Effects of Caffeine and Coffee on Incident Heart Failure in General Population. Role of the CYP1A2 -163C>A Polymorphism

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Abstract— This study investigates in epidemiological setting the effects of chronic caffeine and coffee intake on incident heart failure (HF) across the –163C>A polymorphism of CYP1A2 gene, mediating caffeine metabolism. We studied 1,475 unselected subjects from general population aged 60.0±16.7 years, genotyped for CYP1A2 –163C>A polymorphism and divided into fast (AA homozygous) and slow (C-carriers) caffeine metabolizers. Daily caffeine intake was calculated from a questionnaire and a dietary diary. Events due to HF were recorded during a 12-year follow-up. Multivariate Cox regression adjusted for confounders was used for statistical analysis. In the whole cohort, HF incidence decreased with increasing caffeine intake (hazard ratio, HR, 0.998, 95% confidence intervals, CI, 0.996-0.999, p=0.02). After stratifying by sex and genotype, this effect was still detectable in C-carrier men only (OR 0.994, CI 0.990-0.998, p=0.01). No effect was observed in women and in AA men. Cox estimates were significantly higher for coffee than for caffeine both in the whole cohort and in C-carrier men. At a population level, caffeine intake is protective against HF occurrence in slow-metabolizer men, and innocuous in other subjects. The protective effect of coffee is greater than that of mere caffeine.

Index Terms— caffeine, heart failure, CYP1A2 -163C>A, population

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I. INTRODUCTION

Coffee is the second most consumed beverage after water, and caffeine – its main active component – is the most used recreational compound worldwide [1]. Some studies were conducted by administering coffee or caffeine to volunteers in experimental setting [2,3], but such experiments cannot answer the question about the possible effects of long-term intake of coffee or caffeine in everyday life. Some epidemiological studies tried to associate coffee consumption to the occurrence of particular conditions or events [4-10]. Their results were in the majority of cases disappointing or frankly contrasting [6,7].

Incidence of heart failure (HF) in relation to caffeine or coffee consumption has been limitedly studied, very rarely in subjects from general population [11]-[13] and never taking into account the genetic control of caffeine fate in human body, which is largely determined by the –163C>A polymorphism of the CYP1A2 gene codifying for the CYP1A2 enzymatic protein [14]. This polymorphism can not be disregarded [15], as it has been shown than the effects of caffeine can be very different in the so-called "fast metabolizers" (AA homozygous) and "slow metabolizers" (carrying the C allele) [16]. Fortunately, these two categories are almost equally represented in general population, allowing good statistical analysis.

The present study is aimed at investigating in epidemiological setting 1) if caffeine intake influences the incidence of HF, 2) if the effects of coffee are different from those of mere caffeine, and 3) if these effects depend on the –163C>A polymorphism of the CYP1A2 gene. For these purposes, the setting represented by a large population-based epidemiological study was chosen.

II. METHODS

A. Study cohort and general protocol

The study cohort was represented by 1,475 unselected men and women aged 60.0±16.7 years (range 19.4 to 93.9) living in an area of about 550 km^2 in North-East Italy and sharing homogeneous lifestyle [17], randomly taken from general population in the frame of a population-based epidemiological study whose protocol has been diffusely described elsewhere [17-21]. In brief, all adults of the above-mentioned geographic area identified through the Register's Office were called by letter, and 1,475 of them adhered to the protocol, gave informed consent and were recruited and screened by a sole staff in *ad hoc* surgeries.

Height (in m) and weight (in kg) were measured using mechanical devices, with the subjects wearing light indoor clothing and no shoes [22]. Body mass index was calculated in kg/m^2 from the weight / squared height ratio. Left ventricular hypertrophy was diagnosed on the basis of the Sokolow-Lyon or Cornell criteria [23],[24]. All subjects underwent a Rose's questionnaire [25] concerning personal data, lifestyle, smoking, quality of life and personal and familial anamnesis. Diabetes [26] and the New York Heart Association (NYHA) class [27] were determined according to current guidelines.

Blood pressure was measured in triplicate by trained medical doctors by means of an automatic device. The last measurement was used in data analysis to minimize any alarm reaction.

