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Prognostic cardiovascular cut-off values of dietary caffeine in a cohort of unselected men and women from general population

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Cut-off values;
Population

Abstract *Background and aims:* Among an unselected cohort of men and women from general population (n = 1.668), the prognostic effects of being over the cut-off of all-source dietary caffeine intake were studied.
Methods and results: Prognostic cut-off values for coronary events, incident heart failure (HF), cerebrovascular events (CBV) and arrhythmic events (ARR) were found by means of the receiver-operating-characteristic curves method. Those for HF (>230 mg/day), for CBV (>280 mg/day) and for ARR (>280 mg/day) were confirmed in multivariate Cox analysis adjusted for age, body mass index, circulating thyroid hormone, diabetes mellitus, arterial hypertension, smoking, dietary intake of ethanol, basal heart rate, low-density-lipoprotein cholesterol, forced expiratory volume in 1 s and β-blocking therapy. Being over these cut-off values was associated to a reduced hazard ratio during the follow-up in the whole cohort (HR 0.678, 95%CI 0.567–0.908, p = 0.009 for HF; 0.651, 95%CI 0.428–0.994, p = 0.018 for CBV; 0.395, 95%CI 0.395–0.933, p = 0.022 for ARR) and in men (0.652, 0.442–0.961, p = 0.029; 0.432, 0.201–0.927, p = 0.03; 0.553, 0.302–1.000, p = 0.05, respectively) but not in women. The caffeine-induced risk decrease observed in the whole cohort is therefore entirely attributable to men. In the case of HF, heart rate entered the risk equation in a positive manner without rejecting caffeine. The *−163C>A* polymorphism of the CYP1A2 gene, codifying for ability to metabolize caffeine, introduced in sensitivity analysis, did not alter the prognostic models.
Conclusion: Men introducing >230 mg/day caffeine show a reduced risk of HF, and those introducing >280 mg/day a reduced risk of CBV and ARR independent of genetic pattern.

1. Introduction

Several studies focused on the effect of caffeine on cardiovascular (CV) outcome, both administering caffeine and analysing the effects of caffeine intake in human groups [1–4]. The results of these studies were often disappointing or frankly contrasting [3–9].

Coffee rather than dietary caffeine (DC) from all sources (coffee, cocoa, tea, cola and energizing beverages) was

Acronyms: ARR, arrhythmic events; BBT, beta-blocking therapy; BMI, body mass index; CBV, cerebrovascular events; CI, confidence intervals; CV, cardiovascular; DC, dietary caffeine; FT4, circulating thyroxin; FEV1, forced expiratory volume in 1 s; HF, heart failure; HR, hazard ratio; HRT, heart rate; LDLC, low-density-lipoprotein serum cholesterol; ROC, receiver operating characteristics.

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mainly used to this purpose. Only a limited number of studies were performed in the frame of general population.

DC is usually taken into consideration by researchers and clinicians as a continuous variable, while no clear prognostic cut-off value of DC is available, as their detection requires data from general population which are within the reach of few researchers only.

The present study is aimed 1) at defining in epidemiological setting and in population-based context the cut-off values of DC, if any, having a CV prognostic value, 2) to confirm them in men and women in multivariate analyses, and 3) to establish whether DC, when taken in doses higher to the cut-off, really influences the incidence of CV outcome, namely coronary, cerebrovascular, heart failure and arrhythmic events. In sensitivity analysis, other aims are at defining whether these effect depend on an effect of DC on heart rate (HRT) [10] or on β-blocking therapy (BBT) [11], and/or on the *−163C>A* polymorphism of the CYP1A2 gene [12].

2. Methods

2.1. Study population

The present analysis is based on unselected men and women living in North-East Italy and sharing a homogeneous lifestyle, randomly taken from the adult general population in the frame of an epidemiological study having genetic analysis and a very good phenotype and with a median follow-up period of 13.9 years (interquartile range from 6.7 to 17.2 years) [12,13]. In brief, all individuals aged ≥18 years residing in Northern Italy in an area of about 700 km^2 were identified through the Register Office and invited by letter and then by phone call to take part in the study, irrespective of any personal characteristics. The 1668 who a) accepted, b) gave informed consent, and c) had all the variables necessary for the analysis constituted the population-based non-selected cohort object of the present study. All subjects underwent CV assessment, anthropometric measurements, blood test and a detailed anamnestic questionnaire. A dietary diary was compiled in the week following the visit according to the methods described by our group [14].

