E. AMPD1 (C34T) polymorphism and clinical outcomes in patients undergoing myocardial revascularization. Int J Cardiol 2005;101:191–5.
33. Cornelis MC, El-Sohemy A, Campos H. Genetic polymorphism of CYP1A2 increases the risk of myocardial infarction. J Med Genet 2004;41:758–62.
34. Soma M, Nakayama T, Satoh M, Uwabo J, Rahmutula D, Takahashi Y, Fukuda N, Watanabe Y, Izumi Y, Kanmatsuse K. A T1083C polymorphism in the human adenosine A2a receptor gene is not associated with essential hypertension. Am J Hypertens 1998;11:1492–4.
35. Fredholm BB. Astra Award Lecture. Adenosine, adenosine receptors and the actions of caffeine. Pharmacol Toxicol 1995;76:93–101.
36. Hartley TR, Lovallo WR, Whitsett TL. Cardiovascular effects of caffeine in men and women. Am J Cardiol 2004;93:1022–6.
37. Umemura T, Ueda K, Nishioka K, Hidaka T, Takemoto H, Nakamura S, Jitsuiki D, Soga J, Goto C, Chayama K, et al. Effects of acute administration of caffeine on vascular function. Am J Cardiol 2006;98:1538–41.
38. Corti R, Binggeli C, Sudano I, Spieker L, Hanseler E, Ruschitzka F, Chaplin WF, Luscher TF, Noll G. Coffee acutely increases sympathetic nerve activity and blood pressure independently of caffeine content: role of habitual versus nonhabitual drinking. Circulation 2002;106:2935–40.
39. LaCroix AZ, Mead LA, Liang KY, Thomas CB, Pearson TA. Coffee consumption and the incidence of coronary heart disease. N Engl J Med 1986;315:977–82.
40. Lopez-Garcia E, van Dam RM, Willett WC, Rimm EB, Manson JE, Stampfer MJ, Rexrode KM, Hu FB. Coffee consumption and coronary heart disease in men and women: a prospective cohort study. Circulation 2006;113:2045–53.
41. Panagiotakos DB, Pitsavos C, Chrysohoou C, Kokkinos P, Toutouzas P, Stefanadis C. The J-shaped effect of coffee consumption on the risk of developing acute coronary syndromes: the CARDIO2000 case-control study. J Nutr 2003;133:3228–32.
42. Silletta MG, Marfisi R, Levantesi G, Boccanelli A, Chieffo C, Franzosi M, Geraci E, Maggioni AP, Nicolosi G, Schweiger C, et al. Coffee consumption and risk of cardiovascular events after acute myocardial infarction: results from the GISSI (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico)–Prevenzione trial. Circulation 2007;116:2944–51.
43. Wilson PW, Garrison RJ, Kannel WB, McGee DL, Castelli WP. Is coffee consumption a contributor to cardiovascular disease? Insights from the Framingham Study. Arch Intern Med 1989;149:1169–72.
44. Chait LD. Factors influencing the subjective response to caffeine. Behav Pharmacol 1992;3:219–28.
45. Evans SM, Griffiths RR. Dose-related caffeine discrimination in normal volunteers: individual differences in subjective effects and self-reported cues. Behav Pharmacol 1991;2:345–56.
46. Wu JN, Ho SC, Zhou C, Ling WH, Chen WQ, Wang CL, Chen YM. Coffee consumption and risk of coronary heart diseases: a meta-analysis of 21 prospective cohort studies. Int J Cardiol 2009;137:216–25.
47. Heinonen P, Koulu M, Pesonen U, Karvonen MK, Rissanen A, Laakso M, Valve R, Uusitupa M, Scheinin M. Identification of a three-amino acid deletion in the alpha2B-adrenergic receptor that is associated with reduced basal metabolic rate in obese subjects. J Clin Endocrinol Metab 1999;84:2429–33.
48. Zhang H, Li X, Huang J, Li Y, Thijs L, Wang Z, Lu X, Cao K, Xie S, Staessen JA, et al. Cardiovascular and metabolic phenotypes in relation to the ADRA2B insertion/deletion polymorphism in a Chinese population. J Hypertens 2005;23:2201–7.