Daily caffeine intake was calculated adding that deriving from coffee, tea, chocolate and cola [14] examining both the anamnestic report at the screening and a 7-day dietary diary. Mean individual amount of daily caffeine (mg/day) taken in normal life was used in statistical analysis. According to the same dietary questionnaires, intake of ethanol (g/day) was calculated [21] adding that deriving from wine, beer, liquors and aperitifs. The number of cigarettes smoked per day was ascertained from a questionnaire. This was necessary because both ethanol and smoke are known to influence the expression of CYP1A2 gene [28].

B. Assessment of events

Incidence of cardiovascular events was obtained from the Italian Register's Office according to the international standardized classifications commonly used for epidemiological purposes, and double-checked by referring to hospitals, retirement homes or physicians' files. Fatal and non-fatal events due to HF were defined according to the World Health Organization International Statistical Classification of Diseases-10 (ICD-10), using the codes from I50.1 to I50.4, or I50.9.

C. Ethics

The investigation conformed to the Declaration of Helsinki and institutional guidelines, and was approved by the Ethics Committees of the University of Padua, of the University Hospital of Verona, and of the Local Health Units No. 4 and No. 20 of the Veneto Region (Italy). Each subject gave and signed informed consent including treatment of genetic data.

D. Genotyping

At screening, 6 ml of whole blood were collected in EDTA tubes for the determination of genotype. DNA was extracted using MagNA Pure 96 DNA and viral NA small volume and large volume kits, according to the protocol provided by the manufacturer (Roche Diagnostics GmbH, Penzberg, Germany). Primers and probes for specific allelic discrimination analysis of CYP1A2 polymorphism were included in the Polymerase Chain Reaction (PCR) assay. The primers and probes used were as follows: forward primer 5'-TTT CCA gCT CTC AgA TTC TgT gAT, reverse primer 5'-ggA TAC CAg AAA gAC TAA gCT CCA TC, CYP1A2*1F probe 5' 6FAM-TCT gTg ggC ACA ggA CgC ATg g, CYP1A2*1A probe 5' HEX-CTC TgT ggg CCC Agg ACg CAT as described by dbSNP reference number (rs762551) [29]. Purified DNA was amplified in a real-time PCR reaction in the LightCycler 480 with Gene Scanning software version 1.5.1 Roche. All the reactions were performed in 96-well plates. 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 in pre-incubation, 45 cycles at 95°C for primer-dependent amplification and 66.5°C for annealing.

E. Statistics

Preliminary power analysis showed that 329 subjects per cell were sufficient to show effects with a power of 0.90 and a test level of 0.10 for β error and of 0.05 for α error, assuming a putative difference of 7 percent points in HF incidence between highest and lowest caffeine consumers. This difference was chosen *a priori* on the basis of on preliminary tests performed in our Laboratory, as to our knowledge no data on the effects of increasing daily doses of caffeine on HF in general population are available in literature. Therefore, the cohort of 1,475 subjects recruited for the present study appeared to be adequate also after stratification into quartiles of 369 cases each. Linearity assumption of continuous variables was ascertained for each variable by the residuals method and normality assumption by the Kolmogorov-Smirnov one-sample test.

Continuous variables were expressed as mean ± standard deviation, and compared with analysis of variance. Preliminary logarithmization was preformed for those that were putatively not independent from each other. Continuous variables were compared with the analysis of variance and categorical variables with the χ^2 test.

Cox proportional hazard model was used to find the variables having a prognostic role on HF incidence (<0.10 to enter and remove) and to calculate the hazard ratios (HR) with 95% confidence intervals (CI). An exploratory analysis of the full model demonstrated that age, sex, ethanol intake, smoking, diabetes, systolic and diastolic blood pressure, heart rate, waist-to-hip ratio, left ventricular hypertrophy, NYHA class and history of HF were potential predictors of incident HF during the follow-up. They were therefore used as covariables in the Cox models, together with daily caffeine intake or – separately – with daily servings of coffee.

As the *–163C>A* polymorphism of CYP1A2 gene notoriously controls the metabolism and therefore the effects of caffeine [1],[14], an interaction term between caffeine intake and genotype was introduced as a covariable in the above-mentioned multiple analyses. According to current literature [30],[31], subjects carrying the C allele (C-carriers) were considered together both in univariate analysis for comparison to AA homozygous and in multivariate analysis for the determination of the predictors of incident HF. As sex was a significant predictor, Cox analyses were also performed including the dichotomic covariable «C-carrier or AA homozygous» separately in men and women.

