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13. ABSTRACT *(Maximum 200*

This career development award is allowing me to increase my expertise in the area of hormones, hormonally-related factors and risk of breast cancer. I proposed to prospectively assess plasma hormone levels, alcohol use and body fat distribution in relation to breast cancer risk. In year two of this award, I completed the analyses of plasma hormone levels and breast cancer. We observed significant positive associations between several plasma estrogens and plasma prolactin in relation to risk. Two manuscripts have resulted from this work: the first (assessing sex steroid hormone levels) is "in press" at JNCI and the second manuscript (assessing prolactin levels) was just submitted to JNCI. The analyses of body fat distribution also have been completed. This manuscript, for which I am the senior author, was recently submitted to JAMA for publication.

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FOREWORD

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Susan E Hand 7/23/98

PI - Signature Date

TABLE OF CONTENTS

I. INTRODUCTION

A. Purpose

My long-term career objective is to help resolve outstanding questions concerning endogenous and exogenous hormones and breast cancer. Specifically, I wish to address the relationships between specific postmenopausal and premenopausal hormone levels and breast cancer risk. Also, I wish to examine which breast cancer risk factors operate through a hormonal mechanism, how these factors interact and how they can be modified. The funded analyses will not only give me invaluable experience in the area of hormones and breast cancer, thus providing the background necessary to conduct independent research, but will contribute significantly towards current knowledge in this area.

I proposed addressing the following hypotheses:

1. Plasma sex hormone levels and risk of breast cancer among postmenopausal women:

a. **Estrogens:** Total estradiol, percent free estradiol, percent bioavailable estradiol (the sum of percent free and percent albumin-bound estradiol), estrone, and estrone sulfate each increase risk of breast cancer.

b. **Androgens:** Androstenedione, testosterone, dehydroepiandrosterone (DHEA), and dehydroepiandrosterone sulfate (DHEAS) each increase breast cancer risk.

c. **Prolactin** increases risk of breast cancer.

2. Alcohol intake and breast cancer risk.

a. Moderate alcohol consumption increases the risk of breast cancer and this risk varies by when in life exposure occurs.

b. This increased risk of breast cancer varies by levels of other risk factors including age, menopausal status, body mass index and family history.

3. Body fat distribution (as assessed by the waist-to-hip ratio) and breast cancer risk.

a. Higher waist-to-hip ratios increase breast cancer risk.

b. This increase in risk varies by level of other breast cancer risk factors including age, menopausal status, body mass index, and family history.

As part of my Career Development Award, I also proposed attending several endocrinology courses at Harvard Medical School. In year 2 of this award I addressed hypotheses (1) and (3), thus the background and significance for these specific hypotheses is provided below.

B. Background and Significance

1. Plasma Hormones and Breast Cancer. The epidemiology of breast cancer suggests an etiologic role for endogenous sex hormones: reproductive factors including age at first birth, parity, age at menarche and menopause, and possibly lactation influence breast cancer risk (1). Hormonal manipulations such as anti-estrogens and adrenal suppression have been useful in the

treatment of breast cancer. Furthermore, estrogens and prolactin promote mammary tumors in animals (2).

The relation between plasma estrogens and the risk of breast cancer has received the greatest attention. Estradiol, generally considered the most biologically active endogenous estrogen, circulates in blood bound either to sex hormone binding globulin (SHBG) or albumin, or unbound ("free" estradiol). Data from several previous case-control studies (3,4,5,6) but not others (7,8,9) suggest that high levels of estradiol increase the risk of breast cancer. Higher free estradiol levels among cases were observed prospectively (10) and in several case-control studies (4,7,9,11,12) but not in others (8,13,14,15). Recently, in the largest prospective analysis to date, (130 cases and 260 controls) Toniolo et al. (16) reported a significant 2 to 4 fold increased risk of breast cancer among women in the top versus bottom quartile of plasma estradiol, % free estradiol and % bioavailable estradiol levels. Estrone is the predominant estrogen and the source of much of the circulating estradiol in postmenopausal women. Higher estrone levels were significantly associated with increased breast cancer risk in the study by Toniolo (16) and in one of the larger case-control studies (17); a nonsignificant positive association was noted in several other case-control studies (7,6). No association was found in other studies (8,13,14,18,19).

In a prospective analysis, postmenopausal women who developed breast cancer had a nonsignificant elevation in plasma testosterone and androstenedione compared to controls (18); in a second analysis with 42 mixed incident and prevalent cases no association was noted (13). In a prospective analysis, Gordon et al (20) reported that, relative to controls, cases had a significant 33% higher level of plasma dehydroepiandrosterone (DHEA) and a nonsignificant 16% increase in DHEAS. In several case-control studies, strong positive associations have been observed between levels of plasma testosterone and breast cancer risk among postmenopausal women (17,21,22,23). The biologic mechanism behind an association with androgen levels is less clear but might be related to their conversion to estrogens.

Strong evidence links prolactin to the induction and progression of mammary carcinoma in the rat and mouse (24). In addition, prolonged reductions in prolactin occur after first pregnancy (25) and prolactin levels are higher in women at increased risk of breast cancer due to first pregnancy over the age of 35 years (26), nulliparity (27), and family history (28). In postmenopausal women, prolactin was associated with increased risk of breast cancer in the only prospective study (29) as well as several (30,31), but not all (6,24), case-control studies.

2. Body Fat Distribution and Breast Cancer Risk. Abdominal obesity (as measured by the waist-to-hip ratio), independent of body mass, has been hypothesized to increase risk of breast cancer, perhaps through an increase in either total or bioavailable steroid hormone levels (32,33). To date, few studies have examined this hypothesis. In recent prospective (34,35) and case-control studies (36,37), a positive association has been reported (RR=1.4-1.8). In a third prospective study (38), no association was found although this study was small with only 23 cases. In the Iowa Women's cohort, the association between waist-to-hip ratio and breast cancer risk was strongest in older women with a higher body mass index (46) and among women with a

family history of breast cancer (39). Bruning et al (49) reported that waist-to-hip ratio, rather than body mass index, increased breast cancer risk in postmenopausal women, while in premenopausal women the opposite was true. To my knowledge, the interrelationship of these variables has not been assessed in other studies.

C. Previous Work

1. The Nurses' Health Study (NHS) Cohort.

In 1976, 121,700 female U.S. registered nurses between the ages of 30 and 55 years completed the initial NHS questionnaire forming the NHS cohort. The population is predominantly white, reflecting the ethnic background of women entering nursing in the U.S. in the 1950's and 1960's. The cohort is approximately 1.2% African-American, 0.6% Hispanic, 0.8% Asian, 17% Southern European or Mediterranean, 7% Scandinavian, 60% other Caucasion, and 4% other ancestry. The cohort has been followed by mailed questionnaires sent every two years; nonrespondants to questionnaires are telephoned. Follow-up, calculated as a percentage of total possible follow-up time, was over 92% in 1990.

Since the study began in 1976, extensive information has been collected on exposures that will be important covariates in the proposed analyses. These include height, weight, age at menarche and menopause, age at first birth, parity, lactation, oral contraceptive and postmenopausal hormone use, smoking history, physical activity, history of benign breast disease, family history of breast cancer and dietary intake. In 1980, a 61-item dietary questionnaire was sent to participants; this questionnaire has been studied intensively for reproducibility and validity (40,41). More extensive dietary questionnaires were included in the 1984, 1986, and 1990 follow-up; over 80 nutritional parameters are measured.

Nonfatal breast cancer cases are reported on the questionnaire and by telephone interview. These women are asked to grant us permission to review their medical records to confirm the self-reported diagnosis and to further classify the cancer by histologic type, size, receptor and nodal status. Approximately 99% of reported breast cancer diagnoses are confirmed upon medical record review. To identify cases among non-respondents, the National Death Index is searched and death certificates obtained. Incident cancers identified from death certificates require medical records to be classified as confirmed cancers.

2. Biochemical Markers in the Nurses' Health Study

In 1989 and 1990, we obtained blood samples from 32,826 NHS participants (NIH CA 49449; Frank Speizer, PI). Each woman was sent a blood collection kit which contained all the needed instructions and supplies to have blood drawn and mailed back to our laboratory. We enclosed a questionnaire which requested information on the date and time the sample was drawn, time since last meal, current menopausal status, and recent hormone use. Each participant made arrangements for the blood sample to be drawn, generally by her physician, a colleague, or a local laboratory. The blood samples were returned to our laboratory via overnight courier; 97% of the samples arrived within 26 hours of being drawn. Of over 64,000 blood tubes received, only 75 tubes were broken in transit.

Upon arrival in our laboratory, the blood samples were centrifuged and blood components aliquotted into plasma, white blood cell, and red blood cell components. Cryotubes are stored in 17 liquid nitrogen freezers. All nitrogen freezers are connected to an electronic alarm system and are monitored 24 hours a day. For added security, each participant's sample is stored in 3 different freezers.

