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Association between dietary intake of acrylamide and increased risk of mortality in women: Evidence from the E3N prospective cohort

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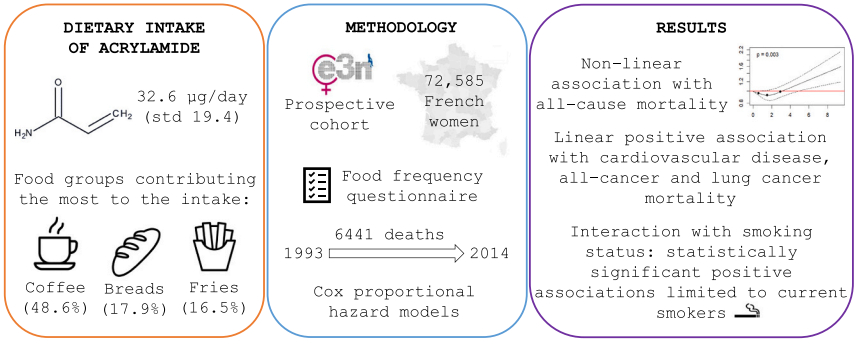
HIGHLIGHTS

- We found a non-linear association between acrylamide intake and all-cause mortality.
- This intake was linearly and positively associated with cardiovascular mortality.
- We also found linear positive associations with all-cancer and lung cancer mortality.
- When stratifying on smoking status, associations were limited to current smokers.

GRAPHICAL ABSTRACT



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ABSTRACT

Acrylamide is an organic compound classified as probably carcinogenic to humans because of sufficient evidence in animals but not in humans. Other health risks associated with acrylamide intake are still not fully elucidated. We aimed to study the relationship between acrylamide dietary intake and mortality in the E3N (Etude Epidémiologique auprès de femmes de l'Education Nationale) French cohort.

We studied 72,585 women of the E3N prospective cohort, which completed a food frequency questionnaire in 1993. The E3N food consumption database and the food contamination database obtained from the second French total diet study were used to estimate participants' average daily acrylamide dietary intake. We estimated the associations between acrylamide dietary intake and all-cause or cause-specific mortality using Cox proportional hazard models.

During follow-up (1993–2014), we identified 6441 deaths. The mean acrylamide dietary intake was 32.6 µg/day, with coffee consumption as principal contributor (48.6 %). In the fully adjusted model, we found a non-linear association between acrylamide dietary intake and all-cause mortality and a linear positive association with cardiovascular disease (HR per one STD increment [95%CI]: 1.11 [1.02; 1.21]), all-cancer (HR [95%CI]: 1.05 [1.01; 1.10]) and lung cancer (HR [95%CI]: 1.22 [1.09; 1.38]) mortality, while we observed no association with breast (HR [95%CI]: 0.94 [0.86; 1.03]) and colorectal (HR [95%CI]: 1.12 [0.97; 1.29]) cancer mortality.

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We highlighted an interaction between acrylamide dietary intake and smoking status in the models for all-cause and all-cancer mortality: when stratifying on smoking status, statistically significant positive associations were observed only in current smokers.

This study on a large prospective cohort following more than 70,000 women for over 20 years suggests that higher acrylamide dietary intakes are associated with an increased risk of mortality. Therefore, it is essential to keep reducing acrylamide contamination and prevent dietary intake of acrylamide, especially among smokers.

1. Introduction

Acrylamide is an organic and soluble compound. Occupational exposure was first studied, as acrylamide is mostly used as polyacrylamide in various industries, such as water treatment, soil stabiliser, paper, cosmetics, textile, food packaging, or tobacco (EFSA, 2015; Koszucka et al., 2020). Acrylamide is also found in cigarette smoke. In 2002, acrylamide was discovered in food and described as a heat-induced contaminant that appears at low moisture levels and during high-temperature (>120 °C) cooking methods, such as baking, roasting or frying (EFSA, 2015). It is mostly formed in plant-based foods during the Maillard reaction between the asparagine amino acid and reducing sugars (Mottram et al., 2002), but several other processes can induce acrylamide formation (Keramat et al., 2011). In the general non-smoking population, diet is the main source of exposure to acrylamide with the highest levels of acrylamide frequently found in potato-fried products, bread, biscuits, and coffee or coffee substitutes. In smokers, cigarette smoke may represent a more important source of acrylamide than diet (EFSA, 2015).

In 1994, with sufficient evidence from animal studies, but limited in human studies, acrylamide was classified as probably carcinogenic to humans (group 2A) by the International Agency for Research on Cancer (IARC) (IARC, 1994). Although acrylamide's carcinogenicity in humans remains uncertain, the European Food Safety Authority (EFSA) reported margins of exposure (MOEs) that suggest a risk of neoplastic effects based on animal evidence (EFSA, 2015). Moreover, despite inconsistent epidemiological results on whether or not exposure to acrylamide through diet increases the risk of human cancers, the American Food and Drug Administration (FDA) recommended that industries and consumers both reduce acrylamide formation in food (FDA, 2022). In 2019, acrylamide was identified as a high-priority agent recommended for a re-evaluation of its classification because of new evidence on its mechanisms and effects in human cancers (Marques et al., 2019). Acrylamide is also known to be neurotoxic in animals and humans (especially through occupational exposure) (Erkekoglu and Baydar, 2014), reprotoxic, and toxic for development (EFSA, 2015).

A Danish study estimated that dietary exposure to acrylamide is annually responsible for the loss of 1.8 years of healthy life per 100,000 inhabitants (Jakobsen et al., 2016). Only one epidemiological study investigated the dietary intake of acrylamide in relation to cancer mortality in elderly Chinese and identified an increased risk of all-cancer, digestive and respiratory cancer mortality (Liu et al., 2017). To the best of our knowledge, no previous study investigated the association between acrylamide dietary intake and all-cause or cardiovascular disease (CVD) mortality.

The main goal of the present study was to investigate the association between acrylamide dietary intake and the risk of all-cause and cause-specific mortality in the E3N (Etude Epidémiologique auprès de femmes de l'Education Nationale) French prospective cohort.

2. Material and methods

2.1. E3N cohort

The E3N cohort is an ongoing prospective cohort established in 1990 that includes 98,995 women born between 1925 and 1950 who were members of the MGEN, a health insurance for workers of the French national education system. Anthropometric, lifestyle, and health characteristics were collected every 2 to 3 years through self-administered questionnaires. Only 3 % of the E3N women are lost to follow-up (Clavel-Chapelon et al., 1997; Clavel-Chapelon and E3N Study Group, 2015). All participants gave consent to this study, that was approved by the CNIL (the French National Commission for Data Protection and Privacy).

2.2. Dietary questionnaire

The third questionnaire (Q3), sent in 1993, was a semi-quantitative food frequency questionnaire (FFQ) containing 208 food items and divided in two parts. The first part focused on the frequency and amount of food and drinks consumed during the previous year, and was organised in eight meal occasions according to French dietary habits. The second part asked more detailed questions about the food items belonging to the food groups included in the first part. The validity and reproducibility of this dietary questionnaire were previously confirmed (van Liere et al., 1997).

We estimated the daily food intakes by multiplying the quantity of food consumed by the frequency of eating occasions. Daily nutrient intakes were derived from the French food composition table of the CIQUAL, the French Information Centre on Food Quality (ANSES, 2013).

2.3. Ascertainment of acrylamide dietary intake

Food contamination levels were derived from the second French total diet study (TDS2) performed by the Anses, the French Agency for Food, Environmental and Occupational Health & Safety (ANSES, 2011). Briefly, between 2007 and 2009, 20,280 food items were purchased in 8 French regions in order to analyse the concentrations of more than 400 contaminants in 1352 composite samples prepared as consumed (i.e., pealing, frying, etc.), according to French cooking habits (Sirot et al., 2009). In particular, acrylamide was analysed in 192 samples prepared from 2280 common French products known to contribute to acrylamide dietary intake, such as bread, breakfast cereals, croissant-like pastries, biscuits, chocolate-based products, potato crisps, French fries, or coffee (Sirot et al., 2012).

