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Ultra-processed food consumption, plasma metabolite profile, and risk of all-cause and cause-specific mortality in a population-based cohort

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Summary

Background & aims: Epidemiological evidence on ultra-processed food (UPF) and cause-specific mortality remains limited and mixed. Molecular mechanisms underlying UPF intake and mortality remain unexplored. This study aimed to evaluate the associations between UPF consumption, metabolic signatures, and all-cause, premature, and cause-specific mortality.

Methods: This study included 27670 participants (mean age 58.1 years) from the Malmö Diet and Cancer (MDC) cohort study. Consumption of UPF was assessed using a food frequency questionnaire and a 7-day food diary. In a subset of the MDC (n=879), the associations of UPF with 991 plasma metabolites were investigated. An elastic net regression model was used to establish the metabolic signature of UPF. Cox proportional hazards regression model was used to determine the association between UPF intake, metabolic signature, and mortality risk.

Results: During a median follow-up of 23.3 years, a total of 11333 participants died. UPF intake showed a nonlinear positive association with all-cause mortality, with more pronounced associations found in females ($P_{interaction}$= 0.044); in females, UPF was linked to a higher mortality risk in a linear manner, while the association was J-shaped in males. Each standard deviation (SD) increment in UPF intake was associated with an increased risk of premature mortality (HR, 1.06; 95% CI, 1.03–1.09), cardiovascular disease (CVD) mortality (HR, 1.05; 95% CI, 1.01–1.08) or respiratory disease mortality (HR, 1.08; 95% CI, 1.01-1.15), but not cancer mortality. The metabolic signature for

UPF consumption (with 93 metabolites) was positively associated with all-cause mortality risk (HR per 1 SD, 1.23; 95% CI, 1.06-1.42).

Conclusions: Our results suggest that higher UPF intake is associated with increased risk of all-cause, premature, CVD, and respiratory disease mortality, with the association varying across sex for all-cause mortality. The plasma metabolic signature of UPF showed a positive association with all-cause mortality.

Keywords: Ultra-processed food; mortality; prospective cohort; NOVA classification; Metabolites

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; FFQ, food frequency questionnaire; HR, hazard ratio; CI, confidence interval; MDC, Malmö Diet and Cancer; MET, metabolic equivalent task; PCI, processed culinary ingredients; SD, standard deviation; UPF, ultra-processed food.

1. Introduction

Ultra-processed foods (UPF), according to the NOVA food classification system, are food products made by a series of industrialized processes and contain multiple ingredients and additives, with little or no whole foods[1]. These food products are usually palatable, ready to consume, inexpensive, and highly marketed. The varieties and amounts of UPF have expanded substantially in the global food system in recent decades[2, 3]. UPF contributes a large proportion of Western diets, accounting for almost 60% of energy intake in the United States[4]. In European countries, the energy proportion from UPF intake ranges from 14% to 44%[5]. High consumption of UPF is associated with poor diet quality[6]. Additionally, chemicals from UPF manufacturing and packaging have been linked to oxidative stress, inflammation, and changes in gut microbiota in experimental studies [7-9].

Since 2019, emerging cohort studies[10-21], but not all[22, 23], have shown positive associations between UPF intake and all-cause mortality. However, evidence on UPF and cause-specific mortality remains limited and mixed, and the association with premature mortality has not been studied. Furthermore, evidence from the Swedish population remains limited to date. UPF covers a broad range of foods that vary widely in composition and nutritional quality[24]. Analyzing the risks associated with UPF subgroups can help tailor and prioritize policy guidance around UPF consumption[25]. However, the associations of specific UPF subgroups with mortality risk have only been examined in two previous studies[14, 21].

Traditional dietary instruments like food frequency questionnaires (FFQs) and dietary recalls are subjective and prone to measurement and recall bias. The metabolic signature of dietary intake has emerged as a valuable tool in nutritional research for its objective nature, reflecting metabolic responses to diet, and enhancing understanding of biological mechanisms[26, 27]. The metabolite profiles for diet patterns (e.g., Mediterranean diet) or specific foods (e.g., legumes) have been identified[28, 29]. However, the only study on the metabolic signature of UPF consumption covered 232 candidate metabolites and was conducted among British children[30]. The molecular mechanisms underlying UPF intake and mortality remain unexplored.

Therefore, we aimed to investigate the association of UPF intake and seven UPF subgroups with all-cause mortality, premature mortality, mortality from cancer, CVD, and respiratory disease in the Malmö Diet and Cancer (MDC) cohort, a large prospective cohort with 23.3 years of follow-up. The metabolic signature of UPF consumption was identified, and its association with mortality risk was assessed.

2. Methods

2.1 Study population

The MDC is a prospective cohort study initiated in 1991 in Malmö, Sweden. Until 1996 the recruitment was completed, and of the 74318 invited participants, 30446 individuals aged 45 to 73 years took part in the baseline examination. Participants visited the study center twice at baseline. During the first visit, a self-administered questionnaire regarding lifestyle and socioeconomic factors, a food diary, and a FFQ

were explained and distributed to participants, and anthropometric measurements were performed by trained personnel. Approximately two weeks later, the returned questionnaires were reviewed and a diet history interview was conducted. Details of this cohort have been described elsewhere[31, 32]. The study was approved by the Ethical Committee at the Medical Faculty at Lund University (approval number: LU 51/90) and all participants provided written informed consent.

We include participants with complete dietary information (n = 28098). Those with missing data on seven covariates were excluded (n=428), leaving 27670 participants for the analysis of UPF intake and mortality risk. A random subset of participants from the MDC was invited to join the Malmö Diet and Cancer Cardiovascular Cohort (MDC-CC) between 1991 and 1994. This sub-cohort consisted of 6103 participants, of whom 5543 provided blood samples after standardized overnight fasting. Data on blood lipids and lipoprotein subfractions was available for 4059 participants. For the analysis of plasma metabolites, the study sample was restricted to 879 participants with available metabolomics data. Details are shown in the flowchart (Supplementary Figure 1).