II. BODY OF REPORT

In year two of this award, my original plan was to complete the alcohol and breast cancer analyses and to attend a second endocrinology course. However, for several reasons, I completed analyses originally scheduled for years 3 and 4 instead. During this past year I completed the hormone analyses which I began in year 1 (this was done because the hormone results were available considerably earlier than I had predicted). Because of the amount of data and the extensive statistical analysis needed, I completed two manuscripts, rather than just one as originally proposed. The first manuscript, addressing the relationships between plasma sex steroids and breast cancer risk, is currently "in press" at the Journal of the National Cancer Institute (JNCI). These results were also presented at the 1998 meeting of the Society for Epidemiologic Research. The second manuscript, which addresses the relation between plasma prolactin levels and breast cancer, has just been submitted to JNCI. A summary of these findings is provided below and the full manuscripts are included as an Appendix. In year 2, I also had the opportunity to work with a post-doctoral fellow on the relationships between waist-to-hip ratio and breast cancer risk. I oversaw her analyses, was her mentor in conducting and interpreting these data, and am the senior author of the resulting manuscript which was recently submitted to the Journal of the American Medical Association (JAMA). A summary of these findings is also provided below and the submitted manuscript is included as an appendix. In year 3 of this award, I expect to complete the work originally proposed for years 1 and 2 (i.e., complete the alcohol and breast cancer analyses).

1. Plasma hormone levels and risk of breast cancer.

(a) Experimental Methods.

(i). Identification and preparation of specimens for analysis. All documented cases of incident breast cancer occuring from after the blood collection up to June 1, 1994 serve as cases for this analysis. For each breast cancer case with a blood sample, two control subjects were selected at random from among individuals of the same age ($\pm$1 yr) and who gave blood at the same time ($\pm$1 month) who were at risk of disease at the time the case occurred (42). Women with a prior history of cancer were excluded from these analyses. I also matched by menopausal status and by time of day the blood sample was drawn. There were 156 cases and 312 controls in total. Samples were pulled from the freezers, aliquotted and sent to the laboratories for analysis.

(ii). Measurement of Hormone Levels. With the exception of prolactin, all hormone analyses were conducted at Nichols Laboratory, San Juan Capistrano, California. Prolactin was assayed at the laboratory of Dr. C. Longcope at the University of Massachusetts Medical Center. The samples were labelled by number only, and matched case-control pairs were handled identically and together, shipped in the same batch, and assayed in the same analytical run. The

order within each case-control pair was determined at random. Aliquots from the pooled quality control specimens were analyzed periodically by each laboratory to monitor quality control. These aliquots were indistinguishable from the real specimens, and were interspersed among them without the knowledge of the laboratory personnel. All between-assay coefficients of variation were ≤15%.

(b). Results and Discussion: Steroid hormones and breast cancer risk.

We observed significant positive relationships with plasma estradiol, estrone, and estrone sulfate, with women in the top quartile having a two-fold higher risk of breast cancer than women in the lowest plasma quartile (Table 1). For DHEAS, women in the top 75% of levels appeared to have an increase in breast cancer risk compared to women with the lowest levels. In contrast to the only previous large prospective study where % free and % bioavailable estradiol were strongly related to risk (25), we observed no substantial association, although we could not rule out a 2-fold difference in risk. Our results did not differ materially after excluding the 30 breast cancer cases that had been diagnosed within one year of blood collection. We also used measurement error correction techniques to correct our relative risk estimates for the random within person fluctuation in hormone levels observed in postmenopausal women (see Table 4 of the manuscript included in the Appendices).

Table 1. Relative risk of breast cancer (and 95% CI) by category of plasma hormone levels among postmenopausal women in the Nurses' Health Study. (156 cases/ 312 controls)

	Multivariate RR for quartile categories				
Hormone	1	2	3	4	95% CI (4 vs 1)
Estradiol (pg/ml)	1.0	1.15	1.11	1.89	(1.05-3.41)
% free estradiol	1.0	0.69	1.04	1.44	(0.79-2.64)
% bio estradiol	1.0	0.82	0.77	1.25	(0.71-2.20)
Estrone (pg/ml)	1.0	1.47	1.44	1.95	(1.05-3.62)
Estrone Sulfate (pg/ml)	1.0	0.98	1.11	2.21	(1.21-4.03)
Androstenedione (ng/dl)	1.0	1.28	1.92	1.48	(0.78-2.80)
Testosterone (ng/dl)	1.0	1.14	1.09	1.42	(0.74-2.73)
DHEA (ng/dl)	1.0	0.66	0.76	1.10	(0.60-2.02)
DHEAS (ug/dl)	1.0	2.21	1.60	2.17	(1.12-4.20)

*Controlling for family history, age at menarche and menopause, parity, age at first birth and BMI at age 18.

We also evaluated these relationships among women who never used postmenopausal hormones prior to blood collection (84 cases/193 controls), as our hormone measure would best reflect their long-term exposure (Table 2). In these analyses, we used unconditional logistic regression, controlling for the matching factors, to maximize our sample size. The relationships with a

number of the hormones were markedly strengthened, particularly for estradiol, estrone sulfate, and DHEAS (see below). The associations between hormone levels and cancer risk among past hormone users were null or weakly positive (e.g., top vs. bottom quartile for estrone sulfate= 1.0 (0.39-2.6); DHEAS = 1.3 (0.5-3.4)) although the confidence limits were very wide.

Table 2: Multivariate relative risk of breast cancer by plasma hormone level, among women who had not used postmenopausal hormones before blood collection (84 cases / 193 controls)

	Quartile categories					
Hormone	1	2	3	4	95% CI (4 vs 1)	p-value for trend
Estradiol	1.0	2.12	1.50	3.75	(1.62-8.71)	0.004
% free estradiol	1.0	0.48	1.06	1.38	(0.62-3.11)	0.05
% bioavailable estradiol	1.0	0.74	0.72	1.69	(0.74-3.85)	0.06
Estrone	1.0	0.86	1.57	2.97	(1.23-7.17)	0.01
Estrone Sulfate	1.0	1.33	1.37	4.66	(1.95-11.15)	0.006
Androstenedione	1.0	1.46	2.06	1.98	(0.78-5.03)	0.80
Testosterone	1.0	0.66	1.21	1.43	(0.59-3.45)	0.08
DHEA	1.0	0.81	0.50	1.19	(0.51-2.81)	0.58
DHEAS	1.0	4.91	5.19	5.33	(1.93-14.77)	0.005

Most steroid hormones are intercorrelated, e.g. the Spearman correlation for estradiol with estrone, testosterone, and DHEAS were 0.67, 0.45, and 0.27, respectively. Whether estradiol is the primary or only hormone associated with risk, or other steroids have independent associations is unknown (e.g., estrone sulfate could provide a ready source of estradiol intracellularly as breast epithelial cells have both aromatase and sulfatase activity). When estradiol was included in a statistical model with one other hormone, the estimates for estradiol were only slightly weaker while the relative risks for testosterone were substantially attenuated (top vs bottom quartile: RR=1.11 [0.53-2.30]) and those for for estrone (RR=1.50; 0.64-3.53), estrone sulfate (RR=1.88; 0.91-3.87) and DHEAS (RR=1.93; 0.97-3.82) were only modestly reduced - however, in all analyses the confidence intervals for each widened considerably. Thus several of the hormones may have independent predictive power.

(c). Results and Discussion: Prolactin levels and breast cancer risk.

In this analysis, we included all incident breast cancer cases who were postmenopausal at blood collection (regardless of postmenopausal hormone use) and their matched controls. Cases and controls were closely matched on time of day of blood draw and fasting status - important because prolactin has a strong circadian variation (52) and increases substantially with a noontime meal (43).

The median prolactin level in the 306 cases was significantly higher than that in 408 controls (9.0

versus 7.9 ng/ml; p=0.01). Breast cancer risk rose linearly with increasing category of prolactin level (RR's by quartile: 1.0, 1.05, 1.45, 2.03; p trend=0.02). RR estimates were somewhat stronger among invasive cases only (top vs bottom quartile RR=2.63 [1.54-4.50]), and were similar when cases diagnosed in the first two years after blood collection were excluded. Controlling for plasma IGF-I levels or plasma steroids did not alter the observed positive relationship. This is the first large prospective study of this relation and the results support a potentially important association between prolactin levels and subsequent risk of breast cancer.

2. Waist-to-hip ratio and breast cancer risk.

(a). Experimental Methods.

