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Relation of Serum Levels of Testosterone and Dehydroepiandrosterone Sulfate to Risk of Breast Cancer in Postmenopausal Women

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The authors examined the relation between postmenopausal serum levels of testosterone and dehydroepiandrosterone sulfate (DHEAS) and subsequent risk of breast cancer in a case-control study nested within the New York University Women's Health Study cohort. A specific objective of their analysis was to examine whether androgens had an effect on breast cancer risk independent of their effect on the biologic availability of estrogen. A total of 130 cases of breast cancer were diagnosed prior to 1991 in a cohort of 7,054 postmenopausal women who had donated blood and completed questionnaires at a breast cancer screening clinic in New York City between 1985 and 1991. For each case, two controls were selected, matching the case on age at blood donation and length of storage of serum specimens. Biochemical analyses were performed on sera that had been stored at −80°C since sampling. The present report includes a subset of 85 matched sets, for whom at least 6 months had elapsed between blood donation and diagnosis of the case. In univariate analysis, testosterone was positively associated with breast cancer risk (odds ratio (OR) for the highest quartile = 2.7, 95% confidence interval (CI) 1.1–6.8, $p < 0.05$, test for trend). However, after including % estradiol bound to sex hormone-binding globulin (SHBG) and total estradiol in the statistical model, the odds ratios associated with higher levels of testosterone were considerably reduced, and there was no longer a significant trend (OR for the highest quartile = 1.2, 95% CI 0.4–3.5). Conversely, breast cancer risk remained positively associated with total estradiol levels (OR for the highest quartile = 2.9, 95% CI 1.0–8.3) and negatively associated with % estradiol bound to SHBG (OR for the highest quartile = 0.05, 95% CI 0.01–0.19) after adjustment for serum testosterone levels. These results are consistent with the hypothesis that testosterone has an indirect effect on breast cancer risk, via its influence on the amount of bioavailable estrogen. No evidence was found of an association between DHEAS and risk of breast cancer in postmenopausal women. *Am J Epidemiol* 1997;145:1030–8.

androgens; breast neoplasms; DHEAS; estrogens; testosterone

A possible role of androgens in the development of breast cancer in postmenopausal women was first suggested by Grattarola et al. (1). Mechanisms by which androgens may increase breast cancer risk were reviewed by Secreto et al. (2, 3) and Bernstein and Ross (4). Androgens may act directly, by stimulating breast cell proliferation through binding to androgen receptors or by stimulating the synthesis of growth factors inside the breast epithelium. Androgens may also act indirectly through their conversion to estrogens, which are known to stimulate breast cell proliferation (5): aromatization of androstenedione and testosterone in peripheral tissues is the main source of estrogens in postmenopausal women. In addition, it is well established that testosterone binds to sex hormone-binding globulin (SHBG) with greater affinity than estradiol. Testosterone may thus indirectly increase the risk of breast cancer by decreasing the fraction of estradiol bound to SHBG and thereby increasing the nonbound fraction, which is thought to be the fraction available to breast cells (6). Finally, it has been suggested that testosterone inhibits hepatic secretion of SHBG (7), which could also result in a decreased fraction of estradiol bound to SHBG.

Several case-control studies have reported on the association of plasma or serum levels of testosterone with risk of breast cancer in postmenopausal women. Most such studies (2, 8–11, Bruning et al., unpub-

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Abbreviations: CI, confidence interval; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; FSH, follicle-stimulating hormone; NYU, New York University; SHBG, sex hormone-binding globulin.

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lished data) although not all studies (12) observed higher levels of testosterone in cases than in controls. Among the three prospective studies which examined the relation of serum levels of testosterone with risk of postmenopausal breast cancer, one found a significant positive association (13), one found a nonsignificant positive association (14), and the third reported no association (15).

Dehydroepiandrosterone (DHEA) is the androgen produced by the adrenal in largest quantity. The physiologic roles of DHEA and of its sulfate (DHEAS), which is thought to be produced exclusively by the adrenal cortex (16), are unknown. They are considered weak androgens, but also appear to have estrogenic properties (17). It has been proposed that DHEA and DHEAS protect against breast cancer in premenopausal women, but increase breast cancer risk in postmenopausal women (17, 18). These conflicting actions could be reconciled by a recent hypothesis: in premenopausal women, DHEA would have an antiestrogenic effect by binding competitively to estrogen receptors, whereas, in postmenopausal women, DHEA would bind to vacant estrogen receptors and enhance estradiol-like effects, thereby stimulating tumor growth (19).

