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

SHORT REPORT: Acrylamide and glycidamide hemoglobin adduct levels and endometrial cancer risk: a nested case-control study in non-smoking postmenopausal women from the EPIC cohort

Authors: Mireia Obón-Santacana[1], Heinz Freisling[2], Petra H. Peeters[3,4], Leila Lujan-Barroso[1], Pietro Ferrari[2], Marie-Christine Boutron-Ruault[5,6,7], Sylvie Mesrine[5,6,7], Laura Baglietto[8,9], Renee Turzanski-Fortner[10], Verena A Katzke[10], Heiner Boeing[11], J. Ramón Quirós[12], Elena Molina-Portillo[13,14], Nerea Larrañaga[14,15], María-Dolores Chirlaque[14,16,17], Aurelio Barricarte[14,18,19], Kay-Tee Khaw[20], Nick Wareham[21], Ruth C. Travis[21], Melissa A. Merritt[4], Marc J. Gunter[4], Antonia Trichopoulou[22], Pagona Lagiou[22,23], Androniki Naska[22,23], Domenico Palli[24], Sabina Sieri[25], Rosario Tumino[26], Valentina Fiano[27], Rocco Galassom[28], H.B. Bueno-de-Mesquita[4,29,30,31], N. Charlotte Onland-Moret[32],Annika Idahl[33,34], Eva Lundin[35], Elisabete Weiderpass[36,37,38,39], Hubert Vesper[40], Elio Riboli[4], Eric J Duell[1].

1. Unit of Nutrition and Cancer. Cancer Epidemiology Research Program, Catalan Institute of Oncology (ICO-IDIBELL), Barcelona, Spain
2. Dietary Exposure Assessment Group, International Agency for Research on Cancer, Lyon, France
3. Department of Epidemiology, Julius Center for Health Sciences and Primary Care,University Medical Center Utrecht, Utrecht, The Netherlands
4. Department of Epidemiology & Biostatistics, School of Public Health, Imperial College London, London, United Kingdom
5. Inserm, CESP Centre for Research in Epidemiology and Population Health, U1018, Lifestyle, genes and health: integrative trans-generational epidemiology, Villejuif, France.
6. Universite Paris Sud, Villejuif, France
7. Institut Gustave-Roussy (IGR), Villejuif, France
8. Cancer Epidemiology Centre, Cancer Council of Victoria, Melbourne, Australia.
9. Centre for Epidemiology and Biostatistics, School of Population and Global Health, University of Melbourne, Australia.
10. Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany
11. Department of Epidemiology, German Institute of Human Nutrition, Potsdam-Rehbruecke, Nuthetal, Germany
12. Public Health Directorate, Asturias, Spain
13. Escuela Andaluza de Salud Pública. Instituto de Investigación Biosanitaria ibs.GRANADA. Hospitales Universitarios de Granada/Universidad de Granada, Granada, Spain
14. CIBER Epidemiology and Public Health CIBERESP, Spain
15. Public Health Division of Gipuzkoa, Regional Government of the Basque Country, Spain
16. Department of Epidemiology, Regional Health Council, Murcia, Spain
17. Department of Health and Social Sciences, Murcia University, Murcia, Spain
18. Navarra Public Health Institute, Pamplona, Spain
19. Navarra Institute for Health Research (IdiSNA) Pamplona, Spain

20. University of Cambridge School of Clinical Medicine, Cambridge, United Kingdom
21. Cancer Epidemiology Unit, Nuffield Department of Population Health University of Oxford, Oxford, United Kingdom
22. Hellenic Health Foundation, Athens, Greece
23. Department of Hygiene, Epidemiology and Medical Statistics, University of Athens Medical School, Athens, Greece
24. Molecular and Nutritional Epidemiology Unit, Cancer Research and Prevention Institute – ISPO, Florence-Italy
25. Epidemiology and Prevention Unit, Department of Preventive & Predictive Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy
26. Cancer Registry and Histopathology Unit, "Civic - M.P.Arezzo" Hospital, ASP Ragusa, Italy
27. Unit of Cancer Epidemiology – CERMS, Department of Medical Sciences University of Turin, Turin, Italy
28. Unit of Clinical Epidemiology, Biostatistics and Cancer Registry, IRCCS Centro di Riferimento Oncologico di Basilicata, Rionero in Vulture, Potenza, Italy
29. Department for Determinants of Chronic Diseases (DCD), National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands
30. Department of Gastroenterology and Hepatology, University Medical Centre, Utrecht, The Netherlands
31. Department of Social & Preventive Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
32. Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands
33. Department of Clinical Sciences, Obstetrics and Gynecology, Nutritional Research Umeå University, Umeå, Sweden
34. Department of Public Health and Clinical Medicine, Nutritional Research Umeå University, Umeå, Sweden
35. Department of Medical Biosciences, Pathology Umeå University, Umeå, Sweden
36. Department of Community Medicine, Faculty of Health Sciences, University of Tromsø, The Arctic University of Norway, Tromsø, Norway.
37. Department of Research, Cancer Registry of Norway, Oslo, Norway.
38. Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden
39. Genetic Epidemiology Group, Folkhälsan Research Center, Helsinki, Finland
40. Centers for Disease Control and Prevention, Atlanta, USA

