

CYP1A2 genotype modifies the association between coffee intake and the risk of hypertension

Paolo Palatini^a, Giulio Ceolotto^a, Fabio Ragazzo^a, Francesca Dorigatti^a,
Francesca Saladini^a, Italia Papparella^a, Lucio Mos^b, Giuseppe Zanata^c and
Massimo Santonastaso^d

Objectives The longitudinal relationship between coffee use and hypertension is still controversial. Cytochrome P450 1A2 (CYP1A2) is the main responsible enzyme for the metabolism of caffeine. The aim of the present study was to investigate the effect of coffee intake on the risk of developing hypertension needing antihypertensive treatment in individuals stratified by CYP1A2 genotype.

Design We assessed prospectively 553 young White individuals screened for stage 1 hypertension. Coffee intake was ascertained from regularly administered questionnaires. Incident physician-diagnosed hypertension was the outcome measure. Genotyping of CYP1A2 SNP was performed by real time PCR.

Results During a median follow-up of 8.2 years, 323 individuals developed hypertension. For carriers of the slow *1F allele (59%), hazard ratios of hypertension from multivariable Cox analysis were 1.00 in abstainers (reference), 1.72 (95%CI, 1.21–2.44) in moderate coffee drinkers ($P=0.03$), and 3.00 (1.53–5.90) in heavy drinkers ($P=0.001$). In contrast, hazard ratios for coffee drinkers with the rapid *1A/*1A genotype were 0.80 (0.52–1.23, $P=0.29$) for moderate drinkers and 0.36 (0.14–0.89, $P=0.026$) for heavy drinkers. In a two-way ANCOVA, a gene \times coffee interactive effect was found on follow-up changes in systolic ($P=0.000$) and diastolic ($P=0.007$)

blood pressure. Urinary epinephrine was higher in coffee drinkers than abstainers but only among individuals with slow *1F allele ($P=0.001$).

Conclusion These data show that the risk of hypertension associated with coffee intake varies according to CYP1A2 genotype. Carriers of slow *1F allele are at increased risk and should thus abstain from coffee, whereas individuals with *1A/*1A genotype can safely drink coffee. *J Hypertens* 27:000–000 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Journal of Hypertension 2009, 27:000–000

Keywords: caffeine, coffee, CYP1A2, hypertension, polymorphism

Abbreviations: AACE, American Association of Clinical Endocrinologists; BMI, body mass index; BP, blood pressure; CIs, confidence intervals; CYP1A2, Cytochrome P450 1A2; HARVEST, Hypertension and Ambulatory Recording VEnetia Study; HRs, hazard ratios

^aClinica Medica 4, University of Padova, Padova, ^bTown Hospital, San Daniele del Friuli, Udine, ^cTown Hospital, Pordenone and ^dTown Hospital, Vittorio Veneto, Italy

Correspondence to Professor Paolo Palatini, MD, Clinica Medica 4, University of Padova, via Giustiniani, 2- 35128 Padova, Italy
Tel: +39 049 8212278; fax: +39 049 8754179; e-mail: palatini@unipd.it

Received 30 May 2008 Revised 26 January 2009

Accepted 16 March 2009

Introduction

As caffeinated coffee has been reported to increase blood pressure (BP) in cross-sectional studies [1–4], prospective studies examining the association between coffee consumption and risk of hypertension have been inconclusive. Two meta-analyses of randomized controlled trials whose duration ranged from one to 12 weeks, demonstrated a small BP increase for coffee intake [5,6]. The effect of coffee consumption on the risk of developing hypertension in the long term has been explored in a few prospective studies, which provided conflicting results [7–9]. In a cohort of young White men from the United States, coffee use was associated with risk of hypertension, but the association was no longer significant after adjustment for confounders [7]. In a more recent analysis of the Nurses' Health Studies I and II of 155 594 the United States normotensive women, regular coffee intake was not associated with increased risk of hypertension [8]. At variance with those results, coffee

abstinence was found to be associated with lower risk of hypertension in the Doetinchem Cohort Study [9].

