Daily Coffee Drinking Is Associated with Lower Risks of Cardiovascular and Total Mortality in a General Italian Population: Results from the Moli-sani Study

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ABSTRACT

Background: An inverse relationship between coffee intake and mortality has been observed in several population cohorts, but rarely within Mediterranean countries. Moreover, the biological pathways mediating such an association remain unclear.

Objectives: We assessed the associations between coffee consumption and total and cause-specific mortality and examined the mediating roles of N-terminal pro B–type natriuretic peptide (NTproBNP), high-sensitivity Troponin I, blood glucose, lipid metabolism, and selected biomarkers of inflammation and renal function.

Methods: We longitudinally analyzed data on 20,487 men and women (35–94 years old at baseline) in the Moli-sani Study, a prospective cohort established in 2005–2010. Individuals were free from cardiovascular disease (CVD) and cancer and were followed-up for a median of 8.3 years. Dietary data were collected by a 188-item semi-quantitative FFQ. Coffee intake was standardized to a 30-mL Italian espresso cup size. HRs with 95% CIs were calculated by multivariable Cox regression.

Results: In comparison with no/rare coffee consumption (up to 1 cup/d), HRs for all-cause mortality across categories of coffee consumption (>1 to ≤2, >2 to ≤3, >3 to ≤4 and >4 cups/d) were 0.79 (95% CI, 0.65–0.95), 0.84 (95% CI, 0.69–1.03), 0.72 (95% CI, 0.57–0.92), and 0.85 (95% CI, 0.62–1.12), respectively. For CVD mortality, a nonlinear (P for non-linearity = 0.021) J-shaped association was found (magnitude of the relative reduction = 37%; nadir at 3–4 cups/d). Circulating levels of NTproBNP explained up to 26.4% of the association between coffee and all-cause mortality, while systolic blood pressure was likely to be on the pathway between coffee and CVD mortality, although to a lesser extent.

Conclusions: In this large cohort of Italian adults, moderate consumption (3–4 cups/d) of Italian-style coffee was associated with lower risks of all-cause and, specifically, of CVD mortality. Among the known biomarkers investigated here, NTproBNP likely mediates the relationship between coffee intake and all-cause mortality. J Nutr 2020;0:1–10.

Keywords: coffee consumption, mortality risk, cardiovascular mortality, general population, Mediterranean diet

Introduction

Coffee is among the most commonly consumed nonalcoholic beverages worldwide and contains various antioxidants and phenolic compounds, such as flavonoids and caffeine, some of which have been shown to exert beneficial effects for health (1–3). The relationships between coffee intake and the risks of disease/mortality have been widely investigated in a number of epidemiological settings, most of which indicate a favorable role of regular coffee intake on a variety of health outcomes, including cardiovascular disease (CVD), mortality, and cancer (4, 5). Regarding cardiovascular health, the currently available evidence on CVD effects related to habitual coffee consumption is largely reassuring, suggesting that coffee can be included as part of a healthy diet for the general public and also for those with an increased cardiovascular risk or CVD (6).

More recently, a meta-analysis of 40 prospective cohort studies found lower risks of mortality from all causes and from CVD in a comparison of the highest versus lowest coffee consumption categories, with similar results across various subpopulations by characteristics of subjects, including age,
sex, overweight status, alcohol drinking, smoking status, and caffeine content consumed (7).

Although the mechanisms of action are not entirely understood, the health-promoting properties of coffee are largely attributed to its rich phytochemistry, including caffeine, chlorogenic acid, caffeic acid, and hydroxyhydroquinone (8), and to its antioxidant compounds, which may explain the potential benefits of coffee against those conditions associated with a chronic state of subclinical inflammation, such as CVD and cancer (9, 10).

Though largely consumed among Mediterranean populations, coffee has generally not been included among the food items used to obtain Mediterranean dietary scores (11), nor has coffee consumption been extensively investigated among Mediterranean countries; the relative paucity of studies renders it difficult to provide definitive evidence on whether an antioxidant-rich beverage may have potential health benefits among populations already consuming a high-antioxidant diet, such as the Mediterranean diet (MD).

The primary aim of this study was to prospectively assess the association of coffee consumption with total and cause-specific mortality in a large Italian population of adult men and women; as a secondary aim, we examined several biological mechanisms that might explain the relationship between coffee intake and mortality by analyzing the possible contributions of selected biomarkers of different biological processes that predispose individuals to CVD onset or progression. Finally, we assessed whether the inclusion of coffee consumption would improve risk predictions associated with a traditional Mediterranean Diet Score (MDS).

The enrollment phase of the Moli-sani Study was supported by unrestricted research grants from the Pfizer Foundation (Rome, Italy), the Italian Ministry of University and Research (MIUR, Rome, Italy)—Programma Triennale di Ricerca, Decreto number 1588, and Instrumentation Laboratory, Milan, Italy. The follow-up phase of the Moli-sani Study (assessment of incident cases) was partially supported by the Italian Association for Cancer Research (AIRC) “SxMILLE” (HYPERCAN Study, number 12237) and the Italian Ministry of Health (Principal Investigator, Gd; Co–Principal Investigator, SC; grant number RF-2018-12367074). The present analyses were partially supported by a grant to MB from the Italian Ministry of Health 2013 (grant number GR-2013-02356060), by the Hypercan Study, AIRC “SxMILLE” number 12237, and by FOR FESR (Programmi Operativi Regionali -Fondo Europeo di Sviluppo Regionale) 2014–2020: DD number 459 of 27/11/2018; SATIN (Sviluppo di Approcci Terapeutici Innovativi per patologie neoplastiche resistenti ai trattamenti) and by a grant to L.I. as a partner of BiomarCaRE (Biomarkers for Cardiovascular Risk Assessment in Europe) by European Commission Seventh Framework Programme FP7/2007–2013 (HEALTH-F2-2011-279813). The funders had no role in the study design, collection, analysis, and interpretation of data, in writing the manuscript; or in the decision to submit the article for publication. All authors were and are independent from funders.

