The Association of Plasma DHEA and DHEA Sulfate with Breast Cancer Risk in Predominantly Premenopausal Women

Shelley S. Tworoger,1,3 Stacey A. Misser,1,2,3 A. Heather Eliassen,1,3 Donna Spiegelman,4 Elizabeth Folkerd,5 Mitch Dowsett,5 Robert L. Barbieri,2 and Susan E. Hankinson1,3

1Channing Laboratory, Department of Medicine and 2Department of Obstetrics, Gynecology, and Reproductive Medicine, Brigham and Women’s Hospital and Harvard Medical School; Departments of 3Epidemiology and 4Biostatistics, Harvard School of Public Health, Boston, Massachusetts; and 5Academic Department of Biochemistry, Royal Marsden Hospital, London, United Kingdom

Abstract

Concentrations of adrenal androgens are positively associated with postmenopausal breast cancer risk; however, results in premenopausal women are conflicting. Therefore, we conducted a prospective nested case-control study within the Nurses’ Health Study II cohort to examine the relationship of DHEA and DHEA sulfate (DHEAS) with breast cancer risk in predominantly premenopausal women. Blood samples were collected from 1996 to 1999. The analysis included 317 cases of breast cancer diagnosed after blood collection and before June 1, 2003; for each case, two controls were matched on age, fasting status, time of day and month of blood collection, race/ethnicity, and timing of blood draw within the menstrual cycle. No associations were observed between DHEA or DHEAS levels and breast cancer risk overall [in situ and invasive; DHEA relative risk (RR), top versus bottom quartile, 1.2; 95% confidence interval (95% CI), 0.8-1.8, P_trend = 0.53; DHEAS RR, 1.3; 95% CI, 0.9-2.0; P_trend = 0.07]. However, both DHEA and DHEAS were positively associated with estrogen receptor–positive/progesterone receptor–positive breast cancer (DHEA RR, 1.6; 95% CI, 0.9-2.8, P_trend = 0.09; DHEAS RR, 1.9; 95% CI, 1.1-3.3, P_trend = 0.02). We observed a significant interaction by age, with an RR for DHEAS of 0.8 (95% CI, 0.4-1.5, P_trend = 0.62) for women <45 years old and 2.0 (95% CI, 1.2-3.5, P_trend = 0.003) for women ≥45 years old; results were similar for DHEA. Our results suggest that adrenal androgens are positively associated with breast cancer among premenopausally premenopausal women, especially for estrogen receptor–positive/progesterone receptor–positive tumors and among women over age 45 years. (Cancer Epidemiol Biomarkers Prev 2006;15(5):967–71)

Introduction

In vitro and animal evidence suggests that the adrenal androgens, DHEA and its sulfate (DHEAS), are important in the development of breast cancer; however, the mechanisms underlying this association are unclear (1). DHEA and DHEAS inhibit growth of some breast cancer cell lines while stimulating growth in others (2); DHEA can directly activate estrogen receptor α (ER-α) expression in MCF7 breast cancer cell lines (3). One hypothesis suggests that in a high estrogen setting (e.g., in premenopausal women), DHEA and DHEAS exhibit antiestrogenic effects, but in a low estrogen setting (e.g., in postmenopausal women) they exhibit weak estrogenic effects (4, 5). Epidemiologic data suggest a positive association with postmenopausal breast cancer (6), but both positive and inverse associations have been observed in younger women (7-11). Therefore, we evaluated the relationship of DHEA and DHEAS with breast cancer in predominately premenopausal women in a prospective nested case-control study within the Nurses’ Health Study II cohort.

Materials and Methods

Study Population. The Nurses’ Health Study II was established in 1989, when 116,609 female registered nurses, ages 25 to 42 years, completed a questionnaire. The cohort has been followed biennially since inception to update exposure variables and ascertain newly diagnosed disease. The racial/ethnic breakdown is 94% White, 2% Asian, 2% African-American, and 2% Hispanic.

