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

Mireia Obón-Santacana[1], Petra H.M. Peeters[2,3], Heinz Freisling[4], Laure Dossus[5,6,7], Françoise Clavel-Chapelon[5,6,7], Laura Baglietto[8,9], Helena Schock[10], Renée T. Fortner[10], Heiner Boeing[11], Anne Tjønneland[12], Anja Olsen[12], Kim Overvad[13], Virginia Menéndez[14], Maria-José Sanchez[15,16], Nerea Larrañaga[16,17], José María Huerta Castaño[16,18], Aurelio Barricarte[16,19], Kay-Tee Khaw[20], Nick Wareham[21], Ruth C. Travis[22], Melissa A. Merritt[2], Antonia Trichopoulou[23,24], Dimitrios Trichopoulos[23,24,25], Philippos Orfanos[23,26], Giovanna Masala[27], Sabina Sieri[28], Rosario Tumino[29], Paolo Vineis[2,30], Amalia Mattiello[31], H.B. Bueno-de-Mesquita[2,32,33,34], N. Charlotte Onland-Moret[3], Elisabeth Wirfält[35], Tanja Stocks[35,36], Annika Idahl[37], Eva Lundin[38], Guri Skeie[39], Inger T. Gram[39], Elisabete Weiderpass[39,40,41,42], Elio Riboli[2], and Eric J. Duell[1]

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.*

[1]Unit of Nutrition, Environment, and Cancer, Cancer Epidemiology Research Program, Catalan Institute of Oncology (ICO-IDIBELL), Barcelona, Spain. [2]Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, United Kingdom. [3]Department of Epidemiology, Julius Center for Health Sciences and Primary Care, University Medical Center, Utrecht, the Netherlands. [4]Dietary Exposure Assessment Group, International Agency for Research on Cancer, Lyon, France. [5]Inserm, Centre for research in Epidemiology and Population Health (CESP), U1018, Nutrition, Hormones, and Women's Health team, Villejuif, France. [6]Univ Paris Sud, UMRS 1018, Villejuif, France. [7]IGR, Villejuif, France. [8]Cancer Epidemiology Centre, Cancer Council of Victoria, Melbourne, Australia. [9]Centre for Epidemiology and Biostatistics, School of Population and Global Health, University of Melbourne, Australia. [10]Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany. [11]Department of Epidemiology, German Institute of Human Nutrition, Potsdam-Rehbruecke, Nuthetal, Germany. [12]Danish Cancer Society Research Center, Copenhagen, Denmark. [13]Department of Public Health, Section for Epidemiology, Aarhus University, Aarhus, Denmark. [14]Public Health Directorate, Asturias, Spain. [15]Escuela Andaluza de Salud Pública, Instituto de Investigación Biosanitaria de Granada (Granada.ibs), Granada, Spain. [16]Consortium for Biomedical Research in Epidemiology and Public Health (CIBER Epidemiología y Salud Pública-CIBERESP), Madrid, Spain. [17]Public Health Division of Gipuzkoa-BIODONOSTIA, Basque Regional Health Department, Spain. [18]Department of Epidemiology, Murcia Regional Health Council, Murcia, Spain. [19]Navarre Public Health Institute, Pamplona, Spain. [20]University of Cambridge School of Clinical Medicine, Cambridge, United Kingdom. [21]MRC Epidemiology Unit, University of Cambridge, Cambridge, United Kingdom. [22]Cancer Epidemiology Unit, Nuffield Department of Population Health, University of Oxford, Oxford, United Kingdom. [23]Hellenic Health Foundation, Athens, Greece. [24]Bureau of Epidemiologic Research, Academy of Athens, Athens, Greece. [25]Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts. [26]Department of Hygiene, Epidemiology, and Medical Statistics, University of Athens Medical School, Goudi, Athens, Greece. [27]Molecular and Nutritional Epidemiology Unit, Cancer Research and Prevention Institute – ISPO, Florence, Italy. [28]Epidemiology and Prevention Unit,

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

Fondazione IRCSS Istituto Nazionale dei Tumori, Milano, Italy. [29]Cancer Registry and Histopathology Unit, "Civic - M.P. Arezzo" Hospital, ASP Ragusa, Italy. [30]Human Genetics Foundation, Torino, Italy. [31]Dipartimento di Medicina Clinica e Chirurgia, Federico II University, Naples, Italy. [32]National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands. [33]Department of Gastroenterology and Hepatology, University Medical Centre, Utrecht, the Netherlands. [34]Department of Social & Preventive Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia. [35]Department of Clinical Sciences, Nutrition Epidemiology, Lund University, Malmö, Sweden. [36]Umeå University, Department of Perioperative and Surgical Sciences, Sweden. [37]Department of Clinical Sciences, Obstetrics and Gynecology and Department of Public Health and Clinical Medicine, Nutritional Research, Umeå University, Umeå, Sweden. [38]Department of Medical Biosciences, Pathology, Umeå University, Umeå, Sweden. [39]Department of Community Medicine, Faculty of Health Sciences, UiT The Artic University of Norway, Tromsø, Norway. [40]Cancer Registry of Norway, Oslo, Norway. [41]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden. [42]Department of Genetic Epidemiology, Folkhälsan Research Center, Helsinki, Finland.

