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CLINICAL RESEARCH
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Ultra-processed food intake and all-cause and cause-specific mortality in individuals with cardiovascular disease: the Moli-sani Study

Marialaura Bonaccio [1]*, Simona Costanzo [1], Augusto Di Castelnuovo [2], Mariarosaria Persichillo [1], Sara Magnacca[2], Amalia De Curtis [1], Chiara Cerletti[1], Maria Benedetta Donati[1], Giovanni de Gaetano [1], and Licia Iacoviello [1,3]; for the Moli-sani Study Investigators[†]

[1]Department of Epidemiology and Prevention, IRCCS NEUROMED, Via dell'Elettronica, 86077 Pozzilli, IS, Italy; [2]Mediterranea Cardiocentro, Via Orazio, Napoli 80122, Italy; and [3]Department of Medicine and Surgery, Research Center in Epidemiology and Preventive Medicine (EPIMED), University of Insubria, Varese, Italy

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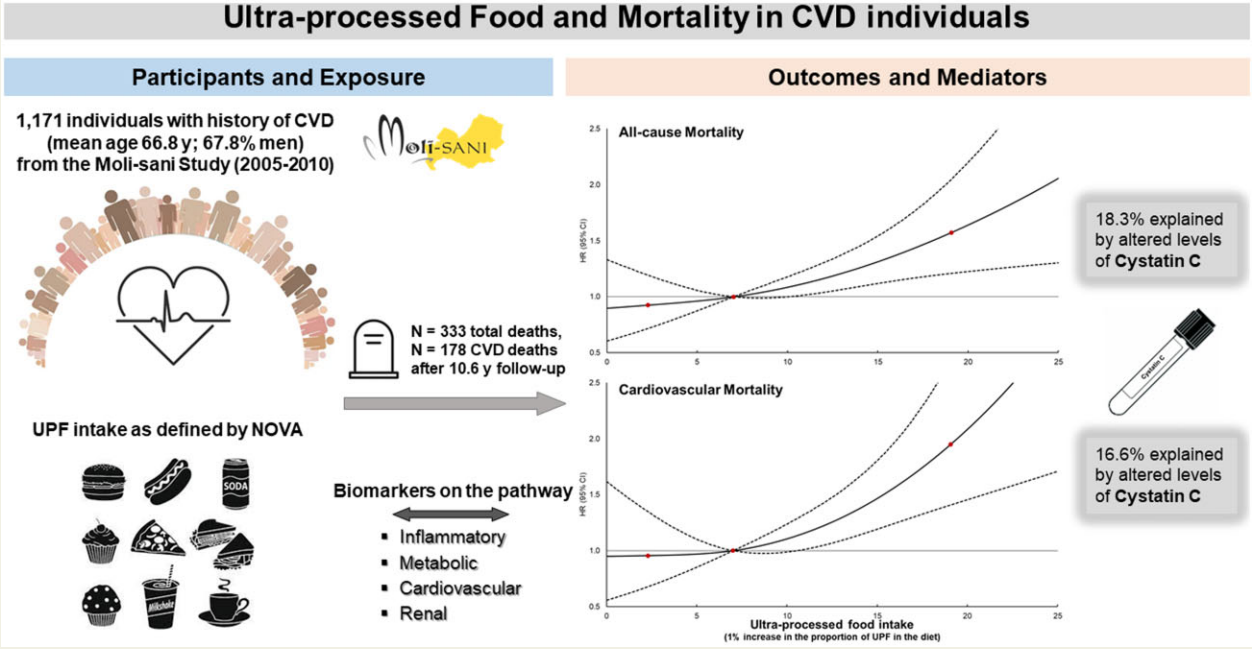
Graphical Abstract



* Corresponding author: Tel: +39 0865 915243, Email: marialaura.bonaccio@moli-sani.org
† Moli-sani Study Investigators are listed in the Supplementary material online, *Appendix*.

Aims To evaluate the association of ultra-processed food (UPF) intake and mortality among individuals with history of cardiovascular disease (CVD) and analyse some biological pathways possibly relating UPF intake to death.

Methods and results Longitudinal analysis on 1171 men and women (mean age: 67 ± 10 years) with history of CVD, recruited in the Moli-sani Study (2005–10, Italy) and followed for 10.6 years (median). Food intake was assessed using a food frequency questionnaire. UPF was defined using the NOVA classification according to degree of processing and categorized as quartiles of the ratio (%) between UPF (g/day) and total food consumed (g/day). The mediating effects of 18 inflammatory, metabolic, cardiovascular, and renal biomarkers were evaluated using a logistic regression model within a counterfactual framework. In multivariable-adjusted Cox analyses, higher intake of UPF (Q4, ≥11.3% of total food), as opposed to the lowest (Q1, UPF <4.7%), was associated with higher hazards of all-cause (hazard ratio [HR]: 1.38; 95% confidence interval (CI): 1.00–1.91) and CVD mortality (HR: 1.65; 95% CI: 1.07–2.55). A linear dose–response relationship of 1% increment in UPF intake with all-cause and CVD mortality was also observed. Altered levels of cystatin C explained 18.3% and 16.6% of the relation between UPF (1% increment in the diet) with all-cause and CVD mortality, respectively.

Conclusion A diet rich in UPF is associated with increased hazards of all-cause and CVD mortality among individuals with prior cardiovascular events, possibly through an altered renal function. Elevated UPF intake represents a major public health concern in secondary CVD prevention.

Keywords Ultra-processed food • Secondary cardiovascular prevention • Mortality • Biomarkers

Introduction

The health impact of food processing has become a relevant and timely topic given the increasing volume of industrially processed food worldwide. Processed food constitutes a large part of the world's food consumption; the proportion of food that is ultra-processed is almost 60% in the USA and in the UK,[1,2] 42% in Australia,[3] and about 24% and 17% in Mediterranean countries such as Spain[4] and Italy,[5] respectively. The term ultra-processed foods (UPF) indicates formulations generally including five or more and usually many ingredients, mostly of cheap industrial sources of dietary energy and nutrients plus additives, using a series of processes and containing minimal whole foods.[6]

Within epidemiological research, NOVA (a name, not an acronym) is the most widely used food classification based on degree of processing and was originally conceived to overcome limitations pertaining to traditional nutrition approaches focussed exclusively on nutrients.[6,7]

Recently, a direct association between consumption of UPF and risk of developing cardiovascular disease (CVD) was described in the well-established Framingham Offspring cohort.[8] Such results align with recent evidence that diets rich in UPF likely lead to higher hazard of mortality and disease in the general population,[9,10] possibly through mechanisms that include poor nutritional content of these foods, food structure, cosmetic additives, neo-formed compounds, and contact materials.[11] Whether consumption of UPF predicts mortality and CVD outcomes in individuals with a history of CVD remains to be established.