III. RESULTS

A. Univariate descriptive statistics

Characteristics of the cohort. The general characteristics of the cohort, also stratified by sex, are shown in Table 1. Caffeine intake was in average 240.3±116.4 mg/day and came from coffee for 89.1% (214.1±109.5 mg/day in average, CI 208.5 to 219.7), from tea for 10.2% (24.6±37.1 mg/day in average, CI 21.6 to 15.4), and from other sources (chocolate or cola) for 0.7% (1.4±3.9 mg/day in average, CI 1.2 to 1.6).

Table 1. General characteristics of the population, also stratified by sex. Mean ± standard deviation (95% confidence intervals in brackets). NYHA: New York Heart Association; BP: arterial blood pressure; HF: heart failure: LVH: left ventricular hypertrophy.

Items	All (n=1,475)	Men (n=669)	Women (n=806)
Age (years)	60.0±16.7 (59.2-60.9)	58.2±16.4 (56.9-59.4)	61.5±16.9 (60.4-62.7)
NYHA class	1.12±0.45 (1.10-1.15)	1.09±0.40 (1.06-1.12)	1.15±0.49 (1.12-1.19)
Waist-to-hip ratio	0.90±0.09 (0.89-0.90)	0.93±0.08 (0.93-0.94)	0.87±0.08 (0.86-0.87)
Systolic BP (mmHg)	156.1±26.1 (154.7-157.4)	155.9±24.0 (154-157.8)	156.2±27.7 (154.2-158.1)
Diastolic BP (mmHg)	88.3±11.4 (87.7-88.9)	89.8±10.9 (89.0-90.7)	87.0±11.6 (86.2-87.9)
Heart rate (bpm)	69.4±10.6 (68.9-69.9)	67.2±10.5 (66.4-68.0)	71.2±10.3 (70.5-71.9)
Smoking (cigarettes/day)	1.6±4.5 (1.3-1.8)	2.2±5.6 (1.8-2.6)	1.0±3.1 (0.8-1.2)
Ethanol intake (g/day)	27.0±31.6 (25.4-28.6)	43.5±38.4 (40.6-46.4)	13.3±13.8 (12.4-14.3)
Caffeine intake (mg/day)	240.3±116.4 (234.4-246.3)	241.3±117 (232.4-250)	239.5±115.8 (231.5-247.5)
History of HF (0: no; 1: yes)	38 (2.58%)	23 (3.44%)	15 (1.86%)
LVH (0: no; 1: yes)	714 (52.50%)	308 (50.91%)	406 (53.77%)
Diabetes (0: no; 1: yes)	279 (18.9%)	138 (20.6%)	141 (17.5%)

CYP1A2/–163C>A polymorphism. In the whole population the distribution of the *–163C>A* polymorphism of CYP1A2 gene was AA 44.1%, AC 43.3% and CC 12.6% and respected the Hardy-Weinberg equilibrium. After stratification by sex, the distribution was 46.9% AA, 40.4% AC and 12.7% CC in men, and 41.7% AA, 45.8% AC and 12.5% CC in women. The C-carriers were 825 (55.9% of the whole cohort), i.e. 470 women and 355 men.

Incidence of HF. Incident events are summarized in Table 2. During the 12 years of follow-up, 125 new cases of HF were recorded (8.5%). Incidence of HF was similar in men (8.8%) and women (8.2%, p=0.7), and higher in subjects labelled as having history of HF in comparison to those free from this trait (18.4% vs. 8.2%, p<0.05). Independent of

caffeine and coffee consumption, incidence of HF was similar in the AA and in the C-carriers (9.4 vs. 7.7%, p=0.26).

Table 2. Incidence of non-fatal and fatal cardiovascular events. Percents in brackets. CAD: coronary artery disease; TIA: transient ischaemic attack.

Cardiovascular events	All (n=1,475)		
	Total	Non-fatal	Fatal
All	*695 (47.1)*	*557 (37.8)*	*138 (9.3)*
Heart failure	125 (8.5)	100 (6.8)	25 (1.7)
Arterial hypertension	136 (9.2)	134 (9.1)	2 (0.1)
CAD	136 (9.2)	80 (5.4)	56 (3.8)
Stroke or TIA	103 (7.0)	71 (4.8)	32 (2.2)
Dysrythmias	110 (7.4)	108 (7.3)	2 (0.1)
Other and minor	85 (5.8)	64 (4.4)	21 (1.4)

Effects of caffeine and coffee. Considering caffeine deriving from any source and independent of the genetic pattern, HF incidence decreased with increasing daily caffeine intake (Figure 1, upper panel; p<0.00001).