Between 1996 and 1999, 29,611 cohort members, ages 32 to 54 years, provided a blood sample (described in ref. 12). Briefly, premenopausal women who had not taken hormones, been pregnant, or breastfed within 6 months (n = 18,521) answered a short questionnaire and provided timed blood samples on the 3rd to 5th day of their menstrual cycle (folllicular draw) and 7 to 9 days before the anticipated start of their next cycle (luteal draw). Follicular plasma was aliquoted by the participant 8 to 24 hours after collection and frozen. All other women (n = 11,090) provided a single 30 mL, untimed blood sample. Luteal and untimed samples were shipped via overnight courier, processed by our laboratory, and separated into plasma, RBC, and WBC components. Samples have been stored in continuously monitored, liquid nitrogen freezers since collection. Menopausal status determination for women providing untimed samples has been described previously (12) and is based on whether the woman’s periods have ceased, ovarian status, smoking status, and age. Follow-up of the blood cohort was 98% in 2003. The study was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women’s Hospital.

Cases had no previously reported cancer diagnosis and were diagnosed with breast cancer after blood collection but before June 1, 2003. Overall, 317 cases of breast cancer were reported and confirmed by medical record review (n = 298) or verbal confirmation by the nurse (n = 19). Given the 99% confirmation rate on medical record review, these latter cases were included. Mean time from blood draw to diagnosis was 31 months (range, 1-87). Each case was matched to two controls on age (±2 years), menopausal status at blood collection and diagnosis, month/year of blood draw (±2 months),
ethnicity (African-American, Asian, Hispanic, Caucasian, other), luteal day of the blood collection (timed samples only, date of next period-date of luteal draw, ±1 day), and, for each blood draw, time of day (±2 hours) and fasting status (<2, 2-4, 5-7, 8-11, and 12-24 hours). For each matching variable, >90% of matches were exact.

**Laboratory Assays.** DHEA, DHEAS, and progesterone were assayed in luteal and untimed samples by two of the authors (E.F. and M.D.). DHEA was assayed by RIA (Diagnostic Systems Laboratories, Webster, TX), whereas DHEAS and progesterone were measured by chemiluminescent immunoassay using the Immulite autoanalyzer (Diagnostic Products Corp., Llanberis, United Kingdom; ref. 8). Estradiol (in timed samples only) and testosterone were assayed at Quest Diagnostic’s Nichols Institute (San Juan Capistrano, CA) as previously described (13). These hormones are stable in whole blood not processed for 24 to 48 hours (14).

Case-control sets were assayed together. Samples, assayed in two batches, were ordered randomly and labeled to mask case-control status. Each batch included replicate plasma samples to assess interassay coefficient of variation (6-12%).

**Statistical Analysis.** We considered all women (timed and untimed) together, using batch-specific quartile cutoffs for DHEA and cutoff points across batches for DHEAS (based on control distributions), because, although the correlations from a subset of 12 samples run in both batches were >0.95, the mean concentrations differed by batch for DHEA, suggesting some laboratory drift. We did not identify any statistical outliers (15). Some samples had missing hormone values related to technical difficulties or low volume; the sample size varied by hormone (see Table 1; estradiol: 185 cases/368 controls, testosterone: 302 cases/605 controls). We used mixed-effects regression to test log-transformed hormone differences between matched cases and controls.

We used conditional logistic regression to estimate relative risks (RR) and 95% confidence intervals (95% CI; ref. 16). To maximize statistical power, RRs and 95% CIs for various case groups (invasive only, estrogen receptor (ER) positive/progesterone receptor (PR) positive time between blood draw and diagnosis) and stratified by age at blood (<45 years, <50 years, <65 years, and >65 years), body mass index (BMI) at blood (<24 versus ≥24 kg/m²), family history of breast cancer, history of benign breast disease, and oral contraceptive use history (never/previous years) were calculated. We considered adjustment for estradiol and progesterone separately.

We used conditional logistic regression to estimate relative risks (RR) and 95% confidence intervals (95% CI; ref. 16). To maximize statistical power, RRs and 95% CIs for various case groups (invasive only, estrogen receptor (ER) positive/progesterone receptor (PR) positive time between blood draw and diagnosis) and stratified by age at blood (<45 years, <50 years, <65 years, and >65 years), body mass index (BMI) at blood (<24 versus ≥24 kg/m²), family history of breast cancer, history of benign breast disease, and oral contraceptive use history (never/previous years) were calculated. We considered adjustment for estradiol and progesterone separately.

