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Cross-Sectional Study on Acrylamide Hemoglobin Adducts in Subpopulations from the European Prospective Investigation into Cancer and Nutrition (EPIC) Study

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Acrylamide exposure was investigated in subgroups of the EPIC study population (510 subjects from 9 European countries, randomly selected and stratified by age, gender, and smoking status) using hemoglobin adducts of acrylamide (HbAA) and its primary metabolite glycidamide (HbGA). Blood samples were analyzed for HbAA and HbGA by HPLC/MS/MS. Statistical models for HbAA and HbGA were developed including body mass index (BMI), educational level, and physical activity. A large variability in acrylamide exposure and metabolism between individuals and country groups was observed with HbAA and HbGA values ranging between 15−623 and 8−377 pmol/g of Hb, respectively. Both adducts differed significantly by country, sex, and smoking status. HbGA values were significantly lower in high alcohol consumers than in moderate consumers. With increasing BMI, HbGA in nonsmokers and HbAA in smokers decreased significantly. In the assessment of potential health effects related to acrylamide exposure, country of origin, BMI, alcohol consumption, sex, and smoking status should be considered.

KEYWORDS: Acrylamide; glycidamide; European Prospective Investigation into Cancer; smoking; alcohol consumption; gender

INTRODUCTION

Acrylamide is an environmental chemical found in tobacco smoke (*1*) and food (*2*). It is neurotoxic in humans and animals (*3*–*5*) and a suspected human carcinogen (*6*). Acrylamide is metabolized to glycidamide, which, in contrast to acrylamide, forms DNA adducts (*7*). Glycidamide is mutagenic in vivo and in vitro (*8*) and clastogenic in vivo (*9, 10*); thus, it is considered to be the actual mutagenic and carcinogenic agent.

The glycidamide-to-acrylamide ratio in humans was found to be highly variable between individuals (*11–15*), indicating a high interindividual variability in the metabolism of acrylamide to glycidamide.

The lifelong exposure of most of the population to acrylamide through food and smoking raised concerns about its toxicological effects. These concerns led to the need for further information about the actual exposure in the general population.

Several studies have been performed to estimate the acrylamide exposure in the general population, especially from food consumption (for a review see ref *16*). Uncertainties, such as food consumption surveys designed for unrelated research or lack of information on acrylamide levels in home cooked food, were described for these estimations. Most of these assessments did not provide information on acrylamide exposure related to tobacco smoke exposure, which significantly affects the overall acrylamide exposure in the general population as shown in previous studies (*14, 17–19*), nor do they provide information on glycidamide levels in individuals.

Other studies have used hemoglobin adducts of acrylamide and glycidamide as biomarkers of acrylamide exposure to determine the internal exposure dose (*11, 18, 20–22*). These biomarkers provide information about the amount of acrylamide exposure accumulated during 4 months (*23*). Several studies used these biomarkers for exposure assessment in the general population (*14, 17–19, 24, 25*). They found different levels of acrylamide exposure, which suggested differences in exposures between the populations investigated. However, the population groups as well as the analytical methods used in these studies differed among each other, which causes difficulties in comparing adduct levels between studies and in people from different countries.

The purpose of this cross-sectional study was to determine the acrylamide exposure in a subgroup of the European Prospective Investigation into Cancer and Nutrition (EPIC) study population by measuring hemoglobin adducts of acrylamide (HbAA) and its metabolite glycidamide (HbGA) as biomarkers. This study aimed at evaluating the variability of acrylamide exposure at the individual level and at the country group level, as well as identifying factors affecting this variability.

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MATERIALS AND METHODS

The EPIC is a multicenter prospective cohort study designed to investigate the relationship between nutrition and cancer. Details on the design and methods of the EPIC study have been described previously (*26, 27*). In brief, the study includes 519,978 participants (366,521 women and 153,457 men, aged 35−70 years) in 23 centers located in 10 European countries, to be followed for several decades to determine cancer incidence and cause-specific mortality. At enrollment, between 1992 and 2000, information was collected through nondietary questionnaires on lifestyle variables and country-specific dietary questionnaires designed to capture normal, long-term dietary intakes. Anthropometric measurements were taken, and blood samples and sample aliquots were prepared for long-term storage.

In this study, the sample consisted of 510 persons from 9 European countries (Sweden, Denmark, United Kingdom, The Netherlands, France, Germany, Italy, Greece, and Spain), 60 persons (30 men and 30 women) per country, except France, where only samples from women were collected. Within the groups of males and females, 15 persons were smokers and 15 were nonsmokers on the basis of questionnaire data. Study subjects were further randomized by age (age ranges were 41−60 years in men and 43−60 years in women). The IARC and CDC ethics committees approved the study, and the study was conducted according to the guidelines of the Helsinki declaration.