Results from case-control studies of DHEA and DHEAS conducted in postmenopausal women have been mixed (2, 20–22). The three prospective cohort studies which examined the relation of testosterone with breast cancer risk in postmenopausal women also measured DHEAS; one study (23) reported no association, whereas the two others (13, 24) observed a nonsignificant positive association.

We report here on the relation between postmenopausal serum levels of testosterone and DHEAS and subsequent risk of breast cancer in a case-control study nested within a prospective cohort, the New York University (NYU) Women's Health Study. We previously reported a positive association between postmenopausal serum fractions of bioavailable estrogens and risk of breast cancer in this study population (25). A specific objective of our analysis was to examine whether serum levels of androgens have an effect on breast cancer risk independent of their influence on serum levels and biologic availability of estrogens.

MATERIALS AND METHODS

The NYU Women's Health Study cohort

Between March 1985 and June 1991, the NYU Women's Health Study enrolled a cohort of 14,275 women aged 34–65 years at the Guttman Breast Diagnostic Institute, a breast cancer screening center in New York City. Details concerning subject recruitment have been published elsewhere (25, 26). The current report is limited to the 7,054 cohort members who were postmenopausal at the time of enrollment. Participants were classified as postmenopausal if they reported either: 1) no menstrual cycles during the preceding 6 months, 2) a total bilateral oophorectomy, or 3) a hysterectomy without complete oophorectomy prior to natural menopause and were 52 years of age or older. Cohort members donated 30 mL of blood and completed a self-administered questionnaire at enrollment. Blood was drawn prior to breast examination, between 9:00 A.M. and 3:00 P.M. in nonfasting women. After centrifugation, serum samples were immediately stored at −80°C for subsequent biochemical analyses. Women who had taken hormonal medications in the 6 months preceding their visit were not eligible.

Nested case-control study

Cases of breast adenocarcinoma were identified primarily through active follow-up of the cohort and were confirmed by review of individual clinical and pathology records (25). For each case diagnosed in a woman who was postmenopausal at enrollment, two controls were selected at random from the risk set of women who were alive and free of disease at the time of diagnosis of the case, and who matched the case on age at enrollment (±6 months), date of initial blood donation (±3 months), and menopausal status. As of October 1991, 130 members of the postmenopausal cohort had been identified who had received a diagnosis of breast cancer prior to January 1, 1991. Serum assays of follicle-stimulating hormone (FSH) were conducted to confirm the postmenopausal status of all the cases and their selected controls: three controls, who had reported the absence of menstrual cycles in the 6 months prior to enrollment, had FSH levels below 17.5 IU/liter, which was less than the minimal level compatible with postmenopausal status for our assay. They were nonetheless included in the analysis, since excluding them did not materially affect risk estimates. Estrogen assays (total estradiol, % estradiol free, and % estradiol bound to SHBG) were performed for all matched sets. For logistical reasons, androgen assays were carried out in a subset of 118 matched sets. Excluded from the analyses reported here are 33 matched sets for whom diagnosis of the case occurred 6 months or less after blood donation, six controls who reported treatment with corticosteroids in the 6 months prior to blood donation, and one control whose estrogen assays were done on a different day than the matching case. As a result, 85 cases (83 invasive and 2 noninvasive intraductal) and 163 controls are included in the present report.

Laboratory methods

For androgen assays, serum samples that had not been previously defrosted were shipped in dry ice to the Netherlands Cancer Institute and analyzed in two batches. Samples from a case and her matched controls were always analyzed in the same batch. All assays were performed in duplicate with the laboratory personnel blinded to the case or control status of the samples. Reference sera were included for each assay in several places within each batch.

