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

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

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

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Abstract

Background—Acrylamide was classified as “probably carcinogenic to humans (group 2A)” by the International Agency for Research on Cancer. Epithelial ovarian cancer (EOC) is the fourth cause of cancer mortality in women. Five epidemiological studies have evaluated the association

between EOC risk and dietary acrylamide intake assessed using food frequency questionnaires, and one nested case–control study evaluated hemoglobin adducts of acrylamide (HbAA) and its metabolite glycidamide (HbGA) and EOC risk; the results of these studies were inconsistent.

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

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

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

Impact—It is unlikely that dietary acrylamide exposure increases ovarian cancer risk; however, additional studies with larger sample size should be performed to exclude any possible association with EOC risk.

Introduction

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

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

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

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

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

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

Materials and Methods

Study population and data collection

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

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

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

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

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

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

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

Measurement of acrylamide and glycidamide hemoglobin adducts

Blood samples were sent to the Center for Disease Control and Prevention (CDC) Protein Biomarker Laboratory (Atlanta, USA) to measure HbAA and HbGA. Details of the methodology can be found elsewhere (7, 26). Briefly, 300 mL of red blood cells were hemolyzed and analyzed using HPLC/tandem mass spectrometry (HPLC/MS-MS). Laboratory personnel were blinded to the case–control status of participants, and blood samples were analyzed in a randomized manner. Concentrations of HbAA and HbGA were reported relative to the amount of hemoglobin (pmol per g of Hb), and two independent measures were performed for each sample. The lower limits of detection for this method are

3 pmol/g of Hb for HbAA, and 4 pmol/g of Hb for HbGA. All of the HbAA and HbGA measurements were within the limits of detection. In this study, 42 of the 751 blood samples were sent in duplicate to the laboratory to independently assess the reproducibility of the hemoglobin adduct measures, which had intraclass correlation coefficients of 0.94 for HbAA and 0.92 for HbGA. The percent coefficient of variation (CV) was estimated using log-transformed (log2) values, and was 9.9 for HbAA and 12.0 for HbGA.

Statistical methods

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

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

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

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

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

Results

Description of the study population

The present nested case–control study was based on 334 incident EOC cases (of which 191 were classified as serous) and 417 controls. A large proportion of cases and controls were from the United Kingdom and the Netherlands (Table 1). Among cases, the median (quartile range) of HbAA and HbGA levels were 42.2 (33.9–54.4) and 37.0 (28.5–49.5), respectively, whereas controls had HbAA and HbGA levels of 43.1 (33.8–54.8) and 35.4 (26.0–49.9), respectively (Table 1). Cases were slightly younger than controls (58.4 years vs. 59.2 years), tended to have higher BMI values (26.4 vs. 25.8 kg/m^2), a higher proportion of HRT users (27.8% vs. 18.9%), and were less likely to take OC (35.6% vs. 41.7%). There were no major differences between cases and controls regarding age at menopause, age at first menstrual period, and parity (Table 1). The median interval between the date of blood draw and the date at diagnosis for cases was 6.2 years.

Overall EOC and serous EOC risk

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

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

Sensitivity analyses were conducted using conditional logistic regression models, which included 261 cases and 416 controls, to estimate ORs of EOC for each biomarker level. Overall, no statistically significant association were observed; nonetheless, results showed similar patters compared to the ones obtained using unconditional logistic regression models (Table 2).

Effect measure modification in EOC

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

Discussion

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

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

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

Blood samples from both EPIC and the NHS/NHSII studies were measured in the same laboratory using the same protocol. Among cases, the median adducts levels presented in the NHS/NHSII study were 63.8, 49.5, and 112.6 pmol/g Hb, whereas in this study median adducts levels were lower at 42.2, 37.0, and 79.3 pmol/g Hb for HbAA, HbGA, and HbAA +HbGA, respectively. To avoid possible confounding by tobacco smoking, the NHS/NHSII study restricted the analyses to nonsmoking women at the time of blood extraction (230 cases vs. 460 controls), and categorized exposures in tertiles based on the distribution in nonsmoking controls; however, referent group cutpoints were higher for HbAA, HbGA, and HbAA+HbGA (0–52.3, 0–40.2, and 0–95.7 pmol/g Hb, respectively) compared with those presented in this study (≤36.5, ≤29.6, and ≤66.2 pmol/g Hb, respectively; tertile data not shown in tables). The minimum detectable OR_{Q5} at 80% power in our study was 1.65, which is similar to the minimum detectable OR (1.78) reported by the NHS/NHSII study.