Cytochrome P450 1A2 (CYP1A2) is the main responsible enzyme for the metabolism of caffeine [10]. An A \rightarrow C substitution at position 734 (CYP1A2*1F) in the CYP1A2 gene decreases enzyme activity and inducibility [11,12]. Carriers of the variant *1F allele are 'slow' caffeine metabolizers, whereas individuals homozygous for the CYP1A2*1A allele are 'rapid' caffeine metabolizers [11,12]. Recently, it has been demonstrated that coffee consumption increases the risk of myocardial infarction only among individuals with slow metabolizer genotype [13], whereas among those with the *1A/*1A genotype coffee even decreases the risk. Whether CYP1A2 genotype modifies the association between coffee intake and risk of hypertension is not known. Thus, the aim of the present study was to examine whether CYP1A2 genotype modifies the long-term association between

intake of caffeinated coffee and risk of hypertension needing antihypertensive treatment in the participants of the HARVEST (Hypertension and Ambulatory Recording VEnetia Study), a prospective longitudinal study of young individuals screened for stage 1 hypertension [14].

Methods

Study population

The HARVEST is a prospective study of 18–45 year old individuals, initiated in 1990, investigating the origin of hypertension with regard to physiological [14], genetic [15], and clinical [16] characteristics. Never treated individuals screened for stage 1 hypertension (systolic BP between 140 and 159 mmHg and/or diastolic BP between 90 and 99 mmHg) were enrolled. Patients with diabetes mellitus, nephropathy, and cardiovascular disease were excluded [14–16]. The 553 individuals taking part in this subproject were all those recruited and followed up in the four HARVEST centers, which agreed to participate in the genetic study [15]. Their characteristics are shown in Table 1. The higher prevalence of men among our study participants (72.5%) confirms previous observations of a much higher prevalence of men in the young segment of the hypertensive population [17]. Patients' recruitment was obtained with the collaboration of the local general practitioners who were instructed during local meetings. Consecutive patients with the earlier-mentioned clinical characteristics seen in the offices of the general practitioners and willing to participate in the study were eligible for recruitment and were sent to the referral centers. Blood and urine samples are periodically collected and taken to the coordinating office in Padova, where they are processed. The study was approved by the HARVEST Ethics Committee and the Ethics Committee of the University of Padova, and written informed consent was given by the participants.

Procedures

At baseline, all individuals underwent physical examination, anthropometry, blood chemistry, and urine analysis.

Participants completed questionnaires about their lifestyle, including coffee consumption, physical activity, alcohol use and cigarette smoking. Coffee intake was defined according to the number of caffeine-containing coffees drunk per day. The caffeine content per cup of 'expresso' Italian coffee, which was the most abundantly consumed type of coffee by the HARVEST participants, averages 100 mg [18]. Decaffeinated coffee was not taken into account. Tea and other caffeinated drinkings were not taken into account in the present study being unusual and irregular in this area of Italy (Venetia). A positive family history of hypertension was defined as one or two parents having hypertension and/or taking antihypertensive treatment [15]. Details about the interview, lifestyle assessment and criteria used for subjects' classification according to lifestyle were reported elsewhere [14–16]. Baseline BP was the mean of six readings obtained during two visits performed 2 weeks apart [14–16]. BMI was considered as an index of adiposity (weight divided by height squared). Individuals with the metabolic syndrome were identified by applying AACE criteria [19]. In 298 participants, urine was collected over 24 h for epinephrine and norepinephrine assessment by a HPLC method [14]. Urine specimens were frozen (–20°C) and sent to the Coordinating Office at the University of Padova, where they were processed [14].

Genotyping

Genomic DNA was extracted from whole blood through the NucleoSpin Blood kit (Macherey-Nagel, Düren, Germany). Primers and probes for allelic discrimination analysis of CYP1A2 polymorphism, designed from sequences derived from the GenBank database using Primer 3 (provided by the Whitehead Institute Cambridge, Massachusetts, USA) and Operon's Oligo software (Operon Technologies Inc., Alameda, California, USA), were as follows: forward primer AGAGAGCCA GCGTTCATGTT, reverse primer CTGATGCGTGT TCTGTGCTT, CYP1A2*1F probe (FAM-labelled)-5'-TCTGTGGGCCCCAGGA-3' (BLACK HOLE1), CYP 1A2*1A (TEXAS RED labelled)-5'-TCTGTGGGCA