Total testosterone was measured by a solid-phase radioimmunoassay (Coat-A-Count, Diagnostic Products Corp., Los Angeles, California) not requiring extraction or chromatography. The mean intra-assay coefficient of variation in the range of measurement was 6.2 percent. The inter-assay coefficients of variation were, respectively, 11 percent at 1.67, 2.2 percent at 10.00, and 7.0 percent at 22.05 nmol/liter.

DHEAS was measured directly in diluted serum as we have reported previously (27) using an antiserum against DHEA which showed a 42 percent crossreactivity with DHEAS. As DHEA was present in serum in concentrations at least 10 times lower than DHEAS, it had a negligible influence on the DHEAS values, which were read from a DHEAS standard plot. The mean intra-assay coefficient of variation in the range of measurement was 3.3 percent. The inter-assay coefficients of variation were, respectively, 10 percent at 1.68, 9 percent at 2.97, 10 percent at 5.51, and 8 percent at 15.18 μmol/liter.

Total estradiol was measured by standard radioimmunoassay (Pantex, Inc., Santa Monica, California). Percent estradiol bound to SHBG and % estradiol free were measured with a concanavalin A-sepharose binding and an ultrafiltration method, respectively, as reported previously (25).

Statistical methods

When treated as continuous, total estradiol, testosterone, and DHEAS were $\log_e$-transformed to reduce departures from the normal distribution. The paired t test was used to compare hormone levels of the cases to the mean hormone levels of their matched controls.

To compute odds ratios, hormonal measurements were categorized into quartiles, using the frequency distribution of the cases and the controls combined. Because the androgen assays were performed in two batches, quartile cut-points were calculated separately for each batch. The weighted averages of the cut-points are reported in the tables.

The data were analyzed using conditional logistic regression (28). Odds ratios were computed relative to the lowest quartile. Regression analyses were also performed on the continuous hormonal variables. Likelihood ratio tests were used to assess the statistical significance of overall associations, linear trends, and deviations from linearity. All p values are two-sided.

One objective of the analysis was to examine concurrently the effect of androgens and estrogens. Therefore, we report on the effect of adding androgen variables to models containing estrogen variables, and vice-versa. When adding estrogen variables to models containing androgen variables, % SHBG-bound estrogen was entered first because it was the estrogen variable most strongly associated with breast cancer risk in multivariate models (25).

Hormone levels in this study were assessed from a single blood donation. For some hormones, however, a single measurement may not provide a reliable estimate of a woman's long-term average level, the exposure of interest, because of intrinsic fluctuations in the hormone over time and laboratory measurement error. In addition, different hormones are measured with varying amounts of error. For example, the reliability coefficients of total estradiol, % estradiol bound to SHBG, and DHEAS were estimated to be 0.51, 0.94, and 0.75, respectively, in our study population (29). The reliability of testosterone was not assessed in our study, but estimates from the literature range from 0.74 (30) to 0.88 (31). We were concerned that these differences might distort our results regarding the relative importance of the hormones. We therefore applied the method of Armstrong et al. (32) for correcting logistic regression parameter estimates of continuous variables for measurement error in case-control data. For total estradiol, % estradiol bound to SHBG, and DHEAS, we used within-subject variances which we had previously estimated (29). For testosterone, we used the within-subject variance estimate provided by Hankinson et al. (Susan Hankinson, Harvard University School of Public Health, personal communication, 1996). We assumed that the different hormonal variables had independent measurement errors.

We examined the effect of Quetelet index (weight (kg)/height $(m)^2$) on the androgen-breast cancer associations, because the rate of conversion of androgens to estrogens increases with Quetelet index (33), and because the known positive association of Quetelet index with risk of breast cancer was confirmed in our data (25). The effect of other known risk factors (age at menarche, parity, age at first full-term pregnancy, age at menopause, history of breast cancer in a first-degree relative, history of a benign breast condition, history of total oophorectomy, lifetime months of lactation, and smoking history) on the androgen-breast cancer associations was also examined in multivariate

conditional logistic analyses. The inclusion of covariates other than Quetelet index in the statistical analyses did not materially affect the results and are therefore not presented. In addition, the exclusion from the analysis of the six cases and 13 controls who had a total oophorectomy prior to enrollment in the study had no material impact on the results (data not shown). Results are therefore presented including these patients.

