

Research Article

Acrylamide Hemoglobin Adduct Levels and Ovarian Cancer Risk: A Nested Case–Control StudyJing Xie^{1,3}, Kathryn L. Terry^{2,3}, Elizabeth M. Poole¹, Kathryn M. Wilson^{1,3}, Bernard A. Rosner^{1,4}, Walter C. Willett^{1,3,5}, Hubert W. Vesper⁶, and Shelley S. Tworoger^{1,3}**Abstract**

Background: Acrylamide is a probable human carcinogen formed during cooking of starchy foods. Two large prospective cohort studies of dietary acrylamide intake and ovarian cancer risk observed a positive association, although two other studies reported no association.

Methods: We measured acrylamide exposure using red blood cell acrylamide and glycidamide hemoglobin adducts among women in two large prospective cohorts: the Nurses' Health Study and Nurses' Health Study II. Between blood collection and 2010, we identified 263 incident cases of epithelial ovarian cancer, matching two controls per case. We used logistic regression models to examine the association between acrylamide exposure and ovarian cancer risk, adjusting for matching factors, family history of ovarian cancer, tubal ligation, oral contraceptive use, body mass index, parity, alcohol intake, smoking, physical activity, and caffeine intake.

Results: The multivariate-adjusted relative risk (RR) of ovarian cancer comparing the highest versus lowest tertile of total acrylamide adducts was 0.79 (95% CI, 0.50–1.24, *P* trend = 0.08). The comparable RR of ovarian cancer among non-smokers at blood draw was 0.85 (95% CI, 0.57–1.27, *P* trend = 0.14). The association did not differ by tumor histology (serous invasive versus not), *P* for heterogeneity = 0.86. Individual adduct types (acrylamide or glycidamide) were not associated with risk.

Conclusions: We observed no evidence that acrylamide exposure as measured by adducts to hemoglobin is associated with an increased risk of ovarian cancer.

Impact: Our finding indicates that acrylamide intake may not increase risk of ovarian cancer. *Cancer Epidemiol Biomarkers Prev*; 22(4); 653–60. ©2013 AACR.

Introduction

Acrylamide is a chemical compound that forms naturally during the cooking of starchy foods (1). Moderate levels of acrylamide (5–50 mg/kg) have been found in heated protein-rich foods and higher levels (150–4,000 mg/kg) in carbohydrate-rich foods, such as French fries, potato chips, baked goods, and cold breakfast cereal (2). Coffee also is an important source of acrylamide, which is

formed during roasting of the beans. High levels also are found in cigarette smoke (3–5). In addition, small exposures to acrylamide could occur through drinking water, cosmetics, and passive smoking (6, 7).

Acrylamide was classified as "probably carcinogenic to humans" on the basis of positive cancer bioassay results by International Agency for Research on Cancer in 1994 (8). Evidence included that acrylamide was efficiently biotransformed to a chemically reactive genotoxic metabolite (glycidamide) in both rodents and humans (9), and in rodents this compound led to the development of certain cancers, particularly hormone-sensitive cancers (10, 11). In addition to genotoxic effects, acrylamide may bind to proteins related to maintenance of hormonal balance including human estrogen receptor and sex hormone-binding globulin (9).

Before 2002, acrylamide was thought to be mainly an industrial exposure; upon discovery of its formation in foods during cooking, many efforts have been made to estimate dietary exposures and to study whether this level of exposure is associated with cancer risk in human populations. Daily intake of acrylamide may be as high as a few tens of micrograms in western populations (12–14). Studies of acrylamide intake, assessed by food frequency questionnaire (FFQ) and risk of cancer at a variety

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of sites have been carried out since 2002, with generally null results (9, 15–27).

However, 2 large prospective studies observed positive associations between dietary acrylamide intake and risk of ovarian cancer (12, 28), although one other prospective study and a hospital-based case–control study observed no association (13, 14). Given the hypothesis that acrylamide may affect sex hormones, the positive findings for ovarian cancer are of particular interest. To further examine this association, we used a biomarker of exposure, acrylamide and glycidamide adducts to hemoglobin, to conduct a nested case–control study assessing the relationship between acrylamide exposure and ovarian cancer risk within the prospective Nurses' Health Study (NHS) and NHSII cohorts.