The associations significantly differed by age (Pinteraction-DHEA = 0.02 and DHEAS = 0.04; Table 3). For DHEA, the RR was 0.6 (top versus bottom quartile, 95% CI, 0.3-1.3; Ptrend = 0.16) among women <45 years and was 1.9 (95% CI, 1.1-3.3; Ptrend = 0.02) among women >45 years. For DHEAS, the comparable results were 0.8 (95% CI, 0.4-1.5; P = 0.04) and 2.0 (95% CI, 1.2-3.5; P = 0.03), respectively. Adjusting for estradiol or testosterone levels did not substantially alter the results.

**Results**

A higher proportion of cases versus controls had a family history of breast cancer and a history of benign breast disease (Table 1). Differences for other risk factors were generally small, but in the expected direction. Median DHEAS levels were modestly higher for cases than controls (P = 0.08), but DHEA levels were similar between groups (P = 0.46).

Modest, nonsignificant associations were observed between DHEA or DHEAS and breast cancer risk among all women or premenopausal women with an ovulatory cycle and a normal luteal phase length (Table 2). Both hormones were positively associated with ER+/PR+ breast cancer (DHEA RR, top versus bottom quartile, 1.6; 95% CI, 0.9-2.8; P trend = 0.09; DHEAS RR, 1.9; 95% CI, 1.1-3.3; P trend = 0.02). Neither hormone seemed to be associated with ER–/PR+ tumors (n = 34 cases, comparable RR = 1.1 for both, P trend > 0.40). Adjustment for estradiol did not change the results (data not shown); however, adjustment for testosterone attenuated the RRs for ER+/PR+ tumors (DHEA RR, 1.2; 95% CI, 0.7-2.2; Ptrend = 0.60 and DHEAS RR, 1.4; 95% CI, 0.8-2.6; Ptrend = 0.21).

The associations significantly differed by age (P interaction-DHEA = 0.02 and DHEAS = 0.04; Table 3). For DHEA, the RR was 0.6 (top versus bottom quartile, 95% CI, 0.3-1.3; P trend = 0.16) among women <45 years and was 1.9 (95% CI, 1.1-3.3; P trend = 0.02) among women >45 years. For DHEAS, the comparable results were 0.8 (95% CI, 0.4-1.5; P trend = 0.62) and 2.0 (95% CI, 1.2-3.5; P trend = 0.03), respectively. Adjusting for estradiol or testosterone levels did not substantially alter the results.
Table 2. RR (95% CI) of breast cancer according to quartile of plasma DHEA and DHEAS levels

<table>
<thead>
<tr>
<th>Plasma hormone</th>
<th>No. cases/controls</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>$P_{\text{trend}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHEA (ng/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cutpoints*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All (simple)</td>
<td>314/631</td>
<td>1.0</td>
<td>1.0</td>
<td>1.2</td>
<td>1.1</td>
<td>0.02</td>
</tr>
<tr>
<td>All (multivariate)</td>
<td>314/631</td>
<td>1.0</td>
<td>1.1</td>
<td>1.2</td>
<td>1.2</td>
<td>0.05</td>
</tr>
<tr>
<td>Premenopausal</td>
<td>208/422</td>
<td>1.0</td>
<td>1.2</td>
<td>1.1</td>
<td>1.0</td>
<td>0.13</td>
</tr>
<tr>
<td>Invasive</td>
<td>217/631</td>
<td>1.0</td>
<td>0.8</td>
<td>1.3</td>
<td>1.2</td>
<td>0.24</td>
</tr>
<tr>
<td>ER+/PR+</td>
<td>142/631</td>
<td>1.0</td>
<td>1.1</td>
<td>1.6</td>
<td>1.6</td>
<td>0.09</td>
</tr>
<tr>
<td>DHEAS (ug/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cutpoints*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All (simple)</td>
<td>315/630</td>
<td>1.0</td>
<td>0.9</td>
<td>1.2</td>
<td>1.3</td>
<td>0.06</td>
</tr>
<tr>
<td>All (multivariate)</td>
<td>315/630</td>
<td>1.0</td>
<td>0.9</td>
<td>1.2</td>
<td>1.3</td>
<td>0.06</td>
</tr>
<tr>
<td>Premenopausal</td>
<td>208/421</td>
<td>1.0</td>
<td>0.7</td>
<td>1.1</td>
<td>1.3</td>
<td>0.08</td>
</tr>
<tr>
<td>Invasive</td>
<td>218/630</td>
<td>1.0</td>
<td>0.9</td>
<td>1.2</td>
<td>1.4</td>
<td>0.05</td>
</tr>
<tr>
<td>ER+/PR+</td>
<td>143/630</td>
<td>1.0</td>
<td>1.1</td>
<td>1.4</td>
<td>1.9</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*Cutpoints shown are based on all controls; however, for DHEA, batch-specific cutpoints used were as follows: batch 1 = 499, 499 to 679, 680 to 884, and batch 2 = 1357, 358 to 522, 523 to 723, 724.