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Supplemental Tables 1–7 and Supplemental Figure 1 are available from the “Supplementary data” link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/

Address correspondence to LI (e-mail: licia.iacoviello@neuromed.it). Abbreviations used: CRP, C-reactive protein; CVD, cardiovascular disease; EPIC, European Prospective Investigation into Cancer and Nutrition; ICD-9, International Classification of Diseases, Ninth Edition; IDI, integrated discrimination improvement; IHD, ischemic heart disease; MD, Mediterranean diet; MDS, Mediterranean diet score; NRI, Net reclassification index; NTproBNP, N-terminal pro B-type natriuretic peptide; PA, physical activity; WBC, white blood cell count.

Methods

Study population

The Moli-sani Study is a large, population-based cohort study that, in 2005–2010, randomly recruited 24,325 men and women aged ≥35 years from the adult general population of the Molise region, a central-southern area of Italy, from city hall registries by multistage sampling. The exclusion criteria were pregnancy, disturbances in mental or decision-making impairments, ongoing poly traumas, or coma.

Details of the study are provided elsewhere (12). For the purpose of the present analyses, we excluded subjects with a history of CVD (n = 1188) or missing data on CVD (n = 366), with a history of cancer (n = 697) or missing data on cancer (n = 87), reporting implausible energy intakes (<800 kcal/d in men and <500 kcal/d in women or >4000 kcal/d in men and >3500 kcal/d in women; n = 371), with unreliable dietary or medical questionnaires (n = 953 and n = 60, respectively), lost to follow-up (n = 22), without information on cause-specific death (n = 15), or with missing information for covariates (n = 77). Those participants with missing values of 1 or more covariates (missing data: diabetes, n = 281; hypertension, n = 378; and hyperlipidaemia, n = 206) were kept in the data set and treated as missing reporting, but were excluded in sensitivity analysis. The final sample was of 20,487 individuals (Supplemental Figure 1).

The cohort was followed-up for mortality until 31 December 2015. Overall and cause-specific mortality rates were assessed by the Italian mortality registry (Registro Nominativo Cause Morte, ReNaM registry), validated by Italian death certificates (Istituto Nazionale di Statistica, ISTAT), and coded according to the International Classification of Diseases, Ninth Edition (ICD-9).

CVD mortality included deaths from diseases of the circulatory system when the underlying cause of death included ICD-9 codes 390–459. ICD-9 codes 430–438 were used to define a specific cause of death for cerebrovascular disease and ICD-9 codes 410–414 and 429 were used to define a specific cause of death for ischemic heart disease (IHD). Cancer was considered as the cause of death when using ICD-9 codes 140–208. Noncardiovascular/noncancer causes of death were included in an “other-cause mortality” group.

The Moli-sani Study complies with the Declaration of Helsinki and was approved by the Ethical Committee of the Catholic University Medical School in Rome, Italy. All participants provided written informed consent.

Dietary assessment

Dietary intake was assessed by an interviewer-administered, semi-quantitative, European Prospective Investigation into Cancer and Nutrition (EPIC) FFQ that was validated and adapted to the Italian population to assess participants’ diet during the past 12 months.

The FFQ includes 188 food items, classified into 74 predefined food groups on the basis of similar nutrient characteristics or culinary usage (13). Participants were asked to indicate the number of times a given item was consumed (per day, week, month, or year), from which the frequency of consumption was calculated. The quantity of food consumed was assessed by asking the participant to select 1 among several images of different food portions or by using a predefined standard portion when no image was available.

Frequencies and quantities of each food were then linked, using specifically designed software (14), to Italian Food Tables (15) to obtain estimates of daily intakes of macro- and micro-nutrients plus energy.

For coffee, the questionnaire inquired about the consumption frequency of caffeinated and decaffeinated coffee, the 2 recipes mainly consumed in Italy, in the form of espresso or percolated (mocha) coffee.

The question asked for coffee was “How many cups of coffee do you usually drink?”, with possible answers being number of cups per day, week, or month, or never/almost never. Subsequently, participants were asked to indicate the type of coffee more usually consumed: that is, Italian espresso (30 mL), mocha coffee (50 mL), or decaffeinated coffee (30 mL). Cappuccino and café latte were assumed to contain 20% coffee and 80% milk. We calculated total coffee consumption as the sum of all these types of coffee.
For the data analysis, we standardized the amount of coffee consumed to a 30-mL cup size, which is the standard cup size of an Italian espresso according to the Italian dietary guidelines (16).

Coffee consumption was categorized as up to 1 cup/d, >1 to \( \leq 2 \) cups/d, >2 to \( \leq 3 \) cups/d, >3 to \( \leq 4 \) cups/d, and >4 cups/d.

Added sugar was the quantity of sugar (g/d) added to beverages (i.e., milk, coffee, tea, or yogurt).

