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

Obón-Santacana, M; Kaaks, R; Slimani, N; Lujan-Barroso, L; Freisling, H; Ferrari, P; Dossus, L; Chabbert-Buffet, N; Baglietto, L; Fortner, R T; 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, R C; Lu, Y; Merritt, M A; Trichopoulou, A; Benetou, V; Trichopoulos, D; Saieva, C; Sieri, S; Tumino, R; Sacerdote, C; Galasso, R; Bueno-de-Mesquita, H B; Wirfält, Elisabet; Ericson, Ulrika; Idahl, A; Ohlson, N; Skeie, G; Gram, I T; Weiderpass, E; Onland-Moret, N C; Riboli, E; Duell, E J

Published in:
British Journal of Cancer

DOI:
10.1038/bjc.2014.328

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

M Obón-Santacana[1], R Kaaks[2], N Slimani[3], L Lujan-Barroso[1], H Freisling[3], P Ferrari[4], L Dossus[5,6,7], N Chabbert-Buffet[5,6,7,8], L Baglietto[9,10], R T Fortner[2], H Boeing[11], A Tjønneland[12], A Olsen[12], K Overvad[13], V Menéndez[14], E Molina-Montes[15], N Larrañaga[15,16], M-D Chirlaque[15,17], E Ardanaz[15,18], K-T Khaw[19], N Wareham[20], R C Travis[21], Y Lu[22], M A Merritt[22], A Trichopoulou[23,24], V Benetou[25], D Trichopoulos[23,24,26], C Saieva[27], S Sieri[28], R Tumino[29], C Sacerdote[30,31], R Galasso[32], H B Bueno-de-Mesquita[33,34,35], E Wirfält[36], U Ericson[37], A Idahl[38,39], N Ohlson[40], G Skeie[41], I T Gram[41], E Weiderpass[41,42,43,44], N C Onland-Moret[45], E Riboli[22] and E J Duell*[,1]

*[1]Unit of Nutrition, Environment and Cancer, Cancer Epidemiology Research Program, Catalan Institute of Oncology (ICO-IDIBELL), Avda Gran Via Barcelona 199-203, 08908L'Hospitalet de Llobregat, Barcelona, Spain; [2]Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 581, Heidelberg 69120, Germany; [3]Dietary Exposure Assessment Group, International Agency for Research on Cancer, 150 Cours Albert Thomas, Lyon 69372, France; [4]Nutritional Epidemiology Group, International Agency for Research on Cancer, 150 Cours Albert Thomas, Lyon 69372, France; [5]Inserm, Centre for research in Epidemiology and Population Health (CESP), U1018, Nutrition, Hormones and Women's Health team, F-94805 Villejuif, France; [6]Paris-Sud University, UMRS 1018, F-94805 Villejuif, France; [7]Institut Gustave Roussy, F-94805 Villejuif, France; [8]Obstetrics and Gynecology Department AP-HP, Hopital Tenon, F-75020 Paris, France; [9]Cancer Epidemiology Centre, Cancer Council Victoria, Melbourne, VIC, Australia; [10]Centre for Molecular, Environmental, Genetic, and Analytic Epidemiology, The University of Melbourne, Melbourne, VIC, Australia; [11]Department of Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Arthur-Scheunert-Allee 114/116, Nuthetal 14558, Germany; [12]Danish Cancer Society Research Center, Strandboulevarden 49, Copenhagen 2100, Denmark; [13]Department of Public Health, Section for Epidemiology, Aarhus University, Nordre Ringgade 1, Aarhus 8000, Denmark; [14]Public Health and Participation Directorate, Ciriaco Miguel Vigil 9, Asturias 33009, Spain; [15]CIBER Epidemiology and Public Health CIBERESP, Melchor Fernández Almagro 3-5, Madrid 28029, Spain; [16]Public Health Division of Gipuzkoa-BIODONOSTIA, Basque Regional Health Department, Avda. Navarra, 4, San Sebastian 20013, Spain; [17]Department of Epidemiology, Murcia Regional Health Authority, Ronda de Levante, 11, Murcia 30008, Spain; [18]Navarre Public Health Institute, Polígono de Landaben C/F, Pamplona 31012, Spain; [19]University of Cambridge School of Clinical Medicine, Robinson Way, Cambridge CB2 0SR, UK; [20]MRC Epidemiology Unit, University of Cambridge, 184 Hills Road, Cambridge CB2 8PQ, UK; [21]Cancer Epidemiology Unit, University of Oxford, Old Road Campus, Oxford OX3 7LF, UK; [22]Department of Epidemiology & Biostatistics, School of Public Health, Imperial College London, Norfolk Place, London W2 1PG, UK; [23]Hellenic Health Foundation, 13 Kaisareias Street, Athens GR-115 27, Greece; [24]Bureau of Epidemiologic Research, Academy of Athens, 23 Alexandroupoleos Street, Athens GR-115 27, Greece; [25]Department of Hygiene, Epidemiology and Medical Statistics, University of Athens Medical School, 75M. Asias Street, Goudi GR-115 27, Athens, Greece; [26]Department of Epidemiology, Harvard School of Public Health, 677 Huntington Avenue, Boston, MA 02115, USA; [27]Molecular and Nutritional Epidemiology Unit,