HbAA and HbGA were measured in 300 μL of hemolysed erythrocytes and analyzed by HPLC/tandem mass spectrometry (HPLC/MS/MS) as described previously (*13, 28*). In brief, the N-terminal valines of hemoglobin with acrylamide and glycidamide attached were cleaved from the protein chain using modified Edman reaction with pentafluorophenyl isothiocyanate as Edman reagent (*29*). The resulting Edman products (AA-Val-PFPTH and GA-Val-PFPTH) were extracted using liquid−liquid extraction and analyzed by HPLC/MS/MS. Calibrators, reagent blanks, and quality-control materials were processed the same way as the samples. Two independent measurements per sample were performed. Hemoglobin adduct concentrations were reported relative to the amount of hemoglobin used in the analysis. The detection limits for this method were 3 and 4 pmol/g of Hb for HbAA and HbGA, respectively. The interday variabilities (n = 20 days) of this method expressed as percent coefficient of variation were on average 13% for HbAA and 14% for HbGA determined with three blood pools each measured in duplicate (HbAA and HbGA concentrations in pmol/g of Hb: pool 1, 182 and 151; pool 2, 119 and 101; pool 3, 45.1 and 37.3). Octapeptides with the same amino acid sequence as the N terminus of the β-chain of hemoglobin and with acrylamide and glycidamide attached at the valine (AA-VHLTPEEK and GA-VHLTPEEK) were synthesized by Bachem (King of Prussia, PA) and used as standards. The corresponding stable isotope-labeled compounds [AA-Val(^{13}C5 ^{15}N)-HLTPEEK and GA-Val(^{13}C5 ^{15}N)-HLTPEEK] were synthesized by the same company and used as an internal standard. PFPITC was obtained from Fluka (St. Louis, MO), and formamide was obtained from USB (Cleveland, OH). All other reagents were purchased from Sigma (St. Louis, MO). Blood samples were analyzed in a randomized manner to minimize systematic analytical differences between country groups.

Data sets for each variable were assessed for normality of data distribution using the Shapiro−Wilk test. Acrylamide and glycidamide adduct values showed skewed distributions and were log-transformed before statistical analysis. Analysis of variance (ANOVA) techniques were used for statistical modeling (SAS version 9.1, with type III sums of squares). A multivariate model was built for each of the outcome variables HbAA, HbGA, and glycidamide-to-acrylamide adduct ratio, for nonsmokers and smokers separately. First, a model with all main effects and lower order interactions was taken into account. The final model was built through stepwise elimination of nonsignificant (p > 0.05) model parameters. Nonsignificant main effect terms were kept in the model if they were involved in any significant interaction or if their inclusion in the model changed parameter estimates of other significant main effect variables by >10%. The following variables were considered: sex, number of cigarettes smoked (in smokers only), highest level of education (in two categories: no school, primary school, technical school; and secondary school and university), age at recruitment, a combined total physical activity index (two categories: inactive

Table 1. Mean, Median, Selected Percentiles, and Minima and Maxima of Hemoglobin Adducts of Acrylamide and Glycidamide in the Study Population

		acrylamide adducts (pmol/g of Hb)				glycidamide adducts (pmol/g of Hb)			
	N	mean	5th	median	95th	mean	5th	median	95th
total population	510	92.5	28.1	62.6	244	72.2	22	51.9	179
nonsmokers									
total	255	48.4	24.2	42.5	88.3	43.3	18.3	39.9	83.3
male	120	50.1	24.1	43.0	97.8	42.0	15.6	38.7	80.0
female	135	46.9	24.2	41.7	85.2	44.4	21.0	40.6	86.7

		acrylamide adducts (pmol/g of Hb)				glycidamide adducts (pmol/g of Hb)			
country	*N*	mean	min	median	max	mean	min	median	max
France	15	51.1	26.7	39.7	92.0	39.4	20.5	36.0	60.0
Italy	30	46.5	21.0	45.2	80.7	44.5	14.4	42.0	92.5
Spain	30	43.1	23.1	42.2	69.6	41.1	13.4	41.1	74.6
United Kingdom	30	74.3	33.5	64.8[a]	177	67.0	26.9	58.4[b]	151
The Netherlands	30	57.3	25.3	48.6[c]	171	51.0	24.1	49.6[d]	76.8
Greece	30	45.5	14.5	40.8	150	42.4	10.4	39.6	112
Germany	30	40.2	14.7	39.6	92.4	37.3	11.2	34.1	96.6
Sweden	30	40.2	20.2	35.4	96.7	33.8	14.6	34.0	68.5
Denmark	30	38.9	17.1	37.0	97.9	30.8	7.8	26.6	100