The design of the present nested case–control study is one of the major strengths, as we wanted to evaluate the dietary contribution to acrylamide biomarker levels and EOC risk, and avoid confounding from tobacco smoking and hormonal oscillations. Dietary

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

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

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References

1. Tareke E, Rydberg P, Karlsson P, Eriksson S, Tornqvist M. Acrylamide: a cooking carcinogen? Chem Res Toxicol. 2000; 13:517–22. [PubMed: 10858325]

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. [PubMed: 12166997]
3. Zodl B, Schmid D, Wassler G, Gundacker C, Leibetseder V, Thalhammer T, et al. Intestinal transport and metabolism of acrylamide. Toxicology. 2007; 232:99–108. [PubMed: 17267090]
4. Friedman M. Chemistry, biochemistry, and safety of acrylamide: a review. J Agric Food Chem. 2003; 51:4504–26. [PubMed: 14705871]
5. Fennell TR, Sumner SC, Walker VE. A model for the formation and removal of hemoglobin adducts. Cancer Epidemiol Biomarkers Prev. 1992; 1:213–9. [PubMed: 1306107]
6. Vesper HW, Caudill SP, Osterloh JD, Meyers T, Scott D, Myers GL. Exposure of the U.S. population to acrylamide in the National Health and Nutrition Examination Survey 2003–2004. Environ Health Perspect. 2010; 118:278–83. [PubMed: 20123601]
7. Vesper HW, Slimani N, Hallmans G, Tjonneland A, Agudo A, Benetou V, et al. Cross-sectional study on acrylamide hemoglobin adducts in sub-populations from the European Prospective Investigation into Cancer and Nutrition (EPIC) Study. J Agric Food Chem. 2008; 56:6046–53. [PubMed: 18624432]
8. 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. [PubMed: 20170357]
9. Ferlay, J., Soerjomataram, I., Ervik, M., Dikshit, R., Eser, S., Mathers, C., et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013. [cited 2015 Jun 30]. Available from: http://globocan.iarc.fr
10. World Cancer Research Fund/American Institute for Cancer Research. Continuous Update Project Report. Food, Nutrition, Physical Activity, and the Prevention of Ovarian Cancer. 2014. [Internet]. [cited 2015 May 5]. Available from: http://www.dietandcancerreport.org/cup/cup_resources.php
11. Aune D, Navarro Rosenblatt DA, Chan DSM, Abar L, Vingeliene S, Vieira AR, et al. Anthropometric factors and ovarian cancer risk: a systematic review and nonlinear dose-response meta-analysis of prospective studies. Int J Cancer. 2015; 136:1888–98. [PubMed: 25250505]
12. Lahmann PH, Cust AE, Friedenreich CM, Schulz M, Lukanova A, Kaaks R, et al. Anthropometric measures and epithelial ovarian cancer risk in the European Prospective Investigation into Cancer and Nutrition. Int J Cancer. 2010; 126:2404–15. [PubMed: 19821492]
13. 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. [PubMed: 21678398]
14. Secretan B, Straif K, Baan R, Grosse Y, El Ghissassi F, Bouvard V, et al. A review of human carcinogens. Part E: Tobacco, areca nut, alcohol, coal smoke, and salted fish. Lancet Oncol. 2009; 10:1033–4. [PubMed: 19891056]
15. Fortner RT, Ose J, Merritt MA, Schock H, Tjønneland A, Hansen L, et al. Reproductive and hormone-related risk factors for epithelial ovarian cancer by histologic pathways, invasiveness and histologic subtypes: Results from the EPIC cohort. Int J Cancer. 2015; 137:1196–208. [PubMed: 25656413]
16. Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA. A prospective study of dietary acrylamide intake and the risk of endome-trial, ovarian, and breast cancer. Cancer Epidemiol Biomarkers Prev. 2007; 16:2304–13. [PubMed: 18006919]
17. 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. [PubMed: 19223560]
18. 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. [PubMed: 20693310]
19. Obón-Santacana M, Peeters PHM, Freisling H, Dossus L, Clavel-Chapelon F, Baglietto L, et al. Dietary intake of acrylamide and epithelial ovarian cancer risk in the european prospective investigation into cancer and nutrition (EPIC) cohort. Cancer Epidemiol Biomarkers Prev. 2015; 24:291–7. [PubMed: 25300475]

