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Acrylamide exposure and incidence of breast cancer among postmenopausal women in the Danish Diet, Cancer and Health study

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Acrylamide, a probable human carcinogen, is formed in several foods during high-temperature processing. So far, epidemiological studies have not shown any association between human cancer risk and dietary exposure to acrylamide. The purpose of this study was to conduct a nested case control study within a prospective cohort study on the association between breast cancer and exposure to acrylamide using biomarkers. N-terminal hemoglobin adduct levels of acrylamide and its genotoxic metabolite, glycidamide in red blood cells were analyzed (by LC/MS/MS) as biomarkers of exposure on 374 breast cancer cases and 374 controls from a cohort of postmenopausal women. The adduct levels of acrylamide and glycidamide were similar in cases and controls, with smokers having much higher levels (~3 times) than nonsmokers. No association was seen between acrylamide-hemoglobin levels and breast cancer risk neither unadjusted nor adjusted for the potential confounders HRT duration, parity, BMI, alcohol intake and education. After adjustment for smoking behavior, however, a positive association was seen between acrylamide-hemoglobin levels and estrogen receptor positive breast cancer with an estimated incidence rate ratio (95% CI) of 2.7 (1.1–6.6) per 10-fold increase in acrylamide-hemoglobin level. A weak association between glycidamide hemoglobin levels and incidence of estrogen receptor positive breast cancer was also found, this association, however, entirely disappeared when acrylamide and glycidamide hemoglobin levels were mutually adjusted.

Key words: breast cancer; acrylamide; glycidamide; biomarkers; epidemiology

Acrylamide is classified as a probable human carcinogen[1] and is also a human neurotoxin, most recently demonstrated in the Hallandsås accident where humans as well as animals were intoxicated by the compound.[2] Three major sources of human acrylamide exposure has been identified, industrial, smoking and thermally processed food. Acrylamide is a common industrial chemical and is primarily used in the production of polyacrylamide and in grouting agents. Human exposure from industrial sources has mainly occurred as a result of workplace exposure. Combustion of tobacco releases acrylamide and tobacco smoke is the major source of acrylamide exposure among smokers.[3] More recently, it was discovered that acrylamide is formed at μg/kg levels during high-temperature processing, such as cooking, frying, roasting and baking of carbohydrate-rich foods.[4,5] Acrylamide is formed mainly from the Maillard reaction between the amino acid asparagine and saccharides such as glucose.[6–8] The average acrylamide exposure from food has been estimated to around 0.5 μg/kg bodyweight/day in Sweden, Norway and the Netherlands.[9]

The majority of ingested acrylamide is absorbed in the body, while absorption is less efficient following inhalation or dermal exposure.[10–12] Acrylamide is a small water-soluble molecule, which is rapidly distributed into all body tissues, including mammary tissue.[12–16]

The metabolism of acrylamide follows 2 main pathways, epoxidation and conjugation with glutathione. Acrylamide is oxidized *in vivo* to the epoxide glycidamide, and in mice it has been demonstrated that P450 2E1 is the primary enzyme responsible for the epoxidation.[17–19] Both acrylamide and glycidamide are conjugated with glutathione and eliminated as mercapturic acid derivatives in the urine, the major excretion route of acrylamide metabolites in humans.[10,20,21]

Both acrylamide and glycidamide are reactive compounds that form adducts with proteins, including hemoglobin. Glycidamide, in the contrary to acrylamide is mutagenic and also forms adducts with the DNA bases in appreciable amounts.[14,15,22–25] Glycidamide is generally considered the causative genotoxic metabolite of acrylamide.[26]