2.2 Dietary assessment of UPF intake

The food intake was assessed using a modified diet history method, consisting of a 7-day food diary, a 168-item semiquantitative FFQ, and a 45-60 minutes dietary interview. This method was validated using an 18-day weighted food record, with energy-adjusted Pearson correlation coefficients for most foods ranging from 0.50 to 0.80[33, 34]. In the food diary, participants were asked to record their daily meals

(usually cooked lunch and dinner), cold beverages, and dietary supplements for 7 consecutive days. FFQ was used to assess the regularly consumed foods over the previous year, mainly breakfast and snacks. A dietary interview was conducted to quantify the food amount in the food diary, check for the food overlap between FFQ and food diary, and collect the details about how foods and dishes in the food diary were prepared. We adopted 48 (FFQ), 180 (food dairy, old method), and 75 (food dairy, new method) sets of food photographs to accurately assess the quantity of food. Food information from the food diary and FFQ was summarized into the average daily intake for each food item (g/day). Food intake was further converted into energy and nutrient intake with the use of computer software and the Swedish Food Database PC KOST2-93 of the Swedish National Food Administration.

The UPF intake was assessed by the NOVA classification that groups each food item into one of the four food groups based on the levels and purpose of industrial processing[1]: 1) unprocessed or minimally processed foods, e.g., fruits, vegetables, eggs, milk, and unprocessed meat; 2) processed culinary ingredients (PCI), e.g., oils, butter, and sugar; 3) processed foods, e.g., canned fish, cheese, high-fiber bread and cereals; and 4) UPF, e.g., savory snacks, reconstituted meat products, pastries, cakes, cookies, and soft drinks. In this study, we focused on the fourth category UPF. The examples of the food items in each category are listed in Supplementary Table 1. We further categorized the UPF into 7 mutually exclusive subgroups, including starchy

118 foods and breakfast cereals, beverages (i.e., soft drinks), sugary products, fats and
119 sauces, meat and fish, dairy products, and salty snacks.

120 Four items (i.e., “crispbread, 10-20% fiber”, “crispbread, > 20% fiber”, “bread, ≥
121 6.0% fiber”, “marmalade, honey, jam, puree”) with inconsistencies among researchers
122 in the classification were assigned to the most likely group. To assess the impact of this
123 inconsistency, we adopted an alternative categorization of the UPF in sensitivity
124 analysis. Specifically, "marmalade, honey, jam, puree" was reclassified from the UPF
125 group to the processed culinary ingredients group, while the three bread food items
126 were reclassified from processed foods to the UPF group.

127 **2.3 Measurement of metabolites**

128 Overnight fasting blood samples were collected at baseline, and separated plasma
129 was stored at -80°C until analysis. A total of 1372 biochemicals were measured by a
130 well-validated untargeted liquid chromatography coupled to tandem mass spectrometry
131 (LC-MS/MS) on the Metabolon Platform (Morrisville, NC, USA). These biochemicals
132 included 835 named metabolites, 268 unnamed metabolites, and 269 xenobiotics.
133 Metabolites with more than 75% missing values were excluded, while xenobiotics with
134 missing values were imputed with 0. All metabolites were log-transformed, and values
135 beyond ±5 standard deviations (SD) from the mean were set at the 5 SD threshold. After
136 exclusions, 991 metabolites remained for metabolomic analysis.

137 **2.4 Measurement of blood lipids and lipoprotein subfractions**

Total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) concentrations were determined using a DAX 48 automatic analyzer (Bayer AB, Göteborg, Sweden) with reagents and calibrators provided by the instrument supplier. Low-density lipoprotein cholesterol (LDL-C) was derived from the Friedewald formula. Lipoprotein subfractions were analyzed via ion mobility analysis. Intra- and interassay coefficients of variation for LDL particles were less than 1.0%. More details on the assessment of biomarkers and quality control have been described previously[35].

2.5 Outcome ascertainment

The outcomes were all-cause mortality, premature mortality, and cause-specific mortality from cancer, CVD, and respiratory disease. Deaths and emigrations were identified through the Swedish National Tax Agency, Statistics in Sweden, and the National Board of Health and Welfare. Cause-specific mortality was based on the Swedish Cause of Death Register. Cancer, CVD, and respiratory disease death were defined according to the following codes from the ninth and tenth revisions of the International Classification of Diseases (ICD): 140–239 (ICD-9) and C, D00-D48 (ICD-10) for cancer death, 390–459 (ICD-9) and I (ICD-10) for CVD death, and 460–519 (ICD-9) and J (ICD-10) for respiratory disease death. Premature death was defined as deaths that occurred before the age of 75 years[36]. We followed up all participants from the date of completing the baseline survey until the death, emigration, or

158 December 31, 2018, whichever occurred first. The rate of loss to follow-up due to
159 emigration was 0.8% (n = 212).

160 **2.6 Assessment of covariates and other variables**

161 Information on age and sex was collected from the Swedish registry using a
162 personal identification number. The recruitment of the participants took more than five
163 years and the follow-up ended at a fixed date, so the year of participant recruitment
164 (quartiles) was considered as a covariate in this study. Weight and height were measured
165 by trained personnel at baseline examination. Body mass index (BMI) was constructed
166 by weight and height and further divided into four groups (<18.5, 18.5-24.9, 25–29.9,
167 and ≥30 kg/m^2). A self-administered questionnaire at baseline was used to collect data
168 on marital status (married or others), smoking habits (current, former, or never),
169 educational level (elementary, primary and secondary, upper secondary, further
170 education without a degree, and university degree), whether lived alone (yes or no).
171 Alcohol consumption was divided into six categories (zero intake in both food diary
172 and FFQ, and sex-specific quintiles for those who reported drinking). Metabolic
173 equivalent task (MET) hours per week, derived from the duration of 17 different leisure-
174 time physical activities, was categorized into five groups (< 7.5, 7.5–15, 15–25, 25–50,
175 and > 50 MET-hour/week). The heredity score of cancer or CVD was generated based
176 on the self-reported family history of the disease. If one of the participant's relatives
177 (father, mother, and siblings) has the disease, one point is assigned to the heredity score.
178 The diet quality index, based on the Swedish dietary guidelines, was calculated as the