Table 1 Clinical characteristics of the study individuals by CYP1A2 genotype

Variable	Total (N = 553)	CYP1A2 genotype	
		*1A/*1A (N = 226)	*1A/*1F+*1F/*1F (N = 327)
Age (years)	33.2 (8.6)	32.9 (8.6)	33.5 (8.6)
Sex (m/f, %)	72.5/27.5	72.1/27.9	72.8/27.2
Parental hypertension (no/yes, %)	37.8/62.2	41.7/58.3	35.2/64.8
BMI (kg/m ²)	25.2 (3.5)	25.2 (3.5)	25.3 (3.6)
Baseline systolic blood pressure (mmHg)	145.9 (10.5)	144.9 (10.3)	146.6 (10.6)
Baseline diastolic blood pressure (mmHg)	93.4 (5.6)	93.2 (6.0)	93.6 (5.3)
Coffee intake (no intake/moderate/heavy, %)	26.9/62.9/10.2	26.1/64.6/9.3	27.5/61.8/10.7
Physical activity (no/yes, %)	59.9/40.1	58.4/41.6	60.9/39.1
Cigarette smoking (no/yes, %)	80.5/19.5	78.8/21.2	81.7/18.3
Oral contraceptive use in women (no/yes, %)	85.4/14.6	87.3/12.7	84.1/15.9
Alcohol drinking (no/0-50 g/day/> 50 g/day, %)	53.2/41.6/5.2	52.7/42.0/5.3	53.5/41.3/5.2
Metabolic syndrome (%)	23.9	21.7	25.4

Values are given as mean (SD) or percentage. All differences between genotypes are nonsignificant.

CAGGA-3' (BLACK HOLE2), as described by dbSNP reference number (rs762551) on the National Center for Biotechnology Information (NCBI) Web site [20] and we defined the allele containing the 'A' nucleotide as CYP1A2*1A. Purified DNA (2 μ l) was amplified in a real-time PCR reaction in the iCycler iQTM system (BIO-RAD, Hercules, California, USA). All the reactions were performed in 96-well plates, using the iQ Supermix (BIO-RAD). Positive controls, genotyped by direct sequencing, were included in each run, together with a negative control containing no DNA template. Taqman reactions were thermocycled as follows: 95°C for 3 min to denature, 40 cycles at 95°C for 30 s for denaturing and 60°C for 1 min for annealing and extension.

Follow-up and study end-point

Office BP and lifestyle habits were assessed monthly during the first 3 months of follow-up, then after 6 months, and every 6 months thereafter. In the HARVEST study, the endpoint was development of hypertension eligible for antihypertensive medication after a period of lifestyle modification. After baseline examination, individuals were given general information about nonpharmacological measures by the HARVEST investigators, following the suggestions of current guidelines on the management of hypertensive patients [21–25]. To ensure homogeneous counselling by doctors participating in the study, training in current international guidelines was provided to them throughout the study duration. As the criteria for treatment were revised several times by the Scientific Committees from 1990 to 2006 [21–24], the definition of the end point changed accordingly. Before 1999, the criteria for treating low-risk individuals with stage 1 hypertension, such as those enrolled in the HARVEST study, were primarily based on the BP level [21,22]. In 1999, stratification of patients according to global cardiovascular risk became crucial for deciding whether antihypertensive drug treatment was needed [23]. However, being the participants in this study young to middle-aged individuals mostly at low-cardiovascular risk, also after 1999 most end points were based on the BP level.

Individuals who do not meet the criteria for treatment are checked at 6-month intervals unless they dropout. Enrolment of individuals with *1A/*1A genotype versus those with *1A*1F + *1F/*1F genotype and of coffee drinkers versus abstainers was equally distributed throughout the years. The definition of sustained hypertension is based on at least six clinic BP readings taken on two subsequent visits within 1 month [14–16]. The second end-point visit was performed immediately before starting antihypertensive treatment. Thus, final BP was the average of the last six clinic readings before starting treatment in the individuals who reached the endpoint and was the average of the three readings obtained at the last available visit in

the individuals who remained untreated. Other details on follow-up procedures were reported elsewhere [14–16].