The E3N food consumption database and the TDS2 food contamination database were then merged to estimate for each participant the acrylamide dietary intake (in μg of acrylamide/day), by summing, over all food items, the product of the quantity consumed of the food item (in g of food/day) and the concentration of acrylamide in the food item (in μg of acrylamide/g of food) (Mancini et al., 2020).

We estimated acrylamide dietary intake according to the lower bound (LB) scenario, in which the concentration values below the limit of detection (LOD, 4 μg/kg) were replaced by 0, and the concentration values below the limit of quantification (LOQ, 10 μg/kg) were replaced by the LOD (Sirot et al., 2012). As there were few values that were not detected (ND) or not quantified (NQ) (respectively 11 % and 15 % of 192 analysed samples), estimates of acrylamide dietary intakes according to the LB scenario were similar to those derived from the middle-bound (MB) scenario where ND and NQ values are replaced by ½ LOD and ½ LOQ, respectively.

2.4. Ascertainment of mortality

We identified deaths that occurred during the follow-up period, i.e., from the baseline of the study considered as the date of completion of the dietary questionnaire sent in 1993 to the most recent update of the mortality database linked to the cohort (i.e., November 2014). The vital status of the participants was obtained from the MGEN, postal services, municipal registries, physicians, or next of kin. Causes of death were coded by the French centre of epidemiology on medical causes of death (Inserm-CépiDc) according to the 9th (before 2000) or the 10th (after 2000) international classification of diseases (ICD). The ICD9 140–208 and ICD10 C00-C97 codes were used for all-cancer mortality, ICD9 390–459 and ICD10 I00-I99 for CVD mortality, ICD9 174 and ICD10 C50 for breast cancer mortality, ICD9 162 and ICD10 C33-C34 for lung cancer mortality and ICD9 153, 154.0–154.1 and ICD10 C18-C20 for colorectal cancer mortality.

2.5. Study population

We included all the women who answered the dietary questionnaire sent in 1993 ($N = 74,522$). We then excluded 1491 women with extreme values of energy intakes, i.e., below the 1st or above the 99th percentiles of the ratio of energy intake on energy requirements (corresponding to the basal metabolic rate based on sex, age and weight multiplied by the physical activity level (Schofield, 1985)), and 446 women lost to follow-up. Finally, for all-cause mortality analyses, our study population included 72,585 women. For all-cancer or CVD mortality analyses, the study population was composed of 72,416 women as we additionally excluded 169 participants with unknown cause of death. Finally, for specific-cancers mortality analyses, 178 women were further excluded because of unknown primary location of cancer leading to death, resulting in a study population of 72,238 participants. The flow chart is presented in **Supplementary fig. 1**.

2.6. Statistical analyses

2.6.1. Descriptive analyses

We described the characteristics (mean and standard deviation (STD) for continuous variables, number and proportion for categorical variables) of the overall population, and according to vital status and quartiles of acrylamide dietary intake.

We analysed the distribution of acrylamide dietary intake and identified its main contributing food groups whose correlations with acrylamide dietary intake were estimated using Spearman rank correlation coefficients.

2.6.2. Main analyses

Cox proportional hazard models with age (continuous, years) as the time scale were used to estimate hazard ratios (HR) and their 95 % confidence intervals (CI) for the association between acrylamide dietary intake and the risk of all-cause, CVD, all-cancer, and specific-cancers (breast, lung or colorectal) mortality. Each participant was followed-up from the age of dietary assessment to the age at death, age at the last completed E3N questionnaire, or the end of the follow-up period (i.e., the last update of the mortality database, in November 2014), whichever occurred first. For cause-specific mortality analyses, we censored all other causes of death at the age at death.

Acrylamide dietary intake, the main exposure variable, was divided by its STD to estimate HR for one STD increment.

Adjustment covariates were derived from the literature and we built a directed acyclic graph (DAG, http://www.dagitty.net, **Supplementary fig. 2**) that shows the hypothetical causal structure of the variables. Confounders were defined using the 2nd questionnaire (Q2) sent in 1992 in order to respect temporality, given that the dietary questionnaire (Q3) sent in 1993 assessed the last 12 months' dietary consumptions, except for physical activity that was defined at Q3 (as it was not collected at Q2).

We started by fitting an unadjusted model (model 1) and then adjusted our main analyses (model 2) on BMI at Q2 (continuous, kg/m^2), birth cohort ($\leq$1930, (1930–1935], (1935–1940], (1940–1945], >1945), education level (<12 years, 12 to 14 years, >14 years), smoking status at Q2 (never or former smoker, current smoker), physical activity at Q3 (continuous, MET-hours/week), menopausal status combined with recent (i.e., during the last year) use of menopausal hormone therapy (MHT) at Q2 (premenopausal, menopausal and recent MHT use, menopausal and no recent MHT use, menopausal and no information on whether and when MHT was used), and dietary characteristics all measured at Q3: total energy intake excluding energy from alcohol and lipids (continuous, kcal/day), alcohol intake (continuous, g of ethanol/day), and lipids intake (continuous, g/day).

In order to examine dose-effect relations and departures from linearity, we used restricted cubic splines functions for all continuous variables and examined whether they improved the main model's fit compared to a model including a single linear term; continuous variables were modelled using restricted cubic splines if the *p*-value of the non-linearity test was below 0.1, and using a linear variable otherwise. For the main exposure variable, we compared several models with an increasing number of knots: 3 nodes at the 10th, 50th and 90th percentiles, 4 nodes at the 5th, 35th, 65th and 95th percentiles or 5 nodes at the 5th, 27.5th, 50th, 72.5th and 95th percentiles; we selected the model (and the related number of knots) with the smallest Akaike information criterion (AIC) value. For all other continuous covariates (i.e. BMI, physical activity, total energy intake excluding energy from alcohol and lipids, alcohol intake, lipids intake, and coffee consumption), we used 4 knots by default as suggested by Harrell (Harrell, 2001), at the 5th, 35th, 65th and 95th percentiles. Moreover, we also performed all main analyses with the main exposure variable in quartile groups to facilitate comparisons between models and to estimate the association for the highest exposure category compared to the lowest (as reference). Linear trends across categories were examined by modelling the median value of each category as a continuous variable.

As cigarette smoke was also a source of exposure to acrylamide, we tested the multiplicative interaction between acrylamide dietary intake and smoking status, and we performed stratified analyses on smoking status if the *p*-value for the interaction test was <0.1.

Finally, as coffee is the main contributing food group to acrylamide dietary intake and as its consumption may also be associated with mortality, we repeated our analyses for non-coffee acrylamide dietary intake (i.e., acrylamide coming from all food groups except coffee), with an additional adjustment on coffee consumption (continuous, mL/day; model 3). We also tested the multiplicative interaction between non-coffee acrylamide dietary intake and smoking status. When the *p*-value for the interaction test was <0.1, we stratified analyses on smoking status.

We imputed covariates with less than 5 % of missing values with the median for continuous variables and with the modal category for categorical variables. We created an "unknown value" category when there were more than 5 % of missing values (menopausal status and recent MHT use).

We considered a *p*-value strictly lower than 0.05 as statistically significant. We used the Statistical Analysis System software package version 9.4 to build the database (SAS Institute, Cary, North California), and R version 4.1.2 to perform the statistical analyses.