Materials and Methods

Study population

The NHS is a prospective cohort study established in 1976, when 121,700 U.S. female registered nurses, 30 to 55 years of age, completed an initial mailed questionnaire about their lifestyle factors, behaviors, and medical history. The NHSII is a prospective cohort established in 1989, when 116,430 U.S. female nurses, 25 to 42 years of age completed an initial questionnaire. Both cohorts have been followed by biennially mailed questionnaires to update exposure information, lifestyle factors, and ascertain nonfatal incident diseases since establishment. Women completed a semiquantitative FFQ every 2 to 4 years since 1980 and 1991 in the NHS and NHSII, respectively. In this study, we used dietary exposures from the 1990 FFQ for NHS and 1999 FFQ for NHS II, because they were the closest to the time of blood collection for each cohort. Deaths in both cohorts were reported by family or postal authorities. We also searched for names of nonresponders in the National Death Index (29, 30).

Between 1989 and 1990, 32,826 NHS participants (aged 43–70 years) provided blood samples with a short questionnaire (31). Similarly, from 1996 to 1999, 29,611 NHS II participants (aged 32–54 years) provided blood samples and a short questionnaire (32). In brief, women in both subcohorts had their blood drawn and shipped overnight on ice to our laboratory, where the blood was processed 24 to 36 hours after collection, and separated into plasma, red blood cells, and white blood cells. Samples were stored in liquid nitrogen freezers after collection. To assess the impact of the delay in processing on acrylamide adducts, we compared acrylamide adduct levels in blood processed immediately versus 24 or 48 hours after collection ($N = 12$). The correlations for delayed versus immediate processing were >0.98 for acrylamide and glycidamide, suggesting that these adducts are stable with delayed processing.

Measurement of exposure and laboratory assays

To measure hemoglobin adducts of acrylamide and glycidamide, erythrocytes were sent to the Protein Biomarker Laboratory (Clinical Chemistry Branch, Centers

for Disease Control and Prevention Atlanta, GA). Measurements were automated using the optimized Edman reaction described by Vesper and colleagues using HPLC-MS/MS (33). The reaction products of acrylamide and glycidamide with the N-terminal valine of the hemoglobin protein chains were measured as *N*-[2-carbamoylethyl]valine-pentafluorophenylhydantion (PFPTH) derivative and *N*-[2-hydroxycarbonyl-ethyl]valine-pentafluorophenylhydantion (PFPTH) derivative for acrylamide and glycidamide adducts, respectively, and results were reported as pmol adduct per gram of total hemoglobin. Total hemoglobin concentration was measured from cyanmethemoglobin, which is formed from methemoglobin by reaction with cyanide (CMH solution) with the manufacturer's assay kit. The resulting red-colored complex has peak absorption at 540 nm. Details of the assay have been published previously (34, 35).

Laboratory personnel were blinded to case status and matched cases and controls were assayed in the same batch. The interbatch coefficient of variation from masked replicate samples in each batch were 8.7% for acrylamide adducts and 11.9% for glycidamide adducts. Samples were processed in 2 batches.

Assessment of covariates

Information on covariates was collected from the biennial questionnaires and the questionnaire completed at blood collection. Participants provided information on height at the cohort baseline. Family history of ovarian cancer was ascertained in 1992 (NHS) and 2001 (NHSII). Tubal ligation was assessed in 1994 and 1997, respectively. Alcohol and caffeine intake were measured in 1990 (NHS) and 1999 (NHSII) on the FFQ. Smoking and weight was ascertained at the time of blood collection in both cohorts. Physical activity was measured in 1992 (NHS) and 1997 (NHSII), respectively. Parity, age at first birth, menopausal status, postmenopausal hormone use, and oral contraceptive use were assessed biennially. For covariates with multiple measurements, we used information from the questionnaire completed closest to the date of blood collection.

Identification of ovarian cancer cases

We identified incident ovarian cancer cases by self-report on biennial questionnaires, reports from family members, the National Death Index, and the US Postal Service. For reported ovarian cancer cases, we obtained medical records or records from cancer registries for confirmation. We confirmed 263 ovarian cancer cases diagnosed after blood collection but before 1 June 2010 for NHS and 1 June 2009 for NHSII. Cases were matched to 2 controls on age at blood draw, time of day of blood draw, month of blood draw, fasting status, menopausal status at blood draw and diagnosis, and postmenopausal hormone use at blood draw. Although smoking is an important source of acrylamide exposure, we did not match for this factor in the nested case–control study as it is not a strong risk factor

for ovarian cancer; however, we carefully considered smoking in the statistical analysis. The Institutional Review Board of the Brigham and Women's Hospital approved this analysis, and all participants provided informed consent.