Although high consumption of UPF has been reportedly associated with an increased risk of metabolic conditions, such as obesity, diabetes, hyperlipidaemia,[12] and a decline in renal function,[13] only few epidemiological studies so far have addressed longitudinally whether such altered conditions possibly mediate the relation between UPF and adverse health outcomes.[9]

To fill this knowledge gap, we first aimed to disentangle the association of UPF intake with all-cause and cause-specific mortality among individuals with a history of CVD by analysing data from a large Italian cohort recruited within the Moli-sani Study, taking advantage of a long follow-up period; second, we tested blood biomarkers (e.g. markers of renal function) and other known CVD risk factors (e.g. blood pressure) as possible parameters on the pathway between UPF and all-cause and cause-specific mortality.

Methods

Study population

We analysed data from the Moli-sani Study, a prospective cohort study for the investigation of risk factors for chronic diseases, where we had previously documented an increased death rate associated with UPF intake.[9] During 2005–10, 24 325 individuals residing in Molise, a region of central-southern Italy, aged ≥35 years were randomly enrolled from city hall registries by a multistage sampling. Exclusion criteria were pregnancy at the time of recruitment, disturbances in mental or decision-making impairments, current poly-traumas or coma, or refusal to sign the informed consent. Thirty percentage of subjects refused to participate and were generally older and had a higher prevalence of CVD and cancer than other participants. Further details of the study design are available elsewhere.[14]

From the initial study sample, 1320 reported at enrolment a previous diagnosis of CVD, including angina, myocardial infarction, revascularization procedures, peripheral artery disease, and cerebrovascular events. Each self-reported CVD event was confirmed if at least one of the following criteria was fulfilled: (i) the participant reported the date of admission to the hospital; (ii) reported drug use for ischaemic vascular disease; and (iii) presented medical records of ischaemic vascular disease diagnosis.

Participants were excluded from the present analyses if they reported missing data on diet, implausible energy intake (<800 or >4000 kcal/day in men and <500 or >3500 kcal/day in women) or extreme food intake (<0.5th and >99.5th centiles of total food eaten, and >99.5th of UPF

consumed), dietary or medical questionnaires judged as unreliable by interviewers, or missing data on cause-specific death.

We finally analysed 1171 subjects with CVD at baseline. Supplementary material online, *Figure 1* shows the flowchart for selection of study participants.

Outcome ascertainment

The Moli-sani Study cohort was followed up for mortality until 31 December 2018. Cause-specific mortality was assessed by the Italian mortality registry, validated by Italian death certificates (ISTAT form), and coded according to the International Classification of Diseases (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 specific cause of death for cerebrovascular disease, ICD-9 codes 410–414 and 429 for ischaemic heart disease (IHD).

Cancer death was considered when the underlying cause of death included ICD-9 codes 140–208. Non-cardiovascular/non-cancer causes of death were included in the 'other cause mortality' group.

The Moli-sani Study was approved by the Ethical Committee of the Catholic University (Rome, Italy) and participants provided written informed consent.

Dietary assessment

Food intake during the year before enrolment was assessed by an interviewer-administered semi-quantitative EPIC food frequency questionnaire (FFQ) validated and adapted to the Italian population,[15] for a total of 188 food items that were classified into 83 pre-defined food groups on the basis of similar nutrient characteristics or culinary usage.

Using a specifically designed software,[16] frequencies and quantities of each food were linked to Italian Food Tables[17] to obtain estimates of daily intake of macro- and micro-nutrients plus energy.

To estimate UPF, we used the NOVA classification[6] that groups foods into four categories representing levels of processing: (i) fresh or minimally processed foods (e.g. fruit, meat, milk); (ii) processed culinary ingredients (e.g. oils, butter); (iii) processed foods (e.g. canned fish); or (iv) UPF containing predominantly industrial substances and little or no whole foods (e.g. carbonated drinks, processed meat, snacks). For the purpose of these analyses, we used the fourth UPF category.

We summed up the amount consumed (g/day) of each food group from the fourth category of NOVA (a total of 22 foods and beverages) and calculated the proportion (%) of UPF in the total weight of food and beverages consumed (g/day) by creating a weight ratio.

Such approach is more appropriate than energy ratio since it better accounts for non-nutritional factors pertaining to food processing (e.g. neo-formed contaminants, additives, and alterations to the structure of raw foods).[18,19] Participants were then divided into quartiles based on the proportion of UPF food consumed over the total food intake. The full list of foods categorized according to the NOVA classification is available as Supplementary material online, *Table 1*.

Adherence to the traditional Mediterranean diet was defined through the Mediterranean diet score (MDS) developed by Trichopoulou *et al.*[20]

Covariate assessment

Personal history of cancer was self-reported and confirmed by medical records. Participants were considered to have diabetes, hypertension, or hyperlipidaemia at baseline if they were taking disease-specific drugs.

Leisure-time physical activity was expressed as daily energy expenditure in metabolic equivalent task-hours for sport, walking, and gardening. Height and weight were measured and body mass index (BMI) was calculated as kg/m^2.

Subjects were classified as never, current, or former smokers (quit at least 1 year ago). Education was based on the highest qualification attained and was categorized as up to lower secondary (approximately ≤8 years of study), upper secondary school (>8 ≤ 13), and post-secondary education (>13). Housing tenure was classified as rented, 1 dwelling ownership, and >1 dwelling ownership.

Selection and assessment of biomarkers of CVD risk

Key biological mechanisms through which UPF intake may adversely affect health include, among others, altered serum lipid concentrations, inflammation, oxidative stress, dysglycaemia, insulin resistance, and hypertension.[12]

The selection of biomarkers reflecting different underlying pathways to CVD incidence and progression,[21,22] as potential mediators of an association between UPF intake and mortality was done by subject area knowledge according to the following criteria: (i) previously studied for their relevance in pathways predisposing to CVD; (ii) shown in epidemiologic studies to be related to CVD or mortality; and (iii) already investigated in the Moli-sani Study cohort.

Blood samples were collected at baseline (2005–10) in participants who had fasted overnight and had refrained from smoking for at least 6 h; lipids (total cholesterol, HDL-cholesterol, triglycerides) and blood glucose were assayed in serum samples by enzymatic reaction methods using an automatic analyser (ILab 350, Instrumentation Laboratory, Milan, Italy) and quality control for lipids and glucose was obtained by a commercial standard (SeraChem® 1 and 2). The coefficients of variability were, respectively, 4.9% and 5.2% for blood cholesterol; 3.2% and 3% for HDL-cholesterol; 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 in-house serum pool and commercial laboratory standard; inter-day coefficients of variability for CRP were 5.5% and 4.2%.

Haemocromocytometric analysis was performed by cell count (Coulter HMX, Beckman Coulter, IL, Milan, Italy) within 3 h of blood collection. Quality control was performed by using three different levels of standards: Abnormal 1 (Abn1, a pathologically high control), Abnormal 2 (Abn2, a pathologically low control), and Normal (Coulter HMX, Beckman Coulter). Coefficient of variability for white blood cells was 6.2%, 3.3%, and 3.0% for Abn1, Abn2, and Normal, respectively. Apolipoprotein A1, apolipoprotein B100, 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 research project (EUFP7, HEALTH-F2-2011-278913) 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.[21]

Statistical analyses

Baseline characteristics are presented as means (±standard deviations) for quantitative traits and number and percentages for categorical variables. Positively skewed variables were log transformed before analysis. Differences in the distribution of baseline covariates according to UPF quartiles were calculated using generalized linear models adjusted for age, sex, and energy intake (GENMOD procedure for categorical variables and GLM procedure for continuous variables in SAS software; *Table 1*).

