Assessment of the Relation between Biomarkers for Smoking and Biomarkers for Acrylamide Exposure in Humans

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Abstract

Smoking is an important source of acrylamide exposure in the general population. We assessed the relationship between hemoglobin adducts of acrylamide (HbAA) and glycidamide (HbGA) as biomarkers of acrylamide exposure and plasma cotinine (PC) as biomarkers of tobacco smoke exposure in 94 men and 67 women. The median (5th-95th percentile) biomarker concentrations (pmol/g Hb) in the group of individuals with PC concentrations of ≤10 ng/mL were 51 (29-155) and 34 (16-117) for HbAA and HbGA, respectively. They were significantly lower than those in the group of individuals with PC concentrations of >10 ng/mL [194 (87-403) and 107 (41-215) for HbAA and HbGA, respectively]. In individuals with PC concentrations of <1 ng/mL, HbAA and HbGA were similar to those observed in the group with PC values of ≤10 ng/mL. The intersubject variability was profoundly smaller in the group with PC values of ≤10 ng/mL compared with the group with PC values of >10 ng/mL. Although HbAA and HbGA could be categorized into distinguishable groups using PC concentration ranges commonly used to categorize presumed smokers and nonsmokers, no significant relationship was observed between these two biomarkers and PC within each group. The different exposure periods reflected by these biomarkers and the resulting different susceptibility to short-term variations in exposure patterns may in part explain these observations. The findings suggest that tobacco smoke exposure in individuals with PC values of <1 ng/mL has only a minimal effect on HbAA and HbGA. (Cancer Epidemiol Biomarkers Prev 2007;16(11):2471–8)

Introduction

Acrylamide is an industrial chemical used mainly in the production of polyacrylamide. Because of its suspected carcinogenicity in humans (1) and its neurotoxicity in both humans and animals (2-4), the assessment of acrylamide exposure and its health effects have been the subject of occupational studies for many years (5-8). Acrylamide is also an environmental chemical found in tobacco smoke (9) and food (10). Recent findings that acrylamide is formed in a variety of foods commonly eaten by a large portion of the population (11-15) created the need to assess the overall acrylamide exposure of the general population to define the extent of exposure and the possible associations between this exposure and health effects such as cancer. Because smoking is known to be a major contributor to the overall acrylamide exposure in the general population and because it is a major risk factor for certain cancers, assessing the effect of smoking on the overall acrylamide exposure in the general population is important.

Hemoglobin adducts of acrylamide and glycidamide, its primary metabolite, were successfully developed as biomarkers of acrylamide exposure and used to investigate the health effects of this chemical on humans and animals (16-20). Assessing acrylamide exposure using these biomarkers provides comprehensive information about the amount of acrylamide that has entered a person's body over the previous 2 to 3 months, even when the actual exposure event has passed. The assessment does not, however, provide information about the exposure source. Therefore, studies have been done assessing the effect of different sources of exposure, such as smoking, on biomarkers of acrylamide exposure.

Early studies focusing on occupational exposure found that smokers had higher biomarker concentrations of acrylamide exposure than did nonsmokers (18, 19). These findings were later confirmed in studies of the general population (21-23). The results of these studies showed mean acrylamide biomarker concentrations as being four to five times higher in smokers compared with nonsmokers. However, the concentration ranges of these biomarkers in the smoker and nonsmoker groups showed considerable overlap, thus making it difficult to distinguish whether an individual's acrylamide exposure was related mainly to smoking or to other sources such as food.

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Assessments of acrylamide in tobacco smoke showed that mainstream cigarette smoke contains 1 to 2 μg of acrylamide per cigarette (9). The results of one study that compared acrylamide biomarker values with the number of cigarettes smoked showed that one cigarette raised the acrylamide adduct level by 3.4 pmol (22). In most of the studies on acrylamide exposure, smoking behavior was assessed through questionnaires. However, the intake of the constituents in tobacco smoke depends on factors such as the brand and composition of the cigarette being smoked, puffs taken per cigarette, puff volume, and depth and duration of inhalation (24, 25). Because this information is typically not available from questionnaires, it is difficult to assess the effect of actual tobacco exposure on biomarker concentrations of acrylamide exposure. Furthermore, the above studies assessed the effect of active smoking but did not address the effect of low level tobacco smoke exposure on biomarkers of acrylamide exposure such as those commonly observed in people exposed to second hand smoke (SHS).