2.6.3. Sensitivity analyses

We tested models 2 and 3 with the dietary intake of acrylamide or non-coffee acrylamide, respectively, modelled with restricted cubic spline functions irrespectively of the non-linearity test, to visually assess non-linear trends beyond statistical significance and make comparisons between all the studied mortality outcomes. Moreover, participants with prevalent chronic disease at baseline may have adopted a healthier diet after diagnosis. Thus we performed a sensitivity analysis in which we

additionally adjusted the main model (model 2) on diagnosis of diabetes before inclusion (yes, no; for all causes of death), diagnosis of cancer before inclusion (yes, no; for all-cause and cancer mortality), diagnosis of high cholesterol before inclusion (yes, no; for all-cause and CVD mortality) and diagnosis of hypertension before inclusion (yes, no; for all-cause and CVD mortality). Furthermore, we performed two other sensitivity analyses (on model 2 for all-cause mortality): first, we used the energy adjustment residual method by substituting the main exposure variable with the residual of the regression of acrylamide dietary intake on energy (Willett et al., 1997); second, we explored the potential influence of reverse causation by excluding participants who died or were censored during the first five years of follow-up.

3. Results

3.1. General characteristics

During an average of 19 years of follow-up (STD 4.1), we identified 6441 deaths, including 3473 from cancer, 896 from CVD, 953 from breast cancer, 364 from lung cancer, and 317 from colorectal cancer.

The mean acrylamide dietary intake in our study population was 32.6 μg/day (STD 19.4) (Table 1). Its median was 29.5 μg/day (IQR 18.9–42.2). The graph of acrylamide dietary intake distribution is presented in **Supplementary fig. 3**.

Characteristics of the study population overall and according to quartiles of acrylamide dietary intake are shown in Table 1, and according to vital status in **Supplementary table 1**. Compared to participants in the 1st quartile of acrylamide dietary intake, participants in the 4th quartile were younger (51.4 vs 54.4 years) and more frequently overweight or obese (20.5 % vs 16.3 %), current smokers (18.1 % vs 8.2 %), and premenopausal (60.3 % vs 43.2 %). They also had a higher intake of energy (1453.2 vs 1210.7 kcal/day), alcohol (14.1 vs 8.9 g ethanol/day), lipids (101.2 vs 77.8 g/day), and coffee (642.2 vs 89.1 mL/day).

The food group that contributed the most to acrylamide dietary intake was coffee (48.6 %), which was highly correlated with acrylamide intake (spearman rank correlation coefficient 0.79) (**Supplementary table 2**). Other contributing food groups were bread and salty cereal products (17.9 %) and starch food (16.5 %, most of the acrylamide coming from French fries) (**Supplementary fig. 4**).

3.2. Acrylamide dietary intake and mortality

The associations between acrylamide dietary intake and all-cause or cause-specific mortality (models 1 and 2) are presented in Table 2 and in Fig. 1. We observed a non-linear association between acrylamide dietary intake and all-cause mortality, which was inverse up to the median after which the association became positive (p-value for the overall association = 0.003). Setting the minimum value of acrylamide dietary intake as reference, we observed a statistically significant increased risk of all-cause mortality above a threshold of 4.8 STD of acrylamide dietary intake. For all other outcomes, we observed linear associations. We highlighted a positive and statistically significant association between acrylamide dietary intake and all-cancer mortality (HR [95%CI] per one STD: 1.05 [1.01; 1.10]), CVD mortality (HR [95%CI]: 1.11 [1.02; 1.21]) and lung cancer mortality (HR [95%CI]: 1.22 [1.09; 1.38]). We observed no statistically significant association with breast cancer mortality (HR [95%CI]: 0.94 [0.86; 1.03]) and colorectal cancer mortality (HR [95%

Table 1
Characteristics[a] of the study population overall and among each quartile group of acrylamide dietary intake in the E3N cohort (N = 72,585).

		Acrylamide dietary intake (μg/day)			
	All	Q1	Q2	Q3	Q4
	(N = 72,585)	(N = 18,146)	(*N* = 18,146)	(N = 18,147)	(N = 18,146)
Acrylamide dietary intake (μg/day)	32.6 (19.4)	12.2 (4.5)	24.2 (3.0)	35.3 (3.6)	58.5 (17.2)
Coffee acrylamide dietary intake (μg/day)	17.7 (15.8)	4.0 (4.3)	11.7 (6.6)	19.4 (8.1)	35.6 (18.1)
Non-coffee acrylamide dietary intake (μg/day)	14.9 (10.3)	8.2 (4.3)	12.5 (6.3)	15.9 (7.8)	22.9 (13.7)
Age (years)	52.9 (6.7)	54.4 (7.0)	53.4 (6.8)	52.6 (6.5)	51.4 (6.1)
Follow-up duration (years)	19.0 (4.1)	18.9 (4.2)	19.0 (4.1)	19.1 (4.1)	19.0 (4.2)
Follow-up duration (person-years)	1,378,046	342,238	345,541	346,134	344,133
BMI (kg/m^2)	22.7 (3.1)	22.5 (3.1)	22.7 (3.1)	22.7 (3.1)	23.0 (3.2)
<18.5	2645 (3.6)	825 (4.6)	672 (3.7)	616 (3.4)	532 (2.9)
[18.5–25)	56,794 (78.2)	14,373 (79.2)	14,301 (78.8)	14,221 (78.4)	13,899 (76.6)
≥25	13,146 (18.1)	2948 (16.3)	3173 (17.5)	3310 (18.2)	3715 (20.5)
Birth cohort					
≤1930	7295 (10.1)	2635 (14.5)	2032 (11.2)	1561 (8.6)	1067 (5.9)
(1930–1935]	9996 (13.8)	3082 (17.0)	2752 (15.2)	2415 (13.3)	1747 (9.6)
(1935–1940]	14,710 (20.3)	3864 (21.3)	3840 (21.2)	3642 (20.1)	3364 (18.5)
(1940–1945]	17,811 (24.5)	4152 (22.9)	4178 (23.0)	4658 (25.7)	4823 (26.6)
>1945	22,773 (31.4)	4413 (24.3)	5344 (29.5)	5871 (32.4)	7145 (39.4)
Education level					
<12 years	8190 (11.3)	1947 (10.7)	1975 (10.9)	2076 (11.4)	2192 (12.1)
12 to 14 years	38,408 (52.9)	9604 (52.9)	9692 (53.4)	9616 (53.0)	9496 (52.3)
>14 years	25,987 (35.8)	6595 (36.3)	6479 (35.7)	6455 (35.6)	6458 (35.6)
Smoking status					
Never or former	63,425 (87.4)	16,666 (91.8)	16,139 (88.9)	15,753 (86.8)	14,867 (81.9)
Current	9160 (12.6)	1480 (8.2)	2007 (11.1)	2394 (13.2)	3279 (18.1)
Physical activity (MET-hours/week)	46.4 (43.4)	46.6 (43.4)	46.5 (43.3)	46.3 (43.0)	46.2 (43.7)
Menopausal status and recent use of MHT					
Premenopausal	37,313 (51.4)	7849 (43.2)	8850 (48.8)	9674 (53.3)	10,940 (60.3)
Menopausal and recent MHT use	10,047 (13.8)	2692 (14.8)	2752 (15.2)	2474 (13.6)	2129 (11.7)
Menopausal and no recent MHT use	21,671 (29.9)	6569 (36.2)	5635 (31.1)	5138 (28.3)	4329 (23.9)
Menopausal and no information on whether and when MHT was used	3554 (4.9)	1036 (5.7)	909 (5.0)	861 (4.7)	748 (4.1)
Energy intake (excluding energy from alcohol and lipids, kcal/day)	1329.0 (356.6)	1210.7 (325.4)	1298.1 (333.8)	1353.8 (347.0)	1453.2 (374.0)
Alcohol consumption (g ethanol/day)	11.6 (13.9)	8.9 (12.4)	11.1 (13.3)	12.3 (13.7)	14.1 (15.7)
Lipid consumption (g/day)	88.9 (27.0)	77.8 (22.7)	85.6 (24.2)	91.1 (25.6)	101.2 (29.6)
Coffee intake (mL/day)	332.6 (282.2)	89.1 (103.2)	230.8 (137.3)	368.3 (161.7)	642.2 (310.2)

[a] Mean (std) for continuous variables, N (%) for categorical variables.