Correspondence to: Eric J. Duell, Unit of Nutrition and Cancer, Cancer Epidemiology Research Program, Catalan Institute of Oncology, Avda Gran Via 199-203, 08907 L'Hospitalet del Llobregat, Barcelona, Spain. Phone: +34 93 260 7401; Fax: +34 93 260 7787. Email: eduell@iconcologia.net

Short title: Biomarkers of acrylamide and endometrial cancer risk.

Keywords: hemoglobin adduct, acrylamide, glycidamide, endometrial cancer, EPIC

Abbreviations used: 24hDR, 24-h dietary recall; BMI, body mass index (kg/m^2); CI, confidence interval; DQ; dietary questionnaire; EC, endometrial cancer; EPIC, European Prospective Investigation into Cancer and Nutrition; FFQ, food frequency questionnaire; HbAA, hemoglobin adducts of acrylamide; HbGA, hemoglobin adducts of glycidamide; HbAA+HbGA, sum of hemoglobin adducts of acrylamide and glycidamide; HbGA/HbAA, ratio of hemoglobin adducts of glycidamide and acrylamide; HPLC/MS/MS, high-performance liquid chromatography–tandem mass spectrometry; HRT, hormone replacement therapy; IARC, International Agency for Research on Cancer; ICC, intraclass correlation coefficient; LRT, likelihood ratio test; LOD, limits of detection; NHS, Nurses' Health Study; OC, oral contraceptive; OR, odds ratio; SHS, second-hand smoke .

Article category: Short report

Abstract

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

Novelty and impact of the work: In this first epidemiologic study assessing the association between hemoglobin adduct biomarkers of acrylamide and EC risk, there was no evidence of an increased risk.

Introduction

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

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

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

Cancer of the corpus uteri is the fourth most common incident cancer in European and North American women. The most common type of corpus uteri cancer is endometrial cancer (EC). The 5-year survival rate of EC is high, ranging from 65 to 85%[11]. EC has been classified into type-I and type-

II tumors; type-I EC is mostly endometrioid adenocarcinoma, and is characterized as an estrogen-dependent tumor. In contrast, type-II EC is usually serous carcinoma, is thought to be estrogen-independent, usually diagnosed in elderly women, and generally has an unfavorable prognosis[12,13]. Epidemiological data suggest that obesity, diabetes, low physical activity, long-term exposure to estrogens, and a history of polycystic ovary syndrome are risk factors for developing EC, and type-I EC in particular[14]. Combined oral contraceptive (OC) use, and tobacco smoking are consistently associated with lower risk of EC[14]. Further, a recent EPIC study observed an inverse association between coffee consumption and EC risk[15].

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

Material and Methods

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

The EPIC methodology has been published elsewhere[22]. Recruitment began between 1992-1998, and participants reported information on dietary habits (referring to the twelve months before

recruitment) assessed through country-specific, validated dietary questionnaires (DQs). Additionally, information on tobacco smoking, education, physical activity, anthropometric measures and reproductive factors was also obtained at recruitment. Blood samples were collected at recruitment for approximately 80% of the EPIC cohort (385,747 of over 500,000 participants). Samples that were stored at the IARC bio-bank were kept in liquid nitrogen (-196^{o}C); whereas blood samples from Umeå were stored at local repositories in freezers (-80^{o}C). The study was approved by the IARC ethical review boards and/or all local ethics committees.