*Correspondence: Dr EJ Duell; E-mail: eduell@iconcologia.net

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

Cancer Research and Prevention Institute—ISPO, Ponte Nuovo, Via delle Oblate n.2, Florence 50141, Italy; [28]Epidemiology and Prevention Unit, Fondazione IRCSS Istituto Nazionale dei Tumori, Via Venezian, 1, Milano 20133, Italy; [29]Cancer Registry and Histopathology Unit, "Civic-M.P.Arezzo" Hospital, Via Civile, Ragusa 97100, Italy; [30]Unit of Cancer Epidemiology, AO Citta' della Salute e della Scienza-University of Turin and Center for Cancer Prevention (CPO-Piemonte), Via Santena 7, 10126 Turin, Italy; [31]Human Genetics Foundation (HuGeF), Via Nizza 52, 10126 Turin, Italy; [32]Unit of Clinical Epidemiology, Biostatistics and Cancer Registry IRCCS, Referral Cancer Center of Basilicata, Rionero in Vulture (Pz), Italy; [33]National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands; [34]Department of Gastroenterology and Hepatology, University Medical Centre, Heidelberglaan 100, Utrecht 3584 CX, The Netherlands; [35]The School of Public Health, Imperial College London, Norfolk Place, London W2 1PG, UK; [36]Department of Clinical Sciences, Nutrition Epidemiology, Lund University, Box 117, Malmö 205 02, Sweden; [37]Department of Clinical Sciences, Diabetes and Cardiovascular Disease, Genetic Epidemiology, Lund University, Clinical Research Centre, Box 117, Malmö 205 02, Sweden; [38]Department of Clinical Sciences, Obstetrics and Gynecology, Umeå University, 1A, 9 tr, Kirurgcentrum, 952, Umeå 901 85, Sweden; [39]Department of Public Health and Clinical Medicine, Nutritional Research, Umeå University, 1A, 9 tr, Kirurgcentrum, 952, Umeå 901 85, Sweden; [40]Departament of Medical Biosciences, Pathology, Umeå University, 1A, 9 tr, Kirurgcentrum, 952, Umeå 901 85, Sweden; [41]Department of Community Medicine, Faculty of Health Sciences, University of Tromsø, Breivika N-9037, Norway; [42]Department of Research, Cancer Registry of Norway, P.O. box 5313 Majorstuen Oslo, N-0304 Oslo, Norway; [43]Department of Medical Epidemiology and Biostatistics, Karolinska Institute, Box 281, Stockholm 171 77, Sweden; [44]Public Health Research Center, Public Health Association, Topeliusgatan 20 (PB 211), 00250 Helsinki, Finland and [45]Julius Center for Health Sciences and Primary Care, University Medical Center, Huispost Str. 6.131, 3508GA Utrecht, The Netherlands

