

## Relationship of Serum Dehydroepiandrosterone (DHEA), DHEA Sulfate, and 5-Androstene-3 $\beta$ ,17 $\beta$ -diol to Risk of Breast Cancer in Postmenopausal Women

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### Abstract

**Laboratory evidence suggests a role for dehydroepiandrosterone (DHEA) and its metabolite 5-androstene-3 $\beta$ ,17 $\beta$ -diol (ADIOL) in mammary tumor growth. Serum DHEA also has been related to breast cancer in postmenopausal women, but the relationship of ADIOL to risk has not been evaluated previously. To assess the relationship of serum DHEA, its sulfate (DHEAS), and ADIOL with breast cancer risk in postmenopausal women, we conducted a prospective nested case-control study using serum from the Columbia, MO Breast Cancer Serum Bank. Cases included 71 healthy postmenopausal volunteers not taking replacement estrogens when they donated blood and who were diagnosed with breast cancer up to 10 years later (median, 2.9 years). Two randomly selected controls, who also were postmenopausal and not taking estrogens, were matched to each case on exact age, date ( $\pm 1$  year), and time ( $\pm 2$  h) of blood collection. Significant (trend  $P = 0.02$ ) gradients of increasing risk of breast cancer were observed for increasing concentrations of DHEA and ADIOL, and women whose serum levels of these hormones were in the highest quartiles were at a significantly elevated risk compared to those in the lowest; their risk ratios were 4.0 [95% confidence interval (CI), 1.3–11.8] and 3.0 (95% CI, 1.0–8.6), respectively. The relationship of DHEAS to breast cancer was less consistent, but women whose serum DHEAS**

**concentration was in the highest quartile also exhibited a significantly elevated risk ratio of 2.8 (95% CI, 1.1–7.4). Results of this prospective study support a role for the adrenal androgens, DHEA, DHEAS, and ADIOL, in the etiology of breast cancer.**

### Introduction

In the Washington County, MD, cohort, serum levels of DHEA<sup>2</sup> were elevated in postmenopausal women who subsequently were diagnosed with breast cancer (1), but they were depressed in premenopausal women who developed breast cancer (2). Furthermore, in postmenopausal breast cancer patients, 24-h mean plasma levels of DHEA and DHEAS were raised, but in premenopausal patients they were lowered (3). Similarly, DHEA has been reported to stimulate 7,12-dimethylbenz(a)anthracene-induced mammary tumor growth in oophorectomized rats but to decrease tumor progression in intact animals with higher circulating estradiol levels (4). The apparent dual character of the action of DHEA may be due to its metabolite ADIOL, which *in vitro* can act as an estrogen or androgen depending on the estradiol concentration of the medium. ADIOL binds to the estrogen receptor and at physiological concentrations has been shown to stimulate proliferation of estrogen-sensitive MCF-7 breast cancer cells grown in estradiol-deficient medium (5–7). However, when MCF-7 cells were grown in estradiol-rich medium, estrogen receptors were occupied and ADIOL acted as an androgen, inhibiting cell proliferation (8). Because serum estradiol concentrations of post-menopausal women are low relative to premenopausal women, the opposite associations of DHEA with breast cancer reported previously for pre- and postmenopausal women could be explained by its metabolite ADIOL. We, therefore, used the Columbia, MO, Breast Cancer Serum Bank established as part of the National Cancer Institute's Biological Markers Project to evaluate the relationship of DHEA, its sulfate (DHEAS), and ADIOL with the subsequent development of breast cancer in postmenopausal women.

### Materials and Methods

The study, which utilized a prospective nested case-control design, has been described in detail previously (9). Participants were volunteers identified through three sources: the Breast Cancer Detection Demonstration Project; Women's Cancer Control Program at the Cancer Research Center, the University of Missouri Health Sciences Center; and the Ellis Fischel Cancer Center (Columbia, MO). A total of 7224 women who initially were free of breast cancer donated blood to the bank on

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<sup>2</sup> The abbreviations used are: DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; ADIOL, 5-androstene-3 $\beta$ ,17 $\beta$ -diol; FSH, follicle-stimulating hormone; RR, risk ratio; CI, confidence interval; ACTH, adrenocorticotrophic hormone.

one or more occasions. Recruitment into the cohort was ongoing between 1977 and 1987, although over 90% of the women first gave blood in 1980 or earlier. Active follow-up by mail continued until 1989, but 70% of the cohort were last contacted in 1982–1983, at least partly because of funding changes. At the time of last contact, 91% of the total cohort were alive and free of breast cancer, 2% had been diagnosed with breast cancer, and 5% were dead from a cause other than breast cancer. Pathology reports were obtained for all women who reported a positive breast biopsy or mastectomy on follow-up.