Acrylamide has been reported carcinogenic in animal studies following oral dosing. In 2 long-term studies in rats, significant increases of tumors occurred in the mammary glands of female rats, in testes of male rats and thyroid gland in both male and female rats.[27,28] Additionally, in the study of Johnson *et al*.,[27] significant increases of tumors were reported in the central nervous system, uterus, clitoral gland and oral tissue. The predominance of endocrine-related cancers has led to the hypothesis that the carcinogenic effect of acrylamide is caused by a nongenotoxic mode of action.[29] A number of population-based epidemiological studies have examined the carcinogenic effect of dietary acrylamide intake in relation to bowel, kidney, bladder, renal cell, colorectal, oral, esophageal, laryngeal, ovarian and prostate cancer. None of these studies has shown any significant association between acrylamide exposure and cancer.[30–33] Two studies have examined the relationship between acrylamide intake and breast cancer. One cohort study consisting of 43,404 Swedish women, showed in the highest quintile a small increase in the relative risk of breast cancer compared to the lowest quintile. But the effect was not significant and there was no evidence of a linear dose response.[34] The second study, an Italian/Swiss case-control study consisting of 2,900 cases and 3,122 controls showed no carcinogenic effect of increased acrylamide intake.[33] All the epidemiological studies so far have estimated the acrylamide intake from food frequency questionnaires.

Acrylamide and glycidamide are reactive molecules that form adducts with the N-terminal amino acid in haemoglobin, valine (AA-Hb and GA-Hb). The concentration of acrylamide bound to the N-terminal amino acid in hemoglobin is strongly correlated to the exposure of acrylamide,[3,35–37] while its glycidamide analog correlates to glycidamide DNA adducts,[37] and is considered a biomarker for the genotoxic dose reflecting the individual ability to activate acrylamide to glycidamide. Measurement of AA-Hb and

Abbreviations: AA-Hb: acrylamide bound to the N-terminal amino acid in hemoglobin, valine; BMI: body mass index; CI: confidence intervals; ER+/−: estrogen receptor positive/negative; GA-Hb: glycidamide bound to the N-terminal amino acid in hemoglobin, valine; HRT: hormone replacement therapy; IRR: incidence rate ratio; LC/MS: liquid chromatography mass spectrometry; LOQ: limit of quantification.

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GA-Hb, therefore, is a good measure of an individuals' average exposure to acrylamide and glycidamide within the 4 months lifetime of an erythrocyte.[35,38]

The present study is, to the authors' knowledge, the first to associate cancer incidence with exposure to acrylamide and glycidamide using AA-Hb and GA-Hb as biomarkers.

We have measured the concentration of AA-Hb and GA-Hb using LC/MS/MS analysis as biomarkers for exposure of acrylamide and glycidamide, respectively, in stored blood samples from 374 breast cancer cases and an equivalent number of matched controls originating from the Danish prospective cohort study, Diet, Cancer and Health. In the subsequent data analyses, potential confounding factors as well as smoking behavior were taken into consideration. Additionally, information concerning estrogen receptor status of the breast tumors was included in the analysis as acrylamide has been shown in animal studies to give mainly endocrine-related cancers.

Material and methods

Cohort

The Danish Diet, Cancer and Health study is a prospective cohort study, established with the primary aim of studying the etiologic role of diet on cancer risk. Between December 1993 and May 1997, 79,729 women were invited to participate in the study. We invited all women who lived in greater Copenhagen and Aarhus and who fulfilled the following criteria: ages between 50 and 64 years, born in Denmark, and not registered with a previous diagnosis of cancer in the Danish Cancer Registry. A total of 29,875 women, corresponding about 37% of those invited, were enrolled into the cohort.[39]

All participants signed an informed consent at study baseline. The Diet, Cancer and Health study and the present substudy was approved by the regional ethical committees on human studies in Copenhagen and Aarhus and by the Danish Data Protection Agency.

All cohort members attended 1 of 2 established study centers, and each participant filled in a food frequency questionnaire and a lifestyle questionnaire. The lifestyle questionnaire included questions about reproductive factors, health status, social factors and lifestyle habits. From this questionnaire, we obtained information about smoking status (present/former/never), duration of smoking (years), tobacco use at baseline (gram/day), years of school education (short: $\leq$7 years, medium: 8–10 years, or long: $>$10 years), parity (parous/nulliparous, number of births and age at birth of first child), use of hormone replacement therapy (HRT) (never, past, current) and duration of HRT. Anthropometric data were obtained by professional staff members. Body mass index (BMI) was calculated as weight (kg) per height (m) squared. Information on alcohol intake was obtained from the food frequency questionnaire.

