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Ultra-processed food consumption is associated with increased risk of all-cause and cardiovascular mortality in the Moli-sani Study

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ABSTRACT

Background: Consumption of ultra-processed food (UPF) is gaining growing attention in relation to disease/mortality risk, but less is known on the main nutritional factors or biological mechanisms potentially underlying such associations.

Objectives: We aimed to assess the association between UPF and mortality risk in a large sample of the Italian adult population and test which nutritional factors were on the pathway of this relation. Established risk factors for cardiovascular disease (CVD) were analyzed as potential biological mechanisms linking UPF to mortality.

Methods: Longitudinal analysis was conducted on 22,475 men and women (mean ± SD age: 55 ± 12 y) recruited in the Moli-sani Study (2005–2010, Italy) and followed for 8.2 y. Food intake was assessed using a semiquantitative FFQ. UPF was defined using the NOVA classification according to degree of processing, and UPF intakes were categorized as quartiles of the ratio (%) of UPF (g/d) to total food consumed (g/d).

Results: Individuals reporting the highest intake of UPF (Q4, >14.6% of total food), as opposed to the lowest (Q1, UPF < 6.6%), experienced increased risks of CVD mortality (HR: 1.58; 95% CI: 1.23, 2.03), death from ischemic heart disease (IHD)/cerebrovascular disease (HR: 1.52; 95% CI: 1.10, 2.09), and all-cause mortality (HR: 1.26; 95% CI: 1.09, 1.46). High sugar content explained 36.3% of the relation of UPF with IHD/cerebrovascular mortality, whereas other nutritional factors (e.g., saturated fats) were unlikely to be on the pathway. Biomarkers of renal function accounted for 20.1% of the association of UPF with all-cause mortality, and 12.0% for that of UPF with CVD mortality.

Conclusions: A high proportion of UPF in the diet was associated with increased risk of CVD and all-cause mortality, partly through its high dietary content of sugar. Some established biomarkers of CVD risk were likely to be on the pathway of such associations. These findings should serve as an incentive for limiting consumption of UPF, and encouraging natural or minimally processed foods, as several national nutritional policies recommend. *Am J Clin Nutr* 2021;113:446–455.

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Supplemental Figure 1, Supplemental Methods, Supplemental Tables 1 and 2, and Supplemental Appendix 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/ajcn/.

The Moli-sani Study Investigators are listed in Supplemental Appendix 1.

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Abbreviations used: CVD, cardiovascular disease; ICD, International Classification of Diseases; IHD, ischemic heart disease; Lp(a), lipoprotein a; MDS, Mediterranean Diet Score; UPF, ultra-processed food.

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Introduction

The volume of industrially processed foods has increased dramatically over the last few decades, leading to a gradual substitution of traditional diets that feature whole or minimally processed foods and involve home-cooking. Diets rich in prepared food products are reportedly associated with obesity and chronic disease onset (1, 2). Because their global consumption dominates the food supply in high-income countries and they are on the rise in growing economies (3), understanding the health impact of these foods has become a relevant and timely topic.

During the last decade, several food processing classification systems were conceived, among which the most prominent is NOVA, which categorizes foods based on the extent and purpose of the industrial processes applied to preserve, extract, modify, or create them, rather than in terms of nutrient content (4).

The NOVA classification ultimately provides 4 main classes of food and beverages, the last of which is represented by the ultra-processed food (UPF) group. This comprises products (e.g., snacks, drinks, ready meals) "created mostly or entirely from substances extracted from foods or derived from food constituents with little if any intact food, which often contain flavours, colours and other additives that imitate or intensify the sensory qualities of foods or culinary preparations made from foods" (4). Such foods are highly convenient (ready-to-consume), attractive (hyper-palatable), inexpensive, have a long shelf-life, and are highly competitive with foods that are naturally ready to consume and freshly prepared dishes and meals (4).

Individual-level consumption data indicate that UPF comprised 60% of energy intake, and contributed ∼90% of the energy from added sugars in the United States (5), whereas in European countries the proportion of daily energy intake from UPF ranges from 24.4% to 36% (6–8).

To date, only 1 US and 3 large European cohort studies have tested the association of UPF with health outcomes, and all found a substantial increase in the risk of mortality, cardiovascular disease (CVD), and cancer (8–13), but none has evaluated the potential mechanisms through which UPF may be detrimental to human health.

To date it is still unclear whether processing itself actually matters for health (2), although evidence from mechanistic studies suggests that other aspects of food health potentially introduced during food processing (e.g., food structure that influences satiety) should be considered beyond nutrient composition (14, 15).

The aims of this study are *1*) to evaluate the association of UPF with all-cause and cause-specific mortality in a large sample of Italian men and women from the Moli-sani Study cohort; *2*) to examine the contribution of main nutrients (e.g., sugar, saturated fats) contained in the UPF as possibly mediating its relation with mortality risk; and *3*) to test which known CVD risk factors are likely to be on the pathway between UPF and mortality.

Methods

Study population

Data are from the Moli-sani Study, a population-based cohort of 24,325 men and women aged ≥35 y established in 2005–2010 in the Molise region, a southern Italian region. The recruitment process, characteristics, and study methods in this population-based cohort have been described previously (16) (**Supplemental Methods**).

For the purpose of the present analyses, participants were excluded if they reported implausible energy intakes (<800 or >4000 kcal/d in men and <500 or >3500 kcal/d in women, $n = 771$; and using the 0.5th and 99.5th centiles as limits for allowable total energy intake), their dietary or medical questionnaires were judged as unreliable ($n = 955$ and $n = 235$, respectively), subjects were lost to follow-up ($n = 23$), were missing information on diet ($n = 100$) or cause-specific death ($n = 45$), or were missing information for educational level ($n = 39$), smoking status ($n = 29$), housing tenure ($n = 57$), and BMI ($n = 17$). Those individuals with missing values for ≥1 covariates were kept in the data set and treated as null reporting, but were excluded in the sensitivity analysis. We finally analyzed 22,475 subjects (**Supplemental Figure 1**).

