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Background: Three prospective studies have evaluated the association between dietary acrylamide intake and endometrial cancer (EC) risk with inconsistent results. The objective of this study was to evaluate the association between acrylamide intake and EC risk: for overall EC, for type-I EC, and in never smokers and never users of oral contraceptives (OCs). Smoking is a source of acrylamide, and OC use is a protective factor for EC risk.

Methods: Cox regression was used to estimate hazard ratios (HRs) for the association between acrylamide intake and EC risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. Acrylamide intake was estimated from the EU acrylamide monitoring database, which was matched with EPIC questionnaire-based food consumption data. Acrylamide intake was energy adjusted using the residual method.

Results: No associations were observed between acrylamide intake and overall EC ($n = 1382$) or type-I EC risk ($n = 627$). We observed increasing relative risks for type-I EC with increasing acrylamide intake among women who both never smoked and were non-users of OCs (HR_{Q5vsQ1}: 1.97, 95% CI: 1.08–3.62; likelihood ratio test (LRT) P-value: 0.01, $n = 203$).

Conclusions: Dietary intake of acrylamide was not associated with overall or type-I EC risk; however, positive associations with type I were observed in women who were both non-users of OCs and never smokers.

Acrylamide is a known neurotoxin in humans, and a carcinogen in animals (Friedman, 2003; LoPachin and Gavin, 2008; Hogervorst *et al*, 2010). In 1994, based on animals studies, as well as evidence found in humans, the International Agency for Research on Cancer (IARC) classified acrylamide as 'probably carcinogenic' to humans (IARC group 2A; IARC, 1994). In 2002, Swedish researchers discovered acrylamide in some heat-treated carbohydrate-rich foods (Tareke *et al*, 2002), and further research concluded that acrylamide is formed during common cooking procedures (predominantly through the Maillard reaction), such as frying, grilling, and baking (Friedman, 2003). In the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, the main determinants of estimated dietary intake of acrylamide based on 24-h dietary recall (DR) were bread, crisp bread, rusks, coffee, fried potatoes, cakes, biscuits, and cookies (Freisling *et al*, 2013). Acrylamide is also a component of cigarette smoke, thus, smoking is an important source of exposure (Boettcher *et al*, 2005; Vesper *et al*, 2008).

Acrylamide is metabolised via the Cyp2e1 enzyme system to glycidamide, a chemically reactive epoxide and mutagen in animals (Doroshyenko *et al*, 2009; Hogervorst *et al*, 2010). After acrylamide administration, hormone-related (including uterine tumours) and other tumours (e.g., oral tissues) have been observed in rats (Johnson *et al*, 1986).

Endometrial cancer (EC) is the fourth most common cancer diagnosed in European women, but mortality is relatively low with a 5-year survival rate varying from 65 to 85% (Cook *et al*, 2006; Ferlay *et al*, 2013). There is considerable international variation in incidence as well as mortality, and both rates increase dramatically with age (Cook *et al*, 2006; Ferlay *et al*, 2013; Jamison *et al*, 2013). Established risks factors for EC are obesity, low physical activity, history of polycystic ovary syndrome, and greater lifetime exposure to estrogens (Kaaks *et al*, 2002; Cook *et al*, 2006). The use of oral contraceptives (OCs, containing both oestrogen and progestin in the formula) is well established to lower the risk of developing EC (Cook *et al*, 2006; Gierisch *et al*, 2013). There is evidence that tobacco smoking also reduces the risk of EC (Terry *et al*, 2004; Cook *et al*, 2006); however, an EPIC study reported an increased risk of EC in premenopausal women who smoked (Al-Zoughool *et al*, 2007). Endometrial cancer is generally classified into two types: type-I EC are mostly endometrioid adenocarcinomas and are associated with unopposed oestrogen exposure; and type-II EC tumours are mainly serous carcinomas, are believed to be oestrogen independent, and have poor prognosis (Amant *et al*, 2005; Setiawan *et al*, 2013).

Three prospective epidemiological studies have assessed the relationship between dietary intake of acrylamide and EC risk. The Netherlands Cohort Study (NLCS) observed a positive association

between acrylamide intake and EC risk, especially in never smokers (Hogervorst *et al*, 2007). Likewise, the Nurses' Health Study (NHS) reported an increased relative risk among women with the highest acrylamide intake (Wilson *et al*, 2010); however, no associations between acrylamide intake and EC were observed in the Swedish Mammography Cohort (SMC; Larsson *et al*, 2009).

The present study evaluated the association between questionnaire-based dietary intake of acrylamide and the risk of overall EC (type I, type II, and undefined) and type-I EC tumours, using data from 301 113 EPIC cohort participants. Subgroup analyses among never-smoking women and never users of OCs were performed with the aim to eliminate the influence of smoking (both a source of acrylamide and a protective factor) and the protective effect of OCs on EC risk.

METHODS

Study population. The EPIC study was initiated between 1992 and 1998 in 23 centres from 10 European countries with the aim to investigate the relationships between nutrition and lifestyle factors, and cancer and other chronic diseases. All participants gave written informed consent. Ethical review boards from the IARC and local centres participating in EPIC approved the study. The EPIC methodology has been reported in detail by Riboli *et al* (2002).

The EPIC study includes 521 330 participants, of which 367 903 are women. A total of 66 790 women were excluded from the current analyses because they were diagnosed with cancer before recruitment ($n = 19\,853$), had a hysterectomy ($n = 35\,116$), had incomplete follow-up data ($n = 2896$), had no lifestyle or dietary information ($n = 2877$), and no information on dietary intake of acrylamide at baseline ($n = 3$), or had an extreme ratio of energy intake to energy required ($n = 6045$); resulting in 301 113 participants for this analysis.

Identification of endometrial cancer cases. Information on cancer incidence was obtained through population cancer registries, or via a combination of methods: health insurance records, cancer and pathology registries, and active follow-up (France, Germany, Naples, and Greece). Subjects were followed until cancer diagnosis (except non-melanoma skin cancer), emigration, death, or until the end of follow-up (dates varied between centres, from December 2004 to June 2010).