2.2. Ethics

The study was performed according to the Declaration of Helsinki for Human Research (41st World Medical Assembly, 1990). The processing of the patients' personal data collected in this study comply with the European Directive on the Privacy of Data. All data to be collected, stored and processed are anonymized, and all study-related documents are retained in a secure location. No personal information is stored on local personal computers. Approval was sought from the Ethical Committee of the University Hospital of Padua and from the 4 Local Health Unit 4 of the Veneto Region (Italy). Each subject gave and signed informed consent to the study, also including treatment of genetic data.

2.3. Detection and calculation of variables

Daily DC intake was calculated from the formula: $caffeine_{mg/day} = 80 \times coffee_{cups/day} + 5 \times decaffeinated\ coffee_{cups/day} + 22.6 \times teacups/day + 40 \times cola_{drinks/day} + 30 \times chocolate_{portions/day} + 80 \times energizer\ beverages_{drins/day}$.

Daily ethanol intake from was calculated from: $ethanol_{g/day} = 0.85 \times (0.12 \times wine_{ml/day} + 0.05 \times beer_{ml/day} + 0.42 \times liquors_{ml/day} + 0.11 \times aperitifs_{ml/day})$, where 0.85 is ethanol density. Arterial blood pressure was measured in triplicate with an Omron M3 Comfort IntelliWrap (https://www.omron-healthcare.it/it/homepage) after 10-min rest in clinostatic posture, and averaged. Arterial hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or current treatment with antihypertensive drugs. Body weight was measured by an electronic balance in light underwear, and body mass index (BMI) was calculated in kg/m^2 from the ratio weigh/heith^2. Diabetes mellitus was diagnosed if blood glucose was >126 mg/dl at fast or >200 mg/dl in the post-prandial period or >200 mg/dl in casual measurement, or if glycated haemoglobin was >65%, or in presence of antidiabetic treatment. Low-density-lipoprotein cholesterol (LDLC) was calculated with the Friedewald formula. Forced expiratory values in the first minute (FEV_1) was measured with a dry spirometer and individualized to each subject according to percent of theoretical value.

2.4. Outcomes

Vital status and events were monitored for 20 years. The date and cause of death was obtained from the Register Office, that is very precise in Italy. Incident events due to acute myocardial infarction, angina pectoris, heart failure, stroke, transient ischemic attack, arrhythmic events and hypertensive complications were taken into consideration during the follow-up (see Table 1s in Supplemental Materials for ICD10 codes). Events were double-checked with hospital and physicians' files.

2.5. Statistics

The SAS package version 9.4 (SAS Institute, Cary, NC) was used for statistical analysis. A preliminary power analysis based on differences from stratified values of daily DC intake for $\alpha = 0.05$ and power $(1\text{-}\beta) = 0.80$ was performed. To our knowledge, no study exists about possible cut-off values of DC discriminating subjects into doomed to and not doomed to develop CV events in general population. Consequently, based on previous work of our research staff, we considered 1 mg/day DC as a possible difference able to stratify subjects according to the above mentioned outcome. Power analysis showed that the number of subjects in our wide cohort of general population represented a sample largely sufficient to avoid β error.

As caffeine notoriously increases basal HRT [10], the determinants of HRT were previously examined by logistic regression analysis having HRT as dependent variable and sex, age, diabetes, hypertension, circulating thyroxin (FT4),

Table 1 General characteristics of the cohort. Mean values ± standard deviation (SD), and 95% confidence intervals (95% CI) are shown or percentage where appropriate.

Variables	All (n = 1678)	Men (n = 751)	Women (n = 917)	*p* men vs. women
Age (years)	59.6 ± 17.1 (58.8–80.5)	57.7 ± 16.7 (56.5–58.9)	61.3 ± 17.3 (60.1–62.4)	<0.0001
BMI (kg/m^2)	26.6 ± 4.4 (26.1–26.5)	26.7 ± 3.7 (26.5–27.0)	25.9 ± 4.8 (25.6-26-3)	<0.0001
Ethanol intake (g/day)	32.6 ± 36.8 (30.9–34.4)	51.6 ± 42.9 (48.5–54,7)	17.0 ± 17.1 (15.8–18.1)	<0.0001
Caffeine intake (mg/day)	183.2 ± 122.3 (177.3–189.0)	180.1 ± 123.9 (177.2–195.0)	180.8 ± 121.2 (173.0–188.7)	0.4
Basal heart rate (bpm)	69.6 ± 10.5 (69.1–70.1)	67.4 ± 10.5 (66.7–68.2)	71.4 ± 10.2 (70.8–72.1)	<0.0001
LDLC (mg/dl)	149.9 ± 38.2 (148.0–151.8)	146.5 ± 35.6 (143.9–149.1)	152.6 ± 40.1 (150.0–155.3)	0.001
Circulating FT_4 (ng/l)	13.1 ± 2.3 (12.9-13.2)	12.5 ± 2.2 (12.3-12.7)	13.5 ± 2.3 (13.3–13.7)	<0.0001
FEV_1 (% individual theoretical values)	81.7 ± 19.8 (80.7–78.9)	77.5 ± 19.0 (75.1-78.9)	85.1 19.7 (83.7–86.4)	<0.0001
Diabetes mellitus (%)	196 (11.7)	95 (12.6)	101 (11.0)	0.3
Smoking (%)	263 (15.8)	134 (17.9)	129 (14.1)	<0.0005
Arterial hypertension (%)	1313 (78.7)	621 (82.7)	692 (75.5)	<0.0001
β-blocking therapy (%)	66 (4.0)	25 (3.3)	41 (4.5)	0.2