Statistical analyses

We categorized 3 continuous exposure variables, including total acrylamide (sum of acrylamide and glycidamide adducts) as well as the individual acrylamide and glycidamide adducts, into tertiles based on the exposure distributions in controls. We used conditional logistic regression models to estimate relative risks (RRs) and 95% confidence intervals (CI) of ovarian cancer for each exposure tertile, conditioning on matching factors (and assay batch, since a case and its matched controls were assayed in the same batch) and adjusting for covariates including height (continuous), family history of ovarian cancer (yes, no), tubal ligation (yes, no), oral contraceptive use (never, <1 year, 1–5 years, 5+ years), BMI (continuous), parity (yes, no), number of children (continuous), average number of alcoholic drinks per week (continuous), smoking (never, past and <15 years since quitting, past and ≥ 15 years since quitting, current), physical activity (<18 MET/week, ≥ 18 MET/week), and caffeine intake (continuous). We also additionally considered adjustment for smoking intensity (pack-years of smoking) among current and ever smokers, but this was not included in the final model as it did not change the results.

To additionally control for smoking, we conducted analyses restricted to nonsmokers (and secondarily in never smokers). To do this, we categorized exposures based on the distributions in controls who were nonsmokers (or never smokers) at blood collection. We used unconditional logistic regression models adjusting for all the matching factors and the covariates mentioned above plus assay batch. To test potential effect modification by menopausal status (pre- vs. postmenopausal), postmenopausal hormone use at blood draw (yes vs. no) among postmenopausal women, age (<55 vs. ≥ 55 years), and BMI (<25 vs. ≥ 25) we included multiplicative interaction terms in multivariate models and assessed statistical significance using the likelihood ratio tests.

We used linear trend tests to examine possible trends across natural log-transformed continuous adduct levels and obtained trend *P*-values using the Wald statistic. We used polytomous logistic regression models to analyze whether the associations between exposures and ovarian cancer were different by histologic subtype (serous invasive versus other). *P*-values for interaction were obtained using likelihood ratio tests, comparing models allowing the associations with the exposure variable of interest to vary versus models not allowing the association to vary (36); we allowed age, parity, and smoking to vary in both models as these factors may be differently associated by histology (36).

We did not identify any statistical outliers using the generalized extreme studentized deviate many-outlier

detection approach (37). We also assigned half the value of the limit of detection to any samples with values less than limit of detection ($n = 12$ for glycidamide). We used SAS 9.3 software (SAS Institute, Cary, NC) or STATA 12.1 software (StataCorp.) for all analyses and used 2-sided *P*-values.

Results

We confirmed 263 incident cases of epithelial ovarian cancer during follow-up. The median interval between blood collection and diagnosis was 9.9 years. Cases were slightly more likely than controls to be current smokers (12.6% vs. 10.7%, respectively) or past smokers (39.9% vs. 38.1%), and had a higher prevalence of family history of ovarian cancer (6.8% vs. 2.1%; Table 1). Controls were more likely than cases to be parous (93.2% vs. 88.2%, respectively), have more pregnancies (3.3 vs. 3.0), and have had a tubal ligation (20.6% vs. 16.4%). Median acrylamide and glycidamide adduct levels were 112.6 pmol/g hemoglobin (Hb) among cases and 113.9 pmol/g Hb among controls. Median levels in current smokers at blood draw ($n = 88$) were 276.5 pmol/g Hb, compared to 108 pmol/g Hb among nonsmokers ($n = 690$). There were 79 serous cases versus 156 nonserous cases, 217 invasive cases versus 46 borderline cases, and 129 cases diagnosed within 10 years versus 134 cases diagnosed more than 10 years after blood draw.