Dietary assessment

Food intake during the year before enrolment was assessed by an interviewer-administered semiquantitative European Prospective Investigation into Cancer and Nutrition (EPIC) FFQ validated and adapted to the Italian population (17, 18), for a total of 188 food items that were classified into 74 predefined food groups on the basis of similar nutrient characteristics or culinary usage.

Using specifically designed software (19), frequencies and quantities of each food were linked to Italian Food Tables (20) to obtain estimates of daily intake of macro- and micronutrients plus energy.

We used the NOVA classification (4) to categorize each food item into 1 of the following categories indicating levels of industrial food processing: *1*) fresh or minimally processed foods (e.g., fruits and vegetables, meat and fish); *2*) processed culinary ingredients (e.g., honey, butter); *3*) processed foods with salt, sugar, or oil (e.g., canned or bottled vegetables and legumes, canned fish); and *4*) UPFs containing predominantly industrial substances and little or no whole foods (e.g., carbonated drinks, processed meat) (**Supplemental Table 1**).

To estimate UPF we summed the amount consumed (g/d) of each food group included in the fourth category of the NOVA classification (a total of 15 food groups and 3 beverages), and then calculated the proportion (%) of UPF in the total weight of food and beverages consumed (g/d) by creating a weight ratio. Such an approach is more appropriate than using an energy ratio because it better accounts for nonnutritional factors pertaining to food processing (e.g., neoformed contaminants, additives, and alterations to the structure of raw foods) (11, 12). Participants were then divided into quartiles based on the proportion of their total food intake constituted by UPF. Adherence to the traditional Mediterranean diet was defined through the Mediterranean Diet Score (MDS) developed by Trichopoulou et al. (21).

Outcome ascertainment

The Moli-sani Study cohort was followed up for mortality until 31 December, 2015. Overall and cause-specific mortality were assessed by the Italian mortality registry (ReNCaM registry), validated by Italian death certificates (ISTAT form), and

TABLE 1 Baseline characteristics of the study population by quartiles of UPF intake in the Moli-sani Study cohort[1]

Characteristic	Quartiles of UPF intake				
	Q1	Q2	Q3	Q4	*P* value
Median [min–max], weight ratio in %	4.8 [0.0–6.6]	8.2 [6.7–9.9]	12.1 [10.0–14.5]	18.5 [14.6–50.8]	—
Subjects	5618 (25.0)	5619 (25.0)	5619 (25.0)	5619 (25.0)	—
Age, y	62 ± 11	57 ± 11	53 ± 11	50 ± 11	<0.001
Men	59.3	49.4	42.5	39.3	<0.001
Urban residence	66.1	66.0	68.0	69.1	<0.001
Educational level					<0.001
Up to lower secondary	63.2	53.8	48.1	43.4	
Upper secondary	27.1	33.6	37.7	41.6	
Postsecondary	9.8	12.6	14.3	15.1	
Housing tenure					<0.001
Rent	7.4	7.8	8.8	11.0	
1 dwelling ownership	83.6	82.9	82.4	80.5	
>1 dwelling ownership	8.9	9.3	8.8	8.5	
Smoking status					<0.001
Nonsmokers	44.8	48.7	51.9	53.2	
Current	20.8	22.3	23.1	25.0	
Former	34.4	29.0	25.0	21.6	
BMI, kg/m^2	28.2 ± 4.6	28.2 ± 4.7	28.0 ± 4.7	27.6 ± 4.9	<0.001
Leisure-time physical activity, METs-h/d	3.7 ± 4.4	3.5 ± 3.9	3.5 ± 3.8	3.3 ± 3.7	<0.001
CVD					0.12
No	89.4	92.8	95.3	95.5	
Yes	8.5	5.8	3.4	3.2	
Unknown	2.2	1.4	1.4	1.3	
Cancer					0.28
No	95.3	96.2	96.3	96.8	
Yes	4.2	3.4	3.5	2.9	
Unknown	0.5	0.4	0.2	0.3	
Diabetes					<0.001
No	89.2	93.5	96.3	97.2	
Yes	9.4	5.3	2.6	1.7	
Unknown	1.4	1.3	1.0	1.1	
Hypertension					0.08
No	59.8	68.6	74.9	80.8	
Yes	37.7	29.9	23.8	17.9	
Unascertained	1.4	1.0	0.7	0.7	
Unknown	1.1	0.5	0.6	0.6	
Hyperlipidemia					<0.001
No	86.2	90.5	94.0	94.8	
Yes	12.4	8.5	5.4	4.6	
Unknown	1.3	1.1	0.6	0.6	
Dietary factors					
MDS (score range: 0–9)	4.9 ± 1.6	4.5 ± 1.6	4.3 ± 1.6	3.8 ± 1.6	<0.001
Good adherence to Mediterranean diet (MDS ≥ 6)	34.1	28.2	23.4	16.9	<0.001
Fruits and nuts, g/d	392 ± 220	371 ± 197	347 ± 182	304 ± 170	<0.001
Vegetables, g/d	178 ± 78	164 ± 67	155 ± 68	151 ± 67	<0.001
Cereals, g/d	227 ± 99	216 ± 92	202 ± 87	182 ± 85	<0.001
Legumes, g/d	29 ± 22	28 ± 21	27 ± 21	25 ± 19	<0.001
Fish, g/d	45 ± 28	46 ± 26	45 ± 26	43 ± 26	<0.001
MUFAs:SFAs ratio	1.51 ± 0.34	1.40 ± 0.27	1.35 ± 0.24	1.29 ± 0.23	<0.001
Milk and dairy products, g/d	178 ± 123	185 ± 118	188 ± 114	188 ± 119	<0.001
Meat and meat products, g/d	101 ± 42	105 ± 43	106 ± 44	102 ± 45	<0.001
Alcohol intake, g/d	25 ± 27	17 ± 20	13 ± 17	9 ± 13	<0.001
Energy intake, kcal/d	1947 ± 567	2048 ± 552	2119 ± 531	2196 ± 557	<0.001
Carbohydrate, % total energy intake	48 ± 8	48 ± 7	48 ± 6	49 ± 6	<0.001
Sugar, g/d	83 ± 30	87 ± 31	92 ± 31	103 ± 37	<0.001
Protein, % total energy intake	16.1 ± 2.5	16.4 ± 2.1	16.4 ± 2.0	16.0 ± 1.9	<0.001
Fat, % total energy intake	31 ± 6	33 ± 5	34 ± 5	34 ± 5	<0.001
Saturated fat, % total energy intake	10.7 ± 2.6	11.6 ± 2.4	12.2 ± 2.4	12.7 ± 2.4	<0.001
Saturated fat, g/d	24 ± 8	27 ± 8	28 ± 9	30 ± 10	<0.001