		acrylamide adducts (pmol/g of Hb)				glycidamide adducts (pmol/g of Hb)			
smokers	*N*	mean	5th	median	95th	mean	5th	median	95th
total	255	137	44.0	121	285	101	33.8	92.8	198
male	120	140	45.4	127	280	97.4	33.7	84.8	193
female	135	134	43.7	117	294	104	35.3	100	219

		acrylamide adducts (pmol/g of Hb)				glycidamide adducts (pmol/g of Hb)			
country	*N*	mean	min	median	max	mean	min	median	max
France	15	70.3	43.7	61.7[e]	138	59.4	29.3	51.2	124
Italy	30	134	34.6	120	274	106	35.8	95.6	279
Spain	30	95.3	43.2	95.7	168	71.8	20.3	68.3[f]	150
United Kingdom	30	157	32.1	131	623	119	27.5	118	377
The Netherlands	30	152	39.3	131	367	107	27.3	109	342
Greece	30	122	38.5	117	247	106	33.4	105	198
Germany	30	157	32.1	137	371	102	25.4	96.1	247
Sweden	30	142	32.2	129	293	108	24.0	95.4	242
Denmark	30	167	44.2	170	421	111	34.6	96.6	273

[a] Value significantly different ($p < 0.0001$) from all other country groups except French group. [b] Value significantly different ($p < 0.007$) from all other country groups except Dutch group. [c] Value significantly different ($p < 0.003$) from Danish, Swedish and German groups. [d] Value significantly different ($p < 0.05$) from Spanish, German and Danish groups group. [e] Value significantly different ($p < 0.05$) from all other country groups. [f] Value significantly different ($p < 0.03$) from all other country groups.

and moderately inactive; moderately active and active), alcohol consumption at recruitment (quartiles of g of ethanol/day: <1.2; 1.2−5.5; 5.5−14.4; >14.4), BMI at recruitment, and country. Cigarette consumption was grouped by quartiles (cigarettes/day: <7; 7−15; 16−20; >20). The parameters used in the final model are listed in **Table 3**. With the model obtained, corrections for multiple comparisons were performed using the Tukey−Kramer method (*30*).

RESULTS

Overall Population. The range of acrylamide biomarker values (pmol/g of Hb) in the group of 510 persons was 14.5−623 for HbAA and 7.8−377 for HbGA (**Table 1**.) The range of glycidamide-to-acrylamide adduct ratios was 0.18−1.67 (**Table 2**.) The glycidamide-to-acrylamide adduct ratio adjusted by age, sex, country, and smoking status increased significantly ($p < 0.0001$) with decreasing acrylamide adducts. When dividing the HbAA concentrations into quintiles (**Figure 1**), we observed significantly lower ratios in the highest HbAA quintile than in the three lowest HbAA quintiles ($p < 0.003$).

In the study population, HbAA and HbGA values differed significantly by country ($p < 0.0001$) and smoking status ($p < 0.0001$), with higher HbAA values in smokers than nonsmokers (**Table 3**). For both biomarkers, a significant interaction between the country and smoking status variables was found. Furthermore, HbAA was inversely associated with BMI ($p < 0.003$); HbGA was inversely associated with alcohol consumption ($p < 0.0001$). The glycidamide-to-acrylamide adduct ratio differed significantly by country ($p < 0.03$) and sex ($p < 0.0001$; lower values in men than in women) and was inversely associated with smoking status ($p < 0.0001$) and alcohol consumption ($p < 0.0001$).

Nonsmoker Group. In the nonsmoker group, the British group had significantly higher median HbAA ($p < 0.0001$) and HbGA ($p < 0.007$) values than other countries, except the Dutch and French groups for HbAA and the Dutch group for HbGA (**Table 1**). The differences between individuals in each country group (difference between maximum and minimum values in pmol/g of Hb) ranged for HbAA between 46.5 (Spanish group) and 146 (Dutch group) and for HbGA between 52.7 (Dutch group, French group with women only, 39.5) and 124 (British group). The glycidamide-to-acrylamide ratios (**Table 2**) ranged

Table 2. Mean, Median, Selected Percentiles, and Minima and Maxima of the Glycidamide-to-Acrylamide Adducts Concentration Ratios in the Study Population

		glycidamide-to-acrylamide adduct ratio			
	N	mean	5th	median	95th
total population	510	0.84	0.45	0.85	1.20
nonsmokers					
total	255	0.91	0.53	0.91	1.31
male	120	0.85	0.48	0.86	1.14
female	135	0.96	0.59	0.95	1.36

