UNIVERSITAT DE BARCELONA

Dietary intake and biomarkers of acrylamide exposure and risk of endometrial and ovarian cancer

A molecular epidemiologic study in the European Prospective Investigation into Cancer and Nutrition

Mireia Obón Santacana

RESULTS

5. RESULTS

The present chapter introduces the five publications presented in this thesis, which have been published in international journals. Each publication is preceded by a brief summary in Catalan.

Table 10. Impact factor, category, and journal rank of the articles presented in this thesis

Articles Reference[1]	IF[2]	Category & Journal rank[2]
Obón-Santacana, M. *et al.* Dietary intake of acrylamide and endometrial cancer risk in the European Prospective Investigation into Cancer and Nutrition cohort. *Br. J. Cancer* **111,** 987–997 (2014).	4.836	Oncology - Quartile 1
Obón-Santacana, M. *et al.* Dietary intake of acrylamide and epithelial ovarian cancer risk in the european prospective investigation into cancer and nutrition (EPIC) cohort. *Cancer Epidemiol. Biomark. Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol.* **24,** 291–297 (2015).	4.125	Public, environmental & occupational health - Quartile 1
Obón-Santacana, M. *et al.* Dietary and lifestyle determinants of acrylamide and glycidamide hemoglobin adducts in non-smoking postmenopausal women from the EPIC cohort. *Eur. J. Nutr.* (2016). doi:10.1007/s00394-016-1165-5	3.467	Nutrition & dietetics – Quartile 1
Obón-Santacana, M. *et al.* Acrylamide and glycidamide hemoglobin adduct levels and endometrial cancer risk: A nested case-control study in nonsmoking postmenopausal women from the EPIC cohort. *Int. J. Cancer* **138,** 1129–1138 (2016).	5.085	Oncology - Quartile 1
Obón-Santacana, M. *et al.* Acrylamide and Glycidamide Hemoglobin Adducts and Epithelial Ovarian Cancer: A Nested Case-Control Study in Nonsmoking Postmenopausal Women from the EPIC Cohort. *Cancer Epidemiol. Biomark. Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol.* **25,** 127–134 (2016).	4.125	Public, environmental & occupational health - Quartile 1

IF; Impact Factor

1. Ordered by thesis presentation
2. Accessed date: 10 June 2016 (Journal Citation Reports ® of the ISI Web of Knowledge℠, Thompson Reuters)

5.1 Publication 1: Dietary intake of acrylamide and endometrial cancer risk in the European Prospective Investigation into Cancer and Nutrition cohort

5.1.1 Resum

L'associació entre la ingesta d'acrilamida i el risc de patir càncer d'endometri només s'ha avaluat en tres estudis prospectius, els resultats dels quals han estat inconsistents.

L'objectiu del present article era estudiar l'associació entre la ingesta d'acrilamida i el risc de càncer d'endometri (càncer d'endometri general i càncer d'endometri de tipus-I). Degut a que l'hàbit de fumar està considerat com una font molt important d'acrilamida, aquesta associació també es va avaluar entre les dones no fumadores. D'altra banda, és àmpliament reconegut que l'ús d'anticonceptius orals (aquells que combinen estròsgens i progesterona) disminueix el risc de càncer d'endometri, i és per aquest motiu, que també es va avaluar aquesta associació en dones que no van prendre anticonceptius orals.

Després d'un seguiment d'11 anys, es van identificar 1,382 casos de càncer d'endometri (CE), dels quals 627 van ser de tipus-I. Es va utilitzar el model de regressió de Cox per estimar els *hazard ratios* (HR) i els intervals de confiança (95% CI) resultants de l'associació entre la ingesta d'acrilamida i el risc de CE en l'estudi EPIC. L'estimació de la ingesta d'acrilamida es va obtenir a partir de la base de dades europea que monitoritza els nivells d'acrilamida i es va harmonitzar amb les dades de consum alimentari (basats en qüestionaris de dieta) de l'estudi EPIC. La ingesta d'acrilamida es va ajustar per l'energia total utilitzant el mètode residual.

En aquest estudi no es va observar cap associació entre la ingesta d'acrilamida i el risc de patir CE (tant general com de tipus-I). Tanmateix, es va observar un increment del risc relatiu de patir CE de tipus-I a mesura que els nivells d'ingesta d'acrilamida augmentaven entre aquelles dones que eren no fumadores i que no havien pres anticonceptius orals (HR $_{Q5vsQ1}$: 1.97, 95% IC: 1.08-3.62; *P*-valor LRT:0.01, n=203).

Així doncs, basat en l'estudi EPIC, la ingesta d'acrilamida no està associada amb el risc de CE en general i de tipus-I. No obstant, es va observar una associació positiva i estadísticament significativa (pel que fa al CE de tipus-I) en aquelles dones no fumadores i que no eren usuàries d'anticonceptius orals.

Paper 1

Obón-Santacana M, Kaaks R, Slimani N, Lujan-Barroso L, Freisling H, Ferrari P, Dossus L, Chabbert-Buffet N, Baglietto L, Fortner RT, Boeing H, Tjønneland A, Olsen A, Overvad K, Menéndez V, Molina-Montes E, Larrañaga N, Chirlaque M-D, Ardanaz E, Khaw K-T, Wareham N, Travis RC, Lu Y, Merritt MA, Trichopoulou A, Benetou V, Trichopoulos D, Saieva C, Sieri S, Tumino R, Sacerdote C, Galasso R, Bueno-de-Mesquita HB, Wirfält E, Ericson U, Idahl A, Ohlson N, Skeie G, Gram IT, Weiderpass E, Onland-Moret NC, Riboli E, Duell EJ

Dietary intake of acrylamide and endometrial cancer risk in the European Prospective Investigation into Cancer and Nutrition cohort

British Journal of Cancer. 111, 987–997 (2014).

FULL PAPER

BJC

British Journal of Cancer (2014) 111, 987–997 | doi: 10.1038/bjc.2014.328

Keywords: acrylamide; endometrial cancer; type-I endometrial cancer; cohort; nutrition

Dietary intake of acrylamide and endometrial cancer risk in the European Prospective Investigation into Cancer and Nutrition cohort

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Received 14 February 2014; revised 12 May 2014; accepted 14 May 2014; published online 17 June 2014

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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 $\geqslant 26$ cigarettes per day, former cigarette smokers who quit >20 years, 11–20 years, or $\leqslant 10$ 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 $\geqslant 56$ years, surgical menopause, postmenopausal women with missing age at menopause), parity (nulliparous, 1, 2, $\geqslant 3$, parous but with missing number of full-term pregnancies), and age at menarche (<12, 12, 13, 14, and $\geqslant 15$ 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 ($<25\,kg\,m^{-2}$, $\geqslant 25\,kg\,m^{-2}$), OC use (never, ever), HRT use (never, ever), baseline menopausal status (premenopausal, perimenopausal, 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' $\geqslant 21\,\mu g$ 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 $10–41\,\mu g$ per day ($0.2–0.6\,\mu g$ 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 HR (95% CI)[b]	1382 0.98 (0.92–1.05)	277 1.00 (ref)	271 1.05 (0.86–1.29)	298 1.11 (0.90–1.36)	250 0.88 (0.71–1.10)	286 0.98 (0.78–1.25)	0.53	
Cases diagnosed ⩾2 years after recruitment								
N cases HR (95% CI)[b]	1186 0.98 (0.91–1.05)	240 1.00 (ref)	217 0.97 (0.78–1.20)	268 1.12 (0.89–1.39)	215 0.85 (0.67–1.08)	246 0.95 (0.74–1.23)	0.52	
Overall EC – stratified analyses								
Smoking status								
Never smokers								
N cases HR (95% CI)[c]	747 0.97 (0.89–1.05)	147 1.00 (ref)	142 1.03 (0.79–1.34)	153 1.04 (0.79–1.36)	132 0.82 (0.61–1.10)	173 1.01 (0.75–1.38)	0.90	
Ever smokers[d]								0.20
N cases HR (95% CI)[c]	587 0.98 (0.89–1.08)	123 1.00 (ref)	118 1.08 (0.80–1.45)	135 1.23 (0.91–1.66)	110 0.96 (0.69–1.33)	101 0.86 (0.60–1.24)	0.23	
OC use								
Non-OC users								
N cases HR (95% CI)[e]	800 1.03 (0.94–1.12)	180 1.00 (ref)	155 1.07 (0.83–1.38)	165 1.09 (0.84–1.42)	127 0.83 (0.62–1.11)	173 1.17 (0.86–1.58)	0.51	
OC users								0.03
N cases HR (95% CI)[e]	547 0.92 (0.83–1.02)	94 1.00 (ref)	111 1.05 (0.76–1.46)	121 1.16 (0.83–1.61)	117 0.97 (0.68–1.39)	104 0.79 (0.53–1.15)	0.08	
Smoking status combined with OC use								
Never smokers and non-OC users								
N cases HR (95% CI)[f]	477 1.02 (0.92–1.13)	106 1.00 (ref)	90 1.05 (0.76–1.44)	94 1.08 (0.77–1.50)	75 0.82 (0.57–1.18)	112 1.28 (0.88–1.85)	0.24	
Ever smokers[d] and non-OC users								
N cases HR (95% CI)[f]	299 1.02 (0.89–1.17)	68 1.00 (ref)	58 1.09 (0.73–1.65)	68 1.28 (0.84–1.95)	47 0.87 (0.55–1.39)	58 0.98 (0.60–1.60)	0.65	
Never smokers and OC users								0.04
N cases HR (95% CI)[f]	253 0.89 (0.77–1.03)	39 1.00 (ref)	49 1.03 (0.64–1.67)	52 0.98 (0.60–1.61)	54 0.83 (0.50–1.40)	59 0.73 (0.42–1.26)	0.13	
Ever smokers[d] and OC users								
N cases HR (95% CI)[f]	277 0.93 (0.80–1.08)	54 1.00 (ref)	58 1.10 (0.71–1.69)	63 1.22 (0.78–1.90)	60 1.07 (0.67–1.71)	42 0.76 (0.44–1.30)	0.22	
Alcohol intake								
Never drinkers								
N cases HR (95% CI)[b]	253 1.06 (0.91–1.24)	70 1.00 (ref)	59 0.95 (0.62–1.46)	38 0.72 (0.44–1.18)	35 0.59 (0.35–1.00)	51 1.03 (0.60–1.76)	0.76	
Ever drinkers								0.07
N cases HR (95% CI)[b]	1129 0.97 (0.90–1.04)	207 1.00 (ref)	212 1.10 (0.87–1.39)	260 1.27 (1.00–1.61)	215 0.96 (0.75–1.24)	235 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	
$\geqslant 25\,kg\,m^{-2}$								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 (⩽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 HR (95% CI)[h]	253 0.88 (0.74–1.04)	67 1.00 (ref)	54 1.12 (0.72–1.74)	52 1.12 (0.70–1.78)	45 1.00 (0.61–1.64)	35 0.68 (0.37–1.22)	0.17	
Perimenopausal								0.05
N cases HR (95% CI)[h]	268 1.05 (0.89–1.23)	51 1.00 (ref)	56 1.08 (0.69–1.70)	73 1.29 (0.82–2.04)	44 0.83 (0.50–1.39)	44 1.18 (0.67–2.10)	0.90	
Postmenopausal[i]								
N cases HR (95% CI)[h]	861 1.01 (0.93–1.10)	159 1.00 (ref)	161 1.05 (0.80–1.38)	173 1.06 (0.80–1.40)	161 0.84 (0.62–1.13)	207 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	LRT *P*-value[a]
Final model – Type I								
N cases HR (95% CI)[b]	627 0.98 (0.90–1.07)	105 1.00 (ref)	111 1.00 (0.74–1.35)	125 1.04 (0.77–1.42)	122 0.87 (0.63–1.21)	164 0.97 (0.69–1.36)	0.79	
Cases diagnosed ⩾2 years after recruitment								
N cases HR (95% CI)[b]	556 0.96 (0.87–1.06)	98 1.00 (ref)	93 0.89 (0.65–1.23)	117 1.04 (0.76–1.43)	107 0.84 (0.60–1.19)	141 0.93 (0.65–1.32)	0.75	
Type I – stratified analyses								
Smoking status								
Never smokers								
N cases HR (95% CI)[c]	350 1.06 (0.95–1.19)	56 1.00 (ref)	54 0.97 (0.63–1.48)	67 1.14 (0.74–1.74)	69 0.97 (0.62–1.51)	104 1.25 (0.79–1.98)	0.21	
Ever smokers[d]								0.01
N cases HR (95% CI)[c]	257 0.90 (0.78–1.03)	44 1.00 (ref)	51 1.02 (0.64–1.63)	55 1.00 (0.62–1.62)	50 0.80 (0.48–1.34)	57 0.70 (0.41–1.19)	0.09	
OC use								
Non-OC users								
N cases HR (95% CI)[e]	347 1.10 (0.99–1.23)	65 1.00 (ref)	56 0.96 (0.64–1.45)	65 1.09 (0.71–1.67)	58 0.90 (0.57–1.42)	103 1.40 (0.89–2.22)	0.06	
OC users								0.01
N cases HR (95% CI)[e]	273 0.83 (0.71–0.95)	39 1.00 (ref)	54 0.97 (0.62–1.51)	59 0.93 (0.59–1.47)	63 0.79 (0.49–1.28)	58 0.57 (0.34–0.96)	0.01	
Smoking status combined with OC use								
Never smokers and non-OC users								
N cases HR (95% CI)[f]	203 1.17 (1.02–1.34)	35 1.00 (ref)	29 1.03 (0.58–1.81)	36 1.28 (0.72–2.27)	35 1.12 (0.61–2.06)	68 1.97 (1.08–3.62)	0.01	
Ever smokers[d] and non-OC users								
N cases HR (95% CI)[f]	134 1.04 (0.86–1.26)	26 1.00 (ref)	25 0.99 (0.51–1.91)	27 0.99 (0.50–1.98)	21 0.76 (0.36–1.62)	35 1.01 (0.47–2.19)	0.98	
Never smokers and OC users								0.01
N cases HR (95% CI)[f]	145 0.89 (0.73–1.09)	20 1.00 (ref)	25 0.76 (0.40–1.45)	31 0.83 (0.44–1.59)	33 0.68 (0.35–1.35)	36 0.59 (0.29–1.21)	0.17	
Ever smokers[d] and OC users								
N cases HR (95% CI)[f]	120 0.75 (0.60–0.94)	18 1.00 (ref)	25 1.02 (0.52–1.99)	27 1.00 (0.50–1.98)	29 0.84 (0.41–1.72)	21 0.45 (0.20–1.00)	0.02	
Alcohol intake								
Never drinkers								
N cases HR (95% CI)[b]	103 1.23 (1.02–1.47)	28 1.00 (ref)	19 0.76 (0.40–1.44)	13 0.61 (0.29–1.28)	17 0.93 (0.46–1.89)	26 1.77 (0.86–3.64)	0.07	
Ever drinkers								0.01
N cases HR (95% CI)[b]	524 0.93 (0.85–1.03)	77 1.00 (ref)	92 1.09 (0.77–1.54)	112 1.19 (0.83–1.69)	105 0.90 (0.61–1.31)	138 0.91 (0.62–1.35)	0.30	
Body mass index								
$<25\,kg\,m^{-2}$								
N cases HR (95% CI)[g]	256 0.86 (0.74–1.00)	43 1.00 (ref)	48 0.88 (0.56–1.38)	62 1.11 (0.71–1.73)	53 0.78 (0.48–1.27)	50 0.56 (0.33–0.96)	0.02	
$\geqslant 25\,kg\,m^{-2}$								0.28
N cases HR (95% CI)[g]	371 1.06 (0.95–1.18)	62 1.00 (ref)	63 1.12 (0.75–1.69)	63 0.99 (0.64–1.52)	69 0.92 (0.59–1.44)	114 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 HR (95% CI)[h]	120 0.78 (0.62–0.99)	28 1.00 (ref)	25 0.89 (0.48–1.64)	26 0.91 (0.49–1.71)	24 0.78 (0.40–1.53)	17 0.52 (0.24–1.13)	0.09	
Perimenopausal								0.01
N cases HR (95% CI)[h]	120 0.88 (0.70–1.12)	24 1.00 (ref)	25 0.77 (0.41–1.43)	32 0.91 (0.49–1.68)	20 0.67 (0.33–1.36)	19 0.59 (0.26–1.31)	0.22	
Postmenopausal[i]								
N cases HR (95% CI)[h]	387 1.07 (0.96–1.18)	53 1.00 (ref)	61 1.24 (0.81–1.89)	67 1.25 (0.81–1.95)	78 1.09 (0.69–1.72)	128 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 $<25\,\mathrm{kg\,m^{-2}}$; 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.

ACKNOWLEDGEMENTS

The author's responsibilities were as follows: ER, RK, NS, LL-B, HF, PF, LD, NC-B, LB, RTF, HB, A Tjønneland, AO, VM, EM-M, NL, M-DC, EA, K-TK, NW, RCT, YL, MAM, A Trichopoulou, VB, DT, CS, SS, RT, CS, RG, HBB-d-M, E Wirfält, UE, AI, NO, GS, ITG, E Weiderpass, and NCO-M: designed and conducted the multicenter EPIC cohort study. NS, EJD, RK, and MO-S: conducted the research. MO-S: analysed the data. MO-S and EJD: wrote the manuscript. MO-S and EJD: had primary responsibility for the final content. MO-S is affiliated with the University of Barcelona. All authors read and approved the final version of the manuscript. This work was partially supported by the Wereld Kanker Onderzoek Fonds (WCRF NL) (grant WCRF 2011/442) and by the Health Research Fund (FIS) of the Spanish Ministry of Health (Exp PI11/01473). The coordination of EPIC is financially supported by the European Commission (DG-SANCO) and the International Agency for Research on Cancer. The national cohorts are supported by the Health Research Fund (FIS) of the Spanish Ministry of Health, Regional Governments of Andalucía, Asturias, Basque Country, Murcia (no. 6236), Navarra and the Catalan Institute of Oncology, La Caixa (BM 06-130), Red Temática de Investigación Cooperativa en Cáncer (RD12/0036/0018; RD06/0020/0091) (Spain); Danish Cancer Society (Denmark); Ligue contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l'Education Nationale, Institut National de la Santé et de la Recherche Médicale (INSERM, France); Deutsche Krebshilfe, Deutsches Krebsforschungszentrum (DKFZ) and Federal Ministry of Education and Research (Germany); the Hellenic Health Foundation (Greece); Associazione Italiana per la Ricerca sul Cancro (AIRC) and National Research Council (Italy); Dutch Ministry of Public Health, Welfare and Sports (VWS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF) and Statistics Netherlands (The Netherlands); Nordic Center of Excellence in Food, Nutrition and Health-Helga (Norway); Swedish Cancer Society, Swedish Scientific Council and Regional Government of Skåne and Västerbotten (Sweden); Cancer Research UK, Medical Research Council (United Kingdom).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DISCLAIMER

None of the funding agencies had a role in the design, implementation, analysis or interpretation of study results.

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5.2 Publication 2: Dietary intake of acrylamide and epithelial ovarian cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort

5.2.1 Resum

L'Agència Internacional d'Investigació del Càncer (IARC) va classificar, l'any 1994, l'acrilamida com a 'probable carcinogen' pels humans (Grup 2A). L'acrilamida va ser descoberta l'any 2002 en alguns aliments rics en hidrats de carboni que havien estat tractats tèrmicament. L'associació entre la ingesta d'acrilamida i el risc de càncer d'ovari epitelial (COE) ha estat estudiada prèviament en un estudi de casos i controls i en tres estudis prospectius. Els resultats derivats d'aquests quatre estudis son incongruents i, a mes a més, no van poder examinar aquesta associació segons els diferents tipus histològics de COE.

Aquest estudi prospectiu, que inclou 325,006, està emmarcat dins l' Estudi Prospectiu Europeu sobre Càncer i Nutrició (EPIC). Després d'un seguiment d'11 anys, es van diagnosticar 1,191 casos de COE. Es va utilitzar el model de regressió de Cox per estimar els *hazard ratios* (HR) i els intervals de confiança (95% CI) resultants de l'associació entre la ingesta d'acrilamida i el risc de COE. La ingesta d'acrilamida es va ajustar per l'energia total utilitzant el mètode residual, i es va analitzar tant en forma continua (per 10 μg/dia) com en quintils. Quan es va estudiar l'associació segons els diferents tipus histològics de COE, l'acrilamida es va analitzar en quartils.

La mediana de la ingesta d'acrilamida basal en aquest estudi va ser de 21.3 μg/dia. No es van trobar associacions ni cap evidència de dosis-resposta entre la ingesta d'acrilamida i el risc de patir COE ($HR_{per\ 10\mu g/dia}$: 1.02, 95% CI: 0.96-1.09; HR_{Q5vsQ1}: 0.97; 95% CI:0.76-1.23). Tampoc es va observar cap associació quan es van analitzar per separat els diferents subtipus histològics de COE (582 serosos, 118 endometrioides i 79 tumors mucinosos).

Resumint: aquest estudi no va evidenciar que la ingesta d'acrilamida (basada en qüestionaris d'ingesta alimentària) estigués associada amb el risc de desenvolupar COE. Es requereixen més estudis que utilitzin una estimació de la ingesta d'acrilamida més fiable, com per exemple biomarcadors, per concloure que l'acrilamida no està associada amb el càncer d'ovari.