(*Continued*)

TABLE 1 (*Continued*)

Characteristic	Quartiles of UPF intake				
	Q1	Q2	Q3	Q4	*P* value
Monounsaturated fat, % total energy intake	15.6 ± 3.4	16.0 ± 3.0	16.2 ± 2.8	16.1 ± 2.6	0.023
Polyunsaturated fat, % total energy intake	3.3 ± 0.7	3.5 ± 0.6	3.6 ± 0.6	3.7 ± 0.6	<0.001
Dietary cholesterol, mg/d	290 ± 91	313 ± 96	327 ± 98	341 ± 115	<0.001
Fiber intake, g/d	21 ± 7	21 ± 6	20 ± 6	19 ± 6	<0.001
Sodium, mg/d	2279 ± 821	2335 ± 824	2359 ± 830	2288 ± 862	<0.001
Biomarkers of CVD risk					
C-reactive protein, mg/L	1.49 (1.45, 1.53)	1.53 (1.49, 1.57)	1.50 (1.46, 1.54)	1.50 (1.46, 1.55)	0.49
Leukocyte count, $\times 10^9$/L	6.0 (6.0, 6.1)	6.0 (6.0, 6.1)	6.0 (6.0, 6.0)	6.0 (6.0, 6.1)	0.88
Blood glucose, mg/dL	101 (101, 102)	100 (99, 100)	98 (98, 99)	97 (97, 98)	<0.001
Insulin, pmol/L	50.1 (49.4, 50.8)	53.0 (52.3, 53.7)	52.8 (52.1, 53.5)	52.1 (51.4, 52.8)	<0.001
C-peptide, ng/mL	1.53 (1.51, 1.55)	1.62 (1.60, 1.64)	1.61 (1.58, 1.63)	1.57 (1.55, 1.60)	<0.001
Blood cholesterol, mg/dL	215 ± 42	214 ± 42	213 ± 41	209 ± 40	<0.001
HDL cholesterol, mg/dL	59 ± 15	57 ± 14	57 ± 15	56 ± 15	<0.001
Triglycerides, mg/dL	115 (113, 116)	114 (113, 116)	112 (110, 113)	109 (108, 110)	<0.001
ApoB100, g/L	1.58 ± 32	1.54 ± 31	1.54 ± 32	1.54 ± 32	<0.001
ApoA1, g/L	0.99 ± 0.24	0.99 ± 0.24	0.98 ± 0.24	0.97 ± 0.24	<0.001
Lp(a), mg/dL	17.7 ± 18.3	17.8 ± 18.5	17.5 ± 18.4	17.7 ± 18.4	0.90
Cystatin C, mg/dL	0.94 (0.94, 0.95)	0.96 (0.95, 0.96)	0.96 (0.96, 0.97)	0.98 (0.97, 0.98)	<0.001
Creatinine, mg/dL	0.79 (0.79, 0.80)	0.80 (0.80, 0.80)	0.80 (0.80, 0.80)	0.81 (0.81, 0.81)	<0.001

[1] $n = 22{,}475$. Values are percentages, *n* (%), means ± SDs, or geometric means (95% CIs) unless otherwise specified. UPF intake is a weight ratio expressed as % of total food intake in g/d. Means of BMI, leisure-time physical activity, dietary data, biomarkers, blood cholesterol, HDL cholesterol, apoA1, apoB100, and Lp(a) were adjusted for age, sex, and energy intake. *P* values were obtained using generalized linear models for both continuous and categorical variables adjusted for age, sex, and energy intake. CVD, cardiovascular disease; Lp(a), lipoprotein a; MDS, Mediterranean Diet Score; MET, metabolic equivalent task; UPF, ultra-processed food.

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 and ICD-9 codes 410–414 and 429 were used for ischemic heart disease (IHD). Cancer death was considered when the underlying cause of death included ICD-9 codes 140–208.

Noncardiovascular/noncancer causes of death were included in the "other cause mortality" group.

Statistical analysis

Baseline characteristics are presented as means ± SDs for quantitative traits and *n* (%) for categorical variables. Positively skewed variables were log transformed before analysis. For biomarkers of CVD risk, ≤5% of values were missing and were imputed to the median value.