LDLC: low-density-lipoprotein cholesterol. FT_4: circulating free thyroxine; FEV_1: forced expiratory volume in the 1st second.

smoking, β-blocking therapy, ethanol intake and DC intake as confounders.

Linearity assumption of continuous variables was ascertained 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. Variables putatively not independent from each other were previously logarithmized. Categorical variables were compared by means of the Pearson $\chi 2$ test. The null hypothesis was always rejected for values of $p < 0.05$.

The receiver operating characteristic (ROC) curves method was used to search for prognostic cut-off of DC for each CV event in the whole database and by sex. Dietary caffeine was used as basic variable and CV events as dichotomic classification variable. The De Long et al. method [15] was used. Ratio of cases in the positive group (prevalence), sensitivity and specificity were calculated. ROC curves were generated in the whole database, and prognostic cut-off values were identified as the curve point nearest to the 100% of axis of the ordinates. In practical terms, this was made by identifying the DC value associated to the highest values of the sum sensitivity + specificity. Youden's index defined for all points of ROC curves was used as a criterion for selecting the optimum cut-off. The area under the curve was also shown for each ROC curves analysis [16].

The cut-off values of DC identified by mean of the ROC curves were used as independent variables in separate multivariate Cox analyses using each event as dichotomous dependent variable and the term *DC>cut-off* as the independent covariables, and adjusting for the confounders already identified in preliminary analysis (age, sex, basal HRT, BMI, LDLC, FEV_1, BBT, ethanol intake, diabetes mellitus, arterial hypertension, smoking and blood pressure). When sex resulted to be a significant confounder, the analyses were repeated in both sexes separately. A cut-off value identified *via* the ROC curves method was considered as valid if accepted in the model, being the null hypothesis rejected, otherwise it was considered a false cut-off. The corresponding HR with 95% confidence intervals (CI) were obtained from each analysis. Outcome curves according to the Kaplan-Meier non-parametric estimator of limit product were produced. Log-rank tests were used to assess differences between curves.

Since both DC and BBT notoriously influence CV risk mainly through an effect on HRT, the analyses were repeated in sensitivity adding separately the interaction terms *DCxHRT*, as well as *DCxBBT* and *DCxHRTxBBT*.

In sensitivity analyses, the role of the *−163C>A* polymorphism of the CYP1A2 gene was investigated by including it as AA (43.9%), AC (43.4%) and CC (12.7%), and as AA (43.9%) and AC+CC (66.1%) [17] in the Cox analyses described above. According to current literature [18], subjects carrying the C allele (C-carriers, i.e. slow metabolizers) were also considered together for comparison to AA homozygous.

3. Results

3.1. Descriptive statistics of caffeine as a continuous variable

3.1.1. General data

The general characteristics of the 1668 subjects aged 59.63 ± 17.1 years, also stratified by sex, are shown in Table 1.

During 20,370 person-years of follow-up, 632 participants experienced CV events (31 per 1000 age-adjusted person-years), 281 men (31.5 per 1000 age-adjusted person-years) and 351 women (30.6 per 1000 age-adjusted person-years).

In logistic regression model adjusted for the confounders listed above, HRT was predicted by age (p = 0.038, p = 0.039 and p = 0.005 in the whole cohort, in men and in women), by caffeine intake (p = 0.001, p = 0.003 and p = 0.001, respectively) and by ethanol intake (p = 0.01, p = 0.012 and p = 0.014, respectively).

3.1.2. Search and validation of cut-off values

The cut-off values of DC for the CV events are shown in the left panels of Table 2. The ROC curves produced plausible

Table 2 For each cardiovascular event, the prognostic cut-off values of caffeine intake (mg/day) with 95% confidence intervals (CI) are detected and validated, both in the whole cohort and after stratification in men and women.