CI]: 1.12 [0.97; 1.29]).

We observed a statistically significant interaction between acrylamide dietary intake and smoking status for analyses on all-cause ($p_{interaction}$ = 0.017) and all-cancer ($p_{interaction}$ = 0.008) mortality. In analyses stratified by smoking status, the association between acrylamide dietary intake and all-cause mortality was not statistically significant in never or former smokers (*p*-value for the overall association = 0.282). On the contrary, we observed a non-linear association in current smokers which showed a statistically significant increase in risk above a threshold of 5.6 STD of acrylamide dietary intake when using the minimum value of this intake as reference (*p*-value for the overall association <0.001). We observed similar results for all-cancer

Table 2
Hazard ratios (95 % CI) estimated by Cox multivariable regression models for the association between acrylamide dietary intake and mortality risk in the E3N cohort (*N* = 72,585).

		N deaths	Model 1	Model 2
			HR [95 % CI][a]	HR [95 % CI][a]
All-cause mortality (*N* = 72,585)	Acrylamide dietary intake (spline with 3 knots)	6441		
	p-value for the overall association		<0.001	0.003
	p-value for the non-linearity test		<0.001	0.006
	Quartiles of acrylamide intake (min-max, μg/day)			
	Q1 (0.0–18.9)	1857	Ref	Ref
	Q2 (18.9–29.5)	1626	0.97 [0.90; 1.03]	0.97 [0.91; 1.04]
	Q3 (29.5–42.2)	1560	1.01 [0.95; 1.08]	1.01 [0.95; 1.09]
	Q4 (42.2–276.2)	1398	1.07 [1.00; 1.15]	1.04 [0.96; 1.12]
	p-linear trend		0.028	0.204
All-cancer mortality (N = 72,416)	Acrylamide dietary intake (linear term)	3473	1.07 [1.03; 1.12]	1.05 [1.01; 1.10]
	p-value		<0.001	0.024
	p-value for the non-linearity test		0.067	0.125
	Quartiles of acrylamide intake (min-max, μg/day)			
	Q1 (0.0–18.9)	916	Ref	Ref
	Q2 (18.9–29.5)	875	1.02 [0.93; 1.11]	1.01 [0.92; 1.11]
	Q3 (29.5–42.2)	871	1.07 [0.98; 1.18]	1.06 [0.96; 1.16]
	Q4 (42.2–276.2)	811	1.12 [1.01; 1.23]	1.07 [0.96; 1.18]
	p-linear trend		0.013	0.166
CVD mortality (N = 72,416)	Acrylamide dietary intake (linear term)	896	1.11 [1.02; 1.20]	1.11 [1.02; 1.21]
	p-value		0.013	0.018
	p-value for the non-linearity test		0.293	0.261
	Quartiles of acrylamide intake (min-max, μg/day)			
	Q1 (0.0–18.9)	259	Ref	Ref
	Q2 (18.9–29.5)	226	1.01 [0.85; 1.21]	1.03 [0.86; 1.23]
	Q3 (29.5–42.2)	217	1.12 [0.93; 1.34]	1.15 [0.96; 1.39]
	Q4 (42.2–276.2)	194	1.30 [1.08; 1.57]	1.33 [1.09; 1.63]
	p-linear trend		0.004	0.003
Breast cancer mortality (N = 72,238)	Acrylamide dietary intake (linear term)	953	0.95 [0.88; 1.03]	0.94 [0.86; 1.03]
	p-value		0.211	0.185
	p-value for the non-linearity test		0.100	0.141
	Quartiles of acrylamide intake (min-max, μg/day)			
	Q1 (0.0–18.9)	271	Ref	Ref
	Q2 (18.9–29.5)	246	0.94 [0.79; 1.12]	0.95 [0.80; 1.13]
	Q3 (29.5–42.2)	222	0.88 [0.73; 1.05]	0.88 [0.73; 1.06]
	Q4 (42.2–276.2)	214	0.90 [0.75; 1.08]	0.90 [0.74; 1.09]
	p-linear trend		0.226	0.227
Lung cancer mortality (N = 72,238)	Acrylamide dietary intake (linear term)	364	1.28 [1.15; 1.42]	1.22 [1.09; 1.38]
	p-value		<0.001	0.001
	p-value for the non-linearity test		0.172	0.124
	Quartiles of acrylamide intake (min-max, μg/day)			
	Q1 (0.0–18.9)	84	Ref	Ref
	Q2 (18.9–29.5)	88	1.11 [0.82; 1.50]	1.07 [0.79; 1.44]
	Q3 (29.5–42.2)	90	1.21 [0.90; 1.62]	1.13 [0.84; 1.54]
	Q4 (42.2–276.2)	102	1.52 [1.14; 2.04]	1.34 [0.98; 1.83]
	p-linear trend		0.003	0.053
Colorectal cancer mortality (N = 72,238)	Acrylamide dietary intake (linear term)	317	1.15 [1.01; 1.30]	1.12 [0.97; 1.29]
	p-value		0.035	0.113
	p-value for the non-linearity test		0.188	0.205
	Quartiles of acrylamide intake (min-max, μg/day)			
	Q1 (0.0–18.9)	73	Ref	Ref
	Q2 (18.9–29.5)	80	1.19 [0.86; 1.63]	1.16 [0.85; 1.60]
	Q3 (29.5–42.2)	84	1.34 [0.98; 1.84]	1.31 [0.95; 1.80]
	Q4 (42.2–276.2)	80	1.46 [1.06; 2.01]	1.39 [0.99; 1.96]
	p-linear trend		0.016	0.049

Model 1: Adjustment on age (years) as time-scale.
Model 2: M1 + BMI (kg/m^2), physical activity (MET-hours/week), birth cohort (≤1930, (1930–1935], (1935–1940], (1940–1945], >1945), education level (<12 years, 12 to 14 years, >14 years), smoking status (never or former smoker, current smoker), menopausal status and recent MHT use (premenopausal, menopausal and recent MHT use, menopausal and no recent MHT use, menopausal and no information on whether and when MHT was used), energy intake (excluding energy from alcohol and lipids, kcal/day), lipids consumption (g/day), and alcohol consumption (g ethanol/day).

[a] We present the results of linear associations between acrylamide dietary intake and mortality risk with HR [95 % CI] per one standard deviation, *p*-value and *p*-value for the non-linearity test, and of non-linear associations with *p*-values for the overall association and for the non-linearity test.

mortality: HR [95%CI]: 1.01 [0.96; 1.06] in never or former smokers, and HR [95%CI]: 1.24 [1.13; 1.37] in current smokers (Table 3 and Fig. 2). Therefore, the association between acrylamide dietary intake and all-cause or all-cancer mortality risk was modified by smoking status in such a way that only current smokers experienced an increased risk.

Finally, in analyses based on non-coffee acrylamide dietary intake (model 3), the associations with mortality were not statistically significant, except for a non-linear association borderline with statistical significance with all-cause mortality (p-value for the overall association = 0.066) and a suggestive linear and positive association with colorectal cancer mortality (HR [95%CI]: 1.14 [1.00; 1.30]) (Table 4 and Fig. 3). We highlighted a statistically significant interaction between non-coffee acrylamide dietary intake and smoking status only for analyses on all-cause mortality ($p_{interaction}$ = 0.031). When stratifying the analyses on smoking status, the association between non-coffee acrylamide dietary intake and all-cause mortality was not statistically significant in never or former smokers (p-value for the overall association = 0.555), while we observed a non-linear association in current smokers, which showed a statistically significant increased risk above a threshold of 6.4 STD of non-coffee acrylamide dietary intake when using the minimum value of this intake as reference (p-value for the overall association 0.005) (Table 5 and Fig. 4). Consequently, the association between non-coffee acrylamide dietary intake and the risk of all-cause mortality was modified by smoking status such that only current smokers presented an increased risk.