The proportional hazards assumption was assessed using weighed Schoenfeld residuals, and no violation was identified (*P*-value for global

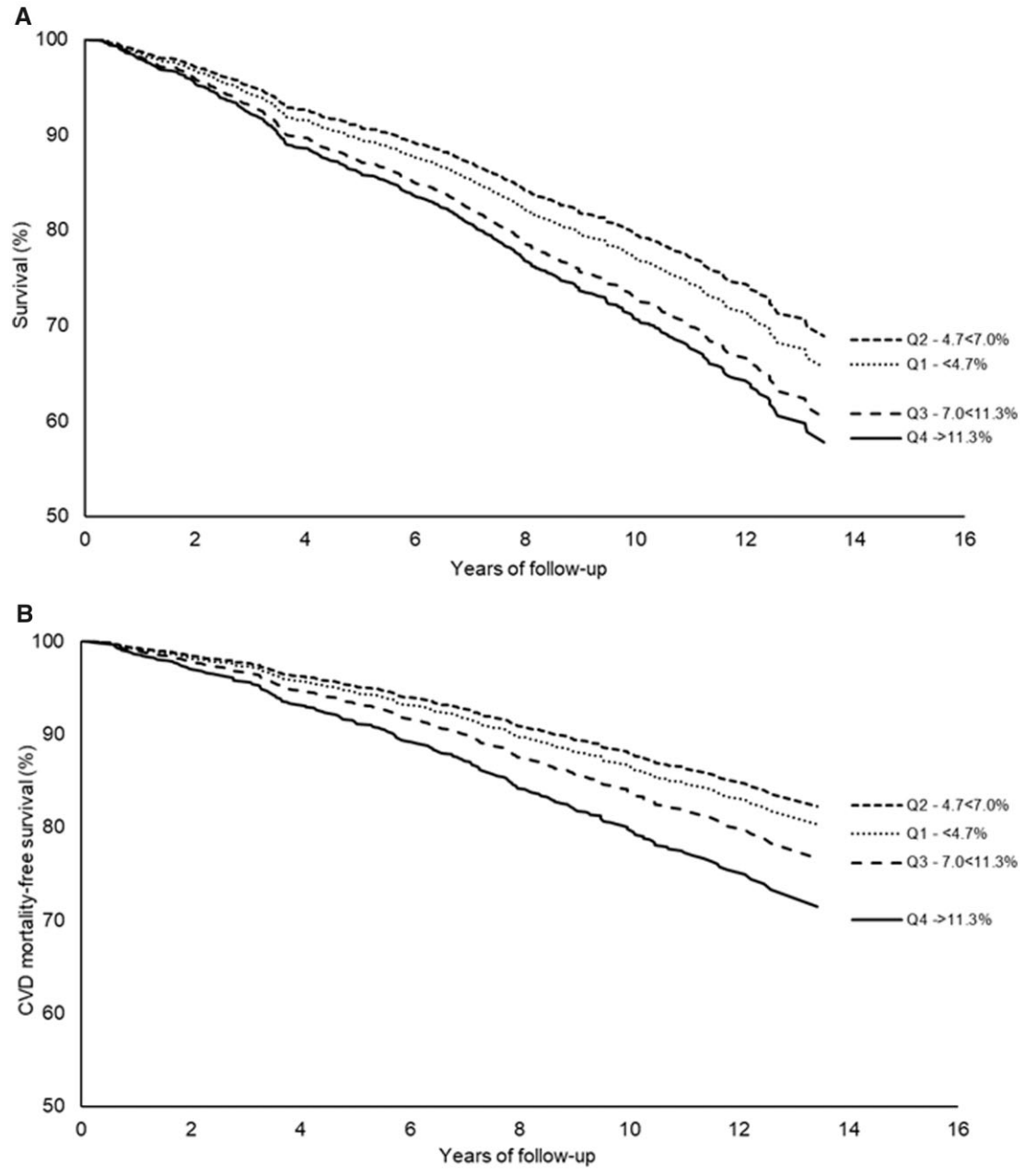


Figure 1 Multivariable-adjusted survival curves for (*A*) all-cause and (*B*) cardiovascular mortality across quartiles of ultra-processed food intake among 1171 individuals with history of cardiovascular disease from the Moli-sani cohort, generated using the first imputed dataset. The other imputed datasets were similar and thus omitted. Survival estimates were obtained from the multivariable model adjusted for sex, age, energy intake, educational level, housing tenure, smoking, leisure-time physical activity, body mass index, history of cancer, diabetes, hypertension, hyperlipidaemia, residence, and the Mediterranean diet score.

test = 0.26). Rate estimates for all-cause and cause-specific mortality were expressed as hazard ratios (HRs) with 95% confidence interval (95% CI) and calculated by using Cox proportional hazards models with time-on-study as the time scale and adjusting for baseline age as covariate in the model. Multivariable-adjusted HRs were calculated across quartiles of UPF, as well as considering the UPF as a continuous variable (1% increase in the proportion of UPF in the diet).

Participants contributed person-time until date of death, date of emigration or loss to follow-up, or end of follow-up, whichever occurred first. Participants who died from another cause than the one under study were included and censored at the date of the competing death event.

Potential confounders were defined a priori and identified based on existing literature, rather than deferring to statistical criteria.[23]

The following models were ultimately fitted: (i) crude model; (ii) age, sex, and energy intake adjusted; (iii) Model 1 including sex, age (continuous), energy intake (continuous), educational level (up to lower secondary, upper secondary, post-secondary), housing tenure (rent, 1 dwelling ownership, >1 dwelling ownership), smoking (never, current, former

Table I Baseline characteristics of the study population by quartiles of ultra-processed food intake (weight ratio expressed as % g/day) among individuals with history of cardiovascular disease from the Moli-sani Study cohort (*n* = 1171)