Tumour morphology was specified for 664 (48%) cases, of which 627 (93%) were classified as type I (endometrioid adenocarcinomas), and 37 (7%) as type II (serous, or clear cell, or squamous adenocarcinomas; Tavassoli and Devilee, 2003). Overall EC comprises type I, type II, and cases that were undefined for histology. Tumours were classified as C54 according to the International Classification of Diseases, 10th revision.

Dietary and acrylamide intake assessment. Information on diet was assessed at baseline (with timeframe referring to the previous 12 months) through country-specific, validated dietary questionnaires (DQ; Riboli *et al*, 2002). The development of the acrylamide database in EPIC has been previously described (Freisling *et al*, 2013; Obon-Santacana *et al*, 2013). To summarise, the EPIC acrylamide database is a compilation of the information acquired to a large extent from the European Community Institute for Reference Materials and Measurements (IRMM). The average acrylamide levels for specific foods in the IRMM database were obtained through a combination of methods based on either liquid or gas chromatography coupled to mass spectrometry. All food items with acrylamide data derived from the IRMM database were classified according to EPIC-Soft food classification (Voss *et al*, 1998; Slimani *et al*, 2000). The reported foods on the DQ and, when available, their relevant description (e.g., baked potatoes) were matched with the corresponding foods in the acrylamide

database. Information on cooking methods for acrylamide sources was available for potatoes (except in Italy), bread, and breaded meats. If an exact match was not possible, the food was linked to the mean of all foods of the respective food group in the acrylamide database (Freisling *et al*, 2013; Obon-Santacana *et al*, 2013).

Lifestyle and reproductive information assessment. At baseline, questionnaires were used to collect data on tobacco smoking, education, physical activity, and menstrual and reproductive factors (i.e., age at first menstrual period, ever use of OCs, ever use of hormone replacement therapy (HRT)). Baseline menopausal status was self-reported for each woman in most centres, and in case of incomplete data, an algorithm was developed based on the age at recruitment: women were classified as premenopausal if their baseline ages were < 46 years, or reported having menstrual cycles the year before recruitment; perimenopausal if their ages were between 46 and 55 years, or had irregular menses the year before recruitment; and postmenopausal if their ages were > 56 years, or had bilateral ovariectomy (surgical menopause), or had < 4 menstrual cycles in the past year before recruitment (Riboli *et al*, 2002).

Height, weight, and waist or hip circumference were measured at baseline by trained personnel for all EPIC participants, except for most participants in France, Norway and Oxford cohorts, where height and weight were self-reported. Umeå and Norway did not record data on waist or hip circumference, and only some participants from France have information on waist (29%) and hip circumference (29%; Riboli *et al*, 2002).

Statistical analysis. Proportional hazards models (Cox regression) were used to estimate hazards ratio (HR) and 95% confidence intervals (95% CI) for overall EC risk in relation to dietary intake of acrylamide. Analyses were also performed separately for risk of type-I EC. Analyses for type-II EC cases were not carried out due to small sample sizes ($n = 37$). All multivariate models had age as the time scale and were stratified by study centre to control for centre effects (i.e., questionnaire design and follow-up procedures), and by age at recruitment (in 1-year categories) as the primary time variable.

All estimates of acrylamide intake in these analyses were energy adjusted using the residual method (Willett, 1998; Ferrari *et al*, 2013). One continuous variable and one categorical variable for dietary intake of acrylamide were evaluated in Cox models: average daily intake in 10 μg increments (10 μg per day), and quintiles of intake (μg per day) based on the distribution in the full EPIC cohort of women.

The following variables were included as known risk factors or potential confounders in these analyses: body mass index (BMI, kg m^{-2}), smoking status (never smokers, current pipe or cigar or occasional smokers, current cigarette smokers: 1–15, 16–25, or ⩾ 26 cigarettes per day, former cigarette smokers who quit > 20 years, 11–20 years, or ⩽ 10 years before recruitment), history of diabetes (no, yes), OC use (never, ever), HRT use (never, ever), baseline menopause status combined with age at menopause (premenopausal, perimenopausal, postmenopausal with: < 45, 45–49, 50–52, 53–55, and ⩾ 56 years, surgical menopause, postmenopausal women with missing age at menopause), parity (nulliparous, 1, 2, ⩾ 3, parous but with missing number of full-term pregnancies), and age at menarche (< 12, 12, 13, 14, and ⩾ 15 years). Variables for education level (none, primary, technical/professional, secondary, and higher education), physical activity using the Cambridge index (Wareham *et al*, 2003), alcohol intake (non-drinkers, drinkers of 0–6, > 6–12, > 12–24, and > 24 g per day), total fat (g per day), total fibre (g per day), vegetables (g per day), and fruits, nuts and seeds consumption (g per day) were evaluated, but were not included in final models because they did not change effect estimates > 10%. Missing values for specific variables were categorised as 'unknown' and were included in the

analyses. All statistical models presented in this study were further adjusted for total energy intake (per 1000 kcal per day).

Analyses of effect-measure modification were carried out by known EC risk factors (BMI, menopausal status, and HRT use), by known protective factors (OC use, and smoking status), by geographical region, and by factors that may affect the activity of Cyp2e1 (alcohol intake, and BMI; Wilson et al, 2009; Freisling et al, 2013). The following subgroups were examined: BMI ($<25 \, \text{kg m}^{-2}$, $\geq 25 \, \text{kg m}^{-2}$), OC use (never, ever), HRT use (never, ever), baseline menopausal status (premenopausal, peri-menopausal, and postmenopausal), smoking status (never, current, or former smokers), and alcohol intake (never, ever drinkers). For region-specific analyses, countries were classified as northern (France, UK, The Netherlands, Germany, Sweden, Denmark, and Norway) and southern (Italy, Spain, and Greece); and by median acrylamide-intake level ('high' $\geq 21 \, \mu$g per day and 'low' $<21 \, \mu$g per day) in the EPIC cohort.