In the study centers, 30 ml of blood (nonfasting, collected in citrated and plain Venojects) was drawn from each participant. The samples were spun and divided into 1-ml tubes of plasma, serum, erythrocytes and buffy coat. All samples were processed and frozen within 2 hr at -20°C. At the end of the day of collection, all samples were stored in liquid nitrogen vapor (max. -150°C).

Of the initial 29,875 women, we excluded 326 who later were reported to the Danish Cancer Registry with a cancer diagnosed before the visit to the study clinic. In addition, 8 women were excluded from the study because they did not fill in the lifestyle questionnaire. Because the present analysis aimed at women who were postmenopausal at study entry, we further excluded 4,844 women, including 4,798 who were considered premenopausal because they had reported at least 1 menstruation no more than 12 months before entry and no use of HRT, 9 women who gave a lifetime history of no menstruation and 37 women who did not answer the questions about current or previous use of HRT, leaving 24,697 postmenopausal women for study.

Cohort members were identified from their unique personal identification number, which is allocated to every Danish citizen by the Central Population Registry. All the postmenopausal cohort members were linked to the Central Population Registry to obtain information on vital status and immigration. Information on cancer occurrence among cohort members was obtained through record linkage to the Danish Cancer Registry, which collects information on all cases of cancer in Denmark.[40] The completeness of the Danish Cancer Registry to identify all women with breast cancer is described in Jensen *et al*.[41]

Linkage was performed by use of the personal identification number. Each cohort member was followed-up for breast cancer occurrence from date of entry, *i.e*., date of visit to the study center until the date of diagnosis of any cancer (except for nonmelanoma skin cancer), date of death, date of emigration, or 31 December, 2000, whichever came first. Incident breast cancer was diagnosed in 434 women during the follow-up period.

A registry exclusively concerning breast cancer also exists in Denmark and information on estrogen receptor (ER), α subtype, status was obtained by linkage with the Danish Breast Cancer Cooperative Group, which holds records on a range of details for $\sim$90% of breast cancers diagnosed in Denmark.[42] A standardized immunohistochemical method was used in all medical centers. The cut-off level used to define positive ER status was 10% or more positive cells. Information on progesterone receptor status is not registered consistently in the register and it was thus not possible to consider this receptor.

Matching of cases and controls

In view of the size of the cohort, concentrations of AA-Hb and GA-Hb could not be determined for all of the women. We, therefore, used a nested case-control design, with 1 control selected for each of the 434 cases. The control was cancer-free at the exact age at diagnosis of the case and was further matched on age at inclusion into the cohort (half-year intervals), certainty of postmenopausal status (known/probably postmenopausal) and use of HRT at inclusion into the cohort (current/former/newer). The probably postmenopausal group includes women that were hysterectomised or used HRT at baseline such that postmenopausal status could not be established based on information on menostasis. These women were assumed to be peri- or postmenopausal, as HRT is rarely administered to women without menopausal symptoms. Furthermore, the median (5–95%) age at diagnosis (or at censoring for the controls) in the probably postmenopausal group was 60 (53–68) years, making it very likely that the women had gone into menopause. Of the 434 pairs (868 women; 434 cases and 434 controls), 18 pairs were excluded owing to lack of blood sample for case or control or sample loss during the adduct analyses, and 42 pairs were excluded because information was missing for either the case or the control about one or more of the potentially confounding variables (smoking (status, duration and tobacco use), reproductive events (number and ages at births), duration of HRT use, length of school education, alcohol intake or BMI), leaving 374 case-control pairs for the study.