Data analysis

The present study was performed in the 589 individuals for whom BP and information on lifestyle habits were available both at baseline and final assessments and who had at least 6 months of follow-up. Thirty-six individuals who were past coffee drinkers or drank less than 1 cup/day were excluded leaving a total of 553 individuals for analysis. For individuals lost to follow-up, the last available BP values were taken into account. Results are presented using a dominant *1F allele model with *1A/*1F and *1F/*1F genotypes combined, because both groups have a similar rate of caffeine metabolism [11,12]. Participants were grouped into three categories of coffee drinking, nondrinkers (none), moderate drinkers (one to three cups daily) and heavy drinkers (four or more cups daily), a classification used in previous analyses [4,24]. Current smokers were those who reported smoking one or more cigarettes per day. Four categories of alcohol drinking were considered (0 g, <50 g, 50–100 g, >100 g of alcohol/day) [14]. As there were only two individuals in the more than 100 g/day alcohol class, the two upper classes of alcohol were subsequently combined. The significance of differences in categorical variables was assessed with the χ^2 test. Differences between individuals with CYP1A2 *1A/*1A genotype and carriers of *1F allele were assessed with unpaired *t* test adjusting for age and sex. The distribution of clinical variables were compared across classes of coffee consumption by ANCOVA adjusting for age and sex. We evaluated potential gene \times coffee interactions on baseline catecholamines and follow-up changes in systolic and diastolic BPs in a two-way ANCOVA after adjustment for age and sex. Changes in BPs were adjusted also for follow-up time and baseline BPs. For catecholamines, significance was given for log-transformed data. Within each CYP1A2 group, the risk of hypertension related to coffee intake was assessed in multivariable Cox proportional hazards models adjusting for sex, age, BMI, family history for hypertension, duration of hypertension, physical activity, smoking status, alcohol consumption, and baseline BP. Analyses were also performed within strata of alcohol use and smoking status. Coffee intake was also modelled as a time-dependent categorical variable in Cox proportional hazards analysis. No violations to the proportional hazards assumption were detected by inspection of survival curves. Estimates of hazard ratio and corresponding two-sided 95% confidence intervals (CIs) relating coffee consumption to risk of hypertension were computed from the Cox models. A two-tailed probability value less than 0.05 was considered significant. Data are presented as mean \pm SD unless specified. Analyses were performed using Statistica version 6 (Stat Soft, Inc, Tulsa, Oklahoma, USA) and Systat version 10 (SPSS Inc., Evanston, Illinois, USA).

Results

Twenty-seven percent of participants did not drink coffee, 62.9% were moderate coffee drinkers (1–3 cups/day), and 10.1% were heavy coffee drinkers (≥ 4 cups/day). Genotypes frequencies (*1A*1A=41%, *1A*1F=43%, *1F*1F=16%) were in agreement with the Hardy–Weinberg equilibrium ($\chi^2=0.81$, $P=0.36$). The proportion of *1F carriers did not differ between the categories of coffee intake ($P>0.7$, Table 1). Coffee drinkers were older ($P=0.000$) and slightly heavier ($P=0.072$) than abstainers, and were more likely to smoke cigarettes ($P=0.005$) and drink alcohol ($P=0.000$). Coffee intake was not related to gender and baseline BP. Demographic and risk factor characteristics of participants divided by genotypes are presented in Table 1. Baseline systolic BP was slightly higher in carriers of *1F allele than in homozygous for the *1A allele but the difference was of borderline significance ($P=0.063$). The other clinical variables did not differ between the groups. Plasma glucose, total cholesterol, high-density lipoprotein cholesterol, and triglycerides did not differ across categories of coffee consumption or CYP1A2 genotypes (data not shown).

Follow-up

During a median follow-up of 8.2 years only 24 individuals (4.3%) changed their habits. Of these, 11 individuals (2%) started to drink coffee or increased coffee consumption, whereas 13 individuals (2.3%) quit or reduced coffee use. During the follow-up, 323 individuals (58.4%) developed sustained hypertension needing antihypertensive treatment whereas 230 individuals did not meet the criteria for treatment. In the whole cohort, only small changes in clinic BP were observed during the follow-up ($-1.2 \pm 15.2/0.7 \pm 9.9$ mmHg). In the individuals who did not reach the endpoint BP at follow-up end declined to $133.6 \pm 8.1/85.3 \pm 6.1$ mmHg. In the individuals stratified by CYP1A2 genotype, an increased incidence of hypertension was found in coffee drinkers compared with abstainers among the carriers of the slow *1F allele (64.6 versus 47.8%, respectively, $P=0.006$) but not among the individuals homozygous for the *1A allele (55.1 versus 59.3%, respectively, P = not significant.). In a two-way ANCOVA, both CYP1A2 genotype and coffee were independent predictors of the follow-up increase in systolic BP with a highly significant interactive effect of the two variables on the BP change (Table 2). An inter-