Sensitivity analyses were additionally performed excluding all cases diagnosed during the first 2 years of follow-up, with the aim to avoid possible influences of preclinical disease on dietary habits including intakes of acrylamide.

To evaluate dose-response trends, the median value for each acrylamide quintile was estimated and included in a score test. Statistical significance of effect-measure modification was evaluated using a LRT and based on the continuous acrylamide intake variable. The proportional hazards (PHs) assumption was tested in STATA (College Station, Texas, USA) using Schoenfeld residuals (Schoenfeld, 1982), and it was met for type-I EC analyses; however, it was violated for overall EC analyses. Variables responsible for the PH violation were: OC use, HRT use, and smoking status; thus, stratified analyses by these variables were also performed for overall EC risk, and the PH assumption was subsequently met. All analyses were performed using SAS v. 9.1 (Cary, NC, USA).

RESULTS

Basic information on cohorts members. The average acrylamide intake in the EPIC subcohort of women was $24 \pm 13 \, \mu$g per day

($0.4 \pm 0.2 \, \mu$g per kg body weight per day), and the 10th–90th percentile range was 10–41 μg per day (0.2–0.6 μg per kg body weight per day). Denmark, followed by the UK and The Netherlands, had the highest mean and median dietary acrylamide intakes, while Italy had the lowest acrylamide intake (Table 1). In total, after 11 years of follow-up there were 1382 first primary EC cases, of which 627 were classified as type-I EC, 37 type-II EC, and 718 cases that were not specified with regard to histology (Table 1).

Women with the highest acrylamide-intake levels tended to have the highest intakes of energy, total fats, total carbohydrates, vegetables, and coffee. Women with the highest intake levels tended to be premenopausal, have a higher proportion of OC use and with longer duration, and were more often current smokers or former smokers at baseline (Table 2). In contrast, women classified in the lower quintiles tended to be postmenopausal, non-consumers of alcohol and tobacco, and to have lower levels of physical activity (Table 2). There were few differences across acrylamide intake quintiles by age, age at first menstrual period, age at menopause, BMI, or waist-to-hip ratio (Table 2).

Overall EC risk and type-I EC risk. No association was observed between acrylamide intake and overall EC (Table 3) or type-I EC risk (Table 4). Similar results were found when we restricted the analyses to cases diagnosed 2 years after recruitment (Tables 3 and 4), or when known type-I and type-II EC were combined in the same analysis (data not shown). Further, an analysis among EC cases that could not be classified into type-I or type-II EC was also carried out, but no associations were observed (data not shown). Most of the stratified analyses performed with overall EC (type I, type II, and undefined) cases indicated no heterogeneity between subgroups (Table 3). When stratified analyses by OC use, and by OC use and smoking were performed, statistically significant LRT P-values were observed; however, neither the continuous nor the categorical acrylamide variable suggested an association with disease risk (Table 3).

Effect-measure modification by OC use and smoking in type-I EC. Subgroup analyses for known type-I EC were also stratified by smoking status, OC use, menopausal status, HRT use, BMI, and geographical region. None of the HRs in never smokers or ever

Table 1. Estimated dietary intake of acrylamide and EC cases by country in the EPIC subcohort of women

Country	Cohort sample	Person-years	EC cases N (%)	Type-I cases N (%)	Type-II cases N (%)	Cases undefined by type N (%)	Acrylamide (μg per day) Mean ± s.d.	Acrylamide[a] (μg per day) Mean ± s.d.	Acrylamide (μg per kg body weight per day) Mean ± s.d.
France	60 702	629 899	276 (20.0)	79 (12.6)	3 (8.1)	194 (27.0)	20.4 ± 8.8	18.3 ± 6.6	0.4 ± 0.2
Italy	27 760	310 816	132 (9.6)	48 (7.7)	1 (2.7)	83 (11.6)	10.9 ± 6.1	8.8 ± 5.7	0.2 ± 0.1
Spain	22 783	275 042	102 (7.4)	48 (7.7)	3 (8.1)	51 (7.1)	20.6 ± 12.1	21.3 ± 10.3	0.3 ± 0.2
United Kingdom	46 068	513 816	170 (12.3)	74 (11.8)	5 (13.5)	91 (12.7)	33.1 ± 15.3	33.4 ± 13.1	0.5 ± 0.3
The Netherlands	22 140	260 499	107 (7.7)	59 (9.4)	5 (13.5)	43 (6.0)	31.2 ± 13.7	31.7 ± 12.1	0.5 ± 0.2
Greece	13 967	136 097	18 (1.3)	4 (0.6)	1 (2.7)	13 (1.8)	19.2 ± 9.1	19.8 ± 7.2	0.3 ± 0.1
Germany	23 321	231 579	82 (5.9)	67 (10.7)	4 (10.8)	11 (1.5)	24.5 ± 11.2	25.3 ± 9.7	0.4 ± 0.2
Sweden	26 375	349 308	183 (13.2)	1 (0.2)	4 (10.8)	178 (24.8)	22.4 ± 9.7	23.6 ± 8.2	0.3 ± 0.2
Denmark	24 473	269 910	182 (13.2)	140 (22.3)	9 (24.3)	33 (4.6)	35.6 ± 11.7	35.5 ± 10.2	0.5 ± 0.2
Norway	33 524	326 296	130 (9.4)	107 (17.1)	2 (5.4)	21 (2.9)	17.9 ± 6.5	20.6 ± 5.8	0.3 ± 0.1
Total	301 113	3 303 262	1382	627	37	718	23.7 ± 13.0	23.7 ± 12.0	0.4 ± 0.2

Abbreviations: EC = endometrial cancer; EPIC = European Prospective Investigation into Cancer and Nutrition; s.d. = standard deviation.
[a]Energy adjusted using the residual method.