Analysis of AA- and GA-hemoglobin adducts in blood

The analysis of the blood samples was conducted according to the method described in Bjellaas *et al*.[43] In short, the globin was purified from the blood samples and stored at -20°C until analysis. Globin (20 mg) was subjected to a modified Edman reaction where phenyl isothiocyanate reacts with the N-terminal amino acid (valine) in hemoglobin, undergoes cyclization and decouples from the hemoglobin molecule releasing a phenylthiohydantoin derivative of N-alkylated valine adducts. The released phenylthiohydantoins were purified by solid phase extraction and analyzed on a LC ion-trap MS using multiple reaction monitoring.

The limit of quantification (LOQ) of the analysis was determined from the standard deviation of blanks (LOQ = 10 $\times$ SD/slope of calibration curve) to 2.4 pmol/g globin and 6.8 pmol/g for

TABLE I – BASELINE CHARACTERISTICS OF 374 BREAST CANCER CASES AND THEIR 374 MATCHED CONTROL SUBJECTS AT BASELINE, IN THE DANISH DIET, CANCER AND HEALTH STUDY

	Cases ($N = 374$)						Controls ($N = 374$)	
	All Median (5–95%)		ER+ Median (5–95%)		ER− Median (5–95%)		All Median (5–95%)	
AA-Hb (pmol/g globin)	47	(20–209)	48	(20–213)	40	(21–197)	47	(18–205)
GA-Hb (pmol/g globin)	26	(9–99)	27	(9–96)	23	(10–102)	28	(9–99)
GA-Hb samples below LOQ	2%		3%		0%		3%	
Smoking								
Present	33%		34%		32%		37%	
Former	25%		24%		26%		25%	
Never	42%		42%		42%		38%	
Smoking duration (years)[1]	31	(4–45)	31	(3–45)	32	(5–44)	32	(3–46)
Tobacco use (g/day)[2]	15	(3–25)	15	(3–25)	15	(2–20)	15	(4–30)
Alcohol intake (g/day)	11	(0–44)	11	(1–45)	13	(1–45)	10	(1–42)
BMI	25	(20–34)	25	(20–34)	25	(19–35)	25	(20–33)
Age at first birth	23	(18–32)	23	(18–32)	23	(18–32)	23	(18–31)
Number of births	2	(0–3)	2	(1–4)	2	(1–3)	2	(0–4)
Nulliparous	14%		15%		13%		12%	
Duration of HRT use in years[3]	6	(0.5–20)	5	(0.5–20)	6	(0–5–20)	5	(0.5–21)
School education								
$\leq$ 7 years	29%		28%		28%		34%	
8–10 years	48%		50%		47%		49%	
$\geq$ 11 years	24%		22%		25%		18%	

[1]Among ever smokers.–[2]Among current smokers.–[3]Among ever users of HRT.

the AA-Hb and GA-Hb, respectively. In total, 42 batches were run. Case controls were always analyzed in the same batch. All samples were injected onto the LC/MS in triplicate.

Statistical methods

Because of the study design using incidence density sampling of controls with match on age at diagnosis, conditional logistic regression analyses lead to estimation of breast cancer incidence rate ratios (IRR).[44]

The associations between AA-Hb and GA-Hb concentrations and breast cancer were evaluated as crude IRR as well as adjusted for the potential confounders: parity (entered as 2 variables; the categorical variable parous/nulliparous and the quantitative variable number of births), age at birth of first child, length of school education (low, medium, high), duration of HRT use, BMI and alcohol intake. Furthermore estimation was done taking smoking behavior into account to investigate the dose-response relationship at different levels and from different sources. Smoking is an important source of acrylamide exposure causing smokers and nonsmokers to have very different levels of exposure. Separate estimates among smokers and nonsmokers were calculated with and without further adjustment for amount of tobacco currently smoked, former smoking and duration of smoking. It was tested whether the associations between adduct levels and breast cancer risk were similar among smokers and nonsmokers and if so a common estimate adjusted for smoking status was calculated. Besides the associations with total breast cancer, associations with ER positive (ER+) breast cancer and ER negative (ER−) breast cancer were considered in separate analyses. Two-sided 95% confidence intervals (95% CI) for the IRR were calculated based on Wald's test of the Cox regression parameter, that is, on the log IRR scale. Tests of interaction were performed using the likelihood ratio test statistics.