3.3. Sensitivity analyses

When representing all the associations between acrylamide dietary intake and mortality using restricted cubic splines, irrespective of the statistical significance of the non-linearity test, we observed similar associations compared to the main analyses. When comparing spline graphs of model 2 (total acrylamide dietary intake as main exposure variable) with the spline graphs of model 3 (non-coffee acrylamide dietary intake as main exposure variable), we observed different shapes of associations, especially for CVD, breast cancer, lung cancer and colorectal cancer mortality risk. Indeed, we observed higher HRs in model 3 compared to those in model 2 for CVD, breast and colorectal cancer mortality, while on the contrary, HRs are lower in model 3 for lung cancer mortality compared to those observed in model 2 (**Supplementary table 3** and **Supplementary fig. 5**).

When additionally adjusting model 2 on diagnosis of diabetes, cancer, high cholesterol or hypertension before inclusion, results didn't change compared to those of model 2 (**Supplementary table 4** and **Supplementary fig. 6**).

Finally, we obtained similar results for the relation between acrylamide dietary intake and all-cause mortality after adjusting model 2 for energy with the residual method (**Supplementary table 5** and **Supplementary fig. 7**) and after excluding participants who died or were censored during the first five years of follow-up (**Supplementary table 6** and **Supplementary fig. 8**).

4. Discussion

In this study, we observed a non-linear association between acrylamide dietary intake and all-cause mortality. We also highlighted positive linear associations between acrylamide dietary intake and CVD, all-cancer and lung cancer mortality, and no associations with breast and colorectal cancer mortality. Moreover, we found no association between non-coffee acrylamide dietary intake and mortality, except for a non-linear association with all-cause mortality and a linear and positive association with colorectal cancer mortality, both borderline with statistical significance. We highlighted an interaction between acrylamide dietary intake and smoking status for all-cause and all-cancer mortality analyses, and an interaction between non-coffee acrylamide dietary intake and smoking status for all-cause mortality analysis only, whereby the association between high acrylamide intake and mortality was restricted to current smokers.

In our study population, the estimated mean acrylamide dietary intake (32.6 μg/day) was in line with a review of 101 studies including 68 studies from 26 countries (Timmermann et al., 2021). In another French study based on a different population of women, the estimated mean dietary intake of acrylamide (30.1 μg/day) was very similar to our estimates (Bellicha et al., 2022). Finally, our estimate of mean acrylamide dietary exposure (0.55 μg/kg bw/day) was consistent with Anses' estimation, of 0.43 μg/kg bw/day in French adults (ANSES, 2011), and of the same magnitude as EFSA's estimation for the European population (0.5 μg/kg bw/day) (EFSA, 2015).

Acrylamide is easily absorbed and distributed in the human organism and highly metabolised, mostly in the liver. Acrylamide is then transformed by the cytochrome P4502E1 (CYP2E1) in glycidamide, an epoxide known for its mutagenic properties. Glycidamide can bind to DNA to form DNA adducts, leading to genotoxicity and carcinogenicity (EFSA, 2015). At least 6 % of an ingested acrylamide dose is converted to the glycidamide epoxide, which is known to be more genotoxic than acrylamide (Koszucka et al., 2020). Acrylamide can also be harmful through other pathways, such as disruption of the endocrine system or the production of reactive oxygen species (ROS) leading to oxidative stress (Adani et al., 2020; Benisi-Kohansal et al., 2021; EFSA, 2015; Koszucka et al., 2020; Liu et al., 2017). Finally, acrylamide and glycidamide can form covalent adducts with haemoglobin. In addition, they can be detoxified by conjugation with glutathione (GSH), leading to mercapturic acids, which are then excreted in the urine.

Our results highlighted a non-linear association between acrylamide dietary intake and all-cause mortality, with an increased mortality risk at the highest acrylamide dietary intakes. No previous study investigated the association between acrylamide dietary intake and all-cause mortality. Only one article studied the associations between several haemoglobin biomarkers of acrylamide and all-cause mortality (Huang et al., 2018). The authors reported that, in non-smokers, an acrylamide haemoglobin biomarker (HbAA) was associated with an increased risk of all-cause mortality, while a glycidamide biomarker (HbGA) was inversely associated with all-cause mortality. In contrast, no association was found in smokers. However, this study was based on an internal exposure assessment that encompasses all sources of acrylamide exposure, and therefore the results were not directly comparable with our findings. Finally, we can hypothesise that the non-linear association found between acrylamide dietary intake and all-cause mortality was due to potential compensation mechanisms of the organism until a threshold above which harmful effects of acrylamide can be observed.

Our findings also suggested a positive association between acrylamide dietary intake and CVD mortality. We found no study on acrylamide dietary intake and CVD mortality and only few studies investigated the association between acrylamide exposure estimated through blood or urinary biomarkers and CVD or CVD mortality (Huang et al., 2018; Wang et al., 2022; Zhang et al., 2018). Nevertheless, the results of these studies are inconsistent. Moreover, they are not directly comparable with ours. However, the authors of the most recent study concluded that acrylamide exposure (estimated from urinary biomarkers) may affect CVD partly from oxidative stress, inflammation, and TGF-β1 mechanisms, which may explain our results (Wang et al., 2022).

Concerning cancer mortality, we observed a positive association with all-cancer mortality, which was consistent with another study on Chinese elderly (Liu et al., 2017). This study reported a positive association between dietary intake of acrylamide and respiratory cancer mortality, which is in line with the positive association we observed with lung cancer mortality. However, Liu et al. also found a positive association with digestive cancer mortality while we did not see a significant association with colorectal cancer mortality in our study. This difference may be explained by the fact that the studied outcome is not exactly the same. Finally, we did not identify other studies investigating acrylamide intake and specific-cancers mortality, such as breast cancer mortality.

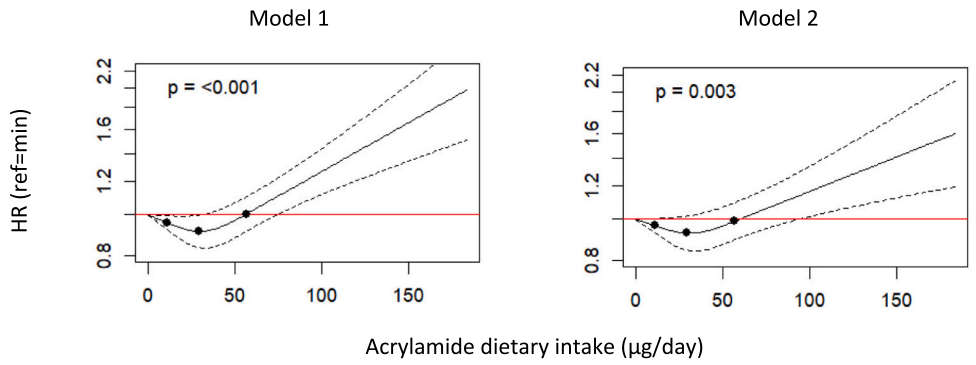

Fig. 1. Restricted cubic splines of acrylamide dietary intake in models 1 and 2 in association with all-cause mortality risk in the E3N cohort (N = 72,585). The minimum value of the main exposure variable is taken as reference. The "p" represents the p-value for the overall association. Solid lines indicate HR, and dashed lines indicate 95 % CI. The points represent percentiles as follows: 3 nodes at the 10th, 50th and 90th percentiles, 4 nodes at the 5th, 35th, 65th and 95th percentiles, or 5 nodes at the 5th, 27.5th, 50th, 72.5th and 95th percentiles.