Overall, we did not observe a statistically significant association between total acrylamide (i.e., the sum of acrylamide and glycidamide adducts) hemoglobin adducts and ovarian cancer risk (Table 2). Compared to women with total adduct concentrations of ≤ 99 pmol/g, the RRs were 0.83 (95% CI, 0.56–1.24) for women with >99–134.1 pmol/g and 0.79 (95% CI, 0.50–1.24) for women with >134.1 pmol/g (*P* trend = 0.08; Table 2). When adducts were analyzed separately, no positive association was observed for glycidamide adducts (*P* trend = 0.19), and a suggestive inverse association was noted for acrylamide adducts (*P* trend = 0.05). Results from age-adjusted models were similar to those from multivariate-adjusted models (data not shown).

Because tobacco smoking is an important source of acrylamide exposure, we conducted an analysis restricted to women who were not current smokers at blood draw. Again, no associations were observed for total acrylamide adducts (*P* trend = 0.13), acrylamide adducts (*P* trend = 0.06), or glycidamide adducts (*P* trend = 0.36). The comparable RRs were 0.84 (95% CI, 0.55–1.27), 0.85 (95% CI, 0.56–1.30), and 0.80 (95% CI, 0.52–1.23), respectively. Results restricted to never smokers were similar to those among nonsmokers (data not shown).

We assessed associations using quartiles of exposure, although the number of cases was small in each group. Most results were similar to main analyses, except the RR was 0.47 (95% CI, 0.26–0.83) for women with acrylamide concentration >82.9 pmol/g (4th quartile) compared to women with ≤ 52.2 pmol/g (1st quartile; Supplementary Table). We also assessed associations comparing the top 9

Table 1. Selected characteristics of study participants in a nested case-control study of ovarian cancer in the NHS and NHSII

	Cases (N = 263)	Controls (N = 515)
Median (10–90 percentile)		
Total adducts (sum of acrylamide and glycidamide; pmol/g Hb)	112.6 (72.0–209.7)	113.9 (74.1–226)
Acrylamide adducts (pmol/g Hb)	63.8 (42.1–119)	62.2 (43.5–130)
Glycidamide adducts (pmol/g Hb)	49.5 (29.2–88.5)	51.1 (29.4–92.6)
Mean (SD)		
Age ^a (years)	55.1 (7.5)	55.2 (7.5)
Height (m)	1.65 (0.06)	1.64 (0.07)
BMI (kg/m ²)	25.2 (5.3)	25.2 (4.5)
Duration of OC use among ever OC users (years)	2.0 (3.3)	2.4 (3.6)
Parity among parous women (number)	3.0 (1.4)	3.3 (1.5)
Alcohol intake (number of drinks/week)	3.5 (4.8)	3.2 (4.4)
Physical activity (MET-hr/week)	21.6 (22.0)	21.7 (22.8)
Caffeine intake (mg/day)	244.4 (209.2)	251.5 (216.5)
Percentage		
Current smoker	12.6	10.7
Past smoker	39.9	38.1
Parous	88.2	93.2
Family history of ovarian cancer	6.8	2.1
Tubal ligation	16.4	20.6
Postmenopausal ^a	57.0	56.9
Postmenopausal hormone use among postmenopausal or unknown menopausal status women ^a	48.0	45.6
White race	99.2	99.6
Study participants from NHS	84.0	84.3

^aMatching factor.

deciles of exposure to the bottom decile and no associations were observed (data not shown).

No differences in association were observed when restricting to invasive cases (data not shown). Similar results were observed for serous versus nonserous ovarian cancer cases, *P* for heterogeneity = 0.86, when considering cases diagnosed within 10 years from blood draw versus more than 10 years, *P* for interaction = 0.79, and when removing caffeine intake from multivariate models (results not shown). No effect modification was observed for age, menopausal status at blood draw, postmenopausal hormone use (PMH) at blood draw (*P*-values for interaction ≥ 0.30). For BMI at blood draw, there was a suggestion of an interaction with acrylamide (*P*-value for interaction = 0.03). However, the confidence intervals were wide as there were 164 cases in the BMI < 25 stratum and 99 cases in the BMI ≥ 25 stratum and there was no apparent trend of association in either strata (*P* trend = 0.23 for women with BMI < 25 and 0.58 for women with BMI ≥ 25).

Discussion

Ours is the first study to examine acrylamide hemoglobin adducts and risk of epithelial ovarian cancer. The median adducts levels measured in our study are in

accordance with the range of previously published levels among smokers and nonsmokers both in the United States and Europe (4, 38–40). We did not observe an association between a biomarker of acrylamide intake, hemoglobin adducts of acrylamide and glycidamide, and ovarian cancer risk in a large nested case-control study of U.S. women. Furthermore, no associations were noted in nonsmokers or never smokers or for serous invasive tumors.