Adherence to the traditional MD was evaluated by the MDS developed by Trichopoulou et al. (11). The score was obtained by assigning 1 point each to foods considered healthy (fruits and nuts, vegetables, legumes, fish, cereals; MUFA to SFA ratio) whose consumption was above the sex-specific medians of intake of the Moli-sani study population for participants apparently free from CVD, cancer, and diabetes, and then the score was applied to the whole population; foods presumed to be detrimental (meat and dairy products) were scored positively if their consumption was below the median. All other intakes received 0 points. For ethanol, men who consumed 10–50 g/d and women who consumed 5–25 g/d received 1 point; otherwise, the score was 0. The MDS ranged from 0 to 9 (the latter reflecting maximal adherence) and scores were categorized according to poor (0–3), average (4–5), and good adherence (6–9) to the MD.

Assessment of covariates

At baseline, information on socio-demographic variables, lifestyles, and medical history were obtained by interviewer-administered questionnaires.

The education variable was based on the highest qualification attained and was categorized as up to lower secondary (approximately \( \leq 8 \) years of study), upper secondary education (8–13 years of study), and post-secondary and higher (\( >13 \) years of study). Housing status was classified as rented, 1-dwelling ownership, or \( >1 \)-dwelling ownership.

Subjects were classified as never, current, or former smokers (quit at least 1 year ago) and, among ever smokers, the number of cigarettes smoked per day was also assessed. Leisure-time physical activity (PA) for sports, walking, and gardening was dichotomized as \( <30 \) or \( \geq 30 \) min/d.

Waist circumference (in cm) was measured between the twelfth rib and the iliac crest, and hip circumference (in cm) was measured around the buttocks. The waist-to-hip ratio was then calculated, and abdominal obesity was defined as a waist-to-hip ratio \( \geq 0.90 \) for men and \( \geq 0.85 \) for women (17).

Participants were considered to have diabetes, hypertension, or hyperlipidemia at baseline if they were taking disease-specific drugs.

Selection and assessment of biomarkers of CVD risk

The biomarkers tested in the present study were selected on the basis of whether they were: 1) previously studied for their relevance in pathways pertaining to CVD; 2) shown in epidemiologic studies to be related to CVD or mortality; and 3) already introduced in the analyses of the Moli-sani Study cohort.

Each of these biomarkers reportedly reflects different underlying pathways to disease incidence and progression (18, 19).

Blood samples were collected at baseline in participants who had fasted overnight and had refrained from smoking for at least 6 hours; lipids (total cholesterol, HDL, triglycerides) and blood glucose were assayed in serum samples by enzymatic reaction methods using an automatic analyzer (ILab 350, Instrumentation Laboratory, Milan, Italy); quality control for lipids and glucose was obtained by a commercial standard (SeraChem1 and SeraChem2). For SeraChem1 and SeraChem2, the coefficients of variability were 4.9% and 5.2% respectively for blood cholesterol; 3.2% and 3% for HDL; 5.2% and 5.3% for triglycerides; and 4.7% and 4.1% for blood glucose.

High sensitivity C-reactive protein (CRP) was measured in fresh serum samples by a particle-enhanced immune-turbidimetric assay (ILab 350, Instrumentation Laboratory, Milan, Italy). Quality control for CRP was maintained using an in-house serum pool and a commercial laboratory standard whose inter-day coefficients of variability were 5.5% and 4.2%, respectively.

A hemocromocytometric analysis was performed by cell count (Coulter HMX, Beckman Coulter, IL, Milan, Italy) within 3 hours of blood collection. Quality control was performed using 3 different levels of standards: Abnormal 1, a pathologically high control; Abnormal 2, a pathologically low control; and Normal (Coulter HMX, Beckman Coulter). Coefficients of variability for white blood cells (WBC) were 6.2%, 3.3%, and 3.0% for Abnormal 1, Abnormal 2, and Normal, respectively. N-terminal pro-B-type natriuretic peptide (NTproBNP), high-sensitivity assay Troponin I, apoA, apoB100, lipoprotein a, markers of renal function (cystatin C, creatinine), insulin, C-peptide, and serum vitamin D were measured subsequently on thawed samples stored frozen in liquid nitrogen at the biological bank of the Moli-sani study, in the framework of the collaborative BiomarCaRE (Biomarker for Cardiovascular Risk Assessment in Europe) research project, whose primary objective is to assess the value of established and emerging biomarkers for CVD risk prediction by using data from 23 cohorts across Europe (18).

The low-grade inflammation (INFILA) score was constructed by summarizing synergistic effects of inflammatory biomarkers (20). Briefly, deciles of each biomarker level (CRP, WBC, platelets, and the granulocyte-to-lymphocyte ratio) were generated. For all 4 components, being in the highest deciles (7–10) gave a score that increased from 1 to 4, while being in the lowest deciles (1–4) was negatively scored from −4 to −1. Being in deciles 5 or 6 was scored as 0 points. In such a way, the INFILA score ranged from between −16 and 16 and came up as the sum of the 4 biomarkers. An increase in the score represented an increase in low-grade inflammation intensity.

Statistical analysis

Baseline characteristics of the participants by categories of daily coffee intake were presented as means and SDs or as percentages for categorical variables. Differences in the distribution of baseline covariates were calculated using an ANOVA adjusted for age, sex, and energy intake (GENMOD procedure for categorical variables and general linear model procedure for continuous variables in SAS software; Table 1). Positively skewed variables were log transformed before analysis.

Risk estimates for all-cause and cause-specific deaths were expressed as HRs with 95% CIs and were calculated using Cox regression models with time-on-study on the time scale and adjusting for baseline age as a covariate in the model.

