Environmental Toxins and Breast Cancer on Long Island. I. Polycyclic Aromatic Hydrocarbon DNA Adducts[1]

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Abstract

Polycyclic aromatic hydrocarbons (PAH) are potent mammary carcinogens in rodents, but their effect on breast cancer development in women is not clear. To examine whether currently measurable PAH damage to DNA increases breast cancer risk, a population-based case-control study was undertaken on Long Island, NY. Cases were women newly diagnosed with *in situ* and invasive breast cancer; controls were randomly selected women frequency matched to the age distribution of cases. Blood samples were donated by 1102 (73.0%) and 1141 (73.3%) of case and control respondents, respectively. Samples from 576 cases and 427 controls were assayed for PAH-DNA adducts using an ELISA. The geometric mean (and geometric SD) of the log-transformed levels of PAH-DNA adducts on a natural scale was slightly, but nonsignificantly, higher among cases [7.36 (7.29)] than among controls [6.21 (4.17); $P = 0.51$]. The age-adjusted odds ratio (OR) for breast cancer in relation to the highest quintile of adduct levels compared with the lowest was 1.51 [95% confidence interval (CI), 1.04–2.20], with little or no evidence of substantial confounding (corresponding multivariate-adjusted OR, 1.49; 95% CI, 1.00–2.21). There was no consistent elevation in risk with increasing adduct levels, nor was there a consistent association between adduct levels and two of the main sources of PAH, active or passive cigarette smoking or consumption of grilled and smoked foods. These data indicate that PAH-DNA adduct formation may influence breast cancer development, although the association does not appear to be dose dependent and may have a threshold effect.

Introduction

As in other areas of the United States, breast cancer is the most commonly reported cancer among women who reside on Long Island, NY. The high incidence rates observed in this geographic region[3] (1) coupled with the local community's long-term concern about the health and ecological effects of environmental pollution (2), have culminated in grass roots support for the hypothesis that environmental pollutants are involved in the development of breast cancer. This hypothesis is supported by laboratory-based scientific evidence, as discussed in several recent reviews (3, 4), although environmental agents other than radiation (5) or use of alcohol (6, 7) have not been consistently linked to breast cancer development in humans.

Candidate chemicals to consider as possible breast carcinogens include PAH[4] (8, 9), a group of compounds with two or more conjoined aromatic rings, that are well-established mammary carcinogens in rodents (3). Although some PAH compounds have been categorized by the Environmental Protection Agency as probable or possible human carcinogens (10), carcinogenic effects on the breast in women have not been clearly demonstrated.

PAH compounds are ubiquitous in the environment and major sources of human exposure are from combustion products of fossil fuels and cigarette smoking, and in grilled and smoked foods (11). These compounds are lipophilic (12) and are, thus, stored in fat tissue in humans, including breast fat. PAH bind to DNA, and a number of methods have been developed to measure the presence of this DNA damage in

Received 6/29/01; revised 4/22/02; accepted 4/25/02.
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[1] Supported in part by Grant UO1NCI/NIEHS 66572 from the National Cancer Institute and the National Institute of Environmental Health, by the Babylon Breast Cancer Coalition, and by gift monies from private individuals.
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[3] Cancer incidence and mortality by county, 1992–1996, New York State (1999). Internet address: http://www.health.state.ny.us/nysdoh/cancer/volume1.htm.
[4] The abbreviations used are: PAH, polycyclic aromatic hydrocarbons; BPDE-I, r-7,t-8-dihyrody-t-9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene; BMI, body mass index; OR, odds ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor; DMBA, dimethylbenzanthracene.

target tissue as well as in mononuclear cells (13, 14). Measures of DNA adducts, therefore, reflect both exposure to PAH and the body's metabolic response to exposure (13).

An early ecological study reported an increase in breast cancer rates among communities exposed to creosote, which includes multiple compounds including PAH, through contamination of the water supply (15). Several small studies (16–19) that followed were successful in documenting a potential role for PAH in breast carcinogenesis in women, measuring DNA adducts using the ^{32}P postlabeling method (20). The first two studies(16, 17), which were based on samples of 10 and 24 subjects, respectively, demonstrated that DNA adducts are detectable in a proportion of normal human breast tissue. The third (18), based on a sample of 19 subjects, found that in about 30% of cancer cases the DNA-adduct pattern that was detected resembled patterns that have been associated with smoking exposure. The fourth study (19), which was based on normal breast tissue derived from a sample size of 87 breast cancer patients and 29 mammoplasty noncancer controls, noted a significantly higher level of aromatic DNA adducts in the cancer patients than in the controls. The most recent study results (21) are based on an immunhistochemical assay (22) that used archived tissue blocks from 100 breast cancer cases and 105 benign breast disease controls; the authors reported a 2-fold increase in risk associated with elevated PAH-DNA adduct levels in this New York City sample. Results of these investigations are suggestive, but do not clearly establish an association between PAH exposure and breast cancer risk, however, because of the small number of subjects studied (which yields unstable study results), the employment of a sensitive but nonspecific laboratory method in most of the studies, and the lack of a control group or controls that lacked internal consistency (*e.g.,* they did not represent the population from which the cases arose), which could yield biased study results (23).

The study reported here is based on data collected as part of the Long Island Breast Cancer Study Project, a large population-based case-control investigation that was motivated by community concerns for the effects of the environment on breast cancer risk (24). With blood samples obtained from newly diagnosed breast cancer cases and population-based controls, PAH-DNA adducts in mononuclear cells were measured using a competitive ELISA method (25), which is a more feasible laboratory approach in a large-scale epidemiological study than methods used in previous reports. An additional goal was to explore whether the association between adduct levels and breast cancer risk varied by cigarette smoking or grilled and smoked food consumption, which are among the primary sources of PAH exposure for U.S. residents.