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	253	67	54	52	45	35		
HR (95% CI)[h]	0.88 (0.74–1.04)	1.00 (ref)	1.12 (0.72–1.74)	1.12 (0.70–1.78)	1.00 (0.61–1.64)	0.68 (0.37–1.22)	0.17	
Perimenopausal								0.05
N cases	268	51	56	73	44	44		
HR (95% CI)[h]	1.05 (0.89–1.23)	1.00 (ref)	1.08 (0.69–1.70)	1.29 (0.82–2.04)	0.83 (0.50–1.39)	1.18 (0.67–2.10)	0.90	
Postmenopausal[i]								
N cases	861	159	161	173	161	207		
HR (95% CI)[h]	1.01 (0.93–1.10)	1.00 (ref)	1.05 (0.80–1.38)	1.06 (0.80–1.40)	0.84 (0.62–1.13)	1.03 (0.76–1.40)	0.99	

Abbreviations: BMI = body mass index; CI = confidence interval; EC = endometrial cancer; EPIC = European Prospective Investigation into Cancer and Nutrition; HR = hazards ratio; HRT = hormonal replacement therapy; LRT = likelihood ratio test; OCs = oral contraceptives.

[a]All LRT *P*-values for effect measure modification are based on the continuous acrylamide intake variable (per 10 μg per day).

[b]Stratified by age at recruitment, centre, smoking status, OC use, and HRT use. Adjusted for total energy intake (per 1000 kcal per day), BMI, prevalent diabetes, menopause status combined with age at menopause, parity, and age at menarche.

[c]Stratified by age at recruitment, centre, OC use, and HRT use. Adjusted for total energy intake (per 1000 kcal per day), BMI, prevalent diabetes, menopause status combined with age at menopause, parity, and age at menarche.

[d]Ever smokers: former and current smokers.

[e]Stratified by age at recruitment, centre, smoking status, and HRT use. Adjusted for total energy intake (per 1000 kcal per day), BMI, prevalent diabetes, menopause status combined with age at menopause, parity and age at menarche.

[f]Stratified by age at recruitment, centre, and HRT use. Adjusted for total energy intake (per 1000 kcal per day), BMI, prevalent diabetes, menopause status combined with age at menopause, parity, and age at menarche.

[g]Stratified by age at recruitment, centre, smoking status, OC use, and HRT use. Adjusted for total energy intake (per 1000 kcal per day), prevalent diabetes, menopause status combined with age at menopause, parity, and age at menarche.

[h]Stratified by age at recruitment, centre, smoking status, OC use, and HRT use. Adjusted for total energy intake (per 1000 kcal per day), BMI, prevalent diabetes, parity, and age at menarche.

[i]Includes surgical menopause.

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

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

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

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

The association between acrylamide intake and type-I EC risk among OC users and non-users was also evaluated by smoking status. Women who at baseline reported being never smokers and non-users of OCs (including 203 type-I EC cases) were at the highest risk of developing type-I EC, when acrylamide was evaluated both as a continuous variable and in quintiles ($HR_{10\ \mu g\ per\ day}$: 1.17, 95% CI: 1.02–1.34; HR_{Q5vsQ1}: 1.97, 95% CI: 1.08–3.62; *P*-value for trend: 0.01; Table 4). Otherwise, associations between dietary acrylamide intake and type-I EC were below the null value in ever smokers (current and former smokers) and OC users ($HR_{10\ \mu g\ per\ day}$: 0.75, 95% CI: 0.60–0.94; Table 4). The LRT *P*-value of the contrast between 'never smokers/non-OC users', 'ever smokers/non-OC users', 'never smokers/OC users', and 'ever smokers/OC users' for the continuous acrylamide intake variable was 0.01 (Table 4).