Table 3
Hazard ratios (95 % CI) estimated by Cox multivariable regression models for the association between acrylamide dietary intake and mortality risk, stratified on smoking status, in the E3N cohort (N = 72,585).

		N deaths	Never or former smoker		N deaths	Current smoker
			HR [95 % CI][a]			HR [95 % CI][a]
All-cause mortality (N = 72,585)	Acrylamide dietary intake (spline with 3 knots)	5493		Acrylamide dietary intake (spline with 5 knots)	948	
	p-value for the overall association		0.282	*p*-value for the overall association		<0.001
	p-value for the non-linearity test		0.112	*p*-value for the non-linearity test		0.024
	Quartiles of acrylamide intake (min-max, µg/day)			Quartiles of acrylamide intake (min-max, µg/day)		
	Q1 (0.0–18.9)	1682	Ref	Q1 (0.0–18.9)	175	Ref
	Q2 (18.9–29.5)	1414	0.96 [0.90; 1.04]	Q2 (18.9–29.5)	212	0.99 [0.81; 1.21]
	Q3 (29.5–42.2)	1319	1.00 [0.93; 1.08]	Q3 (29.5–42.2)	241	1.10 [0.90; 1.34]
	Q4 (42.2–276.2)	1078	1.00 [0.92; 1.09]	Q4 (42.2–204.4)	320	1.25 [1.03; 1.52]
	p-linear trend		0.829	*p*-linear trend		0.007
All-cancer mortality (N = 72,416)	Acrylamide dietary intake (linear term)	2936	1.01 [0.96; 1.06]	Acrylamide dietary intake (linear term)	537	1.24 [1.13; 1.37]
	p-value		0.618	*p*-value		<0.001
	p-value for the non-linearity test		0.362	*p*-value for the non-linearity test		0.235
	Quartiles of acrylamide intake (min-max, µg/day)			Quartiles of acrylamide intake (min-max, µg/day)		
	Q1 (0.0–18.9)	829	Ref	Q1 (0.0–18.9)	87	Ref
	Q2 (18.9–29.5)	758	1.00 [0.90; 1.10]	Q2 (18.9–29.5)	117	1.06 [0.80; 1.40]
	Q3 (29.5–42.2)	733	1.03 [0.93; 1.15]	Q3 (29.5–42.2)	138	1.16 [0.88; 1.53]
	Q4 (42.2–276.2)	616	1.01 [0.90; 1.13]	Q4 (42.2–204.4)	195	1.36 [1.04; 1.78]
	p-linear trend		0.712	*p*-linear trend		0.009

Model: Adjustment on age (years) as time-scale, BMI (kg/m^2), physical activity (MET-hours/week), birth cohort (≤1930, (1930–1935], (1935–1940], (1940–1945], >1945), education level (<12 years, 12 to 14 years, >14 years), menopausal status and recent MHT use (premenopausal, menopausal and recent MHT use, menopausal and no recent MHT use, menopausal and no information on whether and when MHT was used), energy intake (excluding energy from alcohol and lipids, kcal/day), lipids consumption (g/day), and alcohol consumption (g ethanol/day).

[a] We present the results of linear associations between acrylamide dietary intake and mortality risk with HR [95 % CI] per one standard deviation, p-value and p-value for the non-linearity test, and of non-linear associations with p-values for the overall association and for the non-linearity test.

In general, the lack of association with breast and colorectal cancer mortality we observed may be explained in different ways. For breast cancer, even if acrylamide is known to be carcinogenic in the mammary gland in rodents, the given doses of acrylamide in animal studies are frequently much higher than the estimated doses in the general human population, and thus acrylamide dietary intake may not have appreciable effects on breast cancer risk at the levels of exposure estimated in the E3N cohort. In addition, non-significant results may be due to the fact that coffee (the main contributor to acrylamide dietary intake) contains components with opposite effects on breast and colorectal cancer (such as caffeine, phenolic acids, melanoidins, and the diterpenes kahweol and cafestol), which could attenuate the potential positive association with acrylamide (Nehlig and Cunha, 2020). More generally, the beneficial health effects of coffee but also other components of the diet (such as red berries, wasabi, garlic or other food items containing antioxidants) may mask or attenuate the true association between acrylamide dietary intake and health (Koszucka et al., 2020; Nehlig and Cunha, 2020).

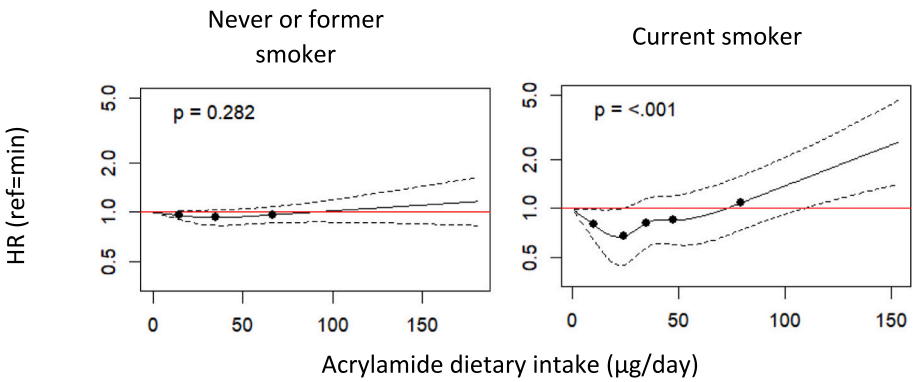

Fig. 2. Restricted cubic splines of acrylamide dietary intake in association with all-cause mortality risk, stratified on smoking status, in the E3N cohort (N = 72,585). The minimum value of the main exposure variable is taken as reference. The "p" represents the *p*-value for the overall association. Solid lines indicate HR, and dashed lines indicate 95 % CI. The points represent percentiles as follows: 3 nodes at the 10th, 50th and 90th percentiles, 4 nodes at the 5th, 35th, 65th and 95th percentiles, or 5 nodes at the 5th, 27.5th, 50th, 72.5th and 95th percentiles.

Although coffee is an important confounder, we did not adjust our main analyses (model 2) on coffee intake, because it was highly correlated with dietary intake of acrylamide (spearman rank correlation coefficient 0.79). We addressed this issue by considering non-coffee acrylamide dietary intake as the exposure, while adjusting on coffee intake (model 3). Our aim was to test the effect of acrylamide independently of the effects of the other substances in coffee or in general with other factors related to coffee consumption. For CVD mortality analyses, we observed a different shape of the spline graph with higher HR in model 3 than in model 2. This change may be due to the fact that we observed a more prominent residual protective effect of coffee in model 2 than in model 3. Indeed, coffee is known to be inversely associated with CVD and CVD mortality (Hou et al., 2022; Kim et al., 2019; Poole et al., 2017). For all studied outcomes, the association with non-coffee acrylamide was not statistically significant, except for a non-linear association for all-cause mortality and a positive association for colorectal cancer mortality, both of borderline statistical significance. Based on these results, we can assume that non-coffee acrylamide intake has no effects on these outcomes per se, and/or that non-coffee acrylamide intake was too low and homogenous among participants to allow to observe any effect.