Materials and Methods

The Long Island Breast Cancer Study Project was undertaken to determine whether environmental factors, specifically including exposure to PAH, are associated with breast cancer risk among women on Long Island. This population-based, case-control study was conducted in the counties of Nassau and Suffolk in New York State, as mandated by Congress in 1993 (Public Law 103-43). The investigation was undertaken after approval from participating institutional review boards and in accordance with an assurance filed with, and approved by, the United States Department of Health and Human Services. Details of the study methods have been described previously (24).

Study Subjects. Women who were residents of Nassau and Suffolk counties, spoke English, and were newly diagnosed with *in situ* or invasive breast cancer between August 1, 1996, and July 31, 1997, were eligible as cases. There were no age or race restrictions. Potentially eligible cases were identified through daily or weekly contact with the pathology departments of the 31 institutions in the Long Island-New York City area. Verification of the diagnosis and consent for recruitment was obtained from the diagnosing physician for 90.5% of potentially eligible case women. Potentially eligible control women, who were frequency matched to cases by 5-year age group, were identified using random digit dialing (RDD; Ref. 26) for those under age 65 years, and Health Care Financing Administration (HCFA) rosters for those age 65 years or greater. For the 59.2% of potentially eligible control women who were under age 65 years at identification, the screening response rate for the RDD component was 77.9%.

Interviews were completed for 1508 (82.1%) of eligible case women and 1556 (62.7%) of eligible control women. The primary reasons for nonresponse among cases and controls, respectively, included refusal or break off (cases and controls: 12.4% and 21.6%, respectively) and too ill, cognitively impaired, or deceased (4.1% and 4.7%, respectively). Interview response rates (see ref. 24) include, in the denominator, 25 potentially eligible case women and 193 potentially eligible control women who were never located or had moved out of the area, and, thus, final study eligibility was never determined. If such women are omitted from the denominator, then the overall interview response rates increase to 83.2% and 68.0%, respectively. The true response rates probably lie between the two sets of estimates. Study subjects ranged in age from 24 to 96 years, and response to the interview varied with the age of the subject, with 88.9% of cases and 76.1% controls under age 65 years participating *versus* 71.6% and 43.3%, respectively, of those 65 years of age and older. As reported previously (24), respondents ranged in age from 24 to 96 years; 93% were white, 5% were black, and 2% were other; and 4% identified themselves as Hispanic ethnicity, regardless of race.

Data Collection. Signed informed consent was obtained from respondents before data collection. The main interview consisted of a 2-h, interviewer-administered questionnaire. Respondents were asked about their pregnancy history; occupational history; residential history; their use of pesticides in their home or on a farm; electrical appliance use; lifetime history of consumption of smoked or grilled foods; medical history; family history of cancer; body size changes by decade; recreational physical activities; cigarette smoking; alcohol use; menstrual history; use of exogenous hormones; and demographic characteristics. As reported previously (24), established risk factors for breast cancer that were found to increase risk among Long Island women include lower parity, late age at first birth, little or no breast feeding, and family history of breast cancer.

Among case and control respondents who participated in the main interview, 73.0% and 73.3%, respectively, donated a nonfasting blood sample. Because of concern regarding analytic results based on blood samples collected after the commencement of chemotherapy among cases (27), a sample of case women with invasive breast cancer who provided a prechemotherapy blood sample donated a second, postchemotherapy blood sample (n = 155, or 98.1% of the women approached). At the time of the blood collection, respondents were asked to self-complete a specimen checklist that inquired about the date of a woman's last menstrual period (if she was still menstruating); selected foods consumed over the past several weeks; medications used over the past several days; cigarette smoking over the past several days; and for cases, breast cancer treatment undergone to date.

Table 1 Number of blood samples selected for the PAH-DNA adduct assays by case-control status and by reason for selection, Long Island Breast Cancer Study Project, 1996–1997

	Subject status	Cases (*n*)			Controls (*n*)	Total (*n*)
		Invasive	*In situ*	All		
(A)	Respondent to main questionnaire			1508	1556	3064
(B)	Blood donor			1102	1141	2243
(C)	Blood samples selected for PAH-DNA adduct assay (= E + F)	566	129	695	424	1119
(D)	Reason for selection:					
(E)	Random sample[a]	398	128	526	400	926
(F)	Specifically selected (= G + H + I + J + K)	168	1	169	24	193
(G)	African American not randomly selected	5			22	27
(H)	Selected as African American, but white	1			2	3
(I)	Selected as *in situ*, but invasive	42				42
(J)	Selected as invasive, but *in situ*		1			1
(K)	Donated second sample	120				120
(L)	Blood samples included in most statistical analyses (= C − K)[b]	446	129	575	424	999

[a] All of the samples donated by *in situ* cases with sufficient amounts of blood/DNA were selected for analyses (see "Materials and Methods").
[b] Excludes second samples (see "Materials and Methods").

In a previous analysis (24), established risk factors for breast cancer that were also found to increase breast cancer risk among Long Island residents included lower parity, late age at first birth, little or no breast feeding, and family history of breast cancer. Similar results were found for the analyses restricted to respondents who donated blood, and for those with DNA available for these analyses (data not shown). Factors that were found to be associated with a decreased likelihood that a respondent would donate blood include increasing age (1% decrease for each yearly increase in age) and past smoking (25% decrease); factors associated with an increased probability include white (65% increase) or other race (74% increase), alcohol use (28% increase), ever breastfed (47% increase), ever use of hormone replacement therapy (63% increase), and ever had a mammogram (51% increase). Case-control status was not a predictor of blood donation among respondents (24).