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

DISCUSSION

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

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

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

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

Abbreviations: BMI = body mass index; CI = confidence interval; EPIC = European Prospective Investigation into Cancer and Nutrition; HR = hazards ratio; HRT = hormonal replacement therapy; LRT = likelihood ratio test; OCs = oral contraceptives.

[a]All LRT *P*-values for effect measure modification are based on the continuous acrylamide intake variable (per 10 μg per day).

[b]Stratified by age at recruitment and centre. Adjusted for total energy intake (per 1000 kcal per day), BMI, smoking status, prevalent diabetes, OC use, HRT use, menopause status combined with age at menopause, parity, and age at menarche.

[c]Stratified by age at recruitment and centre. Adjusted for total energy intake (per 1000 kcal per day), BMI, prevalent diabetes, OC use, HRT use, menopause status combined with age at menopause, parity, and age at menarche.

[d]Ever smokers: former and current smokers.

[e]Stratified by age at recruitment and centre. Adjusted for total energy intake (per 1000 kcal per day), BMI, smoking status, prevalent diabetes, HRT use, menopause status combined with age at menopause, parity, and age at menarche.

[f]Stratified by age at recruitment and centre. Adjusted for total energy intake (per 1000 kcal per day), BMI, prevalent diabetes, HRT use, menopause status combined with age at menopause, parity, and age at menarche.

[g]Stratified by age at recruitment and centre. Adjusted for total energy intake (per 1000 kcal per day), smoking status, prevalent diabetes, OC use, HRT use, menopause status combined with age at menopause, parity, and age at menarche.

[h]Stratified by age at recruitment and centre. Adjusted for total energy intake (per 1000 kcal per day), BMI, smoking status, prevalent diabetes, OC use, HRT use, parity, and age at menarche.

[i]Includes surgical menopause.

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

The relation between dietary intake of acrylamide and EC risk has been previously published in three prospective cohort studies. Both the NLCS and NHS studies found statistically significantly increased relative risks: the NLCS among never-smoking women, and the NHS in the entire cohort (Hogervorst *et al*, 2007; Wilson *et al*, 2010). Although the NLCS and NHS studies did not evaluate the association between acrylamide intake and type-I EC specifically, about 80% of EC cases are thought to be type-I endometrioid tumours (Amant *et al*, 2005); thus, the majority of the cases in the previous publications were likely type-I EC cases. Only the SMC study observed no associations between acrylamide intake and EC risk (Larsson *et al*, 2009), and this could be due to the smaller baseline ranges of acrylamide intake in that study. The median acrylamide intake for the reference group in the SMC was 16.9 μg per day, and for the highest intake category was 32.5 μg per day, whereas in EPIC, the median for the reference group was 9.3 μg per day, and for the highest intake category was 44.0 μg per day. All three previous studies presented statistical models adjusted for OC use, but none reported analyses stratified by OC use.

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

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

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

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

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.

REFERENCES

Al-Zoughool M, Dossus L, Kaaks R, Clavel-Chapelon F, Tjonneland A, Olsen A, Overvad K, Boutron-Ruault MC, Gauthier E, Linseisen J, Chang-Claude J, Boeing H, Schulz M, Trichopoulou A, Chryssa T, Trichopoulos D, Berrino F, Palli D, Mattiello A, Tumino R, Sacerdote C, Bueno-de-Mesquita HB, Boshuizen HC, Peeters PH, Gram IT, Braaten T, Lund E, Chirlaque MD, Ardanaz E, Agudo A, Larranaga N, Quiros JR, Berglund G, Manjer J, Lundin E, Hallmans G, Khaw KT, Bingham S, Allen N, Key T, Jenab M, Cust AE, Rinaldi S, Riboli E (2007) Risk of endometrial cancer in relationship to cigarette smoking: results from the EPIC study. *Int J Cancer* **121**(12): 2741–2747.