Table 3. Multivariate RR (95% CI) of breast cancer according to quartile of plasma DHEA and DHEAS levels, stratified by age

<table>
<thead>
<tr>
<th>Plasma hormone</th>
<th>No. cases</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>$P_{\text{trend}}$</th>
<th>$P_{\text{interaction}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHEA (ng/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All women*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age &lt;45 y</td>
<td>134</td>
<td>1.0</td>
<td>0.8</td>
<td>0.9</td>
<td>0.6</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Age ≥45 y</td>
<td>180</td>
<td>1.0</td>
<td>1.0</td>
<td>1.3</td>
<td>1.7</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Premenopausal women†</td>
<td>114</td>
<td>1.0</td>
<td>0.7</td>
<td>0.8</td>
<td>0.5</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Age &lt;45 y</td>
<td>94</td>
<td>1.0</td>
<td>1.7</td>
<td>1.3</td>
<td>2.0</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Age ≥45 y</td>
<td>133</td>
<td>1.0</td>
<td>0.7</td>
<td>1.0</td>
<td>0.8</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>Premenopausal women†</td>
<td>182</td>
<td>1.0</td>
<td>0.8</td>
<td>1.5</td>
<td>2.0</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Age &lt;45 y</td>
<td>113</td>
<td>1.0</td>
<td>0.6</td>
<td>0.8</td>
<td>0.6</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Age ≥45 y</td>
<td>95</td>
<td>1.0</td>
<td>0.6</td>
<td>1.5</td>
<td>2.5</td>
<td>0.04</td>
<td></td>
</tr>
</tbody>
</table>

*Number of cases <45 years by quartile for DHEA are 22, 35, 39, and 38, and for DHEAS are 24, 29, 37, and 43. Number of cases ≥45 years by quartile for DHEA are 51, 39, 46, and 44, and for DHEAS are 51, 36, 43, and 52.
†Excluding women with anovulatory cycles (progesterone <400 ng/dL) or whose luteal phase day was <3 or >21. Number of cases <45 years by quartile for DHEA are 19, 30, 33, and 32, and for DHEAS are 20, 24, 32, and 37. Number of cases ≥45 years by quartile for DHEA are 20, 27, 24, and 23, and for DHEAS are 21, 13, 28, and 33.

Discussion

We found that DHEA and DHEAS were modestly, but not significantly, associated with breast cancer in predominantly premenopausal women; both hormones were positively associated with risk of ER+/PR+ tumors. We also observed a possible trend toward an inverse association in women <45 years old and a significant positive association among women ≥45 years old.

Biological data of DHEA and DHEAS effects on breast tumors are conflicting. DHEA and DHEAS are primarily produced by the adrenal gland, although DHEA can be produced in the ovaries (19), and are androgen and estrogen precursors (20, 21). DHEA and DHEAS inhibit breast cell proliferation possibly through epidermal growth factor (26), although they can stimulate proliferation, especially in a low-estrogen environment (1, 3, 22, 24, 25). Thus, DHEA and DHEAS may act as estrogen agonists in a high estrogen environment, but act as weak estrogen agonists in a low-estrogen environment (1, 2, 27). A second hypothesis posits that women who develop breast cancer have a blunted age-related decline in DHEA and DHEAS levels (1, 17, 29, 29); that is, compared with healthy controls, women who develop breast cancer in early- to mid-premenopausal years have similar or lower DHEA or DHEAS levels.
levels but those diagnosed in late-premenopausal and postmenopausal years have higher levels (1).