The donated biological specimens were shipped overnight to Columbia University in the City of New York, with the blood samples (which were collected in lavender-top tubes) at room temperature. Processing and aliquoting of the biological samples occurred for most subjects within 24 h of collection. Samples were stored at −80°C and bar-code labeled with the subjects' randomly assigned study identification numbers, with all laboratory personnel blinded to the case and control status of the specimens.

Subjects Selected for Laboratory Assays of PAH-DNA Adducts. As shown in Table 1, from among the study participants who donated a blood sample with a blood volume of greater than 25 ml (the volume judged to be sufficient for DNA isolation), 398 cases with invasive breast cancer and 400 controls were randomly selected for the PAH-DNA analyses. In addition, all of the samples with sufficient blood volume donated by African-American subjects who were not selected during the random selection process were assayed ($n = 5$ cases and 22 controls). Furthermore, all of the 129 cases diagnosed with *in situ* disease who had a blood sample with sufficient blood volume were assayed. For those randomly selected subjects for whom the 100 μg or more of DNA necessary to complete the PAH analyses could not be isolated, a replacement was randomly selected from the pool of eligible samples from which the original sample was chosen. Non-randomly chosen samples with sufficient blood volume (*e.g.*, additional African American and all *in situ* cases) could not be replaced. For study efficiency, the laboratory activities began prior to the completion of data collection, and some samples were specifically selected because they were believed to be donated by subjects who were African-American or cases who had been diagnosed with *in situ* disease. However, by the end of the field activities when data were more complete and accurate, the selected blood samples were determined to have been donated by whites (1 case and 2 controls) or to have invasive disease (42 cases). Blood samples from 120 case women with a second blood draw had sufficient volume for DNA isolation and the PAH analyses. Thus, satisfactory laboratory assays of PAH-DNA adducts were completed for 575 cases (446 diagnosed with invasive breast cancer and 129 cases with *in situ* breast cancer) and 424 controls. In addition, the second blood sample donated by 120 cases diagnosed with invasive breast cancer were also analyzed.

Laboratory Assays. Mononuclear cells were separated by Ficoll (Sigma Chemical Co., St. Louis, MO) and washed twice with PBS. Pelleted cells were frozen at −80°C until DNA isolation by standard phenol and chloroform isoamyl alcohol extraction and RNase treatment. PAH diol epoxide-DNA adducts were analyzed by competitive ELISA, using methods described previously (25, 27). Briefly, 96 microwell plates (Corning, Acton, MA) were coated with 2 ng of BPDE-I-DNA (5 adducts/10^3nucleotides). A previously characterized rabbit antiserum (28) was used at 1:500,000 dilution. A standard curve was constructed by mixing 50 μl diluted antiserum with BPDE-I-DNA (1.5 adduct/10^6nucleotides) in carrier nonmodified calf thymus DNA such that 50 μl contained 3–200 fmol BPDE-I-deoxyguanosine adduct in 50 μg of DNA. Test samples were assayed at 50 μg/well after sonication and denaturation by boiling for 3 min and cooling on ice. Goat antirabbit IgG-alkaline phosphatase (Boehringer Mannheim, Indianapolis, IN) was used at 1:750 dilution, and the substrate was *p*-nitrophenyl phosphate [100 μl of 50 μg/ml 0.1 M diethanolamine (pH 9.6). Absorbance at 405 nm was read on a MR 5000 reader (Dynatech Laboratories).

Samples were run with the laboratory blinded to case-control status in duplicate and mean values used for the determination of percentage inhibition. For analytical purposes, those samples with <15% inhibition were considered nondetectable and assigned a value of 10^8, an amount midway between the lowest positive value and zero. A positive control run with multiple batches of samples ($n = 11$) had a mean (±SD) of 20.3 (±5.0) with a CV of 25%. As an additional quality control, 10% of samples were reassayed after recoding. There

was no significant difference in mean adduct levels between the two analyses [mean difference = −0.60 (SD = 2.43); $P = 0.60$ by paired t test].

Statistical Analyses. PAH-DNA adduct levels (expressed per 10^8 nucleotides) were log transformed on a natural scale, which successfully normalized the observed distribution. Initial statistical analyses included comparison of the means of case and control PAH-DNA adduct levels in blood using the unpaired Student t test. Unconditional logistic regression (29) was used to estimate the risk of breast cancer in relation to PAH-DNA adduct levels, with adjustments made for the frequency-matching variable age (continuous) and for other potential confounding factors. Adduct levels were evaluated as a continuous variable, as a dichotomous variable, or were categorized in quantiles (including exploration of tertiles, quartiles, quintiles, and deciles, with each quantile coded as a designer variable). Covariates considered as potential confounders include: age at menarche, parity, number of live births, lactation, months of lactation, age at first birth, number of miscarriages, history of fertility problems, BMI at reference, BMI at age 20, alcohol intake, active and passive cigarette smoking, first-degree family history of breast cancer, history of benign breast disease, oral contraceptive use, hormone replacement use, race, ethnicity, education, religion, marital status, season of blood donation, total years of residence on Long Island, and age first moved to Long Island. The final multivariate-adjusted models shown include those factors that remained in a best fitting model, which was developed by starting with a saturated model and then excluding covariates that did not improve the overall fit, as measured by the −2 log likelihood ratio test (30). Cigarette smoking and consumption of smoked and grilled foods have been reported as two important contributors to body levels of PAH-DNA adducts (13). These factors were, therefore, considered as antecedents in the causal pathway for breast cancer development and have not been included in the multivariate models constructed to adjust for potential confounding.

