

# Reproductive Factors, Hormone Use, and Risk for Lung Cancer in Postmenopausal Women, the Nurses' Health Study

Christina S. Baik<sup>1</sup>, Gary M. Strauss<sup>1</sup>, Frank E. Speizer<sup>2,3</sup>, and Diane Feskanich<sup>2</sup>

## Abstract

**Background:** There is increasing evidence suggesting that female hormones may play a significant role in lung cancer development. We evaluated the associations between reproductive factors, exogenous hormone use, and lung cancer incidence in the Nurses' Health Study.

**Methods:** We assessed age at menopause, age at menarche, type of menopause, parity, age at first birth, postmenopausal hormone (PMH) use, and past oral contraceptive use in 107,171 postmenopausal women. Cox models were used to estimate the hazard ratios for each exposure, adjusting for smoking and other covariates.

**Results:** We identified 1,729 lung cancer cases during follow-up from 1984 to 2006. Menopause onset before 44 years of age (hazard ratio, 1.39; 95% confidence interval, 1.14-1.70) and past oral contraceptive use for >5 years (hazard ratio, 1.22; 95% confidence interval, 1.05-1.42) were associated with increased lung cancer risk. These associations were strongest in current smokers and small cell histology. In never smokers, increased parity was associated with decreased risk among parous women ( $P$  trend = 0.03), whereas in current smokers, older age at first birth was associated with increased risk ( $P$  trend = 0.02). PMH use was not associated with overall lung cancer incidence. However, nonsignificant results of increased risk in adenocarcinoma were seen with current PMH use.

**Conclusions:** Our findings suggest female hormones may influence lung carcinogenesis, although the effect is likely modest, varied by histologic subtype, and altered by smoking.

**Impact:** Further investigation of the pathophysiology of female hormones in lung cancer subtypes and their interaction with smoking will lead to better understanding of lung carcinogenesis. *Cancer Epidemiol Biomarkers Prev*; 19(10); 2525-33. ©2010 AACR.

## Background

Lung cancer is the leading cause of cancer mortality in U.S. women. The American Cancer Society estimates that 70,490 women will die of lung cancer in 2009, which exceeds combined breast and colorectal cancer mortality in women (1). There is increasing evidence that lung cancers in women are biologically distinct from those of men, with the observation that they exhibit different distribution of histologic subtypes and molecular characteristics. Women are more likely to develop adenocarcinoma compared with men, especially among never smokers (2, 3). In comparison with men, women with lung cancer are

also more likely to be lifelong nonsmokers (4). Moreover, adenocarcinoma in women are more likely to harbor epidermal growth factor receptor mutations (5, 6) and thus more likely to respond to epidermal growth factor receptor tyrosine kinase inhibitors (7-9).

These gender differences raise the question of whether female hormones could play a role in lung carcinogenesis. In fact, estrogen- $\beta$  and progesterone receptors have been found on lung cancer cells (10, 11), and in a preclinical study by Stabile et al. (10, 12), a significant increase in cellular proliferation was seen in lung cancer-derived cell lines when they were incubated with  $\beta$ -estradiol, and this was inhibited by antiestrogens. Similar results have been seen in lung adenocarcinoma mouse models in which ovariectomized mice treated with estradiol developed higher tumor counts and volume compared with untreated mice (13).

These findings suggest that a woman's reproductive history and exogenous hormone use could affect her risk for developing lung cancer. Several epidemiologic studies have investigated the association between postmenopausal hormone (PMH) use and lung cancer incidence. However, results have been conflicting. Although several studies reported decreased risk with hormone replacement therapy (14-18), others reported no effect (19-22) and a few others reported a trend toward increased risk (23-25). Many have also investigated the effect of reproductive

**Authors' Affiliations:** <sup>1</sup>Division of Hematology-Oncology, Tufts Medical Center; <sup>2</sup>Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School; <sup>3</sup>Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts

**Note:** Presented in part at the American Society of Clinical Oncology Annual Meeting, 2009, Orlando, Florida.

**Corresponding Author:** Christina S. Baik, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue N/M3-A410, P.O. Box 19024, Seattle, WA 98109. Phone: 857-998-2861 and 206-667-2975; Fax: 206-667-4142. E-mail: chrisbaik@hotmail.com and cbaik2@u.washington.edu

doi: 10.1158/1055-9965.EPI-10-0450

©2010 American Association for Cancer Research.

factors, but these results have been inconsistent (16, 19, 20, 22-29). Most of these studies were retrospective in design, and varying adjustment for covariates may have contributed to the inconsistent results. Thus, our aim was to conduct a prospective and comprehensive analysis of reproductive factors and exogenous hormone use in relation to lung cancer incidence in the Nurses' Health Study (NHS). We further evaluated these associations separately by smoking status and histologic subtypes.

## Materials and Methods

### Study population

The NHS was established in 1976 with 121,700 female U.S. nurses of ages between 30 and 55 years who responded to the initial mailed questionnaire. The women were asked questions about their medical history and lifestyle, which included detailed reproductive and hormone use information, as well as smoking history. Follow up questionnaires have been sent every 2 years to update information about exposure status and to identify newly diagnosed medical conditions, and the response rate has been at least 90% for each cycle.

We have previously shown that fruit and vegetable consumption was associated with lower risk for lung cancer among women (30). Thus, we used 1984 as the baseline year because this was the first cycle in which diet assessment with extensive questions on fruits and vegetables was available. At baseline, we included women who were postmenopausal and had not reported a diagnosis of cancer (except nonmelanoma skin cancers) and women entered analysis during follow-up when they reached menopause. Women were classified as postmenopausal at the age when natural menopause or bilateral oophorectomy occurred. For women whose periods stopped after a hysterectomy or unilateral oophorectomy, we classified them as postmenopausal at the age of 54 years if a current smoker or 56 years if a nonsmoker, which were the ages by which 90% of NHS participants with a natural menopause had become postmenopausal.

The NHS is approved by the Brigham and Women's Hospital Institutional Review Board in Boston, Massachusetts. This investigation was also approved by Tufts University Institutional Review Board in Boston, Massachusetts.