Differences in the distribution of baseline covariates according to UPF quartiles were calculated using generalized linear models adjusted for age, sex, and energy intake (using the GENMOD procedure for categorical variables and GLM procedure for continuous variables in the SAS software) (**Table 1**).

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

Multivariable-adjusted HRs were calculated across quartiles of UPF, as well as considering UPF as a continuous variable (per 5% increase in the proportion of UPF in the diet).

Based on previous literature and biological plausibility, 4 models were fitted: crude model; a model adjusted for age, sex, and energy intake; model 1, which included sex, age (continuous), energy intake (continuous), educational level (up to lower secondary, upper secondary, postsecondary), housing tenure (rent, 1 dwelling ownership, >1 dwelling ownership), smoking (never, current, former smokers), BMI (continuous), leisure-time physical activity (continuous), cancer (no, yes, unknown), CVD (no, yes, unknown), diabetes (no, yes, unknown), hypertension (no, yes, unknown), hyperlipidemia (no, yes, unknown), and residence (urban, rural); and model 2, which was as per model 1 and further controlled for MDS.

The multivariable Model 1 served as the reference for the mediation analysis used to quantify the contribution of major nutritional factors present in industrially processed foods, i.e., sugar (g/d), SFAs (g/d), dietary cholesterol (g/d), dietary sodium (mg/d), and energy content (kcal/d), which were alternately included in Model 1.

We tested biomarkers of renal function (cystatin C, creatinine), glucose metabolism (blood glucose, insulin, C-peptide), lipid metabolism {total blood cholesterol, HDL cholesterol, triglycerides, apoA1, apoB100, and lipoprotein a [Lp(a)]}, and inflammatory markers (C-reactive protein and white blood cell count) as potential mediators of the association of UPF consumption with mortality risk by using Model 2 (including MDS) as the reference.

For the mediation analysis we used the %MEDIATE macro in SAS (22) which calculates the point and interval estimates of the percentage of exposure effect explained by ≥1 intermediate variables, with 95% CIs and *P* values.

We conducted subgroup analyses to test the robustness of our findings by analyzing potential effect modification of the association of UPF (per 5% increase in the proportion of UPF in the diet) with mortality outcomes by various risk factors: age (35–64 and ≥65 y), sex, socioeconomic strata (education and housing tenure), lifestyles (smoking status, leisure-time physical activity, MDS), and health conditions/disease at baseline (BMI, CVD, cancer, diabetes, hypertension, and hyperlipidemia), within a healthy sample (without CVD, cancer, and diabetes at baseline) and after excluding subjects with follow-up shorter than 2 y. Appropriate multiplicative terms for testing interaction were included in the multivariable models to test for a difference of effect of the UPF across subgroups.

To test for a potential nonlinear, continuous relation between UPF and mortality risk, we used multivariable Cox regression analysis with UPF intake modeled as restricted cubic splines (3 knots at 5%, 50%, and 95% of the UPF distribution) (23) and used the median value of the weight ratio (= 2) as the reference value. The data analysis was generated using SAS/STAT software version 9.4, of the SAS System for Windows© 2009 (SAS Institute).

Results

Study participants reported a median of 10% (IQR: 6.6%–14.6%) of dietary intake as UPF, and a total of 181.5 g/d of UPF intake. Processed meat (19.8%), pizza (16.8%), and cakes/pies (13.4%) were the foods contributing most to the total of UPF consumed (Supplemental Table 1).

High consumers of UPF (>14.6% of total food consumed, i.e., the lower cutoff value of the upper quartile) were more likely to be women, tended to be younger and to have a higher educational level, and reported fewer risk factors and baseline chronic diseases/health conditions than less frequent consumers (Table 1).

High consumption of UPF was associated with lower adherence to the Mediterranean diet; higher energy intake, fat, sugar, dietary cholesterol, and sodium; but lower intake of fiber (Table 1).

During a median follow-up of 8.2 y (IQR: 7.3–9.3 y; 184,816 person years), 1216 all-cause deaths occurred of which 439 were attributed to CVD, 255 to IHD/cerebrovascular disease, 477 to cancer, and 300 were deaths from other causes.

In crude models, higher UPF intake was progressively associated with lower all-cause and cause-specific mortality risk owing to the strong patterning of age and sex across UPF categories; in multivariable-adjusted model 1, HRs for all-cause, CVD, and IHD/cerebrovascular mortality associated with high UPF consumption were 1.32 (95% CI: 1.15, 1.53), 1.65 (95% CI: 1.29, 2.11), and 1.63 (95% CI: 1.19, 2.25), respectively (**Table 2**, Model 1). The inclusion of MDS in the model slightly attenuated the associations whereas no relation between UPF and cancer mortality was found, whereas an increased risk of mortality from other causes was observed in association with increased UPF intake (Table 2, Model 2).

The multivariable dose-response analysis showed a direct linear dose–response relation between a 5% increase in the proportion of UPF in the diet and risk of all-cause and CVD mortality (**Figure 1**).

Among nutrients present in large quantities in UPF, sugar content was likely to account for the largest proportion of the relation between UPF and mortality risk (23.2%, 18.0%, and 36.3% for all-cause, CVD, and IHD/cerebrovascular death risks, respectively) (**Table 3**); the high content of dietary cholesterol explained 9.3% of the association with all-cause mortality risk, whereas the mediating role of energy content of UPF was limited to IHD/cerebrovascular mortality risk that was reduced by 21.1%, although this reduction was not statistically significant (Table 3). Globally, major nutrients and energy density contained in the UPFs investigated here explained ≤41.3% of the relation between UPF and IHD/cerebrovascular mortality risk, whereas they poorly accounted for the other 2 outcomes (Table 3).