To identify potential effect modification, ORs for breast cancer in relation to detectable PAH-DNA adduct levels within strata of selected covariates were calculated using multivariate logistic regression models. The −2 log likelihood ratios from unconditional logistic regression models with and without cross-product terms were compared (30) to formally evaluate, on a multiplicative scale, potential effect modification by cigarette smoking (never/ever passive only/ever active only/ever active + passive), recent consumption of grilled and smoked foods (beef + lamb + pork + poultry + fish intake within the most recent decade, continuous), alcohol intake (ever/never), menopausal status (pre-/postmenopausal), length of residence on Long Island (<15 years/15+ years), and age at reference date (<65 years/65+ years). To determine the risk of breast cancer with cases categorized by stage of disease (*in situ* or invasive), or by ER and PR status, unordered polytomous logistic regression (30) was performed.

Cigarette smoking definitions are based on self-reported data from the main questionnaire, which queried each subject about her exposures before the reference date (the date of diagnosis for cases and date of identification for controls), and the specimen checklist, which queried the respondent about their exposures in the past few days (and thus assessed exposure after the reference date). A current active cigarette smoker was defined as a regular smoker within the last 12 months before the reference date; a former active smoker was defined as a regular smoker who reported quitting more than 12 months before the reference date; a passive smoker was defined as either a current or former smoker or nonsmoker who reported ever living with an active smoker; and a never smoker was a nonsmoker who also did not report living with an active smoker. Discrepancies between the checklist and main questionnaire were infrequent. If a subject was categorized as a never or former smoker based on the main questionnaire, but on the checklist reported smoking ($n = 9$) or data were missing ($n = 9$), the subjects was omitted from the PAH analyses. Further, if the subject was categorized as a current smoker on the main questionnaire, but on the checklist reported not smoking in the past few days ($n = 30$) or data were missing ($n = 1$), then the subject was maintained in the PAH analyses as a current smoker.

Definitions of consumption of grilled and smoked foods were constructed based on data collected in the main questionnaire, which focused on historical consumption by decade before the reference date, and in the specimen checklist, which focused on consumption in the 4 weeks before blood collection. In the main questionnaire, women were queried about their consumption patterns over 6 decades of life (<20 years, 20–29 years, 30–39 years, 40–49 years, 50–59 years, 60+ years) for four different groups of PAH-containing foods: smoked beef, lamb, and pork; grilled or barbecued beef, lamb, and pork; smoked poultry or fish; and grilled or barbecued poultry or fish. The average of the 6 decades was calculated to derive an average lifetime consumption of the four PAH food groups. For each of the 24 groups (6 decades of consumption × four food groups), values were missing for less than 2% of respondents; and for the four derived averages, values were missing for 9–10% of respondents. The vast majority of missingness was restricted to a single decade of reported consumption. Imputations for the missing values were derived by multiple regression (29) using data from subjects with complete data to predict the missing variables in each of the 24 groups. In addition, because of concerns about possible cohort effects, regressions were performed separately by the decade of age at interview. Therefore, for example, to predict average consumption of smoked beef, lamb, and pork between ages 20–29 years, for women in their 30s at reference, multiple regression was conducted using subjects in their 30s at reference to construct a model: beef consumption during ages 20–29 years = α (constant) + β_1 (beef consumption under age 20 years) + β_2 (beef consumption during ages 30–39 years) + ϵ (error). The regression coefficients, β_1 and β_2, were then used to impute beef consumption for all subjects in their 30s who were missing only beef consumption during the decade 20–29 years of age. These steps were repeated for other subjects missing only one interval of consumption within each food category. To correct for artificially minimized SEs for the ORs produced when using imputations, the SEs obtained using imputed data were inflated back to the lower sample-size level. This imputation strategy reduced the amount of missing consumption data in the following manner: lifetime grilled or barbecued beef, lamb, or pork consumption from 9.48% to 2.68%; lifetime intake of grilled or barbecued poultry or fish from 9.60% to 3.27%; lifetime smoked beef, lamb or pork consumption from 8.55% to 2.75%; lifetime intake of smoked poultry or fish from 9.77% to 4.72%; and lifetime consumption of all four types of food combined from 14.77% to 5.24%. The ORs obtained from this imputed data set were not materially different from those obtained from the data set in which missing consumption values were simply dropped, although, as expected, CIs were wider for the latter data set (data not shown). Sensitivity analyses revealed that substitution of more crudely derived imputations, *e.g.*, either the highest or lowest observed values, did not substantially affect the observed ORs (data not shown). Thus, the results based on models

Table 2 Adjusted ORs for breast cancer and 95% CIs in relation to PAH-DNA adduct levels, Long Island Breast Cancer Study Project, 1996–1997

Quantile of PAH-DNA adduct level per 10^8 nucleotides (range)	Cases (*n*)	Controls (*n*)	Age-adjusted ORs (95% CI)	Multivariate-adjusted ORs[a] (95% CI)
Quantile 1 (nondetects)	148	134	1.00	1.00
Quantile 2 (0 to ≤7.7237)	105	72	1.31 (0.89–1.92)	1.45 (0.97–2.17)
Quantile 3 (>7.7237 to ≤14.4212)	112	73	1.36 (0.93–1.98)	1.48 (0.99–2.21)
Quantile 4 (>14.4212 to ≤21.9357)	88	73	1.09 (0.74–1.62)	1.01 (0.67–1.52)
Quantile 5 (>21.9357)	122	72	1.51 (1.04–2.20)	1.49 (1.00–2.21)

[a] Adjusted for age at reference date (continuous), race, history of infertility problems, season of blood donation, religion, parity (continuous), total months of lactation (continuous), BMI at age 20 (continuous), first-degree family history of breast cancer, age at first birth (centered, continuous).

with the more precisely estimated regression coefficients imputed for the missing values are shown.