### Case ascertainment

Lung cancers were reported by the participants or identified on their death certificates, and they were subsequently confirmed by hospital records and pathology reports. The cases were classified as confirmed only if a pathology report indicated that the lesion was a primary lung tumor. The confirmed lung cancer cases were then classified by predominant histologic subtype. Of the self-reported cases, 88% were confirmed with medical records, and an additional 6% were confirmed by death certificates. All self-reported lung cancers were included in the primary analyses because results were very similar when limited to cases confirmed by medical records.

Analyses by histologic subtype included only those confirmed by medical record review.

During the 22-year follow up, 1,729 incident lung cancer cases were identified. Of the 1,505 cases for which histology information was available, 47% were adenocarcinoma, 18% were small cell carcinoma, 17% were squamous cell carcinoma, 5% were large cell carcinoma, 10% were unspecified non-small cell lung cancer, and 3% were other histologies, including carcinoid and sarcoma. The histology distribution is comparable with the Surveillance Epidemiology and End Results data reported for female lung cancer (31).

There were 8% never smokers, 45% former smokers and 47% current smokers among the cases as determined by their smoking status on the biennial questionnaire before lung cancer diagnosis. Of the 138 cases among never smokers, adenocarcinoma was the predominant histologic subtype in 81 cases (62%), and there were no cases with small cell histology.

### Reproductive and hormonal exposures

The exposure variables assessed in this analysis were age at menarche, age at menopause, type of menopause, parity, age at first birth, oral contraceptive (OCP), use and PMH use.

Age at menarche was reported on the initial 1976 questionnaire, and number of pregnancies lasting >6 months was assessed from 1976 through the 1984 questionnaire and reconfirmed in 1996. In each biennial questionnaire, women were asked whether their menstrual periods had stopped, age at which they stopped, and whether the reason was natural or surgical. PMH and OCP use were first assessed on the initial 1976 questionnaire and were updated in each biennial questionnaire, which asked details about current or past use, duration of use, and type of hormones used. At each 2-year follow up cycle, we calculated total duration of use and time since last use. We stopped collecting additional OCP information in 1984 when <1% of the premenopausal women were currently using the medication. Missing information about exposure variables was included as a separate category.

### Smoking exposure and other covariates

Participants were asked on the initial questionnaire whether they were current or former smokers and, if so, their age at initiation. Former smokers were asked the age at which they discontinued smoking, and current smokers were asked to report their average number of cigarettes smoked per day. Participants with missing smoking status were excluded at baseline. Smoking status and quantity of cigarettes have been updated every 2 years. In 1982, participants were asked for information about environmental tobacco exposure, including whether one or both parents smoked, number of years living with someone who smoked, and whether exposed to smoke at work and/or at home. Other covariates included body mass index (BMI), calculated each questionnaire cycle from current weight and from height reported at

baseline, and dietary intake assessed in alternate cycles with a food frequency questionnaire that included 15 fruits and 30 vegetables. The validity of the FFQ has been assessed and published previously (32, 33).

### Statistical analysis

We accumulated person-years beginning at the return of the 1984 questionnaire or the first questionnaire on which a participant was classified as postmenopausal. Follow-up ended with a report of lung or any other cancer other than nonmelanoma skin cancers, death, or end of follow-up on June 1, 2006. Person-years contributed to exposure categories in each biennial follow-up cycle based upon the most recent questionnaire information. Women did not contribute person-years in cycles when their smoking status was unknown.

Cox proportional hazards models were used to estimate hazard ratios of lung cancer in each exposure category compared with a reference category. For continuous variables, *P* values for linear trend were calculated. The

multivariate models included all assessed risk factors and covariates. Risk estimates were obtained separately from analyses stratified by smoking status (never, former, current) and histology subtypes (adenocarcinoma, squamous cell carcinoma, and small cell carcinoma).

### Results

The characteristics of participants over follow-up are shown in Table 1 by PMH and OCP use. We analyzed 107,171 postmenopausal women from 1984 to 2006, during which a total of 1,590,432 person-years were accumulated and in which 14% were current smokers, 41% were former smokers, and 45% were never smokers. The participants were primarily Caucasian, and their mean age over follow-up was 63 years (range, 38-87 y). After adjusting for age, current PMH users were more likely to have used OCP, more likely to have had surgical menopause, less likely to be current smokers, and had a lower mean BMI. Women who used OCP for >5 years were less