For this analysis, follow-up began in 1986, when waist and hip circumferences were provided by 50,828 women, and continued through May 31, 1994. We excluded women who reported any cancer other than nonmelanoma skin cancer prior to 1986, thus resulting in 47,382 women in the analytic cohort. Only women with a diagnosis of incident invasive breast cancer were included in these analyses (in situ cancer cases were excluded). We previously evaluated the validity of self-reported waist and hip measurements (ref). In a subsample of 140 participants living in the Boston area, technician measurements were found to be highly correlated with self-reports (Pearson r's for waist=0.89, hip=0.84, waist/hip=0.70). Relative risks were used as the measure of association. We used proportional hazards analyses to adjust simultaneously for age and other potential confounders. Tests for trend were performed using the categories of waist, hip circumferences or waist/hip ratio as a single continuous variable and assigning the median value to each category. All tests of statistical significance were two-sided.

(b). Results and conclusions.

In age-adjusted analysis among premenopausal women, waist circumference and WHR were not associated with risk of breast cancer. Hip circumference was significantly inversely associated with premenopausal breast cancer risk. The relative risks were not changed materially in the primary multivariate model which simultaneously adjusted for other breast cancer risk factors. The multivariate RR was 0.60 (95% CI, 0.37-0.98) for the upper versus bottom quintile of hip circumference (p trend=0.02). After accounting for BMI, both waist and WHR were positively, but not significantly, related to risk of premenopausal breast cancer. Women in the fifth quintile of waist circumference had a RR of 1.74 (95% CI, 0.74-4.07) compared with women in the first quintile. The RR was 1.43 (95% CI, 0.86-2.37) for the highest quintile of WHR compared to the lowest quintile.

In the age-adjusted analyses among postmenopausal women, waist, hip circumference and waist/hip ratio were positively and significantly associated with risk of postmenopausal breast cancer. The associations did not change materially after adjusting for multiple established risk factors. The positive relation between waist circumference and risk of breast cancer was monotonic (p for trend = 0.007) and the multivariate RR was 1.05 (95% CI, 1.02-1.09) for every 2 inch increase in waist circumference. Compared with women in the first quintile of waist circumference, women in the fifth quintile had a multivariate relative risk of 1.34 (95% CI, 1.05-1.72). After further accounting for BMI in 1986, this association was somewhat attenuated

(RR=1.26, 95% CI, 0.88-1.81) and no longer significant. A similar slightly weaker association was seen between waist/hip ratio and overall risk of postmenopausal breast cancer (p for trend = 0.005). The RR was 1.28 (95% CI, 1.02-1.61) for the highest quintle of WHR compared to the lowest quintile in the primary multivariate model, but was attenuated slightly after further accounting for BMI in 1986 (RR=1.22, 95% CI, 0.96-1.55). Among women who never used PMH, there was a stronger positive association between waist circumference and postmenopausal breast cancer. The multivariate RRs for the 4th and 5th quintiles of waist were 1.52 (95% CI, 1.02-2.27) and 1.88 (95% CI, 1.25-2.85) compared to the first quintile. The associations were only slightly attenuated after further adjusting for BMI in 1986.

In conclusion, we found waist circumference to be associated with a moderately increased risk of breast cancer, especially among postmenopausal women who never used hormone replacement therapy. This adverse impact on breast cancer appears to be in part independent of overall adiposity, and to be mediated largely through greater exposure to endogenous estrogens.

3. Overall Conclusions.

Our plasma hormone analyses represent the most detailed and extensive prospective analyses to date of the relationships between hormone levels and breast cancer risk. The data suggest important positive associations between several plasma estrogens, DHEAS, prolactin and subsequent risk of breast cancer among postmenopausal women. With further follow-up, we hope to further delineate the dose-response relationships and to further clarify the relationship among women who never used postmenopausal hormone therapy. In regards to waist-to-hip ratio and breast cancer risk, there indeed appears to be a moderate positive relationship that is independent of overall adiposity. This was again most clearly observed among women who never used postmenopausal hormone therapy. Reductions in overall adiposity by exercise and dietary changes are likely to decrease risk of postmenopausal breast cancer, possibly in part by reducing central adiposity.

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APPENDICES

1. Plasma Sex Steroid Hormone Levels and Risk of Breast Cancer in Postmenopausal Women. Susan E. Hankinson, Walter C. Willett, JoAnn E Manson, Graham A. Colditz, David J. Hunter, Donna Spiegelman, Robert L. Barbieri, Frank Speizer - Unpublished data (distribute to government agencies only)

2. Plasma Prolactin Levels and Subsequent Risk of Breast Cancer in Postmenopausal Women. Susan E. Hankinson, Walter C. Willett, Dominique S.Michaud, JoAnn E. Manson, Graham A. Colditz, Christopher Longcope, Bernard Rosner, Frank E. Speizer - Unpublished data (distribute to government agencies only)

3. Waist Circumference, Waist/Hip Ratio and Risk of Breast Cancer in Women. Zhiping Huang, Walter C. Willett, Graham A. Colditz, David J. Hunter, JoAnn E Manson, Bernard Rosner, Frank E. Speizer, Susan E. Hankinson. Unpublished data (distribute to government agencies only)

Plasma sex steroid hormone levels and risk of breast cancer in postmenopausal women

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Abstract

Background: A positive relationship between plasma estrogens and breast cancer risk in postmenopausal women has generally been observed, but most studies have been small and few have evaluated specific estrogen fractions. Plasma androgen levels also have been assessed in few studies and results have been inconsistent. **Methods:** We evaluated the relationships between plasma sex steroid hormone levels and risk of postmenopausal breast cancer in a case-control study nested within the Nurses' Health Study. Blood samples were collected in 1989-1990; among postmenopausal women not using hormone replacement therapy at blood collection, 156 women were diagnosed with breast cancer after blood collection but before June 1, 1994. Two controls were selected per case, matched on age, menopausal status, month and time of day of blood collection and fasting status. **Results:** We observed significant positive associations with risk of breast cancer for circulating levels of estradiol (top versus bottom quartile comparisons: multivariate relative risk (RR)= 1.91; 95% CI=1.06-3.46), estrone (RR=1.96; 95% CI=1.05-3.65), estrone sulfate (RR=2.25; 95% CI=1.23-4.12), and dehydroepiandrosterone sulfate (RR=2.15; 95% CI=1.11-4.17). We found no substantial associations with either percent free or percent bioavailable estradiol, androstenedione, testosterone, or dehydroepiandrosterone. The positive relationships were stronger among women with no previous use of hormone replacement therapy (top versus bottom quartile comparisons: for estradiol RR=3.53; 95% CI=1.55-8.03, estrone RR=2.85; 95% CI=1.23-6.61, estrone sulfate RR=4.34; 95% CI=1.87-10.1, and dehydroepiandrosterone sulfate RR=4.15; 95% CI=1.57-11.0). **Conclusion:** Our data, in conjunction with past epidemiologic and animal studies, provide strong evidence for a

causal relationship between postmenopausal estrogen levels and risk of breast cancer. Additional studies are needed before concluding which specific estrogen fractions are most important.

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Introduction.

Substantial indirect evidence supports a central role for endogenous hormones in breast cancer development (1). Reproductive factors such as early age at menarche, late age at menopause and nulliparity are associated with an increased risk of breast cancer. The rate of increase in age-specific breast cancer incidence rates slows at menopause, a time when endogenous estrogen levels decrease dramatically. In postmenopausal women, obesity (2) and use of postmenopausal hormone therapy (3), both positively correlated with plasma estrogen levels, also are positively related to breast cancer risk. Estrogens also induce mammary tumors in animals (4). Androgens may influence breast cancer risk either directly (5) or indirectly, through their conversion to estradiol (6, 7).

The relationships between postmenopausal plasma hormone levels and risk of breast cancer have been evaluated in six previous prospective studies (8, 9, 10, 11, 12, 13). For estrogens, the overall evidence supports a positive association (14) . However, in most studies, only one or two of the major circulating estrogens have been evaluated and, with one exception (8), the studies have been small with only 15 to 71 breast cancer cases. For plasma androgens, the data are more limited and results inconsistent.

To evaluate these relationships in detail, we conducted a prospective nested case-control study within the Nurses' Health Study cohort. We evaluated circulating estrogens and androgens in relation to risk of breast cancer. We also calculated estimates of effect that accounted for laboratory measurement error and the random within-person fluctuation in hormone levels over time (15).

Methods.

Study Population. The Nurses' Health Study (NHS) cohort was established in 1976 when 121,700 female registered nurses 30 to 55 years of age completed and returned a mailed questionnaire. The cohort continues to be followed every two years by questionnaire to update exposure status and to identify cases of newly diagnosed disease. Data have been collected on many breast cancer risk factors including height, weight, age at menarche and menopause, age at first birth, parity, postmenopausal hormone use, diagnosis of benign breast disease and family history of breast cancer.

