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


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 quintiles. 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 (HR10 μg/d: 1.02; 95% CI, 0.96–1.09; HR350μg/d: 0.97; 95% CI, 0.76–1.23). No differences were seen when invasive EOC subtypes (582 serous, 118 endometrioid, and 79 mucinous tumors) were analyzed separately. This study did not provide evidence that acrylamide intake, based on food intake questionnaires, was associated with risk for EOC in EPIC. Additional studies with more reliable estimates of exposure based on biomarkers may be needed. Cancer Epidemiol Biomarkers Prev; 24(1): 291–7. © 2014 AACR.

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Introduction

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

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

One case-control study and 3 prospective cohort studies have evaluated the association between dietary acrylamide intake and epithelial ovarian cancer (EOC), but results were inconsistent. Both the Italian case-control study (8) and the prospective Swedish Mammography Cohort (SMC; ref. 9) study reported null associations, the Nurses’ Health Study (NHS) suggested an increased risk for serous tumors [HRQ5vsQ1, 1.58; 95% confidence interval (CI), 0.99–2.52; Ptrend = 0.04] and for serous invasive tumors (HRQ5vsQ1, 1.67; 95% CI, 0.99–2.81; Ptrend = 0.04; ref. 10), whereas the Netherlands Cohort Study (NLCS) reported positive associations for overall EOC (HRQ5vsQ1, 1.78; 95% CI, 1.10–2.88; Ptrend = 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 factors 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 adenoscarcinomas, carcinomas, and cystadenocarcinoma), endometrioid (n = 118, 11%), mucinous (n = 79, 7%), clear cell (n = 51, 5%), and other tumors (n = 16, 1%).

Acrylamide intake assessment

Details of the EPIC acrylamide database have been previously published (17, 18). Briefly, a harmonized acrylamide database was compiled using mean acrylamide levels in foods mainly derived from the EU monitoring database maintained by the European Community Institute for Reference Materials and
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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), 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.
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 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/m²), 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 (23.3±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 3 prospective 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,
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Table 3. HRs and 95% CIs for estimated dietary intake of acrylamide (energy-adjusted using the residual method) and EOC in EPIC

<table>
<thead>
<tr>
<th>Energy-adjusted acrylamide intake, μg/d</th>
<th>Quintiles</th>
<th>Trend test P&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10–14.6</td>
<td>14.7–19.6</td>
</tr>
<tr>
<td>EOC cases</td>
<td>191</td>
<td>221</td>
</tr>
<tr>
<td>HR (95% CI)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.02 (0.96–1.09)</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>Borderline</td>
<td>96</td>
<td>15</td>
</tr>
<tr>
<td>HR (95% CI)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.90 (0.71–1.13)</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>Invasive</td>
<td>1095</td>
<td>206</td>
</tr>
<tr>
<td>HR (95% CI)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.03 (0.97–1.10)</td>
<td>1.00 (ref)</td>
</tr>
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</table>

| Serous cases                           | 582       | 124                    | 103       | 102       | 152       | 121       | 0.72       |
| HR (95% CI)<sup>b</sup>                | 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) |  |
| Not otherwise specified                | 249       | 28                     | 45        | 38        | 64        | 74        |            |
| HR (95% CI)<sup>b</sup>                | 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 | 831      | 152                    | 148       | 140       | 196       | 195       | 0.52       |
| HR (95% CI)<sup>c</sup>                | 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) |  |
| Endometrioid                           | 118       | 27                     | 20        | 19        | 29        | 23        |            |
| HR (95% CI)<sup>b</sup>                | 1.12 (0.93–1.36) | 1.00 (ref)            | 0.77 (0.49–1.40) | 0.67 (0.33–1.34) | 1.01 (0.51–1.98) | 0.72 (0.34–1.55) | 0.61       |
| Clear cell                             | 51        | 6                      | 8         | 13        | 12        | 12        |            |
| HR (95% CI)<sup>c</sup>                | 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  | 169       | 33                     | 28        | 32        | 41        | 35        |            |
| HR (95% CI)<sup>c</sup>                | 1.05 (0.89–1.23) | 1.00 (ref)            | 0.89 (0.50–1.57) | 0.88 (0.49–1.58) | 1.07 (0.59–192) | 0.76 (0.40–1.45) | 0.45       |
| Mucinous                               | 79        | 16                     | 11        | 13        | 19        | 20        |            |
| HR (95% CI)<sup>b</sup>                | 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       |

<sup>a</sup>All P values for trend are based on the quintile medians.

<sup>b</sup>Stratified by age at recruitment and center. Adjusted for total energy intake (1,000 kcal/d), BMI (kg/m²), smoking status (never smokers, current pipe or cigar or occasional smokers, current cigarette smokers: 1–5, 6–19, or ≥20 cigarettes per day, former cigarette smokers: 1–19, or ≥20 cigarettes per day, or ≥10 years before recruitment), OC use (never, ever, unknown), parity, postmenopausal status, age at menopause, and number of pregnancies, parity (nulliparous, 1, 2, 3, parous with missing number of full-term pregnancies, unknown). and the correlation coefficient between DQs and a single 24-hour DR in EPIC was low (0.35 and 0.17 for crude and adjusted correlation coefficient, respectively; ref. 22). In addition, studies that evaluated correlation coefficients between acrylamide intake (based on DQs) and biomarkers of exposure measured as hemoglobin adducts have reported mixed results, with correlation ranging from 0.08 to 0.43, and with most of the studies falling on the lower end of the range, including EPIC (22–27). Thus, we included energy intake in all regression models, as based on a previous analysis in EPIC, acrylamide intake estimates improved after this adjustment (22). Second, misclassification of acrylamide exposure may exist, as information on cooking methodology was not available in some EPIC centers. Finally, we acknowledge that measurement error may be present in our dietary acrylamide estimates as a harmonized acrylamide database was used, and because DQs in EPIC were not specifically designed to assess dietary acrylamide exposure; nonetheless to reduce the impact of measurement error, estimates were energy-adjusted using the residual method (19), and all models were stratified by center with the intention to partially account for the variation in dietary patterns across the 10 EPIC countries.

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

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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

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