Women included in the current study were restricted to those who had at least 4 ml of blood remaining in the bank and who, at the time of blood collection, had no history of cancer other than nonmelanoma skin cancer, were not diagnosed with benign breast disease within the past 2 years, were postmenopausal, and did not report taking replacement estrogens. Women were classified initially as postmenopausal if they reported natural menopause, bilateral oophorectomy, or radiation to the ovaries prior to blood collection or were at least 51 years of age at blood collection with a history of hysterectomy without oophorectomy. Final determination of menopausal status was based on serum FSH levels. Any woman with a FSH less than 35 mIU/ml was considered potentially premenopausal, and her reported date of last menses, age, and hormonal profile were reviewed to determine eligibility.

Of the 3375 women who met these criteria, 72 subsequently were diagnosed with histologically confirmed breast cancer. For each of these cases, two controls were selected from among the eligible women using incidence-density sampling. Controls were alive and free of cancer (except nonmelanoma skin) at the age of the case's diagnosis and were matched to the case on exact age at blood collection and on the date ( $\pm 1$  year) and time ( $\pm 2$  h) of the blood draw. Two controls who met the matching criteria could not be identified for 12 cases. For these cases, matching criteria were relaxed as follows: (a) age,  $\pm 1$  year ( $n = 8$ ); (b) blood draw,  $\pm 2$  years ( $n = 3$ ); and (c) age,  $\pm 4$  years and blood draw date  $\pm 2$  years and time  $\pm 4$  h ( $n = 1$ ). Following review of FSH results, one case and four controls were dropped because they were premenopausal, and one control was dropped because her hormone profile was consistent with exogenous estrogen use. This left 66 case-control sets with two controls and 5 case-control sets with one control for analysis.

Serum specimens were collected, and clinical data, including age, height, weight, menstrual and reproductive histories, smoking, medication (including hormone) use, and family history of breast cancer, were obtained by self-report or medical record review after obtaining informed consent. Approximately 10 ml of serum was collected from each woman using standard procedures. Blood was chilled immediately and serum was separated and aliquoted into glass vials within 2 h of collection. Serum was shipped on dry ice to the Mayo Foundation repository, where it was maintained at  $-70^{\circ}\text{C}$  until analysis. Blood was stored for a median of 16 years prior to analysis for both cases and controls.

Serum from each case and her matched control(s) were grouped and analyzed together in the same batch. DHEA, DHEAS, and ADIOL were measured by specific RIAs. A direct RIA kit (ICN Biomedicals, Inc., Costa Mesa, CA) was used to quantify DHEAS, after dilution of the serum samples. DHEA and ADIOL were extracted with hexane:ethyl acetate (3:2) and then chromatographed on celite impregnated with ethylene glycol prior to RIA. Elution of DHEA was carried out with 25% toluene in isoctane, and elution of ADIOL was carried out with 100% toluene. Separation of antibody-bound and -unbound steroids in the RIA was achieved by use of dextran-coated charcoal. Internal standards [ $^3\text{H}$ ]DHEA and [ $^3\text{H}$ ]ADIOL, 1000 dpm ( $< 3$  pg of

each)] were added to follow procedural losses that averaged 25%. Intra- and interassay coefficients of variation of  $\log_e$ -transformed hormone levels in blind replicate quality-control samples included in each batch ranged between 1.0–4.1% and 2.5–6.1%, respectively, for all three hormones.

Geometric mean hormone levels for cases and controls were compared using Student's *t* tests (10). The relationship of serum hormones to breast cancer risk for the matched sets were evaluated using conditional logistic regression (11). Women were stratified into quartiles based on their hormone levels relative to the distribution of hormone values in controls and a set of categorical (dummy) variables was included in models. RRs were estimated as the antilogs of the regression coefficients. Models also were fit using quartile medians to test for trends. To adjust for known breast cancer risk factors, time since menopause, height, weight, parity, and family history of breast cancer were included in models. Time since menopause was modeled as a set of categorical variables using the distribution in controls to define cut points, parity was modeled as parous *versus* nulliparous, and height and weight were modeled as continuous variables. To evaluate the joint effects of two hormones simultaneously, women were categorized into three groups as follows: (a) those in the lowest quartile for both hormones, (b) those in the highest quartile for both hormones, and (c) all other women. Models were then fit using categorical (dummy) variables. Interactions of hormone levels with age (a matching criterion) and breast cancer risk factors included in adjusted models were tested by including cross products terms in models. Because of small numbers, analyses stratified by time from blood collection to cases' diagnoses were unadjusted and participants were categorized into tertiles rather than quartiles. All analyses were performed using SAS Statistical Software (12).