**Table 1.** Age-adjusted characteristics by PMH and past OCP use among postmenopausal women in the NHS; 1984 to 2006

|   | PMH use    |            |            | OCP duration |            |            |
|---|------------|------------|------------|--------------|------------|------------|
|   | Never      | Past       | Current    | Never        | <5 y       | >5 y       |
| Age (y)   | 62.2 (7.0) | 65.8 (7.2) | 61.7 (7.3) | 65.5 (6.8)   | 60.9 (7.4) | 61.5 (7.0) |
| PMH (%)   |            |            |            |              |            |            |
| Never   |            |            |            | 33           | 22         | 23         |
| Past  |            |            |            | 23           | 28         | 26         |
| Current   |            |            |            | 27           | 40         | 42         |
| PMH duration (y)                                      |            | 4.8 (5.4)  | 10.3 (7.5) |              |            |            |
| Past OCP use (%)                                      | 33         | 45         | 48         |              |            |            |
| Nulliparous (%)                                       | 5          | 6          | 6          | 7            | 4          | 3          |
| Parity*   | 3.3 (1.5)  | 3.1 (1.4)  | 3.1 (1.3)  | 3.2 (1.5)    | 3.2 (1.4)  | 3.2 (1.4)  |
| Age at first birth (y)                                | 25.5 (3.5) | 25.1 (3.4) | 25.0 (3.2) | 25.5 (3.5)   | 25.2 (3.4) | 24.7 (3.1) |
| Age at menarche (y)                                   | 12.6 (1.4) | 12.5 (1.4) | 12.6 (1.4) | 12.6 (1.4)   | 12.5 (1.4) | 12.6 (1.4) |
| Age at menopause (y)                                  | 50.3 (3.8) | 48.3 (5.2) | 48.8 (5.0) | 49.0 (4.9)   | 49.1 (4.6) | 49.7 (4.0) |
| Natural menopause (%)                                 | 79         | 56         | 46         | 59           | 56         | 64         |
| Surgical menopause (%)                                | 12         | 35         | 44         | 28           | 33         | 26         |
| Smoking (%)   |            |            |            |              |            |            |
| Never   | 44         | 41         | 45         | 46           | 42         | 42         |
| Past  | 39         | 43         | 44         | 39           | 45         | 44         |
| Current   | 17         | 16         | 11         | 15           | 13         | 15         |
| BMI (kg/m <sup>2</sup> )                              | 26.5 (5.1) | 26.1 (4.9) | 25.2 (4.3) | 26.1 (4.8)   | 25.9 (4.8) | 25.6 (4.7) |
| Fruit intake (servings per day)                       | 1.9 (0.7)  | 1.9 (0.7)  | 1.9 (0.7)  | 1.9 (0.7)    | 1.9 (0.7)  | 1.9 (0.7)  |
| Vegetable intake (servings per day)                   | 2.2 (0.6)  | 2.2 (0.6)  | 2.3 (0.6)  | 2.2 (0.6)    | 2.3 (0.6)  | 2.2 (0.6)  |
| Regular smoking exposure at work (%) <sup>†</sup>     | 25         | 25         | 24         | 22           | 26         | 29         |
| Regular smoking exposure at home (%) <sup>†</sup>     | 21         | 20         | 17         | 18           | 26         | 22         |
| Both parents smoked (%) <sup>†</sup>                  | 13         | 15         | 15         | 12           | 17         | 17         |
| Living >20 y with someone who smoked (%) <sup>†</sup> | 36         | 32         | 28         | 32           | 29         | 32         |

NOTE: Values are presented as means or percentages. SDs are presented in parentheses when indicated.

\*Among parous women only.

<sup>†</sup>Smoking exposure at home and work assessed at baseline only.

**Table 2.** Hazard ratios of lung cancer by reproductive factors and PMH use in postmenopausal women; 1984 to 2006

|                        | All women<br>(n = 1,729) |                  | Never smokers<br>(n = 138) |                  | Former smoker<br>(n = 782) |                  | Current smokers<br>(n = 809) |                  |
|------------------------|--------------------------|------------------|----------------------------|------------------|----------------------------|------------------|------------------------------|------------------|
|                        | n                        | HR (95% CI)      | n                          | HR (95% CI)      | n                          | HR (95% CI)      | n                            | HR (95% CI)      |
| Age at menopause       |                          |                  |                            |                  |                            |                  |                              |                  |
| <44                    | 229                      | 1.39 (1.14-1.70) | 18                         | 1.42 (0.69-2.95) | 78                         | 1.08 (0.79-1.48) | 133                          | 1.76 (1.32-2.34) |
| 44-47                  | 258                      | 1.06 (0.89-1.26) | 15                         | 0.88 (0.43-1.80) | 111                        | 1.03 (0.79-1.34) | 132                          | 1.16 (0.89-1.50) |
| 48-49                  | 244                      | 1.0              | 16                         | 1.0              | 115                        | 1.0              | 113                          | 1.0              |
| 50-51                  | 587                      | 1.04 (0.88-1.22) | 52                         | 1.17 (0.64-2.15) | 261                        | 0.97 (0.76-1.23) | 274                          | 1.16 (0.91-1.48) |
| ≥52                    | 386                      | 0.94 (0.79-1.10) | 37                         | 0.78 (0.43-1.44) | 204                        | 0.96 (0.76-1.22) | 145                          | 0.99 (0.77-1.28) |
| P trend                |                          | 0.0004           |                            | 0.22             |                            | 0.33             |                              | 0.001            |
| Age at menarche        |                          |                  |                            |                  |                            |                  |                              |                  |
| ≤11                    | 377                      | 1.04 (0.91-1.19) | 29                         | 0.85 (0.53-1.36) | 181                        | 1.17 (0.96-1.43) | 167                          | 0.97 (0.79-1.19) |
| 12                     | 421                      | 0.97 (0.85-1.10) | 32                         | 0.79 (0.50-1.24) | 190                        | 1.02 (0.84-1.24) | 199                          | 0.98 (0.81-1.19) |
| 13                     | 523                      | 1.0              | 48                         | 1.0              | 227                        | 1.0              | 248                          | 1.0              |