In addition, when stratifying the analyses on smoking status, associations were statistically significant only in current smokers. This differential effect among smokers and non-smokers has been inconsistently observed in previous studies. Indeed, a recent systematic review and meta-analysis on cancer risk highlighted an interaction between acrylamide intake and smoking status only for lung cancer with an increased risk limited to smokers, while no interaction between acrylamide and smoking status was observed for other cancer sites (Filippini et al., 2022). Nevertheless, the authors underline that these results have to be interpreted with caution due to the limited number of studies and the high heterogeneity. On the contrary, other studies highlighted a significant and positive association among non-smokers that was not significant among smokers (Bongers et al., 2012; Hirvonen et al., 2010). As suggested by Filippini et al., this could be explained by the fact that tobacco smoke may saturate the enzyme involved in the epoxidation of acrylamide to glycidamide, thus the conversion of acrylamide to the genotoxic glycidamide could be more effective in non-smokers than in smokers (Filippini et al., 2022; Schettgen et al., 2004). This hypothesis is not totally in contradiction with our results since it may still partly explain the association that we observed in smokers. For low acrylamide dietary intake, tobacco smoking could prevent the biotransformation of acrylamide to glycidamide, but only up to a certain threshold beyond which the epoxidation of acrylamide restarts. Another hypothesis to explain the difference between smokers and non-smokers when studying the association between acrylamide dietary intake and all-cause or all-cancer mortality could be that as smokers are exposed to acrylamide from both diet and cigarette smoking, they can reach more easily a threshold above which acrylamide is harmful to human health. We can thus assume that in our study population, the overall exposure to acrylamide in smokers is probably higher than in non-smokers, leading to appreciable association with all-cause or all-cancer mortality. In addition, we observed a significant interaction between acrylamide dietary intake and smoking status for all-cancer mortality analyses, while it was not significant when studying breast, lung or colorectal cancer mortality individually, probably due to limited statistical power. Moreover, since our analyses were not adjusted for coffee and because coffee and tobacco are positively correlated, the observed interactions between acrylamide dietary intake and smoking status may reflect an interaction between acrylamide dietary intake and coffee. We tested the interaction between non-coffee acrylamide dietary intake and smoking status, and observed a statistically significant interaction only for all-cause mortality. In light of these results, the interaction between acrylamide and tobacco remains to be clarified.

When interpreting our results, some limitations need to be taken into account. The E3N cohort includes volunteer women who are not representative of middle-aged French women, as they are leaner and they have a higher school education level (Clavel-Chapelon and E3N Study Group, 2015). However, this does not prevent from comparing women with high and low levels of acrylamide dietary intake. In addition, the dietary consumptions of the E3N participants and the estimation of their acrylamide dietary intake may have been impacted by the time gap between the dietary questionnaire (1993) and collection of food contamination data (2007–2009). However, we can assume that the dietary consumptions of middle-aged women are stable over time, as suggested by Thorpe et al. (Thorpe et al., 2019). Moreover, since its discovery in food in 2002, acrylamide levels in foods have decreased especially in potato-fried products and coffee (ANSES, 2011). Therefore, in 1993, our study population was probably exposed at higher concentrations of acrylamide than what we have estimated, and this potential exposure classification bias could have led to an attenuation of the real association. Furthermore, acrylamide contamination levels are strongly related to cooking processes (EFSA, 2015), but we do not have specific information on the individual cooking habits in the E3N cohort. Indeed, for French fries intake, the level of acrylamide depends on the reducing sugars level in the potato, the oil, the temperature and the duration of the frying (Sirot et al., 2012). However, the TDS2 provided acrylamide levels measured in food prepared as consumed, according to French cooking habits (Sirot et al., 2012). We can assume that for a given consumer, the acrylamide level in French fries will vary from one time to

another, and thus in a year, the TDS2 acrylamide level will be a good estimation of the mean level of acrylamide in all the French fries consumed. Moreover, the E3N FFQ contained questions on the consumption of French fries independently of the consumption of potatoes from other cooking methods. Finally, it would have been interesting to better assess acrylamide exposure coming from tobacco smoking (for example including quantitative information on the smoking habits such as type, amount and frequency of tobacco consumed), or to measure levels of acrylamide's internal biomarkers of exposure, but this type of information was not available in our study. In general, residual confounding cannot be completely ruled out.

Our study also benefits from several strengths, including its prospective design. With more than 70,000 women followed over 19 years, it was possible to study the long-term health effects of a low level and chronic acrylamide dietary intake. The large study population ensures a good statistical power. The robustness of the results is comforted by the

Table 4
Hazard ratios (95 % CI) estimated by Cox multivariable regression models for the association between non-coffee acrylamide dietary intake and mortality risk in the E3N cohort (N = 72,585).

		N deaths	Model 3
			HR [95 % CI][a]
All-cause mortality (N = 72,585)	Non-coffee acrylamide dietary intake (spline with 4 knots)	6441	
	p-value for the overall association		0.066
	p-value for the non-linearity test		0.040
	Quartiles of non-coffee acrylamide intake (min-max, μg/day)		
	Q1 (0.0–7.8)	1943	Ref
	Q2 (7.8–12.8)	1715	1.03 [0.96; 1.10]
	Q3 (12.8–19.6)	1468	0.97 [0.90; 1.04]
	Q4 (19.6–209.3)	1315	0.98 [0.90; 1.06]
	p-linear trend		0.395
All-cancer mortality (N = 72,416)	Non-coffee acrylamide dietary intake (linear term)	3473	1.01 [0.97; 1.06]
	p-value		0.531
	p-value for the non-linearity test		0.344
	Quartiles of non-coffee acrylamide intake (min-max, μg/day)		
	Q1 (0.0–7.8)	982	Ref
	Q2 (7.8–12.8)	902	1.01 [0.92; 1.11]
	Q3 (12.8–19.6)	831	0.99 [0.90; 1.10]
	Q4 (19.6–209.3)	758	0.98 [0.87; 1.09]
	p-linear trend		0.586
CVD mortality (N = 72,416)	Non-coffee acrylamide dietary intake (linear term)	896	1.07 [0.98; 1.17]
	p-value		0.129
	p-value for the non-linearity test		0.238
	Quartiles of non-coffee acrylamide intake (min-max, μg/day)		
	Q1 (0.0–7.8)	289	Ref
	Q2 (7.8–12.8)	255	1.11 [0.93; 1.32]
	Q3 (12.8–19.6)	175	0.89 [0.73; 1.09]
	Q4 (19.6–209.3)	177	1.15 [0.92; 1.42]
	p-linear trend		0.506
Breast cancer mortality (N = 72,238)	Non-coffee acrylamide dietary intake (linear term)	953	1.02 [0.94; 1.12]
	p-value		0.588
	p-value for the non-linearity test		0.440
	Quartiles of non-coffee acrylamide intake (min-max, μg/day)		
	Q1 (0.0–7.8)	240	Ref
	Q2 (7.8–12.8)	268	1.20 [1.00; 1.43]
	Q3 (12.8–19.6)	229	1.07 [0.88; 1.30]
	Q4 (19.6–209.3)	216	1.07 [0.87; 1.33]
	p-linear trend		0.897
Lung cancer mortality (N = 72,238)	Non-coffee acrylamide dietary intake (linear term)	364	1.03 [0.89; 1.18]
	p-value		0.714
	p-value for the non-linearity test		0.284
	Quartiles of non-coffee acrylamide intake (min-max, μg/day)		
	Q1 (0.0–7.8)	106	Ref
	Q2 (7.8–12.8)	91	0.97 [0.73; 1.30]
	Q3 (12.8–19.6)	92	1.04 [0.77; 1.40]
	Q4 (19.6–209.3)	75	0.93 [0.66; 1.31]
	p-linear trend		0.744
Colorectal cancer mortality (N = 72,238)	Non-coffee acrylamide dietary intake (linear term)	317	1.14 [1.00; 1.30]
	p-value		0.050
	p-value for the non-linearity test		0.486
	Quartiles of non-coffee acrylamide intake (min-max, μg/day)		
	Q1 (0.0–7.8)	90	Ref
	Q2 (7.8–12.8)	70	0.85 [0.62; 1.17]
	Q3 (12.8–19.6)	75	0.98 [0.71; 1.36]
	Q4 (19.6–209.3)	82	1.18 [0.83; 1.68]
	p-linear trend		0.208

Model 3: Adjustment on age (years) as time-scale, BMI (kg/m^2), physical activity (MET-hours/week), birth cohort (≤1930, (1930–1935], (1935–1940], (1940–1945], >1945), education level (<12 years, 12 to 14 years, >14 years), smoking status (never or former smoker, current smoker), menopausal status and recent MHT use (premenopausal, menopausal and recent MHT use, menopausal and no recent MHT use, menopausal and no information on whether and when MHT was used), energy intake (excluding energy from alcohol and lipids, kcal/day), lipids consumption (g/day), and alcohol consumption (g ethanol/day), and coffee consumption (mL/day).