Paper 2

Obón-Santacana M, Peeters PH, Freisling H, Dossus L, Clavel-Chapelon F, Baglietto L, Schock H, Fortner RT, Boeing H, Tjonneland A, Olsen A, Overvad K, Menéndez V, Sanchez M-J, Larranaga N, Huerta Castaño JM, Barricarte A, Khaw K-T, Wareham N, Travis RC, Merritt MA, Trichopoulou A, Trichopoulos D, Orfanos P, Masala G, Sieri S, Tumino R, Vineis P, Mattiello A, Bueno-de-Mesquita HB, Onland-Moret NC, Wirfalt E, Stocks T, Idahl A, Lundin E, Skeie G, Gram IT, Weiderpass E, Riboli E, Duell EJ

Dietary intake of acrylamide and epithelial ovarian cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort

Cancer Epidemiology Biomarkers & Prevention. 24, 291–297 (2015).

Short Communication

Cancer Epidemiology, Biomarkers & Prevention

Dietary Intake of Acrylamide and Epithelial Ovarian Cancer Risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) Cohort

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Abstract

Acrylamide, classified in 1994 by the International Agency for Research on Cancer (IARC) as "probably carcinogenic" to humans, was discovered in 2002 in some heat-treated, carbohydrate-rich foods. The association between dietary acrylamide intake and epithelial ovarian cancer risk (EOC) has been previously studied in one case–control and three prospective cohort studies which obtained inconsistent results and could not further examine histologic subtypes other than serous EOC. The present study was carried out in the European Prospective Investigation into Cancer and Nutrition (EPIC) subcohort of women (n = 325,006). Multivariate Cox proportional hazards models were used to assess the association between questionnaire-based acrylamide intake and EOC risk. Acrylamide was energy-adjusted using the residual method and was evaluated both as a continuous variable (per 10 μg/d) and in quintiles; when subgroups by histologic EOC subtypes were analyzed, acrylamide intake was evaluated in quartiles. During a mean follow-up of 11 years, 1,191 incident EOC cases were diagnosed. At baseline, the median acrylamide intake in EPIC was 21.3 μg/d. No associations and no evidence for a dose–response were observed between energy-adjusted acrylamide intake and EOC risk ($HR_{10\mu_{g/d}}$, 1.02; 95% CI, 0.96–1.09; HR_{Q5vsQ1}, 0.97; 95% CI, 0.76–1.23). No differences were seen when invasive EOC subtypes (582 serous, 118 endometrioid, and 79 mucinous tumors) were analyzed separately. This study did not provide evidence that acrylamide intake, based on food intake questionnaires, was associated with risk for EOC in EPIC. Additional studies with more reliable estimates of exposure based on biomarkers may be needed. *Cancer Epidemiol Biomarkers Prev; 24(1); 291–7. ©2014 AACR.*

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Introduction

Acrylamide has been classified as "probably carcinogenic to humans" by the International Agency for Research on Cancer (IARC; group 2A) since 1994 (1); however, public health concern increased when Swedish researchers reported acrylamide in common carbohydrate-rich foods treated at high temperatures (e.g., fried potatoes, potato crisps, bread, and crisp bread; ref. 2). In the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, the major dietary sources of acrylamide (based on a 24-hour dietary recall; DR) came from bread, rusks, coffee, potatoes, cakes, biscuits, and cookies (3). An important nondietary source of exposure is cigarette smoking. It is known that smokers have higher mean circulating acrylamide hemoglobin adducts levels than nonsmokers (4).

Hormone-related tumors and other tumors have been identified in rodents after oral administration of acrylamide (5). In humans, acrylamide is neurotoxic, and it has been hypothesized that it may also have hormonal effects (6); however, acrylamide is thought to play a role in cancer risk by means of its metabolite glycidamide. The conversion of acrylamide to glycidamide (a chemically reactive epoxide and mutagen in animals) is mediated by the Cyp2e1 enzyme system (7).

One case–control study and 3 prospective cohort studies have evaluated the association between dietary acrylamide intake and epithelial ovarian cancer (EOC), but results were inconsistent. Both the Italian case–control study (8) and the prospective Swedish Mammography Cohort (SMC; ref. 9) study reported null associations, the Nurses' Health Study (NHS) suggested an increased risk for serous tumors [HR_{Q5vsQ1}, 1.58; 95% confidence interval (CI), 0.99–2.52; $P_{trend} = 0.04$] and for serous invasive tumors (HR_{Q5vsQ1}, 1.67; 95% CI, 0.99–2.81; $P_{trend} = 0.04$; ref. 10), whereas the Netherlands Cohort Study (NLCS) reported positive associations for overall EOC (HR_{Q5vsQ1}, 1.78; 95% CI, 1.10–2.88; $P_{trend} = 0.02$; ref. 11). The NHS included in the analyses both borderline and invasive tumors, whereas in the NLCS and SMC studies, all borderline tumors were excluded. The Italian case–control study did not report associations by tumor invasiveness (8–11).

The present study evaluated the association between questionnaire-based intake of acrylamide and the risk of overall EOC. Given that there are risk factor and clinical behavior differences between histologic subtypes (12–14), we also evaluated the association between acrylamide intake and serous, endometrioid, and mucinous subtypes and tumor invasiveness. Secondary objectives were to determine whether this association differed by smoking status (with the intention to remove acrylamide exposure due to smoking), oral contraceptive (OC) use (a strong protective factor for EOC risk; ref. 15), and other baseline participant characteristics.

Materials and Methods

Study population

The EPIC study enrolled participants between 1992–1998 in 23 centers from 10 European countries. All participants signed an informed consent, and ethical review boards from the IARC and local centers authorized the study. The EPIC methodology has been described in detail by Riboli and colleagues. Participants reported information on lifestyle, reproductive, and anthropometric factors at baseline. Dietary intake was also assessed at baseline through validated country-specific dietary questionnaires (DQ; ref. 16).

The EPIC study recruited 521,330 participants, of which 367,903 are women. Women were excluded from the current analyses because they had prevalent cancer other than nonmelanoma skin cancer ($n = 19{,}853$), had a bilateral oophorectomy ($n = 10{,}404$), had incomplete follow-up data ($n = 2{,}896$), had no lifestyle or dietary information ($n = 3{,}239$), no information on dietary intake of acrylamide at baseline ($n = 3$), or had an extreme ratio of energy intake to energy required ($n = 6{,}502$); resulting in 325,006 participants for this analysis.

Follow-up was estimated until cancer diagnosis (except nonmelanoma skin cancer), emigration, death, or until the end of follow-up (centers dates vary from December 2004 to June 2010).

Incident EOC was assessed via population cancer registries or via a combination of methods (health insurance records, cancer and pathology registries, and active follow-up; ref. 16). Incident EOC included ovarian, fallopian tube, and primary peritoneal cancers, classified according to the International Classification of Diseases 10th revision as C56.9, C57.0, and C48, respectively.

Overall EOC comprised borderline ($n = 96$; 8%) and invasive tumors ($n = 1{,}095$; 92%). Invasive EOC were classified as serous ($n = 582$, 53%), not otherwise specified (NOS; $n = 249$, 23%; NOS included adenocarcinomas, carcinomas, and cystadenocarcinoma), endometrioid ($n = 118$, 11%), mucinous ($n = 79$, 7%), clear cell ($n = 51$, 5%), and other tumors ($n = 16$, 1%).

Acrylamide intake assessment

Details of the EPIC acrylamide database have been previously published (17, 18). Briefly, a harmonized acrylamide database was compiled using mean acrylamide levels in foods mainly derived from the EU monitoring database maintained by the European Community Institute for Reference Materials and

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doi: 10.1158/1055-9965.EPI-14-0636

Measurements (IRMM; http://ec.europa.eu/food/food/chemicalsafety/contaminants/acrylamide_en.htm). The DQ items, and when available, their specific description (e.g., "baked potatoes") were matched with the acrylamide database.

Statistical analysis

Cox proportional hazards models were used to estimate HRs and 95% CIs for acrylamide intake and EOC risk. Acrylamide intake was energy-adjusted using the residual method (19) and was analyzed both as a continuous variable (10 μg/d; average daily intake in 10-μg increments) and as quintiles of intake (μg/d) based on the distribution of acrylamide intake in the EPIC subcohort of women at baseline. Analyses were also performed by histologic subtypes. Because of the number of cases, quartiles of acrylamide intake (μg/d) were used to analyze subgroups by histologic subtype.

All models had age at the time scale and were stratified by study center to control for center effects (i.e., questionnaire design and follow-up procedures) and by age at recruitment (1-year categories).

Multivariable models were adjusted for body mass index (BMI), smoking status, OC use, baseline menopausal status combined with age at menopause, parity, age at menarche, and energy intake. If needed, missing values were categorized and included as a separate category in the analyses. Additional covariates were evaluated but were not included in models because they did not change the HR by >10%: age at first menstrual period (years), duration of using OC (years), hormone replacement therapy (HRT) use (yes, no, unknown), duration of using HRT (years), alcohol (nonconsumers, consumers), education level (none, primary, technical/professional, secondary, and higher education), physical activity using the Cambridge index (20), waist-to-hip ratio, total fats (g/d), total carbohydrates (g/d), vegetables (g/d), and coffee (mL/d).

Stratified analyses were carried out by smoking status (an important source of acrylamide), OC use (a protective factor for EOC risk), alcohol intake, and BMI (which may both affect the activity of Cyp2e1, important in acrylamide metabolism; ref. 3), and by geographical region (Northern: France, the United Kingdom, The Netherlands, Germany, Sweden, Denmark, and Norway; Southern: Italy, Spain, and Greece). Sensitivity analyses excluding the first 2 years of follow-up were performed with the aim to minimize the influence of preclinical disease on dietary habits.

The median value for each acrylamide quartile or quintile was estimated and included in a score test to evaluate dose–response trends. The proportional hazards (PH) assumption, assessed using Schoenfeld residuals (21), was met for all the analyses. All analyses were performed using SAS v. 9.1; STATA was used to test the PH assumption.

Results

After a mean follow-up of 11 years, there were 1,191 incident EOC cases. In the present subcohort, the median acrylamide intake at baseline was 21.3 μg/d, and the 25th to 75th percentile range was 14.7–30.4 μg/d (mean and SD acrylamide intake: 23.8 ± 13.0 μg/d). The highest median intakes were found in Denmark, the United Kingdom, and the Netherlands, whereas Italy and Norway had the lowest median intakes (Table 1). The mean age at diagnosis was 61 years. Description of baseline characteristics of the current cohort of women can be found in Table 2.

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

Country	Cohort sample	Person-years	Acrylamide, μg/d	Acrylamide[a], μg/d	Acrylamide, μg/kg body weight/d	EOC cases	Invasive EOC cases by histologic subtype					
			Median (QR)	Median (QR)	Median (QR)	*n* (%)	Serous *n* (%)	Mucinous *n* (%)	Endometrioid *n* (%)	Clear cell *n* (%)	NOS *n* (%)	Others *n* (%)
France	65,538	680,305	19.2 (14.3–25.2)	17.7 (14.0–21.9)	0.3 (0.2–0.4)	159 (13.4)	97 (16.7)	15 (19.0)	14 (11.9)	2 (3.9)	9 (3.6)	6 (37.5)
Italy	29,277	327,642	9.7 (6.5–13.8)	8.6 (5.4–11.8)	0.2 (0.1–0.2)	104 (8.7)	56 (9.6)	8 (10.1)	14 (11.9)	3 (5.9)	15 (6.0)	1 (6.3)
Spain	23,508	283,562	18.4 (11.9–26.9)	19.5 (14.1–26.2)	0.3 (0.2–0.4)	68 (5.7)	32 (5.5)	3 (3.8)	10 (8.5)	5 (9.8)	6 (2.4)	2 (12.5)
United Kingdom	50,858	567,697	30.6 (22.4–40.9)	31.2 (24.2–39.7)	0.5 (0.3–0.7)	211 (17.7)	73 (12.5)	11 (13.9)	15 (12.7)	14 (27.5)	74 (29.7)	4 (25.0)
The Netherlands	26,074	306,436	29.1 (21.3–38.4)	29.7 (23.1–38.0)	0.4 (0.3–0.6)	105 (8.8)	55 (9.5)	6 (7.6)	10 (8.5)	4 (7.8)	20 (8.0)	—
Greece	14,376	140,157	17.6 (12.9–23.4)	18.9 (15.3–23.1)	0.3 (0.2–0.3)	37 (3.1)	12 (2.1)	1 (1.3)	3 (2.5)	2 (3.9)	17 (6.8)	1 (6.3)
Germany	26,571	264,226	22.4 (16.9–29.6)	23.6 (19.1–29.7)	0.3 (0.2–0.4)	82 (6.9)	53 (9.1)	7 (8.9)	8 (6.8)	—	9 (3.6)	—
Sweden	26,375	349,308	20.6 (15.8–26.9)	22.6 (18.7–27.0)	0.3 (0.2–0.4)	137 (11.5)	50 (8.6)	14 (17.7)	10 (8.5)	8 (15.7)	53 (21.3)	2 (12.5)
Denmark	27,403	302,433	34.5 (27.5–42.3)	34.7 (28.4–41.5)	0.5 (0.4–0.6)	140 (11.8)	76 (13.1)	8 (10.1)	18 (15.3)	8 (15.7)	30 (12.0)	—
Norway	35,026	340,876	17.4 (13.6–21.5)	20.2 (16.8–23.7)	0.3 (0.2–0.3)	148 (12.4)	78 (13.4)	6 (7.6)	16 (13.6)	5 (9.8)	16 (6.4)	—
Total	325,006	3,562,642	21.3 (14.7–30.4)	21.9 (16.0–29.8)	0.3 (0.2–0.5)	1,191	582	79	118	51	249	16

Abbreviations: NOS, not otherwise specified; QR, quartile range (25th–75th percentile).
[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 (325,006 women)

	Energy-adjusted acrylamide intake, μg/d				
	<14.6	**14.7–19.6**	**19.7–24.4**	**24.5–32.3**	**32.4–222.4**
Participants	65,001	65,001	65,002	65,001	65,001
EOC cases	221	207	219	280	264
Energy-adjusted acrylamide intake[a], μg/d	10.8 (7.6–13.0)	17.2 (16.0–18.4)	21.9 (20.7–23.1)	27.7 (25.9–29.8)	39.5 (35.4–45.9)
Age at recruitment[a]	51.0 (45.5–57.1)	50.4 (45.3–56.9)	50.2 (44.5–56.6)	50.6 (43.8–57.5)	51.7 (43.5–58.0)
Age at menopause[a,b]	50.0 (47.0–52.0)	50.0 (47.0–52.0)	50.0 (46.0–52.0)	50.0 (46.0–52.0)	50.0 (46.0–52.0)
Menopausal status at baseline (%)					
Premenopausal	35.0	34.1	36.1	37.4	36.9
Postmenopausal	45.2	43.8	42.6	44.2	47.5
Perimenopausal	19.8	22.1	21.3	18.5	15.7
Ever use of OCs (%)					
Yes	49.07	55.63	58.11	61.38	64.89
Unknown	0.64	2.42	4.32	3.70	1.71
Parity (%)					
Nulliparous	12.2	11.9	12.6	16.0	19.4
1 child	17.58	14.61	13.65	13.39	13.39
2 children	41.57	39.94	38.64	36.52	36.02
≥3 children	25.35	27.29	26.36	25.03	23.64
Parous but with missing number of full-term pregnancies	0.4	0.9	1.6	3.3	5.3
Unknown	2.9	5.4	7.1	5.7	2.3
Smoking status (%)					
Never	59.9	60.0	55.4	52.3	49.6
Former	19.5	20.9	23.0	24.3	25.4
Current	18.5	15.6	18.9	21.3	23.8
Unknown	2.2	3.4	2.8	2.1	1.2
Cigarettes per day[a,b] (smokers only)	11.0 (6.0–20.0)	10.0 (8.0–20.0)	10.0 (10.0–20.0)	10.0 (10.0–20.0)	15.0 (10.0–20.0)
Time since quitting smoking[a,b,c], y	12.5 (6.5–20.0)	14.5 (7.0–22.0)	14.5 (6.5–22.0)	14.5 (6.5–22.0)	14.0 (6.0–22.5)
BMI[a], kg/m^2	24.3 (21.9–27.4)	23.8 (21.6–26.8)	24.0 (21.8–27.0)	24.1 (21.9–27.1)	24.3 (22.0–27.3)
Energy[a], kcal/d	2,033.7 (1,684.4–2,444.0)	1,803.9 (1,487.8–2,167.6)	1,750.3 (1,441.8–2,113.0)	1,813.6 (1,509.0–2,172.1)	1,966.1 (1,655.0–2,335.1)

[a]Median and quartile range (25th–75th percentile).
[b]Percentage of women missing the following: age at menopause, 66%; number of cigarettes per day, 55%; and time since quitting smoking, 55%.
[c]Only in former smokers.

No associations were observed between energy-adjusted dietary intake of acrylamide and risk of EOC overall or by histologic subtypes (Table 3). Moreover, there was no evidence for linear dose–response trends (Table 3). Results remained unchanged when we excluded from the analyses those cases diagnosed during the first 2 years of follow-up (data not shown).

None of the stratified analyses by smoking status (never, ever smokers) or by OC use (never, ever users) showed an association between EOC risk and acrylamide intake. Likewise, no association was observed when subgroups by alcohol intake (never, ever drinkers), BMI (<25, ≥25 kg/m^2), or geographical region were evaluated. The same pattern was seen when these associations were analyzed for different histologic subtypes (serous, endometrioid, and mucinous tumors). Furthermore, to increase statistical power, we also evaluated serous tumors combined with tumors that were not specified (NOS) and endometrioid tumors with clear cell tumors; however, the estimates did not vary.

All models were also evaluated using acrylamide intake without energy adjustment using the residual method, and results were similar to those presented in Table 3 (data not shown).

Discussion

The present study did not find an association between acrylamide intake and EOC risk overall or in any of the histologic subtypes that were evaluated. Relative risks also remained unchanged when subgroups were analyzed.

The relation between dietary acrylamide intake and EOC risk has been previously evaluated in one case–control and 3 prospective cohort studies. Our results are in agreement with the Italian case–control (8) and SMC studies (9); moreover, average daily acrylamide intakes (23.33 ± 17.65 and 24.6 ± 7.6 μg/d, respectively) in these 2 studies were similar to the average reported in the current EPIC subcohort (23.8 ± 13.0 μg/d). In contrast to our findings, increased relative risks were observed in high acrylamide consumers in 2 cohort studies: the NLCS for the entire cohort and among never smoking women (11) and the NHS for serous tumors (10). It is noteworthy that compared with the present EPIC subcohort, both the NLCS and the NHS had similar acrylamide intake medians in the lowest quintiles (9.5 and 8.7 μg/d, respectively) to EPIC (9.8 μg/d); however, median intakes in the highest quintiles (36.8 and 25.1 μg/d, respectively) were somewhat lower than in EPIC (41.0 μg/d).

Strengths of this study are the prospective cohort design and the large sample size compared with previous studies which included 1,031 (8), 195 (11), 368 (9), and 416 (10) cases. This enabled us to further investigate specific histologic subtypes, such as serous and endometrioid tumors; nevertheless, we were unable to perform exhaustive analyses for clear cell and mucinous tumors. There are other limitations that should be noted. First, the estimation of dietary acrylamide consumption was based on DQs,

Table 3. HRs and 95% CIs for estimated dietary intake of acrylamide (energy-adjusted using the residual method) and EOC in EPIC

	Energy-adjusted acrylamide intake, μg/d						
		Quintiles					
	10-μg increments	<14.6	14.7–19.6	19.7–24.4	24.5–32.3	32.4–222.4	Trend test P^a
EOC							
n cases	1,191	221	207	219	280	264	
HR (95% CI)[b]	1.02 (0.96–1.09)	1.00 (ref)	0.89 (0.72–1.11)	0.87 (0.70–1.09)	1.08 (0.87–1.34)	0.97 (0.76–1.23)	0.73
Borderline							
n cases	96	15	19	27	23	12	
HR (95% CI)[b]	0.90 (0.71–1.13)	1.00 (ref)	1.29 (0.60–2.76)	1.75 (0.83–3.69)	1.55 (0.71–3.42)	0.82 (0.32–2.08)	0.56
Invasive							
n cases	1,095	206	188	192	257	252	
HR (95% CI)[b]	1.03 (0.97–1.10)	1.00 (ref)	0.87 (0.69–1.08)	0.81 (0.64–1.02)	1.04 (0.83–1.31)	0.97 (0.75–1.24)	0.60
Invasive							
Serous							
n cases	582	124	103	102	132	121	
HR (95% CI)[b]	0.98 (0.89–1.07)	1.00 (ref)	0.78 (0.59–1.05)	0.72 (0.53–0.98)	0.94 (0.69–1.28)	0.84 (0.60–1.17)	0.72
Not otherwise specified							
n cases	249	28	45	38	64	74	
HR (95% CI)[b]	1.09 (0.97–1.23)	1.00 (ref)	1.44 (0.83–2.50)	1.10 (0.61–1.96)	1.54 (0.88–2.69)	1.63 (0.92–2.90)	0.11
Serous combined with not otherwise specified							
n cases	831	152	148	140	196	195	
HR (95% CI)[b]	1.02 (0.95–1.10)	1.00 (ref)	0.90 (0.70–1.17)	0.79 (0.60–1.03)	1.05 (0.81–1.37)	1.00 (0.75–1.33)	0.52
Endometrioid							
n cases	118	27	20	19	29	23	
HR (95% CI)[b]	1.12 (0.93–1.36)	1.00 (ref)	0.77 (0.40–1.49)	0.67 (0.33–1.34)	1.01 (0.51–1.98)	0.72 (0.34–1.55)	0.61
Clear cell							
n cases	51	6	8	13	12	12	
HR (95% CI)[b]	0.92 (0.69–1.23)	1.00 (ref)	1.42 (0.42–4.73)	1.77 (0.54–5.80)	1.42 (0.41–4.91)	1.03 (0.29–3.74)	0.61
Endometrioid combined with clear cell							
n cases	169	33	28	32	41	35	
HR (95% CI)[b]	1.05 (0.89–1.23)	1.00 (ref)	0.89 (0.50–1.57)	0.88 (0.49–1.58)	1.07 (0.59–1.92)	0.76 (0.40–1.45)	0.45
Mucinous							
n cases	79	16	11	13	19	20	
HR (95% CI)[b]	1.17 (0.95–1.44)	1.00 (ref)	0.68 (0.29–1.60)	0.69 (0.29–1.65)	1.11 (0.48–2.55)	1.33 (0.54–3.28)	0.21

[a]All *P* values for trend are based on the quintile medians.