On the basis of previous literature and biological plausibility, 2 models for the association of coffee with the mortality risk were fitted: Model 1 was adjusted for age (continuous), sex, and energy intake (kcal; continuous); Model 2 was adjusted as in Model 1 and further controlled for education (up to lower secondary school; upper secondary school; postsecondary/higher), housing (rented or 1- or >1-dwelling ownership), residence (urban/rural), leisure-time PA (<30 min/d, smoking never, current, former smokers), number of cigarettes per day (<5, >5 to \( \leq 10 \), >10 to \( \leq 20 \), >20, and missing data), abdominal obesity (no/yes/missing), diabetes (no/yes/missing), hyperlipidaemia (no/yes/missing), hypertension (no/yes/missing), MDS (continuous), tea intake (nonconsumers, \( >0 \) to \( \leq 2 \) cups/week, >2 to \( \leq 4 \) cups/week, and >4 cups/week), and added sugar (g/d; continuous). Missing values of hypertension, hyperlipidaemia, and diabetes were included in the model as dummy variables, similar to the way valid categories were represented.

For a biomarker to be considered as mediating the association of coffee intake and mortality, it had to be associated with both the exposure and the outcome (at least 1 of those analyzed) with a \( P \) value \( <0.20 \), in accordance with predefined mediation principles (21, 22). These criteria were tested in distinct multivariable regression models for each potential mediator individually (Supplemental Table 1) and through Cox models that included coffee consumption as a covariate (Supplemental Table 2).

The multivariable Model 2 served as the reference for the mediation analysis used to estimate the contribution of each set of potential mediators, which were alternately excluded in Model 2.

We performed our analysis on 18,861 subjects (after exclusion of those individuals with missing data on any of the biomarkers) to...
TABLE 1  Selected characteristics of participants at baseline in the Moli-sani Study cohort (n = 20,487) across categories of daily coffee intake

<table>
<thead>
<tr>
<th>Coffee intake, cups/d</th>
<th>≤1</th>
<th>&gt;1 to ≤2</th>
<th>&gt;2 to ≤3</th>
<th>&gt;3 to ≤4</th>
<th>&gt;4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants, n(%)</td>
<td>3714 (18.1)</td>
<td>4436 (21.7)</td>
<td>4680 (22.8)</td>
<td>3747 (18.3)</td>
<td>3910 (19.1)</td>
</tr>
<tr>
<td>Age, years</td>
<td>58.1 ± 12.8</td>
<td>57.3 ± 11.9</td>
<td>55.7 ± 11.6</td>
<td>54.2 ± 10.9</td>
<td>51.3 ± 9.3</td>
</tr>
<tr>
<td>Men</td>
<td>47.8</td>
<td>47.2</td>
<td>46.5</td>
<td>44.1</td>
<td>49.3</td>
</tr>
<tr>
<td>Educational level</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Up to lower secondary</td>
<td>58.9</td>
<td>54.7</td>
<td>51.5</td>
<td>45.7</td>
<td>44.2</td>
</tr>
<tr>
<td>Upper secondary</td>
<td>30.7</td>
<td>33.3</td>
<td>34.9</td>
<td>39.5</td>
<td>40.6</td>
</tr>
<tr>
<td>Postsecondary</td>
<td>10.4</td>
<td>12.0</td>
<td>13.6</td>
<td>14.8</td>
<td>15.2</td>
</tr>
<tr>
<td>Housing</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Rent</td>
<td>8.1</td>
<td>7.8</td>
<td>8.6</td>
<td>9.1</td>
<td>11.0</td>
</tr>
<tr>
<td>&gt;1 dwelling ownership</td>
<td>84.0</td>
<td>82.9</td>
<td>82.8</td>
<td>81.8</td>
<td>80.2</td>
</tr>
<tr>
<td>Smoking status</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Nonsmokers</td>
<td>61.2</td>
<td>55.6</td>
<td>50.8</td>
<td>48.1</td>
<td>39.2</td>
</tr>
<tr>
<td>Current smokers</td>
<td>12.3</td>
<td>17.3</td>
<td>21.9</td>
<td>27.2</td>
<td>41.2</td>
</tr>
<tr>
<td>Former smokers</td>
<td>26.5</td>
<td>27.1</td>
<td>27.3</td>
<td>24.7</td>
<td>23.6</td>
</tr>
<tr>
<td>Number of cigarettes smoked per day1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>≤5</td>
<td>33.6</td>
<td>30.5</td>
<td>22.0</td>
<td>21.1</td>
<td>15.1</td>
</tr>
<tr>
<td>&gt;5 to ≤10</td>
<td>24.8</td>
<td>25.0</td>
<td>24.1</td>
<td>23.5</td>
<td>19.2</td>
</tr>
<tr>
<td>&gt;10 to ≤20</td>
<td>31.4</td>
<td>36.7</td>
<td>43.4</td>
<td>44.9</td>
<td>48.1</td>
</tr>
<tr>
<td>&gt;20</td>
<td>9.4</td>
<td>7.0</td>
<td>9.7</td>
<td>9.6</td>
<td>17.1</td>
</tr>
<tr>
<td>Physical activity ≥30 min/d</td>
<td>65.5</td>
<td>65.6</td>
<td>63.8</td>
<td>63.2</td>
<td>61.6</td>
</tr>
<tr>
<td>Abdominal obesity</td>
<td>75.1</td>
<td>73.6</td>
<td>72.8</td>
<td>72.2</td>
<td>70.1</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3.8</td>
<td>4.8</td>
<td>3.8</td>
<td>4.0</td>
<td>3.4</td>
</tr>
<tr>
<td>Hypertension</td>
<td>30.9</td>
<td>29.4</td>
<td>24.7</td>
<td>20.7</td>
<td>15.2</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>6.1</td>
<td>6.0</td>
<td>5.4</td>
<td>5.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Mediterranean diet score</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Poor: 0–3</td>
<td>25.9</td>
<td>29.4</td>
<td>30.5</td>
<td>32.7</td>
<td>35.0</td>
</tr>
<tr>
<td>Average: 4–5</td>
<td>44.6</td>
<td>43.2</td>
<td>44.0</td>
<td>44.5</td>
<td>43.6</td>
</tr>
<tr>
<td>Good: 6–9</td>
<td>29.5</td>
<td>27.4</td>
<td>25.5</td>
<td>22.8</td>
<td>21.4</td>
</tr>
<tr>
<td>Added sugar, g/d</td>
<td>4.2 (4.5)</td>
<td>7.2 (5.6)</td>
<td>9.7 (7.1)</td>
<td>12.1 (8.7)</td>
<td>17.6 (14.6)</td>
</tr>
<tr>
<td>Tea intake, cups/week</td>
<td>1.4 (3.5)</td>
<td>0.9 (2.5)</td>
<td>0.7 (2.1)</td>
<td>0.7 (2.2)</td>
<td>0.6 (2.2)</td>
</tr>
<tr>
<td>Alcohol consumption, g/d</td>
<td>17.4 (22.9)</td>
<td>18.2 (23.7)</td>
<td>18.3 (21.5)</td>
<td>14.5 (19.9)</td>
<td>14.5 (19.3)</td>
</tr>
</tbody>
</table>