active effect of CYP1A2 genotype and coffee was found also for diastolic BP. No effect of coffee or CYP1A2 genotype was found on adjusted follow-up changes in body weight, plasma glucose, total cholesterol, high-density lipoprotein cholesterol, or triglycerides. In a Cox proportional hazards analysis assessing the risk of hypertension needing antihypertensive treatment associated with coffee drinking in the whole group, coffee was a weak predictor of outcome ($P=0.04$). However, when participants were stratified by CYP1A2 genotype, after taking into account age, sex, parental hypertension, hypertension duration, physical activity, smoking status, alcohol intake, BMI, and BP at baseline, the increased risk of hypertension associated with coffee intake was observed only among carriers of the slow *1F allele (Fig. 1). In this group, the hazard ratio (95% CI) of hypertension was 1.72 (95% CI 1.21–2.44, $P=0.028$) for 1 to 3 cups/day and was 3.00 (95% CI 1.53–5.90, $P=0.001$) for 4 cups/day or more, as compared with abstainers. Among the participants who were homozygous for the rapid *1A allele there was even an inverse association between coffee intake and hypertension. The hazard ratios were 0.80 (95% CI, 0.52–1.23, $P=0.29$) for 1–3 cups/day and 0.36 (95% CI 0.14–0.89, $P=0.026$) for 4 cups/day or more. Inclusion of oral contraceptive use in women into the regression model did not materially alter the results. When coffee intake during follow-up was included in the model as a time-dependent covariate, or when more recent coffee intake was taken into account, results were similar to those seen for baseline coffee drinking. The association between coffee intake and risk of hypertension was not influenced by gender, smoking status at baseline, or alcohol use in either CYP1A2 group.

Urinary catecholamines according to coffee use and CYP1A2 genotype

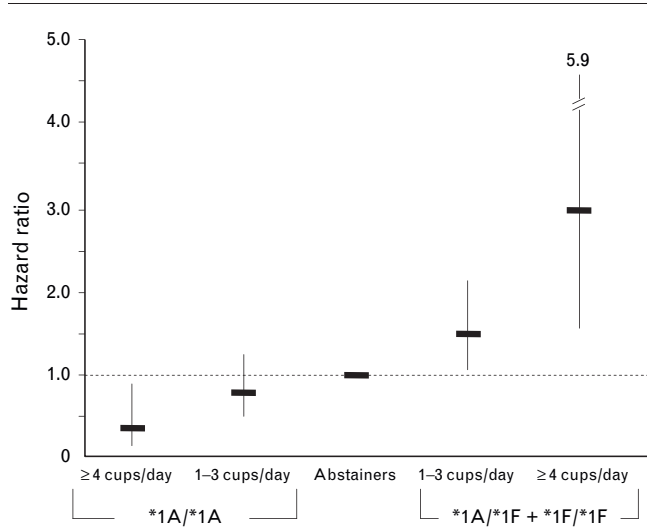
Urinary epinephrine was higher among coffee drinkers than nondrinkers ($P=0.012$). However, the between-category difference in urinary epinephrine was significant among carriers of the slow *1F allele and not among individuals with the *1A/*1A genotype (Fig. 2). In a two-way ANCOVA, an interactive effect of coffee and CYP1A2 genotype was observed on urinary epinephrine ($P=0.050$). Similar results were found for urinary norepinephrine (Fig. 2), but the between-category differences did not attain the level of statistical significance for either CYP1A2 group.