[b]Stratified by age at recruitment and center. Adjusted for total energy intake (1,000 kcal/d), 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, 11–20, or ≤10 years before recruitment), OC use (never, ever, unknown), menopause status combined with age at menopause (premenopausal, perimenopausal, postmenopausal with: <45, 45–49, 50–52, 53–55, ≥56 years, postmenopausal women with missing age at menopause), and parity (nulliparous, 1, 2, ≥3, parous but with missing number of full-term pregnancies, unknown).

and the correlation coefficient between DQs and a single 24-hour DR in EPIC was low (0.35 and 0.17 for crude and adjusted correlation coefficient, respectively; ref. 22). In addition, studies that evaluated correlation coefficients between acrylamide intake (based on DQs) and biomarkers of exposure measured as hemoglobin adducts have reported mixed results, with correlation ranging from 0.08 to 0.43, and with most of the studies falling on the lower end of the range, including EPIC (22–27). Thus, we included energy intake in all regression models, as based on a previous analysis in EPIC, acrylamide intake estimates improved after this adjustment (22). Second, misclassification of acrylamide exposure may exist, as information on cooking methodology was not available in some EPIC centers. Finally, we acknowledge that measurement error may be present in our dietary acrylamide estimates as a harmonized acrylamide database was used, and because DQs in EPIC were not specifically designed to assess dietary acrylamide exposure; nonetheless to reduce the impact of measurement error, estimates were energy-adjusted using the residual method (19), and all models were stratified by center with the intention to partially account for the variation in dietary patterns across the 10 EPIC countries.

This is the third questionnaire-based study to conclude that acrylamide intake is not associated with risk for EOC. Recently, the NHS conducted the first epidemiologic study that assessed the association between acrylamide measured as hemoglobin adducts and EOC risk but failed to replicate the positive associations observed when acrylamide intake was based on food frequency questionnaires (28). Additional studies with biomarkers of internal dose with a larger number of cases should be carried out; however, based on our data and the previous inconsistent findings in the literature, acrylamide appears unlikely to play a major role in ovarian cancer carcinogenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

None of the funding agencies had a role in the design, implementation, analysis, or interpretation of study results.

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Grant Support

This work was partially supported by the Wereld Kanker Onderzoek Fonds (WCRF NL; 2011/442, E.J. Duell) and by the Health Research Fund (FIS) of the Spanish Ministry of Health (Exp PI11/01473, E.J. Duell). The coordination of EPIC is financially supported by the European Commission (DG-SANCO) and the International Agency for Research on Cancer. The national cohorts are supported by the Health Research Fund (FIS) of the Spanish Ministry of Health, Regional Governments of Andalucía, Asturias, Basque Country, Murcia (no. 6236), Navarra and the Catalan Institute of Oncology, La Caixa (BM 06-130), Red Temática de Investigación Cooperativa en Cáncer (RD12/0036/0018; RD06/0020/0091; Spain); Danish Cancer Society (Denmark); Ligue contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l'Education Nationale, Institut National de la Santé et de la Recherche Médicale (INSERM; France); Deutsche Krebshilfe, Deutsches Krebsforschungszentrum (DKFZ) and Federal Ministry of Education and Research (Germany); the Hellenic Health Foundation (Greece); Associazione Italiana per la Ricerca sul Cancro (AIRC) and National Research Council (Italy); Dutch Ministry of Public Health, Welfare, and Sports (VWS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF), and Statistics Netherlands (The Netherlands); Nordic Center of Excellence in Food, Nutrition, and Health Helga (Norway); Swedish Cancer Society, Swedish Scientific Council and Regional Government of Skåne and Västerbotten (Sweden); Cancer Research UK (C570/A16491, R.C. Travis; 14136, K.T. Khaw) and Medical Research Council (G1000143, K.T. Khaw; United Kingdom).

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Received June 6, 2014; revised September 8, 2014; accepted September 22, 2014; published OnlineFirst October 9, 2014.

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5.3 Publication 3: Dietary and lifestyle determinants of acrylamide and glycidamide hemoglobin adducts in non-smoking postmenopausal women from the EPIC cohort

5.3.1 Resum

L'acrilamida va ser classificada com a substància 'probablement cancerígena' pels humans a l'any 1994 per l'Agència Internacional d'Investigació del Càncer (IARC). L'interès científic va augmentar quan es va detectar acrilamida en aquells aliments que eren rics en midó, d'origen vegetal i que havien estat processats a altes temperatures.

L'objectiu d'aquest estudi era identificar quins grups d'aliments i de variables d'estils de vida eren determinants en els nivells en sang dels adductes d'acrilamida i glicidamida en l'hemoglobina (HbAA i HbGA, respectivament). La població d'estudi va ser de 801 dones post-menopàusiques i no fumadores provinents de 8 països de l'Estudi Prospectiu Europeu sobre Càncer i Nutrició (EPIC).

Els biomarcadors d'exposició interna es van mesurar en eritròcits (recol·lectats a l'inici de l'estudi) mitjançant la tècnica HPLC/MS/MS. En aquest estudi transversal es van avaluar quatre variables dependents: HbAA, HbGA, la suma total d'adductes (HbAA+HbGA) i el seu quocient (HbGA/HbAA). Es van utilitzar models de regressió lineal simples i multivariats per identificar els determinants de les quatre variables dependents. Totes les variables dependents (excepte el quocient) i totes les variables independents es van transformar logarítmicament (log_2) per millorar la seva normalitat. La mediana (percentil 25 i 75) dels adductes HbAA i HbGA van ser de 41.3 (32.8-53.1) pmol/g Hb i 34.2 (25.4-46.9) pmol/g Hb, respectivament.

Els principals grups d'aliments que determinaven els nivells de HbAA, HbGA i HbAA+HbGA van ser les galetes, les galetes salades i els pastissos/pa de pessic. El consum d'alcohol i l'índex de massa corporal van ser els principals determinants de la variable HbGA/HbAA. El percentatge total de variabilitat explicada per HbAA, HbGA, HbAA+HbGA, i HbGA/HbAA va ser del 30%, 26%, 29%, i 13%, respectivament.

En aquest estudi, els factors dietètics i d'estil de vida van explicar una proporció moderada de la variació dels adductes de l'acrilamida en dones post-menopàusiques i no fumadores provinents l'estudi EPIC.

Paper 3

Obón-Santacana M, Lujan-Barroso L, Freisling H, Cadeau C, Fagherazzi G, Boutron-Ruault MC, Kaaks R, Fortner RT, Boeing H, Ramón Quirós J, Molina-Montes E, Chamosa S, Castaño JM, Ardanaz E, Khaw KT, Wareham N, Key T, Trichopoulou A, Lagiou P, Naska A, Palli D, Grioni S, Tumino R, Vineis P, De Magistris MS, Bueno-de-Mesquita HB, Peeters PH, Wennberg M, Bergdahl IA, Vesper H, Riboli E, Duell EJ.

Dietary and lifestyle determinants of acrylamide and glycidamide hemoglobin adducts in non-smoking postmenopausal women from the EPIC cohort

European Journal of Nutrition. 2016 Feb 5. [Epub ahead of print]

Eur J Nutr
DOI 10.1007/s00394-016-1165-5

ORIGINAL CONTRIBUTION

Dietary and lifestyle determinants of acrylamide and glycidamide hemoglobin adducts in non-smoking postmenopausal women from the EPIC cohort

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Received: 24 July 2015 / Accepted: 22 January 2016

Abstract

Purpose Acrylamide was classified as 'probably carcinogenic' to humans in 1994 by the International Agency for Research on Cancer. In 2002, public health concern increased when acrylamide was identified in starchy, plant-based foods, processed at high temperatures. The purpose of this study was to identify which food groups and lifestyle variables were determinants of hemoglobin adduct concentrations of acrylamide (HbAA) and glycidamide (HbGA) in 801 non-smoking postmenopausal women from eight countries in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort.

Methods Biomarkers of internal exposure were measured in red blood cells (collected at baseline) by high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) . In this cross-sectional analysis, four dependent variables were evaluated: HbAA, HbGA, sum of total adducts (HbAA + HbGA), and their ratio (HbGA/HbAA). Simple and multiple regression analyses were used to identify determinants of the four outcome variables. All dependent variables (except HbGA/HbAA) and

Electronic supplementary material The online version of this article (doi:10.1007/s00394-016-1165-5) contains supplementary material, which is available to authorized users.

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Published online: 05 February 2016

all independent variables were log-transformed (log_2) to improve normality. Median (25th–75th percentile) HbAA and HbGA adduct levels were 41.3 (32.8–53.1) pmol/g Hb and 34.2 (25.4–46.9) pmol/g Hb, respectively.

Results The main food group determinants of HbAA, HbGA, and HbAA + HbGA were biscuits, crackers, and dry cakes. Alcohol intake and body mass index were identified as the principal determinants of HbGA/HbAA. The total percent variation in HbAA, HbGA, HbAA + HbGA, and HbGA/HbAA explained in this study was 30, 26, 29, and 13 %, respectively.

Conclusions Dietary and lifestyle factors explain a moderate proportion of acrylamide adduct variation in non-smoking postmenopausal women from the EPIC cohort.

Keywords Acrylamide · Glycidamide · Hemoglobin adducts · Biomarkers · Diet · Nutrition

Introduction

Acrylamide was identified in food in 2002 and is mainly formed through the Maillard reaction whereby a carbonyl compound (a reducing sugar, such as glucose or fructose) reacts with the amino group of asparagine processed at high temperatures (>120 °C, i.e., frying, baking, or roasting) [1, 2]. Nevertheless, acrylamide has also been found in foods cooked at temperatures lower than 100 °C (e.g., prune juice) [3]. Thus, levels of acrylamide in foods depend on factors such as temperature and length of cooking time, water content, and the amount of both reducing sugars and asparagine levels present in foods [4]. Freisling et al. [5] assessed the principal food group determinants of acrylamide intake based on a 24-h dietary recall (24hDR) in 13,486 men and 23,508 women from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, and identified bread, crisp bread, rusks, coffee, and potatoes.

Once acrylamide is consumed, it is absorbed in the gastrointestinal tract and, via the circulation, is distributed to peripheral tissues [6, 7]. In the body, acrylamide is metabolized to glycidamide mainly by the cyp2e1 enzyme complex and is conjugated with reduced glutathione for elimination. Acrylamide is neurotoxic in animals and in humans, but only glycidamide is considered to have mutagenic and genotoxic properties [1, 8]. As a consequence of animal and in vitro studies, the International Agency for Research on Cancer (IARC) classified acrylamide as 'probably carcinogenic' to humans [9].

Acrylamide and glycidamide can bind to N-terminal valine of hemoglobin (Hb) in red blood cells, and form adducts, both of which are considered valid biomarkers that reflect human internal exposure within the last 120 days (the average life span of erythrocytes) [1, 10]. Tobacco

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smoking is an important source of acrylamide exposure, and smokers have been observed to have mean Hb adduct levels three to four times higher than non-smokers [11–13].

Two previous EPIC studies evaluated biomarkers of acrylamide measured as acrylamide and glycidamide Hb adducts (HbAA and HbGA, respectively). The first study, published by Vesper et al. [14] aimed to determine acrylamide exposure variability (both at the individual and group/country level) in 240 men and 270 women and, at the same time, determine which non-dietary factors could play a role in this variability. The second study, published by Ferrari et al. [15] was conducted with the intention to compare HbAA and HbGA levels with total estimated dietary acrylamide intakes assessed using dietary questionnaires (DQs), and a 24hDR in 240 men and 270 women to estimate the validity of the EPIC dietary acrylamide assessment. The main objective of the present study, which differed from the two former EPIC studies, was to identify which food groups and lifestyle factors (assessed through country-specific DQs and lifestyle questionnaires) were determinants of HbAA and HbGA concentrations in a subgroup of non-smoking postmenopausal women from the EPIC cohort. The relation between intakes of several food items using DQs, and HbAA and HbGA adduct levels has been previously evaluated in three different studies [16–18].

Materials and methods

Study population and data collection

The EPIC study comprises 23 research centers in 10 European countries and was designed to evaluate the relation between nutrition and lifestyle factors and the incidence of cancer and other chronic diseases. The present study includes eight of the 10 participating EPIC countries: France, Germany, Spain, the United Kingdom (UK), Greece, Italy, the Netherlands, and Sweden (Umeå). Norway and Denmark did not participate in this analysis (EPIC-Denmark published their results separately as the Diet, Cancer, and Health cohort) [17].

The methodology of the EPIC study has been previously described [19]; all local ethics committees and/or the IARC ethical review boards approved the study. Briefly, recruitment started between 1992 and 1998, and all EPIC participants provided information on habitual diet through country-specific validated DQs, referring to the year before recruitment. Information on tobacco smoking, physical activity, and education was assessed using country-specific questionnaires. Anthropometric measures (height, weight, and waist or hip circumference) were obtained at baseline by trained personnel; however, participants from France and Oxford (UK) cohorts self-reported their height and weight. Umeå (Sweden) did not collect information on waist or hip circumference, and only 29 % of participants from France had information on these anthropometric measures.

Blood samples (serum, plasma, white blood cells, and erythrocytes) were collected at recruitment for 385,747 of the over 500,000 EPIC participants and were stored in liquid nitrogen (−196 °C) at the central biological bank located at IARC; blood samples from Umeå were kept in freezers (−80 °C) at local repositories [19]. The present study population comprises control women from two published nested case–control studies of acrylamide hemoglobin adducts levels and ovarian and endometrial cancers risk in EPIC [20, 21]. The selection of cases and controls for these two nested case–control studies followed the protocol that has been previously described by Cust et al. and Peeters et al. [22, 23]. Briefly, two controls (free of cancer, with the exception of non-melanoma skin cancer) were randomly selected using an incidence density sampling protocol, and for each case subject (ovarian or endometrial cancer case) at the time of diagnosis. Matching criteria for both cases and controls included study center, menopausal status (premenopausal, postmenopausal, 'undefined'), age at recruitment (±6 months), time of day of blood draw (±1 h), and fasting status (<3, 3–6, >6 h). For the present study, only women who were postmenopausal at blood draw and non-smokers at recruitment were selected, since it has been postulated that acrylamide may disrupt hormonal balance, and it is known that smoking contributes to Hb adduct levels. Postmenopausal status refers to women who reported having had the last menstrual period more than 1 year before recruitment, or when they were more than 55 years old [23]. The category of non-smoking women includes those women who reported being never smokers or having quit smoking five or more years before recruitment.

Thus, a total of 802 non-smoking postmenopausal control women (416 and 386 controls from the ovarian and the endometrial nested case–control studies, respectively) were available for the present study. One participant was excluded from analyses because she did not have information on HbGA adduct level, leaving a total of 801 observations.

Assessment of dietary acrylamide intake

The methodology followed to create the EPIC acrylamide database has been previously described [15, 24]. In brief, the EPIC acrylamide database was assembled using information on average acrylamide levels in foods from an EU monitoring database (maintained by the European Community Institute for Reference Materials and Measurements; IRMM), and the frequency of consumption of these

foods using country-specific DQs and the EPIC-Soft food classification.

Measurement of acrylamide and glycidamide hemoglobin adducts

The methodology to measure HbAA and HbGA in EPIC has been previously described [14, 15]. Briefly, 300 μL of hemolysed erythrocytes was used to measure HbAA and HbGA, and were analyzed by HPLC/tandem mass spectrometry (HPLC/MS/MS) as has been published elsewhere [12, 25]. All blood samples were measured and analyzed in a randomized and blinded manner. Two independent adduct measures per sample were performed. Hemoglobin adduct concentrations were reported as the average of these two measurements relative to the amount of hemoglobin. The detection limits for this method were 3 and 4 pmol/g Hb for HbAA and HbGA, respectively. Additionally, 42 (5 %) blood samples from the same participants were sent in duplicate to evaluate the reproducibility of the hemoglobin adduct measurements.

Statistical methods

All continuous variables included in the analysis were assessed for normality using the Kolmogorov–Smirnov test and were log-transformed ($\log_2$) in order to reduce skewness. To account for zeroes in dietary and lifestyle variables, a $\log_2$ $(x + 0.1)$ transformation was applied. Regarding adduct values, four outcomes were evaluated: log-transformed HbAA adducts ($\log_2$HbAA), $\log_2$HbGA, sum of total adducts [$\log_2$ (HbAA + HbGA)], and HbGA/HbAA ratio. Simple and multiple linear regression analyses were used to assess the associations between each of the four outcome variables and food consumption and lifestyle data.

The following dietary variables and food groups were evaluated to build the HbAA, HbGA, and HbAA + HbGA final models: total energy; total carbohydrates; total fat; total fiber; total proteins; starch; potatoes; 'vegetables'; 'fruits, nuts, and seeds'; 'cereal and cereal products'; 'meat and meat products' [26]; 'cakes and biscuits'; 'flour, flakes, starches, and semolina'; 'pasta, rice, and other grains'; 'bread, crisp bread, and rusks'; 'breakfast cereals'; 'salty biscuits, aperitif biscuits, and crackers'; 'dry cakes and biscuits'; 'bread'; 'pastries'; 'olives'; 'deep frying fats'; 'chocolate, candy, paste, confetti'; 'snacks'; 'bread, and pizza dough'; 'olive oil'; 'coffee'; 'decaffeinated coffee'; and 'tea'. Then, a correlation matrix was performed to identify interdependency between dietary variables. Variables that were not correlated ($r < 0.6$), that were matching factors for both nested case–control studies (country was used instead of center due to the number of observations), and 'type of control' (endometrial, ovarian control) were selected for building the final models. Lifestyle variables such as alcohol intake (g/day) and body mass index (kg/m^2; BMI) were also investigated as they may affect the activity of Cyp2e1 [5, 27].

Stepwise selection was used to build models for HbAA, HbGA, and HbAA + HbGA adduct outcome variables. Matching factor and 'type of control' variables were forced to be included in the stepwise selection, and covariates were included in the model if they met the 0.10 significance level. Stepwise selection was also performed with all food items energy-adjusted using the residual method [28], but according to the Akaike information criterion (AIC) [29], these models were not optimal compared to those without energy-adjusted food items.

Lifestyle variables such as physical activity using the Cambridge index [30], education level (none, primary, technical/professional, secondary, and higher education), history of diabetes (yes, no, unknown), ever use of oral contraceptives (OCs), and ever use of hormone replacement therapy (HRT) were also evaluated; however, HbAA, HbGA, and HbAA + HbGA were not included in final models because they did not have an effect on β-estimates.

The HbGA/HbAA ratio model only included lifestyle variables described above (BMI, physical activity, education level, history of diabetes, OCs use, HRT use, and alcohol intake), since the ratio of HbGA/HbAA may reflect the metabolism of acrylamide to glycidamide.

Intraclass correlation coefficients (ICC) were estimated to evaluate the reproducibility of acrylamide measurements using 42 duplicate blood samples from the same participants [31]. Analyses stratified by alcohol intake (never drinkers, drinkers), by BMI (<25, 25–30, ≥30 kg/m^2), and by European region (Northern countries: the UK, the Netherlands, Germany, Sweden; Southern countries: France, Italy, Spain, Greece) were also performed. A Wilcoxon signed-rank test was used to assess differences in Hb adduct levels by alcohol intake, BMI, and second-hand smoke exposure (SHS). Variables for SHS were not evaluated in final models due to the large number of missing values (>50 %). R square (R^2) values were used to describe the percent variation in Hb adduct levels explained by the independent variables. Partial R^2 values for each of the selected variables in the models were estimated using Type II sums of squares. Pearson's correlation coefficients for continuous variables were also estimated. To test whether the slope of a regression line differed significantly from zero, Student's t statistics and the corresponding P values were used. All statistical tests were evaluated at α-level 0.05.

All analyses were performed using SAS v. 9.1 (Cary, North Carolina, USA). Graphics were created using R v. 3.1.