Values are expressed as percentages for all variables, except for age, added sugar, tea intake, and alcohol consumption, which are presented as means ± SDs. Means and P values are adjusted for sex, age, and energy intake. Coffee intake was standardized to a 30-mL Italian espresso cup size. Tea intake was standardized to a 150-mL cup size.

1 Percentages were calculated based on the total number of smokers (n = 4881).

evaluate how much of the associations between coffee consumption and all-cause or cause-specific mortality would be accounted for by biomarkers representative of different biological processes.

For the mediation analysis, the % MEDIATE macro in SAS (23) was used to calculate the point and interval estimates of the percentage of exposure effect explained by 1 or more intermediate variables, with 95% CIs and P values. Biomarkers were entered into the mediation analysis as continuous variables.

We conducted several analyses to assess the sensitivity of the observed associations to various baseline risk factors: age (35–64 and ≥65 years), sex, socioeconomic strata (education and housing), lifestyles (smoking status, leisure-time PA, MDS), and health conditions at baseline (abdominal obesity, diabetes, hypertension, and hyperlipidaemia); we also conducted an analysis after excluding those subjects who died during the first 2 years of follow-up. Appropriate multiplicative terms for testing interactions were included in the multivariable models to test for a difference of effect of coffee intake across subgroups (Supplemental Table 3).

Possible curvilinear associations between the continuous measure of coffee intake (cups/d) and the all-cause and cause-specific mortality risks were tested via a multivariable Cox model using a restricted cubic spline with 3 knots at fixed percentiles (5th, 50th, and 95th percentiles of the distribution; Figure 1).

In order to see whether the inclusion of coffee would possibly improve risk predictions associated with MDS, we scored coffee consumption as previously done for the food groups already present in the MDS (11). For consistency with the methodology previously used, we calculated the sex-specific medians of coffee intake in our population (free from CVD, cancer, and diabetes) and assigned a value of 1 if consumption was above the sex-specific median (82.9 g/d for women and 84.0 g/d for men); otherwise, the score was 0.

We then obtained a 2-level variable that was included as an additional component in the original MDS, thus originating an MDS “supplemented” with coffee with scores ranging from 0 to 10; to allow for comparisons among scores, the scores were entered into risk analyses, scaled by their SD.

The categorical Net reclassification index (NRI) and the integrated discrimination improvement (IDI) (24) were used to quantify the predictive value of the MDS supplemented with coffee over the traditional MDS. To estimate these metrics, the follow-up time was censored at 10 years and the risk categories chosen for NRI calculation were <10% and ≥10% (Supplemental Table 4).

Finally, we estimated the E-value, a parameter that calculates the minimum strength of association that an unmeasured confounder would need to have with both the exposure and outcome to fully explain away a specific exposure-outcome association (25).
FIGURE 1 Multivariable-adjusted dose-response association of coffee intake (1-cup increment/d) with (A) all-cause, (B) CVD, and (C) IHD/cerebrovascular mortality in the Moli-sani Study cohort (n = 20,487). Risk estimates (HRs with 95% CIs) were obtained from the multivariable model, adjusted for age, sex, energy intake, education, housing, residence, leisure-time PA, smoking, number of cigarettes per day, abdominal obesity, diabetes, hyperlipidemia, hypertension, Mediterranean diet score, tea intake, and added sugar. The dashed lines represent the 95% CIs for the spline model. Participants with no coffee consumption (0 cups/d) served as the reference group. Coffee intake was standardized to a 30-mL Italian espresso cup size. CVD, cardiovascular disease; IHD, ischemic heart disease; PA, physical activity.

The data analysis was generated using SAS/STAT software, Version 9.4, of the SAS System for Windows.

Results

Characteristics of the population by coffee intake

In this Moli-sani population, the mean intake of coffee was 2.8 cups per day (SD ±1.8).

During a median follow-up of 8.3 years (IQR, 7.4–9.3 years), 834 subjects died: 270 (32.4%) from CVD, of whom 152 (18.2%) died from IHD/cerebrovascular causes; 334 (40.0%) from cancer; and 230 (27.6%) from other causes.