In 1989-90 blood samples were collected from 32,826 cohort members (27% of the original cohort) who were 43 to 69 years of age at blood collection. Details regarding the blood collection methods have been previously published (16). Briefly, each woman arranged to have her blood drawn and then shipped, via overnight courier and with an icepack to keep the sample cool, to our laboratory where it was processed and separated into plasma, red blood cell and white blood cell components. Ninety-seven percent of the samples were received in our laboratory within 26 hours of being drawn. The stability of estrogens and androgens in whole blood for 24 to 48 hours has been previously documented (17). Samples have been archived at $\leq$-130°C in continuously monitored liquid nitrogen freezers since collection. As of 1994, the follow-up rate among women who gave a blood sample was 98%. The study was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital.

Both cases and controls in this analysis are women who, at blood collection, were postmenopausal and had not used postmenopausal hormones for at least three months. The

participants were defined as postmenopausal if they reported having a natural menopause or a bilateral oophorectomy, or, for women who reported a hysterectomy with either one or both ovaries remaining, when they were 56 (if a nonsmoker) or 54 (if a current smoker) - years of age when natural menopause had occurred in 90% of the cohort.

Cases were women with no reported cancer diagnosis prior to blood collection and who were diagnosed with breast cancer after blood collection but before June 1, 1994. Overall, 156 cases of breast cancer (140 invasive; 16 in situ) were reported from among the 11,169 women eligible at baseline. All cases were confirmed by medical record review with one exception where the nurse confirmed the diagnosis but the medical record was unavailable - because of the high confirmation rate (99%) upon medical record review, this case was kept in the analysis. Time from blood collection to diagnosis ranged from <1 month to 57 months (mean [S.D.]= 28.7 (15.8) months). Two controls were matched per case by age ($\pm$2 years), month of blood collection, time of day of blood draw ($\pm$2 hours), and fasting status ($\geq$10 hours since a meal versus <10 hours or unknown). 93% of control matches were exact; the most relaxed match was $\pm$ 5 years of age, $\pm$ 3 months and $\pm$ 7 hours for time of blood collection.

Laboratory Analyses. With the exception of estrone sulfate, all analyses were performed by Nichols Institute (San Juan Capistrano, CA). Plasma samples were extracted with hexane:ethyl acetate (4:1, v/v) and the extract was applied to celite columns (celite/ethylene glycol). The steroids were eluted from the columns in the following fractions: fraction 1: 3.5 ml iso-octane (androstenedione); fraction 2: 3.5 ml iso-octane with

10% ethyl acetate (DHEA, testosterone); fraction 3: 3.0 iso-octane with 15% ethyl acetate (estrone) and fraction 4: 5.0 ml iso-octane with 40% ethyl acetate (estradiol), and then assayed by radioimmunoassay (18, 19, 20, 21). Dehydroepiandrosterone sulfate (DHEAS) was assayed by RIA without a prior separation step (22). Percent free estradiol (i.e., percent non-protein bound) was assayed using equilibrium dialysis (23, 24); the percent dialyzable estradiol was calculated as per Vermuelen (24). The percent bioavailable estradiol (i.e., percent free plus percent albumin-bound estradiol) was assayed using an ammonium sulfate precipitation (25, 26). All case/control/control triplets were assayed together; the samples were ordered randomly within a triplet and labeled such that the laboratory could not identify case-control status. Although all members of a triplet were analyzed at the same time, the triplets were analyzed in up to three different batches (sent in 1992, 1993 and 1996).

For estrone sulfate, the first two batches of samples were assayed at the laboratory of Dr. C. Longcope at the University of Massachusetts Medical Center and the third batch at Nichols Institute. In each laboratory, after extraction of estrone, estrone sulfate was assayed by RIA of estrone, following enzyme hydrolysis, organic extraction and separation by column chromatography (27).

In each batch of samples, we interspersed plasma replicates (one replicate per 10 case/control samples) which were labeled to preclude their identification by the assaying laboratory; these replicate samples were used to assess laboratory precision. Within-batch laboratory coefficients of variation ranged from 6% (percent bioavailable estradiol) to 13.6% (DHEA).

The assay detection limit was 2 pg/ml for estradiol, 0.5% for both percent free estradiol and percent bioavailable estradiol, 10 pg/ml for estrone, 50 pg/ml for estrone sulfate (in each laboratory), 3 ng/dl for androstenedione, 1 ng/dl for testosterone, 3 ng/dl for DHEA, and 5 ug/dl for DHEAS. When plasma hormone values were reported as less than the detection limit, we set the value to half this limit (this occurred only for estrone [n=6], estrone sulfate [n=2] and DHEAS [n=2]).

Reproducibility study. Three hundred and ninety NHS participants who gave a first blood sample in 1989-90 were asked to have two additional samples collected over the following two years. The women were postmenopausal, had not used postmenopausal hormones for at least three months and had no previous diagnosis of cancer (except nonmelanoma skin cancer); these criteria were applied at each sample collection. Of the 390 women, 186 (48%) sent two additional samples. A random sample of 80 of these women who had all three samples drawn between 6 a.m. and 12 noon was sent for hormone analysis, at the same laboratories as the main study, and form the basis of the reproducibility study. Additional details regarding this study are provided elsewhere (15).

Data Analyses. We used quartile cutpoints, based on the distribution in the controls, for the purpose of summarizing breast cancer risk according to plasma hormone level. For most of the hormones, the mean and standard deviation of both the control values and the quality control replicates were very similar across batches, thus quartile cutpoints were made according to the distribution in the controls overall. For estrone, estrone sulfate, and DHEA, the median value for the controls varied by as much as 40% between batches, such that quartile cutpoints based on all controls combined resulted in uneven batch-specific

distributions (e.g., the lowest quartile of estrone contained 12% of the controls from the first two batches but 41% of the controls from the third batch). Because the mean value of the quality control replicates in each of the data sets varied in the same manner for these three assays, much (if not all) of this difference appeared due to laboratory drift rather than true differences in hormone levels between the batches. Thus, for these three hormones, we defined batch-specific quartile cutpoints. Additionally, in all analyses, we controlled for batch. For several hormones (e.g., estradiol) the control distribution was unequal across quartiles because of multiple identical hormone values.

One matched set was removed from the analysis because the case's estrogen values were in the premenopausal range (estradiol=411 pg/ml). Individual values more than 2.5 fold higher than the normal range according to the assaying laboratory also were removed; this resulted in the removal of two testosterone values only. In addition, several women did not have a sufficient volume of plasma for all assays. The final number of cases and controls available for each hormone analysis is provided in Table 1.

To test for differences in hormone levels between cases and controls, we used mixed effect regression models for clustered data in order to adjust for possible confounding due to the matching factors and for any residual correlation between cases and controls within the matched set (28). To compare proportions between cases and controls, we employed the Mantel-Haenszel test (29). We used conditional logistic regression analyses to estimate relative risks (odds ratios) and 95% confidence intervals (30). In analyses stratified by prior postmenopausal hormone use, however, we used unconditional logistic regression, controlling for the matching factors, to maximize our sample size. Tests for

trend were conducted by modeling the natural log of the hormone level as a continuous variable and calculating a Wald statistic (31). All p-values are based on two-sided tests. The regression calibration method was used to correct relative risks and 95% confidence intervals for laboratory measurement error and random within-person variability (32, 33, 34, 35). The within-person variance was calculated from the reproducibility study and the between-person variance from the current case-control study (thus intraclass correlation coefficients are slightly different from the previously published values). In these analyses, hormones levels were log transformed to lessen the influence of a small number of high or low values. Because the measurement error correction methods require that the relationship between disease and exposure be linear on a logistic scale, restricted cubic spline models (36) for breast cancer incidence in relation to each log-transformed hormone were fit to the data. Using this technique, as well as formal significance testing criteria for non-linearity, with just one exception (DHEA), none of the hormones showed substantial evidence of departure from a linear relation on the log relative risk scale. DHEA was modeled on its original scale.

Results.

Women ranged in age from 46 to 69 years (mean age=62 years) and had been menopausal for at least one year and up to 40 years (mean=12 years). Compared to controls, cases had an earlier mean age at menarche (12.4 vs 12.7 years), later age at first birth (26.0 vs 25.3) and were more likely to have reported a family history of breast cancer (19 vs 15%), although none of these differences were statistically significant. We observed

that cases had significantly higher plasma levels than controls of estradiol, estrone, estrone sulfate, testosterone, and DHEAS, but no substantial difference in levels of the other steroid hormones (Table 1).

In the simple conditional models, women in the top quartile of plasma estrone and estrone sulfate levels had an approximately two-fold increase in breast cancer risk that was statistically significant (estrone RR=1.77, 95% CI=1.01-3.11; estrone sulfate RR=2.12, 95% CI=1.21-3.71). For DHEAS, women in the top 75% of levels appeared to have an increase in breast cancer risk compared to women with the lowest levels. Modest, and generally nonsignificant positive associations were noted for percent free estradiol, androstenedione and testosterone and breast cancer risk. We observed little association with either percent bioavailable estradiol or DHEA. When we evaluated absolute levels of free and bioavailable estradiol, the associations were similar to those for total estradiol.