Results

Correlation matrix

Total carbohydrates were correlated with total fiber, total proteins, starch, and 'cereal and cereal products,' Total fat was correlated with total proteins. Starch was correlated with 'cereal and cereal products.' Thus, total carbohydrates, total fat, and starch were excluded from the analyses because these were larger groups of foods.

Dietary acrylamide intake and baseline characteristics

The median (25th–75th percentile) acrylamide intake at baseline based on DQ information was 20.3 (13.5–29.9) μg/day. The median (25th–75th percentile) estimated dietary acrylamide in relation to body weight was 0.3 (0.2–0.5) μg/kg body weight/day. The highest median intakes were found in the UK, the Netherlands, and Germany, whereas Italy had the lowest median intake (Table 1).

The median (25th–75th percentile) age in the present subcohort of women was 59 (54–63) years. Means and standard deviations, as well as medians and 25th–75th percentiles, are presented for all dietary and lifestyle variables that were used in all analyses (Supplemental Table 1).

Hemoglobin adducts of acrylamide and glycidamide

The ICC for the present study based on 42 duplicates was 0.96 for HbAA and 0.95 for HbGA.

The median (25th–75th percentile) HbAA and HbGA adduct levels were 41.3 (32.8–53.1) and 34.2 (25.4–46.9) pmol/g of Hb, respectively (Table 1). The highest median HbAA adduct level was observed in the UK, the Netherlands, and Spain, while Greece had the lowest HbAA levels, followed by Italy. Regarding HbGA adduct levels, the highest medians were found in the UK, Spain, and the Netherlands, and the lowest median was also found in Greece and Italy (Table 1). The geometric means for HbAA and HbGA adducts in the total dataset were 5.3 and 5.1 pmol/g Hb, respectively (data not shown). Values for HbAA + HbGA and HbGA/HbAA by EPIC country are also presented in Table 1.

Regarding differences in Hb adduct levels by alcohol intake and by BMI, only the ratio of HbGA/HbAA differed (never vs. ever drinkers, P value <0.0001; <25, 25 to <30, ≥30 kg/m^2, P value <0.0001) (Figs. 1, 2, respectively). No statistically significant differences in Hb adduct levels were observed between women who reported being exposed to SHS ($n = 95$) and who were not exposed ($n = 149$) (data not shown).

Table 1 Estimated dietary acrylamide intake and hemoglobin acrylamide and glycidamide adducts levels by EPIC country in a subgroup of non-smoking postmenopausal women

EPIC country	Acrylamide intake									
	Total observations	Endometrial controls	Ovarian controls	Acrylamide intake (μg/day)	Acrylamide intake (μg/day)[a]	μg/kg body weight/day	HbAA pmol/g of Hb	HbGA pmol/g of Hb	HbAA + HbGA pmol/g of Hb	Ratio of HbGA/HbAA
	n (%)	*n* (%)	*n* (%)	Median (QR)	Median (QR)	Median (QR)	Median (QR)	Median (QR)	Median (QR)	Median (QR)
France	65 (8)	35 (9)	30 (7)	20.0 (14.5–25.6)	17.6 (13.5–21.6)	0.3 (0.2–0.4)	38.5 (34.2–47.3)	29.4 (24.6–36.8)	66.4 (60.1–82.9)	0.7 (0.7–0.8)
Italy	126 (16)	74 (19)	52 (12)	8.6 (5.5–11.5)	7.8 (4.1–10.7)	0.1 (0.1–0.2)	35.8 (26.7–44.9)	28.9 (21.9–38.2)	64.7 (49.8–87.7)	0.8 (0.7–0.9)
Spain	127 (16)	72 (19)	55 (13)	16.6 (9.8–25.9)	19.0 (13.0–25.4)	0.2 (0.1–0.4)	42.4 (33.8–53.8)	39.8 (28.6–48.6)	82.4 (62.9–101.5)	0.9 (0.8–1.0)
United Kingdom	153 (19)	59 (15)	94 (23)	31.4 (23.8–40.4)	30.5 (24.2–39.5)	0.5 (0.3–0.6)	55.6 (44.4–69.0)	47.1 (35.2–61.3)	102.9 (83.1–133.4)	0.8 (0.7–1.0)
The Netherlands	116 (14)	38 (10)	78 (19)	28.8 (19.4–40.0)	30.0 (22.1–40.4)	0.4 (0.3–0.6)	45.3 (37.2–54.4)	38.6 (29.5–51.7)	80.9 (68.0–105.2)	0.9 (0.7–1.0)
Greece	59 (7)	16 (4)	43 (10)	17.7 (14.4–21.9)	19.1 (17.5–22.8)	0.2 (0.2–0.3)	26.4 (21.1–35.3)	22.0 (16.5–31.8)	50.4 (40.1–67.1)	0.9 (0.7–1.0)
Germany	102 (13)	56 (15)	46 (11)	22.8 (18.5–29.5)	24.9 (19.9–29.2)	0.3 (0.2–0.5)	36.2 (29.7–43.8)	29.3 (23.1–38.5)	67.2 (53.0–78.5)	0.8 (0.7–0.9)
Sweden	53 (7)	34 (9)	19 (5)	18.3 (14.8–21.0)	22.6 (20.5–25.0)	0.3 (0.2–0.3)	38.5 (34.9–46.9)	34.6 (25.9–42.2)	72.8 (62.9–87.8)	0.9 (0.8–1.0)
Total	801	384	417	20.3 (13.5–29.9)	21.9 (14.6–29.2)	0.3 (0.2–0.5)	41.3 (32.8–53.1)	34.2 (25.4–46.9)	75.5 (58.5–100.0)	0.8 (0.7–1.0)

EPIC European Prospective Investigation into Cancer and Nutrition, *QR* quartile range, *HbAA* hemoglobin adducts of acrylamide, *HbGA* hemoglobin adducts of glycidamide, *Hb* hemoglobin

[a] Energy-adjusted using the residual method

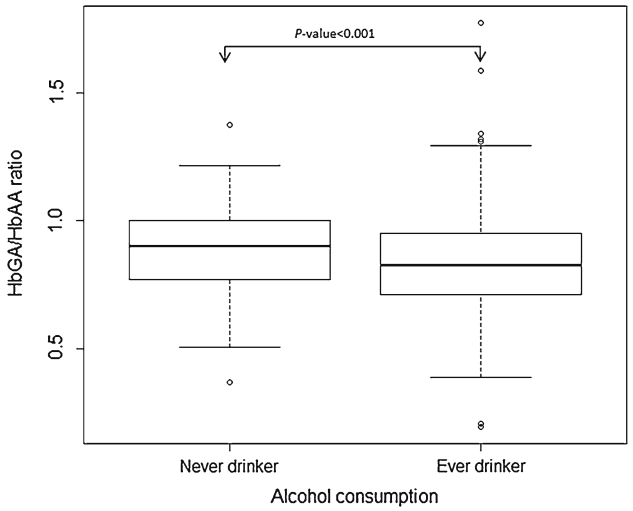

Fig. 1 Box-and-whisker plot of HbGA/HbAA ratio versus alcohol consumption (never drinkers, ever drinkers). *Arrow marks* significant differences between groups. *P* value based on a Wilcoxon rank sum test

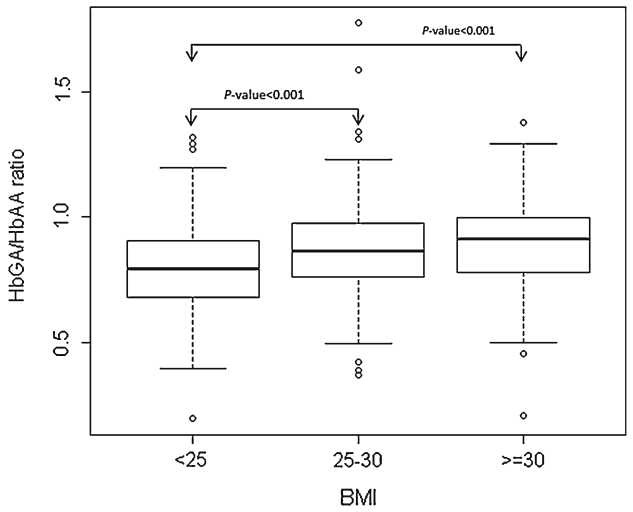

Fig. 2 Box-and-whisker plot of HbGA/HbAA ratio versus body mass index (BMI; <25, 25–30, ≥30 kg/m^2). *Arrow marks* significant differences between groups. *P* values based on a Wilcoxon rank sum test

Simple linear regression analyses

The crude Pearson's correlation coefficient between $\log_2$-acrylamide intake and $\log_2$HbAA was 0.36, between $\log_2$-acrylamide intake and $\log_2$HbGA was 0.35, between $\log_2$-acrylamide intake and $\log_2$ (HbAA + HbGA) was 0.37, and between $\log_2$HbAA and $\log_2$HbGA was 0.86 (all *P* values <0.0001). Log_2HbAA was inversely associated with BMI (*P* value <0.0001) and positively associated with alcohol intake (*P* value = 0.04). A statistically significant inverse association was found between HbGA/HbAA ratio and alcohol intake (*P* value <0.0001), whereas a positive association was observed between HbGA/HbAA ratio and BMI (*P* value <0.0001) (Table 2).

Multiple linear regression analyses

Four multivariable linear regression analyses (all models included country, age at recruitment, date of blood donation, fasting status, and type of control) were performed for each of the four outcome variables with the aim to identify independent determinants of $\log_2$HbAA, $\log_2$HbGA, $\log_2$ (HbAA + HbGA), and HbGA/HbAA (Table 3). Analyses stratified by alcohol intake and by BMI were performed, but no major differences were observed; thus, only overall results are presented.

The HbAA model explained 30 % of the variation in HbAA levels, and the food groups 'salty biscuits, aperitif biscuits, crackers'; 'dry cakes, biscuits'; and 'vegetables' were statistically significant positively associated with $\log_2$HbAA values, whereas 'tea' was inversely associated.

The HbGA model explained 26 % of the variation in HbGA levels, and the following groups were statistically significantly associated with HbGA-log levels: 'salty biscuits, aperitif biscuits, crackers'; 'dry cakes, biscuits'; and 'deep frying fats.' Alcohol intake was inversely associated with $\log_2$HbGA values.

The HbAA + HbGA model explained 29 % of the variation in the sum of total adducts levels, and only 'salty biscuits, aperitif biscuits, crackers'; 'dry cakes, biscuits'; and 'deep frying fats' were significantly associated with the sum of total adducts levels.

The HbGA/HbAA model explained 13 % of the variation in HbGA/HbAA ratio, and BMI was positively associated, whereas alcohol consumption at recruitment was inversely associated with the HbGA/HbAA ratio. No other lifestyle variable explained variation in the ratio.

Multiple linear regression analyses were also performed by European region (data not shown). The three different models from the northern countries explained a higher variation in HbAA, HbGA, and HbAA + HbGA levels than the southern countries; however, the food groups identified as dietary determinants of Hb adduct levels by European region were, in general, similar to those presented in Table 3.

Discussion

The present study was carried out with the aim to identify independent determinants of HbAA, HbGA, HbAA + HbGA, and HbGA/HbAA. We investigated these associations in a cross-sectional study of 801 non-smoking postmenopausal women from the EPIC cohort.

Table 2 Simple linear regression with β-estimates for the association between dietary and lifestyle variables and log-transformed ($\log_2$) HbAA, HbGA, HbAA + HbGA, and HbGA/HbAA

Variables	Log_2HbAA		Log_2HbGA		HbAA + HbGA		HbGA/HbAA	
	β	*P* value	β	*P* value	β	*P* value	β	*P* value
Acrylamide intake (μg/day)	0.20	<0.0001	0.23	<0.0001	0.21	<0.0001	0.02	0.02
Age at recruitment (y)	−0.003	0.26	0.001	0.79	−0.002	0.60	0.002	0.03
Body mass index (kg/m^2)	−0.32	<0.0001	−0.01	0.91	−0.18	0.02	0.17	<0.0001
Alcohol intake (g/day)	0.01	0.04	−0.01	0.09	0.002	0.79	−0.01	<0.0001
Total dietary fiber (g/day)	0.17	<0.0001	0.15	0.002	0.16	0.0001	−0.01	0.53
Total proteins (g/day)	0.15	0.0003	0.18	0.0003	0.17	0.0002	0.01	0.30
Meat and meat products (g/day)	−0.02	0.20	0.00	0.78	−0.01	0.58	0.01	0.01
Potatoes and other tubers (g/day)	0.04	<0.0001	0.05	<0.0001	0.05	<0.0001	0.01	0.16
Vegetables (g/day)	0.05	0.01	0.03	0.17	0.04	0.03	−0.01	0.10
Fruits, nuts, and seeds (g/day)	−0.02	0.21	−0.02	0.32	−0.02	0.25	0.002	0.78
Olives (g/day)	−0.05	<0.0001	−0.05	<0.0001	−0.05	<0.0001	0.001	0.79
Cereal and cereal products (g/day)	−0.02	0.51	−0.02	0.47	−0.02	0.51	0.00003	1.00
Flour, flakes, starches, semolina (g/day)	−0.07	<0.0001	−0.06	<0.0001	−0.06	<0.0001	0.004	0.25
Pasta, rice, other grains (g/day)	−0.02	0.02	−0.03	0.01	−0.02	0.01	−0.002	0.48
Bread, crisp bread, and rusks (g/day)	−0.04	0.01	−0.03	0.10	−0.04	0.03	0.007	0.19
Breakfast cereals (g/day)	0.04	<0.0001	0.03	<0.0001	0.04	<0.0001	−0.002	0.34
Salty biscuits, aperitif biscuits, crackers (g/day)	0.04	<0.0001	0.04	<0.0001	0.04	<0.0001	−0.003	0.21
Dry cakes, biscuits (g/day)	0.02	0.0003	0.03	<0.0001	0.03	<0.0001	0.005	0.02
Pastries (g/day)	−0.02	0.06	−0.04	0.0001	−0.03	0.004	−0.01	<0.0001
Cakes, biscuits (g/day)	0.03	0.0003	0.04	<0.0001	0.03	<0.0001	0.004	0.14
Chocolate, candy, paste, confetti (g/day)	0.03	<0.0001	0.03	<0.0001	0.03	<0.0001	0.001	0.51
Confectionery non-chocolate, candied fruits (g/day)	0.02	0.004	0.03	0.004	0.02	0.003	0.002	0.41
Snacks (g/day)	0.05	<0.0001	0.06	<0.0001	0.05	<0.0001	0.002	0.35
Olive oil (g/day)	−0.04	<0.0001	−0.04	<0.0001	−0.04	<0.0001	0.002	0.33
Deep frying fats (g/day)	0.03	0.04	0.03	0.03	0.03	0.03	0.004	0.36
Tea (ml/day)	0.02	<0.0001	0.02	0.0001	0.02	<0.0001	−0.002	0.06
Coffee (ml/day)	0.02	0.001	0.02	0.01	0.02	0.002	−0.0001	0.98
Decaffeinated coffee (ml/day)	0.02	<0.0001	0.03	<0.0001	0.03	<0.0001	0.002	0.24
Energy intake (kcal/day)	0.13	0.01	0.13	0.02	0.13	0.01	−0.0001	0.99

All independent variables were log-transformed ($\log_2$) to improve normality

HbAA hemoglobin adducts of acrylamide, *HbGA* hemoglobin adducts of glycidamide

The most important determinants of HbAA, HbGA, and HbAA + HbGA adduct levels were 'salty biscuits, aperitif biscuits, and crackers'; and 'dry cakes and biscuits,' whereas alcohol intake and BMI (inversely and positively associated, respectively) were identified as the two main determinants of HbGA/HbAA ratio.

To our knowledge, there are only three published studies that evaluated the relation between specific food group determinants and Hb adducts of acrylamide and glycidamide, and all of them obtained dietary information through DQs [16–18]. The first study was based on a Norwegian population and included 19 men and 31 women ($n = 50$) of which 14 % of the subjects were smokers. The second study comprised 296 female, non-smoking, pre- and postmenopausal women from the Nurses' Health Study II (NHS-II). The Danish study, similar to the present study, was based on postmenopausal women who reported being non-smokers at baseline ($n = 537$).

The current study had the highest estimated intake of acrylamide compared to the Norwegian and the NHS-II studies. The Norwegian study reported a median acrylamide intake of 12.8 μg/day among non-smoking women (in EPIC, 20.3 μg/day), and the NHS-II study reported a mean energy-adjusted intake of 19.3 μg/day (in EPIC, 21.9 μg/day). The Danish study did not report information on overall dietary acrylamide intake.

Table 3 Multiple linear stepwise regression with β-estimates and standard errors for the association between dietary and lifestyle variables and log-transformed (log2) HbAA, HbGA, HbAA + HbGA, and HbGA/HbAA

Hemoglobin adducts	Dietary and/or life style variables	β (SE)	*P* value	Partial R^2	Model R^2
Log_2HbAA	Salty biscuits, aperitif biscuits, crackers	0.03 (0.01)	0.0001	0.01	0.30
	Dry cakes, biscuits	0.02 (0.01)	0.003	0.01	
	Tea	−0.01 (0.005)	0.02	0.01	
	Vegetables	0.05 (0.02)	0.02	0.005	
	BMI	−0.15 (0.07)	0.05	0.004	
	Deep frying fats	0.04 (0.02)	0.08	0.003	
Log_2HbGA	Dry cakes, biscuits	0.03 (0.01)	0.0001	0.02	0.26
	Salty biscuits, aperitif biscuits, crackers	0.03 (0.01)	0.0002	0.01	
	Alcohol at recruitment	−0.02 (0.01)	0.01	0.01	
	Deep frying fats	0.07 (0.03)	0.02	0.01	
	Coffee	0.01 (0.01)	0.06	0.003	
	Tea	−0.01 (0.01)	0.07	0.003	
Log2 (HbAA + HbGA)	Salty biscuits, aperitif biscuits, crackers	0.03 (0.01)	0.0001	0.01	0.29
	Dry cakes, biscuits	0.02 (0.01)	0.0005	0.01	
	Tea	−0.01 (0.005)	0.02	0.005	
	Deep frying fats	0.05 (0.02)	0.03	0.004	
	Fiber	0.07 (0.04)	0.05	0.003	
	Coffee	0.01 (0.01)	0.08	0.003	
HbGA/HbAA	BMI	0.14 (0.03)	<0.0001	0.03	0.13
	Alcohol at recruitment	−0.01 (0.002)	0.00002	0.02	
	Education level				
	None	Reference	–	0.006	
	Primary school completed	−0.01 (0.02)	0.73		
	Technical/professional school	−0.02 (0.03)	0.51		
	Secondary school	−0.02 (0.03)	0.61		
	Higher education	−0.06 (0.03)	0.05		
	Not specified	0.02 (0.05)	0.70		
	Missing	−0.04 (0.07)	0.53		
	Ever use of OCs				
	No	Reference	–	0.003	
	Yes	−0.02 (0.01)	0.12		
	Missing	0.04 (0.06)	0.53		
	Physical activity				
	Inactive	Reference	–	0.003	
	Moderately inactive	0.02 (0.02)	0.28		
	Moderately active	−0.02 (0.02)	0.29		
	Active	0.01 (0.02)	0.77		
	Missing	−0.03 (0.09)	0.74		
	Ever use of HRT				
	No	Reference	–	0.002	
	Yes	0.004 (0.02)	0.81		
	Missing	−0.05 (0.04)	0.22		
	Diabetes				
	No	Reference	–	0.001	
	Yes	−0.02 (0.03)	0.47		
	Missing	−0.03 (0.07)	0.64		

All models are adjusted for country, age at recruitment (years), date of blood donation, fasting status (no, in between, yes), and type of control (endometrial, ovarian control). All independent continuous variables were log-transformed (log_2) to improve normality

SE Standard error, *HbAA* hemoglobin adducts of acrylamide, *HbGA* hemoglobin adducts of glycidamide, *BMI* body mass index (kg/m^2), *HRT* hormonal replacement therapy, *OC* oral contraceptive

Blood samples from both EPIC and NHS-II studies were measured in the same laboratory using the same protocol. Likewise, the Norwegian and the Danish studies shared the same methodology [16, 17]. The median adduct levels for HbAA and HbGA in the present study (41.3 and 34.2 pmol/g of Hb, respectively) were higher than the values reported in the Norwegian (36.8 and 18.2 pmol/g of Hb) and the Danish (35 and 21 pmol/g of Hb) studies; however, the NHS-II study reported the highest values of Hb adducts (43.9 and 49.4 pmol/g of Hb). The NHS-II study also had the highest median values of HbGA/HbAA (1.10), compared to the Norwegian (0.49) and the present (0.8) study.

The correlation between estimated acrylamide intake (based on DQs) and Hb adducts of acrylamide and glycidamide was low (ranging from 0.08 to 0.43) in most studies, including EPIC [15, 16, 32–34]. The NHS-II study reported moderate correlations between acrylamide intake and HbAA or HbGA (0.19–0.35). These differences could be due to errors in dietary assessment methods and to incomplete data on acrylamide content in food composition databases. The NHS-II acrylamide database was mainly based on values from the FDA's Exploratory Analysis of Acrylamide in Foods, but specific foods that were frequently consumed in the study were further analyzed, such as different brands of breakfast cereals, dried food, bread, and potatoes chips among others [18].