sum of five dietary factors: fiber (> 2.4 g/MJ), fruit and vegetables (> 400 g/day), fish (> 300 g/week), added sugar (< 10% energy), and red and processed meat (< 500 g/week)[37]. For each dietary factor, a score of 1 is assigned if the above criterion is met, and otherwise, the score is 0, resulting in an overall score ranging from 0 to 5. We divided the intake of coffee (quartiles) and tea (zero consumers, < 225g/day, and ≥225 g/day) into categorical variables based on their distribution. The variable dietary assessment method (old and new) was created because the dietary interview was shortened from 60 min to 45 min in September 1994. The dates of dietary data collection were categorized into seasons (spring, summer, autumn, and winter). Blood pressure was measured by nurses using a mercury sphygmomanometer. Hypertension was defined as systolic blood pressure ≥ 140mmHg and/or diastolic blood pressure ≥ 90mmHg, or antihypertensive medication usage. Diabetes was identified through self-reported diagnosis, medication usage, or registry records. The use of lipid-lowering medication was self-reported.

Participants were defined as potential energy misreporters when the ratio of their energy intake to basal metabolic rate was outside the 95% CI of the physical activity level, which was estimated from activities at work, leisure time, and household work, as well as time for sleep, self-care, and passive activities[38]. Participants were considered diet changers if they reported “yes” to the question in the baseline questionnaire: “Have you substantially changed your eating habits because of illness or some other reasons?”. In the follow-up survey conducted five years after the baseline

(1997-2001), we obtained the status of diet change by the question: "Have you substantially changed your dietary habits since you participated in the MDC study for the first time?".

2.7 Statistical analyses

The baseline characteristics were presented by quintiles of energy-adjusted UPF intake using mean and standard deviation (SD) for continuous variables and percentage for categorical variables.

Analysis 1 (UPF intake and mortality risk)

Intake of UPF and UPF subgroups were adjusted for total energy intake using the residuals method. We used Cox proportional hazards regression models to investigate the associations of UPF intake and mortality risk, with the first quintile used as a reference. Hazard ratios (HR) and 95% confidence intervals (CI) per one SD increment of UPF intake were also estimated. Covariates were progressively entered into 4 adjusted models; we adjusted age, sex, dietary assessment method, season, total energy intake, and year of participant recruitment in model 1. Model 2 was adjusted for all variables in model 1 plus educational level, leisure time physical activity, smoking status, alcohol consumption, prevalent CVD, cancer or diabetes, heredity score of cancer (0 or >0) or CVD (0 or >0), marital status, whether lived alone, coffee and tea intake. As diet quality index and BMI are possible mediators linking UPF consumption and mortality risk, we further adjusted for all variables in model 2 and diet quality index (model 3), and for BMI in model 4 to observe the changes of association. Model 2 was

considered as the main model. We tested the proportional hazards assumption by Schoenfeld test and visual examination of the Schoenfeld residuals. No violation of the assumption was observed. We investigated potential non-linear associations between UPF intake and mortality risk by fitting models using restricted cubic splines for absolute UPF intake with 3 knots (placed at the 10th, 50th, and 90th percentiles). The P value for nonlinearity was estimated by the Wald test. All covariates were entered into the models in the forms previously described in the covariates assessment section unless otherwise specified.

We evaluated the potential effect modification by repeating model 2 stratified by age, sex, smoking status, alcohol consumption, physical activity, tea intake, coffee intake, and whether lived alone. P for interaction was calculated using the Wald test for cross-product terms (UPF quintiles × stratification variables). We examined the association of UPF subgroups with mortality risk with 7 subgroups simultaneously included in the Cox model. To examine whether heterogeneous associations with mortality risk existed for 7 UPF subgroups, we used the likelihood ratio test to compare two models with one included total UPF intake and the other included total UPF intake plus 7 subgroups; the improved fit for the latter model indicates the existence of heterogeneity.

We performed the following sensitivity analyses: (1) repeating the models after excluding participants with prevalent CVD, cancer, or diabetes (n=3518) at baseline, excluding deaths occurring within the first 10 years of follow-up (n=2279), excluding

energy misreporters (n=5110) or diet changers (n=6741); (2) further adjusted for other three NOVA-defined food groups instead of coffee and tea intake; (3) further adjusted for intake of folate, vitamin D, vitamin C, calcium, iron, potassium, magnesium, selenium, and zinc; (4) using the proportion of UPF weight in total food as exposure; (5) using an alternative categorization of the UPF as exposure; (6) further adjusted for BMI and diet quality index in spline analysis; (7) further adjusted for the EAT-Lancet diet index[39] (derived from 14 food components) instead of diet quality index.

Analysis 2 (UPF intake, plasma biomarkers, and mortality risk)

The metabolites of all four NOVA-defined food groups were analyzed to assess whether these food groups exhibited diverse metabolic signatures and whether these metabolic signatures mirrored the associations observed in food intakes. Given the high dimensionality and collinearity of the metabolomic data, an elastic net regression model was employed for metabolite selection. The hyperparameters were selected through 10-fold cross-validation (R package: "caret"). Participants were randomly assigned into a training set (70%) and a testing set (30%). First, we fitted the elastic net regression models in the training set and then used the coefficients (weights) to construct the metabolic profile score—a weighted sum of the selected metabolites—in the testing set. In the training set, the metabolic profile score was constructed using an elastic net regression model combined with a leave-one-out approach to avoid overfitting. These methods have been used to identify the metabolic signature of dietary patterns and specific food items[28, 29]. Partial correlations of four food groups with biomarkers

were presented in the heatmaps, adjusting for covariates in model 3 (excluding method, coffee, and tea) plus hypertension, diabetes, and lipid-lowering medication. Tertiles were generated for each metabolic profile score, and Cox proportional hazards regression models were used to estimate their associations with all-cause mortality.