	Quartiles of ultra-processed food				
	Q1	Q2	Q3	Q4	*P*-value
Median, min–max (weight ratio, %)	3.4 (0.01 < 4.7)	5.8 (4.7 < 7.0)	8.6 (7.0 < 11.3)	15.0 (11.3 < 35.2)	–
No. of subjects (%)	292 (25.0)	293 (25.0)	293 (25.0)	293 (25.0)	–
Age (years), mean (SD)	68 (9)	68 (9)	66 (10)	64 (11)	<0.0001
Men	75.0	73.4	64.2	58.7	<0.0001
Urban residence	67.5	68.3	67.9	70.0	0.72
Educational level					0.055
Up to lower secondary	64.4	68.6	73.4	62.5	
Upper secondary	26.0	22.9	19.4	30.0	
Post-secondary	9.3	7.5	7.2	7.5	
Missing data	0.3	1.0	0.0	0.0	
Housing tenure					0.052
Rent	5.1	9.2	10.2	10.9	
1 dwelling ownership	83.2	81.6	80.9	79.9	
>1 dwelling ownership	11.3	8.9	8.5	9.2	
Missing data	0.3	0.3	0.3	0.0	
Smoking status					0.022
Non-smokers	31.2	38.6	34.1	42.3	
Current	12.0	10.9	14.3	17.4	
Former	56.8	50.5	51.5	40.3	
BMI (kg/m^2), mean (SD)	29.4 (4.1)	29.6 (4.5)	29.6 (4.7)	29.1 (4.8)	0.41
Leisure-time physical activity (MET-h/day), mean (SD)	3.3 (4.1)	3.1 (3.7)	3.6 (4.6)	3.1 (4.5)	0.52
Cancer					0.096
No	95.2	94.2	92.8	90.8	
Yes	4.5	5.5	6.5	9.2	
Missing data	0.3	0.3	0.7	0.0	
Diabetes					0.64
No	76.7	82.9	81.2	86.0	
Yes	22.3	15.0	16.7	13.6	
Missing data	1.0	2.1	2.1	0.4	
Hypertension					0.96
No	27.4	26.6	30.4	31.4	
Yes	71.6	73.4	68.9	67.6	
Missing data	1.0	0.0	0.7	1.0	
Hyperlipidaemia					0.22
No	44.5	47.1	50.8	55.6	
Yes	52.1	49.8	47.1	42.3	
Missing data	3.4	3.1	2.1	2.1	
Dietary factors, mean (SD)					
MDS	4.9 (1.6)	4.7 (1.6)	4.4 (1.6)	4.2 (1.7)	<0.0001
Good adherence to Mediterranean diet (MDS ≥6; %)	34.6	32.1	25.9	19.1	<0.0001
Fruits and nuts (g/day)	393 (204)	368 (182)	350 (181)	339 (175)	0.0027
Vegetables (g/day)	165 (69)	152 (60)	153 (67)	129 (60)	<0.0001
Cereals (g/day)	215 (92)	210 (87)	192 (80)	176 (85)	<0.0001
Legumes (g/day)	32 (26)	27 (22)	30 (24)	28 (25)	0.13
Fish (g/day)	46 (30)	47 (29)	45 (24)	44 (27)	0.59
MUFA/SFA ratio	1.58 (0.38)	1.47 (0.30)	1.42 (0.26)	1.32 (0.24)	<0.0001
Milk and dairy products (g/day)	179 (117)	185 (101)	188 (113)	186 (102)	0.76
Meat and meat products (g/day)	87 (35)	96 (41)	99 (39)	93 (44)	0.0002
Alcohol intake (g/day)	22 (23)	17 (20)	13 (18)	8 (15)	<0.0001

Continued

Table 1 *Continued*

	Quartiles of ultra-processed food				
	Q1	Q2	Q3	Q4	*P*-value
Energy intake (kcal/day)	1668 (497)	1811 (482)	1891 (565)	1981 (548)	<0.001
Carbohydrate (% total energy intake)	49 (8)	49 (7)	48 (7)	50 (7)	0.010
Sugar (g/day)	77 (28)	76 (28)	82 (33)	94 (33)	<0.0001
Protein (% total energy intake)	16.1 (2.6)	16.7 (2.3)	16.7 (2.2)	16.3 (2.1)	0.0019
Fat (% total energy intake)	30 (6)	32 (5)	34 (5)	33 (6)	<0.0001
Saturated fat (% total energy intake)	10.1 (2.7)	11.0 (2.3)	11.7 (2.2)	12.2 (2.6)	<0.0001
Saturated fat (g/day)	21 (6)	23 (7)	25 (8)	26 (10)	<0.0001
Monounsaturated fat (% total energy intake)	15.2 (3.5)	15.7 (2.9)	16.3 (2.9)	15.7 (2.8)	0.0003
Polyunsaturated fat (% total energy intake)	3.3 (0.7)	3.5 (0.6)	3.6 (0.6)	3.6 (0.6)	<0.0001
Dietary cholesterol (mg/day)	257 (79)	280 (87)	287 (97)	303 (114)	<0.0001
Fibre intake (g/day)	21 (7)	20 (7)	20 (7)	19 (7)	<0.0001
Sodium (mg/day)	2062 (690)	2123 (740)	2130 (760)	2072 (895)	0.16
CVD risk factors, mean (SD)					
C-reactive protein (mg/L)[a]	1.82 (1.60–2.06)	1.90 (1.68–2.14)	1.92 (1.71–2.17)	2.03 (1.80–2.29)	0.66
White blood cell count (x10^9/L)[a]	6.1 (5.9–6.3)	6.1 (5.9–6.3)	6.0 (5.8–6.2)	6.3 (6.1–6.5)	0.13
Granulocyte/lymphocyte ratio	2.16 (1.11)	2.10 (0.91)	2.03 (0.84)	2.20 (0.94)	0.15
Blood glucose (mg/dL)[a]	110 (107–114)	106 (103–110)	108 (105–111)	106 (102–109)	0.27
Insulin (pmol/L)[a]	56.8 (52.9–60.9)	56.7 (53.0–60.7)	57.2 (53.5–61.2)	55.9 (52.3–59.7)	0.97
C-peptide (ng/mL)[a]	1.76 (1.64–1.88)	1.75 (1.63–1.87)	1.79 (1.68–1.91)	1.81 (1.69–1.93)	0.89
Blood cholesterol (mg/dL)	202 (42)	198 (43)	195 (42)	192 (41)	0.051
HDL-cholesterol (mg/dL)	57 (15)	55 (13)	53 (14)	53 (14)	0.0058
Triglycerides (mg/dL)[a]	126 (119–133)	122 (115–129)	130 (123–138)	121 (114–128)	0.21
ApoA (g/L)	1.56 (0.32)	1.52 (0.28)	1.52 (0.30)	1.49 (0.32)	0.10
ApoB100 (g/L)	0.93 (0.24)	0.92 (0.25)	0.93 (0.24)	0.92 (0.24)	0.82
Lp(a) (mg/dL)	23.6 (24.5)	24.2 (24.2)	20.1 (22.0)	22.1 (23.0)	0.18
Cystatin C (mg/L)[a]	1.08 (1.05–1.11)	1.10 (1.07–1.13)	1.13 (1.10–1.16)	1.15 (1.12–1.19)	0.0066
Creatinine (mg/dL)[a]	0.84 (0.82–0.87)	0.85 (0.83–0.88)	0.87 (0.85–0.90)	0.88 (0.85–0.91)	0.14
Systolic BP (mmHg)	150 (21)	147 (19)	151 (22)	150 (22)	0.094
Diastolic BP (mmHg)	81 (10)	80 (9)	80 (10)	82 (10)	0.12
Heart rate (b.p.m.)	66.0 (10.6)	65.1 (10.6)	65.4 (10.0)	67.4 (11.5)	0.0046
Serum vitamin D (ng/mL)	16.9 (8.8)	17.3 (8.7)	16.8 (8.4)	16.6 (8.6)	0.75

Values are reported as percentages unless otherwise specified. BMI, leisure-time physical activity, dietary data, and biomarkers are reported as means (SD) adjusted for age, sex, and energy intake. *P*-values were obtained using generalized linear models both for continuous and categorical dependent variables adjusted for age, sex and energy intake.
ApoA, apolipoprotein A; ApoB100, apolipoprotein B100; BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; HDL, high-density lipoprotein; Lp(a), lipoprotein(a); MDS, Mediterranean diet score; MUFA, monounsaturated fat; SD, standard deviation; SFA, saturated fat.
[a]Geometric means with corresponding 95% confidence intervals, adjusted for age, sex, and energy intake.

smokers), BMI (continuous), leisure-time physical activity (continuous), cancer (no/yes), diabetes (no/yes), hypertension (no/yes), hyperlipidaemia (no/yes), and residence (urban, rural); and (iv) Model 2 as in Model 1 further controlled for MDS (continuous).