The main food group determinants of Hb adducts (HbAA, HbGA, and HbAA + HbGA) in the present study were 'salty biscuits, aperitif biscuits, and crackers'; and 'dry cakes and biscuits,' whereas Freisling et al. [5] reported that 'bread, crisp bread, and rusks'; 'coffee'; and 'potatoes' were the main determinants of dietary acrylamide intake (based on a single 24hDR) in a different subgroup of EPIC men and women. Coffee was selected in the stepwise procedure as one of the food determinants of HbGA, and HbAA + HbGA in the present study, but was not statistically significant. Inverse associations between 'tea' and Hb adduct levels (HbAA, HbGA, and HbAA + HbGA) were observed in the present study. This result may be explained by the possible effect of tea polyphenols, which have been observed to decrease HbAA levels in animals [35]. The variable 'vegetables' was selected as a determinant of $\log_2$HbAA; however, this result might have been confounded by other acrylamide-containing foods, since 'vegetables' includes all forms of cooking methods (including frying and baking). The food groups 'bread, crisp bread, rusks' and 'potatoes' were not selected in any of the models presented in this study; however, the possible effect of 'potatoes,' especially fried potatoes, might have been represented by the variable 'deep frying fats.' It is worth noting that the present study evaluates adduct levels, whereas the Friesling et al.'s study evaluates acrylamide intake based on 24hDR. Further, differences in the results of these two EPIC analyses may reflect differences in the subpopulation studied (e.g., age distribution, sex, menopausal status).

The Norwegian study identified 'potatoes,' 'chips/snacks,' 'crisp bread,' and 'jam/preservatives as dietary determinants of HbAA levels. Furthermore, 'chips/snacks' and 'crisp bread' were also recognized as determinants of HbAA + HbGA. Similar to EPIC, the NHS-II study also identified dietary determinants of HbAA, HbGA, and HbAA + HbGA, but the food groups were different from the determinants observed in the present study. The Danish study reported 'coffee' to be associated with HbAA and HbGA, 'chips' to HbAA, and 'biscuits/crackers' to HbGA levels. Dietary habits are different between the US and European countries, so direct comparison of food groups as determinants of HbAA levels may not be possible.

The proportion of response variation (R^2) explained by food groups and/or lifestyle variables in the present study varied from 13 % in the HbGA/HbAA model to 30 % for HbAA. The Danish study obtained a response variation of 17 and 12 % in the HbAA and HbGA models, respectively; however, the highest response variations explained were obtained in the Norwegian study (48 % in the HbAA, and 37 % in the HbAA + HbGA model) [16, 17]. The NHS-II study did not report this information [18].

The main difference between our study and the other three published studies is that prior analyses only included dietary variables that were suspected to be sources of acrylamide intake. The current study included both known sources of dietary acrylamide together with lifestyle variables, such as alcohol intake and BMI, with the intention to better describe the independent determinants of Hb adducts and their ratio in non-smoking individuals. Diet is complex, and interactions between acrylamide and food ingredients are possible (i.e., acrylamide uptake in humans has been hypothesized to be impaired by a diet rich in proteins) [26]. Likewise, it has been suggested that alcohol intake and BMI may influence the activity of Cyp2e1, the enzyme complex that metabolizes acrylamide to glycidamide. This enzyme is involved in alcohol metabolism mainly when alcohol concentrations in the blood are high. It has been hypothesized that alcohol may compete with acrylamide as a substrate [36]. This could partially explain the results observed in the present study, in which higher alcohol intake was inversely associated with HbGA and HbGA/HbAA, as has been reported in other studies [14, 36]. The mechanism by which BMI may influence acrylamide metabolism is still unclear, but similar to the present study, other studies have observed that BMI was positively associated with the ratio HbGA/HbAA and negatively associated with HbAA [14, 37, 38]. Recently, statistically significant differences in the ratio of HbGA/HbAA between vegetarians and non-vegetarians have been reported, suggesting

that dietary factors may also contribute to acrylamide/glycidamide metabolism [39].

The present study had the largest sample size compared to the previous published papers, and the laboratory methods used to quantify both acrylamide and glycidamide hemoglobin adducts were standardized and followed rigorous quality assurance/quality control [12]. Tobacco is one of the most important sources of acrylamide, and it is well established that cigarette smoke influences Hb adduct levels (smokers have mean HbAA levels at least three to four times higher than non-smokers) [11, 14]. Moreover, it has been hypothesized that acrylamide may influence hormonal homeostasis [40–42]. Thus, the present study was designed to reduce confounding by these factors, and only those women who reported being postmenopausal and never smokers were included in the study. Further, limitations derived from using estimates of dietary acrylamide intake, as described above, were avoided in the present analysis of food group and biomarker levels.

There are some limitations that should be acknowledged: (a) Hb adducts of acrylamide and glycidamide are valid biomarkers for acrylamide exposure and internal dose; however, these adducts reflect levels of acrylamide and glycidamide during the average life span of erythrocytes, which is around 120 days [1, 10], and all dietary and lifestyle variables in EPIC were assessed through DQs that referred to the previous year before recruitment [19]. (b) Cooking methodology, which influences acrylamide levels in food, was not recorded in some EPIC centers; however, relevant information on food preparation was available for potatoes (except in Italy), bread, and breaded meats [15, 24]. (c) Only one baseline blood sample was collected for each participant. (d) Acrylamide content in foods may vary seasonally [43], which was not accounted for in our analyses; nevertheless, all models were adjusted for date of blood extraction to minimize this effect. (e) Ferrari et al. [15] described variations in dietary patterns across EPIC countries, and this may have influenced Hb adduct levels and our prediction models, although all models included country to adjust for country-level effects (i.e., questionnaire design, dietary habits). (f) SHS could not be evaluated in our statistical models due to the large number of missing values (>50 %); however, in a subset of women from the current study, no statistically significant differences in Hb adduct levels were observed between women who were exposed to SHS and who were not exposed to SHS.

To conclude, dietary food group and lifestyle variables explain a moderate proportion of HbAA and HbGA adduct level variation in 801 postmenopausal non-smoking women in the EPIC cohort. The main food group determinants of HbAA, HbGA, and HbAA + HbGA were ‘salty biscuits, aperitif biscuits, and crackers’ and ‘dry cakes and biscuits.’ Alcohol intake and BMI were identified as the principal determinants for the ratio of HbGA/HbAA levels. In this regard, future studies assessing associations between acrylamide and disease risk should take into consideration the use of both biomarkers of acrylamide exposure (HbAA and HbGA), in addition to alcohol intake and BMI.

Acknowledgments This work was supported by the Wereld Kanker Onderzoek Fonds (WCRF NL) [Grant WCRF 2011/442] and by the Health Research Fund (FIS) of the Spanish Ministry of Health [Exp PI11/01473]. The coordination of EPIC is financially supported by the European Commission (DG-SANCO) and the International Agency for Research on Cancer. The national cohorts are supported by the Health Research Fund (FIS) of the Spanish Ministry of Health, Regional Governments of Andalucía, Asturias, Basque Country, Murcia [no. 6236], Navarra and the Catalan Institute of Oncology, La Caixa [BM 06-130], Red Temática de Investigación Cooperativa en Cáncer [RD12/0036/0018; RD06/0020/0091] (Spain); Danish Cancer Society (Denmark); Ligue contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l’Education Nationale, Institut National de la Santé et de la Recherche Médicale (INSERM) (France); Deutsche Krebshilfe, Deutsches Krebsforschungszentrum (DKFZ) and Federal Ministry of Education and Research (Germany); the Hellenic Health Foundation (Greece); Associazione Italiana per la Ricerca sul Cancro (AIRC) and National Research Council (Italy); Dutch Ministry of Public Health, Welfare and Sports (VWS), the Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF) and Statistics Netherlands (the Netherlands); Nordic Center of Excellence in Food, Nutrition and Health-Helga (Norway); Swedish Cancer Society, Swedish Scientific Council and Regional Government of Skåne and Västerbotten (Sweden); Cancer Research UK, Medical Research Council (United Kingdom). MO-S is affiliated with the University of Barcelona.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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5.4 Publication 4: Acrylamide and glycidamide hemoglobin adduct levels and endometrial cancer risk: A nested-case control study in nonsmoking postmenopausal women from the EPIC cohort

5.4.1 Resum

L'acrilamida està classificada per l'Agència Internacional d'Investigació del Càncer (IARC) com a 'probable carcinogen' pels humans (Grup 2A) des de l'any 1994. No va ser fins l'any 2002 que es va descobrir aquest component en aliments rics en hidrats de carboni que havien patit un procés tèrmic. L'associació entre la ingesta d'acrilamida i el càncer d'endometri (CE) s'ha avaluat prèviament en quatre estudis prospectius; tanmateix, no hi ha un resultat clar.

L'objectiu principal d'aquest estudi de casos i controls niat emmarcat en l'Estudi Prospectiu Europeu sobre Càncer i Nutrició (EPIC) era avaluar, per primera vegada, l'associació entre els adductes d'acrilamida i glicidamida en l'hemoglobina (HbAA i HbGA, respectivament) i el risc de desenvolupar CE en dones post-menopàusiques i no fumadores.

Els adductes en l'hemoglobina es van mesurar en eritròcits mitjançant la tècnica de HPLC/MS/MS. Per a realitzar les anàlisis estadístiques es van utilitzar quatre variables d'exposició: HbAA, HbGA, la suma total d'adductes (HbAA+HbGA) i el seu quocient (HbGA/HbAA). Per a avaluar l'associació entre els adductes en l'hemoglobina i el CE es van utilitzar models de regressió logística incondicionals i multivariats, els quals van incloure 383 casos de CE (171 eren de tipus-I) i 385 controls. Les quatre variables d'exposició es van categoritzar en quintils segons la distribució d'exposició dels controls. Cap de les variables d'exposició analitzades van estar relacionades amb el risc de CE ($HR_{HbAA;\ Q5vsQ1}$: 0.84, 95% CI: 0.49-1.48; $HR_{HbGA;\ Q5vsQ1}$: 0.94, 95% CI: 0.54-1.63) o amb el CE de tipus-I. De la mateixa manera, cap dels subgrups estudiats (Índex de massa corporal <25 *vs* ≥25 kg/m^2, consum d'alcohol *vs* no consum d'alcohol, usuàries d'anticonceptius orals *vs* no usuàries d'anticonceptius orals) van suggerir una modificació del risc.

Aquest primer estudi epidemiològic de casos i controls niat va concloure que en 768 dones post-menopàusiques i no fumadores, els biomarcadors en sang d'acrilamida i de glicidamida no estaven associats amb un increment del risc de patir CE general i de tipus-I.

Paper 4

Obón-Santacana M, Freisling H, Peeters PH, Lujan-Barroso L, Ferrari P, Boutron-Ruault MC, Mesrine S, Baglietto L, Turzanski-Fortner R, Katzke VA, Boeing H, Quirós JR, Molina-Portillo E, Larrañaga N, Chirlaque MD, Barricarte A, Khaw KT, Wareham N, Travis RC, Merritt MA, Gunter MJ, Trichopoulou A, Lagiou P, Naska A, Palli D, Sieri S, Tumino R, Fiano V, Galassom R, Bueno-de-Mesquita HB, Onland-Moret NC, Idahl A, Lundin E, Weiderpass E, Vesper H, Riboli E, Duell EJ

Acrylamide and glycidamide hemoglobin adduct levels and endometrial cancer risk: A nested-case control study in nonsmoking postmenopausal women from the EPIC cohort

International Journal of Cancer. 2016 Mar 1;138(5):1129-38. doi: 10.1002/ijc.29853. Epub 2015 Oct 1.

IJC
International Journal of Cancer

Short Report

Acrylamide and glycidamide hemoglobin adduct levels and endometrial cancer risk: A nested case-control study in nonsmoking postmenopausal women from the EPIC cohort

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Key words: hemoglobin adduct, acrylamide, glycidamide, endometrial cancer, EPIC

Abbreviations: 24hDR: 24-h dietary recall; BMI: body mass index (kg m^{-2}); CI: confidence interval; DQ: dietary questionnaire; EC: endometrial cancer; EPIC: European prospective investigation into cancer and nutrition; FFQ: food frequency questionnaire; HbAA: hemoglobin adducts of acrylamide; HbAA+HbGA: sum of hemoglobin adducts of acrylamide and glycidamide; HbGA: hemoglobin adducts of glycidamide; HbGA/HbAA: ratio of hemoglobin adducts of glycidamide and acrylamide; HPLC/MS/MS: high-performance liquid chromatography–tandem mass spectrometry; HRT: hormone replacement therapy; IARC: international agency for research on cancer; ICC: intraclass correlation coefficient; LOD: limits of detection; LRT: likelihood ratio test; NHS: nurses' health study; OC: oral contraceptive; OR: odds ratio; SHS: second-hand smoke

Grant sponsor: Wereld Kanker Onderzoek Fonds (WCRF NL); **Grant number:** WCRF 2011/442; **Grant sponsor:** Health Research Fund (FIS) of the Spanish Ministry of Health; **Grant number:** Exp PI11/01473; **Grant sponsor:** Health Research Fund (FIS) of the Spanish Ministry of Health, Regional Governments of Andalucía, Asturias, Basque Country, Murcia; **Grant number:** 6236; **Grant sponsor:** Navarra and the Catalan Institute of Oncology, La Caixa; **Grant number:** BM 06-130; **Grant sponsor:** Red Temática de Investigación Cooperativa en Cáncer (Spain); **Grant numbers:** RD12/0036/0018; RD06/0020/0091; **Grant sponsors:** European Commission (DG-SANCO); International Agency for Research on Cancer; Danish Cancer Society (Denmark); Ligue contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l'Education Nationale, Institut National de la Santé et de la Recherche Médicale (INSERM) (France); Deutsche Krebshilfe, Deutsches Krebsforschungszentrum (DKFZ); Federal Ministry of Education and Research (Germany); the Hellenic Health Foundation (Greece); Associazione Italiana per la Ricerca sul Cancro (AIRC); National Research Council (Italy); Dutch Ministry of Public Health, Welfare and Sports (VWS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF); Statistics Netherlands (The Netherlands); Nordic Center of Excellence in Food, Nutrition and Health - Helga (Norway); Swedish Cancer Society, Swedish Scientific Council and Regional Government of Skåne and Västerbotten (Sweden); Cancer Research UK, Medical Research Council (United Kingdom)

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DOI: 10.1002/ijc.29853
History: Received 15 June 2015; Accepted 21 July 2015; Online 16 Sep 2015

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Acrylamide, classified in 1994 by IARC as "probably carcinogenic to humans," was discovered in 2002 in some heat-treated, carbohydrate-rich foods. Four prospective studies have evaluated the association between dietary acrylamide intake and endometrial cancer (EC) risk with inconsistent results. The purpose of this nested case-control study, based on the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, was to evaluate, for the first time, the association between hemoglobin adducts of acrylamide (HbAA) and glycidamide (HbGA) and the risk of developing EC in non-smoking postmenopausal women. Hemoglobin adducts were measured in red blood cells by HPLC/MS/MS. Four exposure variables were evaluated: HbAA, HbGA, their sum (HbAA+HbGA), and their ratio (HbGA/HbAA). The association between hemoglobin adducts and EC was evaluated using unconditional multivariable logistic regression models, and included 383 EC cases (171 were type-I EC), and 385 controls. Exposure variables were analyzed in quintiles based on control distributions. None of the biomarker variables had an effect on overall EC ($HR_{HbAA;Q5vsQ1}$: 0.84, 95%CI: 0.49–1.48; $HR_{HbGA;Q5vsQ1}$: 0.94, 95%CI: 0.54–1.63) or type-I EC risk. Additionally, none of the subgroups investigated (BMI < 25 *vs*. $\geq$25 kg m^{-2}, alcohol drinkers *vs*. never drinkers, oral contraceptive users *vs*. non-users) demonstrated effect measure modification. Hemoglobin adducts of acrylamide or glycidamide were not associated with EC or type-I EC risk in 768 nonsmoking postmenopausal women from the EPIC cohort.

What's new?

Acrylamide in food may not lead to endometrial cancer, according to a new report. The carcinogen has provoked public concerns because it can be detected in certain foods. Prospective studies on the relationship between endometrial cancer and dietary acrylamide, however, have produced conflicting results. Taking a different tack, these authors conducted a case-control study, drawing on data from the European Prospective Investigation into Cancer and Nutrition (EPIC). They measured the amounts of certain compounds formed by hemoglobin with acrylamide or glycidamide in nonsmoking, postmenopausal women. Neither of these levels, they report, had any impact on endometrial cancer risk.

The International Agency for Research on Cancer (IARC) classified acrylamide as "probably carcinogenic to humans (group 2A)" based on evidence from animal and *in vitro* studies[1]; however scientific interest did not increase until 2002, when Swedish researchers reported acrylamide concentrations in commonly consumed foods.[2] The principal pathway by which acrylamide is formed in foods is through the Maillard reaction during food processing at temperatures higher than $>120°C$ (*i.e.*, frying or baking),[2,3] but acrylamide has also been observed in foods treated at lower temperatures (*e.g.*, low moisture drying).[4] In the European Prospective Investigation into Cancer and Nutrition (EPIC), the major food contributors to dietary acrylamide intake (based on a 24-hr dietary recall; 24hDR) were bread, crisp bread, rusks, coffee and potatoes.[5]

In the human body, acrylamide is conjugated with reduced glutathione for elimination, or is metabolized to glycidamide through the Cyp2e1 enzyme system. In animal studies, after acrylamide administration, both hormone- and nonhormone-related tumors have been observed.[1] Glycidamide is believed to have mutagenic and genotoxic effects in animals, whereas acrylamide is thought to be neurotoxic both in animals and in humans,[3,6] and may also disrupt hormonal homeostasis.[7,8]

Acrylamide and its metabolite glycidamide can form adducts with hemoglobin (HbAA and HbGA, respectively), which are stable over the lifespan of erythrocytes (~120 days), and thus, have been extensively used as biomarkers of human internal exposure.[3,9] The mean hemoglobin adduct levels in smokers are at least three to four times higher than nonsmokers,[10] and cigarette smoke is considered as one of the major sources of acrylamide exposure. Thus, to assess the impact of dietary acrylamide on health, nonsmokers are considered a more suitable population than smokers.

Cancer of the corpus uteri is the fourth most common incident cancer in European and North American women. The most common type of corpus uteri cancer is endometrial cancer (EC). The 5-year survival rate of EC is high, ranging from 65 to 85%.[11] EC has been classified into type-I and type-II tumors; type-I EC is mostly endometrioid adenocarcinoma, and is characterized as an estrogen-dependent tumor. In contrast, type-II EC is usually serous carcinoma, is thought to be estrogen-independent, usually diagnosed in elderly women, and generally has an unfavorable prognosis.[12,13] Epidemiological data suggest that obesity, diabetes, low physical activity, long-term exposure to estrogens and a history of polycystic ovary syndrome are risk factors for developing EC, and type-I EC in particular.[14] Combined oral contraceptive (OC) use, and tobacco smoking are consistently associated with lower risk of EC.[14] Further, a recent EPIC study observed an inverse association between coffee consumption and EC risk.[15]

To date, four prospective epidemiologic studies, including one from EPIC, have evaluated the association between dietary intake of acrylamide (assessed through dietary questionnaires; DQs) and EC risk.[16–19] Two subsequent meta-analyses concluded that dietary acrylamide intake was not associated with overall EC risk, but increased risk was observed with higher acrylamide intakes in women who were never smokers at baseline.[20,21] To our knowledge, this is the first nested-case control study within a prospective cohort study designed to assess the relation between circulating, red blood cell hemoglobin adducts of acrylamide and glycidamide and overall and type-I EC risk.

Material and Methods

The EPIC study comprises 10 European countries and 23 research centers with the aim to evaluate the association between nutrition and lifestyle factors, cancer and other chronic diseases.[22] The current study includes participants from 8 of the 10 EPIC countries: Denmark, Norway and one center from Sweden (Malmö) did not participate. For each EPIC center, subjects were followed until cancer diagnosis (except non-melanoma skin cancer), emigration, death or end of follow-up, which varied from December 2005 to June 2010).

The EPIC methodology has been published elsewhere.[22] Recruitment began between 1992 and 1998, and participants reported information on dietary habits (referring to the 12 months before recruitment) assessed through country-specific, validated dietary questionnaires (DQs). Additionally, information on tobacco smoking, education, physical activity, anthropometric measures and reproductive factors was also obtained at recruitment. Blood samples were collected at recruitment for ~80% of the EPIC cohort (385,747 of over 500,000 participants). Samples that were stored at the IARC bio-bank were kept in liquid nitrogen ($-196°C$); whereas blood samples from Umeå were stored at local repositories in freezers ($-80°C$). The study was approved by the IARC ethical review boards and/or all local ethics committees.

Blood samples were sent to the Centers for Disease Control and Prevention (CDC) Protein Biomarker Laboratory to measure HbAA and HbGA. Details of the methodology have been previously described.[10] Briefly, adduct levels were measured in 300 μL of hemolysed erythrocytes and analyzed by high-performance liquid chromatography–tandem mass spectrometry (HPLC/MS/MS) in a randomized manner. Additionally, for each sample two independent measurements were performed, and results were reported in pmol per g of Hb. The detection limits (LOD) for this method were 3 and 4 pmol g^{-1} Hb for HbAA and HbGA, respectively.