RESULTS

Some characteristics of the study group are given in table 1. The median age at diagnosis of breast cancer was 61.6 years and the median duration between blood donation and diagnosis was 2.7 years (range 0.5–5.5 years). Known breast cancer risk factors had a similar distribution in this group as in the larger group on which estrogen assays were carried out (25). There were no appreciable differences between cases and controls in age at menarche, parity, age at menopause, and history of prior oophorectomy. Delayed first full-term pregnancy, history of breast cancer in at least one first-degree relative 45 years old or younger and history of a benign breast condition were associated with a nonsignificant increase in risk of breast cancer, while a history of breastfeeding was associated with a nonsignificant protective effect. The median weight and median Quetelet index were significantly higher in cases than in controls.

Table 2 shows the geometric mean levels of testosterone and DHEAS for cases and controls. The mean testosterone level was 21 percent higher in cases than in controls ($p < 0.01$) and the mean DHEAS level was 20 percent higher ($p = 0.10$).

TABLE 2. Geometric mean, geometric standard deviation, and range of serum levels of testosterone and dehydroepiandrosterone sulfate (DHEAS) in breast cancer patients diagnosed at least 6 months after blood donation and their individually matched controls, New York University Women's Health Study, 1985–1990

Hormone	Cases ($n = 85$)	Controls ($n = 163$)
Testosterone (nmol/liter)		
Mean** (SD†)	1.05 (1.79)	0.87 (1.89)
Range	0.20–3.96	0.14–5.96
DHEAS (μmol/liter)		
Mean* (SD†)	2.36 (2.37)	1.96 (2.26)
Range	0.22–14.60	0.12–10.43

* $p = 0.10$, paired t test.
** $p < 0.01$, paired t test.
† SD, standard deviation.

Table 3 reports odds ratios for the association between breast cancer and serum levels of testosterone, total estradiol, and % estradiol bound to SHBG. In univariate analyses, odds ratios showed a significant increase ($p = 0.02$, test for trend) in risk of breast cancer with increasing levels of testosterone: the odds ratios (95 percent CIs) for the second, third, and fourth quartiles relative to the lowest quartile, were 2.4 (1.0–5.6), 3.5 (1.4–8.4), and 2.7 (1.1–6.8), respectively. However, adjusting for % SHBG-bound estradiol, which was the estrogen variable most strongly associated with breast cancer risk, reduced the odds ratios and removed the significant trend. The odds ratios (95% CIs) were 1.5 (0.6–3.7), 2.0 (0.7–5.2), and 1.3

TABLE 1. Characteristics of study subjects, New York University Women's Health Study, 1985–1990

Characteristic	Cases ($n = 85$)	Controls ($n = 163$)
Age (years) at blood donation, median (range)	59.2 (48.9–65.4)	59.1 (48.9–64.9)
Age (years) at diagnosis, median (range)	61.6 (52.2–68.6)	
Age (years) at menarche, median (range)	13 (9–16)	13 (10–17)
No. of full-term pregnancies (%)		
0	24.7	23.9
1	17.6	13.5
>1	57.6	62.6
Age (years) at first full-term pregnancy, median (range)	25 (16–41)	24 (16–43)
Ever breastfeeding (%)	20.8	28.2
Age (years) at menopause, median (range)	51.7 (31.6–57.2)	50.9 (24.9–58.6)
Breast cancer in first-degree relative <45 years old (%)	8.2	3.7
Prior benign breast condition (%)	57.7	46.7
Prior bilateral oophorectomy (%)	7.0	8.0
Height (cm), median (range)	162.6 (149.9–177.8)	162.6 (147.3–177.8)
Weight* (kg), median (range)	70.3 (47.6–122.5)	62.6 (45.4–124.7)
Quetelet's index* (kg/m^2), median (range)	26.1 (19.9–43.6)	24.0 (17.7–44.4)

* $p < 0.001$, paired t test.