Outcome	Detection of cut-off values (ROC curves method)						Validation of cut-off values		
	Youden index J	AUC	Sens (%)	Spec (%)	*P* value	cut-off values	HR (95% CI)	Z statistics	*P* value
Coronary events									
in all	0.145	0.557	79.4	35.1	0.006	>280	0.487 (0.464–0.512)	−2.877	0.004
in men	0.148	0.557	76.4	38.4	0.009	>280	0.487 (0.298–0.797)	−2.877	0.004
in women	0.148	0.556	81.2	32.5	0.07 (N.S.)	>280	N.A.	N.A.	N.A.
Cerebrovascular events									
in all	0.169	0.579	81.8	35.1	0.0004	>280	0.339 (0.262–0.580)	−4.671	<0.0001
in men	0.262	0.616	87.1	39.1	0.0004	>280	0.227 (0.109–0.446)	−4.231	<0.0001
in women	0.112	0.554	70.5	40.6	0.07 (N.S.)	>180	N.A.	N.A.	N.A.
Heart failure events									
in all	0.192	0.611	76.3	42.9	<0.0001	>230	0.432 (0.327–0.571)	−5.907	<0.0001
in men	0.266	0.644	79.7	47.0	<0.0001	>230	0.401 (0.277–0,581)	−4.846	<0.0001
in women	0.152	0.581	63.6	51.6	0.1 (N.S.)	>130	N.A.	N.A.	N.A.
Arrhythmic events									
in all	0.168	0.595	81.2	39.4	<0.0001	>280	0.398 (0.264–0.601)	−4.402	<0.0001
in men	0.203	0.663	81.5	38.8	<0.0001	>280	0.398 (0.335–0.601)	−3.632	<0.0001
in women	0.141	0.557	82.1	31.9	0.1 (N.S.)	>280	N.A.	N.A.	N.A.

Sens: sensitivity. Spec.: specificity. NS: non-significant. N.A.: not applicable because no significant cut-off was detected.

cut-off values for each CV event. Nevertheless, the subsequent validation demonstrated they were confirmed only in men and only for cerebrovascular events (>280 mg/day), for heart failure (>230 mg/day) and for arrhythmias (>280 mg/day) (Table 2, right panels). The other cut-offs values were not validated and were considered as false.

From here on, each analysis was therefore performed only in men for cerebrovascular, heart failure and arrhythmic events; the ROC curves of these cut-offs are shown in Fig. 1.

3.1.3. Outcome in relation to cut-off values of caffeine intake in men

In univariate analysis, incidence of cerebrovascular events, of heart failure and of arrhythmias during follow-up was markedly lower in men who consumed DC>cut-off (Fig. 2).

In multivariate Cox models based on cut-off values having each event as dependent variable, the item *DC>cut-off* as covariables, and age, body mass index, circulating FT4, diabetes mellitus, arterial hypertension, smoking, dietary intake of ethanol, basal HRT, LDLC, FEV1 and BBT as confounders, the term *DC>cut-off* inversely predicted cerebrovascular, heart failure and arrhythmic events in men (Table 3).

The term *DC>cut-off* was not a predictor of coronary events: the cut-off previously found for coronary disease in univariate analysis was therefore a false cut-off.

In sensitivity analysis, introducing in the models the interaction term *DCxHRT* did not influence the statistical significance of the association between DC and cerebrovascular events ($p = 0.035$ in all subjects, $p = 0.002$ in men), while abolished the association of DC with heart failure and arrhythmias. Introducing in the models the interaction term *DCxBBT* never abolished the significant association between DC and outcome ($p = 0.002$ for cerebrovascular events in all subjects, $p = 0.039$ in men; $p = 0.011$ for heart failure in all subjects, $p = 0.018$ in men; $p = 0.025$ for arrhythmias in all subjects, $p = 0.047$ in men). When the models were repeated after including, instead of the previous two, the interaction term *DCxHRTxBBT*, no significance was abolished ($p = 0.047$ for cerebrovascular events in all subjects, $p = 0.041$ in men; $p = 0.012$ for heart failure in all subjects, $p = 0.004$ in men; $p = 0.027$ for arrhythmias in all subjects), but for arrhythmic events in men ($p = 0.3$).