Corresponding Author: Eric J. Duell, Unit of Nutrition, Environment, and Cancer, Cancer Epidemiology Research Program, Catalan Institute of Oncology, Avda Gran Via 199-203, 08907 L'Hospitalet del Llobregat, Barcelona, Spain. Phone: 34-932607401; Fax: 34-932607787; E-mail: eduell@iconcologia.net

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

Measurements (IRMM; http://ec.europa.eu/food/food/chemical-safety/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					
									Serous	Mucinous	Endometrioid	Clear cell	NOS	Others
			Median (QR)		Median (QR)		Median (QR)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*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.

Authors' Contributions

Conception and design: M. Obón-Santacana, P.H.M. Peeters, A. Tjønneland, K. Overvad, A. Barricarte, K.-T. Khaw, R. Tumino, P. Vineis, H.B. Bueno-de-Mesquita, E. Weiderpass, E.J. Duell

Development of methodology: M. Obón-Santacana, A. Barricarte, R. Tumino, H.B. Bueno-de-Mesquita, E. Weiderpass, E.J. Duell

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Obón-Santacana, P.H.M. Peeters, F. Clavel-

Chapelon, H. Boeing, A. Tjønneland, K. Overvad, M.-J. Sanchez, N. Larrañaga, A. Barricarte, K.-T. Khaw, N. Wareham, R.C. Travis, A. Trichopoulou, D. Trichopoulos, P. Orfanos, G. Masala, S. Sieri, R. Tumino, A. Mattiello, H.B. Bueno-de-Mesquita, E. Wirfält, A. Idahl, E. Lundin, G. Skeie, E. Weiderpass, E.J. Duell

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Obón-Santacana, P.H.M. Peeters, H. Freisling, R.T. Fortner, M.A. Merritt, N.C. Onland-Moret, I.T. Gram, E. Weiderpass, E.J. Duell

Writing, review, and/or revision of the manuscript: M. Obón-Santacana, P.H.M. Peeters, H. Freisling, L. Dossus, F. Clavel-Chapelon, L. Baglietto, H. Schock, R.T. Fortner, H. Boeing, A. Tjønneland, A. Olsen, K. Overvad, M.-J. Sanchez, N. Larrañaga, J.M. Huerta Castaño, A. Barricarte, K.-T. Khaw, N. Wareham, R.C. Travis, M.A. Merritt, A. Trichopoulou, D. Trichopoulos, P. Orfanos, G. Masala, S. Sieri, P. Vineis, A. Mattiello, H.B. Bueno-de-Mesquita, N. C. Onland-Moret, E. Wirfält, T. Stocks, A. Idahl, E. Lundin, G. Skeie, I.T. Gram, E. Weiderpass, E. Riboli, E.J. Duell

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Obón-Santacana, H. Boeing, V. Menéndez, M.-J. Sanchez, N. Larrañaga, K.-T. Khaw, R. Tumino, A. Idahl, G. Skeie, E. Weiderpass, E.J. Duell

Study supervision: M. Obón-Santacana, P.H.M. Peeters, R. Tumino, H.B. Bueno-de-Mesquita, E. Weiderpass, E.J. Duell

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).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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