TABLE 3. Odds ratios (OR) and 95% confidence intervals (CI) for the association between breast cancer risk and serum levels of testosterone, total estradiol, and % estradiol bound to sex hormone-binding globulin (SHBG), New York University Women's Health Study, 1985–1990

Hormonal variable by quartiles	Unadjusted OR†	95% CI	Adjusted OR‡	95% CI	Adjusted OR§	95% CI
Testosterone#						
1	1.0		1.0		1.0	
2	2.4	1.0–5.6	1.5	0.6–3.7	1.4	0.6–3.5
3	3.5	1.4–8.4	2.0	0.7–5.2	1.8	0.7–5.0
4	2.7	1.1–6.8	1.3	0.5–3.7	1.2	0.4–3.5
p for trend	*		NS¶		NS	
Total estradiol††						
1	1.0		1.0		1.0	
2	2.0	0.8–5.3	1.8	0.7–4.8	1.7	0.6–4.7
3	4.3	1.8–10.4	3.6	1.4–9.0	2.6	1.0–6.8
4	3.8	1.5–10.3	2.9	1.0–8.3	1.6	0.5–5.8
p for trend	***		*		NS	
% SHBG-bound estradiol‡‡						
1	1.0		1.0		1.0	
2	0.43	0.19–0.98	0.44	0.19–1.01	0.44	0.19–1.05
3	0.19	0.07–0.49	0.20	0.07–0.56	0.21	0.07–0.59
4	0.05	0.01–0.17	0.05	0.01–0.19	0.05	0.01–0.21
p for trend	***		***		***	

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
† Unadjusted, except for matching factors (age and serum storage time).
‡ For testosterone, odds ratios are adjusted for % SHBG-bound estradiol; for total estradiol and % SHBG-bound estradiol, odds ratios are adjusted for testosterone.
§ Adjusted for other hormonal variables in the table.
¶ NS, not significant.
# The cut-points defining quartiles of testosterone were 0.73, 1.02, and 1.45 nmol/mL.
†† The cut-points defining quartiles of total estradiol were 20, 30, and 45 pg/mL.
‡‡ The cut-points defining quartiles of % SHBG-bound estradiol were 34.4, 43.6, and 51.3%.

(0.5–3.7) for second, third, and fourth quartiles, respectively. Adding total estradiol to the model including testosterone and % SHBG-bound estradiol did not significantly improve the fit of the model, although it further reduced the odds ratios (95 percent CIs) to 1.4 (0.6–3.5), 1.8 (0.7–5.0), and 1.2 (0.4–3.5), respectively. Adding % free estradiol or Quetelet index to the model containing testosterone, estradiol, and % estradiol bound to SHBG did not materially affect the odds ratios (data not shown). A strong positive association between breast cancer risk and increasing levels of total estradiol was also present in univariate analysis. This association remained significant after adjusting for testosterone levels, although the odds ratios and the corresponding *p* values were somewhat reduced. The protective effect associated with increasing percentage of SHBG-bound estradiol was hardly affected by adjustment for testosterone levels. In the model including the three hormonal variables, only % estradiol bound to SHBG remained significant. Analysis on continuous variables showed similar results.

Results of the analyses correcting for measurement error were similar to results of the uncorrected analyses with respect to the relative strength of the associations of the hormonal variables with breast cancer risk: the positive association of testosterone was weakened and no longer significant after adjusting for % SHBG-bound estradiol, whereas the positive association of total estradiol became only marginally significant and the negative association of % SHBG-bound estradiol remained highly significant after adjusting for testosterone. In the model including the three variables, only % SHBG-bound estradiol remained significant.

Table 4 reports odds ratios for the association be-

TABLE 4. Odds ratios (OR) and 95% confidence intervals (CI) for the association between breast cancer risk and serum levels of dehydroepiandrosterone sulfate (DHEAS), New York University Women's Health Study, 1985–1990

Quartiles of DHEAS†	OR‡	95% CI	OR§	95% CI
1	1.0		1.0	
2	0.7	0.3–1.5	0.3	0.1–0.9
3	1.0	0.5–2.1	0.5	0.2–1.3
4	1.6	0.7–3.5	0.9	0.4–2.3
p for trend	NS¶		NS	

† The cut-points defining quartiles of DHEAS were 1.33, 2.38, and 3.58 μmol/liter.
‡ Unadjusted, except for matching factors (age and serum storage time).
§ Adjusted for % estradiol bound to sex hormone-binding globulin and total estradiol.
¶ NS, not significant.