Characteristics of the Moli-sani study population according to daily consumption of coffee are reported in Table 1. Higher coffee consumers (>4 cups/d; 19.1%) tended to be younger and to have a higher level of education and were more likely to be smokers but less likely to have diabetes, hypertension, and hyperlipidaemia; they were also poorly adhering to the MD (Table 1).

Coffee intake and mortality risk

In multivariable-adjusted Model 2, those who drank >3 to ≤4 cups/d had a 28% (95% CI, 8–43%) lower risk of all-cause mortality, as opposed to participants with the lowest consumption (up to 1 cup/d), while among heavy consumers
(≥4 cups/d) the mortality risk reduction was no longer significant (Table 2).

When modelling coffee consumption in 1-cup increments per day, the results from main analyses were confirmed (P for association = 0.17; P for non-linearity = 0.47; Figure 1A).

Among those participants with habitual intake of ≥ 3 to ≤ 4 cups/d of Italian-style coffee, the HRs for CVD and IHD/cerebrovascular mortality were 0.58 (95% CI, 0.37–0.91) and 0.55 (95% CI, 0.29–1.03), respectively, while at higher intakes the risk reduction appeared to be lowered (Table 2, Model 2).

Consistently, we found evidence of a nonlinear J-shaped association between coffee consumption and the risk of CVD (magnitude of the relative reduction = 37%; nadir at 3–4 cups/d; P value for overall association = 0.045; P value for nonlinear association = 0.021; Figure 1B), but not for IHD/cerebrovascular mortality (P value for overall association = 0.38; P value for nonlinear association = 0.21; Figure 1C).

No significant inverse associations between the highest (≥ 4 cups/d) as opposed to the lowest (up to 1 cup/d) coffee intakes were found for cancer death (HR = 0.81; 95% CI, 0.52–1.26) or mortality from other causes (HR = 0.71; 95% CI, 0.38–1.31; Table 2; Model 2).

Survival analyses in the general population of the Moli-sani Study, including also participants with CVD and cancer at baseline (n = 22,801), showed HRs for all-cause, CVD, and IHD/cerebrovascular death risks of 0.73 (95% CI, 0.64–0.88), 0.55 (95% CI, 0.39–0.78), and 0.53 (95% CI, 0.34–0.84), respectively, for individuals with habitual intake of 3–4 cups/d in comparison with the lowest intake category (Supplemental Table 5).

**Mediation analysis**

The associations of coffee intake with all potential mediators (selected biomarkers) are shown in Supplemental Table 1. Coffee intake was positively associated with cardiac troponin, biomarkers of glucose metabolism, total blood cholesterol, and apoB100, whereas it was inversely associated with NTproBNP, HDL, and systolic blood pressure (Supplemental Table 1). The HRs for all-cause, CVD, and IHD/cerebrovascular mortality associated with the selected biomarkers, as estimated by including each biomarker in the multivariable-adjusted model, are shown in Supplemental Table 2. Each of these biomarkers was associated with at least 1 of the outcomes under study, with the exception of insulin and HDL (Supplemental Table 2). We finally included in the mediation analysis 5 biomarkers or groups of biomarkers meeting the above mentioned criteria: namely, cardiac troponin, NTproBNP, biomarkers of glucose metabolism, biomarkers of lipid metabolism, and systolic blood pressure. This resulted in a Bonferroni corrected significance threshold α of 0.01.

A mediation analysis on 18,661 subjects indicated that the majority of established biomarkers of CVD did not substantially modify the relationships between coffee consumption and mortality, with the exception of NTproBNP levels, which explained 11.5% (P = 0.0052) and 26.4% (P = 0.0006) of the relationships between the all-cause mortality risk and coffee consumption of ≥ 3 to ≤ 4 cups/d and > 4 cups/d, respectively (Supplemental Table 6).

The risk reduction of CVD mortality associated with consumption of ≥ 3 to ≤ 4 cups/d was slightly accounted for by systolic blood pressure (3.7% reduction; P = 0.0078), a factor also partially explaining the relationship with the risk of IHD/cerebrovascular death (5.0% reduction; P = 0.0032; Supplemental Table 7).

**Additional analyses**

Subgroup analyses are presented in Supplemental Table 3. Coffee intake remained consistently associated with reduced mortality in almost all subgroups. An exception was represented by PA and abdominal obesity, which were likely to modify the magnitude of the inverse associations between coffee consumption and all-cause and CVD mortality risks. Specifically, the associations between a 1-cup increment and all-cause mortality risk were stronger in less physically active subjects (P for interaction = 0.047) and among individuals with abdominal obesity (P for interaction = 0.0068; Supplemental Table 3); those with abdominal obesity were also more likely to have a reduced CVD death risk associated with increased coffee intake, as compared with those participants without abdominal obesity (P for interaction = 0.033).

The percentage of decaffeinated coffee consumers was very low in our population sample (n = 741; 3.6%). The mean caffeine intake was 63.8 mg per day and the main caffeine source was coffee; consequently, caffeine intake was highly correlated with the intake of coffee (Spearman correlation coefficients r = 0.80; P value < .0001) and represents a strong proxy of it. In addition, to account for other food sources of caffeine, all analyses were also adjusted for tea consumption.

NRI and IDI values indicated that the addition of coffee intake into the MDS did not add to the risk prediction model of the traditional MDS (all P values > 0.05; Supplemental Table 4).