We used R version 4.2.1 (R Foundation) in statistical analyses. All tests were 2-sided and the significant level was 0.05.

3. Results

3.1 Baseline characteristics

This study included 27670 participants (60.7% females), with a mean age of 58.1 years (SD, 7.6 years). The mean proportion of UPF in total food is 13.4% (SD, 7.3%). Compared with those in the lowest quintile of UPF intake, participants in the highest quintile group were more likely to be older, female, and never smoker and to drink less alcohol. They also tended to have a higher BMI, and prevalence of cancer, CVD, and diabetes, but they were less likely to have a university degree. In addition, they had a lower diet quality index, and a lower intake of fiber, coffee, and tea (Table 1). The main subgroups contributing to the total UPF were starchy foods and breakfast cereals (percentage, 26.0%), beverages (23.3%), and sugary products (18.4%), fats and sauces (15.5%), and meat and fish (13.6%) (Supplementary Table 2).

3.2 Dietary UPF intake and mortality risk

During a median follow-up of 23.3 years (582853 person-years), a total of 11333 participants died (of cancer, 3938; of CVD, 3709; of respiratory disease, 758), with

3672 dying before age 75 (Table 2). The intake of UPF was positively associated with all-cause mortality in a nonlinear manner ($P_{nonlinear}$=0.022), with increased mortality risk beginning to appear only when UPF intake exceeded the median (Fig. 1a). This association was modified by sex ($P_{interaction}$= 0.04); in females, UPF was associated with a higher mortality risk in a linear manner; while in males, the associations were J-shaped (Fig. 1b). Higher mortality risk with increased UPF intake was more pronounced in females. Relative to the lowest quintile of UPF intake, HR (95% CI) of all-cause mortality was 1.06 (1.00-1.12) for the fifth quintile (Table 2). This association was attenuated markedly with the progressive inclusions of potential mediators (diet quality index and BMI). In the main model, each SD increment in UPF intake was associated with an increased risk of premature mortality (HR, 1.06; 95% CI, 1.03–1.09), CVD mortality (HR, 1.05; 95% CI, 1.01–1.08) or respiratory disease mortality (HR, 1.08; 95% CI, 1.01-1.15). UPF intake was not associated with cancer mortality.

Sex modified the association between UPF and CVD mortality in a similar way as it did the association of UPF with all-cause mortality ($P_{interaction}$= 0.037) (Supplementary Fig. 2). The positive association of UPF intake with premature mortality and respiratory disease mortality was not modified by sex ($P_{interaction}$ > 0.05).

For the UPF subgroups, we observed heterogeneity in the associations with all-cause, premature, and CVD mortality risk ($P_{heterogeneity}$ < 0.001) (Supplementary Fig. 3). Intakes of beverages group and meat and fish group were positively associated with all-cause mortality risk, whereas sugary products showed an inverse association. These

findings were similar in analyses of premature and CVD mortality. Other UPF subgroups were not associated with all-cause, premature, and CVD mortality risk.

In analyses stratified by age, drinking status, coffee intake, tea intake, and whether lived alone, the associations between UPF intake with all-cause, premature, cause-specific mortality risk were similar across strata ($P_{interaction} > 0.05$; Supplementary Table 3). The association with all-cause mortality was weaker in individuals with higher levels of physical activity compared to those who were less physically active ($P_{interaction}=0.014$; data not shown). Increased respiratory disease mortality risk in relation to a higher intake of UPF seems more pronounced in never smokers ($P_{interaction}=0.027$; data not shown). In sensitivity analyses, the elevated mortality risk above the median of UPF intake was robust, while less robust results were observed when UPF intake below the median (Supplementary Fig. 4).

3.3 Plasma metabolites, lipid and lipoproteins, and mortality risk

A total of 93, 49, 23, and 96 metabolites were selected as the metabolic signatures of UPF, unprocessed or minimally processed foods, processed foods, and PCI, respectively. The metabolic signatures were significantly correlated with the corresponding food groups ($r = 0.21–0.32$, $P <0.001$) (Supplementary Fig. 5). The metabolic signature of UPF was positively associated with all-cause mortality risk, with each SD increase in the metabolic profile score linked to a 23% higher mortality risk (HR=1.23; 95% CI, 1.06-1.42; P=0.005) (Table 3). Conversely, the processed foods

signature showed an inverse association with all-cause mortality risk (HR per 1 SD, 0.87; 95%CI, 0.77-0.97; P=0.015).

As shown in the Venn diagram, UPF shared 20 metabolites and 11 metabolites with PCI and unprocessed or minimally processed foods, respectively (Supplementary Fig. 6). Ergothioneine was the only metabolite selected by all four groups of food, which showed positive associations with unprocessed or minimally processed foods and processed foods but inverse associations with UPF and PCI. The perfluorooctanesulfonate (PFOS) showed positive associations with UPF and PCI but an inverse association with unprocessed or minimally processed foods (Supplementary Table 4).

After adjusting for covariates, the top selected metabolites that showed significant negative association with UPF were N2,N5-diacetylornithine, ergothioneine, methyl glucopyranoside (alpha+beta), heneicosapentaenoate (21:5n3), and N-delta-acetylornithine. In contrast, glutamine_degradant, 2-hydroxy-4-(methylthio) butanoic acid, X-18887, gamma-tocopherol/beta-tocopherol, and X-23276 showed a positive association. As compared to UPF, both unprocessed or minimally processed foods and processed foods tend to demonstrate opposite associations with 93 metabolites comprising the metabolic signature of UPF (Fig. 2).