Considering coffee consumption instead of intake of caffeine from any source, HF incidence decreased with increasing daily servings of coffee (Fig. 1, lower panel; p<0.0001).

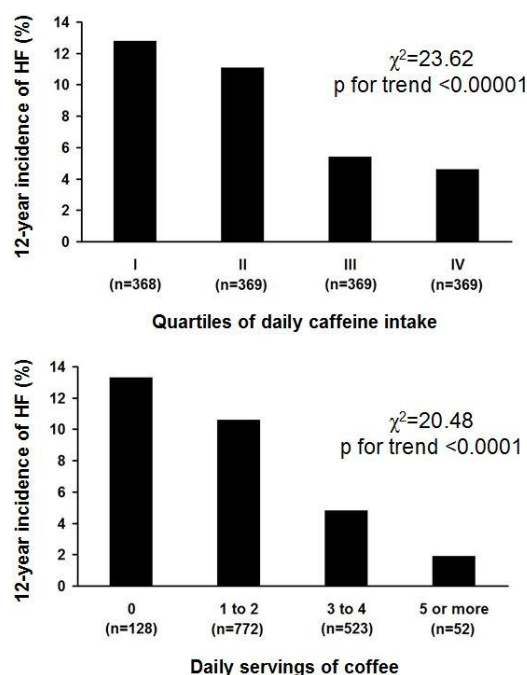Incidence of HF was not associated to intake of tea, chocolate and cola (data not show).

Fig. 1. Incident heart failure by quartiles of caffeine (upper panel) and by cups of coffee (lower panel). A significant trend towards lower incidence is observed with increasing daily caffeine or coffee intake.

B. Multivariate statistics

Cox analysis for any-source caffeine. Caffeine intake had a protective effect on incident HF in the whole population (OR 0.998, CI 0.996 to 0.999, p=0.02). An interaction term between caffeine and the variable «C-carrier or AA homozygous» was accepted in the equation (HR 1.0008, CI

1,0002 to 0.0014, p<0.02); sex resulted to be a significant predictor of HF as well (HR 1.915, CI 1.220 to 3.008, p<0.005). After stratifying according to sex and genotype, a protective effect of caffeine against HF was detectable in C-carrier men, but neither in C-carrier women nor in AA homozygous independent of sex (Fig. 2). Attention was therefore focused on the C-carrier men.
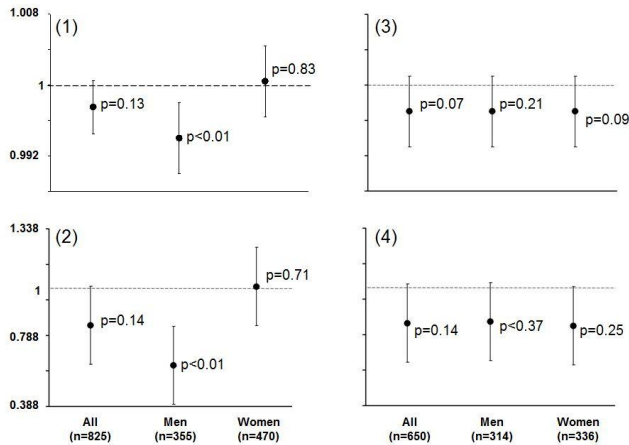

Fig. 2. Adjusted OR with 95% CI of heart failure for caffeine and coffee intake in the different genotypes. Multivariate Cox analysis had incident heart failure as dichotomic dependent variable, mg/day of caffeine [panel (1) in C-carriers, panel (3) in AA homozygous] or serving/day of coffee [panel (2) in C-carriers, panel (4) in AA homozygous] as independent variable, and age, sex, ethanol intake, smoking, diabetes, systolic and diastolic blood pressure, heart rate, waist-to-hip ratio, left ventricular hypertrophy, NYHA class and history of HF as confounders.

Cox analysis for coffee. When the Cox analysis was repeated using daily cups of coffee, the results were similar to those obtained for daily caffeine intake, with a protective effect of increasing coffee consumption in the entire cohort and in the C-carrier men only (Fig. 2).