All quantitative variables were entered linearly or log-linearly into the logistic model, because this is biologically more reasonable than the step functions corresponding to categorization and, furthermore, increases the power of the analyses.[45] The linearity of the associations was evaluated by linear spline models with three boundaries placed at the quartile cut-off points according to the exposure distribution among cases.[46] None of the associations showed departures from linearity. The AA-Hb and GA-Hb concentrations were entered log10 transformed in the models, as the transformed concentration described a more linear association with breast cancer than the untransformed concentrations. This means that the IRR estimates correspond to a 10-fold increase in adduct concentration, which corresponds with the full variation (difference between 5% and 95% percentiles) in the material. All measurements of AA-Hb concentration were above the quantification limit, but 20 (2.7%) of the GA-Hb measurements were below. This group of women was included in the analyses by an indicator variable allowing them to contribute to the estimation of associations with other variables included in the model.

The procedures PHREG in SAS (release 9.1; SAS Institute, Cary, NC) was used for the conditional logistic regression analyses.

Results

The median age at entry into the cohort for the 374 pairs was 57 years (range, 50–65 years). The median (1st–99th percentiles) length of follow-up for the 748 postmenopausal women was 4.2 years, (0.1–6.8 years). Information about the ER status of tumors was obtained for 348 (93%) cases of breast cancer, with 269 of the observed tumors reported to be ER+ and 79 tumors ER−. Information about ER status was not obtained for the remaining 26 cases.

The baseline characteristics of cases and controls are presented in Table I. The concentrations of AA-Hb and GA-Hb were found to be similar among cases and controls, both varying with a factor 11 from the 5% to the 95% percentile. The number of GA-Hb samples below the limit of quantification was low. Among cases a lower proportion were smoking at baseline and a higher proportion had never smoked than among controls, additionally a lower proportion of cases had short education.

Tobacco smoke was the major source of acrylamide exposure (Table II), which is in accordance with previously published studies.[3,35,47] The effect of smoking on adduct levels were evident with smoker levels being 3.5 (AA-Hb) to 2.8 (GA-Hb) times higher than among nonsmokers (Table II). Among nonsmokers, AA-Hb and GA-Hb levels varied with a factor 5 and 6, respectively, from the 5% to the 95% percentile.

Because of the log-transformation, the IRR's correspond to a 10-times increment in concentration and resemble comparisons of the women with the highest and the women with the lowest adduct concentrations. Neither AA-Hb (IRR (95% CI) 0.99 (0.63–1.54)) nor GA-Hb (IRR (95% CI) 0.76 (0.46–1.27)) levels were found to be significantly associated with breast cancer incidence when evaluated in a model without adjustment (Table III). Inclusion of

TABLE II – COHORT ADDUCT LEVELS IN SMOKERS AND NONSMOKERS AMONG THE 374 BREAST CANCER CASES AND 374 CONTROLS IN THE DANISH, DIET, CANCER AND HEALTH STUDY

	AA-Hb (pmol/g globin)		GA-Hb (pmol/g globin)	
	Median (5–95%)		Median (5–95%)	
Controls				
Nonsmoker ($N = 235$)	35 (17–88)		21 (7–53)	
Smoker ($N = 139$)	122 (28–277)		60 (20–126)	
Cases				
Nonsmoker ($N = 249$)	35 (20–96)		21 (9–47)	
ER^+ ($N = 179$)		35 (18–114)		22 (7–50)
ER^- ($N = 54$)		34 (20–76)		19 (10–37)
Smoker ($N = 125$)	125 (36–254)		58 (17–130)	
ER^+ ($N = 90$)		127 (46–251)		58 (20–134)
ER^- ($N = 25$)		116 (36–254)		53 (16–117)
All				
Nonsmoker ($N = 484$)	35 (18–90)		21 (8–49)	
Smoker ($N = 264$)	123 (35–273)		59 (19–128)	