Amant F, Moerman P, Neven P, Timmerman D, Van LE, Vergote I (2005) Endometrial cancer. *Lancet* **366**(9484): 491–505.

Boettcher MI, Schettgen T, Kutting B, Pischetsrieder M, Angerer J (2005) Mercapturic acids of acrylamide and glycidamide as biomarkers of the internal exposure to acrylamide in the general population. *Mutat Res* **580**(1-2): 167–176.

Cibula D, Gompel A, Mueck AO, La VC, Hannaford PC, Skouby SO, Zikan M, Dusek L (2010) Hormonal contraception and risk of cancer. *Hum Reprod Update* **16**(6): 631–650.

Cook LS, Weiss NS, Doherty JA, Chen C (2006) Endometrial Cancer. In *Cancer Epidemiology and Prevention*, Schottenfeld D, Fraumeni Jr JF (eds) pp 1027–1043. Oxford University Press: New York.

Doroshyenko O, Fuhr U, Kunz D, Frank D, Kinzig M, Jetter A, Reith Y, Lazar A, Taubert D, Kirchheiner J, Baum M, Eisenbrand G, Berger FI, Bertow D, Berkessel A, Sorgel F, Schomig E, Tomalik-Scharte D (2009) In vivo role of cytochrome P450 2E1 and glutathione-S-transferase activity for acrylamide toxicokinetics in humans. *Cancer Epidemiol Biomarkers Prev* **18**(2): 433–443.

Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JWW, Comber H, Forman D, Bray F (2013) Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. *Eur J Cancer* **49**(6): 1374–1403.

Ferrari P, Freisling H, Duell EJ, Kaaks R, Lujan-Barroso L, Clavel-Chapelon F, Boutron-Ruault MC, Nailler L, Polidoro S, Mattiello A, Palli D, Tumino R, Grioni S, Knuppel S, Tjonneland A, Olsen A, Overvad K, Orfanos P, Katsoulis M, Trichopoulou A, Quiros JR, Ardanaz E, Huerta JM, Etxezarreta PA, Sanchez MJ, Crowe F, Khaw KT, Wareham NJ, Ocke M, Bueno-De-Mesquita B, Peeters PH, Ericson U, Wirfalt E, Hallmans G, Johansson I, Engeset D, Nicolas G, Gallo V, Norat T, Riboli E, Slimani N (2013) Challenges in estimating the validity of dietary acrylamide measurements. *Eur J Nutr* **52**(5): 1503–1512.

Freisling H, Moskal A, Ferrari P, Nicolas G, Knaze V, Clavel-Chapelon F, Boutron-Ruault MC, Nailler L, Teucher B, Grote VA, Boeing H, Clemens M, Tjonneland A, Olsen A, Overvad K, Quiros JR, Duell EJ, Sanchez MJ, Amiano P, Chirlaque MD, Barricarte A, Khaw KT, Wareham NJ, Crowe FL, Gallo V, Oikonomou E, Naska A, Trichopoulou A, Palli D, Agnoli C, Tumino R, Polidoro S, Mattiello A, Bueno-de-Mesquita HB, Ocke MC, Peeters PH, Wirfalt E, Ericson U, Bergdahl IA, Johansson I, Hjartaker A, Engeset D, Skeie G, Riboli E, Slimani N (2013) Dietary acrylamide intake of adults in the European Prospective Investigation into Cancer and Nutrition differs greatly according to geographical region. *Eur J Nutr* **52**(4): 1369–1380.

Friedman M (2003) Chemistry, biochemistry, and safety of acrylamide. A review. *J Agric Food Chem* **51**(16): 4504–4526.

Gierisch JM, Coeytaux RR, Peragallo UR, Havrilesky LJ, Moorman PG, Lowery WJ, Dinan M, McBroom AJ, Hasselblad V, Sanders GD, Myers ER (2013) Oral contraceptive use and risk of breast, cervical, colorectal, and endometrial cancers: a systematic review. *Cancer Epidemiol Biomarkers Prev* **22**(11): 1931–1943.