20. 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. [PubMed: 16003724]
21. 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. [PubMed: 23417989]
22. 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. [PubMed: 12639222]
23. Peeters PH, Lukanova A, Allen N, Berrino F, Key T, Dossus L, et al. Serum IGF-I, its major binding protein (IGFBP-3) and epithelial ovarian cancer risk: the European Prospective Investigation into Cancer and Nutrition (EPIC). Endocr Relat Cancer. 2007; 14:81–90. [PubMed: 17395977]
24. Hogervorst JG, Fortner RT, Mucci LA, Tworoger SS, Eliassen AH, Hankinson SE, et al. Associations between dietary acrylamide intake and plasma sex hormone levels. Cancer Epidemiol Biomarkers Prev. 2013; 22:2024–36. [PubMed: 23983241]
25. Nagata C, Konishi K, Tamura T, Wada K, Tsuji M, Hayashi M, et al. Associations of acrylamide intake with circulating levels of sex hormones and prolactin in premenopausal Japanese women. Cancer Epidemiol Biomarkers Prev. 2015; 24:249–54. [PubMed: 25352525]
26. Vesper HW, Ospina M, Meyers T, Ingham L, Smith A, Gray JG, et al. Automated method for measuring globin adducts of acrylamide and glycidamide at optimized Edman reaction conditions. Rapid Commun Mass Spectrom. 2006; 20:959–64. [PubMed: 16479554]
27. 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. [PubMed: 12795830]
28. Heinzl H, Kaider A. Gaining more flexibility in Cox proportional hazards regression models with cubic spline functions. Comput Methods Programs Biomed. 1997; 54:201–8. [PubMed: 9421665]
29. 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. [PubMed: 23238529]
30. Mei N, McDaniel LP, Dobrovolsky VN, Guo X, Shaddock JG, Mittelstaedt RA, et al. The genotoxicity of acrylamide and glycidamide in big blue rats. Toxicol Sci. 2010; 115:412–21. [PubMed: 20200216]
31. Spivey A. A matter of degrees: advancing our understanding of acrylamide. Environ Health Perspect. 2010; 118:A160–7. [PubMed: 20359976]
32. Wilson KM, Vesper HW, Tocco P, Sampson L, Rosen J, Hellenas 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. [PubMed: 18855107]

Table 1

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

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

	All EOC cases ***n*** **= 334**	**Invasive serous EOC cases** ***n*** **= 191**	**Controls** ***n*** **= 417**
Yes	93 (27.8)	60 (31.4)	79 (18.9)
Parity[b]			
Unknown	41 (12.3)	27 (14.1)	58 (13.9)
1 child	129 (38.6)	81 (42.4)	161 (38.6)
2 children	99 (29.6)	53 (27.8)	141 (33.8)
≥3 children	48 (14.4)	23 (12.0)	44 (10.6)
Nulliparous	8 (2.4)	4 (2.1)	9 (2.2)
Parous but with missing number of full-term pregnancies	9 (2.7)	3 (1.6)	4 (1.0)

Abbreviation: HbGA, hemoglobin adducts of glycidamide.

[a]Median and quartile range (25th–75th percentile).

[b]Number (*n*) and percent (%).

Table 2

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

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

Abbreviation: HbGA, hemoglobin adducts of glycidamide.

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

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

Table 3

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

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

Abbreviation: HbGA, hemoglobin adducts of glycidamide.

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

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

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

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