Missing data on covariates (see flowchart in Supplementary material online, *Figure 1*) were handled using multiple imputation (SAS PROC MI, followed by PROC MIANALYZE) to maximize data availability for all variables, avoid bias introduced by not-at-random missing data patterns, and achieve robust results over different simulations ($n = 10$ imputed datasets).

After being selected by subject area knowledge, a biomarker was considered as potentially mediating the association of UPF intake with all-cause and cause-specific mortality, if it resulted associated with both the exposure and the outcome, in accordance with predefined mediation principles.[24,25]

These criteria were tested in distinct multivariable regression models for each potential mediator individually (Supplementary material online, *Table 2*) and through Cox models including UPF consumption (continuous) as a covariate (Supplementary material online, *Table 3*).

For the mediation analysis, we used mediation models (SAS PROC CAUSALMED) to assess the potential mediation effect of biomarkers on the association between UPF intake (1% increment of UPF in the diet) and all-cause and CVD mortality in a counterfactual framework.[26]

PROC CAUSALMED estimates causal mediation effects and CIs for the effects based on the maximum likelihood estimates. We utilized 1000 bootstrap resampling to compute CIs of the percentage mediated. The factors adjusted in the mediation analyses were the same as those in the main analyses (Model 2).

To test for a potential non-linear, continuous relationship between UPF and mortality, we used multivariable Cox regression analysis with

UPF intake modelled as restricted cubic splines (three knots at 5%, 50%, and 95% of the UPF distribution)[27] and used the median value of UPF weight ratio (=7.02%) as the reference value.

We calculated the hypothetical population attributable risk, an estimation of the percentage of mortality in the study population during follow-up that theoretically would not have occurred if all individuals had been in the low-risk category, assuming a causal relation.

For these analyses, we compared participants in the highest UPF intake (Q4) with the rest of the population (Q1 + Q2 + Q3).

Statistical tests were two-sided, and *P*-values < 0.05 were considered to indicate statistical significance. The data analysis was generated using SAS/STAT software, version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

The analysed population consisted of 377 women (32.2%) and 794 men (67.8%) with a mean age of 66.8 years (min 38.0 to max 74.1 years).

Study participants reported a median of 7.02% [interquartile range (IQR): 4.7–11.3%] UPF intake in the diet, an average UPF daily intake of 132.0 g/day (IQR: 65.9–172.8 g), and the average energy from UPF was 17.8% (IQR: 12.2–22.0%) of the total calories consumed daily. Food mostly contributing to the total of UPF consumed were processed meat (18.3%), cakes/pies/pastries (11.5%), crispbread/rusks (11.3%), and non-homemade pizza (10.2%) (Supplementary material online, *Table 1*). As compared with subjects with low UPF intake (Q1), those having high proportion of UPF in the diet (Q4) were younger, less frequently men, and less likely former smokers, whereas no substantial differences were found for chronic conditions at baseline.

Adherence to the Mediterranean diet tended to be progressively lower across quartiles of UPF intake, as well as consumption of fruits and nuts, vegetables, cereals, monounsaturated (MUFAs) over saturated fats (SFAs), and alcohol, while meat and meat products intake was higher among those with high UPF intake. Heavy UPF consumers tended to consume more calories, carbohydrates, sugar, SFA, MUFA, polyunsaturated fat, and dietary cholesterol while exhibiting lower consumption of fibre (*Table 1*).

Regarding CVD risk factors, higher UPF was associated with increased concentrations of cystatin C and heart rate, and lower levels of HDL-cholesterol (*Table 1*). Multivariable-adjusted regression analyses showed that UPF intake was significantly associated with higher levels of biomarkers of renal function, as well as with diastolic blood pressure and heart rate, while being inversely related to blood glucose, total blood, and HDL-cholesterol (Supplementary material online, *Table 2*).

After a median follow-up of 10.6 years (IQR: 9.2–11.9 years; 11 536 person-years), a total of 333 all-cause deaths occurred, including 178 CVD, 114 IHD/cerebrovascular, 80 cancer, and 75 other deaths.

In multivariable-adjusted Model 1, participants in the highest quartile of UPF intake (Q4) had a 46% relatively higher hazard of all-cause mortality compared with those in the lowest quartile (HR: 1.46; 95% CI: 1.06–2.00), that was attenuated to 1.38 (95% CI: 1.00–1.91) after controlling for the traditional MDS (*Table 2*).

CVD mortality increased by 65% (95% CI: 1.07–2.55) in the highest UPF quartile as compared with the lowest, independently of the MDS (*Table 2*).

Increased UPF intake was also directly associated with IHD/cerebrovascular death with a hazard of 1.65 (95% CI: 0.96–2.85) and a significant dose–response relation (*P* for trend = 0.021), while no association with cancer death nor mortality from other causes was observed (*Table 2*).

Multivariable-adjusted survival curves for all-cause and CVD mortality across UPF quartiles are well separated and tend to diverge over time (*Figure 1*).

Accordingly, the multivariable dose–response analysis between a 1% increase in the proportion of UPF in the diet and CVD mortality showed a direct linear dose–response relationship (*P* for overall association = 0.0003; *P* for non-linearity = 0.24). In comparison with the reference value of 7.02%, the relative hazard of CVD mortality significantly increased at proportion of UPF > 10.7% and was almost 50% higher when the proportion of UPF reached 15.4% (*Figure 2B*). Similar findings were observed for all-cause mortality (*P* for overall association = 0.0028; *P* for non-linearity = 0.47) (*Figure 2A*).

The population attributable risk for CVD mortality associated with the highest UPF intake (Q4) was 8.8% (95% CI: –7.6 to 9.99%) and 8.7% (95% CI: –0.5 to 9.97%) for all-cause mortality.

Mediation analysis

When analysing biological mechanisms potentially linking UPF to all-cause and CVD mortality, we found that part of such relations was explained by altered renal function, with cystatin C concentrations, respectively, accounting for 18.3% and 16.6% of the excess of all-cause and CVD mortality associated with 1% increase in UPF intake (*Table 3*). Heart rate explained 13% of the relationship between UPF intake and all-cause mortality.