| 14                     | 217                      | 0.99 (0.84-1.16) | 20                         | 1.00 (0.59-1.69) | 94                         | 0.95 (0.74-1.21) | 103                          | 1.08 (0.85-1.37) |
| ≥15                    | 174                      | 1.05 (0.88-1.25) | 9                          | 0.67 (0.32-1.36) | 81                         | 1.08 (0.83-1.39) | 84                           | 1.14 (0.88-1.47) |
| P trend                |                          | 0.84             |                            | 0.88             |                            | 0.43             |                              | 0.29             |
| Type of menopause      |                          |                  |                            |                  |                            |                  |                              |                  |
| Natural                | 982                      | 1.0              | 70                         | 1.0              | 458                        | 1.0              | 454                          | 1.0              |
| 0 Ovaries removed*     | 184                      | 1.19 (0.99-1.42) | 16                         | 1.08 (0.59-1.95) | 81                         | 1.12 (0.86-1.45) | 87                           | 1.30 (0.99-1.70) |
| 1                      | 68                       | 1.29 (0.99-1.67) | 8                          | 1.67 (0.77-3.62) | 33                         | 1.30 (0.89-1.88) | 27                           | 1.17 (0.77-1.76) |
| 2                      | 265                      | 1.06 (0.90-1.25) | 29                         | 1.33 (0.78-2.26) | 109                        | 1.02 (0.80-1.30) | 127                          | 1.03 (0.80-1.32) |
| Parity                 |                          |                  |                            |                  |                            |                  |                              |                  |
| Nulliparous            | 104                      | 0.97 (0.79-1.18) | 14                         | 1.66 (0.94-2.93) | 47                         | 1.08 (0.80-1.46) | 43                           | 0.75 (0.54-1.03) |
| Parous                 | 1,567                    | 1.0              | 124                        | 1.0              | 706                        | 1.0              | 737                          | 1.0              |
| 1-2 Children           | 510                      | 1.0              | 54                         | 1.0              | 209                        | 1.0              | 247                          | 1.0              |
| 3-4                    | 771                      | 1.09 (0.97-1.22) | 54                         | 0.70 (0.48-1.03) | 367                        | 1.29 (1.08-1.53) | 358                          | 1.00 (0.84-1.18) |
| ≥5                     | 278                      | 1.00 (0.86-1.17) | 16                         | 0.50 (0.28-0.88) | 130                        | 1.18 (0.94-1.48) | 132                          | 0.93 (0.75-1.16) |
| P trend†               |                          | 0.87             |                            | 0.03             |                            | 0.16             |                              | 0.30             |
| Age at first birth (y) |                          |                  |                            |                  |                            |                  |                              |                  |
| <26                    | 951                      | 1.0              | 73                         | 1.0              | 436                        | 1.0              | 442                          | 1.0              |
| 26-30                  | 461                      | 1.03 (0.91-1.15) | 36                         | 0.91 (0.60-1.38) | 205                        | 0.95 (0.80-1.13) | 222                          | 1.12 (0.95-1.33) |
| >30                    | 154                      | 1.20 (1.01-1.43) | 15                         | 1.30 (0.73-2.31) | 65                         | 1.10 (0.84-1.44) | 74                           | 1.38 (1.06-1.78) |
| P trend†               |                          | 0.06             |                            | 0.66             |                            | 0.60             |                              | 0.02             |
| PMH use                |                          |                  |                            |                  |                            |                  |                              |                  |
| Never                  | 436                      | 1.0              | 34                         | 1.0              | 184                        | 1.0              | 218                          | 1.0              |
| Past                   | 440                      | 0.91 (0.79-1.04) | 32                         | 0.90 (0.54-1.50) | 201                        | 0.83 (0.67-1.02) | 207                          | 0.96 (0.78-1.18) |
| Current                | 455                      | 0.99 (0.86-1.14) | 46                         | 1.00 (0.61-1.63) | 228                        | 0.96 (0.77-1.18) | 181                          | 1.02 (0.82-1.27) |
| OCP use                |                          |                  |                            |                  |                            |                  |                              |                  |
| Never                  | 1,059                    | 1.0              | 96                         | 1.0              | 471                        | 1.0              | 492                          | 1.0              |
| Past (y)               | 626                      | 1.10 (0.99-1.22) | 39                         | 0.88 (0.58-1.33) | 290                        | 1.15 (0.98-1.35) | 297                          | 1.06 (0.90-1.25) |
| <5                     | 358                      | 1.06 (0.93-1.21) | 26                         | 0.96 (0.59-1.54) | 166                        | 1.13 (0.93-1.37) | 166                          | 1.00 (0.82-1.21) |
| >5                     | 232                      | 1.22 (1.05-1.42) | 10                         | 0.72 (0.36-1.41) | 106                        | 1.25 (1.00-1.56) | 116                          | 1.28 (1.03-1.60) |
| P trend                |                          | 0.07             |                            | 0.33             |                            | 0.07             |                              | 0.17             |