Finally, by the use of E-values, we found that to nullify the reported association, unobserved confounders should be associated with both coffee consumption and mortality by an OR/HR of around 3.0. Of interest, the whole set of known confounders included in our analysis shifted the univariate HR for CVD mortality (HR = 0.29; 95% CI, 0.19–0.44) associated with > 3 to ≤ 4 cups/d to the fully adjusted HR of 0.38 (95% CI, 0.37–0.92; Model 2), acting as a confounder with an E-value of 3.41. Therefore, it is unlikely that 1 (or more) unmeasured factors with an E-value of around 3.0 would exist and create an impact in the way the large, entire set of known confounders considered in our study did.

**Discussion**

In a large, adult, Italian population apparently free from major chronic diseases at baseline, a moderate intake of Italian-style coffee was associated with lower risks of all-cause and CVD mortality. The association with CVD mortality follows a nonlinear J-shaped curve, showing its nadir at 3–4 cups per day. This finding is in line with a previous meta-analysis showing evidence of a nonlinear relationship between coffee consumption and CVD mortality, with the strongest association being observed for 3 cups/d (21% lower risk) (26) and with data from an umbrella review indicating the largest relative risk reduction at intakes of 3 to 4 cups a day versus none (4).

Similarly, a recent meta-analysis on nearly 4 million subjects showed that the lowest relative risks were at intakes of 3.5 cups/d for all-cause mortality and 2.5 cups/d for CVD mortality, while additional intakes were not associated with further reductions in mortality risks (7).
### TABLE 2  Risk of all-cause and cause-specific mortality associated with coffee intake in the Moli-sani Study cohort (n = 20,487)

<table>
<thead>
<tr>
<th>Coffee intake, cups/d</th>
<th>All-cause mortality, n = 234</th>
<th>CVD mortality, n = 270</th>
<th>IHD/cerebrovascular mortality, n = 152</th>
<th>CVD and metabolic-related outcomes, n = 334</th>
<th>Other causes mortality, n = 230</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤1</td>
<td>&gt;1 to ≤2</td>
<td>&gt;2 to ≤3</td>
<td>&gt;3 to ≤4</td>
<td>&gt;4</td>
<td></td>
</tr>
<tr>
<td>A/number of deaths/number of subjects</td>
<td>245/3714</td>
<td>202/4436</td>
<td>190/4680</td>
<td>108/3747</td>
<td>89/3910</td>
</tr>
<tr>
<td>Person-years</td>
<td>30,469</td>
<td>36,367</td>
<td>38,570</td>
<td>31,284</td>
<td>32,727</td>
</tr>
<tr>
<td>Event rates per 10,000 person-years</td>
<td>80.4</td>
<td>95.5</td>
<td>49.3</td>
<td>34.5</td>
<td>27.2</td>
</tr>
<tr>
<td>Model 1 HR (95% CI)¹</td>
<td>1.0</td>
<td>0.85 (0.70–1.02)</td>
<td>0.94 (0.70–1.14)</td>
<td>0.88 (0.68–1.08)</td>
<td>1.11 (0.86–1.43)</td>
</tr>
<tr>
<td>Model 2 HR (95% CI)²</td>
<td>1.0</td>
<td>0.79 (0.65–0.95)</td>
<td>0.84 (0.69–1.03)</td>
<td>0.72 (0.57–0.92)</td>
<td>0.85 (0.62–1.12)</td>
</tr>
<tr>
<td>Model 1 HR (95% CI)³</td>
<td>1.0</td>
<td>0.85 (0.70–1.02)</td>
<td>0.94 (0.70–1.14)</td>
<td>0.88 (0.68–1.08)</td>
<td>1.11 (0.86–1.43)</td>
</tr>
<tr>
<td>Model 2 HR (95% CI)⁴</td>
<td>1.0</td>
<td>0.79 (0.65–0.95)</td>
<td>0.84 (0.69–1.03)</td>
<td>0.72 (0.57–0.92)</td>
<td>0.85 (0.62–1.12)</td>
</tr>
</tbody>
</table>

Data are shown as HRs with 95% CIs. CVD, cardiovascular disease; IHD, ischemic heart disease; PA, physical activity.

¹Model 1 is adjusted for age, sex, and energy intake.
²Model 2 had the same adjustments as Model 1 and was further controlled for education, housing, residence, leisure-time PA, smoking, number of cigarettes per day, abdominal obesity, diabetes, hypertension, adherence to Mediterranean diet, tea intake, added sugar.

Potential explanations for the lack of further risk reduction observed for high coffee consumption may rely on the fact that coffee is a composite brew containing several bioactive compounds whose health effects are in opposite directions. Although it is a major source of antioxidants, coffee contains diterpenes, whose concentrations vary by the type of coffee brew, being intermediate in espresso and coffee made in a Moka pot and negligible in drip-filtered, instant, and percolator coffee (27).

Recently, data from the UK Biobank indicated a stronger direct association between blood lipids and espresso coffee as compared to filtered or instant coffee, which is possibly due to the different content of diterpenes with known lipid-raising effects (28). However, the effect of caffeine on the cardiovascular system depends on various factors, including the metabolic status and presence of illness (29), and may vary according to different genotypes and gene-environment interactions (30, 31).

To the best of our knowledge, this is the first cohort study to analyze the associations of Italian-style coffee and mortality risks in a healthy, adult population. Much of the research so far has evaluated the health effects associated with filtered or boiled coffee, which are the most popular preparation methods in the United States and Scandinavian countries, respectively (32). The favorable effect of coffee intake on the mortality risk was previously documented by other meta-analyses (3, 26, 33) and prospective cohort studies (34). Yet, our data on CVD outcomes were not in accordance with prior findings on over 40,000 Italians analyzed in the EPICOR (long-term follow-up of antithrombotic management Patterns In acute CORonary syndrome patients) Study, which reported an increased CHD risk associated with consumption of >2 cups of Italian-style coffee per day; their results are partially explained by the caffeine content and rapid consumption, resulting in a high peak plasma concentration of caffeine (35).