		glycidamide-to-acrylamide adduct ratio			
country	*N*	mean	min	median	max
France	15	0.85	0.38	0.81	1.63
Italy	30	0.94	0.40	0.93	1.25
Spain	30	0.95	0.43	0.95	1.61
United Kingdom	30	0.92	0.59	0.87	1.38
The Netherlands	30	0.95	0.30	0.98	1.23
Greece	30	0.96	0.39	0.99	1.51
Germany	30	0.94	0.43	0.97[a]	1.67
Sweden	30	0.85	0.58	0.84	1.20
Denmark	30	0.78	0.44	0.80	1.36

		glycidamide-to-acrylamide adduct ratio			
smokers	*N*	mean	5th	median	95th
total	255	0.77	0.43	0.77	1.13
male	120	0.72	0.40	0.70	1.09
female	135	0.82	0.49	0.85	1.15

		glycidamide-to-acrylamide adduct ratio			
country	*N*	mean	min	median	max
France	15	0.85	0.41	0.90[b]	1.22
Italy	30	0.82	0.43	0.82	1.27
Spain	30	0.77	0.36	0.76	1.30
United Kingdom	30	0.79	0.39	0.80	1.16
The Netherlands	30	0.74	0.18	0.76	1.13
Greece	30	0.89	0.58	0.88	1.23
Germany	30	0.66	0.34	0.67	1.02
Sweden	30	0.78	0.33	0.77	1.10
Denmark	30	0.71	0.27	0.69	1.12

[a] Value significantly different ($p < 0.04$) from all other country groups. [b] Value significantly different from Danish, Swedish, German, Italian and Dutch groups.

between 0.30 and 1.67 with differences between individuals being between 0.62 (Swedish group) and 1.24 (German group, French group with women only, 1.25).

In the nonsmoker group, HbAA values differed significantly only by country ($p < 0.0001$, **Table 3**). HbGA values differed significantly by country ($p < 0.0001$) and were inversely associated with alcohol consumption ($p < 0.008$, **Figure 2**) and BMI ($p < 0.05$).The glycidamide-to-acrylamide adduct values differed significantly by country ($p < 0.02$), sex ($p < 0.0001$), and alcohol consumption ($p < 0.0007$), with significant interaction between sex and alcohol consumption. The glycidamide-to-acrylamide adduct ratios in nonsmoking males differed significantly by country ($p < 0.0001$; values in the Danish group were significantly higher than those in the Italian, Spanish, and Dutch groups) and were inversely associated with alcohol consumption ($p < 0.05$). The ratios observed in nonsmoking females were inversely associated with alcohol consumption ($p < 0.03$) and positively associated with BMI ($p < 0.002$, **Table 3**).

Comparison between Nonsmoker Group and Smoker Group. When smokers and nonsmokers were compared (**Table 1**), median hemoglobin adduct values were generally 3−4 times higher in smokers than in nonsmokers. The median glycidamide-to-acrylamide adduct ratio in smokers was on average 13% lower than in nonsmokers (**Table 2**). In both the nonsmoker and smoker groups, the difference between men and women was insignificant for HbAA and HbGA and in smokers for the glycidamide-to-acrylamide adduct ratios. The glycidamide-to-acrylamide adduct ratios in nonsmokers were significantly higher in women than in men ($p < 0.007$). The largest difference between smokers and nonsmokers was in the Danish group. The variability in HbAA values among individuals as expressed in the difference between the 95th and 5th percentile was 4 times higher in smokers than nonsmokers; the variability was about twice as high for HbGA and slightly lower for the glycidamide-to-acrylamide adducts ratio.

Smoker Group. In smokers, the HbAA values observed in the French groups were significantly lower ($p < 0.05$) than those in all other groups except the Spanish group; HbGA values in the Spanish group were significantly lower ($p < 0.03$) than those in other country groups except for the French, German, and Swedish groups (**Table 1**). Differences between individuals in each country group (difference between maximum and minimum in pmol/g of Hb) ranged for HbAA between 125 (Spanish group, French group, 94.3) and 377 (Danish group) and for HbGA between 130 (Spanish group, French group, 94.7) and 350 (British group). The range of glycidamide-to-acrylamide adduct ratio in the French group was significantly higher ($p < 0.05$) than that observed in other country groups, except the Greek, Spanish, and British groups (**Table 2**). The difference between individuals from each country group was between 0.65 (Greek group) and 0.94 (Spanish group).

The HbAA values in smokers differed significantly by country ($p < 0.0007$) and were inversely associated with BMI ($p < 0.007$), and highest level of education ($p < 0.006$) and positively associated with the number of cigarettes smoked ($p < 0.0001$, **Table 3**). The HbGA values differed significantly by country ($p < 0.002$), were inversely associated with education ($p < 0.007$), and were positively associated with the number of cigarettes smoked ($p < 0.0001$, **Table 1**). Persons in the highest quartile of alcohol consumption had higher glycidamide adduct values than persons in the other quartiles ($p < 0.002$, **Figure 2**). The glycidamide-to-acrylamide adduct ratios in smokers differed significantly by country ($p < 0.002$), were positively associated with BMI ($p < 0.006$), and were inversely associated with alcohol consumption ($p < 0.0001$, **Table 3**).