TABLE III – IRRs AND 95% CIs PER 10-FOLD INCREASE IN CONCENTRATIONS OF AA-Hb AND GA-Hb FOR TOTAL BREAST CANCER (374 PAIRS), ER+ BREAST CANCER (269 PAIRS) AND ER− BREAST CANCER (79 PAIRS)

Breast cancer	IRR (95% CI)[1]	p^1	IRR (95% CI)[2]	p^2
All				
Log AA-Hb	0.99 (0.63–1.54)	0.95	1.05 (0.66–1.69)	0.83
Log GA-Hb	0.76 (0.46–1.27)	0.30	0.88 (0.51–1.52)	0.65
Estrogen receptor positive				
Log AA-Hb	1.05 (0.61–1.81)	0.85	1.10 (0.63–1.93)	0.74
Log GA-Hb	0.79 (0.42–1.48)	0.46	0.88 (0.45–1.71)	0.70
Estrogen receptor negative				
Log AA-Hb	0.81 (0.30–2.16)	0.67	0.83 (0.28–2.48)	0.74
Log GA-Hb	0.60 (0.21–1.75)	0.35	0.71 (0.21–2.35)	0.57

[1]Univariate estimates (only adjusted for age and use of HRT due to the matching procedure).–[2]As A, but further adjusted for duration of HRT use (years), age at first birth (years), number of births, BMI (kg/m2), alcohol intake (g/day) and school education (low/medium/high).

potential confounding factors except smoking behavior in the model did not change this result and neither did evaluation with ER specific breast cancer as the outcome.

No significant differences were seen between smokers and nonsmokers regarding the estimated associations between adduct levels and breast cancer risk ($p > =0.2$, Table IV). The common estimate, (IRR (95% CI) 1.5 (0.8–3.0)) indicating a positive association between AA-Hb and breast cancer was further strengthened by inclusion of former smoking, duration of smoking and amount of tobacco currently smoked (IRR (95% CI) 1.9 (0.9–4.0)). Restricting the analyses to the 210 pairs with identical smoking status at baseline corresponding to matching on smoking status did not alter the results (IRR (95%CI) 1.9(0.8–4.5); data not shown.). Among the 64 pairs where both case and control were never smokers at baseline the estimated association between AA-Hb levels and breast cancer when adjusted for potential confounders was IRR (95%) 2.7 (0.3–24) (data not shown).

When the concentration of AA-Hb and GA-Hb were evaluated with ER specific breast cancer as the outcome, all associations were found to be strongest with regard to ER+ breast cancer (Table IV). In the fully adjusted model, women with the highest concentrations of AA-Hb were found to be at 2.7 times increased risk of ER+ breast cancer when compared to women with the lowest concentrations ($p = 0.03$). Neither of the adduct concentrations were found to be associated with ER− breast cancer according to smoking status but all estimates had very broad confidence limits (data not shown). This uncertainty of the estimates is due to the very low statistical power (only 79 ER− cases) and these results must therefore be evaluated with caution.

As glycidamide is an activated form of acrylamide, the adduct levels are known to be highly correlated. The lower part ([5]) of Table IV shows the association with breast cancer when AA-Hb and GA-Hb were included in the same model. Whereas the association between AA-Hb and breast cancer remained constant, and even tended to be strengthened, after adjustment for GA-Hb (except for widened confidence limits), the tendency towards higher breast cancer risk among women with high GA-Hb concentrations disappeared.