Table 2 Follow-up changes in blood pressure in the participants subdivided according to CYP1A2 genotype and coffee consumption. Results of two-way ANCOVA

	Cups/day coffee (*1A/*1A genotype)			Cups/day coffee (*1A/*1F + *1F/*1F genotypes)			P-values		
	0	1–3	≥ 4	0	1–3	≥ 4	Coffee	CYP1A2	Coffee \times CYP1A2
N	59	146	21	90	202	35			
Systolic BP	0.0 \pm 2.2	-0.4 \pm 1.3	-5.9 \pm 2.9	-2.8 \pm 1.7	1.0 \pm 1.1	2.8 \pm 2.3	0.050	0.001	0.000
Diastolic BP	1.3 \pm 1.4	0.4 \pm 0.8	-5.6 \pm 1.9	0.2 \pm 1.1	2.6 \pm 0.7	3.0 \pm 1.5	0.012	0.032	0.007

Data are means \pm SEM, and are adjusted for age, sex, baseline blood pressure, and follow-up length. BP denotes blood pressure in mmHg.

Fig. 1



Adjusted associations between CYP1A2 genotype (homozygous for the fast CYP1A2 *1A allele versus carriers of the slow *1F allele) and incident hypertension in 553 HARVEST participants. All hazard ratios are from multivariable Cox regression and represent risk of hypertension needing antihypertensive treatment for coffee drinkers versus abstainers (reference)*. *Adjusted for sex, age, BMI, family history for hypertension, duration of hypertension, physical activity, smoking status, alcohol consumption, and baseline blood pressure.

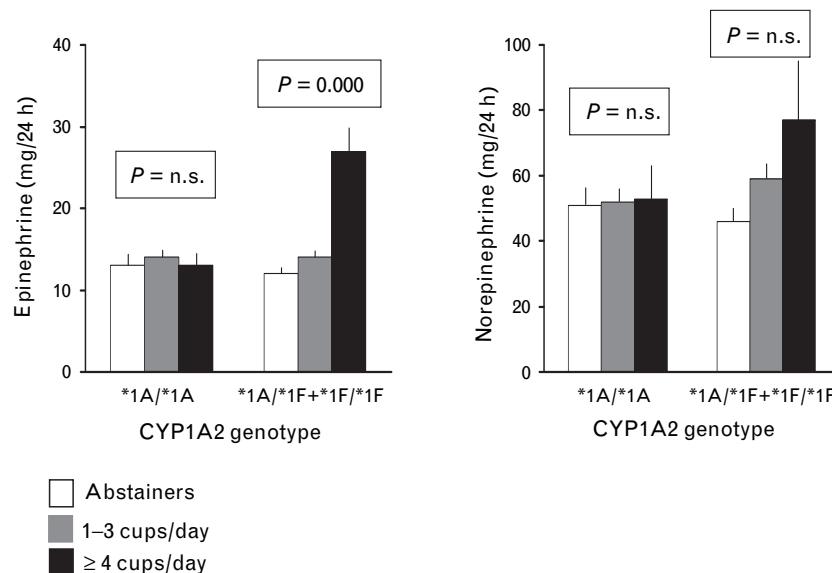
Discussion

In a previous analysis of the whole cohort of the HARVEST participants, we found a nonlinear association between coffee consumption and development of sustained hypertension [25]. We undertook the present

study in a subset of those individuals to investigate whether the CYP1A2 genotype modifies the relationship between coffee intake and risk of developing hypertension needing antihypertensive treatment. We found a linear relationship between coffee use and risk of hypertension among carriers of the *1F variant of the CYP1A2 gene and an inverse association among individuals homozygous for the *1A allele.

All guidelines recommend several dietary changes for preventing development of hypertension [21–24]. Coffee consumption is not included among the nonpharmacological measures, which should be instituted because the adverse effects of coffee on hypertension have constituted a controversial subject for several decades. Meta-analyses examining the relationship between coffee intake and risk of hypertension have observed a positive though weak association among short-term clinical trials [5,6] but not among prospective cohort studies. Two large prospective studies failed to demonstrate an independent association between coffee intake and incidence of hypertension [7,8]. In the study by Klag *et al.* [7] the association of coffee drinking with hypertension incidence ceased to be statistically significant after taking into account parental hypertension and lifestyle factors. In the study conducted in the Nurses' Health Studies I and II, a positive association was found between risk of hypertension and frequency of caffeinated soft drinks [8]. However, coffee was not associated with incident hypertension in either cohort. In a recent report by Funatsu *et al.* [26], coffee intake of more than three cups per day for 4 weeks even lowered BP in a small

Fig. 2



Baseline urinary epinephrine and norepinephrine normalized by 24-h creatinine output, by coffee consumption and CYP1A2 genotype in 298 HARVEST participants. Data are means \pm SEM, and are adjusted for age and sex. Statistical significance was calculated for log-transformed data. Individuals with *1A/*1A genotype, $N = 119$. Individuals carriers of *1F allele, $N = 179$.

group of prehypertensive men. At variance with the above data, in a recent study by Uiterwaal *et al.* [9] abstinence from coffee was found to be associated with lower risk of hypertension compared with moderate coffee drinking. In women, a lower risk of hypertension was found also for heavy consumers [9].