The HbAA and HbGA values increased with increasing daily cigarette consumption. When cigarette consumption was grouped by quartiles, HbAA and HbGA values increased from the first to the third quartile. No significant difference was observed between the third and fourth quartiles (**Figure 3**).

DISCUSSION

In this study, acrylamide exposure data were assessed for the first time in comparable population groups from nine countries using the same analytical methodology. The results show that HbAA and HbGA values differed by country group. These differences were more pronounced in nonsmokers than smokers. The ranges of HbAA and HbGA values were similar to those reported in studies using population groups from individual countries (*17–19, 24, 25, 28*). However, maximum values observed in the British, Dutch, and Greek nonsmoker groups were higher than those commonly observed for nonsmokers.

Table 3. Parameters Significantly Affecting Acrylamide and Glycidamide Adducts and Glycidamide-to-Acrylamide Adduct Ratio in the Study Population

	acrylamide adducts		glycidamide adducts		glycidamide-to-acrylamide adduct ratio	
parameter[a]	nonsmoker estimate[b] (SE) *p* value	smoker estimate (SE) *p* value	nonsmoker estimate (SE) *p* value	smoker estimate (SE) *p* value	nonsmoker estimate (SE) *p* value	smoker estimate (SE) *p* value
intercept	4.279 (0.154) <0.0001	4.963 (0.235) <0.0001	3.648 (0.178) <0.0001	4.352 (0.244) <0.0001	0.468 (0.09) <0.0001	0.425 (0.083) <0.0001
Denmark	−0.619 (0.094) <0.0001	0.116 (0.141) NS[d]	−0.816 (0.107) <0.0101	0.091 (0.147) NS	−0.138 (0.053) 0.0001	−0.023 (0.047) NS
France	−0.407 (0.116) 0.0005	−0.622 (0.173) 0.0004	−0.487 (0.13) 0.0002	−0.401 (0.177) 0.0249	−0.116 (0.066) NS	0.136 (0.06) 0.0246
Germany	−0.587 (0.094) <0.0001	−0.075 (0.139) NS	−0.564 (0.105) <0.0001	−0.146 (0.141) NS	0.031 (0.052) NS	−0.087 (0.046) NS
Greece	−0.521 (0.094) <0.0001	−0.219 (0.142) NS	−0.532 (0.105) <0.0001	−0.081 (0.146) NS	0.005 (0.051) NS	0.071 (0.046) NS
Italy	−0.435 (0.095) <0.0001	−0.059 (0.132) NS	−0.424 (0.106) <0.0001	−0.004 (0.136) NS	−0.001 (0.052) NS	0.04 (0.047 NS
Spain	−0.481 (0.098) <0.0001	−0.247 (0.136) NS	−0.505 (0.109) <0.0001	−0.279 (0.141) 0.0498	−0.012 (0.053) NS	−0.033 (0.047) NS
Sweden	−0.594 (0.092) <0.0001	−0.116 (0.136) NS	−0.671 (0.102) <0.0001	−0.105 (0.139) NS	−0.069 (0.05) NS	−0.016 (0.046) NS
The Netherlands	−0.248 (0.093) 0.0081	−0.015 (0.141) NS	−0.242 (0.103) 0.0193	−0.004 (0.144) NS	0.019 (0.051) NS	−0.011 (0.046) NS
United Kingdom	0	0	0	0	0	0
no school, primary school, technical school	−0.049 (0.048) NS	0.185 (0.071) 0.0096	−0.026 (0.054) NS	0.197 (0.075) 0.0092	NU[e]	NU
secondary school and university	0	0	0	0	NU	NU
BMI	−0.001 (0.006) NS	−0.024 (0.008) 0.0046	0.014 (0.006) 0.0383	−0.015 (0.009) NS	0.012 (0.003) 0.0002	0.009 (0.003) 0.0031
no. of cigarettes smoked per day	NU NU	0.028 (0.003) <0.0001	NU	0.023 (0.003) <0.0001	NU	NU
<1.2 g of ethanol/day	NU	NU	0.203 (0.074) 0.0064	0.351 (0.096) 0.0003	0.064[c] (0.055) NS	0.188 (0.051) 0.0003
1.2−5.5 g of ethanol/day	NU	NU	0.144 (0.073) 0.05	0.074 (0.099) NS	0.156 [c] (0.05) 0.002	0.158 (0.046) 0.0007
5.5−14.4 g of ethanol/day	NU	NU	0.227 (0.072) 0.0017	0.112 (0.095) NS	0.175[c] (0.047) 0.0002	0.036 (0.042) NS
>14.4 g of ethanol/day	NU	NU	0	0	0	0
female	NU	NU	NU	NU	0.167 [c] (0.061) 0.0062	0.014 (0.052) NS
male	NU	NU	NU	NU	0	0