[a] We present the results of linear associations between non-coffee acrylamide dietary intake and mortality risk with HR [95 % CI] per one standard deviation, *p*-value and *p*-value for the non-linearity test, and of non-linear associations with *p*-values for the overall association and for the non-linearity test.

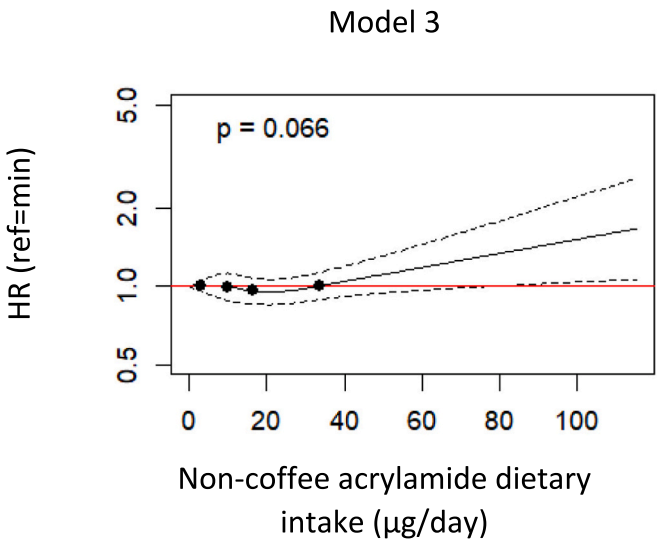

Fig. 3. Restricted cubic splines of non-coffee acrylamide dietary intake in association with all-cause mortality risk in model 3 in the E3N cohort (N = 72,585). The minimum value of the main exposure variable is taken as reference. The "p" represents the *p*-value for the overall association. Solid lines indicate HR, and dashed lines indicate 95 % CI. The points represent percentiles as follows: 3 nodes at the 10th, 50th and 90th percentiles, 4 nodes at the 5th, 35th, 65th and 95th percentiles, or 5 nodes at the 5th, 27.5th, 50th, 72.5th and 95th percentiles.

numerous sensitivity analyses. The mortality database with causes of death was validated by the Inserm-CépiDc. As the dietary questionnaire was previously validated (van Liere et al., 1997), and as food samples were prepared "as consumed" to estimate their contamination level (Sirot et al., 2009), the measurement error of the exposure is supposed to be limited. Finally, in the E3N study much information on lifestyle and characteristics of participants was collected allowing us to take into account several potential confounders.

5. Conclusion

We observed a non-linear association between acrylamide dietary intake and all-cause mortality. This association was only significant in current smokers when stratifying on smoking status. We also found that a higher acrylamide dietary intake was associated with an increased risk of CVD, all-cancer and lung cancer mortality. For all-cancer mortality risk, this association was only significant among current smokers. Moreover, we observed a non-linear association borderline with statistical significance between non-coffee acrylamide dietary intake and all-cause mortality, that was limited to current smokers when stratifying on smoking status. Our results also suggested that a higher non-coffee acrylamide dietary intake increased the risk of colorectal cancer mortality. It is therefore essential to keep reducing acrylamide contamination and to prevent dietary intake of acrylamide, especially among smokers.

CRediT authorship contribution statement

All authors contributed to the study conceptualization and methodology. CM performed statistical analyses. CM wrote the first draft of the manuscript and all authors reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Table 5
Hazard ratios (95 % CI) estimated by Cox multivariable regression models for the association between non-coffee acrylamide dietary intake and all-cause mortality risk, stratified on smoking status, in the E3N cohort (N = 72,585).

	N deaths	Never or former smoker		N deaths	Current smoker
		HR [95 % CI][a]			HR [95 % CI][a]
Non-coffee acrylamide dietary intake (spline with 3 knots)	5493		Non-coffee acrylamide dietary intake (spline with 3 knots)	948	
p-value for the overall association		0.555	*p*-value for the overall association		0.005
p-value for the non-linearity test		0.282	*p*-value for the non-linearity test		0.006
Quartiles of non-coffee acrylamide intake (min-max, µg/day)			Quartiles of non-coffee acrylamide intake (min-max, µg/day)		
Q1 (0.0–7.8)	1640	Ref	Q1 (0.0–7.8)	303	Ref
Q2 (7.8–12.8)	1485	1.03 [0.93; 1.11]	Q2 (7.8–12.8)	230	0.99 [0.83; 1.18]
Q3 (12.8–19.6)	1275	0.99 [0.92; 1.07]	Q3 (12.8–19.6)	193	0.84 [0.69; 1.02]
Q4 (19.6–209.3)	1093	0.97 [0.89; 1.06]	Q4 (19.6–124.0)	222	1.02 [0.84; 1.25]
p-linear trend		0.370	*p*-linear trend		0.994

Model: Adjustment on age (years) as time-scale, BMI (kg/m^2), physical activity (MET-hours/week), birth cohort (≤1930, (1930–1935], (1935–1940], (1940–1945], >1945), education level (<12 years, 12 to 14 years, >14 years), menopausal status and recent MHT use (premenopausal, menopausal and recent MHT use, menopausal and no recent MHT use, menopausal and no information on whether and when MHT was used), energy intake (excluding energy from alcohol and lipids, kcal/day), lipids consumption (g/day), alcohol consumption (g ethanol/day), and coffee consumption (mL/day).

[a] We present the results of linear associations between non-coffee acrylamide dietary intake and mortality risk with HR [95 % CI] per one standard deviation, *p*-value and *p*-value for the non-linearity test, and of non-linear associations with *p*-values for the overall association and for the non-linearity test.

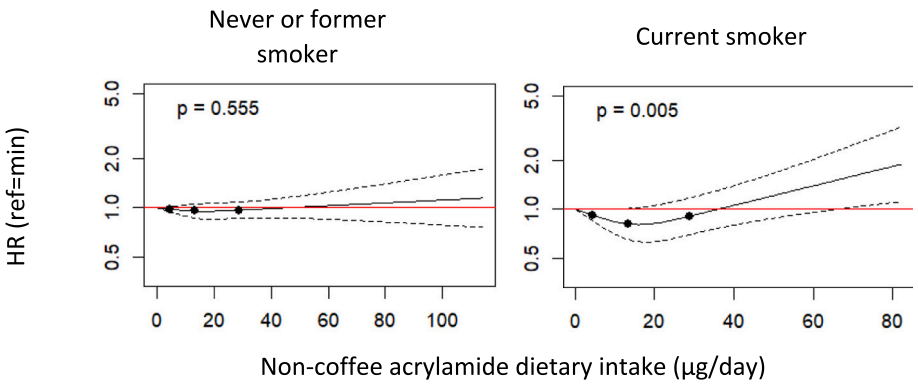

Fig. 4. Restricted cubic splines of non-coffee acrylamide dietary intake in association with all-cause mortality risk, stratified on smoking status, in the E3N cohort (*N* = 72,585). The minimum value of the main exposure variable is taken as reference. The "p" represents the *p*-value for the overall association. Solid lines indicate HR, and dashed lines indicate 95 % CI. The points represent percentiles as follows: 3 nodes at the 10th, 50th and 90th percentiles, 4 nodes at the 5th, 35th, 65th and 95th percentiles, or 5 nodes at the 5th, 27.5th, 50th, 72.5th and 95th percentiles.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.167514.

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