When a number of established breast cancer risk factors were controlled for statistically (see Table 2 footnote), the relationships tended to strengthen somewhat, primarily due to control for age at first birth and body mass index (BMI) at age 18. The association with estradiol was statistically significant (RR=1.91, 95% CI=1.06-3.46). BMI at age 18 was included in these models as it is inversely related to postmenopausal breast cancer risk (2) and thus we expected it could be a confounder. In contrast, when we included BMI at the time of blood collection in each of the models, relative risks for the estrogens were modestly attenuated, as postmenopausal BMI is a major determinant of postmenopausal estrogen levels (16). For example, the relative risk for the top to bottom quartile comparison decreased from 1.91 to 1.69 (95% CI: 0.83-3.42) for estradiol and from

1.96 to 1.75 (95% CI: 0.90-3.38) for estrone.

When we assessed the relationships between plasma hormones and breast cancer after excluding in situ breast cancer cases (n=16), nearly identical relative risks were observed. We also evaluated these relationships after excluding the 30 breast cancer cases that had been diagnosed within one year of blood collection, to assess if the positive associations might be due to an influence of the breast cancer itself on hormone levels. With the exception of percent free and percent bioavailable estradiol, where the relationships were slightly strengthened (top versus bottom quartile comparison, 1.69 (0.86-3.32) and 1.50 (0.79-2.84), respectively) results again did not differ materially.

We next evaluated the hormone/breast cancer relationships according to postmenopausal hormone use prior to blood collection (i.e., never versus past use) (Table 3). We hypothesized that our single hormone measure would best reflect long-term endogenous hormone exposure among the never users, and therefore we might see stronger associations in this group. Because of the small number of cases in each of the groups, we included in the statistical models only the matching factors and other most important covariates (see Table 3 footnote). Among never hormone users, the relationships with the estrogens, particularly estradiol and estrone sulfate, were markedly strengthened (top versus bottom quartile comparisons: estradiol RR=3.53 (1.55-8.03); estrone sulfate RR=4.34 (1.87-10.1). The association with DHEAS also was stronger. In contrast, the relationships among past hormone users were weak (or null) and not statistically significant, although the confidence intervals were wide.

Most of the steroid hormones are positively correlated. For example, the Spearman

correlation for estradiol with estrone, testosterone, and DHEAS were 0.67, 0.45, and 0.27, respectively. Therefore, we evaluated the independent association of each of the hormones with breast cancer risk, among all cases and controls combined, when estradiol also was included in the statistical model. The relative risks for testosterone were substantially attenuated (top versus bottom quartile: RR=1.08 [0.52-2.25]) whereas the relative risks for estrone (RR=1.50; 0.64-3.54), DHEAS (RR=1.90; 0.96-3.77) and estradiol itself were only modestly reduced. When estrone and estrone sulfate were included in the same statistical model, neither was attenuated, although the confidence intervals for each widened considerably.

We next corrected the associations for laboratory error and random within-person variability; in these analyses hormone levels were modeled as continuous variables (Table 4). The relative risk (based on a contrast in hormone levels from the 12.5 to 87.5 percentile of the distribution, corresponding to the medians of the bottom and top quartiles, respectively, as shown in Table 1) for estradiol strengthened considerably, increasing from 1.77 to 2.42. Similarly, the relationships with each of the other hormones strengthened somewhat, although only the relationships with estrone, estrone sulfate, percent free estradiol, DHEAS and testosterone were statistically significant. As in the categorical analyses, the association with testosterone was substantially attenuated after controlling for estradiol.

Discussion.

We observed positive associations between circulating levels of estradiol, estrone,

estrone sulfate, and DHEAS and risk of breast cancer in postmenopausal women. In contrast, we found no substantial associations between either percent bioavailable estradiol, androstenedione or DHEA in relation to breast cancer. The positive relationships were considerably stronger among women with no previous use of hormone replacement therapy after menopause.

Strengths of our study include that it was prospective and relatively large. Additionally, we were able to evaluate nine steroid hormones or hormone fractions, all of which were assayed with good precision. By using multiple hormone measures from a subset of study participants, we were able to correct our relative risk estimates for the random (and largely biologic) variation in hormone levels that can not ordinarily be captured by a single hormone measurement.

Evidence from our study, in conjunction with other recent prospective studies (8, 9, 10, 11, 12) supports a strong predictive role for plasma estradiol levels in relation to breast cancer risk among postmenopausal women. In only one small prospective study has a positive association not been observed (13). Although considerably larger relative risks have been reported for contrasts in levels generally similar to ours (11, 12), these two studies had sample sizes of only 24 and 61 cases respectively, thus their confidence limits broadly overlap ours. Additionally, some of the heterogeneity in relative risks between studies may be due to varying prevalences of past postmenopausal hormone use in study subjects. The magnitude of the associations also might be expected to vary because of different sensitivities and specificities of the laboratory assays used in different studies (37, 38) - this limitation makes the comparison of results between studies difficult and

estimation of the increase in disease risk per unit increase in estradiol levels (such as is done with plasma cholesterol and heart disease risk) currently infeasible.

Free or bioavailable estradiol are hypothesized to be readily available to the breast tissue and thus considered the most biologically active estrogen fraction (39). As such, compared to total estradiol, a stronger relationship between one of these fractions and breast cancer risk might be expected. However, the evidence has not been consistent (8, 9, 40, 41, 42, 43). We noted only a marginally significant positive relationship with percent free estradiol. We also observed no substantial relationship between percent bioavailable estradiol and risk, in contrast to the only previous large prospective study of this issue (RR for top versus bottom quartile comparison=4.4) (8). These differences seem unlikely to be due to confounding, or to different levels of measurement error. Our laboratory coefficient of variation was small and measurement error correction did not increase the estimates appreciably. We previously documented that our blood collection methods did not alter levels of percent free estradiol (17), suggesting that a change in the bioavailable fraction also is unlikely. The average age (59 versus 62 years of age) and lengths of follow-up (5 versus 2.5 years) of Toniolo's and our study populations also were similar. In addition, although the percent bioavailable estradiol values varied substantially between the two studies, results of the two assays are highly correlated. We sent 112 of our control samples for analysis to the laboratory used by Toniolo et al.; the Spearman correlation between the two different percent bioavailable estradiol assays was 0.91. These estrogen fractions have not been evaluated in any other large prospective studies, thus additional assessments are needed.

Estrone sulfate is the most abundant circulating estrogen in postmenopausal women (44, 45) and a major component of some postmenopausal hormone preparations. Although Dorgan et al (10), in the only other prospective study to examine this hormone, observed little association with breast cancer risk, they were unable to rule out an approximately 2-fold increase in risk such as we observed among women in the top 25% of the distribution compared to those in lower exposure categories.

Androgens have been hypothesized to increase breast cancer risk either directly, by increasing the growth and proliferation of breast cancer cells (5), or indirectly, by their conversion to estrogen (6, 7). Testosterone has been positively associated with breast cancer in most (10, 11, 12, 46, 47, 48, 49) but not all (50, 51) previous studies. However, the positive association has tended to weaken after controlling for total estradiol (or another estrogen fraction) (12, 46), similar to our findings, suggesting that increased testosterone levels may have a modest, but indirect, association with breast cancer through its conversion to estradiol.

DHEA and DHEAS are adrenal androgens that decrease substantially with increasing age and have little documented physiologic role (52). DHEA administered to rodents can decrease the risk of spontaneous and chemically-induced cancers (53). However, in postmenopausal women, DHEA has been proposed to act like an estrogen in stimulating cell growth (52), in part because of the estrogenic effect of its major metabolite, 5-androstenediol (54).

DHEAS has been evaluated in relation to breast cancer risk in five previous prospective studies and with one exception (55) (21 cases), nonsignificant positive

associations have been reported (46, 56, 10, 11), although in one study the weak positive association became inverse after controlling for estradiol (46). We observed a positive association, which was essentially independent of estradiol. In the two previous assessments of DHEA and breast cancer (56, 10), a significant positive association was observed; we found no significant association, but can not rule out a modest positive relationship. Overall, these findings should serve to caution against the increasing use of pharmacologic doses of DHEA as an "anti-aging" agent. DHEA and DHEAS are metabolically interconvertible and, after oral administration of DHEA, circulating levels of DHEAS rise substantially (57). Certainly epidemiologic evidence does not support a decreased risk of breast cancer with increasing levels of these androgens, and in fact suggests a possible positive association. Additionally, DHEA supplementation may increase plasma insulin-like growth factor 1 levels (58), a hormone which has recently been associated with risk of breast (59, 60) and prostate (61) cancer.