A subject was defined as postmenopausal if her last menstrual period was more than 6 months before the reference date or if she had both ovaries removed before the reference date (24). If a subject was taking hormone replacement therapy or had a hysterectomy without removal of both ovaries, her menopausal status was initially classified as unknown (11.81% of subjects). To reduce the number of subjects with unknown menopausal status, we used information about the subject's reference age. That is, any smoker with unknown menopausal status was categorized as postmenopausal if her age at reference was ≥54.8 years (90% percentile for natural menopause among smoking controls), and any nonsmoker with unknown menopausal status was categorized as postmenopausal if her age at reference was ≥55.4 years (90% percentile for natural menopause among nonsmoking controls).

The statistical analyses are based on 575 cases (398 who were randomly selected and 171 who were specifically selected) and 424 controls (400 who were randomly selected and 24 who were specifically selected). The analyses were repeated restricting the study sample to those 398 cases and 400 controls that were randomly chosen to have their blood samples assayed for the PAH-DNA adducts. Results from these latter models were nearly identical to the former, and are not shown.

Results

Among the 575 cases and 424 controls who donated a blood sample and who were selected for the PAH-DNA analyses, mean PAH-DNA adducts per 10^8 nucleotides (and corresponding SD) were 16.5 (24.2) for cases and 15.1 (24.2) for controls; the median (and range) for cases and controls were 9.03 (1.00–278.64) and 8.17 (1.00–246.97), respectively. Means (and SD) of the log-transformed data (on a natural scale) were 2.00 (1.39) and 1.83 (1.43) per 10^8 nucleotides among cases and controls, respectively; the corresponding geometric means and geometric SD were 7.36 (7.29) for cases and 6.21 (4.17) for controls. The observed difference in the log means was not statistically significant ($P = 0.51$). All of the additional analyses are based on the log-transformed data.

Table 2 shows the age-adjusted and multivariate-adjusted ORs for breast cancer in relation to PAH-DNA adduct levels categorized in quantiles according to the distribution among controls, with all nondetectable levels placed in the lowest quantile as the referent. Among those with the highest quintile of exposure, as compared with the lowest quintile, the age-adjusted OR was 1.51 (95% CI, 1.04–2.20). There was no further substantial elevation in risk when PAH-DNA adduct levels were categorized in deciles rather than quintiles (data not shown). Because of the large number of women for whom no detectable levels of adducts were observed, we estimated the risk associated with having detectable adduct levels (age-adjusted OR, 1.32; 95% CI, 1.00–1.74, for detectable *versus* nondetectable levels). Repeating the analyses with the sample restricted to those subjects with detectable levels of adducts, which can be interpreted as an indicator of the dose-response among the exposed, the ORs showed little or no increasing risk with increasing adduct formation; for the highest quantile of five categories as compared with the lowest, the OR was 1.13 (95% CI, 0.71–1.81). As shown in Table 2, there was no substantial confounding of the association between PAH-DNA adduct levels and breast cancer risk.

Table 3 shows the multivariate-adjusted ORs for breast cancer in relation to detectable PAH-DNA adducts (1.35 as compared with no detectable levels, 95% CI, 1.01–1.81) and stratified by factors that have potential to modify the association. The multivariate adjusted ORs for breast cancer shown are in relation to detectable adduct levels as compared with nondetectable levels within strata of the potential effect modifier. Risk of breast cancer in relation to detectable adducts did not appear to significantly vary by: menopausal status; the subjects' length of residence on Long Island; whether the study subjects were younger than 65 years of age at reference or 65 years of age and older; or whether the case was diagnosed with *in situ* or invasive disease. Consideration of other cut points for years of residence on Long Island did not materially differ from the results reported (data not shown). Risk significantly varied when cases were categorized by the ER and PR status of the tumor, with an ~60% increase in risk among women with ER+PR+ and ER−PR− tumors and no elevation in risk among those with discordant ERPR status.

Differences between case and control levels of PAH-DNA adducts [geometric means (geometric SDs)] also did not vary substantially with whether the blood sample from the same subjects ($n = 103$ pairs) was collected before [7.22 (4.19)] or after [8.36 (3.67)] the commencement of chemotherapy ($P = 0.45$). Thus, when restricting the analyses to controls and cases who had donated a prechemotherapy sample only ($n = 381$ cases), the ORs (and 95% CI) for breast cancer in detectable PAH-DNA adducts did not vary substantially from the analyses that included case subjects with either pre- or postchemotherapy samples available (data not shown).

Grilled and smoked foods as well as cigarette smoking are reported as important sources of PAH (11). In these data, however, adduct levels among control women did not increase with increasing lifetime average intake of grilled or smoked foods: geometric mean adduct levels per 10^8 nucleotides (and geometric SD) were 7.90 (3.80) for quintile 1; 5.44 (4.12) for quintile 2; 7.67 (4.36) for quintile 3; 6.01 (4.27) for quintile 4; and 5.71 (4.04) for quintile 5 (ANOVA $P = 0.32$). In addition, mean levels were not associated with consumption of grilled or smoked foods during the 4 weeks before donation of the blood

Table 3 Adjusted ORs for breast cancer and 95% CIs in relation to detectable PAH-DNA adduct levels stratified by menopausal status, length of residence in interview home, age at diagnosis, stage, and estrogen receptor and progesterone receptor status. Long Island Breast Cancer Study Project, 1996–1997