Table 2. Estimated total dietary intake of acrylamide (energy adjusted using the residual method) and covariates at baseline used in the analyses: EPIC subcohort (301 113 women)

	Energy-adjusted acrylamide intake (μg per day)				
	⩽14.5	14.6–19.5	19.6–24.2	24.3–32.0	32.1–222.4
Participants (n)	60 222	60 223	60 223	60 223	60 222
Endometrial cancer cases (n)	277	271	298	250	286
Type-I EC cases (n)	105	111	125	122	164
Energy-adjusted acrylamide intake (median; μg per day)	10.7	17.2	21.7	27.4	39.3
Age (years)	51.1 ± 8.4[a]	50.8 ± 9.1	50.1 ± 9.6	49.7 ± 10.6	49.6 ± 11.5
Age at first menstrual period (years)[b]	12.8 ± 1.5	13.1 ± 1.5	13.1 ± 1.5	13.2 ± 1.5	13.2 ± 1.6
Age at menopause (years)[b]	49.3 ± 4.4	49.3 ± 4.5	49.3 ± 4.5	49.4 ± 4.4	49.4 ± 4.3
Menopausal status (%)					
Premenopausal	36.5	35.76	37.8	40.05	40.15
Perimenopausal	18.16	20.55	19.68	16.51	12.92
Postmenopausal[c]	45.34	43.69	42.52	43.44	46.93
Ever use of OCs (%)					
Yes	49.45	55.8	58.12	61.46	65.48
Unknown	0.65	2.51	4.53	4.04	1.8
Duration of using OCs (years)[b]	6.1 ± 6.6	7.4 ± 7.2	7.9 ± 7.4	8.4 ± 7.5	8.7 ± 7.5
Ever use of HRT (%)					
Yes	19.96	22.71	21.94	21.29	22.22
Unknown	3.25	6.69	9.09	9.33	6.37
Duration of using HRT (years)[b]	2.9 ± 3.1	3.4 ± 3.3	3.6 ± 3.6	3.9 ± 4.2	4.2 ± 4.6
Smoking status (%)					
Never	59.49	60.01	55.53	52.35	49.68
Former	19.45	20.8	22.71	23.88	25.15
Current	18.86	15.75	18.88	21.61	23.85
Unknown	2.2	3.44	2.88	2.16	1.31
Cigarettes per day (smokers only)	13.1 ± 8.7	12.5 ± 7.7	12.8 ± 7.5	13.2 ± 7.6	14.0 ± 7.8
Time since quitting[d] (years)	13.7 ± 9.0	15.0 ± 9.6	14.8 ± 9.8	14.9 ± 10.1	14.9 ± 10.5
Prevalent diabetes (%)					
Yes	2.67	2.42	2.0	1.65	1.61
Unknown	1.94	4.42	5.07	4.59	4.64
Alcohol					
Non-consumers (%)	22.56	19.08	16.49	13.51	10.24
Consumers (g per day)	9.2 ± 14.1	7.2 ± 10.9	6.6 ± 10.1	7.6 ± 10.8	8.5 ± 10.9
Education (%)					
Primary school completed	31.48	20.23	21.76	21.93	21.13
Higher education[e]	22.57	25.92	23.91	23.56	21.5
Unknown	1.72	2.69	2.98	4.3	6.31
Physical activity (%)					
Inactive	28.99	21.35	19.13	18.26	17.44
Active	9.49	9.71	11.78	15.93	22.08
Unknown	7.09	18.22	19.71	12.13	4.29
BMI (kg m^{-2})	25.1 ± 4.5	24.6 ± 4.4	24.7 ± 4.3	24.8 ± 4.4	25.0 ± 4.4
WHR[b]	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
Energy (kcal)	2098.2 ± 571.9	1860.1 ± 521.1	1810.3 ± 515.9	1873.8 ± 516.2	2027.5 ± 523.3
Total fats (g per day)	84.8 ± 28.3	74.5 ± 26.3	70.9 ± 25.8	72.6 ± 25.9	78.3 ± 26.4
Carbohydrates (g per day)	224.5 ± 74.2	203.7 ± 63.6	204 ± 62.3	213.0 ± 63.9	232.7 ± 67.3
Vegetables (g per day)	252.9 ± 165.6	232.3 ± 146.5	203.1 ± 133.6	198.8 ± 129.8	204.5 ± 127.7
Coffee (ml per day)	123.6 ± 129.9	228.5 ± 194.4	337.8 ± 240.2	441.8 ± 305.9	643.4 ± 449.3
Bread, crisp bread, and rusks (g per day)	121.1 ± 76.0	114.9 ± 65.9	115.7 ± 66.1	116.6 ± 67.4	124.2 ± 69.1
Potatoes (g per day)	48.6 ± 46.2	70.8 ± 52.9	84.3 ± 57.5	95.1 ± 64.4	105.7 ± 67.5
Cakes and biscuits (g per day)	34.8 ± 37.6	34.8 ± 33.4	38.4 ± 34.3	42.4 ± 38.6	48.3 ± 47.7

Abbreviations: BMI = body mass index; EC = endometrial cancer; EPIC = European Prospective Investigation into Cancer and Nutrition; HRT = hormonal replacement therapy; OCs = oral contraceptives; WHR = waist-to-hip ratio.
[a]Mean ± s.d.
[b]Number of women missing the following; age at first menstrual period: 10 321; age at menopause: 201 651; duration of using OCs: 142 462; duration of using HRT: 278 012; number of cigarettes: 243 668; time since quitting smoking; 236 217; and WHR: 88 717.
[c]Includes surgical menopause.
[d]Only in former smokers.
[e]Higher education includes any university degree or above.