References

1. IARC. IARC working group on the evaluation of carcinogenic risks to humans: some industrial chemicals. Lyon, 15–22 February 1994. IARC Monogr Eval Carcinog Risks Hum 1994;60:1–560.
2. Tareke E, Rydberg P, Karlsson P, Eriksson S, Tornqvist M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. J Agric Food Chem 2002;50:4998–5006.
3. Freisling H, Moskal A, Ferrari P, Nicolas G, Knaze V, Clavel-Chapelon F, et al. Dietary acrylamide intake of adults in the European Prospective Investigation into Cancer and Nutrition differs greatly according to geographical region. Eur J Nutr 2013;52:1369–80.
4. Vesper HW, Bernert JT, Ospina M, Meyers T, Ingham L, Smith A, et al. Assessment of the relation between biomarkers for smoking and biomarkers for acrylamide exposure in humans. Cancer Epidemiol Biomarkers Prev 2007;16:2471–8.
5. Friedman MA, Dulak LH, Stedham MA. A lifetime oncogenicity study in rats with acrylamide. Fundam Appl Toxicol 1995;27:95–105.
6. Hogervorst JG, Baars BJ, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA The carcinogenicity of dietary acrylamide intake: a comparative discussion of epidemiological and experimental animal research. Crit Rev Toxicol 2010;40:485–512.
7. Doroshyenko O, Fuhr U, Kunz D, Frank D, Kinzig M, Jetter A, et al. *In vivo* role of cytochrome P450 2E1 and glutathione-S-transferase activity for acrylamide toxicokinetics in humans. Cancer Epidemiol Biomarkers Prev 2009;18:433–43.
8. Pelucchi C, Galeone C, Levi F, Negri E, Franceschi S, Talamini R, et al. Dietary acrylamide and human cancer. Int J Cancer 2006;118: 467–71.
9. Larsson SC, Akesson A, Wolk A. Long-term dietary acrylamide intake and risk of epithelial ovarian cancer in a prospective cohort of Swedish women. Cancer Epidemiol Biomarkers Prev 2009;18:994–7.
10. Wilson KM, Mucci LA, Rosner BA, Willett WC. A prospective study on dietary acrylamide intake and the risk for breast, endometrial, and ovarian cancers. Cancer Epidemiol Biomarkers Prev 2010;19:2503–15.
11. Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA. A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. Cancer Epidemiol Biomarkers Prev 2007;16:2304–13.
12. Kurian AW, Balise RR, McGuire V, Whittemore AS. Histologic types of epithelial ovarian cancer: have they different risk factors? Gynecol Oncol 2005;96:520–30.
13. Cho KR, Shih I. Ovarian cancer. Annu Rev Pathol 2009;4:287–313.
14. Gram IT, Lukanova A, Brill I, Braaten T, Lund E, Lundin E, et al. Cigarette smoking and risk of histological subtypes of epithelial ovarian cancer in the EPIC cohort study. Int J Cancer 2012;130:2204–10.
15. Tsilidis KK, Allen NE, Key TJ, Dossus L, Lukanova A, Bakken K, et al. Oral contraceptive use and reproductive factors and risk of ovarian cancer in the European Prospective Investigation into Cancer and Nutrition. Br J Cancer 2011;105:1436–42.
16. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2002;5: 1113–24.
17. Obon-Santacana M, Slimani N, Lujan-Barroso L, Travier N, Hallmans G, Freisling H, et al. Dietary intake of acrylamide and pancreatic cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. Ann Oncol 2013;24:2645–51.
18. Obon-Santacana M, Kaaks R, Slimani N, Slimani N, Lujan-Barroso L, Freisling H, Ferrari P, et al. Dietary intake of acrylamide and endometrial cancer risk in the European Prospective Investigation into Cancer and Nutrition cohort. Br J Cancer 2014;111:987–97.
19. Willett WC. Nutritional epidemiology. 3rd ed. New York: Oxford University Press; 2013.
20. Wareham NJ, Jakes RW, Rennie KL, Schuit J, Mitchell J, Hennings S, et al. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Public Health Nutr 2003;6:407–13.
21. Schoenfeld D. Partial residuals for the proportional hazards regression model. Biometrica 1982;69:239–41.
22. Ferrari P, Freisling H, Duell EJ, Kaaks R, Lujan-Barroso L, Clavel-Chapelon F, et al. Challenges in estimating the validity of dietary acrylamide measurements. Eur J Nutr 2013;52:1503–12.
23. Bjellaas T, Olesen PT, Frandsen H, Haugen M, Stølen LH, Paulsen JE. Comparison of estimated dietary intake of acrylamide with hemoglobin adducts of acrylamide and glycidamide. Toxicol Sci 2007;98: 110–7.
24. Wirfalt E, Paulsson B, Tornqvist M, Axmon A, Hagmar L. Associations between estimated acrylamide intakes, and hemoglobin AA adducts in a sample from the Malmo Diet and Cancer cohort. Eur J Clin Nutr 2008; 62:314–23.

25. Kutting B, Uter W, Drexler H. The association between self-reported acrylamide intake and hemoglobin adducts as biomarkers of exposure. Cancer Causes Control 2008;19:273–81.
26. Wilson KM, Vesper HW, Tocco P, Sampson L, Rosén J, Hellenäs KE, et al. Validation of a food frequency questionnaire measurement of dietary acrylamide intake using hemoglobin adducts of acrylamide and glycidamide. Cancer Causes Control 2009;20:269–78.
27. Tran NL, Barraj LM, Murphy MM, Bi X. Dietary acrylamide exposure and hemoglobin adducts–National Health and Nutrition Examination Survey (2003–04). Food Chem Toxicol 2010;48:3098–108.
28. Xie J, Terry KL, Poole EM, Wilson KM, Rosner BA, Willett WC, et al. Acrylamide hemoglobin adduct levels and ovarian cancer risk: a nested case-control study. Cancer Epidemiol Biomarkers Prev 2013;22:653–60.