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

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

The selection of the study population for the present nested case-control study was based on the algorithm that has been previously published by *Cust et al.* and *Peeters et al.*[23]: for each EC case two corresponding controls were randomly selected at the date of diagnosis (subjects free of cancer, with the exception of non-melanoma skin cancer). Cases and controls were matched by study center, menopausal status, age at recruitment (±6 months), date at blood collection (± 1 month), time of the day of blood draw (±1 hour), and fasting status (<3, 3-6, >6 hours). Individual matching was broken in the present study (one control per case) because we only included women who were

non-smokers, defined as women who reported never smoking or who quit smoking ≥5 years before recruitment, and who were postmenopausal at blood draw, defined as women who reported not having menses ≥1 year before recruitment.

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

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

All statistical models were adjusted for matching variables (age at recruitment (years), country, date of blood draw, time of day of the blood draw, and fasting status), and other covariates such as ever use of OC (never, ever), ever use of hormone replacement therapy (never, ever; HRT), parity (nulliparous, 1, 2, ≥3, parous but with missing number of full-term pregnancies), age at menopause (years), body mass index (kg/m^2; BMI), and alcohol intake (non-drinkers, drinkers of 0-6, >6-12, >12-24, and >24 g/day). The following variables were evaluated as potential confounders but were not included in final models because they did not change the risk estimates by >10%: dietary variables (such as coffee, potatoes, biscuits, crackers, and cakes), history of diabetes (yes, no), age at

menarche (<12, 12, 13, 14, ≥15 years), height (cm), weight (kg), hip circumference (cm), waist circumference (cm), physical activity using the Cambridge index[24], and education level (none, primary, technical/professional, secondary, and higher education).

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

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

Results

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

No associations and no evidence for linear dose-response trends were observed between biomarkers of dietary acrylamide exposure and overall EC (highest *vs* lowest quintiles: $HR_{HbAA;Q5vsQ1}$:

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

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

Discussion

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

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

The lack of association between biomarkers of acrylamide exposure and overall and type-I EC risk is in agreement with results we previously reported in the EPIC sub-cohort of women, where hazard ratios were estimated for the association between dietary acrylamide intake (assessed through DQs) and overall EC (n=1382) or type-I EC risk (n=627); nevertheless, in the full cohort analysis, positive associations were reported between acrylamide intake and type-I EC risk in women who were never smokers and non-users of OCs[19]. In the present study, using circulating biomarkers of acrylamide exposure, we did not replicate these results possibly due to the small sample size with tumor histology information (n=171 type-I EC cases). Additionally, the null results based on FFQ data reported by the Swedish Mammography Cohort study[17] are also in line with the results presented in the current study. However, the Netherlands Cohort Study reported hazard ratios for dietary acrylamide intake and risk of EC of 1.29 (95%CI: 0.81-2.07; *P*-trend: 0.18) and 1.99 (95%CI: 1.25-3.52; *P*-trend: 0.03) in the entire cohort and in never smoking women, respectively[16]. The Nurses' Health Study also reported relative risks for dietary acrylamide intake of 1.41 (95%CI: 1.01-1.97; *P*-trend: 0.03) and 1.43 (95%CI: 0.90-2.28; *P*-trend: 0.04) in the entire cohort and in never smoking women[18]. Two recent meta-analyses concluded that higher consumption of dietary acrylamide was significantly associated with overall EC risk in never smoking women; but not in all women combined [20,21]. In the present study of acrylamide and glycidamide biomarkers and EC risk in non-smoking postmenopausal women, we did not observe any evidence for associations with overall or type-I EC risk.

The main strengths of the present nested case-control study are its study design, with the intention to prevent confounding from tobacco smoking and hormonal fluctuations, and the use of prospective information on the main risk factors for EC. The minimum detectable ORs at 80% power in our study were 1.22 and 1.60 for the continuous and categorical variables, respectively. Moreover, measurement errors from using acrylamide intake estimates based on FFQs were avoided, and the quantification of HbAA and HbGA was performed following rigorous quality assurance/quality control laboratory protocols[10]; and all blood samples were drawn from