Stratifying factor	Mean (SD) PAH-DNA adduct levels[a]		Case, *n*	Control, *n*	Multivariate-adjusted[b] OR (95% CI)[c]
	Cases	Controls			
Main effect	16.52 (24.18)	15.14 (24.21)	575	424	1.35 (1.01–1.81)
Menopausal status					
Premenopausal	16.23 (28.10)	14.19 (22.28)	180	142	1.58 (0.94–2.66)
Postmenopausal	16.20 (21.66)	15.92 (25.82)	352	248	1.19 (0.82–1.72)
Length of residence in interview home					
<15 yr	17.42 (24.14)	14.30 (23.58)	65	49	1.74 (0.71–4.26)
15+ yr	16.59 (24.49)	14.93 (23.15)	468	344	1.30 (0.95–1.80)
Age at diagnosis					
<65 yr	16.32 (24.02)	14.12 (20.69)	370	317	1.48 (1.05–2.09)
65+ yr	16.91 (24.56)	18.74 (33.67)	178	90	1.18 (0.64–2.18)
Stage					
In situ	17.41 (22.66)	15.14 (24.21)	129	424	1.50 (0.93–2.42)
Invasive	16.26 (24.62)	15.14 (24.21)	446	424	1.32 (0.97–1.79)
ER and PR status					
ER+/PR+	19.73 (29.93)	15.14 (24.21)	218	424	1.59 (1.06–2.37)
ER+/PR−	14.24 (25.09)	15.14 (24.21)	52	424	0.92 (0.46–1.85)
ER−/PR+	17.82 (23.93)	15.14 (24.21)	18	424	0.92 (0.32–2.60)
ER−/PR−	14.42 (15.66)	15.14 (24.21)	68	424	1.64 (0.87–3.10)

[a] PAH-DNA adduct levels are presented untransformed.
[b] Adjusted for age at reference date (continuous), race, history of infertility problems, season of blood donation, parity (continuous), total months of lactation (continuous), BMI at age 20 (continuous), first-degree family history of breast cancer, age at first birth (centered, continuous).
[c] PAH-DNA adduct levels have been log transformed prior to logistic regression analyses (see "Materials and Methods.").

sample (data not shown). As shown in Table 4, geometric mean PAH-DNA adduct levels among controls also did not vary with current or former active cigarette smoking [6.31 (4.38) for never; 5.92 (3.93) for former; and 6.52 (4.24) for current; ANOVA $P = 0.87$] or, when passive smoking exposure was considered [7.96 (4.46) for never active or passive; 5.87 (4.31) for ever passive only; 4.95 (3.96) for ever active only; and 6.33 (4.04) for ever active and passive; ANOVA $P = 0.48$].

Table 4 also shows the multivariate-adjusted ORs for breast cancer in relation to detectable PAH-DNA adducts stratified by cigarette smoking, alcohol intake, or grilled or smoked food consumption in the most recent decade of life, before the reference date. The ORs shown are for detectable PAH-DNA adduct levels as compared with no detectable levels within the strata of smoking or smoked food consumption. There was little variation in the ORs in relation to adduct levels with active or passive smoking status, alcohol intake, or by quintile of grilled and smoked food consumption.

Discussion

The carcinogenicity of PAH, such as DMBA, on the mammary gland of laboratory animals has been well demonstrated (31), but their role in breast cancer development in women has not been well studied. PAH are bulky carcinogens, and elevated levels of PAH-DNA adducts have been observed in lung cancer cases (32). Furthermore, patterns of p53 mutations seen in the breast resemble those seen in the lung (33). Thus, it is possible that PAHs are breast carcinogens in humans as they are in animal models. In contrast, it is also possible that PAH may have antiestrogenic potential (34), as has been postulated for cigarette smoking (35), which is one of the major sources of PAH exposure in American populations (11, 13). However, it is also unclear whether cigarette smoking has carcinogenic effects or antiestrogenic effects on the breast (36, 37). Because of the biological plausibility of dual effects of PAH on the breast, utilization of a biological marker, such as DNA adducts, that reflects internal dose may help to clarify the issue.

This study is the first large-scale epidemiological study to evaluate whether DNA damage associated with PAH exposure increases the risk of breast cancer. In this population-based, case-control study among women on Long Island, a modest 50% elevation in the risk of breast cancer was noted in relation to the highest quintile of PAH-DNA adduct levels; however, no dose-response effect was observed. These results, coupled with the observed 50% increase in risk noted among women with detectable adducts, suggests that there may be a threshold effect. Results were not strongly confounded by known or suspected risk factors for breast cancer.

Mean PAH-DNA adduct levels among control women did not vary with active or passive cigarette smoking status or within levels of grilled or smoked food consumption, which are reported to be among the largest sources of PAH among Americans. This lack of an association between adduct levels and the major sources of PAH adducts suggest perhaps that the adducts are better indicators of the body's response to the carcinogenic insult, rather than an indicator of exposure level. In other words, the presence of high levels of PAH-DNA adducts may be indicative of a susceptible individual.

We also found elevated risks for breast cancer in relation to adduct levels associated with ER−PR− tumors as well as ER+PR+ tumors, but not for those with discordant receptor status. Smoking has been previously reported to be associated with p53+ breast cancer (37), and p53 expression and ER− are consistently found to occur together (38, 39). In previous studies, smoking has been found to increase the risk of ER− tumors, but not ER+ tumors (40, 41). Thus, our observation of an increase risk with PAH among concordant ERPR status tumors, regardless of whether the status of the receptor was positive or negative, is not entirely consistent with other reports. However, our observation for an ~50% elevation in breast cancer risk associated with DNA adducts is consistent with two previously reported epidemiological studies (19, 21), with one of them showing a doubling in risk (21). A number of issues that may affect interpretation of our findings are discussed below.