NOTE: Stratified analysis by smoking status. Adjusted by: age at menopause, age at menarche, parity, type of menopause, PMH use, OCP use, smoking status, age at start smoking, cigarettes per day, time since quitting, fruit/vegetable intake, BMI, and environmental smoking exposure (parents smoking, years living with someone who smokes, exposure to smoking at work, exposure to smoking at home).

Abbreviation: HR, hazard ratio

\*Hysterectomy only.

†Among parous women only.

likely to be nulliparous, although the mean number of children among parous women was similar across the OCP use duration categories. Current smokers were younger at menopause compared with never smokers, but parity, age at first birth, and age at menarche did

not differ by smoking status (data not shown). The age-adjusted crude incidence of lung cancer in this cohort was 109 per 100,000 person-years.

Among all women in our analysis, onset of menopause before 44 years of age when compared with onset at 48 to

**Table 3.** Hazard ratios of lung cancer histology subtypes by reproductive factors in postmenopausal women; 1984 to 2006

|                        | Adenocarcinoma<br>(n = 706) |                  | Squamous carcinoma<br>(n = 253) |                  | Small cell carcinoma<br>(n = 264) |                  |
|------------------------|-----------------------------|------------------|---------------------------------|------------------|-----------------------------------|------------------|
|                        | n                           | HR (95% CI)      | n                               | HR (95% CI)      | n                                 | HR (95% CI)      |
| Age at menopause       |                             |                  |                                 |                  |                                   |                  |
| <44                    | 80                          | 1.16 (0.84-1.62) | 31                              | 1.12 (0.68-1.84) | 33                                | 1.63 (0.98-2.70) |
| 44-47                  | 117                         | 1.26 (0.95-1.66) | 36                              | 0.79 (0.51-1.23) | 39                                | 1.04 (0.66-1.63) |
| 48-49                  | 89                          | 1.0              | 48                              | 1.0              | 40                                | 1.0              |
| 50-51                  | 260                         | 1.29 (0.99-1.70) | 71                              | 0.69 (0.46-1.02) | 96                                | 1.07 (0.71-1.61) |
| ≥52                    | 152                         | 1.00 (0.78-1.31) | 67                              | 0.89 (0.60-1.31) | 56                                | 0.88 (0.57-1.33) |
| P trend                |                             | 0.21             |                                 | 0.49             |                                   | 0.008            |
| Age at menarche        |                             |                  |                                 |                  |                                   |                  |
| ≤11                    | 165                         | 1.18 (0.96-1.45) | 55                              | 0.94 (0.67-1.32) | 56                                | 1.10 (0.77-1.58) |
| 12                     | 170                         | 0.98 (0.80-1.20) | 57                              | 0.78 (0.56-1.10) | 79                                | 1.29 (0.93-1.80) |
| 13                     | 208                         | 1.0              | 89                              | 1.0              | 71                                | 1.0              |
| 14                     | 91                          | 1.05 (0.82-1.34) | 24                              | 0.62 (0.39-0.98) | 35                                | 1.21 (0.80-1.82) |
| ≥15                    | 72                          | 1.09 (0.83-1.43) | 26                              | 0.92 (0.59-1.44) | 20                                | 0.93 (0.56-1.54) |
| P trend                |                             | 0.56             |                                 | 0.54             |                                   | 0.64             |
| Type of menopause      |                             |                  |                                 |                  |                                   |                  |
| Natural                | 393                         | 1.0              | 160                             | 1.0              | 164                               | 1.0              |
| 0 Ovaries removed*     | 76                          | 1.04 (0.80-1.37) | 32                              | 0.75 (0.43-1.31) | 34                                | 1.48 (0.96-2.26) |
| 1                      | 33                          | 1.36 (0.94-1.98) | 9                               | 1.22 (0.60-2.50) | 7                                 | 0.83 (0.38-1.83) |
| 2                      | 120                         | 1.24 (0.97-1.59) | 16                              | 0.87 (0.55-1.37) | 26                                | 0.66 (0.41-1.07) |
| Parity                 |                             |                  |                                 |                  |                                   |                  |
| Nulliparous            | 44                          | 1.05 (0.77-1.43) | 10                              | 0.60 (0.31-1.14) | 9                                 | 0.56 (0.28-1.10) |
| Parous                 | 637                         | 1.0              | 238                             | 1.0              | 245                               | 1.0              |
| 1-2 Children           | 51                          | 1.0              | 14                              | 1.0              | 91                                | 1.0              |
| 3-4                    | 197                         | 1.10 (0.92-1.31) | 66                              | 1.18 (0.88-1.60) | 114                               | 0.84 (0.64-1.12) |
| ≥5                     | 98                          | 0.86 (0.67-1.10) | 46                              | 1.12 (0.77-1.65) | 40                                | 0.78 (0.53-1.14) |
| P trend†               |                             | 0.22             |                                 | 0.19             |                                   | 0.01             |
| Age at first birth (y) |                             |                  |                                 |                  |                                   |                  |
| <26                    | 391                         | 1.0              | 139                             | 1.0              | 151                               | 1.0              |
| 26-30                  | 181                         | 0.99 (0.83-1.19) | 76                              | 1.15 (0.86-1.53) | 78                                | 1.08 (0.81-1.43) |
| >30                    | 68                          | 1.31 (1.00-1.72) | 22                              | 1.08 (0.68-1.72) | 16                                | 0.75 (0.44-1.29) |
| P trend†               |                             | 0.12             |                                 | 0.43             |                                   | 0.91             |
| OCP duration           |                             |                  |                                 |                  |                                   |                  |
| Never                  | 430                         | 1.0              | 154                             | 1.0              | 157                               | 1.0              |
| Past (y)               | 258                         | 1.05 (0.88-1.24) | 93                              | 1.16 (0.87-1.53) | 101                               | 1.21 (0.92-1.59) |
| <5                     | 160                         | 1.09 (0.90-1.33) | 51                              | 1.09 (0.78-1.53) | 50                                | 0.99 (0.70-1.39) |
| >5                     | 81                          | 0.99 (0.77-1.27) | 37                              | 1.37 (0.93-2.00) | 46                                | 1.71 (1.20-2.44) |
| P trend                |                             | 0.82             |                                 | 0.25             |                                   | 0.08             |

NOTE: Adjusted by age at menopause, age at menarche, parity, type of menopause, PMH use, OCP use, smoking status, age at start smoking, cigarettes per day, time since quitting, fruit/vegetable intake, BMI, and environmental smoking exposure (parents smoking, years living with someone who smokes, exposure to smoking at work, exposure to smoking at home).

\*Hysterectomy only.

†Among parous women only.

**Table 4.** Hazard ratios of lung cancer histology subtypes by PMH use in postmenopausal women; 1984 to 2006

|              | All lung cancer<br>(n = 1,729) |                  | Adenocarcinoma<br>(n = 706) |                  | Squamous carcinoma<br>(n = 253) |                  | Small cell carcinoma<br>(n = 264) |                  |
|--------------|--------------------------------|------------------|-----------------------------|------------------|---------------------------------|------------------|-----------------------------------|------------------|
|              | n                              | HR (95% CI)      | n                           | HR (95% CI)      | n                               | HR (95% CI)      | n                                 | HR (95% CI)      |
| PMH use      |                                |                  |                             |                  |                                 |                  |                                   |                  |
| Never        | 436                            | 1.0              | 171                         | 1.0              | 76                              | 1.0              | 78                                | 1.0              |
| Past         | 440                            | 0.91 (0.79-1.04) | 178                         | 0.93 (0.74-1.16) | 68                              | 0.87 (0.61-1.24) | 71                                | 0.89 (0.63-1.26) |
| Current      | 455                            | 0.99 (0.86-1.14) | 224                         | 1.18 (0.95-1.47) | 52                              | 0.76 (0.52-1.12) | 57                                | 0.86 (0.60-1.25) |
| PMH type     |                                |                  |                             |                  |                                 |                  |                                   |                  |
| Never        | 436                            | 1.0              | 171                         | 1.0              | 76                              | 1.0              | 78                                | 1.0              |
| E            | 486                            | 0.99 (0.85-1.14) | 223                         | 1.09 (0.87-1.37) | 54                              | 0.73 (0.49-1.09) | 65                                | 0.91 (0.63-1.33) |
| E + P        | 249                            | 1.00 (0.85-1.18) | 106                         | 1.07 (0.83-1.38) | 44                              | 1.06 (0.71-1.57) | 33                                | 0.88 (0.57-1.36) |
| PMH duration |                                |                  |                             |                  |                                 |                  |                                   |                  |
| Never        | 436                            | 1.0              | 171                         | 1.0              | 76                              | 1.0              | 78                                | 1.0              |
| E (y)        |                                |                  |                             |                  |                                 |                  |                                   |                  |
| <5           | 139                            | 0.97 (0.80-1.19) | 51                          | 0.91 (0.66-1.25) | 20                              | 0.82 (0.49-1.36) | 26                                | 1.11 (0.70-1.77) |
| ≥5           | 347                            | 0.99 (0.84-1.17) | 172                         | 1.21 (0.94-1.56) | 34                              | 0.66 (0.41-1.05) | 39                                | 0.78 (0.50-1.22) |
| E + P (y)    |                                |                  |                             |                  |                                 |                  |                                   |                  |
| <5           | 85                             | 0.88 (0.70-1.12) | 35                          | 0.91 (0.62-1.32) | 14                              | 0.85 (0.47-1.53) | 14                                | 0.94 (0.52-1.69) |
| >5           | 164                            | 1.08 (0.89-1.30) | 71                          | 1.17 (0.88-1.57) | 30                              | 1.22 (0.78-1.91) | 19                                | 0.85 (0.50-1.44) |