Estrogen (and some androgen) levels in normal breast tissue are generally much higher than levels in plasma, and levels in malignant tissue are higher than those in normal breast tissue (62, 63, 64). These differences may be due to enzyme activities in normal and malignant breast cells which result in the local conversion of androgens to estrogens, estrone sulfate to estrone, and estrone to estradiol (6, 63, 65). Although several reports have indicated there is little if any correlation between plasma and tissue steroid levels (62, 63, 64, 66 ,67) these studies were all small ($n \leq 14$ women) and the correlations were not provided. Given our findings, and those of others, it seems unlikely that these levels are entirely uncorrelated. A low correlation would suggest, however, that

the relationships between tissue hormone levels and breast cancer risk may be stronger than those observed with our plasma surrogates.

Our data, in conjunction with past epidemiologic (1, 2, 3, 8, 9, 10, 11, 12) and animal studies (4), provide strong evidence for a causal relationship between postmenopausal plasma estrogen levels and risk of breast cancer (68). However, additional studies are needed before concluding whether total estradiol or other specific fractions are most important to risk. Testosterone most likely has a modest indirect influence on risk through its conversion to estradiol and increasing evidence suggests a positive relationship between DHEAS and breast cancer risk. Although higher estrogen levels may have both beneficial (69) and adverse effects, reducing the levels or activity of endogenous estrogens may be a promising means for preventing breast cancer in postmenopausal women.

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64. van Landeghe

Table 1. Median and range* of plasma hormone levels for cases and matched controls.

	Cases			Controls			
Hormone	n	median	range	n	median	range	p-value†
Estradiol (pg/ml)	154	8.0	(4-16)	306	7.0	(4-14)	0.04
Percent free estradiol (%)	152	1.60	(1.33-1.85)	303	1.55	(1.33-1.82)	0.12
Percent bioavailable estradiol (%)	154	23.5	(13.9-40.6)	305	23.0	(13.5-37.2)	0.26
Estrone (pg/ml)	154	31	(20-51)	306	28	(17-45)	0.02
Estrone Sulfate (pg/ml)	144	232	(102-593)	288	192	(97-420)	0.02
Androstenedione (ng/dl)	147	62	(35-99)	296	57	(30-103)	0.13
Testosterone (ng/dl)	147	23	(13-44)	299	22	(12-37)	0.05
DHEA (ng/dl)	139	210	(97-434)	272	205	(99-366)	0.48
DHEAS (ug/dl)	153	87	(42-200)	298	79	(34-163)	0.01

* Range given is from median of the bottom quartile (12.5%) to median of the top quartile (87.5%)

† P-values from mixed effects regression model, controlling for matching factors

Table 2. Relative risk (RR) of breast cancer (and 95% confidence intervals) by category of plasma hormone levels among postmenopausal women in the Nurses' Health Study.

	RR (95% CI) for quartile categories				
Hormone	1	2	3	4	p-value*
Estradiol (pg/ml)	≤5	6-7	8-11	≥12	
Cases/Controls	41/97	33/69	36/78	44/62	
Simple RR†	1.0	1.12	1.09	1.73	0.04
Multivariate RR§	1.0	1.17	1.12	1.91	0.03
		(0.64-2.15)	(0.62-2.03)	(1.06-3.46)	
% free estradiol	≤1.43	1.44-1.55	1.56-1.70	≥1.71	
Cases/Controls	38/74	29/79	38/75	47/75	
Simple RR	1.0	0.70	0.98	1.23	0.14
MV RR	1.0	0.71	1.05	1.48	0.05
		(0.37-1.34)	(0.55-1.98)	(0.81-2.72)	
% bioavailable estradiol	≤17.38	17.39-23.0	23.1-31.38	≥31.39	
Cases/Controls	40/76	35/77	31/76	48/76	
Simple RR	1.0	0.84	0.78	1.19	0.26
MV RR	1.0	0.84	0.79	1.27	0.28
		(0.45-1.54)	(0.43-1.44)	(0.72-2.26)	
Estrone (pg/ml)‡					
Cases/Controls	27/70	36/77	38/79	53/79	
Simple RR	1.0	1.24	1.28	1.77	0.02
MV RR	1.0	1.46	1.42	1.96	0.01
		(0.77-2.77)	(0.74-2.75)	(1.05-3.65)	

Table 2 . Cont. Relative risk of breast cancer (and 95% confidence intervals) by category of plasma hormone level among postmenopausal women in the Nurses' Health Study.

	RR (95% CI) for quartile categories				
Hormone	1	2	3	4	p-trend
Estrone Sulfate (pg/ml)‡					
Cases/Controls	29/73	27/71	28/70	60/74	
Simple RR	1.0	0.93	1.04	2.12	0.02
MV RR	1.0	1.01	1.14	2.25	0.01
		(0.51-2.00)	(0.58-2.26)	(1.23-4.12)	
Androstenedione (ng/dl)	≤40	41-57	58-77	≥78	
Cases/Controls	26/73	37/76	45/73	39/74	
Simple RR	1.0	1.33	1.74	1.50	0.14
MV RR	1.0	1.25	1.88	1.46	0.10
		(0.70-2.29)	(1.00-3.54)	(0.77-2.76)	
Testosterone (ng/dl)	≤15	16-22	23-31	>31	
Cases/Controls	33/75	38/79	37/78	39/67	
Simple RR	1.0	1.12	1.10	1.34	0.05
MV RR	1.0	1.12	1.07	1.40	0.04
		(0.60-2.10)	(0.57-2.00)	(0.73-2.70)	
DHEA (ng/dl)‡					
Cases/Controls	43/73	25/68	33/65	38/66	
Simple RR	1.0	0.62	0.90	0.99	0.36
MV RR	1.0	0.64	0.74	1.08	0.31
		(0.33-1.24)	(0.40-1.36)	(0.59-1.98)	
DHEAS (ug/dl)	≤48	49-78.5	79-124	≥125	
Cases/Controls	23/73	48/76	37/75	45/74	
Simple RR	1.0	2.15	1.68	2.10	0.01
MV RR	1.0	2.20	1.62	2.15	0.01
		(1.12-4.29)	(0.84-3.14)	(1.11-4.17)	

* p-value for trend from model with the log hormone level entered as a continuous variable.

† Conditional model controlling for matching factors only

§ Conditional model additionally controlling for BMI at age 18 (<21, 22-22.9, 23-24.9, ≥25 kg/m^2), history of breast cancer (no family history, history in mother or sister), age at menarche (<12 years, 12, 13, ≥ 14), age at first birth/parity (nulliparous, 1-4 children/age at first birth < 25 years, 1-4 child/ age at first birth 25-29, 1-4 children/ age at first birth ≥ 30, 5+ children/ age at first birth <25, 5+ children/ age at first birth ≥25), age at menopause (<45 years, 45-49, 50-55, >55, missing), and past postmenopausal hormone use (continuous in years).

‡ For estrone, cutpoints for batches one and two were <25 pg/ml, 25-32, 33-42, >42 and, for batch three, <18 pg/ml, 18-23, 24-30, and >30. For estrone sulfate, cutpoints for batch one were ≤118 pg/ml, 119-164, 165-227, >227 and, for batches two and three, ≤141 pg/ml, 142-205, 206-299, and >299. For DHEA, cutpoints for batch one were ≤114 pg/ml, 115-162, 163-252, >252 and for batches two and three, ≤159, 160-223, 224-320, and >320.

Table 3. Multivariate relative risk* of breast cancer by plasma hormone level, according to use of postmenopausal hormones prior to blood collection.

	Quartile categories					
Hormone	1	2	3	4	(95% CI)†	p-value§
No use of postmenopausal hormones prior to blood collection ‖						
Estradiol	1.0	2.07	1.52	3.53	(1.55-8.03)	0.003
% free estradiol	1.0	0.52	1.27	1.47	(0.67-3.23)	0.04
% bioavailable estradiol	1.0	0.76	0.77	1.80	(0.83-3.93)	0.11
Estrone	1.0	0.82	1.57	2.85	(1.23-6.61)	0.002
Estrone Sulfate	1.0	1.33	1.36	4.34	(1.87-10.1)	0.002
Androstenedione	1.0	1.35	1.73	1.77	(0.72-4.32)	0.27
Testosterone	1.0	0.62	1.10	1.32	(0.56-3.11)	0.12
DHEA	1.0	0.77	0.48	1.11	(0.48-2.60)	0.92
DHEAS	1.0	3.65	3.57	4.15	(1.57-11.0)	0.005
Use of postmenopausal hormones prior to blood collection #						
Estradiol	1.0	0.78	1.00	1.39	(0.50-3.84)	0.33
% free estradiol	1.0	0.92	0.67	1.31	(0.52-3.25)	0.42
% bioavailable estradiol	1.0	0.97	1.24	1.33	(0.53-3.38)	0.40
Estrone	1.0	1.70	1.28	0.87	(0.33-2.27)	0.85
Estrone Sulfate	1.0	0.82	1.19	1.08	(0.43-2.71)	0.23
Androstenedione	1.0	1.66	1.83	1.08	(0.40-2.90)	0.70
Testosterone	1.0	1.56	1.23	1.61	(0.59-4.37)	0.13
DHEA	1.0	0.64	0.96	1.03	(0.42-2.53)	0.29
DHEAS	1.0	1.73	0.68	1.01	(0.38-2.65)	0.53

* Unconditional logistic regression analyses using same category cutpoints as in Table 2, and controlling for the matching factors, BMI at age 18, age at first birth and parity, with categories as described in Table 2.