Table 4 Adjusted ORs for breast cancer and 95% CIs in relation to detectable PAH-DNA adduct levels stratified by cigarette smoking, alcohol, and by consumption of grilled and smoked foods in most recent decade of life, Long Island Breast Cancer Study Project, 1996–1997

Stratifying Factor	Mean (SD) PAH-DNA adduct levels[a] per 10^8 nucleotides		Case, *n*	Control, *n*	Multivariate-adjusted[b] ORs (95% CI) for detectable PAH-DNA adduct levels[c]
	Cases	Controls			
	16.52 (24.18)	15.14 (24.21)	575	424	1.35 (1.01–1.81)
Active cigarette smoking exposure					
Never	16.17 (24.99)	16.89 (29.53)	235	190	1.39 (0.90–2.15)
Former	18.33 (26.75)	12.94 (17.05)	209	154	1.44 (0.88–2.38)
Current	13.69 (15.08)	15.09 (20.76)	104	62	1.28 (0.62–2.64)
Active and/or passive cigarette smoking					
Never either	12.00 (13.87)	18.79 (24.80)	43	42	0.51 (0.17–1.50)
Ever passive only	17.16 (26.95)	16.22 (30.95)	188	146	1.63 (0.99–2.70)
Ever active only	17.99 (30.33)	11.95 (20.70)	41	32	3.06 (0.66–14.12)
Ever both	16.22 (22.25)	13.97 (18.02)	263	177	1.22 (0.78–1.89)
Alcohol intake					
Never	7.23 (4.08)	6.34 (4.33)	173	147	1.42 (0.86–2.36)
Ever	7.42 (3.98)	6.14 (4.09)	375	260	1.37 (0.95–1.98)
Intake of grilled/BBQ[d] beef, lamb, and pork in most recent decade of life					
Quantile 1	15.06 (23.68)	14.80 (20.48)	122	87	0.69 (0.35–1.35)
Quantile 2	16.49 (17.93)	15.82 (23.02)	91	58	1.43 (0.64–3.20)
Quantile 3	18.50 (24.67)	18.33 (30.57)	123	101	1.29 (0.68–2.42)
Quantile 4	15.00 (15.75)	16.09 (29.19)	89	78	1.78 (0.83–3.85)
Quantile 5	17.39 (33.09)	10.61 (10.88)	115	76	1.26 (0.64–2.49)
Intake of grilled/BBQ poultry and fish in most recent decade of life					
Quantile 1	15.46 (22.06)	15.29 (20.08)	145	104	0.66 (0.35–1.26)
Quantile 2	14.50 (16.57)	15.00 (17.07)	112	88	1.42 (0.70–2.89)
Quantile 3	18.23 (23.78)	21.01 (41.61)	76	51	1.50 (0.64–3.53)
Quantile 4	15.24 (16.66)	14.60 (27.22)	89	67	2.09 (0.91–4.80)
Quantile 5	19.43 (35.81)	13.00 (18.76)	115	89	2.02 (1.08–3.78)
Intake of smoked beef, lamb, and pork in most recent decade of life					
Quantile 1	17.59 (22.35)	15.20 (21.17)	195	148	1.34 (0.78–2.31)
Quantile 2	15.52 (17.63)	17.17 (34.82)	80	47	1.61 (0.69–3.77)
Quantile 3	12.47 (16.69)	15.04 (20.77)	101	90	1.87 (0.94–3.70)
Quantile 4	20.48 (44.06)	21.71 (38.84)	48	41	0.23 (0.06–0.93)
Quantile 5	17.08 (25.17)	10.94 (13.44)	117	77	1.88 (0.97–3.64)
Intake of smoked poultry and fish in most recent decade of life					
Quantile 1	16.33 (22.44)	15.97 (23.35)	355	267	1.45 (1.00–2.09)
Quantile 2	15.48 (17.58)	20.11 (47.92)	44	25	0.82 (0.18–3.64)
Quantile 3	17.57 (34.64)	11.37 (15.23)	87	64	1.39 (0.68–2.83)
Quantile 4	23.83 (32.89)	7.20 (7.38)	15	18	14.62 (0.75–284.21)
Quantile 5	14.18 (13.85)	14.65 (19.59)	40	27	0.34 (0.05–2.23)
Intake of all PAH foods in most recent decade of life					
Quantile 1	17.04 (23.49)	15.58 (20.64)	100	70	0.80 (0.35–1.83)
Quantile 2	14.79 (18.54)	12.42 (16.20)	108	82	1.42 (0.72–2.79)
Quantile 3	14.45 (19.09)	24.44 (41.45)	101	80	0.75 (0.38–1.49)
Quantile 4	18.05 (22.66)	14.94 (19.81)	108	82	2.53 (1.17–5.45)
Quantile 5	18.01 (34.54)	9.90 (10.19)	110	78	1.28 (0.67–2.45)

[a] PAH-DNA adduct levels are presented untransformed.
[b] Adjusted for age at reference date (continuous), race, history of infertility problems, season of blood donation, religion, parity (continuous), total months of lactation (continuous), BMI at age 20, first-degree family history of breast cancer, age at first birth (centered, continuous).
[c] PAH-DNA adduct levels have been log transformed prior to logistic regression analyses (see "Materials and Methods").
[d] BBQ, barbecued.

Subject Selection. Response rates were lower among controls than in cases, especially among women over age 75 years (24). This study had no upper age limit, and comorbidity among the elderly controls and the protective efforts of the subjects' families limited study participation among these older women. If the older respondents in the our study somehow differ systematically from nonrespondents, our results may not be generalizable to older women.

As reported previously (24), the distribution of known risk factors for breast cancer differed among blood donors and nondonors. Factors that were found to be associated with a decreased likelihood that a respondent would donate blood included increasing age and past smoking; factors associated with an increased probability included white or other race, alcohol use, ever breastfed, ever use of hormone replacement therapy, ever use of oral contraceptives, and ever had a mammogram (24). Thus, there is the possibility that bias may have affected our results, although the few factors identified as potential confounders were not related to the factors found to influence the probability of donating a sample. Furthermore, the proportion of eligible subjects who were willing to donate blood was comparable with that reported by other population-based studies with a phlebotomy component (42). Thus, the sample reported here is likely to be similar to a population from other similarly designed epidemiological studies.