## Results

Characteristics of cases and controls are summarized in Table 1. Their median age was 62 years and, except for one control, all participants were white. Although cases were slightly taller than controls, their weights and body mass indexes did not differ. Cases tended to have fewer children than controls, and a slightly larger proportion of cases (20%) than controls (14%) were nulliparous. Menopause occurred naturally in 54 cases (76%), compared to 96 controls (72%), and median times from menopause to blood collection did not differ significantly by case status. A positive family history of breast cancer among mothers, grandmothers, sisters, and blood-related aunts was reported by comparable percentages of cases (34%) and controls (28%).

Distributions of dates of blood collection and diagnosis of breast cancer for cases are shown in Table 2. The time from blood collection to diagnosis ranged from less than 1 to 9.5 years with a median of 2.9 years.

Serum concentrations of DHEA, DHEAS, and ADIOL were significantly correlated with Pearson correlations of log-transformed values among controls ranging between 0.70 and 0.84. As shown in Table 3, mean serum levels of DHEA, DHEAS, and ADIOL all were approximately 20% higher in cases compared to controls.

For DHEA and ADIOL there was a significant gradient of increasing risk of breast cancer with increasing hormone level (Table 4). After adjustment for known breast cancer risk factors, women in the highest quartiles of DHEA and ADIOL showed significant 4- and 3-fold excess risks, respectively, compared to women in the lowest quartiles. The relationship of DHEAS with breast cancer was less clear. Although the trend

Table 1 Characteristics of cases and controls at blood collection

	Cases (n = 71)		Controls (n = 133)		P value <sup>a</sup>
	Median	Percentile 5–95	Median	Percentile 5–95	
Age at blood collection (yr)	61	52–71	62	52–73	0.53
Age at menopause (yr)	50	43–56	50	42–55	0.66
Time since menopause at blood collection (yr)	11.1	2.3–23.5	12.4	2.6–25.7	0.49
Age at menarche (yr)	13	11–16	13	11–16	0.38
Parity	2	0–5	3	0–6	0.06
Age at first pregnancy (yr)	24	18–31	23	18–32	0.83
Height (cm)	163	152–170	160	152–169	0.02
Weight (kg)	68	56–94	65	52–99	0.24
Body mass index (kg/m <sup>2</sup> )	26.0	21.3–32.9	25.2	20.1–37.3	0.44

<sup>a</sup> P values from Wilcoxon rank-sum test.

Table 2 Dates of blood collection and diagnosis for cases (years)

	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988
Blood collection	2	41	19	5	2	1	1	8	2	3	3	2
Diagnosis		3	6	15	6	19	4	8	2	3	3	2

Table 3 Geometric mean serum hormone levels for cases and controls

	Cases (n = 71)		Controls (n = 133)		P value <sup>a</sup>
	Mean	95% CI	Mean	95% CI	
DHEA (nM)	6.57	5.61–7.70	5.34	4.74–6.01	0.04
DHEAS (μM)	2.41	2.03–2.87	2.01	1.80–2.25	0.08
Androstenediol (nM)	1.14	0.98–1.33	0.97	0.88–1.07	0.07

<sup>a</sup> P value (two-sided) from t test.

Table 4 Relationship of serum hormones to breast cancer risk

	Number of participants		Unadjusted <sup>a</sup>		Adjusted <sup>a,b</sup>		Trend P
	Cases	Controls	RR	95% CI	RR	95% CI	
DHEA (nM)							
<3.18	10	32	1.0		1.0		0.02
3.18–5.58	17	33	1.7	0.7–4.5	1.8	0.6–5.3	
5.59–8.94	18	33	1.8	0.7–4.3	2.9	1.0–8.2	
8.95+	25	33	2.5	1.0–6.2	4.0	1.3–11.8	
DHEAS (μM)							
<1.32	13	33	1.0		1.0		0.04
1.32–2.19	19	31	1.4	0.6–3.4	1.6	0.6–4.1	
2.20–3.17	8	34	0.6	0.2–1.8	0.6	0.2–1.9	
3.18+	31	35	2.2	1.0–5.2	2.8	1.1–7.4	
Androstenediol (nM)							
<0.62	10	32	1.0		1.0		0.02
0.62–1.00	16	32	1.6	0.6–4.1	1.2	0.4–3.8	
1.01–1.49	19	32	1.8	0.7–4.4	2.3	0.8–6.6	
1.50+	25	33	2.4	1.0–6.0	3.0	1.0–8.6	

<sup>a</sup> Matched on age, year, and time of day of blood collection.