† 95% CI for top versus bottom quartile comparison

§ P-value for trend from model with log hormone level entered as a continuous variable.

‖ From 71 to 83 cases and 168 to 190 controls, depending upon the specific hormone

\# From 65 to 71 cases and 105 to 118 controls, depending upon the specific hormone

Table 4. Correction of multivariate relative risk (RR)* estimates and 95% confidence intervals (CI) for random within-person measurement error.

Hormone	ICC+	uncorrected RR (95% CI)	corrected RR (95% CI)
Estradiol	0.66	1.77 (1.06-2.93)	2.42 (1.10-5.35)
% free estradiol	0.79	1.69 (1.03-2.80)	1.97 (1.03-3.77)
% bioavailable estradiol	0.87	1.30 (0.82-2.06)	1.36 (0.80-2.31)
Estrone	0.77	1.91 (1.15-3.16)	2.35 (1.20-4.58)
Estrone sulfate	0.83	1.80 (1.14-2.85)	2.04 (1.17-3.58)
Androstenedione	0.64	1.51 (0.89-2.58)	1.95 (0.82-4.63)
Testosterone	0.84	1.65 (1.00-2.71)	1.83 (1.01-3.32)
DHEA	0.53	1.34 (0.89-2.02)	1.75 (0.79-3.85)
DHEAS	0.81	1.94 (1.17-3.24)	2.29 (1.21-4.34)

* Relative risks based on comparing median hormone level in top quartile to median level in bottom quartile (see Table 1 for range in values).

\+ Intraclass correlation coefficient

Plasma prolactin levels and subsequent risk of breast cancer in postmenopausal women.

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running head: prolactin and risk of breast cancer

Abstract

Background. In animal studies, prolactin is important in mammary epithelial development and consistently increases rates of mammary tumor formation. Previous epidemiologic studies of prolactin and breast cancer risk in postmenopausal women have been small and the results inconsistent.

Methods. We conducted a nested case-control study within the prospective Nurses' Health Study cohort. Blood samples were collected from cohort members in 1989-1990; included in this analysis were 306 postmenopausal women diagnosed with breast cancer after blood donation but before June 1994. One or two postmenopausal controls were matched per case by age, postmenopausal hormone use, and time of day and month of blood collection, for a total of 448 controls.

Results. In conditional logistic regression analyses, we observed a significant positive association between prolactin levels and breast cancer risk (top versus bottom quartile comparison: multivariate relative risk=2.03; 95% confidence interval = 1.24-3.31, p-trend=0.01). The relationship was independent of plasma sex steroid hormone levels and was similar after excluding cases diagnosed in the first two years after blood collection.

Conclusions. These prospective data suggest that higher plasma prolactin levels are associated with an increased risk of breast cancer in postmenopausal women.

key words: prolactin, breast cancer, epidemiology, prospective

Introduction.

Prolactin, a polypeptide hormone, is essential for mammary gland development and lactation (1, 2). Whether it influences the risk of breast cancer in women is unclear. In animals, prolactin is important in mammary epithelial development; administration of exogenous prolactin increases rates of mammary tumor formation and suppression of prolactin levels decreases tumor formation (3, 4). Prolactin also increases the growth of both normal and malignant breast cells in vitro (5, 6, 7), although these findings have not been entirely consistent (8, 9).

The epidemiologic data relating plasma prolactin levels to risk of breast cancer have been limited and the results inconsistent. In postmenopausal women, prolactin levels have been associated with an increased risk in several (10, 11), but not all (12, 13, 14), retrospective case-control studies. Because prolactin secretion can be affected by either physical or psychological stress (15, 16, 17), levels in women with breast cancer may not reflect pre-disease levels. To date, only one prospective study of prolactin and breast cancer risk has been reported 18, with just 40 postmenopausal breast cancer cases, and a nonsignificant positive relationship was observed.

To evaluate the relationship between plasma prolactin levels and breast cancer risk in postmenopausal women, we conducted a nested case-control study within the large prospective Nurses' Health Study cohort.

Methods.

Study Population. The Nurses' Health Study (NHS) cohort was established in 1976

when 121,700 U.S. female registered nurses 30 to 55 years of age completed and returned a mailed questionnaire. The cohort continues to be followed every two years by questionnaire to update exposure status and to identify cases of newly diagnosed disease. Data have been collected on breast cancer risk factors including height, weight, age at menarche and menopause, age at first birth, parity, postmenopausal hormone (PMH) use, diagnosis of benign breast disease and family history of breast cancer. Weight, use of PMH and diagnosis of benign breast disease have been updated every two years.

In 1989-90 blood samples were collected from 32,826 cohort members who were 43 to 69 years of age at collection. Details regarding the blood collection methods have been previously published (19). Briefly, each woman arranged to have her blood drawn and shipped, via overnight courier and with an icepack, to our laboratory where it was processed and separated into plasma, red blood cell and white blood cell components. Ninety-seven percent of the samples were received within 26 hours of being drawn. The stability of prolactin in whole blood for 24 to 48 hours has been previously documented (20). Samples have been archived in continuously monitored liquid nitrogen freezers since collection. At blood collection, women were asked if they were currently using antidepressant medications, many of which (e.g., phenothiazines) can increase prolactin levels. Other medications that can alter prolactin levels were not queried. As of 1994, the follow-up rate among women who provided a blood sample was 98%. The study was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital.

Both cases and controls are women who were postmenopausal at blood collection.

Women were defined as postmenopausal if they reported having a natural menopause (no menstrual cycles in the previous 12 months) or a bilateral oophorectomy, or, for women who reported a hysterectomy with either one or both ovaries remaining, when they were 56 (if a nonsmoker) or 54 (if a current smoker) - years of age when natural menopause had occurred in 90% of the cohort.

Cases were women with no reported cancer diagnosis prior to blood collection and who were diagnosed with breast cancer after blood collection but before June 1, 1994. 337 cases of breast cancer were reported. All cases were confirmed by medical record review with one exception where the nurse confirmed the diagnosis but the medical record was unavailable - because of the high confirmation rate (99%) upon medical record review, this case was kept in the analysis. Time from blood collection to diagnosis ranged from <1 month to 57 months (mean=27.8 months). For each case who reported PMH use within three months prior to blood collection ("recent use"), one control was matched per case by age ($\pm$2 years), recent PMH use, month of blood collection ($\pm$1 month), time of day of blood draw ($\pm$2 hours), and fasting status ($\geq$10 hours since a meal versus <10 or unknown). For each case who had not reported recent PMH use at blood collection, two controls were selected using the same matching factors (done to increase our statistical power in analyses using only this case group). Control matches were exact 94% (age), 96% (time of day) and 98% (month) of the time; the most relaxed match was $\pm$5 years of age, $\pm$7 hrs, and $\pm$3 months.

Reproducibility study. Three hundred and ninety NHS participants who gave a first blood sample in 1989-90 were asked to collect two additional samples over the following

two years. The women were postmenopausal, had no prior diagnosis of cancer (except nonmelanoma skin cancer) and no recent PMH use; these criteria were applied at each sample collection. Of the 390 women, 186 (48%) sent two additional samples. A random sample of 80 of these women who had all three samples drawn between 6 a.m. and 12 noon was sent for prolactin analysis, at the same laboratories as the main study, and form the basis of the reproducibility study. Details regarding this study have been published elsewhere (21).

Laboratory Analyses. Prolactin was assayed at Dr. C. Longcope's laboratory at the University of Massachusetts Medical Center. Prolactin was measured using a microparticle enzyme immunoassay (IMx System, Abbott Laboratory, Abbott Park, IL). The assay detection limit was 0.6 ng/ml; none of our values was less than this limit.

All case/control pairs (or case/control/control triplets) were assayed together; the samples were ordered randomly within a pair and labeled such that the laboratory could not identify case-control status. Although all members of a pair were analyzed at the same time, the pairs were analyzed in two different batches (1993 and 1996). In each batch, we interspersed replicate plasma samples, labeled to preclude their identification by the hormone laboratory, to assess laboratory precision. The intra-assay laboratory coefficient of variation was 7.6%.