Regarding biological mechanisms potentially linking UPF to increased death risk, we found that part of such relations was explained by biomarkers of renal function (from 8.4% to 20.1%), whereas all tested CVD risk factors accounted for 22.4% and 9.4% of the excess of all-cause and CVD mortality risk, respectively, associated with UPF intake (**Table 4**).

Subgroup analyses indicated that the magnitude of the association between UPF and all-cause mortality risk was greater among high-risk individuals, i.e., subjects with history of CVD or presence of diabetes at baseline (*P* values for interaction = 0.019 and 0.014, respectively) (**Supplemental Table 2**). Similarly, UPF was likely to be more strongly associated with CVD mortality among these 2 high-risk groups (Supplemental Table 2), and the magnitude of the association between increased consumption of UPF and risk of CVD or IHD/cerebrovascular mortality was greater among individuals showing good adherence to the Mediterranean diet (*P* values for interaction = 0.010 and <0.001, respectively) (Supplemental Table 2).

Finally, by the use of E-values [a parameter that calculates the minimum strength of association that an unmeasured confounder would need to have with both the exposure and outcome, to cancel a specific exposure–outcome association (24)], we found that to nullify the reported association, unobserved confounders should be associated with both UPF consumption and CVD mortality by a HR > 2.0. Interestingly, the whole set of known confounders used in our analysis shifted the age-, sex-, and energy intake–adjusted model HR of 1.50 (95% CI: 1.18, 1.91) to a fully adjusted HR of 1.58 (95% CI: 1.23, 2.03; Model 2, of note, the adjusted HR increased), acting as a confounder with an E-value of 1.29; thus, it is unlikely that 1 (or more) unmeasured factors with an E-value of ∼2.0 would exist and have a similar impact to the large whole set of known confounders considered in our study.

Discussion

Our data from a large Mediterranean cohort indicate that high consumption of UPF is associated with a 58% increased risk of CVD mortality and 52% higher risk of dying from IHD/cerebrovascular causes, independently of known risk factors, including a global assessment of overall diet quality as reflected by adherence to a Mediterranean diet. All-cause mortality risk was also increased by 26% among those consuming

TABLE 2 Associations of all-cause and cause-specific mortality with UPF intake in the Moli-sani Study cohort[1]

	Quartiles of UPF intake			
	Q1	Q2	Q3	Q4
Median [IQR], weight ratio in %	4.8 [3.7–5.8]	8.2 [7.4–9.1]	12.1 [11.0–13.1]	18.5 [16.2–22.3]
Subjects, *n* (%)	5618 (25.0)	5619 (25.0)	5619 (25.0)	5619 (25.0)
All-cause mortality ($n = 1216$)				
Deaths, *n*	492	313	224	187
Person-years, *n*	45,961	45,977	46,258	46,619
Event rates per 10,000 person-years, *n*	107.0	68.1	48.4	40.1
Crude model	1 (ref)	0.64 (0.55, 0.73)	0.45 (0.39, 0.52)	0.37 (0.32, 0.43)
Age-, sex-, and energy intake–adjusted model	1 (ref)	1.07 (0.92, 1.23)	1.17 (1.01, 1.34)	1.28 (1.11, 1.48)
Model 1	1 (ref)	1.08 (0.94, 1.25)	1.21 (1.05, 1.39)	1.32 (1.15, 1.53)
Model 2	1 (ref)	1.07 (0.92, 1.23)	1.17 (1.02, 1.36)	1.26 (1.09, 1.46)
CVD mortality ($n = 439$)				
Deaths, *n*	185	105	71	78
Person-years, *n*	45,961	45,977	46,258	46,619
Event rates per 10,000 person-years, *n*	40.2	22.8	15.3	16.7
Crude model	1 (ref)	0.57 (0.45, 0.72)	0.38 (0.30, 0.48)	0.41 (0.32, 0.52)
Age-, sex, and energy intake–adjusted model	1 (ref)	0.99 (0.78, 1.27)	1.08 (0.84, 1.37)	1.50 (1.18, 1.91)
Model 1	1 (ref)	1.01 (0.79, 1.30)	1.13 (0.88, 1.44)	1.65 (1.29, 2.11)
Model 2	1 (ref)	1.00 (0.78, 1.28)	1.10 (0.86, 1.41)	1.58 (1.23, 2.03)
IHD/cerebrovascular mortality ($n = 255$)				
Deaths, *n*	110	64	38	43
Person-years, *n*	45,961	45,977	46,258	46,619
Event rates per 10,000 person-years, *n*	23.9	13.9	8.2	9.2
Crude model	1 (ref)	0.58 (0.43, 0.79)	0.34 (0.25, 0.47)	0.38 (0.28, 0.52)
Age-, sex-, and energy intake–adjusted model	1 (ref)	1.03 (0.76, 1.42)	0.98 (0.71, 1.34)	1.44 (1.05, 1.97)
Model 1	1 (ref)	1.06 (0.77, 1.46)	1.07 (0.78, 1.47)	1.63 (1.19, 2.25)
Model 2	1 (ref)	1.03 (0.75, 1.42)	1.02 (0.74, 1.41)	1.52 (1.10, 2.09)
Cancer mortality ($n = 477$)				
Deaths, *n*	184	137	91	65
Person-years, *n*	45,961	45,977	46,258	46,619
Event rates per 10,000 person-years, *n*	40.0	29.8	19.7	13.9
Crude model	1 (ref)	0.75 (0.60, 0.93)	0.49 (0.39, 0.61)	0.35 (0.28, 0.43)
Age-, sex-, and energy intake–adjusted model	1 (ref)	1.14 (0.91, 1.43)	1.07 (0.86, 1.34)	0.98 (0.78, 1.23)
Model 1	1 (ref)	1.16 (0.93, 1.46)	1.10 (0.88, 1.39)	1.00 (0.80, 1.26)
Model 2	1 (ref)	1.15 (0.92, 1.44)	1.08 (0.86, 1.36)	0.97 (0.77, 1.22)
Other cause mortality ($n = 300$)				
Deaths, *n*	123	71	62	44
Person-years, *n*	45,961	45,977	46,258	46,619
Event rates per 10,000 person-years, *n*	26.8	15.4	13.4	9.4
Crude model	1 (ref)	0.58 (0.43, 0.77)	0.50 (0.37, 0.67)	0.35 (0.26, 0.47)
Age-, sex-, and energy intake–adjusted model	1 (ref)	1.01 (0.76, 1.35)	1.39 (1.04, 1.86)	1.31 (0.98, 1.76)
Model 1	1 (ref)	1.04 (0.77, 1.39)	1.43 (1.06, 1.92)	1.36 (1.01, 1.83)
Model 2	1 (ref)	1.01 (0.75, 1.35)	1.36 (1.01, 1.83)	1.26 (0.94, 1.69)