Caffeine vs. coffee. In multivariate Cox analysis the estimates of coffee consumption for the whole cohort (-0.170, SE=0.089, p<0.03) and for the C-carrier men (–0.476, SE=0.182, p<0.009) were significantly higher in absolute values than those for caffeine intake (-0.002, SE=0.001, p<0.03; and -0.004, SE=0.001, p<0.008, respectively).

Analysis of C-carriers. Fig. 3 shows the Kaplan-Meier statistics in the C-carriers in relation to intake of caffeine (left panels) and coffee (right panels).
The upper panels include all the C-carriers, while in the lower panels only the men are taken into account. Plausible aggregations and their statistics are indicated.

Cox analysis for tea and other sources of caffeine. When the Cox analysis was repeated using daily cups of tea or other sources of caffeine (chocolate, cola), no predictive role on incident HF was found, independent of the *–163C>A* polymorphism of the CYP1A2 gene, the only direct predictors being age, male sex, higher NYHA class and left ventricular hypertrophy (data not shown). Due to the low number of subjects consuming caffeine from these sources, no stratification by genotype was possible.

IV. DISCUSSION

A. Background

In this unselected sample of 1,475 men and women from general population followed-up for 12 years, both caffeine and coffee intake inversely predicted incident HF. This is in line with previous papers demonstrating a protective effect of coffee [32], and in contrast with other ones showing its possible detrimental [13], neutral [33,34] or J-shaped effect [35].
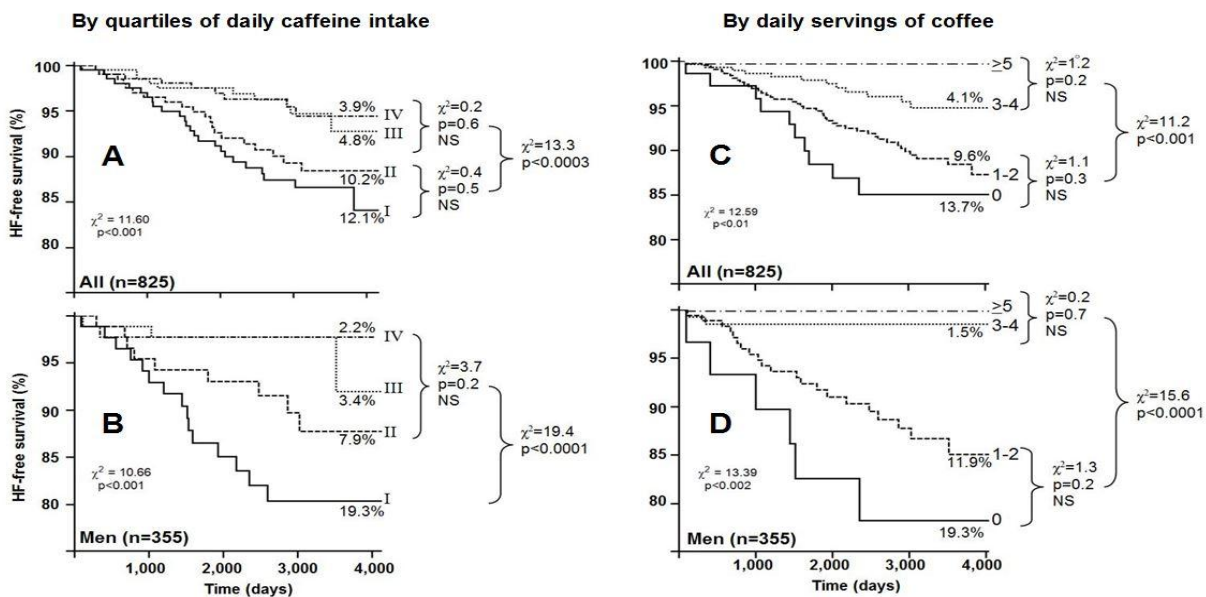Caffeine is the most represented active compound of

Fig. 3. Kaplan-Meier analysis in C-carriers only. *Left panels*. Kaplan-Meier analysis by quartiles of caffeine intake in all the 825 subjects (upper panel) and in the 355 men (lower panel). I, II, II and IV indicate the four quartiles, and percent numbers indicate, for each quartile, the 12-year incidence of heart failure. In all the C-carriers, 210 mg/day caffeine is the cut-off separating the first 2 from the last 2 quartiles. *Right panels*. The same analysis by daily cups of coffee in all (upper panel) and in men (lower panel). 0, 1-2, 3-4 and ≥5 indicate daily servings; % indicate, for each line, the 12-year incidence of heart failure. χ^2 and p for trend are show in the middle of the figure. Two cups/day is the cut-off separating the first two from the last two lines in both panels.

coffee, and coffee is in turn the main source of dietary caffeine worldwide [14]. Nevertheless, whole coffee contains many other substances acting as agonists. In the study presented herein, caffeine and coffee will therefore be discussed separately.