Ninety-five percent of caffeine is detoxified through an initial N³-demethylation catalyzed by CYP1A2, and caffeine is an inducer of the enzyme [10–12]. However, this enzyme has a wide interindividual variability in activity, which is regulated by a genetic polymorphism [27]. Thus, CYP1A2 genotype may have important influences on the pressor effect of coffee. Indeed, our results demonstrated that carriers of the *1F allele, who are slow caffeine metabolizers [11,12], had an increased risk of developing hypertension needing antihypertensive treatment which was proportional to coffee intake. Within the individuals with the fast *1A/*1A genotype an opposite trend was found. The CYP1A2 genotype-related difference was particularly striking among the individuals who drank at least 4 cups/day. If coffee exerts opposite effects on BP according to CYP1A2 genotype, it is not surprising that most studies, including our previous analysis [25], found a nonlinear association between coffee intake and risk of hypertension. In the Klag *et al.* study [7], the relative risk estimates increased only slightly with successive levels of coffee drinking, and decreased somewhat in the heaviest drinkers. In the women of both Nurses' Health Studies cohorts [8], and those of the Uiterwaal *et al.* study [9], even a modest inverse U-shaped relation was found between coffee intake and the incidence of hypertension.

Coffee is a complex 'blend' of a vast number of different bioactive chemicals, any of which may be responsible for its effect on BP [28]. Among these, polyphenols seem to act as protective antioxidants and have other beneficial actions on the cardiovascular system [28]. The polyphenols chlorogenic acid and dihydrocaffeic acid (both found in coffee) have been shown to increase nitric oxide synthase activity in a dose-dependent manner in cultured cells, which is associated with a comparable increase in endothelial nitric oxide synthase protein [29]. In spontaneously hypertensive rats, the development of hypertension was inhibited when rats were fed diets containing 0.5% chlorogenic acid for 8 weeks [30]. A BP-lowering effect of chlorogenic acid has been observed also in human studies. In 28 mild hypertensive individuals who were randomized in a double-blind placebo-controlled study to receive either 140 mg of chlorogenic acid or placebo for 12 weeks, the chlorogenic acid regimen caused a significant 10 mmHg lowering of systolic BP and 7 mmHg lowering of diastolic BP [31]. Thus, there seems to be a Jekyll and Hyde aspect to coffee whose overall action on the cardiovascular system appears to be regulated by the CYP1A2 gene. In individuals with the fast *1A/*1A genotype, the effect of

caffeine on BP seems to be negligible and outweighed by the hypotensive action of polyphenols or other bioactives. In carriers of CYP1A2*1F allele, who are slow caffeine metabolizers, the pressor effect of caffeine seems to prevail. Indeed, in the present study, after 8.2 years of follow-up our heavy coffee drinkers homozygous for the fast *1A/*1A genotype had a 9 mmHg lower BP than their counterparts with the slow *1F allele. This interpretation explains why in a meta-analysis of randomized controlled trials on the effect of intake of coffee and caffeine, BP elevations were reported to be much larger for caffeine than coffee [6]. In the Nurses Health Study I and II [8], caffeinated colas but not coffee were associated with hypertension, which may be due to the lack of polyphenols in the colas and the presence of them in the coffee. This mechanism may explain the differential effect of coffee found also in patients with myocardial infarction according to CYP1A2 genotype. Also in this clinical setting carriers of *1F allele had an increased risk of myocardial infarction with increasing coffee consumption and homozygous for *1A allele had a decrease in risk [13].