Because tobacco use is a major source of acrylamide exposure, we carefully addressed possible confounding by smoking. Since the prevalence of smoking in this cohort is fairly low, we were able to restrict our analyses to nonsmokers and secondarily never smokers at the time of blood collection, and noted no important differences in the acrylamide-ovarian cancer association. In addition, adjustment for pack-years of smoking did not alter the risk estimates. Finally, it is worth noting that smoking is only weakly associated with overall ovarian cancer risk and seems to be positively associated with only the mucinous type (41, 42); thus, its role as a possible confounder is limited in spite of its very strong association with acrylamide adduct levels. However, passive smoking information, which could be a potential confounder, was not collected in our study.

Table 2. Multivariate-adjusted ovarian cancer RR (95% CI) by tertile of hemoglobin adducts of acrylamide and glycidamide in the NHS and NHSII

	Cases	T1	T2	T3	P trend ^a
Total adducts (acrylamide and glycidamide)					
All					
Cases/controls		94/170	84/176	85/169	
Cut-points ^b (pmol/g Hb)		0–99	>99–134.1	>134.1	
RR ^c	263	1.00	0.83 (0.56–1.24)	0.79 (0.50–1.24)	0.08
Serous ^d	156	1.00	0.93 (0.59–1.46)	0.82 (0.49–1.37)	<i>P</i> _{net} ^e
Nonserous ^d	79	1.00	0.96 (0.53–1.75)	0.69 (0.34–1.40)	0.86
Nonsmokers					
Cases/controls		82/152	75/157	73/151	
Cut-points ^f (pmol/g Hb)		0–95.7	>95.8–124.2	>124.2	
RR ^g	230	1.00	0.84 (0.56–1.26)	0.84 (0.55–1.27)	0.13
Acrylamide					
All					
Cases/controls		92/171	84/175	87/169	
Cut-points ^b (pmol/g Hb)		0–54.6	>54.6–73.2	>73.2	
RR ^c	263	1.00	0.86 (0.58–1.26)	0.89 (0.57–1.39)	0.05
Serous ^d	156	1.00	0.97 (0.62–1.54)	0.89 (0.54–1.49)	<i>P</i> _{net} ^e
Nonserous ^d	79	1.00	0.79 (0.42–1.46)	0.83 (0.42–1.63)	0.83
Nonsmokers					
Cases/controls		80/152	75/157	75/151	
Cut-points ^f (pmol/g Hb)		0–52.3	>53.4–68.5	>68.5	
RR ^g	230	1.00	0.87 (0.58–1.31)	0.85 (0.56–1.30)	0.06
Glycidamide					
All					
Cases/controls		82/171	105/174	76/170	
Cut-points ^b (pmol/g Hb)		0–41.9	>41.9–61.6	>61.6	
RR ^c	263	1.00	1.29 (0.88–1.90)	0.80 (0.49–1.29)	0.19
Serous ^d	156	1.00	1.08 (0.69–1.68)	0.71 (0.42–1.21)	<i>P</i> _{net} ^e
Nonserous ^d	79	1.00	1.72 (0.94–3.17)	0.87 (0.41–1.83)	0.37
Nonsmokers					
Cases/controls		75/153	93/156	62/151	
Cut-points ^f (pmol/g Hb)		0–40.2	>40.2–58	>58	
RR ^g	230	1.00	1.14 (0.77–1.71)	0.80 (0.52–1.23)	0.36

^aLinear trend test across log-transformed biomarker levels using the Wald test determined the *P*-trend.

^bCut-points obtained from controls.

^cConditioning on matching factors and adjusting for height, family history of ovarian cancer, tubal ligation, OC use, BMI, parous, number of additional children, average number of alcohol drinks per week, smoking, physical activity, and caffeine intake.

^dPolytomous logistic regression adjusting for matching factors and the same covariates as listed above plus assay batch, allowing age at blood draw and parity to vary between serous and nonserous cases.

^eLikelihood ratio test comparing full model (allowing the exposure association to vary by histology) to the reduced model that held exposure estimates constant across tumor subtype.

^fCut-points obtained from controls who were nonsmokers.