After including in the Cox models also the *−163C>A* polymorphism of the CYP1A2 gene (AA, AC or CC), the results did not change apart for cerebrovascular and arrhythmic events in men, in which statistical significance was touched upon ($p = 0.06$ and $p = 0.053$, respectively). The same was observed by combining the C-carriers (slow metabolizers of caffeine) and obtaining in the same subjects $p = 0.06$ and $p = 0.054$.

4. Discussion

4.1. General results

Although the effects of DC as a continuous variable has been widely and also recently studied in large human groups [8,19], to our knowledge this is the first study taking into account the prognostic value of specific cut-off values of DC in influencing each incident CV event.

The study presented herein, performed in a cohort of unselected men and women of general population, free in everyday life to intake caffeine in form of coffee, chocolate, tea, cola and energizing beverages according to their habit and preference, demonstrate that DC over specific cut-off values determined *via* the ROC curve analysis is associated

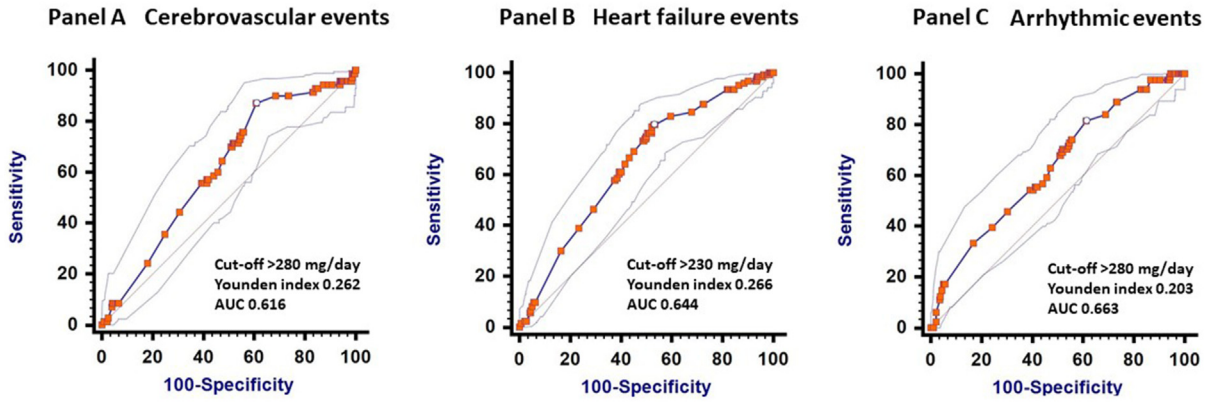

Figure 1 ROC curves analysis for the cut-off of dietary caffeine intake detected and validated for each cardiovascular event in 751 men. Panel A cerebrovascular events, Panel B heart failure and Panel C arrhythmias. AUC: area under the curve.

to lower incidence of CV events in a 20-year follow-up period. The cerebrovascular events and those due to heart failure and arrhythmias were reduced also in multivariate models adjusted for confounders. On the contrary, DC>cut-off was only apparently associated to lower incidence of coronary disease, but this unadjusted crude association was wales and not confirmed after adjustment.