Discussion

The data from this prospective cohort study shows a positive association between AA-Hb level in red blood cells and the risk of breast cancer when comparing women with similar levels of exposure from smoking. In a model adjusted for confounding factors as well as smoking behavior, a 10-fold increase in AA-Hb levels were associated with an 1.9 (0.9–4.0) times higher risk of breast cancer and a 5-fold increase (which corresponds to the range in AA-Hb levels among nonsmokers) were associated with an 1.6 (0.9–2.6) times elevated risk. The estimated associations were stronger when looking at ER+ breast cancer only. Several studies are indicating that the etiology of ER+ and ER− breast cancer are distinct, thereby offering an explanation for this outcome.[48]

The median adduct levels measured in this study is in the range of previously published levels among smokers and nonsmokers.[3,43,47,49,50] Based on data of adduct levels measured in humans and dose of ^{13}C acrylamide published by Fennell *et al.*[36] we calculated the average daily intake of acrylamide in the nonsmokers to 0.57 μg acrylamide/kg bodyweight. This value is in accordance with the average acrylamide intake from food (0.5 μg/kg bodyweight/day), reported from the North European countries; Sweden, Norway and the Netherlands.[9]

Several population based epidemiological studies have examined the association between estimated acrylamide intake determined from reported dietary intake and cancer in various organs, including the breast. None of these studies has shown any significant associations between acrylamide intake and cancer risk.[30,31–34] However, both the power of some of these epidemiological studies as well as their methods of exposure assessments has been

TABLE IV – ASSOCIATIONS BETWEEN LOG-TRANSFORMED AA-Hb AND GA-Hb ADDUCT CONCENTRATIONS FOR TOTAL BREAST CANCER (374 PAIRS) AND ER+ BREAST CANCER (269 PAIRS) ACCORDING TO SMOKING STATUS

Breast cancer	All				Estrogen receptor positive			
	IRR (95% CI) by smoking status at baseline	p^1 equal	IRR (95% CI)	p^2	IRR (95% CI) by smoking status at baseline	p^1 equal	IRR (95% CI)	p^2
Log AA-Hb[3]								
Nonsmokers	1.4 (0.6–3.3)	0.7	1.5 (0.8–3.0)	0.2	1.6 (0.5–4.4)	0.5	1.9 (0.9–4.4)	0.1
Smokers	1.8 (0.7–4.8)				2.6 (0.8–8.8)			
Log GA-Hb[3]								
Nonsmokers	1.0 (0.4–2.7)	0.7	1.2 (0.6–2.4)	0.7	1.1 (0.4–3.5)	0.7	1.3 (0.5–3.1)	0.6
Smokers	1.3 (0.5–3.5)				1.5 (0.5–4.9)			
Log AA-Hb[4]								
Nonsmokers	1.5 (0.6–3.6)	0.3	1.9 (0.9–4.0)	0.08	1.9 (0.7–5.6)	0.3	2.7 (1.1–6.6)	0.03
Smokers	3.1(1.0–9.7)				4.9 (1.2–20)			
Log GA-Hb[4]								
Nonsmokers	1.0 (0.4–2.8)	0.5	1.3 (0.6–2.8)	0.5	1.1 (0.4–3.7)	0.5	1.5 (0.6–3.8)	0.4
Smokers	1.8 (0.6–5.5)				2.2 (0.6–7.9)			
Log AA-Hb[5]								
Nonsmokers	1.5 (0.5–4.5)	0.2	2.0 (0.7–5.5)	0.2	2.2 (0.6–8.3)	0.2	3.2 (0.9–10.8)	0.07
Smokers	3.8 (0.9–15.2)				7.0 (1.2–40)			
Log GA-Hb[5]								
Nonsmokers	0.7 (0.2–2.2)	0.5	0.8 (0.3–2.3)	0.7	0.5 (0.1–2.2)	0.5	0.7 (0.2–2.4)	0.6
Smokers	1.1 (0.3–4.1)				1.0 (0.2–4.6)			

The IRRs correspond to a 10-fold increase in adduct concentration.