Hogervorst JG, Baars BJ, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA (2010) The carcinogenicity of dietary acrylamide intake: a comparative discussion of epidemiological and experimental animal research. *Crit Rev Toxicol* **40**(6): 485–512.

Hogervorst JG, Fortner RT, Mucci LA, Tworoger SS, Eliassen AH, Hankinson SE, Wilson KM (2013) Associations between dietary acrylamide intake and plasma sex hormone levels. *Cancer Epidemiol Biomarkers Prev* **22**(11): 2024–2036.

Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA (2007) A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. *Cancer Epidemiol Biomarkers Prev* **16**(11): 2304–2313.

IARC (1994) 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* **60**: 1–560.

Jamison PM, Noone AM, Ries LA, Lee NC, Edwards BK (2013) Trends in endometrial cancer incidence by race and histology with a correction for the prevalence of hysterectomy, SEER 1992 to 2008. *Cancer Epidemiol Biomarkers Prev* **22**(2): 233–241.

Johnson KA, Gorzinski SJ, Bodner KM, Campbell RA, Wolf CH, Friedman MA, Mast RW (1986) Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats. *Toxicol Appl Pharmacol* **85**(2): 154–168.

Kaaks R, Lukanova A, Kurzer MS (2002) Obesity, endogenous hormones, and endometrial cancer risk: a synthetic review. *Cancer Epidemiol Biomarkers Prev* **11**(12): 1531–1543.

Larsson SC, Hakansson N, Akesson A, Wolk A (2009) Long-term dietary acrylamide intake and risk of endometrial cancer in a prospective cohort of Swedish women. *Int J Cancer* **124**(5): 1196–1199.

LoPachin RM, Gavin T (2008) Acrylamide-induced nerve terminal damage: relevance to neurotoxic and neurodegenerative mechanisms. *J Agric Food Chem* **56**(15): 5994–6003.

Obon-Santacana M, Slimani N, Lujan-Barroso L, Travier N, Hallmans G, Freisling H, Ferrari P, Boutron-Ruault MC, Racine A, Clavel F, Saieva C, Pala V, Tumino R, Mattiello A, Vineis P, Arguelles M, Ardanaz E, Amiano P, Navarro C, Sanchez MJ, Molina ME, Key T, Khaw KT, Wareham N, Peeters PH, Trichopoulou A, Bamia C, Trichopoulos D, Boeing H, Kaaks R, Katzke V, Ye W, Sund M, Ericson U, Wirfalt E, Overvad K, Tjonneland A, Olsen A, Skeie G, Asli LA, Weiderpass E, Riboli E, Bueno-de-Mesquita HB, Duell EJ (2013) Dietary intake of acrylamide and pancreatic cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. *Ann Oncol* **24**(10): 2645–2651.

Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, Charrondiere UR, Hemon B, Casagrande C, Vignat J, Overvad K, Tjonneland A, Clavel-Chapelon F, Thiebaut A, Wahrendorf J, Boeing H, Trichopoulos D, Trichopoulou A, Vineis P, Palli D, Bueno-de-Mesquita HB, Peeters PH, Lund E, Engeset D, Gonzalez CA, Barricarte A, Berglund G, Hallmans G, Day NE, Key TJ, Kaaks R, Saracci R (2002) European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr* **5**(6B): 1113–1124.

Schoenfeld D (1982) Partial residuals for the proportional hazards regression model. *Biometrica* **69**(1): 239–241.