Cotinine is a major proximate metabolite of nicotine and is currently regarded as the best, most specific, and most sensitive biomarker for tobacco smoke exposure. Cotinine allows the assessment of smoking exposure in both active smokers and nonsmokers exposed to SHS. Nonsmokers with low SHS exposures typically have plasma cotinine (PC) concentrations of <1 ng/mL, whereas people heavily exposed to SHS frequently have PC concentrations between 1 and 10 ng/mL (26, 27). Active, customary smokers almost always have concentrations >10 ng/mL and sometimes >500 ng/mL (28).

Although studies showed that active smoking is closely related to concentrations of hemoglobin adducts of acrylamide and glycidamide, and other studies found that PC concentrations are closely related to tobacco smoke exposure, no information is available on the relationship between both biomarkers. In this study, acrylamide biomarkers and cotinine as an index of smoking exposure are assessed to describe the relationship between these biomarkers and to obtain further information about the influence of tobacco smoke exposure on acrylamide biomarker concentrations.

Materials and Methods

Blood samples from 161 individuals—94 men (median age, 36 years; range, 19-64 years) and 67 women (median age, 30; range, 18-66) were obtained from a commercial supplier of human specimens in agreement with the Centers for Disease Control's human subject protection regulations. On the basis of the classification described by Benowitz (28), individuals were categorized into groups according to their PC concentrations: The first group consisted of individuals with PC concentrations of ≥10 ng/mL (abbreviated as HC), who were commonly presumed to be active smokers. The second group (abbreviated as LC) consisted of individuals with PC concentrations of <10 ng/mL, who were presumed to be nonsmokers. The LC group was further divided into individuals with PC concentrations of <1 ng/mL (abbreviated as VLC), who were considered to have low exposure to SHS and individuals with PC concentrations between 1 and 10 ng/mL (MLC), who were considered to have high exposure to SHS.

Hemoglobin adducts of acrylamide and glycidamide were measured in hemolyzed erythrocytes obtained from EDTA-whole blood, whereas cotinine was measured in the plasma obtained from the same sample. For measurement of acrylamide and glycidamide adducts, 300 μL of hemolyzed erythrocytes were diluted with 100 μL of water. The total hemoglobin content of this solution was determined using Drabkins reagent (Stanbio Laboratory). A 350 μL aliquot of the diluted hemolyzate was added to 1,500 μL of formamide. The sample solution was further processed using the modified Edman reaction as described previously (29). In brief, to this solution, we added 100 μL of internal standard solution (20 nmol/L), 20 μL of Edman reagent (pentafluorophenylisothioisocyanate), and 55 μL of acetic acid solution (0.22 mol/L) to adjust the pH to 7.0. After mixing, the sample solution was heated for 2 h at 55°C to carry out the Edman reaction. The sample was then applied to a 48-well filter plate containing ~1 g of Isolute material (Biotage). The Edman products [AA-Val-PFPTH, GA-Val-PFPTH, AA-Val($^{13}C_5^{15}N$)-PFPTH, and GA-Val($^{13}C_5^{15}N$)-PFPTH] were extracted from the sample solution into a 48-well deep well plate using 8 mL of a solvent mixture containing isopropylether, ethylacetate, and toluene (50:40:10 v/v/v). After removing the solvents under vacuum using a GeneVac concentrator, the samples were dissolved in 200 μL of a methanol-water mixture (40:60 v/v) and transferred to a 96-well deep well plate for analysis by high-performance liquid chromatography tandem mass spectrometry. All pipetting steps were done using a Tecan Genesis Freedom liquid handling system with disposable pipette tips. The extraction was done with a Gilson 215 SPE system. Calibrators, reagent blanks, and quality control materials were processed in the same way as the samples.