Discussion

This is possibly the first study to provide evidence that a diet rich in UPF, as assessed by the NOVA classification, is independently associated with an increased hazard for all-cause and CVD mortality among adults with pre-existing cardiovascular events (*Graphical Abstract*).

We confirmed our preliminary data showing that a 5% increase in the proportion of UPF in the diet led to increased all-cause and CVD mortality rates among high-risk individuals such as those with prior CVD or individuals with diabetes over 8.2 years of follow-up.[9]

In this study, we focussed specifically on a sub-population from the Moli-sani Study with pre-existing CVD and took advantage from an extended follow-up of 10.6 years; moreover, the mediation analysis represents a major novelty of this study.

Analyses on this sample of 1171 high-risk individuals extend prior evidence from general populations confirming an increased hazard of disease/mortality associated with regular consumption of highly processed foods.[9,10,18,19]

Recent findings from the Framingham Offspring cohort on 3000 CVD-free subjects showed that each additional daily serving of UPF resulted in an increased risk of CVD incidence and mortality,[8] in line with prior studies.[9,18,28]

The potential adverse health effects of a diet rich in UPF, as reflected by the NOVA classification, in a secondary CVD setting have never been addressed to date; while the health advantages associated with a Mediterranean diet have been supported by high-quality observational studies,[29,30] there is scarce evidence indicating that Western-type diets, typically rich in processed/highly processed food, may increase the risk of secondary CVD events.[31]

The inclusion of baseline adherence to a traditional Mediterranean diet in our analyses only marginally attenuated the magnitude of the association between UPF and all-cause and CVD mortality, suggesting that UPF are an independent risk factor for secondary CVD events.

UPF tend to be nutrient poor while representing a major source of SFA, added sugar, dietary cholesterol, energy density, resulting in an overall lower nutritional quality of the diet[11] that may be partially responsible for the observed detrimental health effects.

Consistently, in our study, people eating larger amounts of UPF exhibited a lower nutritional profile, although no difference in sodium intake was observed and this may be due to the lack of data on salt added during preparation or at the table, or to the fact that UPF include either sweet (e.g. cakes, breakfast cereals, biscuits) or salty products, the latter being more prevalent in the processed food category (e.g. cheese, ham).

Our analyses were controlled for a measure of overall diet quality at baseline, as reflected by the Mediterranean diet, which did not substantially alter the association between UPF and CVD mortality, suggesting that the excess of all-cause and CVD mortality associated with elevated intake of UPF is only partially mediated by an overall low diet quality, in accordance with others.[8]

Besides being nutrient poor, UPF are important sources of cosmetic additives (e.g. glutamates, emulsifiers, sulphites) and neoformed compounds (e.g. acrylamide) resulting from food processing and particularly heat treatments that may promote disease.[32]

In addition, evidence from mechanistic studies suggests that processing itself actually matters for health and, even with identical chemistry, food structure can make a major difference to biological and health outcomes.[33]

Indeed, the greater deconstruction of the original food matrix and the cosmetic additives added to these products have been associated with changes in the composition and metabolic behaviour of the gut microbiota that promote inflammatory diseases.[34]

Finally, UPF are frequently packaged in materials that are a source of phthalates and bisphenols, which are multifunctional synthetic chemicals used to make plastics flexible and durable.[35]

Studies on the cardiovascular toxicity of plasticizers additives point to inflammation, oxidative stress, and hormone imbalance as potential mediators,[36] and experimental studies have established an association between exposure to plastic chemicals and cardiac dysfunction.[37,38] In addition, bisphenols exhibit nephrotoxicity,[39] and long-term exposure to phthalates is associated with nephropathy and exacerbates chronic kidney disease progression.[40]

Regarding biological mechanisms, we found that part of the excess of all-cause and CVD mortality associated with increasing UPF consumption was likely explained by altered renal function as reflected by higher concentrations of cystatin C among heavy UPF consumers as compared with people consuming lower amounts of these foods. These results align with prior observations from the general population of the Moli-sani Study,[9] and are also in agreement with data from a Spanish population of older adults showing that high UPF consumption is associated with 50% increased risk of renal function decline, independently of other risk factors that predispose to renal function impairment.[13]

Noticeably, we failed to observe any association between UPF intake and cancer mortality in line with prior findings from another large prospective cohort study,[41] possibly because cancer mortality is influenced by too many factors, well beyond diet and lifestyles, which also include diagnostic practices and prevention strategies that may vary substantially across socioeconomic strata of the population. It is worth noting that available evidence on the relation between UPF and cancer is inconsistent, with some observational studies suggesting that elevated UPF consumption may lead to an increased risk of certain types of cancer, while not being associated with some other types.[19,42]

Finally, it is worth noting that the average energy intake from UPF in this cohort is in line with estimations from general adult populations in other Mediterranean countries[4,5]; this suggests that, although CVD individuals in our cohort might have already adopted a healthy dietary pattern following lifestyle advice from their cardiologists, the contribution of UPF to their diet is not negligible and deserves particular attention from the public health experts.

Strengths and limitations

To our knowledge, this appears to be the first study evaluating the association of UPF intake in secondary CVD prevention, and testing several potentially mediating pathways, as measured by a large set of biomarkers.

Important strengths of the present study include its prospective design, the examination of numerous biomarkers representative of different pathophysiological processes, and the detailed information on several dietary, lifestyle, and clinical factors to minimize confounding.

Yet our findings should be interpreted in light of several limitations. This is an observational study thus causality cannot be inferred, and although analyses were controlled for several factors, we cannot fully rule out the potential of residual confounding by unmeasured factors. The relatively small sample size, along with quite small mortality cases in some of the categories of UPF, is another critical limitation of this study; however, this is a common feature of studies in a secondary CVD prevention setting.[43]

The FFQ used in this population was not specifically conceived to collect data based on the NOVA classification, thus many food items were not included (e.g. pre-prepared dishes, energy bars, slimming products). Also, dietary data were self-reported and this may lead to recall and selection bias.

Another weakness is that diet and health data were measured at baseline only; thus, potential changes occurred over life course might have modified the strength of the findings; nevertheless, there is some evidence that diet in adulthood tends to remain stable over time[44] as well as most of the biomarkers here tested were not found to vary substantially over time.[45] Also, the FFQ used in this study enables the assessment of long-term dietary intakes, so that the exposure likely precedes the assessment of biomarkers.