^gUnconditional logistic regression adjusting for matching factors and the same covariates listed above plus assay batch.

The lack of association between acrylamide hemoglobin adducts and ovarian cancer risk in our study is in line with a prospective study and a hospital-based case-control study of FFQ-assessed dietary acrylamide intake and ovarian cancer (13, 14). Notably the prospective study did not have baseline information about smoking status, limiting the interpretation of the results. Con-

versely, 2 large prospective studies, including the NHS, reported positive associations for FFQ-assessed acrylamide. The Netherlands Cohort Study observed an RR comparing the highest versus lowest quintile of intake of 1.78 in all women (95% CI, 1.10–2.88, *P* trend = 0.02) and 2.22 in never-smokers (95% CI, 1.20–4.08, *P* trend = 0.01). In the NHS, higher dietary acrylamide intake was

associated with an increased risk for serous ovarian cancer (comparable RR 1.58; 95% CI, 0.99–2.52, P trend = 0.04; refs. 12 and 28). We did not observe an association with serous tumors in this analysis of acrylamide adduct levels. It is not clear whether acrylamide exposure assessed by FFQ or by hemoglobin adducts is more biologically relevant for assessing ovarian cancer risk, thus it is important to consider the possible reasons for the differences in the observed associations.

These inconsistent findings between dietary studies may be because acrylamide comes from various sources of foods with variable concentrations that depend on cooking conditions as well as differences in study design. It is unclear why we previously observed an association for dietary acrylamide intake as measured through an FFQ in the NHS, but did not observe an association using a biomarker of acrylamide exposure. In our previous validation study, the correlation between dietary acrylamide intake and the sum of acrylamide hemoglobin adduct and glycidamide hemoglobin adduct was modest $r = 0.31$ (95% CI, 0.20–0.41; ref. 43). In our current nested case–control study, we observed a similar correlation among nonsmokers adjusting for batch, age, and energy intake of 0.25 (95% CI, 0.18–0.32). This modest correlation was confirmed in other studies as well (44–46). This suggests that while FFQs provide some information on acrylamide intake, there likely is notable measurement error associated with FFQ-assessed intake measures. This may be in part because acrylamide formation in food varies greatly depending on exact preparation and heating methods, or because FFQs do not capture all sources of exposure. Furthermore, the moderately low correlation of dietary acrylamide with adducts levels in hemoglobin may in part be because of differences in how individuals absorb and metabolize acrylamide from food, including cytochrome P450 activity, which is not reflected in the dietary assessment (47, 48); thus, the 2 methods of exposure assessment are not fully comparable. Conversely, another possibility is that circulating acrylamide adducts levels do not adequately reflect exposure at the ovarian tissue level, because the ovarian epithelium has less vasculature than other organs (49).

However, the most plausible reason for the difference in associations observed in the FFQ versus our current study is that the variation in dietary acrylamide intake may be correlated with other lifestyle factors and health behaviors that are associated with an increased risk of ovarian cancer; that is, residual confounding may exist in the dietary studies. Strengths of our study include a relatively large number of cases and objective measurement of acrylamide exposure measured before ovarian cancer diagnosis. Power calculations indicated that the minimal detectable RR for 80% power in our study was 1.78, which is comparable to the associations observed in the dietary studies. In addition, we have prospective data on most known risk factors of ovarian

cancer. We acknowledge that the exposure measurement relied on a single baseline measurement of acrylamide adducts in red blood cells and the follow-up was over a long time period, which may have induced nondifferential misclassification and could potentially bias effect estimates toward the null. However, our previous work reported an intraclass correlation coefficient of 0.77 over 3 years (43), suggesting that the between-person variation is much larger than the within-person variation and acrylamide measurement at one point in time appropriately ranks individuals with respect to their long-term exposure. In addition, no differences in associations were observed based on the timing of diagnosis in relation to blood draw. Because of very small number of cases within other histology subtypes, we had limited power to examine the association by tumor subtypes except for the serous versus nonserous tumors. Residual confounding may be of concern, although we comprehensively controlled or matched for major ovarian cancer risk factors and smoking.

Conclusions

Overall, this study does not support that acrylamide exposure, measured as hemoglobin adducts, is associated with an increased risk of ovarian cancer. Further studies with increased sample sizes and multiple blood measures are needed to confirm our findings.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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