Acrylamide biomarkers were measured by high-performance liquid chromatography tandem mass spectrometry. Chromatographic separation was achieved with a Surveyor HPLC system (ThermoFinnigan) using a Luna C18(2) column (10 cm × 2 mm, 3 μm; Phenomenex) at a temperature of 50°C and an isocratic eluent of methanol and water (63:37 v/v) at a flow rate of 300 μL/min. The injection volume was 50 μL. The tandem mass spectrometry analysis was carried out using a Quantum MS system (ThermoFinnigan) equipped with an atmospheric pressure chemical ionization source. Ionization was done using atmospheric pressure chemical ionization in positive ion mode at 4.5 μA, and at 375°C vaporizer temperature. The mass spectrometry system was operated using single reaction monitoring at 10 eV collision energy of transitions *m/z* 396 → *m/z* 379 for AA-Val-PFPTH, *m/z* 402 → *m/z* 385 for AA-Val($^{13}C_5^{15}N$)-PFPTH, *m/z* 412 → *m/z* 395 for GA-Val-PFPTH, and *m/z* 418 → *m/z* 401 for GA-Val($^{13}C_5^{15}N$)-PFPTH. Hemoglobin adduct concentrations are reported relative to the amount of hemoglobin used.

Octapeptides with the same amino acid sequence as the NH_2-terminal of the beta-chain of hemoglobin and with acrylamide and glycidamide attached at the valine (AA-VHLTPEEK, GA-VHLTPEEK) were synthesized by Bachem and used as calibrators. The corresponding stable isotope–labeled compounds [AA-Val($^{13}C_5^{15}N$)-HLTPEEK and GA-Val($^{13}C_5^{15}N$)-HLTPEEK] were also synthesized by Bachem and used as an internal standard.

Table 1. Acrylamide and glycidamide adduct values in a convenience sample

	Age (y)	Acrylamide adducts (pmol/g Hb)	Glycidamide adducts (pmol/g Hb)	Glycidamide to acrylamide adduct ratio	Cotinine (ng/mL)
Total (N = 161)					
Median	34	109	55	0.61	29
Range	18-66	7-610	4-364	0.03-1.14	N.D.-546
(5th-95th Percentile)	(21-50)	(32-342)	(20-188)	(0.33-0.97)	(0.02-372)
Men (N = 94)					
Median	36	154*	81*	0.55 †	119 ‡
Range	19-64	20-610	4-364	0.03-1.14	N.D.-546
(5th-95th Percentile)	(21-51)	(35-435)	(18-212)	(0.28-0.86)	(0.03-403)
Women (N = 67)					
Median	30	58*	38*	0.69 †	0.11 ‡
Range	18-66	7-399	7-260	0.38-1.13	N.D.-372
(5th-95th Percentile)	(20-46)	(33-247)	(22-160)	(0.46-1.03)	(0.02-304)

*Significant differences between men and women ($P < 0.004$).
†Significant differences between men and women ($P < 0.0001$).
‡Significant differences between men and women ($P < 0.001$).

Methanol, acetic acid, ethylacetate, toluene, and isopropylether were purchased from Sigma. Pentafluorophenylisothioisocyanate was obtained from Fluka, and formamide was obtained from U.S. Biochemical.

PC was measured by high performance liquid chromatography tandem mass spectrometry using a method previously described in detail (30, 31). Aliquots of serum pools with known cotinine contents were included with each group of samples for quality assurance purposes.

An unbalanced ANOVA with type III sums of squares was done using SAS 9.0. Because of the skewed frequency distribution of data, log-converted acrylamide and glycidamide adduct concentrations, as well as PC concentrations, were used in the statistical analysis. To assess the relationship between acrylamide biomarker concentrations and PC concentrations, multivariate models were developed for the HC, LC, MLC, and VLC groups using age, gender, and PC concentrations as main effect variables. Log(cotinine)^2 values were used in the models to be able to detect possible quadratic relationships between both biomarkers. Combinations of these variables were used to assess lower order interactions. Significant interactions were identified by stepwise exclusion of nonsignificant interactions ($P > 0.05$). Receiver operating characteristic (ROC) analyses were done using Analyse-iT with Clinical Laboratory Statistics 1.7.