Identification of EC cases was achieved by means of population cancer registries, or through a combination of methods: health insurance records, cancer and pathology registries and active follow-up. EC cases were classified as C54 according to the International Classification of Diseases, 10th revision.

The selection of the study population for the present nested case-control study was based on the algorithm that has been previously published by Cust *et al.* and Peeters *et al.*[23]: for each EC case two corresponding controls were randomly selected at the date of diagnosis (subjects free of cancer, with the exception of non-melanoma skin cancer). Cases and controls were matched by study center, menopausal status, age at recruitment (± 6 months), date at blood collection (± 1 month), time of the

Table 1. Description of the study population from a nested case-control study of acrylamide biomarkers and EC in the EPIC cohort

	All EC cases $n = 383$	Type-I cases $n = 171$	Controls $n = 385$
HbAA (pmol/g Hb)[1]	39.9 (31.4–52.4)	40.1 (31.4–52.8)	39.4 (32.1–51.1)
HbGA (pmol/g Hb)[1]	34.1 (25.7–44.6)	33 (25.3–46.2)	33.3 (24.6–43.8)
HbAA+HbGA (pmol/g Hb)[1]	74.4 (57.5–97.6)	72.5 (56.8–97.8)	72.8 (57.2–94.5)
HbGA/HbAA (pmol/g Hb)[1]	0.9 (0.7-1.0)	0.8 (0.7-1.0)	0.8 (0.7-1.0)
Age at recruitment (y)[1]	58.0 (53.5–61.4)	57.7 (53.6–61.0)	58.5 (54.3–61.7)
Age at menopause (y)[1]	49.5 (49.5–52.0)	49.5 (49.5–52.0)	49.5 (49.0–52.0)
BMI (kg m^{-2})[1]	27.4 (24.1–31.6)	27.4 (24.4–33.2)	26.1 (23.2–29.3)
Country[2]			
France	33 (8.6)	17 (9.9)	35 (9.1)
Italy	69 (18.0)	24 (14.0)	74 (19.2)
Spain	55 (14.4)	25 (14.6)	72(18.7)
United Kingdom	70 (18.3)	30 (17.5)	60 (15.6)
The Netherlands	56 (14.6)	32 (18.7)	38 (9.9)
Greece	13 (3.4)	3 (1.8)	16 (4.2)
Germany	51 (13.3)	40 (23.4)	56 (14.6)
Sweden	36 (9.4)	0 (0.0)	34(8.8)
Fasting status[2]			
Unknown	1 (0.3)	1 (0.6)	0 (0.0)
<3 hr	150 (39.2)	77 (45.0)	129 (33.5)
3–6 hr	60 (15.7)	34 (19.9)	64 (16.6)
>6 hr	172 (44.9)	59 (34.5)	192 (49.9)
Alcohol consumption[2]			
Non drinker	94 (24.5)	37 (21.6)	93 (24.2)
>0–6 g day^{-1}	168 (43.9)	72 (42.1)	166 (43.1)
>6–12 g day^{-1}	63 (16.5)	32 (18.7)	67 (17.4)
>12–24 g day^{-1}	33 (8.6)	19 (11.1)	38 (9.9)
>24–60 g day^{-1}	25 (6.5)	11 (6.4)	21 (5.5)
Ever use of OC[2]			
Unknown	10 (2.6)	1 (0.6)	8 (2.1)
No	249 (65.0)	102 (59.7)	237 (61.6)
Yes	124 (32.4)	68 (39.8)	140 (36.4)
Ever use of HRT [2]			
Unknown	16 (4.2)	5 (2.9)	15 (3.9)
No	263 (68.7)	114 (66.7)	287 (74.6)
Yes	104 (27.2)	52 (30.4)	83 (21.6)
Parity[2]			
Unknown	61 (4.4)	31 (2.3)	59 (2.3)
1 child	130 (15.9)	62 (18.1)	140 (15.3)
2 children	105 (33.9)	46 (36.3)	131 (36.4)
≥3 children	62 (27.4)	21 (26.9)	42 (34.0)
Nulliparous	8 (16.2)	7 (12.3)	4 (10.9)
Parous but with missing number of full-term pregnancies	17 (2.1)	4 (4.1)	9 (1.0)

[1]Median and quartile range (25th – 75th percentile). [2]Number (*n*) and percent (%).
Abbreviations: EC, endometrial cancer; EPIC, European prospective investigation into cancer and nutrition; HbAA, hemoglobin adducts of acrylamide; HbGA, hemoglobin adducts of glycidamide, BMI, body mass index; OC, oral contraceptive; HRT, hormone replacement therapy.

Table 2. OR and 95% CI for biomarkers of acrylamide exposure and EC risk in a nested case-control study in the EPIC cohort

Exposure cut points		Overall EC				Type 1 EC			
		Cases $n = 383$	Controls $n = 385$	OR (95%CI)	*p*-trend	Cases $n = 171$	Controls $n = 385$	OR (95%CI)	*p*-trend
HbAA	≤29.4	77	74	1.00 (ref)	0.94	33	74	1.00 (ref)	0.94
	29.5–36.1	75	80	0.82 (0.49–1.37)		33	80	0.94 (0.49–1.84)	
	36.2–43.6	74	77	0.96 (0.57–1.61)		36	77	1.21 (0.62–2.36)	
	43.7–54.3	73	77	0.87 (0.51–1.48)		30	77	0.96 (0.49–1.92)	
	>54.3	84	77	0.85 (0.49–1.46)		39	77	0.96 (0.48–1.92)	
	Continuous			1.00 (0.99–1.01)				1.00 (0.99–1.02)	
	Continuous-Log_2			1.00 (0.68–1.47)				1.03 (0.62–1.70)	
HbGA	≤23	56	76	1.00 (ref)	0.74	29	76	1.00 (ref)	0.92
	23.1–29.4	85	78	1.28 (0.76–2.15)		42	78	1.31 (0.68–2.52)	
	29.5–37.6	87	77	1.20 (0.71–2.04)		30	77	1.01 (0.51–2.01)	
	37.7–46.9	75	77	1.06 (0.62–1.83)		29	77	1.03 (0.52–2.06)	
	>46.9	80	77	0.94 (0.54–1.63)		41	77	1.06 (0.53–2.12)	
	Continuous			1.00 (0.98–1.01)				1.00 (0.99–1.01)	
	Continuous-Log_2			0.92 (0.66–1.28)				1.00 (0.66–1.50)	
Sum of HbAA + HbGA	≤53.6	67	77	1.00 (ref)	0.95	34	77	1.00 (ref)	0.97
	53.7–66.3	81	76	1.16 (0.69–1.96)		38	76	1.15 (0.59–2.23)	
	66.4–81.8	78	78	0.99 (0.59–1.67)		30	78	0.91 (0.47–1.78)	
	81.9–100.2	73	77	1.05 (0.61–1.81)		29	77	0.98 (0.49–1.96)	
	>100.2	84	77	0.95 (0.55–1.63)		40	77	0.97 (0.49–1.91)	
	Continuous			1.00 (0.99–1.01)				1.00 (0.99–1.01)	
	Continuous-Log_2			0.97 (0.67–1.41)				1.02 (0.64–1.63)	
Ratio of HbGA/HbAA	≤0.69	62	76	1.00 (ref)	0.16	27	76	1.00 (ref)	0.02
	0.70–0.80	92	78	1.29 (0.78–2.14)		49	78	1.93 (1.01–3.69)	
	0.81–0.88	57	73	0.72 (0.42–1.26)		24	73	0.75 (0.36–1.56)	
	0.89–0.98	73	78	0.79 (0.46–1.35)		29	78	0.81 (0.39–1.68)	
	>0.98	99	80	1.08 (0.64–1.84)		42	80	1.45 (0.73–2.88)	
	Continuous			0.82 (0.26–2.54)				0.99 (0.19–5.05)	

All models are adjusted for age at recruitment, country, fasting status, date at blood collection, time of the day of blood collection, OC use, HRT use, alcohol intake, parity, age at menopause, and BMI.
Abbreviations: OR, odds ratio; CI, confidence interval; EC, endometrial cancer; EPIC, European Prospective Investigation into Cancer and Nutrition; HbAA, hemoglobin adducts of acrylamide; HbGA, hemoglobin adducts of glycidamide.

day of blood draw (±1 hrs), and fasting status (<3, 3–6, >6 hrs). Individual matching was broken in the present study (one control per case) because we only included women who were non-smokers, defined as women who reported never smoking or who quit smoking ≥5 years before recruitment, and who were postmenopausal at blood draw, defined as women who reported not having menses ≥1 year before recruitment.

A total of 771 subjects (385 EC cases and 386 controls) were included in the study. Of these, three had to be excluded due to the lack of information on HbAA ($n = 2$ cases) or HbGA ($n = 1$ control), leaving 383 EC cases and 385 controls included in the final analyses. Only one observation had an HbGA value below the LOD; thus, we assigned half of the corresponding value of the LOD (2 pmol g^{-1} Hb). Tumor histology was available for 372 (97%) cases, of which 171(46%) were classified as endometrioid tumors (type-I), 14 (4%) as serous/clear cell tumors (type-II), and 187 (50%) as other types. "Overall EC" comprises type-I, type-II, and tumors that were classified as others or undefined for histology.

To improve normality of the distributions, all biomarker variables were log-transformed (log_2) and were evaluated as: log_2HbAA, log_2HbGA, sum of total adducts [log_2(HbAA+HbGA)], and HbGA/HbAA ratio. Additionally, these four continuous variables were categorized into quintiles based on the distribution in the control group. Unconditional logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CI). Analyses were also performed separately for type-I EC tumors.

Table 3. Stratified analyses: OR and 95% CI for biomarkers of acrylamide exposure and EC risk in a nested case-control study in the EPIC cohort.

		<25 kg m^{-2}			≥25 kg m^{-2}			Never drinkers			Drinkers			Nonoral contraceptive users			Oral contraceptive users		
	Cutpoints	Cases	Controls	OR (95%CI)[1]	Cases	Controls	OR (95%CI)[1]	Cases	Controls	OR (95%CI)[2]	Cases	Controls	OR (95%CI)[2]	Cases	Controls	OR (95%CI)[3]	Cases	Controls	OR (95%CI)[3]
HbAA	≤30.3	18	21	1.00 (ref)	59	53	1.00 (ref)	21	20	1.00 (ref)	56	54	1.00 (ref)	56	56	1.00 (ref)	19	18	1.00 (ref)
	30.4–37.6	25	32	0.79 (0.29–2.15)	50	48	0.91 (0.49–1.71)	21	18	1.32 (0.47–3.69)	54	62	0.71 (0.39–1.31)	46	48	0.87 (0.47–1.63)	29	30	0.85 (0.30–2.40)
	37.7–45.3	18	40	0.47 (0.17–1.35)	56	37	1.37 (0.72–2.60)	14	22	0.66 (0.22–1.97)	60	55	1.06 (0.58–1.95)	47	45	0.98 (0.52–1.84)	25	30	0.96 (0.34–2.74)
	45.4–56.0	27	27	1.03 (0.35–3.01)	46	50	0.60 (0.31–1.16)	16	20	0.98 (0.31–3.03)	57	57	0.88 (0.47–1.66)	43	43	0.92 (0.47–1.79)	26	31	0.72 (0.25–2.03)
	>56.0	30	34	0.84 (0.29–2.41)	54	43	0.87 (0.44–1.70)	22	13	1.24 (0.36–4.28)	62	64	0.75 (0.40–1.41)	57	45	1.13 (0.58–2.20)	25	31	0.62 (0.21–1.83)
	LRT[4]			0.06						0.42						0.63			
HbGA	≤23	21	26	1.00 (ref)	35	50	1.00 (ref)	7	15	1.00 (ref)	49	61	1.00 (ref)	40	48	1.00 (ref)	16	27	1.00 (ref)
	23.1–29.4	26	37	0.75 (0.30–1.87)	59	41	1.88 (0.98–3.62)	29	18	3.89 (1.11–13.71)	56	60	0.97 (0.54–1.75)	50	52	0.93 (0.49–1.77)	35	24	2.89 (1.08–7.75)
	29.5–37.6	31	36	0.60 (0.24–1.54)	56	41	1.73 (0.87–3.44)	14	22	1.13 (0.30–4.18)	73	55	1.33 (0.74–2.40)	55	41	1.36 (0.70–2.64)	27	33	1.30 (0.48–3.52)
	37.7–46.9	19	27	0.54 (0.19–1.59)	56	50	1.38 (0.71–2.69)	19	23	1.62 (0.44–6.00)	56	54	1.02 (0.55–1.89)	49	49	1.00 (0.51–1.98)	23	27	1.75 (0.62–4.95)
	>46.9	21	28	0.55 (0.19–1.59)	59	49	1.25 (0.63–2.49)	25	15	2.17 (0.54–8.79)	55	62	0.76 (0.41–1.42)	55	47	1.16 (0.58–2.30)	23	29	0.78 (0.27–2.30)
	LRT[4]			0.35						0.05						0.07			
Sum of HbAA + HbGA	≤53.6	20	24	1.00 (ref)	47	53	1.00 (ref)	14	17	1.00 (ref)	53	60	1.00 (ref)	49	55	1.00 (ref)	18	22	1.00 (ref)
	53.7–66.3	24	33		57	43		26	19		55	57		46	45		33	28	

Table 3. Stratified analyses: OR and 95% CI for biomarkers of acrylamide exposure and EC risk in a nested case-control study in the EPIC cohort. (Continued)

		<25 kg m^{-2}			≥25 kg m^{-2}			Never drinkers			Drinkers			Nonoral contraceptive users			Oral contraceptive users		
	Cutpoints	Cases	Controls	OR (95%CI)[1]	Cases	Controls	OR (95%CI)[1]	Cases	Controls	OR (95%CI)[2]	Cases	Controls	OR (95%CI)[2]	Cases	Controls	OR (95%CI)[3]	Cases	Controls	OR (95%CI)[3]
				0.91 (0.34–2.46)			1.41 (0.75–2.65)			2.17 (0.69–6.76)			0.93 (0.50–1.71)			1.19 (0.63–2.27)			1.30 (0.47–3.54)
	66.4–81.8	24	41	0.48 (0.18–1.27)	54	37	1.48 (0.76–2.89)	12	21	0.80 (0.25–2.63)	66	57	1.10 (0.61–2.01)	51	45	1.10 (0.58–2.10)	24	31	0.90 (0.33–2.48)
	81.9–100.2	26	25	1.04 (0.36–2.98)	47	52	0.86 (0.44–1.67)	17	23	1.07 (0.32–3.55)	56	54	1.09 (0.59–2.03)	45	44	1.21 (0.62–2.37)	25	31	0.89 (0.32–2.50)
	>100.2	24	31	0.76 (0.27–2.20)	60	46	1.09 (0.56–2.12)	25	13	1.64 (0.45–6.00)	59	64	0.80 (0.43–1.48)	58	48	1.25 (0.64–2.42)	24	28	0.65 (0.22–1.89)
	LRT[4]			0.09						0.14						0.68			
Ratio of HbGA/HbAA	≤0.69	34	40	1.00 (ref)	28	36	1.00 (ref)	8	13	1.00 (ref)	54	63	1.00 (ref)	38	37	1.00 (ref)	23	37	1.00 (ref)
	0.70–0.80	35	39	1.14 (0.51–2.53)	57	39	1.63 (0.79–3.35)	24	13	2.79 (0.75–10.34)	68	65	1.08 (0.62–1.88)	56	48	1.05 (0.54–2.06)	35	28	2.47 (1.03–5.94)
	0.81–0.88	14	30	0.45 (0.17–1.16)	43	43	1.10 (0.52–2.30)	8	18	0.44 (0.10–1.86)	49	55	0.81 (0.44–1.48)	35	42	0.63 (0.31–1.31)	20	29	1.10 (0.42–2.87)
	0.89–0.98	16	22	0.63 (0.22–1.79)	57	56	1.03 (0.51–2.07)	18	25	0.69 (0.19–2.48)	55	53	0.83 (0.45–1.52)	51	56	0.73 (0.37–1.46)	21	22	1.17 (0.44–3.11)
	>0.98	19	23	0.75 (0.27–2.07)	80	57	1.59 (0.80–3.17)	36	24	1.12 (0.33–3.82)	63	56	1.01 (0.55–1.86)	69	54	1.06 (0.53–2.10)	25	24	1.25 (0.47–3.37)
	LRT[4]			0.76						0.16						0.56			

[1]Adjusted for age at recruitment, country, fasting status, date at blood collection, OC use, HRT use, alcohol intake, parity, and age at menopause. [2]Adjusted for age at recruitment, country, fasting status, date at blood collection, OC use, HRT use, parity, age at menopause, and BMI. [3]Adjusted for age at recruitment, country, fasting status, date at blood collection, HRT use, alcohol intake, parity, age at menopause, and BMI. [4]All LRT *p* values for effect measure modification are based on the categorical exposure adduct variable.

Abbreviations: OR, odds ratio; CI, confidence interval; EC, endometrial cancer; EPIC, European Prospective Investigation into Cancer and Nutrition; HbAA, hemoglobin adducts of acrylamide; HbGA, hemoglobin adducts of glycidamide.

All statistical models were adjusted for matching variables (age at recruitment (years), country, date of blood draw, time of day of the blood draw, and fasting status), and other covariates such as ever use of OC (never, ever), ever use of hormone replacement therapy (never, ever; HRT), parity (nulliparous, 1, 2, ≥3, parous but with missing number of full-term pregnancies), age at menopause (years), body mass index (kg m^{-2}; BMI), and alcohol intake (non-drinkers, drinkers of 0–6, >6–12, >12–24, and >24 g day^{-1}). The following variables were evaluated as potential confounders but were not included in final models because they did not change the risk estimates by >10%: dietary variables (such as coffee, potatoes, biscuits, crackers and cakes), history of diabetes (yes, no), age at menarche (<12, 12, 13, 14, ≥15 years), height (cm), weight (kg), hip circumference (cm), waist circumference (cm), physical activity using the Cambridge index[24] and education level (none, primary, technical/professional, secondary and higher education).

Effect-measure modification was evaluated for established risk factors, and for factors considered to affect the activity of Cyp2e1: BMI (<25 *vs.* ≥25 kg m^{-2}), HRT use (never *vs.* ever users), OC (never *vs.* ever users), and alcohol intake (never *vs.* ever drinkers)[5] using a likelihood ratio test (LRT) based on categorical biomarker variables. For each biomarker quartile, the median was estimated, and was included in a score test to evaluate dose-response trends.

The reproducibility of the hemoglobin adducts measurements was assessed using 43 (5%) duplicate blood samples revealing intraclass correlation coefficients of 0.92 for HbAA and 0.95 for HbGA. All statistical tests were two-sided and statistical significance was set at $p < 0.05$. All analyses were performed using SAS v. 9.1 (Cary, NC).

Results

A large number of cases and controls were from Italy and the United Kingdom, and the major proportion of type-I EC cases were from Germany and The Netherlands (Table 1). The median interval between the dates at blood collection and diagnosis was 6.2 years. Among cases, the median (25th–75th percentile) HbAA and HbGA adducts levels were 39.9 (31.4–52.4) and 34.1 (25.7–44.6) pmol/g Hb, respectively; and in controls 39.4 (32.1–51.1) and 33.3 (24.6–43.8) pmol/g Hb, respectively. As compared with controls, cases were slightly younger, had a slightly higher proportion of heavy drinking (6.5% *vs.* 5.5%), tended to use less OCs (32.4% *vs.* 36.4%) and more HRT (27.2% *vs.* 21.6), had higher median BMI values (27.4 *vs.* 26.1 kg m^{-2}), and were more likely to be nulliparous (16.2% *vs.* 10.9%). Cases and controls had similar ages at menopause.

No associations and no evidence for linear dose–response trends were observed between biomarkers of dietary acrylamide exposure and overall EC (highest *vs.* lowest quintiles: $HR_{HbAA;Q5vsQ1}$: 0.85, 95%CI: 0.49–1.46; $HR_{HbGA;Q5vsQ1}$: 0.94, 95%CI: 0.54–1.63) (Table 2). We also restricted the analyses to known type-I EC cases and no statistically significant associations were observed (Table 2). Associations between biomarkers of exposure and overall or type-I EC risk were also assessed using tertiles, quartiles, and deciles (based on the exposure distribution in the control group), and no significant variations in risk were observed across categories (data not shown).

Subgroup analyses for overall EC were stratified by BMI (<25, ≥25 kg m^{-2}), alcohol intake (never drinkers, ever drinkers), HRT use (never HRT users, ever HRT users; data not shown) and OC use (never OC users, ever OC users). No evidence for effect measure modification was observed in any of the subgroups evaluated (all LRT p values >0.05) (Table 3). Because of the small sample size, stratified analyses for type-I EC were conducted using tertiles, and results indicated no heterogeneity (data not shown).

Discussion

The present nested case-control study within the EPIC cohort is the first epidemiologic study to evaluate the association between biomarkers of acrylamide exposure and endometrial cancer risk. We did not observe any evidence to support the hypothesis that levels of biomarkers of acrylamide and glycidamide exposure measured as hemoglobin adducts (HbAA, HbGA, sum of total adducts and HbGA/HbAA ratio) were associated with the risk of developing overall EC or type-I EC in nonsmoking postmenopausal women. Furthermore, there was no evidence for effect measure modification by BMI, alcohol intake, HRT use or OC use though there was relatively limited power to assess heterogeneity among subgroups.