Table 3. Hazard ratios and 95% confidence intervals for the estimated dietary intake of acrylamide (energy-adjusted using the residual method) and EC risk in EPIC

| | | Energy-adjusted acrylamide intake (µg per day) | | | | | | |
| | | | Quintiles | | | | | |
	10 µg increments	Q1 (≤14.5)	Q2 (14.6–19.5)	Q3 (19.6–24.2)	Q4 (24.3–32.0)	Q5 (32.1–222.4)	Trend test P-value	LRT P-value[a]
Final model – overall EC								
N cases	1382	277	271	298	250	286		
HR (95% CI)[b]	0.98 (0.92–1.05)	1.00 (ref)	1.05 (0.86–1.29)	1.11 (0.90–1.36)	0.88 (0.71–1.10)	0.98 (0.78–1.25)	0.53	
Cases diagnosed ≥2 years after recruitment								
N cases	1186	240	217	268	215	246		
HR (95% CI)[b]	0.98 (0.91–1.05)	1.00 (ref)	0.97 (0.78–1.20)	1.12 (0.89–1.39)	0.85 (0.67–1.08)	0.95 (0.74–1.23)	0.52	
Overall EC – stratified analyses								
Smoking status								
Never smokers								
N cases	747	147	142	153	132	173		
HR (95% CI)[c]	0.97 (0.89–1.05)	1.00 (ref)	1.03 (0.79–1.34)	1.04 (0.79–1.36)	0.82 (0.61–1.10)	1.01 (0.75–1.38)	0.90	
Ever smokers[d]								0.20
N cases	587	123	118	135	110	101		
HR (95% CI)[c]	0.98 (0.89–1.08)	1.00 (ref)	1.08 (0.80–1.45)	1.23 (0.91–1.66)	0.96 (0.69–1.33)	0.86 (0.60–1.24)	0.23	
OC use								
Non-OC users								
N cases	800	180	155	165	127	173		
HR (95% CI)[e]	1.03 (0.94–1.12)	1.00 (ref)	1.07 (0.83–1.38)	1.09 (0.84–1.42)	0.83 (0.62–1.11)	1.17 (0.86–1.58)	0.51	
OC users								0.03
N cases	547	94	111	121	117	104		
HR (95% CI)[e]	0.92 (0.83–1.02)	1.00 (ref)	1.05 (0.76–1.46)	1.16 (0.83–1.61)	0.97 (0.68–1.39)	0.79 (0.53–1.15)	0.08	
Smoking status combined with OC use								
Never smokers and non-OC users								
N cases	477	106	90	94	75	112		
HR (95% CI)[f]	1.02 (0.92–1.13)	1.00 (ref)	1.05 (0.76–1.44)	1.08 (0.77–1.50)	0.82 (0.57–1.18)	1.28 (0.88–1.85)	0.24	
Ever smokers[d] and non-OC users								
N cases	299	68	58	68	47	58		
HR (95% CI)[f]	1.02 (0.89–1.17)	1.00 (ref)	1.09 (0.73–1.65)	1.28 (0.84–1.95)	0.87 (0.55–1.39)	0.98 (0.60–1.60)	0.65	
Never smokers and OC users								0.04
N cases	253	39	49	52	54	59		
HR (95% CI)[f]	0.89 (0.77–1.03)	1.00 (ref)	1.03 (0.64–1.67)	0.98 (0.60–1.61)	0.83 (0.50–1.40)	0.73 (0.42–1.26)	0.13	
Ever smokers[d] and OC users								
N cases	277	54	58	63	60	42		
HR (95% CI)[f]	0.93 (0.80–1.08)	1.00 (ref)	1.10 (0.71–1.69)	1.22 (0.78–1.90)	1.07 (0.67–1.71)	0.76 (0.44–1.30)	0.22	
Alcohol intake								
Never drinkers								
N cases	253	70	59	38	35	51		
HR (95% CI)[b]	1.06 (0.91–1.24)	1.00 (ref)	0.95 (0.62–1.46)	0.72 (0.44–1.18)	0.59 (0.35–1.00)	1.03 (0.60–1.76)	0.76	
Ever drinkers								0.07
N cases	1129	207	212	260	215	235		
HR (95% CI)[b]	0.97 (0.90–1.04)	1.00 (ref)	1.10 (0.87–1.39)	1.27 (1.00–1.61)	0.96 (0.75–1.24)	1.01 (0.77–1.32)	0.54	
Body mass index								
<25 kg m^{-2}								
N cases								
HR (95% CI)[g]	1.01 (0.91–1.12)	1.00 (ref)	0.94 (0.70–1.27)	1.13 (0.83–1.53)	0.92 (0.67–1.28)	0.93 (0.64–1.35)	0.68	
≥25 kg m^{-2}								0.96
N cases								
HR (95% CI)[g]	0.99 (0.90–1.08)	1.00 (ref)	1.29 (0.96–1.73)	1.21 (0.89–1.64)	0.94 (0.68–1.31)	1.12 (0.79–1.57)	0.89	

Table 3. (Continued)

	Energy-adjusted acrylamide intake (μg per day)							
		Quintiles						
	10 μg increments	Q1 ($\leqslant$14.5)	Q2 (14.6–19.5)	Q3 (19.6–24.2)	Q4 (24.3–32.0)	Q5 (32.1–222.4)	Trend test P-value	LRT P-value[a]
Menopausal status								
Premenopausal								
N cases	253	67	54	52	45	35		
HR (95% CI)[h]	0.88 (0.74–1.04)	1.00 (ref)	1.12 (0.72–1.74)	1.12 (0.70–1.78)	1.00 (0.61–1.64)	0.68 (0.37–1.22)	0.17	
Perimenopausal								0.05
N cases	268	51	56	73	44	44		
HR (95% CI)[h]	1.05 (0.89–1.23)	1.00 (ref)	1.08 (0.69–1.70)	1.29 (0.82–2.04)	0.83 (0.50–1.39)	1.18 (0.67–2.10)	0.90	
Postmenopausal[i]								
N cases	861	159	161	173	161	207		
HR (95% CI)[h]	1.01 (0.93–1.10)	1.00 (ref)	1.05 (0.80–1.38)	1.06 (0.80–1.40)	0.84 (0.62–1.13)	1.03 (0.76–1.40)	0.99	