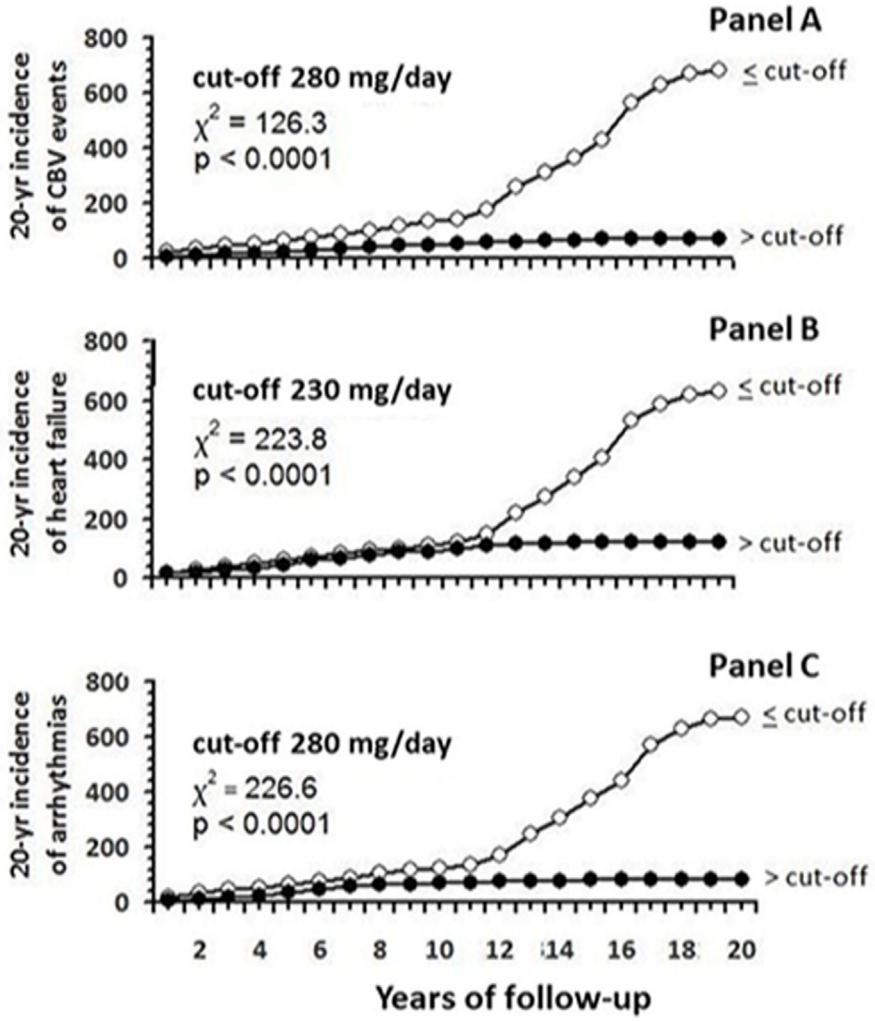

Figure 2 Incidence of cases of cerebrovascular (CBV) events (Panel A), of heart failure (Panel B) and of arrhythmias (Panel C) in 751 men being until or over the specific cut-offs of dietary daily caffeine intake during the 20-year follow-up.

Table 3 Cox analysis having events as depending variables, the term *caffeine intake > cut-off* as covariables, and age, sex when proper, body mass index, circulating thyroxin (FT_4), diabetes mellitus, arterial hypertension, smoking, dietary intake of ethanol, basal heart rate, LDL-cholesterol, Forced expiratory values in the first minute (FEV_1) and β-blocking therapy as confounders. Hazard ratios (HR) and related parameters of being over the cut-off value of caffeine for of each cardiovascular event are shown in men and women, as well as in the whole cohort (1668 homogeneous unselected subjects from general population).

Parameter	HR	95% CI	Z	p-value
Coronary events				
In whole cohort	0.753	0.451−1.260	0.0001	0.1 (NS)
In men	0.813	0.464−1.425	−0.728	0.5 (NS)
In women	0.692	0.413−1.157	−1.407	0.2 (NS)
Cerebrovascular events				
In whole cohort	0.651[a]	0.428−0.994	−2.2002	**0.02**
In men	0.432[b]	0.201−0.927	−2.167	**0.03**
In women	0.767	0.486−1212	−1.142	0.2 (NS)
Heart failure events				
In all cohort	0.678[c]	0.567−0.908	−2.625	**0.009**
In men	0.652[d]	0.442−0.961	−2.177	**0.03**
In women	0.741	0.499−1.10	−0.693	0.09 (NS)
Arrhythmic events				
In whole cohort	0.607[e]	0.395−0.933	−2.289	**0.02**
In men	0.553[f]	0.302−1.000	−1.939	**0.05**
In women	0.652	0.351−1.213	−1.355	0.2 (NS)

[a] Significant confounders: age ($p < 0.0001$), male sex ($p = 0.045$).
[b] Significant confounder: age ($p < 0.0001$).
[c] Significant confounders: age ($p < 0.0001$), male sex ($p < 0.001$), low FEV_1 ($p < 0.001$).
[d] Significant confounders: age ($p < 0.0001$), low FEV1 ($p < 0.0001$).
[e] Significant confounders: age ($p < 0.0001$), male sex ($p = 0.011$), low FEV_1 ($p = 0.002$).
[f] Significant confounders: age ($p < 0.0001$), low FEV_1 ($p = 0.013$).

4.2. Beneficial effects of DC>cut-off on cerebrovascular events in men

In the study presented herein, incidence of cerebrovascular events was dramatically lower in men consuming *DC>cut-off*. The HR of being over the cut-off was 0.651 in the whole cohort and 0.432 in men.

The reasons of this evidence can only be speculative. It is known that xanthines have a potent stimulant effect of cerebral vasoconstriction [20]. Paradoxically, this can be associated to an *inverse intracerebral steal* [21,22] with decrease of cerebral blood flow in the non-ischaemic areas and increase of collateral blood flow surrounding the ischaemic region [23]. This has been experimentally demonstrated in the animal [24].