The higher intake of UPF was related to unfavorable lipid and lipoprotein profiles, indicated by lower levels of large HDL, HDL, and large IDL, but higher levels of large VLDL, small LDL, and TG (Supplementary Fig. 7). The four groups of food showed

346 distinct lipid and lipoprotein patterns, with unprocessed or minimally processed foods
347 showing a reverse pattern compared to UPF.

348 **4. Discussion**

349 In this large population-based prospective cohort, we found nonlinear positive
350 associations of UPF intake with all-cause and CVD mortality; sex modified these
351 associations. High intake of UPF was positively associated with premature mortality
352 and respiratory disease mortality whereas no association was found for cancer mortality.
353 Specific UPF subgroups showed heterogeneous associations with all-cause, premature
354 and CVD mortality. The metabolic signature of UPF was positively associated with all-
355 cause mortality risk. Higher UPF intake was related to unfavorable lipid and lipoprotein
356 profiles.

357 **4.1 UPF intake and mortality risk**

358 Our observed positive association with all-cause mortality was supported by a
359 recent meta-analysis[40] and four subsequent cohort studies[17-19, 21]. However, a
360 large US cohort[23] reported a null association. Of note, this cohort mainly included
361 low-income American adults with a mean BMI of 30.4 kg/m^2, and 24.4% of them had
362 prevalent diabetes; apparent differences in baseline characteristics compared to other
363 studies may interpret this inconsistency. Five previous studies evaluated the dose-
364 response relationship between UPF intake and death risk from all causes or CVD [10,
365 14, 15, 17, 20]. Of those, four studies[10, 15, 17, 20] did not find a significant nonlinear
366 association, whereas a large US cohort (n= 91891)[14] exhibited a J-shaped association

between UPF intake and CVD mortality. The reported associations between UPF and CVD death are less consistent. Four studies[14, 18, 20, 22] showed a significant positive association, while three[10, 11, 17] did not reach statistical significance likely due to their small number of CVD deaths (n=71-649 cases).

We found that positive associations with all-cause and CVD mortality appeared to be stronger in females. Five previous studies[10, 14, 18, 20, 22] performed stratified analysis by sex, with four reporting null interaction, while one, which had the largest number of mortality events, reported a more pronounced association with CVD mortality in females as in our study[14]. This finding is reasonable, given the reported stronger associations of processed meat and beverages with all-cause and CVD mortality in females compared to males[41, 42].

Our observed null association of UPF intake with cancer mortality was consistent with five cohorts from Spain[10], Italy[20], and the US[16, 21, 23] but not with one from the UK biobank cohort[43] which found a 6% increase of cancer death risk for every 10% increment in UPF intake. This inconsistency may not be explained by statistical power, as the number of cancer deaths in our study (3938) and US cohorts (4267 and 13557) [21, 23] is similar to or larger than that in the UK biobank (4009) [43]. Nor can it be attributed to the amount of UPF intake since the mean proportion of UPF in total food in this UK biobank cohort (22.9%) falls within the range of previous studies (ranging from 10.8% to 41.0%). Our observation of a positive association between UPF intake and respiratory disease mortality is supported by two studies based

388 on US cohorts [16, 21]. This study is the first to examine the association between UPF
389 intake and premature mortality.

390 Our results on UPF subgroups were largely consistent with previous studies [14,
391 21]. Our observed increased death risk related to high UPF intake largely contributed
392 by subgroups of beverages and ultra-processed meat products considering their large
393 proportion (36.9%) in total UPF. Sugar-sweetened beverages and processed meat have
394 been shown to be associated with a higher risk of all-cause mortality[44, 45]. Inverse
395 association of sugary products intake with CVD mortality was also supported by these
396 two large US cohorts[14, 21]. There is limited epidemiological evidence regarding
397 sugary products, and further study is required to confirm these findings. Besides, this
398 result should be interpreted with caution considering UPF collected in our baseline may
399 not be entirely equivalent to the current UPF as varieties and compositions of UPF have
400 undergone significant changes over the past decades[2].

401 UPFs are often high in sodium, fat, added sugar, and energy, but low in fiber,
402 vitamins, and micronutrients, which explains our findings. In addition, some commonly
403 used food additives (e.g., emulsifiers and artificial sweeteners) in UPF [8, 46], newly
404 generated compounds during UPF manufacturing (e.g., acrylamide) [7, 47], and
405 contaminants migrated from food packaging (e.g., bisphenol A) [48] may also
406 contribute to the adverse health effects of UPFs.

407 Notably, when UPF intake was below the median, higher UPF intake showed no
408 association (in females) or an inverse association (in males) with all-cause mortality.

409 This may be due to poor health leading to less UPF intake or survival benefits from
410 moderate sugary products consumption. Additionally, less robust association in
411 sensitive analyses suggest that reverse causality, residual confounding, or measurement
412 error might also explain this finding.

413 **4.2 Plasma metabolites, lipid and lipoproteins, and mortality risk**

414 Our analysis of plasma biomarkers provided some novel insights in understanding
415 UPF-mortality association. The identified signatures tend to mirror the relationship
416 between dietary intake and mortality risk, despite the analysis of metabolic signatures
417 being based on the small subset of the MDC. Ergothioneine, a diet-derived amino acid
418 that has strong antioxidant and cytoprotective properties, is found in higher
419 concentrations in specific foods like specialty mushrooms, liver, kidney, beans, and oat
420 bran, rather than in most commonly consumed foods[49]. N2,N5-Diacetylornithine and
421 N-delta-acetylornithine, both amino acids involved in the urea cycle and arginine and
422 proline metabolism, have been positively associated with the consumption of legumes
423 and papaya[29, 50]. N2,N5-Diacetylornithine is also recognized as a plasma biomarker
424 reflecting gut microbiome diversity[51]. Methyl glucopyranoside (alpha + beta) was
425 previously correlated with apple intake and carotene diol (2) was correlated with
426 cruciferous vegetable intake[50]. Overall, these metabolites showed negative
427 associations with UPF intake but positive associations with the intake of plant foods.
428 Some of these metabolites have shown potential health benefits[49], indicating that the

observed mortality risk with higher UPF consumption may be partly due to the accompanied lower intake of some plant foods.