B. Caffeine

In univariate analysis, incidence of new cases of HF decreased significantly with increasing caffeine intake from any source, passing from 12.8% in twelve years in subjects consuming <160 mg/day of caffeine to 4.6% in those consuming >320 mg/day. Not only this, but in multivariate analysis caffeine intake was an inverse predictor of incident HF independent of age, sex, NYHA class, diabetes, arterial blood pressure, smoking habits, ethanol intake, body adiposity, heart rate, caloric intake and even history of HF. Actually, even selecting subjects free from HF at initial screening did not change the model.

The fate of caffeine in the body is notoriously influenced by the _–163C>A_ polymorphism of CYP1A2 gene, that codifies for a hepatic enzyme oxydating caffeine and accounting for about 95% of its clearance [14]. The effects of caffeine intake is therefore different across this polymorphism [14,31,36]. Actually, the effects of caffeine are very different in slow metabolizers (C-carriers) and in fast metabolizers (AA homozygous) [15].

In our experience, no beneficial effects of caffeine and coffee were detectable in AA subjects.

Taking into account the C-carriers, a protective effect against incident HF was only detected in men. It is likely that in slow metabolizers caffeine body pool tends to remain higher due to reduced oxidation, leading to greater effect in all organs (caffeine is widely distributed through the body [14]). Caffeine induces Ca^{++} release from the sarcoplasmic reticulum and inhibits its reuptake [37,38]. As a consequence, the intracellular Ca^{++} decay is slowed down. Intracellular Ca^{++} in turns determines the activation of endothelial nitric oxide synthase, with production of higher quantities of nitric oxide, so increasing muscular contractility [39]. This effect, more evident in slow than in fast metabolizers, could hypothetically lead to chronically better heart function in the former than in the latter. On the other hand, this hypothetical mechanism cannot be the only one involved in protection against incident HF, as other recreational compounds having inotropic effect show no protective action and can on the contrary favour HF occurrence [40].

C. Coffee

Coffee contains many active compounds that, although not directly associated to heart contractility, could play a role in influencing HF occurrence.

In our experience, in univariate analysis incidence of new cases of HF during the follow-up decreased significantly with increasing coffee intake, passing from 13% in subjects abstaining from coffee to 2% in those consuming ≥5 servings/day. Furthermore, in multivariate analysis, daily cups of coffee inversely predicted incident HF independent of age, sex, NYHA class, diabetes, arterial blood pressure, smoking habits, ethanol intake, body adiposity, heart rate, caloric intake and even of history of HF. This multivariate

trend was limited to the C-carrier men. Selecting subjects free from HF at initial screening did not change the model. Our data therefore demonstrate that coffee consumption was not only innocuous or neutral as suggested for instance by Kelly and Granger [41] or – at a population level – by Ahmed et al. [34] and by Lopez-Garcia et al. [42], but even protective, at least in men carrying the C allele of _–163C>A_ polymorphism. Other Authors occasionally found a positive effect of coffee against HF [32], but they had no information about genotype, which made their conclusions less authoritative.

Cox estimates of coffee consumption were significantly higher in absolute values than those of caffeine intake, suggesting that the protective of coffee was greater than that of caffeine. In other words, it is plausible that the additional compounds of coffee could add a surplus value, making coffee more protective than mere caffeine against incident HF.

D. Strength and limitations

The strength of our study is that it is population-based and it is the first one taking into consideration the CYP1A2 – _163C>A_ pattern when analyzing the effects of caffeine and of coffee on incident HF separately in men and women. A limitation is represented by the fact that Italian «espresso» coffee was consumed, so that the results could not be directly applied to other types of coffee or serving. Consumption of tea, chocolate and cola was too low to allow statistical analysis.

E. Conclusions

At a population level, caffeine is protective against HF occurrence in men carrying the C allele of _–163C>A_ CYP1A2, and innocuous in other people. Coffee is more protective than caffeine. The results of the present study are limited to HF, and the reader must be aware that caffeine and coffee could have detrimental effects on other organs and functions [3,9,15,43].

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