tween breast cancer risk and increasing levels of DHEAS. In unadjusted analyses, although the odds ratio in the highest quartile was slightly elevated (odds ratio = 1.6, 95 percent CI 0.7–3.5), there was no trend of increasing risk of breast cancer with increasing levels of DHEAS. The inclusion of estrogen variables or of Quetelet index did not result in a significant trend. The odds ratios for the association between breast cancer risk and DHEAS are shown adjusting for % SHBG-bound estradiol and total estradiol. Inclusion of DHEAS in models containing estrogen variables did not materially affect the associations between estrogen variables and breast cancer risk (data not shown). Correcting for measurement error in the hormonal variables did not alter the results.

Finally, analyses were conducted using only the 56 matched sets with at least 2 years between blood donation and diagnosis of the case. The results were similar to the results of analyses conducted in the larger group, both for testosterone and DHEAS (data not shown).

Table 5 reports the Spearman correlation coefficients for hormone levels and Quetelet index, by case-control status. Note that testosterone was correlated positively with total estradiol (r_S = 0.23 in cases and 0.27 in controls) and negatively with % estradiol bound to SHBG (r_S = −0.27 in cases and −0.33 in controls).

DISCUSSION

In unadjusted analyses (except for matching variables), we observed a statistically significant trend of increasing risk of breast cancer with increasing serum levels of testosterone in postmenopausal women. Because all cases were diagnosed at least 6 months after blood donation (median 2.7 years) and because a similar trend was observed when the analysis was limited to the two-thirds of the cases diagnosed at least 2 years after blood donation, it seems unlikely that the higher levels of testosterone observed in women who subsequently developed the disease compared with controls resulted from the presence of tumors.

Three previous prospective studies (13–15) have examined the association between serum levels of testosterone and breast cancer risk in postmenopausal women. No association was observed in the Rancho Bernardo, California, study (15), in which the age-adjusted mean testosterone levels were 258 pg/ml in 15 cases diagnosed at least one year after blood donation and 261 pg/ml in 400 noncases. However, results from the two other prospective studies (13, 14) are consistent with ours findings. In the Washington County, Maryland, study (14), serum levels were 11 percent higher in 39 cases (mean 304 pg/ml) than in 155 controls (mean 274 pg/ml), although this difference was not statistically significant. Finally, in 24 cases diagnosed during the first 3.5 years of follow-up of a cohort of 4,040 postmenopausal women from northern Italy, the risk ratios (95 percent CIs) for breast cancer associated with the second and third tertiles of testosterone were 4.8 (0.9–25.1) and 7.0 (1.4–36.4), respectively (p for trend = 0.026) (13).

We recently reported a positive association between bioavailable estrogens and subsequent risk of breast cancer in a slightly larger group of postmenopausal women from the NYU Women's Health Study (25). An objective of the present analysis was to examine whether androgens had an effect on breast cancer risk that was independent of their influence on serum levels and biologic availability of estrogen. Results

TABLE 5. Spearman correlation coefficients for androgen and estrogen levels and Quetelet index, New York University Women's Health Study, 1985–1990

	DHEAS†	Total estradiol	% SHBG†-bound estradiol	% free estradiol	Quetelet index
Controls (n = 163)					
Testosterone	0.35***	0.27***	−0.33***	0.25**	0.30***
DHEAS		0.23**	−0.27***	0.25**	0.05
Total estradiol			−0.48***	0.45***	0.43***
% SHBG-bound estradiol				−0.72***	−0.52***
% free estradiol					0.48***
Cases (n = 85)					
Testosterone	0.38***	0.23*	−0.27*	0.12	0.11
DHEAS		0.28*	−0.37***	0.24*	−0.06
Total estradiol			−0.47***	0.19	0.38***
% SHBG-bound estradiol				−0.56***	−0.42***
% free estradiol					0.29**

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
† DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone-binding globulin.

showed that, after including estrogen variables (% SHBG-bound estradiol and total estradiol) in our statistical model, the odds ratios associated with higher levels of testosterone were considerably reduced, and there was no longer a significant trend. A similar result was recently observed in the reanalysis of a population-based case-control study conducted in Sweden (9, 34). Whereas, in univariate analysis, a significant positive association was found between testosterone and breast cancer risk, the association disappeared after controlling for estrone (and androstenedione). On the other hand, Berrino et al. (13) did not observe a reduction of the association between breast cancer risk and levels of testosterone when they adjusted for total estradiol. However, multivariate analysis was hampered by the small sample size of the study (24 cases).