<sup>b</sup> Adjusted for years since menopause, height, weight, family history of breast cancer, and parity.

was significant, and women in the highest quartile of DHEAS were at a significant increased risk of developing breast cancer, women in the third quartile had the lowest risk. These inconsistencies were not an artifact of quartile cut points, because a similar pattern of risks was observed when women were categorized into quintiles. They may, however, have been related to our fairly small sample size, because a trend became apparent when women were categorized into tertiles; compared to

women in the lowest tertile, RRs for women in the upper two tertiles for DHEAS were 1.3 (95% CI, 0.6–2.8) and 2.0 (95% CI, 0.9–4.2), respectively.

Analysis of the joint effects of DHEA and ADIOL on breast cancer risk suggest additivity. Compared to women in the lowest quartile for both hormones, the RR for women in the highest quartile for both was 6.2 (95% CI, 1.7–23.7). Women with all other combinations of levels of these hormones, con-

Table 5 Unadjusted RRs of breast cancer for tertiles of serum hormone levels by time from blood collection to diagnosis in cases

	≤2 years (n = 25 cases)		>2 years (n = 46 cases)	
	RR	95% CI	RR	95% CI
DHEA (nM)				
<4.36	1.0		1.0	
4.36–7.52	2.2	0.5–9.8	0.8	0.3–2.3
7.53+	1.3	0.3–5.3	2.4	1.0–6.0
DHEAS (μM)				
<1.55	1.0		1.0	
1.55–2.87	1.4	0.4–4.9	1.2	0.5–3.2
2.88+	1.3	0.4–4.9	2.3	0.9–5.9
Androstenediol (nM)				
<0.80	1.0		1.0	
0.80–1.28	1.3	0.4–4.8	0.8	0.3–2.1
1.28+	0.8	0.3–2.4	2.2	0.9–5.5

sidered as a single group, had an intermediate RR of 3.4 (95% CI, 1.1–10.6). When similar analyses were performed for DHEAS with each of the other two hormones, RRs for women in the highest quartiles for both hormones did not differ materially from those for the individual hormones shown in Table 4. Compared to women in the lowest quartile for DHEAS and ADIOL, women in the highest quartile for both of these hormones had a RR of 3.5 (95% CI, 1.0–12.0). The RR from a similar analysis for DHEA and DHEAS combined was 3.4 (95% CI, 1.0–11.0).

No significant ( $P \leq 0.05$ ) interactions between hormones and breast cancer risk factors were detected. Furthermore, as shown in Table 5, when we limited analysis to the 46 cases whose blood was collected more than 2 years prior to diagnosis, women in the upper tertile for DHEA, ADIOL, and DHEAS were at the highest risk of developing breast cancer.

Because of concerns about potential bias stemming from incomplete follow-up, we also re-evaluated associations of hormones with breast cancer after truncating the study period at 1982–1983, when follow-up was greater than 90% complete. During this early phase of the study, 53 breast cancers were diagnosed. Risk of breast cancer in this subgroup in relation to serum levels of hormones did not differ materially from those reported for the entire cohort. Increasing gradients of risk with increasing serum levels were apparent for DHEA and ADIOL. Adjusted for years since menopause, height, weight, family history of breast cancer, and parity, RRs by increasing quartile of DHEA were 1.0, 2.3 (95% CI, 0.7–7.3), 4.2 (95% CI, 1.2–14.7), and 4.9 (95% CI, 1.2–20.2). For ADIOL, the analogous RRs were 1.0, 1.1 (95% CI, 0.3–3.6), 2.0 (95% CI, 0.6–6.4), and 2.7 (95% CI, 0.8–8.9). As with the entire cohort, the risk of breast cancer in relation to serum DHEAS was inconsistent, but women in the highest quartile were at greatest risk.

Of the 53 cases diagnosed in 1983 or earlier, 29 (55%) were diagnosed more than 2 years after blood collection. When we restricted analysis to this subset of women, trends in risk across tertiles were not apparent for any of the hormones, but women in the highest tertile for each hormone were at an increased risk of breast cancer. Compared to women in the lowest tertile, RRs for those in the highest were 2.4 (95% CI, 0.7–8.4) for DHEA, 2.3 (95% CI, 0.7–7.5) for ADIOL, and 2.6 (95% CI, 0.9–3.0) for DHEAS.