For over 90% of the sample, history of CVD at baseline was ascertained through medical records or record linkage with the administrative registries (general practitioners, hospital discharge registry);

Table 2 Hazard ratios (95% confidence intervals) for all-cause and cause-specific mortality associated with ultra-processed food intake among individuals with history of cardiovascular disease from the Moli-sani Study cohort (*n* = 1171) using data obtained from multiple imputation

	Quartiles of ultra-processed foods				P for trend
	Q1	Q2	Q3	Q4	
Median, min–max (weight ratio, %)	3.4 (0.01 < 4.7)	5.8 (4.7 < 7.0)	8.6 (7.0 < 11.3)	15.0 (11.3 < 35.2)	
No. of subjects (%)	292 (25.0)	293 (25.0)	293 (25.0)	293 (25.0)	
All-cause mortality (*n* = 333)					
No. of deaths	88	76	85	84	
Person-years	2910	2968	2824	2834	
Event rates per 10 000 person-years	302.4	256.1	301.0	296.4	
Crude model	1 (ref)	0.85 (0.62–1.15)	1.00 (0.74–1.34)	0.99 (0.73–1.33)	0.82
Age-, sex-, and energy intake-adjusted model	1 (ref)	0.87 (0.64–1.19)	1.27 (0.94–1.71)	1.43 (1.05–1.95)	0.0045
Model 1	1 (ref)	0.88 (0.64–1.20)	1.29 (0.95–1.75)	1.46 (1.06–2.00)	0.0035
Model 2	1 (ref)	0.86 (0.63–1.18)	1.24 (0.91–1.69)	1.38 (1.00–1.91)	0.011
Cardiovascular mortality (*n* = 178)					
No. of deaths	45	39	43	51	
Person-years	2910	2968	2824	2834	
Event rates per 10 000 person-years	154.6	131.4	152.3	180.0	
Crude model	1 (ref)	0.85 (0.55–1.31)	0.99 (0.65–1.50)	1.17 (0.78–1.75)	0.33
Age-, sex-, and energy intake-adjusted model	1 (ref)	0.86 (0.56–1.32)	1.25 (0.82–1.91)	1.69 (1.12–2.56)	0.0042
Model 1	1 (ref)	0.90 (0.58–1.40)	1.31 (0.85–2.01)	1.78 (1.16–2.72)	0.0026
Model 2	1 (ref)	0.87 (0.56–1.35)	1.24 (0.80–1.91)	1.65 (1.07–2.55)	0.0083
IHD/cerebrovascular mortality (*n* = 114)					
No. of deaths	28	23	31	32	
Person-years	2910	2968	2824	2834	
Event rates per 10 000 person-years	96.2	77.5	109.8	112.9	
Crude model	1 (ref)	0.81 (0.46–1.40)	1.15 (0.69–1.91)	1.18 (0.71–1.96)	0.31
Age-, sex-, and energy intake-adjusted model	1 (ref)	0.82 (0.47–1.42)	1.46 (0.87–2.44)	1.71 (1.01–2.89)	0.011
Model 1	1 (ref)	0.86 (0.49–1.51)	1.50 (0.89–2.54)	1.80 (1.05–3.08)	0.0081
Model 2	1 (ref)	0.82 (0.47–1.45)	1.40 (0.83–2.38)	1.65 (0.96–2.85)	0.021
Cancer mortality (*n* = 80)					
No. of deaths	21	18	23	18	
Person-years	2910	2968	2824	2834	
Event rates per 10 000 person-years	72.2	60.6	81.4	63.5	
Crude model	1 (ref)	0.84 (0.45–1.57)	1.13 (0.63–2.05)	0.88 (0.47–1.66)	0.96
Age-, sex-, and energy intake-adjusted model	1 (ref)	0.84 (0.44–1.58)	1.33 (0.73–2.42)	1.12 (0.58–2.16)	0.43
Model 1	1 (ref)	0.78 (0.41–1.49)	1.21 (0.66–2.22)	1.06 (0.54–2.07)	0.55
Model 2	1 (ref)	0.76 (0.40–1.45)	1.15 (0.62–2.13)	0.99 (0.50–1.95)	0.70
Other cause mortality (*n* = 75)					
N of deaths	22	19	19	15	
Person-years	2910	2968	2824	2834	
Event rates per 10 000 person-years	75.6	64.0	67.3	52.9	
Crude model	1 (ref)	0.85 (0.46–1.57)	0.89 (0.48–1.64)	0.70 (0.36–1.35)	0.34
Age-, sex-, and energy intake-adjusted model	1 (ref)	0.94 (0.50–1.74)	1.22 (0.65–2.27)	1.17 (0.60–2.30)	0.49
Model 1	1 (ref)	0.98 (0.52–1.84)	1.31 (0.69–2.47)	1.26 (0.63–2.52)	0.36
Model 2	1 (ref)	0.99 (0.52–1.87)	1.33 (0.70–2.53)	1.29 (0.63–2.61)	0.34

Model 1 adjusted for sex, age (continuous), energy intake (continuous), educational level (categorical), housing tenure (categorical), smoking (categorical), body mass index (continuous), leisure-time physical activity (continuous), history of cancer (no/yes), diabetes (no/yes), hypertension (no/yes), hyperlipidaemia (no/yes), and residence (categorical). Model 2 as in Model 1 further adjusted for Mediterranean diet score (continuous).
IHD, ischaemic heart disease.

Table 3 **Biomarkers of cardiovascular risk as possible mediators of the association of ultra-processed food intake with all-cause and cardiovascular mortality among individuals with history of cardiovascular disease from the Moli-sani Study cohort (*n* = 1171) using data obtained from multiple imputation**

	Total effect				Natural direct effect				Natural indirect effect				Percentage mediated			
	OR	Lower 95% CI	Upper 95%CI	*P*-value	OR	Lower 95% CI	Upper 95%CI	*P*-value	OR	Lower 95% CI	Upper 95%CI	*P*-value	%	Bootstrap percentile Lower 95% CI	Bootstrap percentile Upper 95%CI	*P*-value
All-cause mortality																
Cystatin C (mg/L)	1.044	1.013	1.074	0.005	1.036	1.006	1.065	0.019	1.008	1.002	1.013	0.006	18.30	6.89	66.28	0.028
Creatinine (mg/dL)	1.044	1.014	1.074	0.004	1.040	1.011	1.070	0.008	1.004	1.000	1.008	0.065	8.65	0.27	34.44	0.093
Heart rate (b.p.m.)	1.043	1.013	1.073	0.005	1.037	1.008	1.067	0.014	1.005	1.001	1.010	0.016	13.05	2.50	42.29	0.047
CVD mortality																
Cystatin C (mg/L)	1.056	1.021	1.092	0.002	1.047	1.012	1.082	0.008	1.009	1.003	1.015	0.005	16.63	6.16	55.16	0.020
Creatinine (mg/dL)	1.057	1.022	1.092	0.002	1.053	1.018	1.088	0.003	1.004	1.000	1.008	0.068	7.12	0.26	21.59	0.089
Heart rate (b.p.m.)	1.054	1.020	1.089	0.002	1.050	1.016	1.085	0.004	1.004	1.000	1.008	0.042	7.81	1.11	26.09	0.074

Proportion of effect explained by intermediate variables with 95% CI and relevant *P*-value as produced by the PROC CAUSALMED are reported for each potential mediator, in multivariable adjusted logistic regression controlled for sex, age (continuous), energy intake (continuous), educational level (categorical), housing tenure (categorical), smoking (categorical), body mass index (continuous), leisure-time physical activity (continuous), history of cancer (no/yes), diabetes (no/yes), hypertension (no/yes), hyperlipidaemia (no/yes), residence (categorical), and Mediterranean diet score (continuous). The proportion refers to 1% increment of ultra-processed food intake. Mediation analyses were generated using the first imputed dataset. The other imputed datasets were similar and thus omitted.