An umbrella review including meta-analyses of observational studies and randomized controlled trials suggested that coffee may exert beneficial effects on human health, including for some cancers (endometrial, prostate, colorectal, and liver), CVD and metabolic-related outcomes (such as type 2 diabetes and metabolic syndrome), and neurological conditions (such as Parkinson’s disease, Alzheimer’s disease, and depression) (5). Of note, it was shown in the same Moli-sani population studied here that coffee consumption reduced the risk of prostate cancer (36).
In EPIC, high coffee consumers (>3 cups per day) had lower all-cause mortality and digestive disease mortality risks compared to nonconsumers (37), in line with results from African Americans, Japanese Americans, Latinos, and Whites in the Multiethnic Cohort (34).

In our analyses, coffee intake was not associated with cancer death, with these results at variance with other population studies (37, 38). However, in a Mediterranean setting, a recent study suggested an inverse association between coffee consumption and the cardiovascular mortality risk in elderly (65 years old and above) after a 6-year follow-up, but no effects for total or cancer mortality, in line with our findings (39).

Several potential mechanisms could explain the beneficial effects of moderate coffee consumption on health, including increased blood antioxidant levels (40–43), inhibition of DNA methylation by caffeic acid and chlorogenic acid, 2 common catechol-containing coffee polyphenols (44), or an antiproliferative and antimetastatic activity by caffeine (36). Among other mechanisms, improved insulin sensitivity (45–47) and reduced inflammation (48, 49) have been suggested as possible pathways by which coffee consumption may reduce the risk of chronic diseases (9). The mechanisms by which higher coffee consumption is associated with increased mortality remain poorly understood.

We tested some known CVD risk factors, reflecting different underlying pathways to disease incidence and progression, as potentially mediating the relationship between coffee consumption and mortality, but failed to find consistent results with the majority of established biomarkers; in contrast, we found that circulating levels of NTproBNP accounted for a substantial proportion of the relationship between coffee consumption and all-cause mortality. NTproBNP is the N-terminal fragment of the B-type natriuretic peptide, secreted by myocytes as a reaction to several stimuli, including wall stretch (50). High levels of NTproBNP have been associated with increased cardiovascular risks and, more recently, with an increased risk of stroke (51); an intervention study on 14 obese, normotensive subjects (52) observed a significant increase in NTproBNP levels after a very short time period of lifestyle intervention, including a dietary intervention with a hypocaloric diet, thus indicating that this natriuretic peptide is susceptible to dietary exposure. We have now observed for the first time that coffee consumption is associated with lower NTproBNP levels, which possibly accounts for the inverse relationship between coffee intake and all-cause mortality. However, our findings should be considered exploratory and further research is needed to understand the clinical meaning of this association.

We also found that smokers were less likely to experience a CVD mortality risk reduction associated with coffee drinking as compared with nonsmokers, in line with previous studies (5, 21).

Lastly, with respect to a potential improvement of risk predictions deriving from the inclusion of coffee consumption into the traditional MDS, we failed to find significant changes in the discrimination ability of the modified score when supplemented with coffee.

The fact that an exposure significantly associated with an outcome does not lead to improvements in predictive value is a well-known and widespread occurrence, and does not entirely devalue the association between the variable and the outcome (53). More importantly, the association of coffee with the mortality risk was independent of the MD, supporting the notion that minor dietary changes, such as consuming coffee regularly within the usual diet, could be valuable measures for improving health, especially cardiovascular health, independent of the overall diet quality.

**Strengths and limitations of the study**

The major strengths of this study include a large sample size of an apparently healthy, adult population; a prospective cohort design; and considerable numbers of covariates included for analyses.

Moreover, the robustness of our findings was confirmed by sensitivity analyses. To the best of our knowledge, this is among only a few studies investigating the biological mechanisms potentially linking coffee intake to health outcomes in a primary prevention context.

However, there are also a number of caveats. First, given the observational nature of our investigation, causality can only be suggested and residual confounding cannot be fully ruled out. However, the use of E-values indicates a very small impact of potential unmeasured factors on the strength of our associations. Second, only the all-cause mortality outcome could be fully explored, as cause-specific mortality analyses were limited by the relatively small number of events. Lastly, subjects’ information was collected at baseline only; thus, changes that possibly occurred during the follow-up could not be considered.

**Conclusions**

In conclusion, this study shows a beneficial effect of Italian-style coffee consumption (when limited to 4 cups per day) on all-cause and CVD mortality risks, confirming in a large cohort of Italian adults the results of previous epidemiological studies performed in other settings.

The mechanisms through which moderate coffee consumption could lead to a lower mortality risk are still unclear, although an important mediation was found for NTproBNP levels, while other biomarkers tested were unlikely to be on the coffee-mortality prevention pathway. Finally, the inclusion of coffee intake into a traditional score assessing adherence to the MD did not offer any added value to the discrimination ability of the modified score.

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The authors’ responsibilities were as follows—ER, MB, and LI: contributed to the design of the study and interpretation of data; SC, ADeC, and MP: managed data collection; ER, MB, and ADiC: analyzed the data; ER and MB: wrote the manuscript; MBD, CC, GdG, and LI: originally inspired the research and critically reviewed the manuscript; and all authors: read and approved the final manuscript.

**References**