Caffeine (trimethylxanthine) is not currently used in clinical setting to surround ischaemic areas, but theophylline (dimethylxanthine) has a potential use in treatment for acute ischaemic stroke. Some studies with theophylline or aminophylline (theophyllineethylenediamine) at doses similar to those currently used in therapy of asthma have corroborated clinically this line of research showing [25]. Mineharu et al. [26] demonstrated that the consumption of coffee or tea was associated with a reduced risk of CV mortality, but they did not analyse the individual CV events.

With these premises it is plausible that DC over a certain amount (280 mg/day) exerts a beneficial preventing effects on occurrence of cerebrovascular events.

Only a few studies on this topic have been carried out on large numbers of people and even fewer in general population cohorts, and generally did not take into account sex differences, and probably no one the cut-off of DC. Nevertheless, some results agree with ours. Consumption of 1−6 cups of coffee per day was significantly inversely associated with risk of stroke, with the strongest association (17% lower risk) being observed for 3−4 cups/day [27]. Recently in the UK biobank, Zhang et al. found lower incidence of stroke in all participants taking 2 to 3 cups of coffee per day (HR 0.68, 95% CI, 0.59 to 0.79; $P < 0.001$) [28]. Some studies reported a preventive effect of coffee consumption on the onset of diabetes mellitus, which is a risk factor for stroke, without any sex difference [29]. These studies were based on coffee only, not on dietary caffeine from all sources.

The problem is therefore open. We offer here a rigorous analysis of the question at an epidemiological level in a representative sample of general population.

4.3. Beneficial effects of caffeine intake >cut-off on incident heart failure in men

Strangely, the incidence of heart failure in relation to caffeine or coffee consumption has been limitedly studied, and very rarely in subjects from general population [5−7]. Caffeine intake is followed by a conspicuous positive inotropic effect, probably because it induces Ca^{++} release from the sarcoplasmic reticulum and also inhibits its reuptake [30,31]: as a consequence, the intracellular Ca^{++} decay is slowed down, determining in turns the activation of endothelial nitric oxide synthase, with production of higher quantities of nitric oxide, so increasing muscular contractility [32]. Nothing of new, in this.

Our data demonstrated in multivariate models that DC was not only innocuous or neutral, as suggested before [33,34], but even protective against incidence of heart failure, at least in men taking more than 230 mg/die DC from any source. The HR of being over the cut-off was 0.678 in the whole cohort and 0.652 in men. Other Authors occasionally found a positive effect of coffee against heart failure [35], but did not determine a specific cut-off.

This beneficial effect of DC on heart failure probably depends on its effect on HRT. In fact, it was prevented by adding the interaction term *DCxHRT*.

Finally, the effects were confirmed also including as a covariables the *−163C>A* polymorphism of CYP1A2 gene (that codifies for a hepatic caffeine-oxydizer enzyme and accounts for about 95% of its clearance [36,37]). This demonstrates that genetics have no role in reducing incidence of heart failure observed longitudinally in strong caffeine consumers.

4.4. Beneficial effects of DC>cut-off on arrhythmic events in men

Due to its stimulating effects [38], the general belief is that caffeine intake is associated to hyperkinetic dysrhythmias [39,40]. As a consequence, patients prone to suffer from tachycardia or dysrhythmic diseases are usually discouraged to intake caffeine-containing food or beverages. Nevertheless, from the analysis of literature emerges that this belief is nothing more than an opinion, not clearly supported by experimental data. In the present study, incident arrhythmias were less frequent in subjects taking >280 mg/day caffeine, with a HR that of 0.607 in the whole cohort and 0.553 in men.

Another belief is that caffeine consumption increases HRT. For this reason, we have introduced both basal HRT and the interaction term *DCxHRT* in the multivariate models. Neither of these two covariables modified the reduction of arrhythmic events induced by high caffeine consumption.

Finally, as BBT tends to reduce HRT, adjustment for BBT was also added. The interaction term *DCxBBT* did not change the models. Nevertheless, after introducing separately the further interaction term *DCxHRTxBBT*, the beneficial effects of higher doses of DC on arrhythmic events was no longer present, demonstrating that the preventive effects of BBT on arrhythmic risk it is such as to overwhelm even that of DC and that of the interaction between DC and HRT. However, it cannot be ruled out that, being relatively few subjects on beta-blocker treatment, this reduced the number of the sample examined, consequently reducing the statistical power of the analysis.

HRT, when introduced as a confounder, did not change significantly the model, but the interaction terms *DCxHRT* reduced the protective effect of DC on CV outcome, while *DCxHRTxBBT* (where BBT counterbalance the effects of DC of HRT) did not. The conclusion is that the beneficial effect of DC on risk of incident arrhythmias partially depends on its action on HRT.