participants before disease diagnosis. The present study also had limitations: (a) a single blood sample was collected at baseline for each observation, thus, we were not able to measure intra-individual variability in adduct measurements. Hemoglobin adducts of acrylamide and glycidamide reflect exposure to acrylamide within the past 4 months, thus, a single measurement may not capture long-term average exposure in the presence of high intra-individual variability. In a small study of 13 participants Vikström et al. observed high intra-individual variability (up to 2-fold and 4-fold differences in HbAA and HbGA levels, respectively) over a period of 20 months [25]. By contrast, the NHS-II study observed lower intra-individual variability for Hb-adduct measurements (intra-individual correlation= 0.78, 0.80, and 0.77 for HbAA, HbGA, and sum of HbAA+HbGA, respectively) from 45 non-smoking women at two time-points separated by a median of 23 months [26]. (b) Although all models accounted for matching variables as well as known EC risk factors, we cannot exclude the possibility of residual confounding in our analyses. (c) Further, variables for second-hand smoke (SHS) exposure could not be evaluated in statistical models due to the large number of missing values (>50%). In a subset of the present study with available data, no statistically significant differences in Hb-adducts levels were observed between controls who reported not being exposed to SHS (n=80) and controls who were exposed to SHS (n=53) (data not presented). Moreover, two additional studies reported null or negligible effects of SHS on biomarkers of acrylamide exposure [27,28]. (d) Despite having information on tumor histology for 97% of the EC cases (of which 46% were classified as type-I), we were not able to analyze type-II EC due to the small sample size (n=14).

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

Acknowledgments

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

References

1. IARC. IARC working group on the evaluation of carcinogenic risks to humans: some industrial chemicals. Lyon, 15-22 February 1994. *IARC Monogr EvalCarcinogRisks Hum* 1994;60:1–560.

2. Tareke E, Rydberg P, Karlsson P, Eriksson S, Tornqvist M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J AgricFood Chem* 2002;50:4998–5006.

3. Friedman M. Chemistry, biochemistry, and safety of acrylamide. A review. *J AgricFood Chem* 2003;51:4504–26.

4. Becalski A, Brady B, Feng S, Gauthier BR, Zhao T. Formation of acrylamide at temperatures lower than 100°C: the case of prunes and a model study. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* 2011;28:726–30.

5. 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, et al. Dietary acrylamide intake of adults in the European Prospective Investigation into Cancer and Nutrition differs greatly according to geographical region. *EurJNutr* 2013;52:1369–80.

6. LoPachin RM, Gavin T. Acrylamide-induced nerve terminal damage: relevance to neurotoxic and neurodegenerative mechanisms. *JAgricFood Chem* 2008;56:5994–6003.

7. Hogervorst JG, Fortner RT, Mucci LA, Tworoger SS, Eliassen AH, Hankinson SE, Wilson KM. Associations between Dietary Acrylamide Intake and Plasma Sex Hormone Levels. *Cancer Epidemiol Biomarkers Prev* 2013;22:2024–36.

8. Nagata C, Konishi K, Tamura T, Wada K, Tsuji M, Hayashi M, Takeda N, Yasuda K. Associations of acrylamide intake with circulating levels of sex hormones and prolactin in premenopausal Japanese women. *Cancer Epidemiol Biomarkers Prev* 2015;24:249–54.

9. Fennell TR, Sumner SC, Walker VE. A model for the formation and removal of hemoglobin adducts. *Cancer Epidemiol Biomarkers Prev* 1992;1:213–9.

10. 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, et al. Cross-sectional study on acrylamide hemoglobin adducts in subpopulations from the European Prospective Investigation into Cancer and Nutrition (EPIC) Study. *JAgricFood Chem* 2008;56:6046–53.

11. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015;

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

13. Murali R, Soslow RA, Weigelt B. Classification of endometrial carcinoma: more than two types. *Lancet Oncol* 2014;15:e268–78.

14. Cote ML, Alhajj T, Ruterbusch JJ, Bernstein L, Brinton LA, Blot WJ, Chen C, Gass M, Gaussoin S, Henderson B, Lee E, Horn-Ross PL, et al. Risk factors for endometrial cancer in black and white women: a pooled analysis from the Epidemiology of Endometrial Cancer Consortium (E2C2). *Cancer Causes Control* 2015;26:287–96.

15. Merritt MA, Tzoulaki I, Tworoger SS, De Vivo I, Hankinson SE, Fernandes J, Tsilidis KK, Weiderpass E, Tjønneland A, Petersen KEN, Dahm CC, Overvad K, et al. Investigation of Dietary Factors and Endometrial Cancer Risk Using a Nutrient-wide Association Study Approach in the EPIC and Nurses' Health Study (NHS) and NHSII. *Cancer Epidemiol Biomarkers Prev* 2015;24:466–71.

16. Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA. A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. *Cancer Epidemiol Biomarkers Prev* 2007;16:2304–13.