Results

Median concentrations for biomarkers of acrylamide exposure in samples from 161 men and women were 109 pmol/g Hb [95% confidence interval (95% CI), 84-140 pmol/g Hb] and 55 pmol/g Hb (95% CI, 46-76 pmol/g Hb) for acrylamide and glycidamide adducts, respectively (Table 1). The median cotinine concentration was 29 ng/mL (95% CI, 3-80 ng/mL). Acrylamide and glycidamide adduct values were significantly higher in men than in women ($P < 0.004$), whereas the glycidamide-to-acrylamide adduct ratio was significantly higher in women than in men ($P < 0.0001$). PC values were significantly higher in men than in women ($P < 0.001$). The frequency distribution of PC concentrations after logarithmic conversion showed a bimodal pattern with a less pronounced maximum at 0.03 ng/mL and a more pronounced maximum at 245 ng/mL (data not shown). The logarithmic acrylamide and glycidamide adduct values showed a less pronounced bimodal pattern with acrylamide adduct values having maxima at 1.8 and 2.4 (63 and 251 pmol/g Hb) and glycidamide adduct values having maxima at 1.6 and 2.2 (40 and 159 pmol/g Hb; Fig. 1). When we assessed the LC and HC groups separately, we observed no bimodal pattern in the frequency distribution, with the lower maximum of logarithmic acrylamide biomarker concentrations found

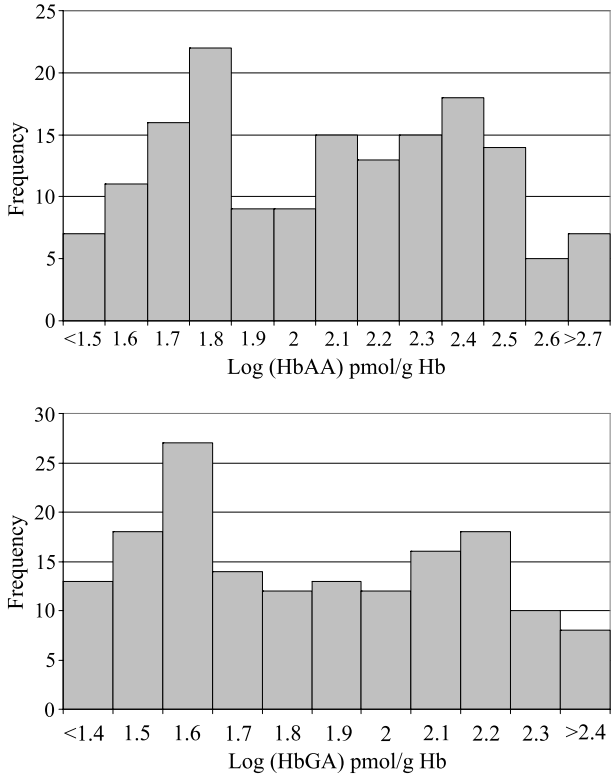

Figure 1. Frequency distribution of acrylamide adduct (*HbAA*) and glycidamide adduct (*HbGA*) concentrations after logarithmic conversion.

in the LC group and the higher maximum found in the HC group.

In the HC group, acrylamide and glycidamide adduct values were three to four times higher than in the LC group (Table 2), and the glycidamide-to-acrylamide adduct ratios were significantly lower in the HC groups than in the LC group ($P < 0.0001$). We observed a slight but nonsignificant increase in the glycidamide-to-acrylamide adduct ratio with decreasing acrylamide adduct concentrations in the LC group; in the HC group, the glycidamide-to-acrylamide adduct ratio increased significantly ($P < 0.04$) with decreasing acrylamide adduct concentration. In both, the HC and LC groups, acrylamide and glycidamide adduct values were nonsignificantly different between men and women. However, the glycidamide-to-acrylamide adduct ratio was significantly higher in women than in men (LC group, $P < 0.005$; HC group, $P < 0.05$).