4.5. Lack of effects of caffeine on incident coronary events in both sexes

It is well known that one of the effects of caffeine on the CV system is the positive inotropic one, which joins the positive chronotropic one in increasing the oxygen consumption of the heart.

The univariate analysis shows that there is an apparent decrease in events in men when caffeine intake goes over the dietary cut-off, but this is only an apparent effect. In fact, an apparent univariate cut-off obtained with the ROC curves method did not pass validation. Not only that, but, in all Cox multivariate analyses on coronary events corrected for covariables, daily caffeine intake was rejected from the models and has no protective role against the development of coronary heart disease.

It is not surprising that caffeine is unable to reduce the incidence of coronary events. It is probable that the inotropic + chronotropic effect of caffeine is such as to overwhelm (in the clinical reality which is not univariate but always multivariate) the potential protective role of the caffeine alkaloids. A similar case occurs with digitalis alkaloids, which increase inotropism but do not improve survival. Furthermore, the chronotropic effect of caffeine too probably contributes to the unwanted increase in heart workload, a long-term counterproductive effect.

4.6. Sensitivity analysis

The polymorphisms of the genes of the CYP group strongly influence CV risk directly or *via* metabolic changes, and have been largely studied [17–19]. The beneficial effects in men were confirmed also including as a further covariable the *−163C>A* polymorphism of CYP1A2 gene that codifies for a hepatic caffeine-oxydizer enzyme and accounts for about 95% of its clearance, demonstrating that this polymorphism has no role in the ability of DC to predict CV events.

4.7. While men only?

In an epidemiological setting it is difficult to establish why an effect is released into a sex and not in the other. There is also to say that the topic is quite controversial, with authors who have found CV effects of xanthines in men only or on the contrary in women only. Genetic, environmental, geographical factors related to the different consumption or - being kinds of comfort - simple rituals can play a role in these differences. We tend to exclude that the *−163C>A* polymorphism of the CYP1A2 gene, to which a great importance is given today, can be called into question, as it has always been rejected by the multivariate models we used. For instance, BMI is positively associated with the consumption of tea for men and women but were inversely associated with the consumption of coffee for men and women, green tea for men and black tea for women. Wedick et al. attribute the lack of effect of caffeine in women to sex hormone-binding globulin and endogenous sex hormone levels [41]. The question is too complex to be addressed in an

epidemiological study, especially because caffeine has metabolic and hepatic effects and the metabolism of men is different from that of women, which can play a role etc. Specific studies will be needed to clarify this point.

4.8 Strengths and limitations

The study provides insights into the associations between caffeine and important CV events in a large Italian prospective cohort followed for 20 years. This may provide some mechanistic insights particularly into the putative role of caffeine. In sensitivity analyses, the role of the −163C>A polymorphism of the CYP1A2 gene was investigated.

There are several limitations to consider when interpreting the study findings. Caffeine intake was based on participant self-reporting, with the attendant risk of reporting bias. However, as the data were collected before incident conditions had developed, the occurrence of recall bias was extremely unlikely. Caffeine consumption at baseline was assumed to remain unchanged throughout the participants' follow-up. Previous studies have shown that the assessment of nutrient intake has a high degree of reproducibility over time. The cohort is a Caucasian population and the findings may not be entirely applicable to the populations of other ethnicities. Lastly, residual and unaccounted confounding including dietary factors may have occurred despite the multivariate adjustment.

5. Conclusions

The results we obtained in a cohort representative of general population show that, in men but not in women, higher voluntary daily consumption of DC from any source reduces significantly and to a considerable extent, in a long follow-up, the incidence of cerebrovascular events, heart failure and arrhythmias. To have preventive effects on events the consumption of caffeine must be higher than a cut-off that corresponds to 280, 230 and 280 mg/day, respectively. As a consequence, a daily consumption >280 mg/day DC should be suggested to men in order to reduce the risk of all these CV events. No prognostic cut-off can be identified – and no advice is possible – in women, and in any sex in the case of coronary events. Specific studies will be mandatory to understand the reason of this sex-specific differences.

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Authors' contributions

E.C. wrote the first draft with inputs from V.T. All authors (E.C. and V.T.) conducted all statistical analyses and had full access to the data. All authors commented on multiple drafts and V.T. submitted the final draft.

Data availability

The data underlying this article will be shared upon reasonable request to the corresponding author.

Conflicts of interest

None declared.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2023.07.006.

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