We were concerned about the impact of measurement error in the hormonal variables on our results. It is well known that in the absence of confounders, nondifferential measurement error in an exposure variable will result in an attenuation of the true exposure/disease relation. When several variables are measured with error, however, the associations of these variables with disease in a multivariate model may be weaker or stronger than the true associations (35). It is reassuring that, in our analysis, correcting for measurement error did not affect the relative strength of the associations of the hormonal variables with risk of breast cancer.

Our results are consistent with the hypothesis that testosterone has an indirect effect on breast cancer risk, through its association with estrogen levels. The fact that % SHBG-bound estradiol was the estrogen variable which caused the greatest reduction in the testosterone-breast cancer odds ratios suggests that the effect of testosterone on the bioavailability of estrogens may be more important than its role as a precursor of estrogens. An increase in the serum levels of testosterone could lead to a decrease in % estradiol bound to SHBG, because testosterone binds to SHBG with greater affinity than estradiol. However, the modeling studies performed by Dunn et al. (36) as well as in vitro experiments (37) indicate that higher concentrations of testosterone would be required to observe such an effect. Inhibition of the hepatic secretion of SHBG by testosterone could also result in a decrease in % SHBG-bound estradiol, because small changes in SHBG concentration can produce an important reduction in the percentage of hormone bound to this protein (38). In support of this hypothesis, a moderate negative correlation between testosterone and SHBG has been reported by some studies (7, 39, 40) although not all studies (34).

A limitation of our study is that only total testosterone was measured. The free and albumin-bound hormone fractions might be more relevant biologically because these fractions are thought to diffuse readily into the cells (41). Indeed, with regard to estrogen, the variable most strongly related to risk of breast cancer was the % SHBG-bound estradiol, which had a protective effect. Thus, we cannot exclude the possibility that the free and albumin-bound fractions of testosterone might have an independent effect on breast cancer risk.

The lack of an association between DHEAS and breast cancer observed here is consistent with the results of the previous prospective studies which examined the role of this hormone in postmenopausal women. Barrett-Connor et al. (23) measured DHEAS levels in a cohort of 534 women, 50–79 years old, among whom 21 subsequently developed breast cancer, and reported no difference between cases and non-cases. In a case-control study nested within a cohort of approximately 13,000 female residents of Washington County, Maryland, Gordon et al. (24) reported that serum levels of DHEA were significantly higher in 30 postmenopausal women who developed breast cancer 9 years or more after blood donation than in 59 matched controls. However, no statistically significant difference in DHEAS levels was observed, although serum levels of DHEAS were slightly higher in the women who developed breast cancer than in the controls. Finally, an increase in the odds ratios for breast cancer was observed with increasing serum levels of DHEAS in an Italian cohort study, the Study of Hormones and Diet in the Etiology of Breast Cancer (ORDET Study), but this trend was not statistically significant (13). Overall, there is little epidemiologic evidence that DHEAS plays an important role in breast cancer development in postmenopausal women.

In conclusion, elevated serum levels of testosterone were found to be associated with subsequent risk of breast cancer in postmenopausal women. However, this association was considerably reduced and no longer significant after taking into account the effect of serum estrogen levels on breast cancer risk, suggesting that androgens act through their influence on the availability of estrogens via SHBG binding and/or as precursors of estrogens. There was no evidence in our study that the adrenal androgen DHEAS plays a role in breast cancer development. In light of these results, additional research to identify factors influencing testosterone levels in healthy postmenopausal women would be of interest. Among life-style factors such as smoking, obesity, diet, alcohol consumption, and exercise, only obesity has been found to be mar-

ginally associated with higher levels of testosterone (42, 43).

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