[1] $n = 22,475$. Values are HRs (95% CIs) unless otherwise indicated. Model 1 adjusted for sex, age (continuous), energy intake (continuous), educational level (categorical), housing tenure (categorical), smoking (categorical), BMI (continuous), leisure-time physical activity (continuous), history of cancer, CVD, diabetes, hypertension, hyperlipidemia, and residence (categorical). Model 2 was as per Model 1 and further adjusted for Mediterranean Diet Score (continuous). CVD, cardiovascular disease; IHD, ischemic heart disease; UPF, ultra-processed food.

high amounts of UPF, whereas no association was observed with cancer death.

Our findings are in accordance with previous data on >100,000 French adults (11) concluding that higher consumption of UPF (highest quartile of the weight ratio) was associated with increased risk of CVDs, ischemic heart disease, and cerebrovascular diseases. Although a direct comparison with other epidemiological studies is difficult owing to different

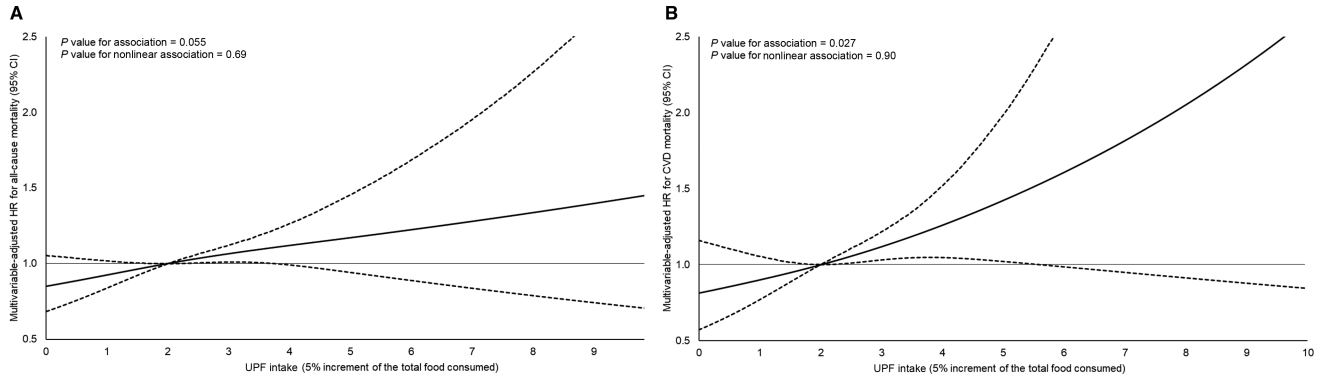

FIGURE 1 Multivariable dose–response association of all-cause (A) and CVD (B) death risks with UPF consumption (per 5% increase in the proportion of UPF in the diet) in the Moli-sani Study cohort (n = 22,475). Risk estimates (HRs with 95% CIs) were obtained from the multivariable model adjusted for sex, age, energy intake, educational level, housing tenure, smoking, leisure-time physical activity, BMI, history of cancer, CVD, diabetes, hypertension, hyperlipidemia, residence, and Mediterranean Diet Score. UPF consumption was considered as a continuous exposure and the reference value for HRs was 2 (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 UPF intake. CVD, cardiovascular disease; UPF, ultra-processed food.

assessments of UPF, it is of note that others provided similar results in terms of increased mortality risk being associated with higher UPF intake (10, 13).

Of interest, in our study the magnitude of the association between UPF and CVD mortality was greater among groups with good adherence to a Mediterranean diet than among those conforming less to a Mediterranean diet; this may be explained by the fact that people who potentially benefit from a Mediterranean diet are more susceptible to losing its health advantages when introducing a detrimental dietary behavior, whereas subjects with poor diet quality are less likely to be damaged by an additional unhealthy behavior such as eating UPF regularly. Also, we found that high-risk individuals, such as those with prior CVD or diabetes, were more likely to experience increased all-cause or CVD mortality risks associated with UPF, highlighting the importance of nutritional strategies more aggressively targeting high-risk individuals. Analysis of the nutritional pathways revealed a leading role for sugar which explained nearly 40% of the association between UPF and IHD/cerebrovascular mortality, and in line with the high energy density hypothesis suggesting that UPFs may promote excess energy intake because of their high energy density (2), energy content was on the UPF–mortality pathway, although limited to IHD/cerebrovascular death.