The intersubject variability, expressed as the difference between the 95th and 5th percentile, was smaller in the LC group (126 pmol/g Hb for acrylamide adducts, 101 pmol/g Hb for glycidamide adducts) than in the HC group (316 pmol/g Hb for acrylamide adducts, 174 pmol/g Hb for glycidamide adducts). In the LC group, the acrylamide adduct values were mostly <63 pmol/g Hb, whereas acrylamide adduct values in the HC group were typically >128 ng/mL. Glycidamide adduct values showed a similar but less pronounced pattern (Fig. 2; Table 2).

In the MLC group, acrylamide biomarker values were mostly at the low levels observed in individuals with PC values <1 ng/mL, but started to increase at PC values of ~7 ng/mL. Also, at PC values of 7 ng/mL, the dispersion of acrylamide biomarker data started to increase to the range observed in the HC group (Fig. 2).

In the VLC group, the median values for acrylamide adducts, glycidamide adducts, and the glycidamide-to-acrylamide adduct ratio were 51 pmol/g Hb (95% CI, 46-57 pmol/g Hb), 34 pmol/g Hb (95% CI, 32-37 pmol/g Hb), and 0.69 (95% CI, 0.63-0.75), respectively (Table 3). No significant differences in biomarkers of acrylamide exposure were found compared with the MLC group. However, the sample size of the MLC group ($n = 12$) was small compared with the sample sizes of the VLC group ($n = 61$).

Because acrylamide biomarker values seemed clearly different in the LC group (PC values <10 ng/mL) as compared with the HC group (PC values ≥10 ng/mL), ROC curves were created to assess whether acrylamide biomarker concentrations could be used to identify individuals with PC values ≥10 ng/mL. The ROC curves produced area under the curve of 0.95 (95% CI, 0.90-0.99), 0.89 (95% CI, 0.84-0.95), and 0.70 (95% CI, 0.62-0.78) for acrylamide adducts, glycidamide adducts, and the glycidamide-to-acrylamide adduct ratio, respectively (Fig. 3). At acrylamide adduct values of 98 pmol/g Hb both the sensitivity and specificity were 89%, at glycidamide adduct values of 51 pmol/g Hb they were

Table 2. Acrylamide and glycidamide adduct values in individuals with PC concentrations <10 and ≥10 ng/mL

		Acrylamide adducts (pmol/g Hb)				Glycidamide adducts (pmol/g Hb)				Glycidamide to acrylamide adduct ratio			
		PC < 10 ng/mL*		PC ≥ 10 ng/mL*		PC < 10 ng/mL		PC ≥ 10 ng/mL		PC < 10 ng/mL		PC ≥ 10 ng/mL	
N		73		88		73		88		73		88	
Median (95% CI)		51 (46-56)		194 (164-215)		34 (31-36)		107 (86-123)		0.67† (0.63-0.74)		0.55† (0.51-0.59)	
Percentiles	Min	7		60		4		17		0.11		0.03	
	5	29		87		16		41		0.42		0.32	
	25	40		128		28		71		0.56		0.46	
	50	51		194		34		107		0.67		0.55	
	75	63		255		41		143		0.81		0.65	
	95	155		403		117		215		1.03		0.92	
	Max	610		584		319		364		1.13		1.14	
		Men	Women	Men	Women	Men	Women	Men	Women	Men	Women	Men	Women
N		25	48	69	19	25	48	69	19	25	48	69	19
Median (95% CI)		49 (40-66)	52 (46-57)	194 (155-215)	182 (129-235)	33 (28-40)	34 (31-37)	100 (82-121)	138 (70-150)	0.56‡ (0.53-0.79)	0.72‡ (0.62-0.79)	0.53§ (0.49 -0.59)	0.61§ (55-74)
Percentiles	Min	20	7	60	72	4	7	17	44	0.11	0.38	0.03	0.46
	5	23	32	88	91	11	21	36	51	0.29	0.46	0.29	0.47
	25	39	41	125	139	27	29	71	74	0.52	0.62	0.42	0.55
	50	49	52	194	182	33	34	100	138	0.56	0.72	0.53	0.61
	75	70	61	263	231	42	40	134	149	0.76	0.86	0.64	0.73
	95	299	97	456	325	162	107	214	195	0.82	1.06	0.92	0.89
	Max	610	178	548	399	319	158	364	260	0.89	1.13	1.14	0.94

*Smoking status was assigned based on categories described by Benowitz (28) with presumed smokers having plasma cotinine values of ≥10 ng/mL and presumed nonsmokers <10 ng/mL.
†Significant differences ($P < 0.0001$).
‡Significant differences ($P < 0.005$).
§Significant differences ($P < 0.05$).