The present study was based on a subgroup of nonsmoking postmenopausal women in the EPIC cohort to address two major concerns. First, tobacco smoking is considered one of the major sources of acrylamide exposure, and it is recognized that smokers have higher levels of acrylamide biomarkers[10]; second, hormonal homeostasis may be disrupted by acrylamide,[7,8] thus, the analyses were performed in nonsmoking postmenopausal women only.

The lack of association between biomarkers of acrylamide exposure and overall and type-I EC risk is in agreement with results we previously reported in the EPIC sub-cohort of women, where hazard ratios were estimated for the association between dietary acrylamide intake (assessed through DQs) and overall EC ($n = 1{,}382$) or type-I EC risk ($n = 627$); nevertheless, in the full cohort analysis, positive associations were reported between acrylamide intake and type-I EC risk in women who were never smokers and non-users of OCs.[19] In the present study, using circulating biomarkers of acrylamide exposure, we did not replicate these results possibly due to the small sample size with tumor histology information ($n = 171$ type-I EC cases). Additionally, the null results based on FFQ data reported by the Swedish Mammography Cohort study[17] are also in line with the results presented in the current study. However, the Netherlands Cohort Study reported hazard ratios for dietary acrylamide intake and risk of EC of 1.29 (95%CI: 0.81–2.07; p-trend: 0.18) and 1.99 (95%CI: 1.25–3.52; p-trend: 0.03) in the entire cohort and in never

smoking women, respectively.[16] The Nurses' Health Study also reported relative risks for dietary acrylamide intake of 1.41 (95%CI: 1.01–1.97; *p*-trend: 0.03) and 1.43 (95%CI: 0.90–2.28; *p*-trend: 0.04) in the entire cohort and in never smoking women.[18] Two recent meta-analyses concluded that higher consumption of dietary acrylamide was significantly associated with overall EC risk in never smoking women; but not in all women combined.[20,21] In the present study of acrylamide and glycidamide biomarkers and EC risk in nonsmoking postmenopausal women, we did not observe any evidence for associations with overall or type-I EC risk.

The main strengths of the present nested case-control study are its study design, with the intention to prevent confounding from tobacco smoking and hormonal fluctuations, and the use of prospective information on the main risk factors for EC. The minimum detectable ORs at 80% power in our study were 1.22 and 1.60 for the continuous and categorical variables, respectively. Moreover, measurement errors from using acrylamide intake estimates based on FFQs were avoided, and the quantification of HbAA and HbGA was performed following rigorous quality assurance/quality control laboratory protocols[10]; and all blood samples were drawn from participants before disease diagnosis. The present study also had limitations: (a) a single blood sample was collected at baseline for each observation, thus, we were not able to measure intra-individual variability in adduct measurements. Hemoglobin adducts of acrylamide and glycidamide reflect exposure to acrylamide within the past 4 months, thus, a single measurement may not capture long-term average exposure in the presence of high intra-individual variability. In a small study of 13 participants Vikström *et al.* observed high intra-individual variability (up to twofold and fourfold differences in HbAA and HbGA levels, respectively) over a period of 20 months.[25] By contrast, the NHS-II study observed lower intra-individual variability for Hb-adduct measurements (intra-individual correlation = 0.78, 0.80, and 0.77 for HbAA, HbGA and sum of HbAA+HbGA, respectively) from 45 nonsmoking women at two time-points separated by a median of 23 months.[26] (b) Although all models accounted for matching variables as well as known EC risk factors, we cannot exclude the possibility of residual confounding in our analyses. (c) Further, variables for second-hand smoke (SHS) exposure could not be evaluated in statistical models due to the large number of missing values (>50%). In a subset of the present study with available data, no statistically significant differences in Hb-adducts levels were observed between controls who reported not being exposed to SHS ($n = 80$) and controls who were exposed to SHS ($n = 53$) (data not presented). Moreover, two additional studies reported null or negligible effects of SHS on biomarkers of acrylamide exposure.[27,28] (d) Despite having information on tumor histology for 97% of the EC cases (of which 46% were classified as type-I), we were not able to analyze type-II EC due to the small sample size ($n = 14$).

In conclusion, this study does not provide evidence of an association between levels of hemoglobin adducts of acrylamide and glycidamide and risks of overall EC and type-I EC.

Acknowledgement

MO-S is affiliated with the University of Barcelona.

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5.5 Publication 5: Acrylamide and glycidamide hemoglobin adduct levels and epithelial ovarian cancer risk: a nested-case control study in nonsmoking postmenopausal women from the EPIC cohort

5.5.1 Resum

L'acrilamida va ser classificada com a substància 'probablement cancerígena' pels humans l'any 1994 per l'Agència Internacional d'Investigació del Càncer (IARC). El càncer d'ovari epitelial (COE) és la quarta causa de mortalitat per càncer en les dones. Cinc estudis epidemiològics han avaluat l'associació entre el risc de COE i la ingesta dietètica d'acrilamida utilitzant qüestionaris de freqüència alimentària, i només un estudi de casos i controls niat ha avaluat l'associació entre el risc de COE i els nivells en sang dels adductes d'acrilamida i glicidamida en l'hemoglobina (HbAA i HbGA, respectivament). Els resultats d'aquests estudis són inconsistents.

Dins el marc de l'Estudi Prospectiu Europeu sobre Càncer i Nutrició (EPIC) es va realitzar un estudi de casos i controls niat en dones post-menopàusiques i no fumadores (334 casos de COE i 417 controls). Es van utilitzar models de regressió logística incondicionals i multivariats per estimar *odds ratios* (OR) i els intervals de confiança (95% CI) per a l'associació entre HbAA, HbGA, la suma total d'adductes (HbAA+HbGA) i el seu quocient (HbGA/HbAA) i el risc de desenvolupar COE i el COE de tipus invasiu-serós.

En general, no es va trobar associacions entre els biomarcadors d'exposició a l'acrilamida (analitzats en quintils) i el risc de COE. Tanmateix, es van observar associacions positives entre algunes categories dels quintils i el risc de COE. Quan es van estudiar les variables HbGA i HbAA+HbGA en relació al risc de desenvolupar COE de tipus invasiu-serós, es van observar ORs elevats però no van ser estadísticament significatius i tampoc van mostrar cap evidència de dosis-resposta ($OR_{HbGA,\ Q5vsQ1}$: 1.91, 95% CI: 0.96-3.81 i $OR_{HbAA+HbGA,\ Q5vsQ1}$: 1.90, 95% CI: 0.94-3.83).

Aquest estudi de casos i controls niat realitzat amb participants de l'estudi EPIC no va observar cap associació clara entre els biomarcadors de l'exposició a l'acrilamida i el risc de desenvolupar COE i el COE de tipus invasiu-serós. És poc probable que la ingesta d'acrilamida augmenti el risc de càncer d'ovari; no obstant això, es requereixen més estudis i, amb mostres més grans, per descartar qualsevol possible associació amb el risc de càncer d'ovari.

Paper 5

Obón-Santacana M, Lujan-Barroso L, Travis RC, Freisling H, Ferrari P, Severi G, Baglietto L, Boutron-Ruault MC, Fortner RT, Ose J, Boeing H, Menéndez V, Sánchez-Cantalejo E, Chamosa S, Castaño JM, Ardanaz E, Khaw KT, Wareham N, Merritt MA, Gunter MJ, Trichopoulou A, Papatesta EM, Klinaki E, Saieva C, Tagliabue G, Tumino R, Sacerdote C, Mattiello A, Bueno-de-Mesquita HB, Peeters PH, Onland-Moret NC, Idahl A, Lundin E, Weiderpass E, Vesper HW, Riboli E, Duell EJ.

Acrylamide and glycidamide hemoglobin adduct levels and epithelial ovarian cancer risk: A nested-case control study in nonsmoking postmenopausal women from the EPIC cohort

Cancer Epidemiology Biomarkers & Prevention. 2016 Jan;25(1):127-34. doi: 10.1158/1055-9965.EPI-15-0822. Epub 2015 Nov 23.

Research Article

Cancer Epidemiology, Biomarkers & Prevention

Acrylamide and Glycidamide Hemoglobin Adducts and Epithelial Ovarian Cancer: A Nested Case–Control Study in Nonsmoking Postmenopausal Women from the EPIC Cohort

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Abstract

Background: Acrylamide was classified as "probably carcinogenic to humans (group 2A)" by the International Agency for Research on Cancer. Epithelial ovarian cancer (EOC) is the fourth cause of cancer mortality in women. Five epidemiological studies have evaluated the association between EOC risk and dietary acrylamide intake assessed using food frequency questionnaires, and one nested case–control study evaluated hemoglobin adducts of acrylamide (HbAA) and its metabolite glycidamide (HbGA) and EOC risk; the results of these studies were inconsistent.

Methods: A nested case–control study in nonsmoking postmenopausal women (334 cases, 417 controls) was conducted within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. Unconditional logistic regression models were used to estimate ORs and 95% confidence intervals (CI) for the association between HbAA, HbGA, HbAA+HbGA, and HbGA/HbAA and EOC and invasive serous EOC risk.

Results: No overall associations were observed between biomarkers of acrylamide exposure analyzed in quintiles and EOC risk; however, positive associations were observed between some middle quintiles of HbGA and HbAA+HbGA. Elevated but nonstatistically significant ORs for serous EOC were observed for HbGA and HbAA+HbGA (OR_{Q5vsQ1}, 1.91; 95% CI, 0.96–3.81 and OR_{Q5vsQ1}, 1.90; 95% CI, 0.94–3.83, respectively); however, no linear dose–response trends were observed.

Conclusion: This EPIC nested case–control study failed to observe a clear association between biomarkers of acrylamide exposure and the risk of EOC or invasive serous EOC.

Impact: It is unlikely that dietary acrylamide exposure increases ovarian cancer risk; however, additional studies with larger sample size should be performed to exclude any possible association with EOC risk. *Cancer Epidemiol Biomarkers Prev; 25(1); 127–34. ©2015 AACR.*

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Introduction

In 1994, the International Agency for Research on Cancer (IARC) classified acrylamide as "probably carcinogenic to humans (group 2A)." Acrylamide is formed in carbohydrate rich foods during common cooking procedures such as frying, baking, or roasting, which involve temperatures usually higher than 120°C (1, 2).

Acrylamide is thought to be absorbed in the gastrointestinal tract mainly through passive transport, and once it is in the body, is metabolized by at least two pathways: via direct conjugation with glutathione for its elimination, or via the Cyp2e1 enzyme system to form glycidamide, a DNA-reactive epoxide (3). Both acrylamide and glycidamide can interact with hemoglobin to form adducts (HbAA and HbGA, respectively) which are considered relevant biomarkers of internal exposure, represent exposure over the life-span of erythrocytes, previous ≈4 months (4, 5), and have been used in multiple epidemiological and experimental studies. In addition to dietary acrylamide intake, tobacco smoking, occupational exposures, and environmental tobacco smoke can also influence levels of HbAA and HbGA (6). It has been observed that smokers have, on average, three to four times higher levels of hemoglobin adducts than nonsmokers (7).

Genotoxic and mutagenic properties have been described in animals after glycidamide exposure. Furthermore, several animal studies observed an increase in the incidence of hormone and nonhormone-related tumors after acrylamide exposure (8).

Almost 90% of malignant ovarian tumors are epithelial ovarian cancer (EOC), which is the seventh most common cancer in women worldwide, and the fourth cause of cancer mortality in women (9). The 5-year survival rate ranges between 30% and 50% depending upon geographic region (10). There is epidemiological evidence that both adult attained height and body mass index (BMI) increase the risk of developing EOC (11, 12), and that tobacco smoking is positively associated with mucinous ovarian cancer (13, 14), whereas oral contraceptive (OC) use and full-term pregnancy are established preventive factors (15).

Four prospective cohort studies and one case–control study have evaluated the association between dietary acrylamide intake (assessed using food frequency questionnaires, FFQ) and EOC risk (16–20). A lack of association was reported in an Italian case–control study (20), the prospective Swedish Mammography Cohort (SMC; ref. 17), and the EPIC cohort (19). The Nurses' Health Study (NHS) observed a nonstatistically significant increased risk only for serous EOC tumors (18). Nevertheless, a prospective study within the Netherlands Cohort Study (NLCS) observed a statistically significant positive association between high consumption of acrylamide and overall EOC risk (16). A nested case–control study was subsequently conducted within the NHS and the NHSII (NHS/NHSII) to examine the relation between acrylamide exposure measured as hemoglobin adducts and EOC risk (21); however, no evidence for any associations for overall EOC or serous EOC risk were observed comparing the highest to the lowest tertile of HbAA and HbGA.

The present nested case–control study was performed in a subgroup of nonsmoking postmenopausal women from the EPIC cohort with the aim to evaluate the association between EOC risk and hemoglobin adducts of acrylamide/glycidamide. Analyses by different EOC histologic subtype and tumor invasiveness were also performed, as well as stratified analyses by known risk and preventive factors in the development of EOC.

Materials and Methods

Study population and data collection

The EPIC study is an ongoing multicenter prospective cohort study which comprises 23 research centers in 10 European countries (France, Italy, Spain, the United Kingdom, The Netherlands, Greece, Germany, Sweden, Denmark, and Norway). Norway, Denmark, and a center from Sweden (Malmö) did not participate in the present nested case–control study. All EPIC study participants signed an informed consent at recruitment (range: 1992–2000), and the study was approved by both the ethical review boards from the IARC, and local ethics committees. Details of the study methodology have been previously described (22).

The EPIC study includes 153,427 men and 367,903 women. At recruitment, participants completed country-specific, validated dietary questionnaires (DQ) with the time frame referring to the previous year. Information on lifestyle factors (such as tobacco smoking, level of education, socioeconomic status, alcohol consumption, and physical activity), anthropometric factors, brief occupational history, and medical history were also assessed at recruitment. Women also reported baseline information on menstrual and reproductive factors [i.e., age at first menstrual period, pregnancy, use of OC, use of hormone replacement therapy (HRT), and menopausal status].

The standardized protocol followed to collect and store blood samples at recruitment has been previously published (22). Briefly, almost 80% of the EPIC participants, of which 226,673 were women, provided a single blood sample. Most of the samples were stored in liquid nitrogen (−196°C) at the IARC bio-bank; however, samples from Sweden (Umeå) were stored in freezers (−80°C) at the Medical Biobank of Northern Sweden.

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doi: 10.1158/1055-9965.EPI-15-0822

Identification of epithelial ovarian cancer cases and selection of the study population

Incident EOC were classified according to the International Classification of Diseases for Oncology (ICD-0-3), and included epithelial borderline tumors (C56.9), invasive epithelial ovarian (C56.9), fallopian tube (C57.0), and primary peritoneal (C48) cancers. Incident EOC were recorded through a combination of methods (health insurance records, cancer and pathology registries, and active follow-up), or via population cancer registries.

Cases and controls for the present nested case–control study were selected according to the methodology described by Peeters and colleagues (23). To summarize, for each case (participant who developed an ovarian, fallopian tube, or peritoneal tumor after the date of blood draw and before the end of follow-up) two controls free of cancer (with the exception of nonmelanoma skin cancer) were randomly selected at the time of diagnosis using a density sampling protocol. Matching criteria included study center, menopausal status (premenopausal, postmenopausal, perimenopausal), age at recruitment (±6 months), time of the day of blood collection (±1 hour), and fasting status (<3, 3–6, >6 hours). For the current study of hemoglobin adducts, one control per case was selected. Because acrylamide may disrupt hormonal levels, and tobacco smoking is an important source of acrylamide exposure (7, 24, 25), this study only included women who at baseline reported being postmenopausal and nonsmokers (thus, individual matching was broken). Postmenopausal women were defined as those who were >55 years old, or who reported not having had any menses during the 12 months before recruitment. Nonsmokers women were defined as those who reported never smoking or having given up smoking ≥5 years before recruitment.

A total of 751 participants (334 EOC cases and 417 controls) were included in the study. EOC comprised both borderline (n = 2, 1%) and invasive tumors (n = 332, 99%). Invasive EOC were classified into subtypes: serous (n = 191, 58%), endometrioid (n = 26, 8%), mucinous (n = 18, 5%), clear cell (n = 12, 3%), not otherwise specified (NOS) which included adenocarcinomas, carcinomas, and cystadenocarcinoma (n = 79, 24%), and others (n = 6, 2%).

Measurement of acrylamide and glycidamide hemoglobin adducts

Blood samples were sent to the Center for Disease Control and Prevention (CDC) Protein Biomarker Laboratory (Atlanta, USA) to measure HbAA and HbGA. Details of the methodology can be found elsewhere (7, 26). Briefly, 300 μL of red blood cells were hemolyzed and analyzed using HPLC/tandem mass spectrometry (HPLC/MS-MS). Laboratory personnel were blinded to the case–control status of participants, and blood samples were analyzed in a randomized manner. Concentrations of HbAA and HbGA were reported relative to the amount of hemoglobin (pmol per g of Hb), and two independent measures were performed for each sample. The lower limits of detection for this method are 3 pmol/g of Hb for HbAA, and 4 pmol/g of Hb for HbGA. All of the HbAA and HbGA measurements were within the limits of detection. In this study, 42 of the 751 blood samples were sent in duplicate to the laboratory to independently assess the reproducibility of the hemoglobin adduct measures, which had intraclass correlation coefficients of 0.94 for HbAA and 0.92 for HbGA. The percent coefficient of variation (CV) was estimated using log-transformed (log2) values, and was 9.9 for HbAA and 12.0 for HbGA.

Statistical methods

Unconditional logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (CI) for the association between biomarkers levels of acrylamide and glycidamide and the risk of EOC. Conditional logistic regression model were also evaluated in a sensitivity analyses.

All statistical models were adjusted for matching factors [age at recruitment (in years), country, time of the day of blood draw, date of blood draw, and fasting status] and covariates including OC use (never, ever, unknown), HRT use (never, ever, unknown), alcohol consumption (nondrinkers, drinkers of 0–6, >6–12, >12–24, and >24 g/day), parity (nulliparous, 1, 2, ≥3, parous but with missing number of full-term pregnancies), age at menopause (years), age at first menstrual period (years), and BMI (kg/m^2). Lifestyle, anthropometric, and reproductive variables such as physical activity using the Cambridge index (27), education level (none, primary, technical/professional, secondary, and higher education), height (cm),weight (kg), hip circumference (cm), waist circumference (cm), duration of using OC (years), duration of using HRT (years), and age at first birth (years) were evaluated as potential confounders, but were not included in final models because they did not change effect estimates >10%.

Restricted cubic splines with 3, 4, and 5 knots were evaluated, and indicated nonmonotonic relations between each of the four biomarker variables and EOC risk. Because the relations were not linear, even when exposure variables were logarithmically ($\log_2$) transformed, results for continuous biomarker variables were not presented (28). For each biomarker quintile, the median was estimated, and was included in a score test to evaluate dose–response trends. The four continuous biomarker variables HbAA, HbGA, sum of total adducts (HbAA+HbGA) and HbGA/HbAA ratio were categorized into quintiles based on the exposure distribution in controls. Biomarker quartiles were evaluated in stratified analyses.

Analyses were also carried out excluding borderline tumors (n = 2), and by histologic subtypes: invasive serous EOC, invasive serous EOC combined with NOS, and nonserous EOC (which included endometrioid, mucinous, clear cell, and NOS tumors).

Effect measure modification was evaluated by BMI (<25 kg/m^2 vs. ≥25 kg/m^2), HRT (never vs. ever users), OC (never vs. ever users), and alcohol intake (never vs. ever drinkers) using a likelihood ratio test (LRT). These variables were selected because they are established risk or preventive factors, or because they may affect the activity of Cyp2e1 (29). All statistical tests were two-sided and evaluated at α-level 0.05. All analyses were performed using SAS v. 9.1.