Abbreviations: BMI = body mass index; CI = confidence interval; EC = endometrial cancer; EPIC = European Prospective Investigation into Cancer and Nutrition; HR = hazards ratio; HRT = hormonal replacement therapy; LRT = likelihood ratio test; OCs = oral contraceptives.
[a]All LRT P-values for effect measure modification are based on the continuous acrylamide intake variable (per 10 μg per day).
[b]Stratified by age at recruitment, centre, smoking status, OC use, and HRT use. Adjusted for total energy intake (per 1000 kcal per day), BMI, prevalent diabetes, menopause status combined with age at menopause, parity, and age at menarche.
[c]Stratified by age at recruitment, centre, OC use, and HRT use. Adjusted for total energy intake (per 1000 kcal per day), BMI, prevalent diabetes, menopause status combined with age at menopause, parity, and age at menarche.
[d]Ever smokers: former and current smokers.
[e]Stratified by age at recruitment, centre, smoking status, and HRT use. Adjusted for total energy intake (per 1000 kcal per day), BMI, prevalent diabetes, menopause status combined with age at menopause, parity and age at menarche.
[f]Stratified by age at recruitment, centre, and HRT use. Adjusted for total energy intake (per 1000 kcal per day), BMI, prevalent diabetes, menopause status combined with age at menopause, parity, and age at menarche.
[g]Stratified by age at recruitment, centre, smoking status, OC use, and HRT use. Adjusted for total energy intake (per 1000 kcal per day), prevalent diabetes, menopause status combined with age at menopause, parity, and age at menarche.
[h]Stratified by age at recruitment, centre, smoking status, OC use, and HRT use. Adjusted for total energy intake (per 1000 kcal per day), BMI, prevalent diabetes, parity, and age at menarche.
[i]Includes surgical menopause.

smokers indicated associations between dietary acrylamide intake and type-I EC risk; however, statistically significant evidence for heterogeneity was observed (LRT P-value: 0.01; Table 4).

Inverse associations were observed for the highest versus the lowest quintile of acrylamide intake (HR_{Q5vsQ1}: 0.57, 95% CI: 0.34–0.96; P-value for trend: 0.01), as well as a continuous variable (HR: 0.83, 95% CI: 0.71–0.95; Table 4). Regarding the HRs obtained in the subgroup of non-OC users, none of them were statistically significant ($HR_{10\ \mu g\ per\ day}$: 1.10, 95% CI: 0.99–1.23; Table 4).

Moreover, the OC-use model was additionally adjusted by duration of OC use (per 2 years of OC use), and the results were similar to those presented without adjustment for this variable (data not shown).

There were some differences in non-dietary variables between OC users and non-users. OC users with the highest acrylamide intake tended to have a higher proportion of former or current smokers, and these women tended to smoke more cigarettes per day than non-users. Further, non-OC users were older than OC users, but with similar age at menopause. With regard to dietary factors, there were no major differences between OC users and non-users (data not shown).

The association between acrylamide intake and type-I EC risk among OC users and non-users was also evaluated by smoking status. Women who at baseline reported being never smokers and non-users of OCs (including 203 type-I EC cases) were at the highest risk of developing type-I EC, when acrylamide was evaluated both as a continuous variable and in quintiles ($HR_{10\ \mu g\ per\ day}$: 1.17, 95% CI: 1.02–1.34; HR_{Q5vsQ1}: 1.97, 95% CI: 1.08–3.62; P-value for trend: 0.01; Table 4). Otherwise, associations between dietary acrylamide intake and type-I EC were below the null value in ever smokers (current and former smokers) and OC

users ($HR_{10\ \mu g\ per\ day}$: 0.75, 95% CI: 0.60–0.94; Table 4). The LRT P-value of the contrast between 'never smokers/non-OC users', 'ever smokers/non-OC users', 'never smokers/OC users', and 'ever smokers/OC users' for the continuous acrylamide intake variable was 0.01 (Table 4).

Other effect-measure modifications in type-I EC. There was no evidence for effect-measure modification by BMI (Table 4), HRT use, or by geographical region (all LRT P-values > 0.12, data not shown); however, evidence for effect-measure modification was found when the analyses were stratified by baseline menopausal status (LRT P-value: 0.01; Table 4), but none of the individual HRs were statistically significant. Likewise, effect-measure modification was observed by alcohol intake (LRT P-value: 0.01), but only the continuous variable in never drinkers showed a statistically significant positive association ($HR_{10\ \mu g\ per\ day}$: 1.23, 95% CI: 1.02–1.47; Table 4).

DISCUSSION

No overall association was observed between dietary intake of acrylamide and overall EC or type-I EC risk; nevertheless, elevated relative risks, as well as P-values for linear trend were observed for the association between dietary intake of acrylamide and type-I EC among women who both never smoked and never used OCs. Statistically significant inverse associations between type-I EC risk and acrylamide intake were observed in OC users, and among OC users and ever smokers.

It is widely published that use of OCs (containing oestrogen and progestin) is protective against EC risk, and this effect is

Table 4. Hazard ratios and 95% confidence intervals for the estimated dietary intake of acrylamide (energy-adjusted using the residual method) and type-I endometrial cancer risk in EPIC