Exposure Measurement. The most optimal laboratory method to measure PAH-DNA adducts is controversial. Although the ELISA technique used in our study requires 25–35 ml of blood, a volume larger than the 10 ml or less required for the postlabeling method, the assay is more quickly conducted, which enhances its use in large epidemiological studies. In addition, laboratory personnel are not exposed to radiation. More importantly, the antiserum used recognizes benzo(*a*)pyrene and structurally related PAH diol epoxide-DNA adducts. Furthermore, the method was successful in identifying increased levels of adducts among lung cancer cases (33). In contrast, the smaller blood volume required for postlabeling is more advantageous for human subject research. In addition, the method is more sensitive than the ELISA because of the high specific activity of ^{32}P. However, postlabeling is not specific for PAH and is a more tedious laboratory assay, which restricts its usefulness for large-scale research. Another available option is the assessment of adducts in archived tissue by immunohistochemical methods. Although this method has the advantage of detection of adducts in the target tissue, one (22) of the two previous studies (21, 22) that has used immunohistochemical methods to detect PAH-DNA adducts in breast tissue was unable to document an association between adduct levels and smoking. Also, control selection in a study that uses the immunohistochemical method of adduct assessment is necessarily restricted to those from whom target tissue can be obtained, which is generally not the source population from which the cases arose; such selection methods can result in study bias (23). It is reassuring to note that, regardless of the method used to detect PAH compounds, our findings, which are based on a large population-based sample of breast cancer cases and controls, are similar to those observed in earlier smaller hospital-based reports (19, 21).

The time frame of exposure measured by the adduct levels is unclear and may vary from a few months to a few years. In earlier studies of lung cancer, total WBCs, with a cell turnover of several months, were used to measure the DNA adducts. With the current use of mononuclear cells, with a cell life as long as 3 years, as the source of DNA for the assay, the relevant exposure time may be longer, but the actual relevant time frame is uncertain. Thus, as in many environmental epidemiology studies, measurement of current levels of an environmental contaminant are assumed to reflect past exposures. Unfortunately, no biomarkers are currently available that reflect long-past exposures to PAH. Current levels of PAH-DNA adducts, however, may be relevant to breast carcinogenesis if PAH affect mechanisms related to later stages of cancer, such as mutations in tumor suppressor genes, like *p53*. Adducts measured at diagnosis are unlikely to be related to earlier, tumor-initiating events, given the long latency of breast cancer.

Measurement of two of the major sources of PAH, cigarette smoking and consumption of grilled and smoked foods, was comprehensive. Assessment of smoking included both active and passive smoking exposures. However, because of time constraints in the main questionnaire, respondents were queried about their lifetime residential or leisure time, but not occupational, passive smoking exposures. Lifetime grilled and smoked food intake was assessed by asking women about their intake of these foods by decade of life. It is, therefore, unlikely that the lack of an association between PAH-DNA adduct levels and smoking or grilled and smoked food intake is associated with poor measurement of these exposures, although exposure misclassification cannot be ruled out. It is possible that the PAH-DNA adduct levels observed in this study (*a*) are associated with other unmeasured sources of PAHs [such as air pollution, or leafy green vegetables in the diet (43)]; (*b*) are associated with some other exposures that may be highly correlated with PAH such as heterocyclic amines; or (*c*) are a surrogate for some other unmeasured factor. Alternatively, the lack of association between measured PAH sources of smoking and diet and adduct levels in these data may reflect variability in individual susceptibility. For example, among those persons with similar exposure levels such as smokers, the subject's individual response to the insult, through individual variation in DNA repair or other mechanisms, influences breast cancer development. To evaluate potential gene-environment interactions, laboratory assays are under way on polymorphisms involved in oxidative stress and DNA repair.

In conclusion, consistent with animal evidence that demonstrates the mammary carcinogenicity of PAH compounds, in this population-based study of women on Long Island an ~50% increase in breast cancer risk was noted in relation to PAH-DNA adducts, which are indicative of recent DNA damage. Our results are also consistent with two previous smaller epidemiological studies (19, 21), which noted higher levels of adducts among breast cancer cases as compared with hospital-based controls. No trend in risk was observed, which is suggestive of a threshold effect. We did not observe a relation between PAH-DNA adduct levels and several major sources of PAH, including cigarette smoking or intake of grilled and smoked foods, which is in contrast to several previous small investigations. However, these findings may suggest that individual differences in the response to similar levels of PAH exposure may be more relevant in breast carcinogenesis. PAH-DNA adducts reflect only recent exposures; therefore, future research in this area should focus on PAH exposure in the more distant past, which is likely to be more useful when assessing breast cancer risk. Biomarkers are not available that reflect exposures in the distant past, and other exposure assessment methods, such as geographic modeling (44), may need to be used when evaluating the breast carcinogenic potential in humans.

Acknowledgments

For their valuable contributions to the Long Island Breast Cancer Study Project, we thank members of the Long Island Breast Cancer Network; the participating hospital and other institutions on Long Island and in New York, NY; the study respondents; the cooperating NIH scientists (Drs. G. Iris Obrams and Gwen Collman); and members of the study's External Advisory Committee: Dr. Leslie Bernstein, Committee chair; Drs. Gerald Ackland, Blake Cady, Dale Sandler, Roy Shore, Gerald Wogan, and Barbara Balaban, breast cancer advocate.

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