[a] Parameters found to significantly affect the particular outcome variable in smokers and nonsmokers in the study population. [b] Parameter (effect) estimates obtained for each parameter after applying the statistical model (log-linear model obtained using ANOVA with type III sums of squares). [c] Significant interaction between sex and alcohol consumption. [d] Nonsignificant ($p > 0.05$). [e] Variable not used in the model for the particular outcome variable (acrylamide and glycidamide adducts and glycidamide-to-acrylamide adduct ratio), because it was not applicable or nonsignificant.

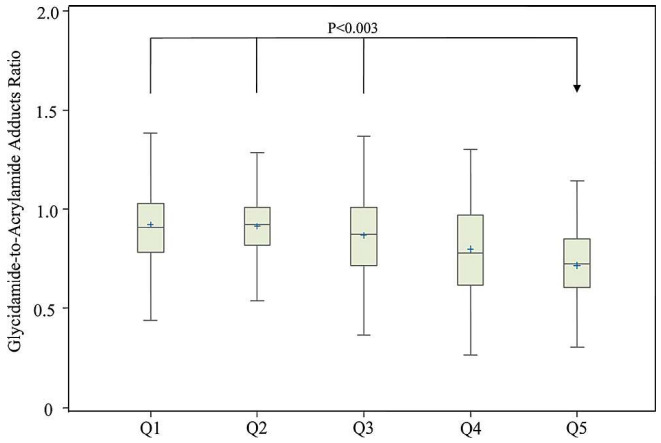

Figure 1. Box-and-whisker plots of glycidamide-to-acrylamide adduct ratio versus quintiles of acrylamide adducts concentrations in the whole study population. (Quintiles in pmol/g Hb: Q1, 14.5−38.9; Q2, 39.0−52.2; Q3, 52.3−81.2; Q4, 81.3−138; Q5, 139−623.) Shown are minimum, maximum, 25th, 75th, median, and average (+) values. Arrow marks significant differences between groups.

Removing these single extreme values did not change the findings of this study. Assuming that acrylamide exposure in nonsmokers was determined mainly by dietary intakes, as suggested in a recent study (*19*), the higher HbAA values in the British and Dutch groups compared to other country groups may indicate higher exposure to acrylamide through food consumption. The diets of the Dutch and British EPIC population were described as relatively high in potatoes compared to other countries in this study (*27*). Fried potato products contain high amounts of acrylamide (*16*), and significant associations between HbAA and fried potato products have been described (*19*). Therefore, the higher acrylamide biomarker levels in these countries may indicate high consumption of fried potato products. Further studies are needed to assess whether combinations of food consumption and food preparation such as frying can explain our observation.

Using rate constants for detoxification of acrylamide and formation of acrylamide adducts, Bergmark (*20*) calculated that an acrylamide adduct level of 30 pmol/g of globin corresponds to an intake of 1.2 μg/kg of bw/day. The range of median HbAA values in nonsmokers in most country groups was 40−49 pmol/g of Hb, with the highest median value at 64.8 pmol/g of Hb. These values would correspond to an acrylamide intake of 1.6−2.0 μg/kg of bw/day, with highest intake at 2.6 μg/kg of bw/day. These estimates are higher than those from food acrylamide databases and food consumption estimates, which estimate mean acrylamide intakes between 0.2 and 1.1 μg/kg of bw/day (*16*). Both approaches for reconstructing the actual acrylamide intake through food have limitations and are still subject to discussions (*16, 17*).

The patterns of HbGA and HbAA values were similar but not parallel, indicating effects of additional factors on acrylamide metabolism. The intersubject variability among countries was typically smaller for HbGA than HbAA. Interestingly, the intersubject variability of HbGA in the Dutch group was among the lowest; for HbAA it was among the highest. HbGA values decreased with high alcohol consumption (>29.96 g of ethanol/day). This effect is significant in nonsmokers and smokers and has not been reported previously. Glycidamide is metabolized from acrylamide by hepatic cytochrome P450 2E1 [CYP2E1 (*31*)], which is activated by alcohol (*32, 33*) in what is thought to be a two-step process. At low blood-alcohol concentrations, CYP2E1 is assumed to have only a minor involvement in alcohol metabolism; however, at high blood alcohol concentrations, CYP2E1 may be actively involved in alcohol metabolism. At this stage, acrylamide may compete with alcohol as substrate for CYP2E1, which could explain the decrease in HbGA values.