However, the adverse association between UPF and mortality risk was only partially accounted for by nutrients present in large quantities in such foods, thus suggesting that nutrient composition alone is not able to fully explain the excess of mortality risk associated with increased consumption of UPF, in line with other studies (10, 13, 25, 26). Moreover, we should acknowledge that a higher proportion of the observed association may be explained by other dietary factors contained in UPFs not explored here.

Our study is apparently the first to investigate the nutrition-related pathways linking UPF to mortality, whereas previously Fiolet et al. (12) failed to find any significant effect of nutritional mediators (e.g., sodium, fats) to explain the relation between UPF and cancer risk, suggesting that other compounds contained in UPF might contribute to explaining the observed associations.

Indeed, evidence accumulating from mechanistic studies suggests that, besides nutritional characteristics, other factors introduced during food processing can have an equally important health effect by promoting, for example, inflammation-related processes through diet–microbiome–host interactions, thus being risk factors for adverse health outcomes themselves (15). In addition, ultra-processing negatively affects both food structure and nutrient composition, leading to unstructured, fractionated, and recombined energy-dense and micronutrient-poor foods (14); food structure, which is highly dependent on processing conditions, is increasingly recognized to play a role in satiety and glycemic responses (27). Finally, there is evidence that food processing can also lead to the loss of some nutrients and phytochemicals naturally present in plant foods (28) which in turn may have adverse health effects, as well as to neoformed compounds related to the heating and processing (29) and industrial chemicals used in some UPF plastic packaging (30) that may result in harm especially to cardiovascular health.

The present study also analyzed the biological mechanisms possibly underlying the association between UPF and health and found that part of the association between increasing UPF intake and mortality was likely mediated by biomarkers of renal function. Habitual consumers of UPF tended to report higher concentrations of biomarkers of renal function which have been associated with increased CVD risk (31), whereas cystatin C, a more sensitive marker of renal function, represents a strong predictor of CVD risk also in the general population (32).

Moreover, both markers were found to be modulated by dietary factors (33, 34) and there is evidence indicating that high consumption of foods rich in sugar is associated with incidence of chronic kidney disease (35). Of note, our results do not indicate a direct association between UPF and BMI, one of the mechanisms that is thought to link UPF to disease/mortality risk (2); however, analyses in many cohort studies did not provide evidence of an

TABLE 3 Macronutrients and nutrients as possible mediators of the association of ultra-processed food intake with all-cause, CVD, and IHD/cerebrovascular mortality in the Moli-sani Study cohort[1]

	All-cause mortality		CVD mortality		IHD/cerebrovascular mortality	
	HR (95% CI)	PTE[2]	HR (95% CI)	PTE[2]	HR (95% CI)	PTE[2]
Model 1	1.32 (1.15, 1.53)	—	1.65 (1.29, 2.11)	—	1.63 (1.19, 2.25)	—
Model 1 + sugar, g/d	1.24 (1.07, 1.44)	23.2% (9.7%, 45.9%), $P < 0.001$	1.51 (1.17, 1.94)	18.0% (7.2%, 38.4%), $P = 0.003$	1.37 (0.98, 1.90)	36.3% (13.8%, 67.0%), $P < 0.001$
Model 1 + saturated fat, g/d	1.32 (1.14, 1.54)	Null	1.64 (1.27, 2.11)	1.3% (0.0%, 99.8%), $P = 0.43$	1.57 (1.12, 2.19)	8.7% (0.8%, 53.0%), $P = 0.18$
Model 1 + dietary cholesterol, mg/d	1.29 (1.11, 1.49)	9.3% (2.4%, 29.9%), $P = 0.048$	1.65 (1.29, 2.13)	Null	1.61 (1.16, 2.24)	3.1% (0.0%, 82.3%), $P = 0.34$
Model 1 + dietary sodium, mg/d	1.31 (1.13, 1.52)	2.7% (0.1%, 56.8%), $P = 0.30$	1.68 (1.31, 2.16)	Null	1.66 (1.20, 2.30)	Null
Model 1 + energy content, kcal/d	1.35 (1.15, 1.58)	Null	1.66 (1.28, 2.16)	Null	1.48 (1.05, 2.09)	21.1% (4.0%, 60.3%), $P = 0.06$
Model 1 + all dietary factors	1.28 (1.09, 1.49)	12.8% (1.6%, 56.5%), $P = 0.14$	1.56 (1.19, 2.03)	11.5% (1.5%, 53.3%), $P = 0.15$	1.33 (0.94, 1.90)	41.3% (11.9%, 78.5%), $P = 0.003$

[1] $n = 22,475$. HRs with 95% CIs are for Q4 compared with Q1 from Model 1 adjusted for sex, age (continuous), energy intake (continuous), educational level (categorical), housing tenure (categorical), smoking (categorical), BMI (continuous), leisure-time physical activity (continuous), history of cancer, CVD, diabetes, hypertension, hyperlipidemia, and residence (categorical). Null = not mediating the effect. CVD, cardiovascular disease; IHD, ischemic heart disease; PTE, percentage of exposure effect.

[2] PTE explained by intermediate variables, with 95% CIs and *P* values.

association between UPF and adiposity (8, 10, 12, 14) perhaps due to reverse bias or selective under-reporting although it is reasonable that any detrimental effect of UPF would not translate to body weight (36).

Strengths and limitations

The prospective population-based design, the inclusion of detailed information on lifestyle factors to accommodate possible confounding, the use of numerous sensitivity analyses to test the robustness of the results, and the relatively long follow-up are the main strengths of this study. To the best of our knowledge, this is the first study to assess nutrients and a variety of biological mechanisms as possibly mediating the relation between UPF intake and mortality.