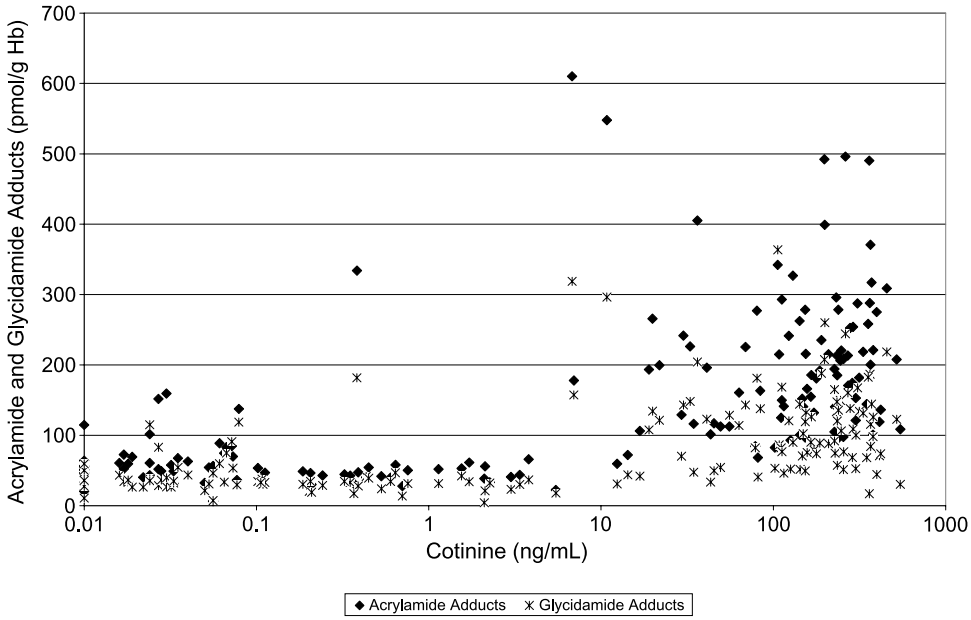

Figure 2. Acrylamide adduct and glycidamide adduct values plotted over PC values in the same subject.

84%, and at a glycidamide-to-acrylamide adduct ratio of 0.61 they were 67%.

In multivariate models, we found that cotinine affected acrylamide adduct values [parameter estimate of the log(cotinine)^2 variable, 0.012; $P < 0.03$] and the glycidamide-to-acrylamide adduct ratios [parameter estimate of the nonquadratic log(cotinine) variable, 0.159; $P < 0.004$] in the LC group only. Gender significantly affected the glycidamide-to-acrylamide adducts ratio in all cotinine groups with men having lower values than women (parameter estimates for men, -0.136; $P < 0.02$ in the VLS group, -0.126; $P < 0.02$ in the LC group, and -0.097; $P = 0.05$ in the HC group). Age affected the glycidamide-to-acrylamide adduct ratios in the VLC group (parameter estimates, -0.008; $P < 0.03$) and HC group (parameter estimates, -0.025; $P < 0.02$) at slightly decreasing values with increasing age.