Smoking was a major contributor to the overall acrylamide exposure and caused a profound and significant elevation in HbAA and HbGA values as well as an increase in the interindividual variability. The high interindividual variability in smokers compared to nonsmokers could be explained by smoking habits, such as the number of cigarettes smoked per day. HbAA and HbGA values showed a slight exponential elevation as the number of cigarettes smoked increased. Cigarette consumption of >15 cigarettes per day, however, did not further elevate HbAA and HbGA values. The reason for this decrease in the adduct formation rate at high cigarette consumption was not fully understood. Similar observations were reported on hemoglobin adducts of 4-aminobiphenyl, a biomarker for tobacco smoke exposure (*34*). Changes in the metabolism of acrylamide with high cigarette consumption such as saturation of metabolic enzymes, as indicated in significantly lower glycidamide-to-acrylamide ratios at high HbAA concentrations, could be one explanation. The observed lower glycidamide-to-acrylamide ratio at high HbAA values was consistent with previous observations in other study populations (*13, 14, 28*).

In smokers, higher educational levels were associated with lower levels of HbAA and HbGA. The outcome was independent of the effects of smoking, which indicates that factors associated with educational level do affect acrylamide biomarker levels. Other factors (e.g., dietary patterns) may explain the differences in these subgroups and should be explored.

The effect of BMI on HbAA and HbGA was not consistent in smokers and nonsmokers and was small compared to the effect of other parameters such as country, alcohol consumption, and smoking; only HbAA decreased in smokers, and only HbGA decreased in nonsmokers. Similarly, the effects of BMI on the glycidamide-to-acrylamide adducts ratios were different for men and women; only in nonsmoking women did the ratio increase with increasing BMI.

These findings, including those related to alcohol consumption, indicate that the extent of glycidamide formation in individuals and resulting potential health risks are difficult to predict using HbAA values alone. Further studies are needed to obtain more detailed information on factors affecting this metabolism. The glycidamide-to-acrylamide adduct ratios observed in this study population are similar to those observed in mice (*9, 35*), suggesting that mice could be appropriate animal models for such studies.

In conclusion, this study showed for the first time significant variability between country groups in acrylamide exposure, especially among nonsmokers. Alcohol consumption, BMI, sex, smoking, and educational level are associated with acrylamide biomarker levels and the metabolism of acrylamide to glycidamide. When health effects associated with acrylamide exposure and metabolism are assessed, these parameters should be considered. It should be noted that the country groups in this study were not representative of the populations of these countries. Therefore, the observations made for one country group could not be generalized for the whole country population. The results suggested that factors other than those investigated (e.g., diet) may explain part of the heterogeneity in the acrylamide exposure, particularly among nonsmokers. Further investigations on the effect of acrylamide exposure from food on biomarkers of acrylamide exposure are ongoing.