	Energy-adjusted acrylamide intake (μg per day)							
		Quintiles						
	10 μg increments	Q1 (⩽14.5)	Q2 (14.6–19.5)	Q3 (19.6–24.2)	Q4 (24.3–32.0)	Q5 (32.1–222.4)	Trend test P-value	LRTP-value[a]
Final model – Type I								
N cases	627	105	111	125	122	164		
HR (95% CI)[b]	0.98 (0.90–1.07)	1.00 (ref)	1.00 (0.74–1.35)	1.04 (0.77–1.42)	0.87 (0.63–1.21)	0.97 (0.69–1.36)	0.79	
Cases diagnosed ⩾2 years after recruitment								
N cases	556	98	93	117	107	141		
HR (95% CI)[b]	0.96 (0.87–1.06)	1.00 (ref)	0.89 (0.65–1.23)	1.04 (0.76–1.43)	0.84 (0.60–1.19)	0.93 (0.65–1.32)	0.75	
Type I – stratified analyses								
Smoking status								
Never smokers								
N cases	350	56	54	67	69	104		
HR (95% CI)[c]	1.06 (0.95–1.19)	1.00 (ref)	0.97 (0.63–1.48)	1.14 (0.74–1.74)	0.97 (0.62–1.51)	1.25 (0.79–1.98)	0.21	
Ever smokers[d]								0.01
N cases	257	44	51	55	50	57		
HR (95% CI)[c]	0.90 (0.78–1.03)	1.00 (ref)	1.02 (0.64–1.63)	1.00 (0.62–1.62)	0.80 (0.48–1.34)	0.70 (0.41–1.19)	0.09	
OC use								
Non-OC users								
N cases	347	65	56	65	58	103		
HR (95% CI)[e]	1.10 (0.99–1.23)	1.00 (ref)	0.96 (0.64–1.45)	1.09 (0.71–1.67)	0.90 (0.57–1.42)	1.40 (0.89–2.22)	0.06	
OC users								0.01
N cases	273	39	54	59	63	58		
HR (95% CI)[e]	0.83 (0.71–0.95)	1.00 (ref)	0.97 (0.62–1.51)	0.93 (0.59–1.47)	0.79 (0.49–1.28)	0.57 (0.34–0.96)	0.01	
Smoking status combined with OC use								
Never smokers and non-OC users								
N cases	203	35	29	36	35	68		
HR (95% CI)[f]	1.17 (1.02–1.34)	1.00 (ref)	1.03 (0.58–1.81)	1.28 (0.72–2.27)	1.12 (0.61–2.06)	1.97 (1.08–3.62)	0.01	
Ever smokers[d] and non-OC users								
N cases	134	26	25	27	21	35		
HR (95% CI)[f]	1.04 (0.86–1.26)	1.00 (ref)	0.99 (0.51–1.91)	0.99 (0.50–1.98)	0.76 (0.36–1.62)	1.01 (0.47–2.19)	0.98	
Never smokers and OC users								0.01
N cases	145	20	25	31	33	36		
HR (95% CI)[f]	0.89 (0.73–1.09)	1.00 (ref)	0.76 (0.40–1.45)	0.83 (0.44–1.59)	0.68 (0.35–1.35)	0.59 (0.29–1.21)	0.17	
Ever smokers[d] and OC users								
N cases	120	18	25	27	29	21		
HR (95% CI)[f]	0.75 (0.60–0.94)	1.00 (ref)	1.02 (0.52–1.99)	1.00 (0.50–1.98)	0.84 (0.41–1.72)	0.45 (0.20–1.00)	0.02	
Alcohol intake								
Never drinkers								
N cases	103	28	19	13	17	26		
HR (95% CI)[b]	1.23 (1.02–1.47)	1.00 (ref)	0.76 (0.40–1.44)	0.61 (0.29–1.28)	0.93 (0.46–1.89)	1.77 (0.86–3.64)	0.07	
Ever drinkers								0.01
N cases	524	77	92	112	105	138		
HR (95% CI)[b]	0.93 (0.85–1.03)	1.00 (ref)	1.09 (0.77–1.54)	1.19 (0.83–1.69)	0.90 (0.61–1.31)	0.91 (0.62–1.35)	0.30	
Body mass index								
<25 kg m^{-2}								
N cases	256	43	48	62	53	50		
HR (95% CI)[g]	0.86 (0.74–1.00)	1.00 (ref)	0.88 (0.56–1.38)	1.11 (0.71–1.73)	0.78 (0.48–1.27)	0.56 (0.33–0.96)	0.02	
⩾25 kg m^{-2}								0.28
N cases	371	62	63	63	69	114		
HR (95% CI)[g]	1.06 (0.95–1.18)	1.00 (ref)	1.12 (0.75–1.69)	0.99 (0.64–1.52)	0.92 (0.59–1.44)	1.34 (0.85–2.10)	0.12	

Table 4. (Continued)								
	Energy-adjusted acrylamide intake (µg per day)							
		Quintiles						
	10 µg increments	Q1 (⩽14.5)	Q2 (14.6–19.5)	Q3 (19.6–24.2)	Q4 (24.3–32.0)	Q5 (32.1–222.4)	Trend test P-value	LRT P-value[a]
Menopausal status								
Premenopausal								
N cases	120	28	25	26	24	17		
HR (95% CI)[h]	0.78 (0.62–0.99)	1.00 (ref)	0.89 (0.48–1.64)	0.91 (0.49–1.71)	0.78 (0.40–1.53)	0.52 (0.24–1.13)	0.09	
Perimenopausal								0.01
N cases	120	24	25	32	20	19		
HR (95% CI)[h]	0.88 (0.70–1.12)	1.00 (ref)	0.77 (0.41–1.43)	0.91 (0.49–1.68)	0.67 (0.33–1.36)	0.59 (0.26–1.31)	0.22	
Postmenopausal[i]								
N cases	387	53	61	67	78	128		
HR (95% CI)[h]	1.07 (0.96–1.18)	1.00 (ref)	1.24 (0.81–1.89)	1.25 (0.81–1.95)	1.09 (0.69–1.72)	1.39 (0.88–2.20)	0.17	