Yet, there are several important limitations too. First of all, the FFQ used in this population was not specifically conceived to collect data based on the NOVA classification, thus many food items have been left out (e.g., preprepared dishes, energy bars, slimming products). Second, the observational nature of the study cannot fully rule out residual or unmeasured confounding. However, the use of E-values suggested a very small impact of potential unmeasured factors on the strength of our associations.

Third, dietary data were based on self-reported information and therefore may be susceptible to error and bias; fourth, our data were gathered from an adult cohort from a small Southern Italian region, which might limit the generalizability of our findings, although our cohort is representative of the whole Italian population. Finally, subjects' information (e.g., dietary and biological data) was collected at baseline only, thus life course changes that possibly occurred during the follow-up might have modified the strength of the findings. We should also acknowledge that the usefulness of the NOVA classification has been recently questioned because this food classification scheme is not based on unequivocal, distinct physical/chemical aspects of foods and has been revised and refined over time (2, 15, 37). However, this classification allows comparison with previous studies and increases the level of evidence.

Conclusions

Our study documented an increased risk of all-cause and CVD mortality in association with high consumption of UPF in a large sample of an adult Mediterranean population.

Our data provide an original insight into the potential biological mechanisms through which UPF might exert its detrimental health effects; in addition, we support previous evidence indicating that the adverse association of UPF with health is unlikely to be fully explained by the nutritional factors contained in these foods, at least by those investigated here. These findings corroborate evidence from previous population studies and support the need for several public health actions, possibly encouraging people to prefer fresh or minimally processed foods and freshly made dishes and meals rather than UPF. Further longitudinal studies on dietary behaviors reflecting the modern food supply are needed to support our associations, but more urgently, there is a clear need for randomized controlled trials to document the effects on health of increasing UPF that are

TABLE 4 Biomarkers of CVD risk as possible mediators of the association of ultra-processed food intake with all-cause, CVD, and IHD/cerebrovascular mortality in the Moli-sani Study cohort[1]

	All-cause mortality		CVD mortality		IHD/cerebrovascular mortality	
	HR (95% CI)	PTE[2]	HR (95% CI)	PTE[2]	HR (95% CI)	PTE[2]
Model 2	1.26 (1.09, 1.46)	—	1.58 (1.23, 2.03)	—	1.52 (1.10, 2.09)	—
Model 2 + biomarkers of inflammation	1.25 (1.08, 1.44)	5.1% (1.3%, 18.0%), $P = 0.051$	1.56 (1.21, 1.99)	3.6% (0.9%, 12.8%), $P = 0.059$	1.49 (1.08, 2.05)	5.1% (1.6%, 15.2%), $P = 0.013$
Model 2 + biomarkers of glucose metabolism	1.24 (1.07, 1.43)	7.3% (2.6%, 19.1%), $P = 0.008$	1.58 (1.23, 2.02)	Null	1.54 (1.12, 2.13)	Null
Model 2 + biomarkers of lipid metabolism	1.24 (1.07, 1.43)	6.6% (2.2%, 18.1%), $P = 0.014$	1.56 (1.22, 2.00)	3.1% (0.7%, 12.6%), $P = 0.075$	1.56 (1.13, 2.15)	Null
Model 2 + biomarkers of renal function	1.20 (1.04, 1.39)	20.1% (8.7%, 39.9%), $P < 0.001$	1.50 (1.17, 1.91)	12.0% (5.6%, 24.1%), $P < 0.001$	1.47 (1.06, 2.02)	8.4% (3.0%, 21.2%), $P = 0.002$
Model 2 + all biomarkers	1.20 (1.03, 1.38)	22.4% (9.2%, 45.8%), $P < 0.001$	1.48 (1.16, 1.90)	9.4% (2.8%, 27.0%), $P = 0.037$	1.52 (1.10, 2.09)	Null

[1] $n = 22,475$. HRs with 95% CIs are for Q4 compared with Q1 from Model 2 adjusted for sex, age (continuous), energy intake (continuous), educational level (categorical), housing tenure (categorical), smoking (categorical), BMI (continuous), leisure-time physical activity (continuous), history of cancer, CVD, diabetes, hypertension, hyperlipidemia, residence (categorical), and Mediterranean Diet Score (continuous). Biomarkers of inflammation include C-reactive protein (mg/L; log) and white blood cell count ($\times 10^9$/L; log). Biomarkers of glucose metabolism include blood glucose (mg/dL; log), insulin (pmol/L; log), and C-peptide (ng/mL; log). Biomarkers of lipid metabolism include blood cholesterol (mg/dL), HDL cholesterol (mg/dL), triglycerides (mg/dL; log), apoA1 (g/L), apoB100 (g/L), and lipoprotein a (mg/dL). Biomarkers of renal function include cystatin C and creatinine (mg/L; log). Null = not mediating the effect. CVD, cardiovascular disease; IHD, ischemic heart disease; PTE, percentage of exposure effect.

[2] PTE explained by intermediate variables, with 95% CIs and P values.

independent from differences in nutrient content or the types of foods consumed (1, 2).

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The authors' responsibilities were as follows—MB: conceived the study and wrote the manuscript; LI and ADiC: contributed to its design and to interpretation of data; SC, ADeC, and MP: managed the data collection and laboratory tests; MB and ADiC: analyzed the data; FS, MBD, CC, GdG, and LI: originally inspired the research and critically reviewed the manuscript; MB and LI: are responsible for the overall content as guarantors and affirm that the article is an honest, accurate, and transparent account of the study being reported, that no important aspects of the study have been omitted, and that any discrepancies from the study as planned have been explained; and all authors: read and approved the final manuscript. The authors report no conflicts of interest.

Data Availability

The data sets analyzed in the current study are not publicly available because of restricted access, but further information about the data sets is available from the corresponding author on reasonable request.

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