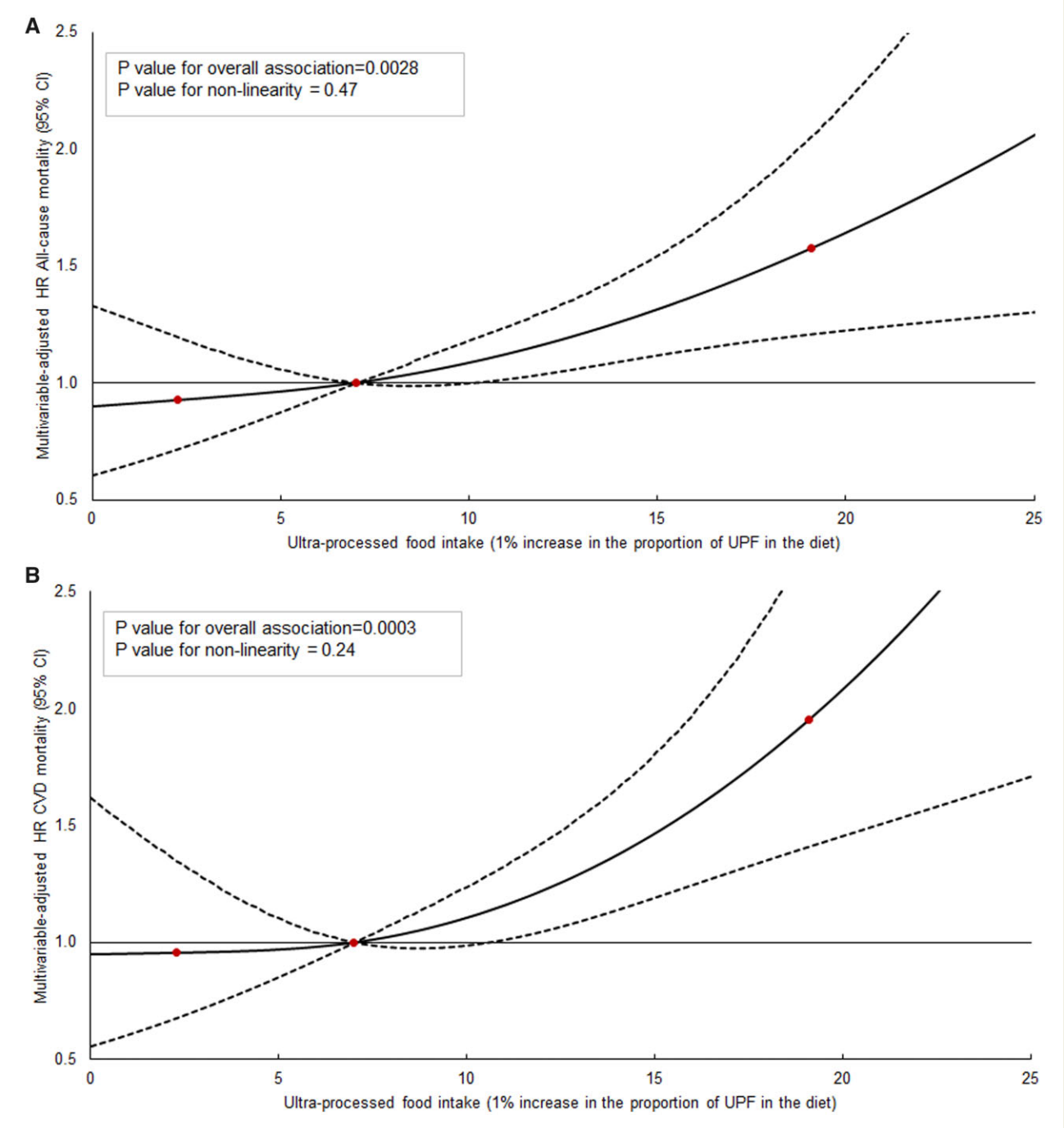


Figure 2 Multivariable dose–response association of (*A*) all-cause and (*B*) cardiovascular mortality with ultra-processed food consumption (1% increase in the proportion of ultra-processed food in the diet) among 1171 individuals with history of cardiovascular disease from the Moli-sani cohort, generated using the first imputed dataset. The other imputed datasets were similar and thus omitted. Hazard ratios with 95% confidence interval were obtained from the multivariable model adjusted for sex, age, energy intake, educational level, housing tenure, smoking, leisure-time physical activity, body mass index, history of cancer, diabetes, hypertension, hyperlipidaemia, residence, and the Mediterranean diet score. Ultra-processed food consumption was considered as a continuous exposure and the reference value for hazard ratios was 7.02% (median value of the exposure). The dashed lines indicate 95% confidence bands. Three knots were used, located at the 5th, 50th, and 95th percentiles of the ultra-processed food intake.

however, for a small proportion of subjects, it was not possible to verify their self-reported admission to the hospital when it was the unique reported criterion to confirm pre-existing CVD.

Moreover, we were unable to address the contribution of non-nutrient factors possibly linking UPF to mortality, such as neo-formed compounds, plasticizers, and food additives.

Finally, the usefulness of the NOVA classification is actually debated since this food classification scheme is not based on unequivocal, distinct physico-chemical aspects of foods and has been revised and refined over time.[46] However, this classification allows comparison with previous studies and increases the level of evidence. Caution is needed in generalizing these findings to other populations.

Conclusions

In this cohort of adult individuals with pre-existing CVD, a high proportion of UPF in the diet is associated with an increased hazard of all-cause and CVD mortality, possibly through mechanisms that include altered renal function. Such association is independent from the overall diet quality at baseline, as reflected by a traditional Mediterranean diet.

Although guidelines for CVD prevention generally emphasize consuming minimally processed foods, such as fruits and nuts, vegetables, whole grains, and fish, they do not explicitly suggest to markedly reduce UPF in the diet[47] and the same stands for guidelines for secondary prevention for patients with pre-existing CVD.[48]

Despite deriving from an observational study in the field of nutrition epidemiology, which is prone to several biases, our data suggest that excessive consumption of UPF may represent a major public health concern in secondary CVD prevention and support the need to stress the importance of limiting UPF in dietary guidelines, as done in some[49] but not in the majority of countries.

Further longitudinal studies with similar designs are warranted to replicate and potentially confirm these findings in different populations with pre-existing CVD.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Authors' contribution

Authorship: LI, MB, SC and GdG conceived and designed the study. MP, SM and AdeC acquired the data. SC managed the data. MB and ADiC analysed the data. MB drafted the manuscript. CC, MBD, GdG and LI critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work ensuring integrity and accuracy.

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Conflict of interest: The Authors declare no conflict of interest.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author. The data are stored in an institutional repository (https://repository.neuromed.it) and access is restricted by the ethical approvals and the legislation of the European Union.

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