17. Larsson SC, Hakansson N, Akesson A, Wolk A. Long-term dietary acrylamide intake and risk of endometrial cancer in a prospective cohort of Swedish women. *IntJCancer* 2009;124:1196–9.

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 EpidemiolBiomarkers Prev* 2010;19:2503–15.

19. Obón-Santacana M, Kaaks R, Slimani N, Lujan-Barroso L, Freisling H, Ferrari P, Dossus L, Chabbert-Buffet N, Baglietto L, Fortner RT, Boeing H, Tjønneland A, et al. Dietary intake of acrylamide and endometrial cancer risk in the European Prospective Investigation into Cancer and Nutrition cohort. *Br J Cancer* 2014;111:987–97.

20. Pelucchi C, Bosetti C, Galeone C, La Vecchia C. Dietary acrylamide and cancer risk: An updated meta-analysis. *Int J Cancer* 2014;

21. Je Y. Dietary acrylamide intake and risk of endometrial cancer in prospective cohort studies. *Arch Gynecol Obstet* 2014;

22. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, Charrondiere UR, Hemon B, Casagrande C, Vignat J, Overvad K, Tjonneland A, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr*

2002;5:1113–24.

23. Cust AE, Kaaks R, Friedenreich C, Bonnet F, Laville M, Tjønneland A, Olsen A, Overvad K, Jakobsen MU, Chajès V, Clavel-Chapelon F, Boutron-Ruault M-C, et al. Metabolic syndrome, plasma lipid, lipoprotein and glucose levels, and endometrial cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Endocr Relat Cancer* 2007;14:755–67.

24. Wareham NJ, Jakes RW, Rennie KL, Schuit J, Mitchell J, Hennings S, Day NE. 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.

25. Vikström AC, Warholm M, Paulsson B, Axmon A, Wirfält E, Törnqvist M. Hemoglobin adducts as a measure of variations in exposure to acrylamide in food and comparison to questionnaire data. *Food Chem Toxicol* 2012;50:2531–9.

26. Wilson KM, Vesper HW, Tocco P, Sampson L, Rosen J, Hellenas KE, Tornqvist M, Willett WC. Validation of a food frequency questionnaire measurement of dietary acrylamide intake using hemoglobin adducts of acrylamide and glycidamide. *Cancer Causes Control* 2009;20:269–78.

27. Vesper HW, Bernert JT, Ospina M, Meyers T, Ingham L, Smith A, Myers GL. Assessment of the relation between biomarkers for smoking and biomarkers for acrylamide exposure in humans. *Cancer Epidemiol Biomarkers Prev* 2007;16:2471–8.

28. Heudorf U, Hartmann E, Angerer J. Acrylamide in children--exposure assessment via urinary acrylamide metabolites as biomarkers. *Int J Hyg Environ Health* 2009;212:135–41.