Results

Description of the study population

The present nested case–control study was based on 334 incident EOC cases (of which 191 were classified as serous) and 417 controls. A large proportion of cases and controls were from the United Kingdom and the Netherlands (Table 1). Among cases, the median (quartile range) of HbAA and HbGA levels were 42.2 (33.9–54.4) and 37.0 (28.5–49.5), respectively, whereas controls had HbAA and HbGA levels of 43.1 (33.8–54.8) and 35.4 (26.0–49.9), respectively (Table 1). Cases were slightly younger than controls (58.4 years vs. 59.2 years), tended to have higher BMI values (26.4 vs. 25.8 kg/m^2), a higher proportion of HRT users (27.8% vs. 18.9%), and were

Table 1. Description of the study population from a nested case–control study of acrylamide biomarkers and EOC in the EPIC cohort

	All EOC cases ***n*** **= 334**	**Invasive serous EOC cases** ***n*** **= 191**	**Controls** ***n*** **= 417**
HbAA pmol/g of Hb[a]	42.2 (33.9–54.4)	42.2 (33.8–56.6)	43.1 (33.8–54.8)
HbGA pmol/g of Hb[a]	37.0 (28.5–49.5)	37.0 (28.1–52.2)	35.4 (26.0–49.9)
HbAA+HbGA pmol/g of Hb[a]	79.3 (62.5–105.4)	82.1 (62.0–107.8)	78.7 (60.6–106.0)
HbGA/HbAA pmol/g of Hb[a]	0.9 (0.7–1.0)	0.9 (0.7–1.0)	0.8 (0.7–1.0)
Age at recruitment (years)[a]	58.4 (53.8–63.4)	57.7 (53.0–62.7)	59.2 (54.4–64.2)
Age at first menstrual period (years)[a]	13.0 (12.0–14.0)	13.0 (12.0–14.0)	13.0 (12.0–14.0)
Age at menopause (years)[a]	49.5 (49.0–52.0)	49.5 (49.0–51.0)	49.5 (48.0–52.0)
BMI (kg/m^2)[a]	26.4 (23.4–29.3)	26.0 (22.8–29.3)	25.8 (23.2–29.5)
Country[b]			
France	32(9.6)	23 (12.0)	30 (7.2)
Italy	43 (12.9)	25 (13.1)	52 (12.5)
Spain	36 (10.8)	21 (11.0)	55 (13.2)
United Kingdom	71 (21.3)	29 (15.2)	94 (22.5)
The Netherlands	59 (17.7)	37 (19.4)	78 (18.7)
Greece	27 (8.1)	10 (5.2)	43 (10.3)
Germany	45(13.5)	33 (17.3)	46 (11.0)
Sweden	21 (6.3)	13 (6.8)	19 (4.6)
Fasting status[b]			
Unknown	3 (0.9)	1 (0.5)	2 (0.5)
<3 hours	169 (50.6)	97 (50.8)	213 (51.1)
3–6 hours	44 (13.2)	23 (12.0)	58 (13.9)
>6 hours	118 (35.3)	70 (36.7)	44 (34.5)
Alcohol consumption[b]			
Non drinker	80 (24.0)	47 (24.6)	93 (22.3)
>0–6	166 (49.7)	95 (49.7)	178 (42.7)
>6–12	35 (10.5)	22 (11.5)	73 (17.5)
>12–24	38 (11.4)	19 (10.0)	50 (12.0)
>24–60	15 (4.5)	8 (4.2)	23 (5.5)
Ever use of OC[b]			
Unknown	6 (1.8)	3 (1.6)	4 (1.0)
No	209 (62.6)	114 (59.7)	239 (57.3)
Yes	119 (35.6)	74 (38.7)	174 (41.7)
Ever use of HRT[b]			
Unknown	12 (3.6)	8 (4.2)	13 (3.1)
No	229 (68.6)	123 (64.4)	325 (77.9)
Yes	93 (27.8)	60 (31.4)	79 (18.9)
Parity[b]			
Unknown	41 (12.3)	27 (14.1)	58 (13.9)
1 child	129 (38.6)	81 (42.4)	161 (38.6)
2 children	99 (29.6)	53 (27.8)	141 (33.8)
≥3 children	48 (14.4)	23 (12.0)	44 (10.6)
Nulliparous	8 (2.4)	4 (2.1)	9 (2.2)
Parous but with missing number of full-term pregnancies	9 (2.7)	3 (1.6)	4 (1.0)

Abbreviation: HbGA, hemoglobin adducts of glycidamide.
[a]Median and quartile range (25th–75th percentile).
[b]Number (*n*) and percent (%).

less likely to take OC (35.6% vs. 41.7%). There were no major differences between cases and controls regarding age at menopause, age at first menstrual period, and parity (Table 1). The median interval between the date of blood draw and the date at diagnosis for cases was 6.2 years.

Overall EOC and serous EOC risk

Four multivariate unconditional logistic regression analyses were performed for the association between each biomarker exposure variable and EOC risk. No associations were observed between HbAA levels analyzed in quintiles and EOC risk. Participants with HbGA levels >52.71 pmol/g of Hb (fifth quintile) were at nonsignificant increased EOC risk (OR_{Q5vsQ1}, 1.63; 95% CI, 0.92–2.86). The sum of total adducts was also analyzed. Compared to women with ≤56.70 pmol/g of Hb (reference group), the ORs for the fourth and fifth quintiles were elevated but none were statistically significant. Participants classified in the second and third quintile of HbAA+HbGA were at higher risk of developing EOC (OR_{Q2vsQ1}, 1.81; 95% CI, 1.06–3.10) and (OR_{Q3vsQ1}, 2.00; 95% CI, 1.16–3.45).

Similar models were also evaluated for invasive serous EOC. Despite not observing any statistically significant associations between biomarker levels (HbAA, HbGA, HbAA+HbGA, and HbGA/HbAA) and serous EOC risk, positive nonstatistically significant associations were observed for upper versus lower quintiles of HbGA and HbAA+HbGA (Table 2). Similar patterns were found when borderline tumors were excluded, when nonserous tumors were evaluated, and when invasive serous and NOS were combined in the same analyses (data not shown).

Sensitivity analyses were conducted using conditional logistic regression models, which included 261 cases and 416 controls, to

Table 2. OR and 95% CI for biomarkers of acrylamide exposure and EOC risk in a nested case–control study in the EPIC cohort

	Overall EOC[a]				Sensitivity analysis[b] Overall EOC				Invasive serous EOC[a]			
Exposure cut-points	Cases $n = 334$	Controls $n = 417$	OR (95% CI)	P_{trend}	Cases $n = 261$	Controls $n = 416$	OR (95% CI)	P_{trend}	Cases $n = 191$	Controls $n = 417$	OR (95% CI)	P_{trend}
HbAA												
≤31.30	60	82	1.00 (ref)		45	81	1.00 (ref)		32	82	1.00 (ref)	
31.31–39.10	80	85	1.25 (0.75–2.10)		60	85	1.25 (0.71–2.20)		47	85	1.29 (0.68–2.45)	
39.11–47.20	60	81	1.01 (0.58–1.76)	0.86	48	81	1.11 (0.61–2.01)	0.94	34	81	0.96 (0.48–1.92)	0.59
47.21–59.20	71	86	1.20 (0.69–2.06)		58	86	1.12 (0.63–2.00)		36	86	1.27 (0.65–2.48)	
>59.21	63	83	1.19 (0.67–2.11)		50	83	1.04 (0.56–1.93)		42	83	1.55 (0.78–3.09)	
HbGA												
≤24.70	51	83	1.00 (ref)		39	82	1.00 (ref)		29	83	1.00 (ref)	
24.71–31.30	62	83	1.23 (0.72–2.11)		46	83	1.05 (0.60–1.85)		36	83	1.37 (0.70–2.67)	
31.31–41.20	91	84	2.14 (1.27–3.60)	0.04	75	84	1.76 (1.01–3.08)	0.06	49	84	2.11 (1.10–4.03)	0.20
41.22–52.70	58	84	1.32 (0.75–2.33)		43	84	0.81 (0.43–1.50)		32	84	1.57 (0.78–3.18)	
>52.71	72	83	1.63 (0.92–2.86)		58	83	1.22 (0.66–2.26)		45	83	1.91 (0.96–3.81)	
Sum of HbAA + HbGA												
≤56.70	48	83	1.00 (ref)		38	82	1.00 (ref)		28	83	1.00 (ref)	
56.71–71.00	77	83	1.81 (1.06–3.10)		54	83	1.41 (0.79–2.52)		43	83	1.67 (0.86–3.26)	
71.01–88.90	80	84	2.00 (1.16–3.45)	0.14	68	84	1.77 (0.98–3.19)	0.28	45	84	2.07 (1.06–4.06)	0.30
88.91–112.60	64	84	1.75 (0.98–3.13)		49	84	1.09 (0.58–2.02)		33	84	1.68 (0.82–3.44)	
>112.61	65	83	1.60 (0.89–2.87)		52	83	1.22 (0.65–2.29)		42	83	1.90 (0.94–3.83)	
Ratio of HbGA/HbAA												
≤0.70	55	83	1.00 (ref)		41	83	1.00 (ref)		33	83	1.00 (ref)	
0.71–0.79	55	81	1.07 (0.62–1.83)		42	81	0.96 (0.53–1.74)		33	81	1.18 (0.61–2.30)	
0.80–0.90	78	89	1.43 (0.85–2.41)	0.46	61	89	1.22 (0.71–2.10)	0.66	43	89	1.43 (0.75–2.74)	0.71
0.91–0.99	69	79	1.53 (0.87–2.67)		59	78	1.37 (0.77–2.45)		40	79	1.59 (0.80–3.16)	
>1.00	77	85	1.40 (0.82–2.39)		58	85	1.01 (0.56–1.81)		42	85	1.42 (0.74–2.74)	

Abbreviation: HbGA, hemoglobin adducts of glycidamide.

[a]Models are adjusted for age at recruitment, country, fasting status, date at blood collection, time of the day of blood collection, OC use, HRT use, alcohol intake, parity, age at menopause, age at first menstrual period, and BMI.

[b]Conditional logistic regression model adjusting for matching factors and OC use, HRT use, alcohol intake, parity, age at menopause, age at first menstrual period, and BMI.

estimate ORs of EOC for each biomarker level. Overall, no statistically significant association were observed; nonetheless, results showed similar patters compared to the ones obtained using unconditional logistic regression models (Table 2).

Effect measure modification in EOC

Although some individual ORs were statistically significant, no consistent evidence for effect measure modification by BMI, alcohol intake, OC use (all LRT *P*-values >0.07; Table 3), or by HRT use (data not shown) was observed.

Discussion

The present nested case–control study was performed to assess the association between circulating hemoglobin adducts of acrylamide and glycidamide exposure and the risk of EOC in nonsmoking postmenopausal women from the EPIC cohort. Overall, our results do not support the hypothesis of an association between acrylamide or glycidamide biomarker levels and EOC risk; although increased risks were observed for some middle quintiles of HbGA and HbAA+HbGA, and nonstatistically significant increased risk for serous EOC was observed for the fifth versus the first quintile of HbGA and HbAA+HbGA. No evidence for effect measure modification was noted when subgroups were analyzed.

Acrylamide is thought be carcinogenic through its reactive epoxide, glycidamide, which forms DNA adducts and induces tumor development in animal models (30). Epidemiologic evidence for an association between dietary acrylamide consumption and EOC risk is controversial. Only two of the five published studies (four prospective studies and one case–control study) found positive associations or suggestive increased risks for the relation between acrylamide (measured using FFQs) and overall EOC or serous EOC (16, 18). The main results of the present nested case–control study are in line with the results presented in the Italian case–control, the SMC, and the EPIC cohort study (17, 19, 20).

A previous nested case–control biomarker study (conducted within the NHS and the NHSII) also concluded that there were no associations between adduct levels (measured as HbAA, HbGA, and HbAA+HbGA) and EOC or serous EOC risk (21). However, most of the effect estimates presented in the NHS/NHSII study were below the null value; unlike those observed in the current EPIC study. Moreover, the NHS/NHSII study included participants who were pre- or perimenopausal, and current or former smokers, whereas this study was based on postmenopausal nonsmoking women, because our aim was to evaluate the effect of dietary acrylamide exposure, and tobacco smoking is widely recognized to influence hemoglobin adduct concentrations (7, 31).

Blood samples from both EPIC and the NHS/NHSII studies were measured in the same laboratory using the same protocol. Among cases, the median adducts levels presented in the NHS/NHSII study were 63.8, 49.5, and 112.6 pmol/g Hb, whereas in this study median adducts levels were lower at 42.2, 37.0, and 79.3 pmol/g Hb for HbAA, HbGA, and HbAA+HbGA, respectively. To avoid possible confounding by tobacco smoking, the NHS/NHSII study restricted the analyses to nonsmoking women at the time of blood extraction (230 cases vs. 460 controls), and categorized exposures in tertiles based on the distribution in

Table 3. Stratified analyses: OR and 95% CI for biomarkers of acrylamide exposure and EOC risk in an EPIC nested case–control study in the EPIC cohort

	Normal and underweight			Overweight and obese			Never drinkers			Drinkers			Nonoral contraceptive users			Oral contraceptive users		
Cutpoints	**Cases**	**Controls**	**OR (95% CI)[a]**	**Cases**	**Controls**	**OR (95% CI)[a]**	**Cases**	**Controls**	**OR (95% CI)[b]**	**Cases**	**Controls**	**OR (95% CI)[b]**	**Cases**	**Controls**	**OR (95% CI)[c]**	**Cases**	**Controls**	**OR (95% CI)[c]**
HbAA																		
≤33.60	35	37	1.00 (ref)	45	67	1.00 (ref)	19	26	1.00 (ref)	61	78	1.00 (ref)	55	74	1.00 (ref)	24	30	1.00 (ref)
33.61–42.70	35	42	1.01 (0.44–2.33)	55	61	1.15 (0.63–2.09)	27	21	1.35 (0.51–3.52)	63	82	1.11 (0.65–1.92)	55	45	1.47 (0.81–2.68)	32	55	0.80 (0.34–1.89)
42.71–54.60	28	50	0.68 (0.29–1.59)	56	55	1.52 (0.81–2.87)	19	28	0.65 (0.22–1.96)	65	77	1.40 (0.80–2.43)	55	58	1.22 (0.66–2.26)	27	46	1.12 (0.44–2.88)
>54.61	39	46	1.23 (0.54–2.84)	41	59	0.93 (0.47–1.82)	15	18	0.77 (0.24–2.52)	65	87	1.19 (0.67–2.12)	44	62	0.85 (0.44–1.66)	36	43	1.60 (0.64–4.00)
LRT[d]			0.07						0.42						0.18			
HbGA																		
≤25.90	35	49	1.00 (ref)	27	54	1.00 (ref)	11	22	1.00 (ref)	51	81	1.00 (ref)	44	62	1.00 (ref)	16	41	1.00 (ref)
26.91–35.20	38	46	1.66 (0.76–3.60)	51	59	1.45 (0.76–2.78)	16	19	1.85 (0.53–6.53)	73	86	1.47 (0.87–2.49)	50	57	1.22 (0.67–2.24)	37	47	2.91 (1.18–7.14)
35.21–49.90	29	42	1.53 (0.67–3.51)	72	63	2.10 (1.08–4.08)	35	26	4.32 (1.32–14.18)	66	79	1.80 (1.04–3.13)	67	57	2.02 (1.09–3.76)	32	47	2.33 (0.90–5.99)
>49.91	35	38	1.82 (0.79–4.22)	47	66	1.29 (0.64–2.63)	18	26	1.39 (0.38–5.03)	64	78	1.62 (0.91–2.89)	48	63	1.11 (0.57–2.15)	34	39	3.33 (1.27–8.77)
LRT[d]			0.60						0.66						0.33			
Sum of HbAA + HbGA																		
≤59.80	34	41	1.00 (ref)	34	62	1.00 (ref)	11	21	1.00 (ref)	57	82	1.00 (ref)	47	71	1.00 (ref)	19	32	1.00 (ref)
59.81–78.70	34	48	1.12 (0.50–2.49)	62	58	1.85 (1.00–3.43)	28	22	2.80 (0.91–8.59)	68	84	1.30 (0.77–2.22)	57	51	1.60 (0.88–2.92)	37	54	1.59 (0.67–3.80)
78.80–106.00	34	45	1.27 (0.55–2.93)	54	59	1.51 (0.79–2.88)	26	28	1.97 (0.59–6.54)	62	76	1.52 (0.87–2.63)	56	51	1.72 (0.93–3.20)	30	51	1.21 (0.48–3.06)
>106.01	35	41	1.48 (0.63–3.50)	47	63	1.23 (0.63–2.43)	15	22	1.01 (0.27–3.85)	67	82	1.46 (0.83–2.58)	49	66	1.06 (0.55–2.07)	33	37	2.14 (0.83–5.53)
LRT[d]			0.34						0.40						0.29			
Ratio of HbGA/HbAA																		
≤0.70	39	66	1.00 (ref)	28	37	1.00 (ref)	3	15	1.00 (ref)	64	88	1.00 (ref)	39	49	1.00 (ref)	26	54	1.00 (ref)
0.71–0.80	39	48	1.99 (0.96–4.15)	37	55	0.88 (0.43–1.78)	21	21	15.55 (1.74–138.79)	55	82	1.02 (0.61–1.72)	46	59	1.02 (0.54–1.91)	30	44	2.10 (0.90–4.87)
0.81–1.00	39	31	3.06 (1.34–7.01)	65	77	1.22 (0.62–2.39)	29	24	17.90 (2.00–160.05)	75	84	1.48 (0.88–2.50)	69	65	1.79 (0.96–3.32)	31	41	2.21 (0.90–5.45)
>1.01	20	30	1.06 (0.44–2.52)	67	73	1.15 (0.59–2.23)	27	33	11.20 (1.27–99.05)	60	70	1.24 (0.73–2.11)	55	66	1.17 (0.63–2.19)	32	35	1.86 (0.77–4.51)
LRT[d]			0.18						0.09						0.61			

Abbreviation: HbGA, hemoglobin adducts of glycidamide.

[a]Adjusted for country, fasting status, date at blood collection, time of the day of blood collection, OC use, HRT use, alcohol intake, parity, age at menopause, and age at first menstrual period.

[b]Adjusted for country, fasting status, date at blood collection, time of the day of blood collection, OC use, HRT use, parity, age at menopause, age at first menstrual period, and BMI.

[c]Adjusted for country, fasting status, date at blood collection, time of the day of blood collection, HRT use, alcohol intake, parity, age at menopause, age at first menstrual period, and BMI.

[d]All LRT *P*-values for effect measure modification are based on the categorical exposure adduct variable.

nonsmoking controls; however, referent group cutpoints were higher for HbAA, HbGA, and HbAA+HbGA (0–52.3, 0–40.2, and 0–95.7 pmol/g Hb, respectively) compared with those presented in this study (≤36.5, ≤29.6, and ≤66.2 pmol/g Hb, respectively; tertile data not shown in tables). The minimum detectable OR_{Q5} at 80% power in our study was 1.65, which is similar to the minimum detectable OR (1.78) reported by the NHS/NHSII study.

The design of the present nested case–control study is one of the major strengths, as we wanted to evaluate the dietary contribution to acrylamide biomarker levels and EOC risk, and avoid confounding from tobacco smoking and hormonal oscillations. Dietary acrylamide exposure assessment using FFQs has been criticized due to its low correlation with hemoglobin adducts of exposure in many epidemiologic studies (correlation range: 0.08–0.43; ref. 19); however, this weakness was avoided because our exposure data were based on hemoglobin adducts levels. Furthermore, HbAA and HbGA levels were measured in blood collected before cancer diagnosis, and following exhaustive quality assurance and quality control laboratory protocols (7, 26). There are some limitations that should be noted: (i) only one blood sample was collected at baseline from each participant, and this did not allow us to estimate intra-individual variation; however, a prior study conducted in 45 women from the NHS-II (who provided two to three blood samples over a period of 1–3 years) suggested that biomarkers of acrylamide intake were reproducible over time (32), (ii) although the EPIC study has prospective information for most of the known EOC risks factors, information on endometriosis and polycystic ovary syndrome could not be accounted for in our statistical analyses since it was not collected, (iii) occupational exposure and environmental tobacco smoke exposure could not be evaluated due to the large number of missing values (>50%) for environmental tobacco smoke, and the low prevalence of occupational exposure information in women, (iv) and despite having a larger number of EOC cases ($n = 334$) than the NHS/NHSII study ($n = 263$), we were unable to perform analyses for EOC subtypes other than serous due to small sample size.

In summary, this nested case–control study within the EPIC cohort failed to observe a clear association between biomarkers of acrylamide exposure (measured as hemoglobin adducts of acrylamide and glycidamide in red blood cells) and the risk of EOC or serous EOC. Additional studies with larger sample size, and pooled analysis of existing studies should be performed to exclude any possible association.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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Grant Support

This work was supported by the Wereld Kanker Onderzoek Fonds (WCRF NL; grant WCRF 2011/442) and by the Health Research Fund (FIS) of the Spanish Ministry of Health (Exp PI11/01473). The coordination of EPIC is financially supported by the European Commission (DG-SANCO) and the International Agency for Research on Cancer. The national cohorts are supported by the Health Research Fund (FIS) of the Spanish Ministry of Health, Regional Governments of Andalucía, Asturias, Basque Country, Murcia (no. 6236), Navarra and the Catalan Institute of Oncology, La Caixa (BM 06-130), Red Temática de Investigación Cooperativa en Cáncer (RD12/0036/0018; RD06/0020/0091; Spain); Danish Cancer Society (Denmark); Ligue contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l'Education Nationale, Institut National de la Santé et de la Recherche Médicale (INSERM) (France); Deutsche Krebshilfe, Deutsches Krebsforschungszentrum (DKFZ) and Federal Ministry of Education and Research (Germany); the Hellenic Health Foundation (Greece); Associazione Italiana per la Ricerca sul Cancro (AIRC) and National Research Council (Italy); Dutch Ministry of Public Health, Welfare, and Sports (VWS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF) and Statistics Netherlands (The Netherlands); Nordic Center of Excellence in Food, Nutrition, and Health -Helga (Norway); Swedish Cancer Society, Swedish Scientific Council and Regional Government of Skåne and Västerbotten (Sweden); Cancer Research UK (grant C570/A16491, R.C. Travis; grant 14136, K.T. Khaw, N.J. Wareham; United Kingdom); Medical Research Council (grant G1000143, K.T. Khaw, N.J. Wareham; grant MC_UU_12015/1, N.J. Wareham; United Kingdom). M. Obón-Santacana is affiliated with the University of Barcelona.

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Received August 4, 2015; revised October 27, 2015; accepted October 28, 2015; published OnlineFirst November 23, 2015.

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