[1]*p*-value for testing similar associations among smokers and nonsmokers.–[2]*p*-value for the common estimate of association.–[3]Adjusted for duration of HRT use (years), age at first birth (years), number of births, BMI (kg/m2), alcohol intake (g/day) and school education (low/medium/high); age and use of HRT via matching; smoking at baseline (yes/no) via stratification (columns 1 and 5) or adjustment (columns 3 and 7).–[4]As A but further adjusted for amount of tobacco smoked at baseline (g/day), past smoking (yes/no) and duration of smoking (years).–[5]As B, but AA-Hb and GA-Hb mutually adjusted.

criticized.[51,52] In all these epidemiological studies the acrylamide intake has been estimated from food frequency questionnaires. Exposure assessment of dietary acrylamide using food frequency questionnaires has been shown to be inaccurate as a measure of the exposure, probably due to a large variation in the acrylamide content within and between foods. This inaccuracy is corroborated by the lack of correlation between AA-Hb levels and estimated dietary intake.[43,50,53,54] AA-Hb levels have however shown to be strongly correlated to the total exposure of acrylamide.[3,35–37] In this study, hemoglobin adducts of acrylamide has been measured as biomarkers of exposure. The finding of a positive association between acrylamide exposure and breast cancer risk after adjustment for smoking behavior may be a result of this more accurate exposure assessment. Though exposure assessment is improved compared to using food frequency questionnaires a major limitation on this type of study is the general uncertainty regarding extrapolating acrylamide exposure from a few month into a lifetime exposure. Improved exposure estimates in future studies could be achieved by taking blood samples from cohort members 2, 3 or 4 times in a year to adjust for seasonal variations. Further the power of the study is limited due to its size, a general problem when conducting laborious biomarker analysis.

Interestingly, the increased breast cancer incidence observed in this study emerges, only after adjustment for smoking suggesting that only acrylamide exposure from other sources is associated with breast cancer. However the association between smoking and breast cancer risk is not completely solved and may depend of the time windows of a women's life, when exposed to tobacco smoking. This may reflect a competition between the carcinogenic and the antiestrogenic effects of smoking.[55] It should be cautioned that heat treatment of food results in the formation of a large number of substances through Maillard reactions and it cannot be excluded that AA-Hb represents a biomarker for additional toxic substances co-occuring with acrylamide in food.

Although not significantly different, there seemed to be a tendency of a stronger association between acrylamide level and breast cancer among smokers than among nonsmokers where the association did not reach statistical significance. This possible difference between smokers and nonsmokers remained or even strengthened after further adjustment for smoking behavior within strata indicating that the part of exposure from smoking is not confounded by unmeasured confounders and making it questionable that the strong effect of smoking adjustment is just a matter of taking into account differences in dose response relationships at different exposure levels. Due to the broad confidence intervals it was not possible to judge whether the observed positive association was the same among smokers and nonsmokers or only existed among smokers. Further and larger studies are needed to confirm and interpret these findings.

Acrylamide is metabolically activated to glycidamide, a genotoxic metabolite. The weaker association between breast cancer risk and concentrations of GA-Hb found in this study, compared to AA-Hb, may suggest that acrylamide can induce cancer by a nongenotoxic mechanism. This is corroborated by the observation that acrylamide induced DNA-synthesis in target organs for tumor development in F344 and Sprague-Dawley rats, but not in nontarget tissues. In addition, acrylamide induced morphological transformations in Syrian hamster embryo cells. In both these studies, inhibition of the P450 enzyme responsible for activation of acrylamide to glycidamide did not affect the outcome of the studies.[56,57] Acrylamide it self is a reactive compound that can alkylate both amino and sulfhydryl groups in proteins.[29] This may result in altered protein functionality (*e.g.*, estrogens receptors) possibly inducing cancer.

In conclusion, in this prospective cohort study we have found a significant positive association between the risk for ER+ breast cancer and AA-Hb adducts in reed blood cells, a biomarker for acrylamide exposure. We encourage further studies to confirm or reject this possible association between acrylamide exposure and breast cancer. Also further research into acrylamides potential to induce cancer by a nongenotoxic mechanism is encouraged.

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