NOTE: Adjusted by: age at menopause, age at menarche, parity, type of menopause, PMH use, OCP use, smoking status, age at start smoking, cigarettes per day, time since quitting, fruit/vegetable intake, BMI, and environmental smoking exposure (parents smoking, years living with someone who smokes, exposure to smoking at work, exposure to smoking at home).

Abbreviations: E + P, estrogen + progestin combination; E, unopposed estrogen.

49 years [hazard ratio, 1.39; 95% confidence interval (95% CI), 1.14-1.70] and OCP use for >5 years when compared with never OCP users (hazard ratio, 1.22; 95% CI, 1.05-1.42) were associated with increased risk for lung cancer incidence after adjusting for smoking, fruit/vegetable intake, BMI, environmental smoking exposure, and other exposure variables (Table 2). The increased risk with younger age at menopause was also seen when restricted to natural menopause (data not shown). When assessed separately by smoking status, these associations were primarily evident in current smokers. With regard to parity, greater number of children was associated with decreased risk among parous women ( $P$  trend = 0.03) in never smokers, whereas older age at first birth was associated with increased risk ( $P$  trend = 0.02) in current smokers. Risk for lung cancer was not associated with PMH use, age at menarche, or type of menopause in the overall study population or within categories of smoking status.

When assessed by histologic subtypes, risk for small cell carcinomas was increased with younger age at menopause ( $P$  trend = 0.008) and >5 years of OCP use (hazard ratio, 1.71; 95% CI, 1.20-2.44) and decreased with greater number of children among parous women ( $P$  trend = 0.01). A decreased risk (hazard ratio, 0.56; 95% CI, 0.28-1.10) was also observed for nulliparous versus parous women, although the small number of nulliparous women in this subgroup make this result unstable (Table 3). Although

no significant associations were seen for squamous cell or adenocarcinoma, an increased risk with older age at first birth seemed limited to adenocarcinoma.

We examined PMH use in more detail in relation to risk for specific lung cancer histologic subtypes (Table 4). No statistically significant results were observed, although patterns were observed. For adenocarcinoma, risk was increased among current PMH users and among those with ≥5 years of use for estrogen-only and estrogen plus progestin formulations. For squamous carcinoma, a trend of increased risk was seen with ≥5 years of estrogen plus progestin use.

## Discussion

In this analysis of reproductive factors and lung cancer incidence, younger age at menopause and longer duration of OCP use were associated with increased risk. These associations were strongest among current smokers and small cell histology, which likely reflects the high proportion of current smokers within this histologic subtype. Increased risk was also seen with fewer children among never smokers and older age at first birth among current smokers.

The literature on hormonal factors and lung cancer has expanded in the past several years, reflecting the increased interest in this field. However, the methods of

study design are heterogeneous across studies, which may explain the inconsistent reports in the literature. Despite the inconsistencies, several studies have reported an inverse association between age at menopause and lung cancer incidence. In a prospective analysis of the Shanghai Women's Health Study with ~75,000 participating Chinese women who were lifetime nonsmokers, the authors reported higher lung cancer risk with younger age at menopause (26). Other population based studies have also reported similar trends albeit not statistically significant (24, 27-29). However, there also are reports of null association (16, 22, 23) and one report of decreased risk with younger age at menopause (25).

About parity, two prospective cohort studies on never smokers have reported an inverse association, in which decreased risk was seen with increasing parity (23, 26). This is consistent with our results, in which the inverse relationship was seen primarily in never smokers. The role of parity in other studies that include smokers is less clear. With regard to the age at first birth, literature is inconsistent with reports of decreased (19, 22) and increased (23) risk with increasing age at first birth, as well as reports of no significant association (16, 26, 28, 29). The association with the use of OCPs is inconsistent as well, with a few reports of decreased risk (22, 24). However, most studies report a null association (16, 19, 20, 25, 26).

Our findings suggest that endogenous hormones during premenopausal years may have a protective role in lung cancer development. This is evident by the increased lung cancer risk with younger age at menopause and longer use of OCPs, in which the contraceptive mechanism is by suppressing ovulation and the release of endogenous hormones (34). The protective role seems to be greater among smokers and a possible mechanism may be the interaction between estrogen and cigarette smoke metabolism. *CYP1A1* and *CYP1B1*, members of the cytochrome P450 family of enzymes involved in phase I drug metabolism, are induced by cigarette smoking, and they activate cigarette smoke carcinogens such as polycyclic aromatic hydrocarbons and also participate in estradiol metabolism (35). Several investigators have hypothesized that this interaction may be the mechanism by which smokers are found to have lower estrogen levels compared with nonsmokers (36-38). Conversely, there are reports of *CYP1A1* and *CYP1B1* upregulation by estrogen- $\alpha$  and estrogen- $\beta$  receptors (35, 39, 40). The effect of estrogen on phase II metabolism enzymes is not clear, although increased glutathione S-transferase (*GSTT1*) mRNA expression has been associated with higher estradiol levels (35). This suggests that estrogen may influence the metabolism of smoking constituents and lead to differential lung cancer outcomes among smokers.