Setiawan VW, Yang HP, Pike MC, McCann SE, Yu H, Xiang YB, Wolk A, Wentzensen N, Weiss NS, Webb PM, van den Brandt PA, van de Vijver K, Thompson PJ, Strom BL, Spurdle AB, Soslow RA, Shu XO, Schairer C, Sacerdote C, Rohan TE, Robien K, Risch HA, Ricceri F, Rebbeck TR, Rastogi R, Prescott J, Polidoro S, Park Y, Olson SH, Moysich KB, Miller AB, McCullough ML, Matsuno RK, Magliocco AM, Lurie G, Lu L, Lissowska J, Liang X, Lacey Jr. JV, Kolonel LN, Henderson BE, Hankinson SE, Hakansson N, Goodman MT, Gaudet MM, Garcia-Closas M, Friedenreich CM, Freudenheim JL, Doherty J, De Vivo I, Courneya KS, Cook LS, Chen C, Cerhan JR, Cai H, Brinton LA, Bernstein L, Anderson KE, Anton-Culver H, Schouten LJ, Horn-Ross PL (2013) Type I and II endometrial cancers: have they different risk factors? *J Clin Oncol* **31**(20): 2607–2618.

Slimani N, Ferrari P, Ocke M, Welch A, Boeing H, Liere M, Pala V, Amiano P, Lagiou A, Mattisson I, Stripp C, Engeset D, Charrondiere R, Buzzard M, Staveren W, Riboli E (2000) Standardization of the 24-hour diet recall calibration method used in the European Prospective Investigation into Cancer and Nutrition (EPIC): general concepts and preliminary results. *Eur J Clin Nutr* **54**(12): 900–917.

Tareke E, Rydberg P, Karlsson P, Eriksson S, Tornqvist M (2002) Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J Agric Food Chem* **50**(17): 4998–5006.

Tavassoli FA, Devilee P (2003) Tumours of the Uterine Corpus. In *World Health Organization Classification Tumours. Pathology and Genetics of Tumours of the Breast and Female Genital Organs*, Tavassoli FA, Devilee P (eds) pp 218–258. IARC Press: Lyon.

Terry PD, Rohan TE, Franceschi S, Weiderpass E (2004) Endometrial Cancer. In *Tobacco and Public Health: Science and Policy*, Boyle P, Gray N, Henningfield J, Seffrin J, Zatonski W (eds) pp 523–545. Oxford University Press: New York.

Vesper HW, Slimani N, Hallmans G, Tjonneland A, Agudo A, Benetou V, Bingham S, Boeing H, Boutron-Ruault MC, Bueno-de-Mesquita HB, Chirlaque D, Clavel-Chapelon F, Crowe F, Drogan D, Ferrari P, Johansson I, Kaaks R, Linseisen J, Lund E, Manjer J, Mattiello A, Palli D, Peeters PH, Rinaldi S, Skeie G, Trichopoulou A, Vineis P, Wirfalt E, Overvad K, Stromberg U (2008) Cross-sectional study on acrylamide hemoglobin adducts in subpopulations from the European Prospective Investigation into Cancer and Nutrition (EPIC) Study. *J Agric Food Chem* **56**(15): 6046–6053.

Voss S, Charrondiere UR, Slimani N, Kroke A, Riboli E, Wahrendorf J, Boeing H (1998) [EPIC-SOFT a European computer program for 24-hour dietary protocols]. *Z Ernahrungswiss* **37**(3): 227–233.

Wareham NJ, Jakes RW, Rennie KL, Schuit J, Mitchell J, Hennings S, Day NE (2003) 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* **6**(4): 407–413.

Willett WC (1998) *Nutritional Epidemiology*. Oxford University Press: New York.

Wilson KM, Balter K, Adami HO, Gronberg H, Vikstrom AC, Paulsson B, Tornqvist M, Mucci LA (2009) Acrylamide exposure measured by food frequency questionnaire and hemoglobin adduct levels and prostate cancer risk in the Cancer of the Prostate in Sweden Study. *Int J Cancer* **124**(10): 2384–2390.

Wilson KM, Mucci LA, Rosner BA, Willett WC (2010) A prospective study on dietary acrylamide intake and the risk for breast, endometrial, and ovarian cancers. *Cancer Epidemiol Biomarkers Prev* **19**(10): 2503–2515.