Our results confirmed previous findings of a positive association between UPF and vitamin E (gamma-tocopherol)[52]. This association is possibly due to tocopherols often being used as an additive to prevent the peroxidation of fats and lipids in foods[53]. PFOS, a compound widely found in commonly consumed foods, was correlated with UPF and PCI but negatively with unprocessed or minimally processed foods; this could be explained by the migration of PFOS from food packaging[54]. Some other top metabolites in the signature, such as glutamine degradant and 2-hydroxy-4-(methylthio) butanoic acid, were rarely linked to foods or health outcomes in previous studies. The only study concerning the metabolic profile of UPF covers 232 candidate metabolites that have minimal overlap with our metabolite panel and was conducted among children[30]. Therefore, most associations of metabolites with UPF and the other three groups of food were first identified in our study, and further replications were warranted.

In MDC-CC, our results, covering more lipids and lipoproteins than most previous studies, supported the previous findings of unfavorable lipid and lipoprotein profiles related to higher intake of UPF[30, 55, 56]; this suggests that lipid metabolism may constitute one of the potential mechanisms underlying the detrimental effects of UPF.

In addition, we observed an inverse association between the processed foods signature and mortality risk; this is plausible as a similar association between intake of processed foods and mortality risk was found in our study (data not shown). Some

450 major food items in processed foods, such as cheese, high-fiber bread, cereals, and
451 crispbread, may contribute to this association. Some metabolites that showed a positive
452 association with processed foods have been linked to food intake in previous studies.
453 The aforementioned ergothioneine and N-delta-acetylornithine showed opposite
454 associations with UPF and processed foods. Ectoine has been found in cheese and is
455 associated with the fermentation process, while 2-aminophenol sulfate has been linked
456 to the intake of wholegrain foods[50, 57]. Taken together, biomarkers seem to capture
457 the information on the intake of several food items in processed foods.

458 **4.3 Strengths and limitations**

459 To our knowledge, this population-based cohort study is the first to integrate a
460 large panel of metabolites (n=991), lipids, and lipoprotein subfractions in assessing the
461 association between UPF intake and mortality risk. Several key strengths of this study
462 include its prospective design, large sample size (27670 participants with 11333 deaths),
463 long follow-up time (a median of 23.3 years), a low rate of loss to follow-up (0.8%),
464 and the use of validated food assessing method combining FFQ and food diary.

465 Several limitations of this study should be noted. First, residual confounding may
466 still exist although a wide range of potential confounders were adjusted. Second,
467 misclassification of UPF is possible since our FFQ and food diary were not specifically
468 developed for assessing UPF intake. Third, the dietary assessment was conducted only
469 once at baseline, which may not represent the long-term UPF intake. Nevertheless, in
470 the five-year follow-up survey, only 16.8% (3740 out of 22262) of the participants

reported substantially altering their diet. Most of these changes were towards a healthier way, including more intake of vegetables (73.9%) and fruits (64.7%), and less meat (43.3%). The common reasons for the change were overweight (32.2%) and hypertension (19.3%). As a result, such a direction of change may lead to an underestimation of our findings. Fourth, our analyses concerning biomarkers were cross-sectional and not validated in external cohorts, so future replications are necessary. Finally, this cohort only recruited participants living in Sweden, which limits the generalizability of these findings to other populations.

4.4 Conclusions

Our findings suggest that UPF intake was positively associated with all-cause, premature, CVD, and respiratory disease mortality, but not with cancer mortality. The risk increase of all-cause and CVD mortality was more apparent in females. Findings from UPF subgroups suggested that special attention should be given to ultra-processed meats and beverages when considering restrictions on UPF intake. Our identified metabolic signature mirrored the association between UPF consumption and mortality risk. The identified metabolites provided insights into understanding UPF-related metabolic variations and underlying mechanisms linking UPF intake and mortality risk. Future validations of these findings are warranted.

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Data availability: Supporting data are available from the corresponding author upon reasonable request but access to data must be granted by the MDC steering committees.

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

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Table 1. Baseline characteristics of study participants by quintiles of energy-adjusted UPF consumption (g/day)[a]

Characteristics	Total	Q1	Q2	Q3	Q4	Q5
Participants, n	27670	5534	5534	5534	5534	5534
Age, years	58.11±7.63	57.09±7.10	58.02±7.60	58.62±7.78	58.55±7.77	58.26±7.78
Female	16787 (60.7)	2855 (51.6)	3626 (65.5)	3665 (66.2)	3536 (63.9)	3105 (56.1)
Body mass index, kg/m^2	25.73±3.96	25.38±3.71	25.53±3.81	25.65±3.97	25.87±4.02	26.21±4.24
Married	18073 (65.3)	3505 (63.3)	3654 (66.0)	3765 (68.0)	3622 (65.5)	3527 (63.7)
Lived alone	6734 (24.3)	1427 (25.8)	1306 (23.6)	1254 (22.7)	1325 (23.9)	1422 (25.7)
University degree	3949 (14.3)	1225 (22.1)	928 (16.8)	672 (12.1)	587 (10.6)	537 (9.7)
Smoking status						
Current	7798 (28.2)	1684 (30.4)	1506 (27.2)	1496 (27.0)	1559 (28.2)	1553 (28.1)
Former	9374 (33.9)	2076 (37.5)	1939 (35.0)	1820 (32.9)	1689 (30.5)	1850 (33.4)
Never	10498 (37.9)	1774 (32.1)	2089 (37.8)	2218 (40.1)	2286 (41.3)	2131 (38.5)
Zero-consumers of alcohol	1737 (6.3)	215 (3.9)	218 (3.9)	359 (6.5)	437 (7.9)	508 (9.2)
High leisure-time physical activity (> 50METh/week)	4454 (16.1)	1002 (18.1)	891 (16.1)	790 (14.3)	819 (14.8)	952 (17.2)
Heredity score of cancer (>0)	12752 (46.1)	2514 (45.4)	2569 (46.4)	2607 (47.1)	2553 (46.1)	2509 (45.3)
Heredity score of CVD (>0)	14728 (53.2)	2818 (50.9)	2943 (53.2)	2956 (53.4)	3065 (55.4)	2946 (53.2)
Conditions at baseline:						
Cancer	1713 (6.2)	292 (5.3)	319 (5.8)	356 (6.4)	380 (6.9)	366 (6.6)
CVD	829 (3.0)	130 (2.4)	138 (2.5)	173 (3.1)	173 (3.1)	215 (3.9)
Diabetes	1209 (4.4)	225 (4.1)	197 (3.6)	264 (4.8)	241 (4.4)	282 (5.1)
Dietary intake:						