Urinary epinephrine and norepinephrine and vanillyl-mandelic acid have been shown to increase after caffeine administration in humans [32,33], and increased sympathetic activity is considered one main mechanism through which caffeine raises BP. In agreement with our previous results [24], in the present study there was a relationship between coffee intake and urinary epinephrine. However, the increase in epinephrine related to coffee consumption was found only among individuals with the CYP1A2*1F allele, whereas no effect of coffee on catecholamines was found among the individuals with the fast *1A/*1A genotype.

The absence of an association between coffee and risk of hypertension in some studies may have been due to a relatively low frequency of *1F carriers in the populations that were examined. In the HARVEST study, the frequency of carriers of the *1F allele was 59%, but lower frequencies have been reported in other populations [34,35].

Our results were obtained in a cohort of individuals screened for stage 1 hypertension with a low cardiovascular risk profile. According to international guidelines, these individuals should be followed for extended periods with nonpharmacological treatment [21–24]. Indeed, in many of the HARVEST participants, clinic BP decreased to below 140/90 mmHg during the first few months of observation, and in the group that did not meet the criteria for treatment mean final clinic BP was 133.6/85.3 mmHg. In the HARVEST study, the diagnosis of sustained hypertension was made on the basis of several visits encompassing an average of over 50 BP measurements per person using uniform criteria. However, a limitation of our study is that the definition of the

endpoint changed during the follow-up because treatment criteria for this category of individuals changed from 1990 to 2006. However, individual's recruitment was well balanced throughout the years across categories of either coffee intake or CYP1A2 genotype. In addition, besides categorical endpoint we also considered absolute changes in BP during the follow-up as outcome measure obtaining consistent results.

Limitations

Several limitations of this study should be noted. In the present study we used a short food frequency questionnaire and the results can only be interpreted as indicators of the dietary habits in general. Therefore, misclassification must be considered. Other weaknesses of our study include the incomplete nature of our questionnaire without information on dietary factors associated with hypertension incidence such as dietary intake of sodium (or measurement of sodium urinary output), potassium, and fiber. However, previous studies have shown that coffee consumption was associated to alcohol use, smoking and physical activity, which were available in our study, and not to other dietary habits [36]. In addition, we did not measure caffeine, polyphenols, or direct markers of CYP1A2 catalytic activity. However, urinary epinephrine concentration, which is related to caffeine intake [25,32,33], was proportional to coffee consumption and was more elevated in *1F allele carriers. These limitations notwithstanding, the major strength of our analyses is that they offer data-linking coffee intake and change in BP over time from a homogeneous cohort of young-to-middle-age individuals included within a narrow BP range. However, because we do not know of any other study on the effects of the CYP1A2 polymorphisms on the BP effects of chronic coffee use, the present findings should be confirmed in other studies.

In conclusion, the present data show that coffee has differential effects on BP among individuals screened for stage 1 hypertension according to CYP1A2 genotype. Abstinence from caffeinated coffee should be included among the dietary changes that should be instituted in prehypertensive or hypertensive patients who are carriers of the *1F allele. In individuals homozygous for the *1A allele coffee consumption can be allowed.

Acknowledgement

Sources of funding: The study was funded by the University of Padova, Padova, Italy, and from the Associazione '18 Maggio 1370', San Daniele del Friuli, Italy.

List of the centers participating in the HARVEST study:

Belluno – Cardiologia: G. Catania, R Da Cortà; Cremona – Divisione Medica: G Garavelli; Dolo – Divisione Medica: F Pegoraro, S Laurini; Mirano – Cardiologia: D D'Este; Padova – Clinica Medica 4: F Dorigatti, V

Zaetta, P Frezza, P Bratti, D Perkovic, C Guarnieri, A Zanier; Pordenone – Centro Cardioreumatologico: G Cignacco, G Zanata; Rovereto – Ala – Divisione Medica: M Mattarei, T Biasion; Rovigo – Cardiologia: P Zonzin, A Bortolazzi; San Daniele del Friuli – Area di Emergenza: L Mos, S Martina, O Vriz; San Donà di Piave – Cardiologia: L. Milani, C Canali; Trento – Divisione Medica: P Dal Ri, S Cozzio; Treviso – Divisione Nefrologia: G Calconi, P. Gatti; Vittorio Veneto – Divisione Medica: M Santonastaso, E Cozzutti, R Garbelotto, A Mazzer. Trial Coordinator: P Palatini.

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