## Discussion

This is the first epidemiological study to evaluate the relationship between serum ADIOL and breast cancer risk in postmenopausal women. Our finding of a significant positive association is consistent with results of *in vitro* laboratory studies that have shown ADIOL to cause proliferation of breast cancer cells grown in estradiol-deficient medium (5–7, 13). The positive association that we observed between DHEA and breast cancer in postmenopausal women is in agreement with results of Gordon *et al.* (1) and is consistent with enhancement by DHEA of 7,12-dimethylbenz(a)anthracene-induced mammary tumorigenesis in oophorectomized rats (4). Because of the small numbers, we were unable to investigate associations of hormones with premenopausal breast cancer.

The age-adjusted incidence of breast cancer among women 50 years of age and older in the cohort was 128 per 100,000 person-years followed, which is less than the average incidence of 289 per 100,000 per year reported for white women of the same age by the Surveillance, Epidemiology, and End Results Program during the period of case ascertainment for the study (14). The lower rate in our cohort may have been due to a lower incidence of breast cancer in the community, a lower incidence among women who volunteered to participate in the study, or incomplete follow-up. If the latter is correct, bias could have been introduced if serum hormones were related to follow-up, and this association differed for women who did and did not develop breast cancer. Because data on response rates were not tabulated during the conduct of the study and these records were destroyed at completion of the contract, statistics on response rates cannot be reported. However, our findings of similar associations between serum hormones and breast cancer over the entire study period and during the early phase, when follow-up was more than 90% complete, suggest that the associations we observed were not biased by incomplete follow-up.

DHEAS is the most abundant steroid in human serum and is secreted solely by the adrenal cortex. The adrenal cortex also is the primary source of serum DHEA and ADIOL as a result of direct secretion and peripheral conversion of adrenal hormones (15–17). Control of secretion of adrenal androgens is not understood completely, but, at least in part, it is regulated by ACTH (16, 17). ACTH is elevated in response to stress (18), which to our knowledge has not been investigated prospectively in relation to breast cancer. ACTH levels also increase in response to alcohol ingestion (19), which has been positively associated with postmenopausal breast cancer risk in numerous epidemiological studies (20–25).

Estradiol has the strongest binding affinity for the estrogen receptor, and as we reported previously (9), serum levels may be important in determining breast cancer risk. ADIOL also binds the estrogen receptor but with 1:25 the affinity of estradiol (5). However, given that the serum concentration of ADIOL is much higher than estradiol in postmenopausal women (20:1 in our controls), circulating ADIOL could be an important source of estrogenic stimulation of breast tumor growth.

Whereas ADIOL, because of its high affinity for the estrogen receptor, could act directly as a breast cancer promoter, DHEA has only poor affinity for the estrogen receptor and is more likely to increase breast cancer risk by conversion to ADIOL or other metabolites (5, 26). DHEA levels have been reported to be higher in blood supplying than in blood draining tumor-bearing breasts (27, 28), and in one study, the arteriovenous gradient in DHEA plasma concentration was positively correlated with tumor DHEA content, suggesting uptake of

DHEA by tumors (28). Some breast tumors have been shown to possess the enzymes required to metabolize DHEA to ADIOL (5–7, 29–31) and estradiol (32, 33). Normal breast tissue also could potentially metabolize DHEA to other androgens or estrogens and stimulate tumor growth in a paracrine fashion.

The relationship of DHEAS with breast cancer risk in our data was less clear than that of DHEA or ADIOL. Although the test for trend was significant and women in the fourth (highest) quartile for DHEAS were at elevated risk for breast cancer, women in the third quartile were at the lowest risk. Furthermore, women with elevated DHEAS in addition to DHEA or ADIOL were not at a greater risk of breast cancer than women with elevated levels of each of these hormones alone, indicating that the suggested association of DHEAS with breast cancer in our data may have been due to its correlation with other hormones. In the study by Gordon *et al.* (1), DHEA, but not DHEAS, was related to breast cancer risk. However, Japanese women, who have a lower breast cancer risk compared to Western women, particularly after menopause, also have lower DHEAS levels (34).

We previously reported positive associations of testosterone and non-sex hormone-binding globulin-bound estradiol with breast cancer in these same women (9). The addition of DHEA, ADIOL, and possibly DHEAS strongly suggests a role for adrenal androgens in the etiology of breast cancer, at least in postmenopausal women.

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