Diet quality index	1.94±1.29	2.52±1.28	2.27±1.26	1.96±1.22	1.66±1.17	1.29±1.11
Energy-adjusted UPF, g/day	379.62±176.76	201.31±60.00	293.23±16.30	347.14±16.02	416.69±26.55	639.71±205.09
Total energy intake, kcal/day	2275.83±653.22	2543.61±660.87	2174.94±582.13	2096.38±593.19	2168.71±621.16	2395.54±693.22
Proportion of UPF weight in total food,%	13.40±7.29	8.25±3.84	9.68±4.11	11.65±4.32	14.77±4.76	22.66±7.84
Coffee, g/day	518.70±394.18	551.89±418.06	516.03±385.23	508.72±380.84	518.99±390.00	497.86±393.75
Tea, g/day	146.15±251.17	171.95±288.47	150.37±252.46	136.42±238.05	133.98±235.93	138.05±234.94
Fiber, g/1000 Kcal	9.37±2.78	9.93±3.27	9.78±2.82	9.41±2.58	9.08±2.49	8.65±2.44
Saturated fat, E%	16.77±3.87	17.68±4.39	16.99±3.85	16.77±3.65	16.42±3.60	16.00±3.57
Added sugars, E%	9.99±4.31	8.03±3.65	8.61±3.48	9.29±3.55	10.44±3.66	13.60±4.67

[a]Variables are presented as mean± SD or n (%).

Table 2 Hazard ratios (95% CIs) for all-cause, premature and cause-specific mortality by energy-adjusted UPF consumption [a]

	Quintiles of UPF consumption					$P_{nonlinear}$ [b]	Continuous[c]	
	Q1	Q2	Q3	Q4	Q5		Per SD increase	P
All cause						0.022		
Cases/person years	2124/118952	2147/118349	2252/116672	2366/115371	2444/113510		—	—
Model 1	1.00	0.96 (0.91-1.02)	0.97 (0.91-1.03)	1.04 (0.98-1.11)	1.10 (1.04-1.17)		1.06 (1.04-1.08)	<0.001
Model 2 (main)	1.00	0.99 (0.93-1.05)	0.98 (0.92-1.04)	1.03 (0.97-1.09)	1.06 (1.00-1.12)		1.03 (1.02-1.05)	<0.001
Model 3	1.00	0.98 (0.92-1.04)	0.96 (0.90-1.03)	1.01 (0.95-1.07)	1.03 (0.97-1.10)		1.03 (1.01-1.05)	0.003
Model 4	1.00	0.98 (0.92-1.04)	0.96 (0.90-1.02)	1.00 (0.94-1.07)	1.02 (0.96-1.09)		1.03 (1.01-1.05)	0.008
Premature mortality						0.177		
Cases	774	664	659	729	846		—	—
Model 1	1.00	0.94 (0.85-1.05)	0.96 (0.86-1.06)	1.05 (0.95-1.17)	1.18 (1.07-1.30)		1.10 (1.07-1.13)	<0.001
Model 2 (main)	1.00	1.00 (0.90-1.11)	0.97 (0.87-1.08)	1.02 (0.92-1.13)	1.10 (0.99-1.21)		1.06 (1.03-1.09)	<0.001
Model 3	1.00	0.99 (0.89-1.10)	0.95 (0.85-1.05)	0.99 (0.89-1.10)	1.05 (0.95-1.17)		1.05 (1.02-1.08)	0.002
Model 4	1.00	0.98 (0.88-1.09)	0.94 (0.84-1.05)	0.99 (0.89-1.10)	1.05 (0.94-1.16)		1.05 (1.01-1.08)	0.004
Cancer						0.932		
Cases	783	797	721	838	799		—	—
Model 1	1.00	1.01 (0.92-1.12)	0.90 (0.81-1.00)	1.06 (0.96-1.17)	1.02 (0.93-1.13)		1.02 (0.99-1.05)	0.287
Model 2 (main)	1.00	1.03 (0.93-1.14)	0.91 (0.82-1.01)	1.05 (0.94-1.16)	1.00 (0.90-1.11)		1.01 (0.97-1.04)	0.757
Model 3	1.00	1.02 (0.92-1.12)	0.89 (0.80-0.99)	1.02 (0.91-1.13)	0.96 (0.87-1.07)		0.99 (0.96-1.03)	0.750
Model 4	1.00	1.01 (0.92-1.12)	0.89 (0.80-0.99)	1.01 (0.91-1.12)	0.96 (0.86-1.07)		0.99 (0.96-1.03)	0.672
CVD						0.091		
Cases	660	691	744	774	840		—	—
Model 1	1.00	0.98 (0.88-1.10)	1.01 (0.90-1.12)	1.07 (0.96-1.19)	1.18 (1.06-1.30)		1.08 (1.05-1.12)	<0.001