Abbreviations: BMI = body mass index; CI = confidence interval; EPIC = European Prospective Investigation into Cancer and Nutrition; HR = hazards ratio; HRT = hormonal replacement therapy; LRT = likelihood ratio test; OCs = oral contraceptives.
[a]All LRT P-values for effect measure modification are based on the continuous acrylamide intake variable (per 10 µg per day).
[b]Stratified by age at recruitment and centre. Adjusted for total energy intake (per 1000 kcal per day), BMI, smoking status, prevalent diabetes, OC use, HRT use, menopause status combined with age at menopause, parity, and age at menarche.
[c]Stratified by age at recruitment and centre. Adjusted for total energy intake (per 1000 kcal per day), BMI, prevalent diabetes, OC use, HRT use, menopause status combined with age at menopause, parity, and age at menarche.
[d]Ever smokers: former and current smokers.
[e]Stratified by age at recruitment and centre. Adjusted for total energy intake (per 1000 kcal per day), BMI, smoking status, prevalent diabetes, HRT use, menopause status combined with age at menopause, parity, and age at menarche.
[f]Stratified by age at recruitment and centre. Adjusted for total energy intake (per 1000 kcal per day), BMI, prevalent diabetes, HRT use, menopause status combined with age at menopause, parity, and age at menarche.
[g]Stratified by age at recruitment and centre. Adjusted for total energy intake (per 1000 kcal per day), smoking status, prevalent diabetes, OC use, HRT use, menopause status combined with age at menopause, parity, and age at menarche.
[h]Stratified by age at recruitment and centre. Adjusted for total energy intake (per 1000 kcal per day), BMI, smoking status, prevalent diabetes, OC use, HRT use, parity, and age at menarche.
[i]Includes surgical menopause.

maintained for years (Amant *et al*, 2005; Cook *et al*, 2006; Cibula *et al*, 2010; Gierisch *et al*, 2013). Likewise, cigarette smoking tends to lower the risk of developing EC, and it is thought to be more pronounced in recent smokers (Cook *et al*, 2006). All the relative risk estimates for type-I EC risk observed among OC users and ever smokers were below the null value; however, because OC use, duration of OC use, and smoking are associated with higher acrylamide intake in EPIC, and are also associated with lower EC risk, residual confounding by these variables may play a role in the observed inverse associations (in OC users and smokers). In addition, OC users, compared to non-OC users, tended to smoke more cigarettes per day and reported less time since having quit smoking. Thus, these baseline characteristics may have partially influenced the results obtained in this subgroup of women. Moreover, it has been hypothesised that acrylamide may have hormonal effects, and the results in non-OC users for type I are potentially compatible with this hypothesis, since type-I EC is considered to be oestrogen driven (Amant *et al*, 2005); nevertheless, this hypothesis has not been substantiated, and other mechanisms (i.e., genotoxicity caused by glycidamide) may be compatible with the results (Hogervorst *et al*, 2010, 2013).

The relation between dietary intake of acrylamide and EC risk has been previously published in three prospective cohort studies. Both the NLCS and NHS studies found statistically significantly increased relative risks: the NLCS among never-smoking women, and the NHS in the entire cohort (Hogervorst *et al*, 2007; Wilson *et al*, 2010). Although the NLCS and NHS studies did not evaluate the association between acrylamide intake and type-I EC specifically, about 80% of EC cases are thought to be type-I endometrioid tumours (Amant *et al*, 2005); thus, the majority of the cases in the previous publications were likely type-I EC cases.

Only the SMC study observed no associations between acrylamide intake and EC risk (Larsson *et al*, 2009), and this could be due to the smaller baseline ranges of acrylamide intake in that study. The median acrylamide intake for the reference group in the SMC was 16.9 µg per day, and for the highest intake category was 32.5 µg per day, whereas in EPIC, the median for the reference group was 9.3 µg per day, and for the highest intake category was 44.0 µg per day. All three previous studies presented statistical models adjusted for OC use, but none reported analyses stratified by OC use.

Some evidence for an inverse association between the highest and lowest acrylamide quintiles and type-I EC risk was observed among women with a BMI < 25 kg m^{-2}; however, neither the continuous variable for acrylamide intake (per 10 µg per day) nor the LRT P-value were statistically significant. A suggestive increased risk for type-I EC was observed in women who reported never drinking alcohol at baseline when the continuous acrylamide variable was evaluated; nevertheless, this result was based on 103 type-I EC cases. Further, suggestive evidence for heterogeneity of the association between dietary acrylamide intake and type-I EC risk was also indicated by smoking status, and by menopausal status at baseline; nevertheless no dose-response trend was observed.

The strengths of our study are that EPIC is one of the largest prospective cohort studies on diet and cancer, and recall bias is unlikely because exposure and diet information were collected years before cancer diagnoses. The present study had more cases than the other three previously published studies ($n = 1382$), and this allowed us to evaluate known type-I EC separately ($n = 627$). The SMC study analysed 687 EC cases (Larsson *et al*, 2009), the NHS study analysed 484 EC cases (Wilson *et al*, 2010), and the NLCS study evaluated 221 (Hogervorst *et al*, 2007).

The present study had the following limitations: some food preparation techniques (e.g., cooking method) that could have contributed to the variability of total acrylamide intake were not assessed in all EPIC centres. In addition, the correlation coefficient between a single 24-h DR in EPIC, and acrylamide intake derived from food intake questionnaires was low: 0.17 (Ferrari *et al*, 2013). This could indicate that a single 24-h DR may not be enough to accurately estimate the average acrylamide intake. Further, the EPIC acrylamide estimates might have been influenced by measurement error; however, all the analyses were adjusted for energy intake since in EPIC and in other populations, it has been observed that the validity of acrylamide estimates improved after energy intake adjustment (Ferrari *et al*, 2013). Another limitation of our study is that 718 EC cases were not classified in any of the EC subtypes; however, as has been previously mentioned, a large proportion ($\approx 80\%$) of endometrial carcinomas are thought to be type I (Amant *et al*, 2005). Finally, it should to be kept in mind that several subgroups have been examined in this study; thus, some of the observed results might be due to chance.

In conclusion, the results of the present study indicate that there were no associations between dietary intake of acrylamide and risk of overall EC or type-I EC; nevertheless, women with elevated acrylamide intake (upper quintile median, 44 μg per day) who both never smoked and never used OCs at baseline, were at higher risk of developing type-I EC relative to women with the lowest intakes. Additional studies with biomarkers of internal dose of acrylamide exposure are needed in order to better understand the associations observed.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DISCLAIMER

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