1 # Tables

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

	All EC cases	Type-I Cases	Controls
	n=383	n=171	n=385
HbAA (pmol/g Hb) [a]	39.9 (31.4-52.4)	40.1 (31.4-52.8)	39.4 (32.1-51.1)
HbGA (pmol/g Hb) [a]	34.1 (25.7-44.6)	33 (25.3-46.2)	33.3 (24.6-43.8)
HbAA+HbGA (pmol/g Hb) [a]	74.4 (57.5-97.6)	72.5 (56.8-97.8)	72.8 (57.2-94.5)
HbGA/HbAA (pmol/g Hb) [a]	0.9 (0.7-1.0)	0.8 (0.7-1.0)	0.8 (0.7-1.0)
Age at recruitment (y) [a]	58.0 (53.5-61.4)	57.7 (53.6-61.0)	58.5 (54.3-61.7)
Age at menopause (y) [a]	49.5 (49.5-52.0)	49.5 (49.5-52.0)	49.5 (49.0-52.0)
BMI (Kg/m²) [a]	27.4 (24.1-31.6)	27.4 (24.4-33.2)	26.1 (23.2-29.3)
Country [b]			
France	33 (8.6)	17 (9.9)	35 (9.1)
Italy	69 (18.0)	24 (14.0)	74 (19.2)
Spain	55 (14.4)	25 (14.6)	72(18.7)
United Kingdom	70 (18.3)	30 (17.5)	60 (15.6)
The Netherlands	56 (14.6)	32 (18.7)	38 (9.9)
Greece	13 (3.4)	3 (1.8)	16 (4.2)
Germany	51 (13.3)	40 (23.4)	56 (14.6)
Sweden	36 (9.4)	0 (0.0)	34(8.8)
Fasting status[b]			
Unknown	1 (0.3)	1 (0.6)	0 (0.0)
<3 hours	150 (39.2)	77 (45.0)	129 (33.5)
3-6 hours	60 (15.7)	34 (19.9)	64 (16.6)
>6 hours	172 (44.9)	59 (34.5)	192 (49.9)
Alcohol consumption [b]			
Non drinker	94 (24.5)	37 (21.6)	93 (24.2)
>0-6 g/day	168 (43.9)	72 (42.1)	166 (43.1)
>6-12 g/day	63 (16.5)	32 (18.7)	67 (17.4)
>12-24 g/day	33 (8.6)	19 (11.1)	38 (9.9)
>24-60 g/day	25 (6.5)	11 (6.4)	21 (5.5)
Ever use of OC[b]			
Unknown	10 (2.6)	1 (0.6)	8 (2.1)
No	249 (65.0)	102 (59.7)	237 (61.6)
Yes	124 (32.4)	68 (39.8)	140 (36.4)
Ever use of HRT [b]			
Unknown	16 (4.2)	5 (2.9)	15 (3.9)
No	263 (68.7)	114 (66.7)	287 (74.6)
Yes	104 (27.2)	52 (30.4)	83 (21.6)
Parity [b]			
Unknown	61 (4.4)	31 (2.3)	59 (2.3)
1 child	130 (15.9)	62 (18.1)	140 (15.3)
2 children	105 (33.9)	46 (36.3)	131 (36.4)
>=3 children	62 (27.4)	21 (26.9)	42 (34.0)
Nulliparous	8 (16.2)	7 (12.3)	4 (10.9)
Parous but with missing number of full-term pregnancies	17 (2.1)	4 (4.1)	9 (1.0)

EC, endometrial cancer; EPIC, European Prospective Investigation into Cancer and Nutrition; HbAA, hemoglobin adducts of acrylamide; HbGA, hemoglobin adducts of glycidamide, BMI, body mass index; OC, oral contraceptive; HRT, hormone replacement therapy.

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 EC risk in a nested case-control study in the EPIC cohort

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

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

All models are adjusted for age at recruitment, country, fasting status, date at blood collection, time of the day of blood collection, OC use, HRT use, alcohol intake, parity, age at menopause, and BMI.

