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

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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.

22 # Introduction

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

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

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

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

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

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

62 Material and Methods

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

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

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

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

85 Identification of EC cases was achieved by means of population cancer registries, or through a
86 combination of methods: health insurance records, cancer and pathology registries, and active
87 follow-up. EC cases were classified as C54 according to the International Classification of Diseases,
88 10th revision.

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

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

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

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

113 All statistical models were adjusted for matching variables (age at recruitment (years), country, date
114 of blood draw, time of day of the blood draw, and fasting status), and other covariates such as ever
115 use of OC (never, ever), ever use of hormone replacement therapy (never, ever; HRT), parity
116 (nulliparous, 1, 2, ≥3, parous but with missing number of full-term pregnancies), age at menopause
117 (years), body mass index (kg/m^2; BMI), and alcohol intake (non-drinkers, drinkers of 0-6, >6-12, >12-
118 24, and >24 g/day). The following variables were evaluated as potential confounders but were not
119 included in final models because they did not change the risk estimates by >10%: dietary variables
120 (such as coffee, potatoes, biscuits, crackers, and cakes), history of diabetes (yes, no), age at

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

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

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

133 Results

134 A large number of cases and controls were from Italy and the United Kingdom, and the major
135 proportion of type-I EC cases were from Germany and The Netherlands (Table 1). The median
136 interval between the dates at blood collection and diagnosis was 6.2 years. Among cases, the
137 median (25th–75th percentile) HbAA and HbGA adducts levels were 39.9 (31.4-52.4) and 34.1 (25.7-
138 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,
139 respectively. As compared with controls, cases were slightly younger, had a slightly higher
140 proportion of heavy drinking (6.5% vs 5.5%), tended to use less OCs (32.4% vs 36.4%) and more HRT
141 (27.2% vs 21.6), had higher median BMI values (27.4 vs 26.1 kg/m^2), and were more likely to be
142 nulliparous (16.2% vs 10.9%). Cases and controls had similar ages at menopause.

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

145 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
146 analyses to known type-I EC cases and no statistically significant associations were observed (Table
147 2). Associations between biomarkers of exposure and overall or type-I EC risk were also assessed
148 using tertiles, quartiles, and deciles (based on the exposure distribution in the control group), and no
149 significant variations in risk were observed across categories (data not shown).

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

156 Discussion

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

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

170 The lack of association between biomarkers of acrylamide exposure and overall and type-I EC risk is
171 in agreement with results we previously reported in the EPIC sub-cohort of women, where hazard
172 ratios were estimated for the association between dietary acrylamide intake (assessed through DQs)
173 and overall EC (n=1382) or type-I EC risk (n=627); nevertheless, in the full cohort analysis, positive
174 associations were reported between acrylamide intake and type-I EC risk in women who were never
175 smokers and non-users of OCs[19]. In the present study, using circulating biomarkers of acrylamide
176 exposure, we did not replicate these results possibly due to the small sample size with tumor
177 histology information (n=171 type-I EC cases). Additionally, the null results based on FFQ data
178 reported by the Swedish Mammography Cohort study[17] are also in line with the results presented in
179 the current study. However, the Netherlands Cohort Study reported hazard ratios for dietary
180 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;
181 *P*-trend: 0.03) in the entire cohort and in never smoking women, respectively[16]. The Nurses' Health
182 Study also reported relative risks for dietary acrylamide intake of 1.41 (95%CI: 1.01-1.97; *P*-trend:
183 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].
184 Two recent meta-analyses concluded that higher consumption of dietary acrylamide was
185 significantly associated with overall EC risk in never smoking women; but not in all women combined
186 [20,21]. In the present study of acrylamide and glycidamide biomarkers and EC risk in non-smoking
187 postmenopausal women, we did not observe any evidence for associations with overall or type-I EC
188 risk.

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

196 participants before disease diagnosis. The present study also had limitations: (a) a single blood
197 sample was collected at baseline for each observation, thus, we were not able to measure intra-
198 individual variability in adduct measurements. Hemoglobin adducts of acrylamide and glycidamide
199 reflect exposure to acrylamide within the past 4 months, thus, a single measurement may not
200 capture long-term average exposure in the presence of high intra-individual variability. In a small
201 study of 13 participants Vikström et al. observed high intra-individual variability (up to 2-fold and 4-
202 fold differences in HbAA and HbGA levels, respectively) over a period of 20 months [25]. By contrast,
203 the NHS-II study observed lower intra-individual variability for Hb-adduct measurements (intra-
204 individual correlation= 0.78, 0.80, and 0.77 for HbAA, HbGA, and sum of HbAA+HbGA, respectively)
205 from 45 non-smoking women at two time-points separated by a median of 23 months [26]. (b)
206 Although all models accounted for matching variables as well as known EC risk factors, we cannot
207 exclude the possibility of residual confounding in our analyses. (c) Further, variables for second-hand
208 smoke (SHS) exposure could not be evaluated in statistical models due to the large number of
209 missing values (>50%). In a subset of the present study with available data, no statistically significant
210 differences in Hb-adducts levels were observed between controls who reported not being exposed
211 to SHS (n=80) and controls who were exposed to SHS (n=53) (data not presented). Moreover, two
212 additional studies reported null or negligible effects of SHS on biomarkers of acrylamide exposure
213 [27,28]. (d) Despite having information on tumor histology for 97% of the EC cases (of which 46% were
214 classified as type-I), we were not able to analyze type-II EC due to the small sample size (n=14).

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

217

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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/m^2) [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 (%).

2

3

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.