Another possible mechanism for the protective effect of premenopausal endogenous hormones may be the role of progesterone in lung cancer development. In a preclinical study by Ishibashi et al. (11), cellular proliferation was inhibited when lung cancer cell lines with progesterone receptors were incubated with progesterone. However, the

literature on progesterone in lung cancer is limited, and the role of progesterone and its interaction with estrogen in lung cancer remains unclear.

With regard to PMH use, we did not detect a significant association between PMH use and overall lung cancer incidence, although differential trends were seen in the various histologic subtypes. Although reproductive factors reflect the effect of hormonal exposure during premenopausal years, the use of hormone replacement therapy exerts its effect during postmenopausal years. As described earlier, the literature on PMH use and lung cancer is conflicting with reports of protective, null, and harmful associations (14-25). More recently, the Women's Health Initiative investigators reported an increase in mortality and a nonsignificant increase in incidence of non-small cell lung cancer in the women who were randomized to the estrogen and progestin combination arm (41). A similar trend has been reported in a prospective cohort study on never smokers, in which most cases were adenocarcinoma (23), and in two other case-control studies, in which the primary outcome was adenocarcinoma (24, 25). In addition, the Vitamins and Lifestyle study recently reported an increased risk for lung cancer incidence with estrogen and progestin use (42). We did not detect a significant increased risk with estrogen and progestin use, although a nonstatistically significant trend of increased risk was detected for squamous and adenocarcinoma. These findings, in addition to the results of our analysis, suggest that the role of PMHs likely differ from that of premenopausal reproductive hormones and that the effect may vary in the different histologic subtypes.

There are many challenges in elucidating the role of hormones in lung carcinogenesis. One of the challenges is the fact that there are many situations in which a hormonal function could be altered. For instance, the action of the same hormone may vary in premenopausal versus postmenopausal women, smokers versus never smokers, and in the pathogenesis of tumors of various histologic subtypes. Furthermore, the effect of hormones may also depend on the receptor status of lung cancer cells. In a study by Schwartz et al. (16), the investigators found a protective association between PMH use and lung cancer, but the association was seen primarily in lung cancers that harbored estrogen receptors. This suggests that the role of reproductive hormones, as well as exogenous hormones, may be restricted to those cancers that possess the receptors.

There are several strengths in this study. First, this study is prospective in design, which avoids the biases associated with retrospective studies, and the large number of cases and participants with long duration of follow-up have allowed us to do various subgroup analyses. Although the number of exposure variables and subgroups in this study increases the likelihood of chance findings, all analyses were hypothesis driven and exposure variables were chosen *a priori*, thus no adjustment was made for multiple comparisons. Another strength

of this study is that information on exposures and smoking variables was updated every 2 years, which reduces misclassification bias. A limitation is the small number of cases among never smokers. Accordingly, the lack of significant findings in never smokers may be due to the small number of cases.

In conclusion, the results of our study add to the increasing body of literature that female hormones may influence lung carcinogenesis. Their role is likely modest, especially in the presence of powerful risk factors such as smoking. However, additional research of this topic is warranted because it may lead to a better understanding of lung cancer biology and possibly to the development of novel therapeutic strategies. To achieve this goal, an integrative approach will be necessary spanning from

molecular and clinical epidemiology research to clinical prevention and therapeutic trials.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

### Grant Support

Grant CA87979 from the NIH and a grant from the Lung Cancer Research Foundation.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 04/30/2010; revised 07/20/2010; accepted 07/21/2010; published OnlineFirst 08/25/2010.