Model 2 (main)	1.00	1.01 (0.90-1.12)	1.00 (0.89-1.11)	1.03 (0.92-1.15)	1.10 (0.99-1.22)		1.05 (1.01-1.08)	0.004
Model 3	1.00	1.00 (0.89-1.11)	0.98 (0.88-1.10)	1.01 (0.90-1.13)	1.07 (0.96-1.20)		1.04 (1.01-1.07)	0.013
Model 4	1.00	0.99 (0.89-1.11)	0.98 (0.88-1.09)	1.00 (0.89-1.12)	1.06 (0.95-1.19)		1.04 (1.00-1.07)	0.024
Respiratory disease						0.197		
Cases	137	134	160	150	177		—	—
Model 1	1.00	0.90 (0.71-1.14)	1.02 (0.81-1.29)	0.99 (0.79-1.26)	1.21 (0.97-1.52)		1.11 (1.04-1.19)	0.001
Model 2 (main)	1.00	0.93 (0.73-1.18)	1.04 (0.82-1.32)	0.99 (0.78-1.26)	1.18 (0.93-1.49)		1.08 (1.01-1.15)	0.017
Model 3	1.00	0.91 (0.71-1.16)	1.01 (0.79-1.28)	0.95 (0.74-1.22)	1.11 (0.87-1.42)		1.07 (1.00-1.14)	0.051
Model 4	1.00	0.91 (0.71-1.16)	0.99 (0.77-1.26)	0.95 (0.74-1.22)	1.11 (0.87-1.42)		1.07 (1.00-1.15)	0.044

[a] Model 1: adjusted for age, sex, dietary assessment version (method), season, total energy intake, and year of participants recruitment. Model 2: model 1 plus educational level, leisure-time physical activity, smoking status, alcohol consumption, prevalent CVD, cancer and diabetes, heredity score of cancer and CVD, marital status, whether lived alone, coffee and tea intake. Model 3: model 2 plus diet quality index. Model 4: model 3 plus BMI.

[b] $P_{nonlinear}$ was estimated using spline analysis in Model 2.

[c] SD of energy-adjusted UPF = 176.76 g/day.

Table 3 Associations of the metabolic profile score of four NOVA-defined food groups with all-cause mortality risk [a]

	Tertiles of metabolic profile score				Continuous	
	T1	T2	T3	P for trend	Per SD increase	P
UPF						
Cases/person years	100/6843	130/6650	156/6126	—	—	—
Model 1	1.00	1.33 (1.00-1.77)	1.83 (1.31-2.56)	<0.001	1.31 (1.14-1.50)	<0.001
Model 2	1.00	1.21 (0.91-1.62)	1.55 (1.10-2.21)	0.013	1.22 (1.06-1.41)	0.007
Model 3	1.00	1.28 (0.95-1.71)	1.63 (1.14-2.32)	0.007	1.23 (1.06-1.42)	0.005
Unprocessed or minimally processed foods						
Cases/person years	135/6500	130/6596	121/6523	—	—	—
Model 1	1.00	1.08 (0.85-1.38)	1.20 (0.93-1.54)	0.158	1.09 (0.98-1.21)	0.108
Model 2	1.00	1.00 (0.78-1.29)	1.06 (0.82-1.37)	0.674	1.04 (0.94-1.16)	0.449
Model 3	1.00	0.95 (0.73-1.22)	1.02 (0.79-1.33)	0.862	1.00 (0.90-1.12)	0.981
Processed foods						
Cases/person years	178/6128	115/6691	93/6799	—	—	—
Model 1	1.00	0.58 (0.46-0.73)	0.60 (0.46-0.78)	<0.001	0.78 (0.70-0.87)	<0.001
Model 2	1.00	0.64 (0.50-0.81)	0.75 (0.57-0.99)	0.014	0.85 (0.76-0.96)	0.007
Model 3	1.00	0.66 (0.52-0.85)	0.78 (0.59-1.03)	0.032	0.87 (0.77-0.97)	0.015
Processed culinary ingredients						
Cases/person years	115/6764	132/6490	139/6364	—	—	—
Model 1	1.00	1.11 (0.86-1.42)	1.08 (0.83-1.40)	0.580	1.09 (0.98-1.21)	0.102

Model 2	1.00	1.02 (0.79-1.32)	0.87 (0.66-1.15)	0.331	0.99 (0.88-1.10)	0.809
Model 3	1.00	1.08 (0.83-1.41)	0.93 (0.70-1.23)	0.615	1.02 (0.92-1.15)	0.667

Model 1: adjusted for age, sex.

Model 2: model 1 plus season, year of participants recruitment, educational level, leisure-time physical activity, smoking status, alcohol consumption, heredity score of cancer and CVD, and total energy intake.

Model 3: model 2 plus hypertension, diabetes, BMI, lipid-lowering medication.

Figure legends

Fig. 1 Dose-response association between UPF and all-cause mortality (a) and stratified associations by sex ($P_{interaction}$=0.044) (b). The median UPF consumption (331.12 g/day) was used as reference level (HR=1). Multivariable model adjusted for age, sex, dietary assessment version (method), season, total energy intake, year of participant recruitment, educational level, leisure-time physical activity, smoking status, alcohol consumption, prevalent CVD, cancer and diabetes, heredity score of cancer and CVD, marital status, whether lived alone, and coffee and tea intake.

Fig. 2 Heatmap showing partial correlations of 93 metabolites constituting the metabolic signature of UPF with four NOVA-defined food groups. The bottom row shows the metabolites' coefficients in the signature of UPF. Covariates in model 3 (except for method, coffee, and tea), plus hypertension, diabetes, and lipid-lowering medication, were adjusted. PCI, processed culinary ingredients. * $P < 0.05$.