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

		<25 kg/m²			≥25 kg/m²			Never drinkers			Drinkers			Non-Oral 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	≤30.3	18	21	1.00 (ref)	59	53	1.00 (ref)	21	20	1.00 (ref)	56	54	1.00 (ref)	56	56	1.00 (ref)	19	18	1.00 (ref)
	30.4-37.6	25	32	0.79(0.29- 2.15)	50	48	0.91(0.49- 1.71)	21	18	1.32(0.47- 3.69)	54	62	0.71(0.39- 1.31)	46	48	0.87(0.47- 1.63)	29	30	0.85(0.30- 2.40)
	37.7-45.3	18	40	0.47(0.17- 1.35)	56	37	1.37(0.72- 2.60)	14	22	0.66(0.22- 1.97)	60	55	1.06(0.58- 1.95)	47	45	0.98(0.52- 1.84)	25	30	0.96(0.34- 2.74)
	45.4-56.0	27	27	1.03(0.35- 3.01)	46	50	0.60(0.31- 1.16)	16	20	0.98(0.31- 3.03)	57	57	0.88(0.47- 1.66)	43	43	0.92(0.47- 1.79)	26	31	0.72(0.25- 2.03)
	>56.0	30	34	0.84(0.29- 2.41)	54	43	0.87(0.44- 1.70)	22	13	1.24(0.36- 4.28)	62	64	0.75(0.40- 1.41)	57	45	1.13(0.58- 2.20)	25	31	0.62(0.21- 1.83)
	LRT[d]			0.06						0.42						0.63			
HbGA	≤23	21	26	1.00 (ref)	35	50	1.00 (ref)	7	15	1.00 (ref)	49	61	1.00 (ref)	40	48	1.00 (ref)	16	27	1.00 (ref)
	23.1-29.4	26	37	0.75(0.30- 1.87)	59	41	1.88(0.98- 3.62)	29	18	3.89(1.11- 13.71)	56	60	0.97(0.54- 1.75)	50	52	0.93(0.49- 1.77)	35	24	2.89(1.08- 7.75)
	29.5-37.6	31	36	0.60(0.24- 1.54)	56	41	1.73(0.87- 3.44)	14	22	1.13(0.30- 4.18)	73	55	1.33(0.74- 2.40)	55	41	1.36(0.70- 2.64)	27	33	1.30(0.48- 3.52)
	37.7-46.9	19	27	0.54(0.19- 1.59)	56	50	1.38(0.71- 2.69)	19	23	1.62(0.44- 6.00)	56	54	1.02(0.55- 1.89)	49	49	1.00(0.51- 1.98)	23	27	1.75(0.62- 4.95)
	>46.9	21	28	0.55(0.19- 1.59)	59	49	1.25(0.63- 2.49)	25	15	2.17(0.54- 8.79)	55	62	0.76(0.41- 1.42)	55	47	1.16(0.58- 2.30)	23	29	0.78(0.27- 2.30)
	LRT[d]			0.35						0.05						0.07			
Sum of HbAA + HbGA	≤53.6	20	24	1.00 (ref)	47	53	1.00 (ref)	14	17	1.00 (ref)	53	60	1.00 (ref)	49	55	1.00 (ref)	18	22	1.00 (ref)
	53.7-66.3	24	33	0.91(0.34- 2.46)	57	43	1.41(0.75- 2.65)	26	19	2.17(0.69- 6.76)	55	57	0.93(0.50- 1.71)	46	45	1.19(0.63- 2.27)	33	28	1.30(0.47- 3.54)
	66.4-81.8	24	41	0.48(0.18- 1.27)	54	37	1.48(0.76- 2.89)	12	21	0.80(0.25- 2.63)	66	57	1.10(0.61- 2.01)	51	45	1.10(0.58- 2.10)	24	31	0.90(0.33- 2.48)
	81.9-100.2	26	25	1.04(0.36- 2.98)	47	52	0.86(0.44- 1.67)	17	23	1.07(0.32- 3.55)	56	54	1.09(0.59- 2.03)	45	44	1.21(0.62- 2.37)	25	31	0.89(0.32- 2.50)
	>100.2	24	31	0.76(0.27- 2.20)	60	46	1.09(0.56- 2.12)	25	13	1.64(0.45- 6.00)	59	64	0.80(0.43- 1.48)	58	48	1.25(0.64- 2.42)	24	28	0.65(0.22- 1.89)
	LRT[d]			0.09						0.14						0.68			
Ratio of HbGA/HbAA	≤0.69	34	40	1.00 (ref)	28	36	1.00 (ref)	8	13	1.00 (ref)	54	63	1.00 (ref)	38	37	1.00 (ref)	23	37	1.00 (ref)
	0.70-0.80	35	39	1.14(0.51- 2.53)	57	39	1.63(0.79- 3.35)	24	13	2.79(0.75- 10.34)	68	65	1.08(0.62- 1.88)	56	48	1.05(0.54- 2.06)	35	28	2.47(1.03- 5.94)
	0.81-0.88	14	30	0.45(0.17- 1.16)	43	43	1.10(0.52- 2.30)	8	18	0.44(0.10- 1.86)	49	55	0.81(0.44- 1.48)	35	42	0.63(0.31- 1.31)	20	29	1.10(0.42- 2.87)
	0.89-0.98	16	22	0.63(0.22- 1.79)	57	56	1.03(0.51- 2.07)	18	25	0.69(0.19- 2.48)	55	53	0.83(0.45- 1.52)	51	56	0.73(0.37- 1.46)	21	22	1.17(0.44- 3.11)
	>0.98	19	23	0.75(0.27- 2.07)	80	57	1.59(0.80- 3.17)	36	24	1.12(0.33- 3.82)	63	56	1.01(0.55- 1.86)	69	54	1.06(0.53- 2.10)	25	24	1.25(0.47- 3.37)
	LRT[d]			0.76						0.16						0.56			

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

a Adjusted for age at recruitment, country, fasting status, date at blood collection, OC use, HRT use, alcohol intake, parity, and age at menopause.

b Adjusted for age at recruitment, country, fasting status, date at blood collection, OC use, HRT use, parity, age at menopause, and BMI.

c Adjusted for age at recruitment, country, fasting status, date at blood collection, HRT use, alcohol intake, parity, age at menopause, and BMI.

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

5