Discussion

We analyzed 161 blood samples from a U.S. blood bank to investigate the relationship between PC as a biomarker of tobacco smoke exposure and hemoglobin adducts of acrylamide and glycidamide as biomarkers of acrylamide exposure. Biomarker concentrations of acrylamide exposure and PC concentrations spanned the range commonly reported for smokers and nonsmokers. The frequency distribution of cotinine concentrations after logarithmic conversion showed a bimodal pattern similar to that reported in the general U.S. population (32). A similar pattern was observed for hemoglobin adducts of acrylamide and glycidamide with the first maximum at 63 and 40 pmol/g Hb and the second maximum at 251 and 158 pmol/g Hb for acrylamide and glycidamide adducts, respectively. The lower maxima were in the range commonly measured in nonsmokers, whereas the higher maxima were commonly measured in smokers. When separating the study samples into individuals with PC <10 ng/mL (LC) and individuals with PC ≥10 ng/mL (HC), the frequency distributions of the acrylamide and glycidamide adducts showed only one maximum with the lower maxima of acrylamide and glycidamide adducts being in the LC group and the higher maxima being in the HC group. The magnitude of differences in hemoglobin adducts of acrylamide and glycidamide between the LC and HC group were similar to those reported in nonsmokers and smokers (21-23). The acrylamide adduct concentration range (5th-95th percentile) determined in the LC and HC groups were also similar to the concentration range reported for smokers and nonsmokers in another population (21).

The findings of this study show that the values of biomarkers of acrylamide exposure can be categorized into two separate groups using PC concentration ranges commonly used to distinguish presumed smokers from presumed nonsmokers. As indicated by the ROC analysis, this categorization cannot only be applied to groups in this study but also to individuals. The limitation of this assessment, however, is in the small number of individuals in the cotinine concentration range of 1 to 10 ng/mL. Therefore, only a distinction between population subgroups or individuals with PC values of <1 ng/mL and those with PC values of ≥10 ng/mL can be done with confidence at this point. Furthermore, because the investigated samples are not representative for the general population, additional studies are needed to confirm these findings.

Although we were able to categorize the values of biomarkers of acrylamide exposure into two distinguishable groups, we observed only a weak relationship between values of biomarkers of acrylamide exposure and PC values within each of these two categories. One reason for this observation might be the different exposure periods these biomarkers represent and the susceptibility to intrasubject and intersubject variability that is associated with these different exposure periods. PC reflects exposure to tobacco smoke over the previous 16 to 18 h (33). These immediate and short-term exposures could result in high intraindividual and

Table 3. Acrylamide and glycidamide adduct values in individuals PC values <1 ng/mL

		Acrylamide adducts (pmol/g Hb)		Glycidamide adducts (pmol/g Hb)		Glycidamide to acrylamide adduct ratio	
N		61		61		61	
Median (95% CI)		51 (46-57)		34 (32-37)		0.69 (0.63-0.75)	
Percentiles	Min	7		7		0.25	
	5	30		17		0.46	
	25	40		29		0.58	
	50	51		34		0.69	
	75	63		41		0.81	
	95	138		91		1.03	
	Max	334		182		1.13	
		Men	Women	Men	Women	Men	Women
N		18	43	18	43	18	43
Median (95% CI)		46 (37-75)	51 (46-57)	33 (27-47)	37 (32-38)	59 (0.52-0.76)	0.72 (0.66-0.79)
Percentiles	Min	20	7	11	7	0.25	0.38
	5	27	32	13	22	0.42	0.48
	25	38	41	27	30	0.52	0.63
	50	46	51	33	35	0.59	0.72
	75	74	61	45	40	0.74	0.84
	95	186	88	98	89	0.83	1.07
	Max	334	138	182	119	0.89	1.13

interindividual variabilities depending on the smoking habits of an individual, the exposure patterns, and the time period between the last exposure and the specimen collection. Hemoglobin adducts of acrylamide and glycidamide reflect exposure to acrylamide over the previous 120 days (34). Therefore, these biomarkers provide more long-term exposure information compared with cotinine, making it less susceptible to short-term variabilities such as smoking habits and exposure patterns.