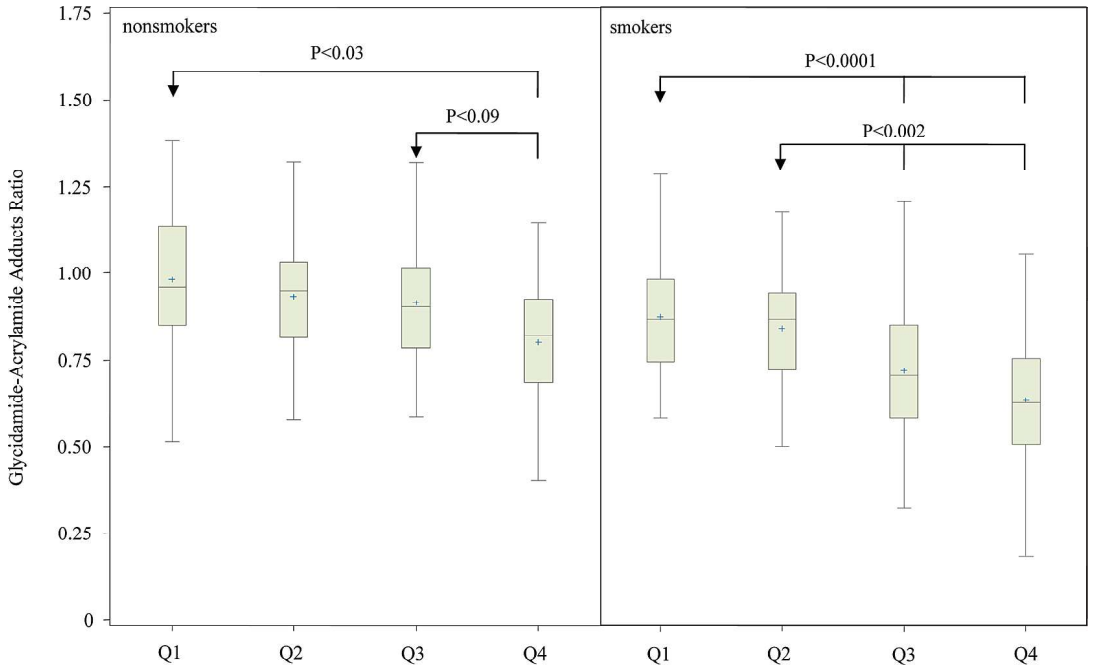

Figure 2. Box-and-whisker plots of glycidamide-to-acrylamide adduct ratios in smokers and nonsmokers versus quartiles of alcohol consumption. (Nonsmokers: Q1, <1.18 g of ethanol; Q2, 1.19−5.45 g of ethanol; Q3, 4.56−14.43 g of ethanol; Q4, >14.43 g of ethanol. Smokers: Q1, >1.7 g of ethanol; Q2, 1.8−9.64 g of ethanol; Q3, 9.65−29.96 g of ethanol; Q4, >29.96 g of ethanol.) Arrow marks significant differences between groups.

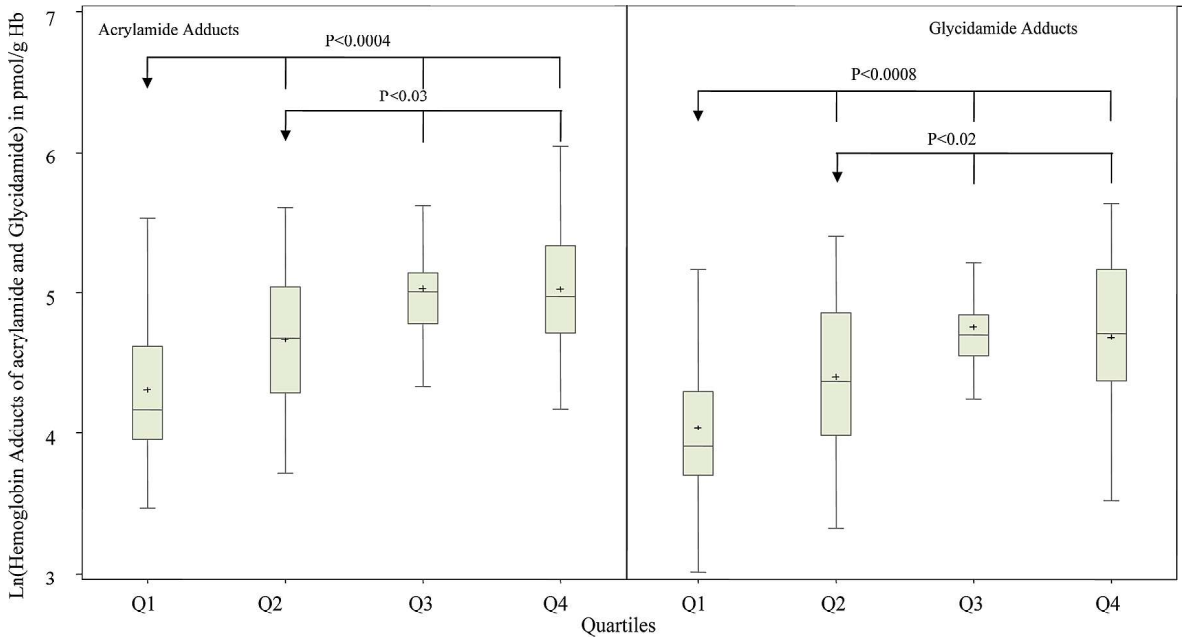

Figure 3. Box-and-whisker plots of log-transformed values of hemoglobin adducts of acrylamide and glycidamide versus quartiles of cigarette consumption among smokers. (Q1, <7 cigarettes/day; Q2, 7−15 cigarettes/day; Q3, 16−20 cigarettes/day; Q4, >20 cigarettes/day.) Shown are minimum, maximum, 25th, 75th, median, and average (+) values. Arrow marks significant differences between groups.

ABBREVIATIONS USED

AA-VHLTPEEK, octapeptide consisting of valine (V), histidine (H), leucine (L), threonine (T), proline (P), glutamic acid (E), and lysine (K) with acrylamide attached to valine; BMI, body mass index; EPIC, European Prospective Investigation into Cancer and Nutrition; GA-VHLTPEEK, octapeptide consisting of valine (V), histidine (H), leucine (L), threonine (T), proline (P), glutamic acid (E), and lysine (K) with glycidamide attached to valine; HbAA, hemoglobin adducts of acrylamide; HbGA, hemoglobin adducts of glycidamide; HPLC/MS/MS, high-performance liquid chromatography with tandem mass spectrometry; SE, standard error.

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