Epidemiologic data generally do not support the hypothesis of different associations by estrogen levels. Case-control studies in premenopausal women in which blood samples were collected after breast cancer diagnosis generally reported no associations (30, 31); however, major surgery can alter levels (32) possibly explaining these results. One case-control study collected serum several weeks after surgery and reported a significant positive association for DHEA (odds ratio, top versus bottom quartile, 2.7; ref. 32).

Results from prospective studies in premenopausal women seem contradictory. Three studies (n = 15-116 cases) reported inverse (10, 11) or nonsignificant positive associations (9). Two larger studies (n > 300 cases) reported no association in older premenopausal women (8) and a significant positive association for DHEAS that was stronger in women <49 years (7). In contrast, we observed a positive association among women ≥45 years old and a suggestion of an inverse association among those <45 years. This was not related to different proportions of ER+/PR+ tumors (<45 years: 67%, ≥45 years: 65%) or follicular/luteal estradiol concentrations (<45 years: 45/117 pg/mL, ≥45 years: 43/114 pg/mL).

Although the reasons for the conflicting results are unclear, large prospective studies generally observed a positive association between DHEA and DHEAS levels and breast cancer in premenopausal women, although this population has high estrogen levels. This suggests that these hormones may not act as an estrogen antagonist in premenopausal women. Conversely, our observed interaction with age supports the hypothesis that women who develop breast cancer have a blunted DHEA and DHEAS decline, such that levels in cases are lower at younger ages and higher at older ages than controls. These results should be interpreted with caution because no similar interaction was observed in other studies (7, 8).

Additional studies are needed to address this issue in detail.

We also observed a stronger association among women with ER+/PR+ tumors, suggesting that DHEA and DHEAS may act directly or indirectly through the ER (33). In vitro data suggest that DHEA can directly activate ERα expression in MCF7 cells (3). Adjustment for testosterone attenuated the observed association of DHEA and DHEAS with ER+/PR+ tumors, suggesting that the association may in part be due to conversion of these hormones to testosterone. However, given the sample size and correlation between DHEA or DHEAS and testosterone (Spearman r ~ 0.5), the independent influences of individual hormones need to be reevaluated with further follow-up.

One limitation of this study is the laboratory drift over time for DHEA, which precluded us from considering absolute DHEA concentrations and risk; however, there was no laboratory drift for DHEAS. Although this study had a relatively large sample size, we had limited power for some subgroup analyses, requiring further follow-up and reevaluation. Although we only had one blood sample, DHEA and DHEAS are relatively stable over time (intraclass correlation = 0.66 and 0.81, respectively), so there is little misclassification (34). Additionally, plasma DHEA levels may reasonably reflect breast tissue levels (35).

In this study of predominantly premenopausal women, we found that DHEA and DHEAS were positively associated with breast cancer risk, particularly for ER+/PR+ tumors and in older women, supporting the hypothesis that breast cancer patients have a blunted age-related decline in these hormones. However, other studies have reported conflicting results about an age interaction (7, 8). Given the positive association of DHEA and DHEAS with postmenopausal breast cancer risk (6), it is important to further explore these relationships in premenopausal women with continued follow-up in this and other cohorts.

Acknowledgments
We thank Drs. Graham Colditz, David Hunter, and Walter Willett for their helpful comments on the manuscript.

References


The Association of Plasma DHEA and DHEA Sulfate with Breast Cancer Risk in Predominantly Premenopausal Women


Updated version
Access the most recent version of this article at:
[http://cebp.aacrjournals.org/content/15/5/967](http://cebp.aacrjournals.org/content/15/5/967)

Cited articles
This article cites 30 articles, 8 of which you can access for free at:
[http://cebp.aacrjournals.org/content/15/5/967.full#ref-list-1](http://cebp.aacrjournals.org/content/15/5/967.full#ref-list-1)

Citing articles
This article has been cited by 6 HighWire-hosted articles. Access the articles at:
[http://cebp.aacrjournals.org/content/15/5/967.full#related-urls](http://cebp.aacrjournals.org/content/15/5/967.full#related-urls)

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.