Specimens in this study were collected without restrictions on smoking habits and tobacco exposure patterns, and without selecting specific time points after the last tobacco smoke exposure. We can therefore assume specimen collection in this study to be random with regards to the time interval of the previous tobacco smoke exposure. Because of the differences in the exposure periods discussed above and the random specimen collection, a high variability in the relationship between PC values and values of biomarkers of acrylamide exposure can be expected and is apparent in the group of individuals with PC values ≥10 ng/mL (Fig. 2). Similar observations made in the area of diabetes between the relation of hemoglobin adducts of glucose (HbA1c) as biomarkers for long-term blood glucose values and randomly collected blood glucose values seem to support this hypothesis (35). Significant relations between HbA1c and blood glucose values were observed when blood glucose was collected under controlled conditions such as fasting morning blood glucose collections, which indicates that more significant correlations between acrylamide biomarkers and PC could be expected if details on previous and current smoking habits are available and taken into consideration.

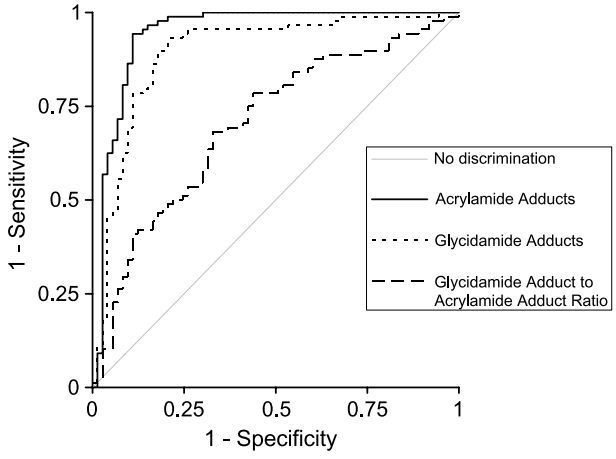

Figure 3. ROC curve for acrylamide adducts, glycidamide adducts, and the ratio of glycidamide-to-acrylamide adducts describing the sensitivity and specificity of these markers for identifying individuals with cotinine values ≥10 ng/mL having higher acrylamide adduct values than individuals with PC values of <10 ng/mL.

In the PC concentration range of 1 to 10 ng/mL, the variability in the relationship between PC values and acrylamide biomarker values decreases drastically with decreasing PC values and remains small in individuals with PC values of <1 ng/mL. This change in acrylamide biomarker concentrations in this PC concentration range is the reason for the significant exponential relationship observed in the LC group between PC values and biomarkers of acrylamide exposure. Individuals with low PC values (1-10 ng/mL) and high acrylamide values commonly observed in individuals with high PC values (≥10 ng/mL) might be smokers who have not smoked for more than a day or two prior to specimen collection and therefore have low cotinine values but still have elevated acrylamide biomarker values. These findings

indicate that biomarkers of acrylamide exposure might have the ability to detect individuals with infrequent smoking habits that cannot be detected with cotinine. A combination of acrylamide biomarkers and PC could provide further insight in individual smoking habits and/or smoking cessations. The consistently low interindividual variability of biomarkers of acrylamide exposure in people with PC concentrations of <1 ng/mL indicates that tobacco smoke exposure in these individuals does not have a profound effect on the values of biomarkers of acrylamide exposure.

We observed gender differences in the glycidamide-to-acrylamide adduct ratio, with women having higher ratios than men, which is consistent with previous findings from animal studies (36). Acrylamide is metabolized to glycidamide mainly through the action of CYP2E1 (37-39), which is affected by genetic factors (40-42) and other exposures such as ethanol and smoking (43, 44). Because the magnitude of these factors could be different in each individual, a high intersubject variability in the glycidamide-to-acrylamide adduct ratio is expected and was observed in our study.

In conclusion, in this first investigation on the relationship between biomarkers of acrylamide exposure and of tobacco smoke exposure, we found that acrylamide and glycidamide adducts could be categorized into distinguishable groups using PC-based categories commonly used to distinguish presumed active smokers and nonsmokers. Biomarkers of acrylamide exposure seem to detect smoking exposures in individuals with infrequent smoking habits or recent smoking cessation that cannot be detected by using PC. The results indicate that tobacco smoke exposure in individuals with PC values of <1 ng/mL, commonly observed in individuals with low SHS exposure, does not have a profound effect on biomarkers of acrylamide exposure.

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