### References

- Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics 2009. *CA Can J Clin Oncol* 2009;59:225-49.
- Radzikowska E, Glaz P, Roszkowski K. Lung cancer in women: age, smoking, histology, performance status, stage, initial treatment and survival. Population study of 20,561 cases. *Ann Oncol* 2002;13:1087-93.
- Patel JD. Lung cancer in women. *J Clin Oncol* 2005;23:3212-8.
- Thun MJ, Lally CA, Glannery JT, Calle EE, Flanders WD, Heath CW. Cigarette smoking and changes in the histopathology of lung cancer. *J Natl Cancer Inst* 1997;89:1580-6.
- Tam IYS, Chung LP, Suen WS, et al. Distinct epidermal growth factor receptor and KRAS mutation patterns in non-small cell lung cancer patients with different tobacco exposure and clinicopathologic features. *Clin Cancer Res* 2006;12:1647-53.
- Shigematsu H, Lin L, Takahashi T, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst* 2005;97:339-46.
- Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;350:2129-39.
- Shepherd FA, Pereira JR, Ciuleanu T, et al. Erlotinib in previously treated non-small cell lung cancer. *N Engl J Med* 2005;353:123-32.
- Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004;304:1497-500.
- Stabile LP, Gaither Davis AL, Gubish CT, et al. Human non-small cell lung tumors and cells derived from normal lung express both estrogen receptor  $\alpha$  and  $\beta$  and show biological responses to estrogen. *Cancer Res* 2002;62:2141-50.
- Ishibashi H, Suzuki T, Suzuki S, et al. Progesterone receptor in non-small cell lung cancer—a potent prognostic factor and possible target for endocrine therapy. *Cancer Res* 2005;65:6450-8.
- Stabile LP, Lyker JS, Gubish CT, Zhang W, Grandis JR, Siegfried JM. Combined targeting of estrogen receptor and the epidermal growth factor receptor in non-small cell lung cancer shows enhanced anti-proliferative effects. *Cancer Res* 2005;65:1459-70.
- Hammoud Z, Tan B, Badve S, Bigsby RM. Estrogen promotes tumor progression in a genetically defined mouse model of lung adenocarcinoma. *Endocr Relat Cancer* 2008;15:475-83.
- Rodriguez C, Spencer H, Deka A, et al. Postmenopausal hormone therapy and lung cancer risk in the Cancer Prevention Study II Nutrition Cohort. *Cancer Epidemiol Biomarkers Prev* 2008;17:655-60.
- Ramnath N, Menezes RJ, Loewen G, et al. Hormone replacement therapy as a risk factor for non-small cell lung cancer: results of a case-control study. *Oncology* 2007;73:305-10.
- Schwartz AG, Wenzlaff AS, Prysak GM, et al. Reproductive factors, hormone use, estrogen receptor expression and risk of non-small-cell lung cancer in women. *J Clin Oncol* 2007;25:5785-92.
- Chen KY, Hsiao CF, Chang GC, et al. Hormone replacement therapy and lung cancer risk in Chinese. *Cancer* 2007;110:1768-75.
- Schabath MB, Wu X, Vassilopoulou-Sellin R, Vaporciyan AA, Spitz MR. Hormone replacement therapy and lung cancer risk: a case-control analysis. *Clin Cancer Res* 2004;10:113-23.
- Kabat GC, Miller AB, Rohan TE. Reproductive and hormonal factors and risk of lung cancer in women: a prospective cohort study. *Int J Cancer* 2007;120:2214-20.
- Elliott AM, Hannaford PC. Use of exogenous hormones by women and lung cancer: evidence from the Royal College of General Practitioners' Oral Contraception Study. *Contraception* 2006;73:331-5.
- Blackman JA, Coogan PF, Rosenberg L, et al. Estrogen replacement therapy and risk of lung cancer. *Pharmacoepidemiol Drug Saf* 2002;11:561-7.
- Kreuzer M, Gerken M, Heinrich J, Kreienbrock L, Wichmann H. Hormonal factors and risk of lung cancer among women? *Int J Epidemiol* 2003;32:263-71.
- Liu Y, Inoue M, Sobue T, Tsugane S. Reproductive factors, hormone use and the risk of lung cancer among middle-aged never-smoking Japanese women: a large-scale population-based cohort study. *Int J Cancer* 2005;117:662-6.
- Wu AH, Yu MC, Thomas DC, Pike MC, Henderson BE. Personal and family history of lung disease as risk factors for adenocarcinoma of the lung. *Cancer Res* 1988;48:7279-84.
- Taioli E, Wynder EL. Re: endocrine factors and adenocarcinoma of the lung in women. *J Nat Cancer Inst* 1994;86:869-70.
- Weiss JM, Lacey JV, Shu X, et al. Menstrual and reproductive factors in association with lung cancer in female lifetime nonsmokers. *Am J Epidemiol* 2008;168:1319-25.
- Zatloukal P, Kubik A, Pauk N, Tomasek L, Petruzella L. Adenocarcinoma of the lung among women: risk associated with smoking, prior lung disease, diet and menstrual and pregnancy history. *Lung Cancer* 2003;41:283-93.
- Brenner AV, Wang Z, Kleinerman RA, et al. Menstrual and reproductive factors and risk of lung cancer among Chinese women, Eastern Gansu Province, 1994-1998. *J Epidemiol* 2003;13:22-8.
- Koushik A, Parent ME, Siemiatycki J. Characteristics of menstruation and pregnancy and the risk of lung cancer in women. *Int J Cancer* 2009;125:2428-33.
- Feskanich D, Ziegler RG, Michaud DS. Prospective study of fruit and vegetable consumption and risk of lung cancer among men and women. *J Natl Cancer Inst* 2000;92:1812-23.
- Ries LAG, Eisner MP. Cancer of the Lung. In: Ries LAG, Young JL, Keel GE, et al, editors. SEER survival monograph: cancer survival among adults: U.S. SEER program, 1988-2001, patient and tumor

- characteristics. Bethesda: National Cancer Institute, SEER Program, NIH Pub. No 07-6215; 2007, p. 73–80.
32. Willett WC, Sampson LS, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol* 1984;122:51–65.
  33. Feskanich D, Rimm EB, Giovannucci EL, et al. Reproducibility and validity of food intake measurements from a semiquantitative food frequency questionnaire. *J Am Diet Assoc* 1993;93:790–6.
  34. Sondheimer SJ. Oral contraceptives: mechanism of action, dosing, safety and efficacy. *Cutis* 2008;81:S19–22.
  35. Spivack SD, Hurteau GJ, Fasco MJ, Kaminsky LS. Phase I and II carcinogen metabolism gene expression in human lung tissue and tumors. *Clin Cancer Res* 2003;9:6002–11.
  36. McDivitt AM, Greendale GA, Stanczyk FZ, Huang MH. Effects of alcohol and cigarette smoking on change in serum estrone levels in postmenopausal women randomly assigned to fixed doses of conjugated equine estrogens with or without a progestin. *Menopause* 2008;15:382–5.
  37. Jensen J, Christiansen C, Rodbro P. Cigarette smoking, serum estrogens, and bone loss during hormone-replacement therapy early after menopause. *N Engl J Med* 1985;313:973–5.
  38. Bjarnason NH, Wilkinson GR. Drug metabolism and variability. *N Engl J Med* 2005;353:955–6.
  39. Tsuchiya Y, Nakajima M, Kyo S, Kanaya T, Inoue M, Yokoi T. Human CYP1B1 is regulated by estradiol via estrogen receptor. *Cancer Res* 2004;64:3119–25.
  40. Han W, Pentecost BT, Bietropaolo RL, Fasco MJ, Spivack SD. Estrogen receptor  $\alpha$  increases basal and cigarette smoke extract-induced expression of CYP1A1 and CYP1B1, but not GSTP1, in normal human bronchial epithelial cells. *Mol Carcinogen* 2005;44:202–11.
  41. Chlebowski RT, Schwartz AG, Wakelee H, et al. Oestrogen plus progestin and lung cancer in postmenopausal women (Women's Health Initiative trial): a post-hoc analysis of a randomised controlled trial. *Lancet* 2009;374:1243–51.
  42. Slatore CG, Chien JW, Au DH, et al. Lung cancer and hormone replacement therapy: association in the Vitamins and Lifestyle Study. *J Clin Oncol* 2010;9:1540–6.