We previously measured insulin-like growth factor 1 (IGF-1) levels among all of the cases and controls (22), as well as steroid hormone levels (estradiol, estrone, percent free estradiol, percent bioavailable estradiol, estrone sulfate, androstenedione, testosterone, dehydroepiandrosterone [DHEA], and dehydroepiandrosterone sulfate [DHEAS]) among the

cases and controls not using postmenopausal hormones at blood collection.

Data Analyses. Prolactin values tended to be higher in the first batch of samples assayed, such that quartile cutpoints based on all controls combined resulted in uneven batch-specific distributions (e.g., the highest quartile contained 41% of batch 1 and 18% of batch 2 controls). Because the mean levels of the quality control samples varied in the same manner between batches, this difference appeared to be due to laboratory variation over time. We therefore defined batch specific cut-points, based on the distribution of the control values in each batch. The quartile cutpoints were 6.4, 9.3, and 13.7 ng/ml for batch 1 and 5.9, 7.6, and 9.7 ng/ml for batch 2.

One matched set was removed from the analysis because the case's estrogen values were in the premenopausal range. In addition, individual prolactin values more than 2.5-fold higher than the normal range (1.8-18 ng/mL) were removed from the analysis (4 cases and 1 control); however, in a secondary analysis which included these participants, findings were essentially unchanged. A number of women either did not have plasma available for laboratory analysis or had implausible values related to initial technical difficulties with the assay, resulting in a loss of 25 cases and 33 controls. One case and 9 controls were excluded because the other members of their matched set did not have prolactin values. Overall, 306 cases (277 invasive, 28 in situ, 1 unknown) and 448 controls were included.

To test for differences in means between cases and controls, we used mixed effect regression models for clustered data, in order to adjust for possible confounding due to the matching factors and for any residual correlation between cases and controls within the matched set (23). To compare proportions between cases and controls, we employed the

Mantel-Haenszel test (24). We used conditional logistic regression analyses to estimate relative risks (odds ratios) and 95% confidence intervals (25). Tests for trend were conducted by modeling prolactin levels continuously and calculating a Wald statistic (26). All p-values were based on two-sided tests.

The regression calibration method was used to correct point and interval estimates of the relative risks for laboratory measurement error and random within-person variation (27, 28, 29, 30). The within-person variance was calculated from the reproducibility study, and the between-person variance from the case-control study thus the intraclass correlation coefficient for prolactin was slightly different from the previously published value (21). Because the measurement error correction methods require that the relationship between exposure and disease be linear on the logistic scale, four knot restricted cubic spline models (31) for breast cancer incidence in relation to log-transformed prolactin levels were fit to the data. Using these graphical techniques as well as significance testing criteria for non-linearity, prolactin levels did not show evidence of departure from linearity (p-value= 0.49).

Results.

At blood collection, women ranged in age from 45 to 69 years (mean age=62 years) and had been menopausal for at least 1 year (mean=12 years) (Table 1). Mean BMI and age at menopause were essentially identical in case and control groups, but this partly reflects the matching of cases and controls on PMH use at blood collection (1:2 case:control ratio if no recent PMH use and 1:1 ratio if recent use). Among non-hormone users at blood collection, cases had a later mean age at menopause and higher mean BMI

than controls, as expected, although these differences were not statistically significant. The median prolactin level in cases was significantly higher than that in controls (9.0 versus 7.9 ng/ml; p=0.01).

We observed a linear increase in breast cancer risk with increasing category of plasma prolactin level (Table 2) (p trend=0.02). Women in the top quartile of plasma prolactin levels had a significant 87% higher risk of breast cancer compared to women with the lowest levels (RR=1.87; 95% CI=1.19-2.94). When we controlled for several established breast cancer risk factors, the relationship strengthened slightly in the top quartile (RR=2.03), primarily due to control for BMI at age 18 and parity. Additionally controlling for duration of use of oral contraceptives did not materially alter these estimates. Qualitatively similar positive associations were observed within each laboratory batch (RRs for increasing quartiles of prolactin levels: Batch 1 [121 cases/176 controls]: 1.0, 1.45, 1.81, 1.83 (95% CI: 0.79-4.23); Batch 2 [185 cases/272 controls]: 1.0, 0.87, 1.53, 2.47 (95% CI: 1.28-4.76)).

Current use of postmenopausal hormones tends to increase circulating prolactin levels. After controlling for age, batch, fasting status, and time of day of blood collection, mean prolactin levels were 8.4 ng/ml (SD=0.99) in nonusers and 9.5 ng/ml (SD=1.03) in hormone users (p=0.01). When we examined the relationship between prolactin and breast cancer separately according to PMH use at blood collection, the relative risks were stronger among women not on hormones (top versus bottom quartile comparisons for women not on PMH: multivariate RR=2.45; 95% CI=1.25-4.79, versus women on PMH: multivariate RR=1.86; 95% CI=0.86-4.04). Among women who reported never using PMH (77 cases

and 181 controls), however, the comparable unconditional relative risk was similar (multivariate RR=1.93).

Additionally controlling for plasma IGF-I levels, a hormone hypothesized to influence risk of breast cancer (32, 33), did not alter the observed relationship. Among the 145 cases and 290 controls who were not using PMH at blood collection, we also were able to control for plasma levels of several sex steroid hormones. None of the steroids substantially altered the relationship between plasma prolactin and breast cancer risk. For example, when controlling for quartile of plasma estradiol levels, the relative risk for the top versus bottom quartile of plasma prolactin changed from 2.45 to 2.35 (95% CI: 1.20-4.61).

We also evaluated this relationship after excluding the 28 cases of in situ breast cancer, and observed that the relative risks were slightly strengthened (Table 2). When we excluded cases who were diagnosed with breast cancer within the first two years of providing their blood sample (and their matched controls), the relative risks were very similar to those observed for all cases and controls combined (top versus bottom quartile comparison RR=2.39; 95% CI=1.24-4.61). Ten cases and 21 controls had reported antidepressant medication use at blood collection; removing these women from the analysis also did not alter the relative risks. In addition, controlling for fasting status more tightly (in two hour increments) did not appreciably alter our findings.

The relative risk associated with having prolactin levels at or above the 87.5 percentile (i.e., median of the top quartile) compared to levels at or below the 12.5 percentile (i.e., median of the bottom quartile) in a continuous model was 1.62 (95% CI=

1.12 - 2.37). The intraclass correlation coefficient for prolactin, measured over a two to three year period, was 0.45. Measurement error correction resulted in a substantially higher relative risk for the same contrast in levels (RR=3.05; 95% CI= 1.27-7.36).

Discussion.

In this prospective analysis, we observed a positive relationship between plasma prolactin and subsequent risk of breast cancer; women in the upper 25% of levels had approximately a two-fold higher risk of breast cancer compared to those in the lower 25% of the distribution. This relationship was similar after controlling for a number of established breast cancer risk factors, plasma estrogen and androgen levels, and IGF-1 levels. After excluding cases diagnosed in the first two years after blood collection, similar relative risks were observed.

Our study is larger than all previous epidemiologic studies of postmenopausal prolactin levels and breast cancer combined, and much larger than the only other published prospective study (n=40 cases) (18). Because prolactin is known to be influenced by both physical and emotional stress (15, 16, 17), the prospective nature of our study is an important strength. The observation of similar relative risks after excluding cases diagnosed in the first two years after blood collection further assures that the relationship is not due to an influence of the breast cancer on hormone levels. Although 9% of our case samples were unavailable for analysis, this is unlikely to have been related to their prolactin levels and thus would not bias our relative risks. Prolactin levels were measured with excellent precision by the laboratory. However, substantial drift in the prolactin values

between the two laboratory batches limited our ability to evaluate absolute levels of prolactin in relation to breast cancer risk; nevertheless, the positive findings were observed in each of the two batches. Circulating prolactin has a strong circadian variation (17), increases substantially with a noontime meal (34) and, postmenopausally, tends to fluctuate more over time (within-woman) than most sex steroid hormones (21). To minimize misclassification related to these factors (which would have attenuated our relative risks), we closely matched our cases and controls on both time of day of blood draw and fasting status and, by using multiple hormone measures from a subset of study participants, we were able to correct our relative risk estimates for the random (and largely biologic) variation in hormone levels that can not be captured by a single hormone measurement.

Postmenopausal prolactin levels have been evaluated in relation to risk of breast cancer in few previous studies. In addition to the potential limitations of case-control studies of prolactin described above, these studies were all small, with the largest including just 66 cases (10). In these studies, either a positive association (10, 11) or essentially no association (13, 14 ,12) between prolactin and breast cancer has been observed. In the only previous prospective study (18), women in the top quintile of prolactin levels were at a nonsignificant 63% higher risk of breast cancer compared to those in the bottom quintile, results comparable to our findings. Epidemiologic data on premenopausal prolactin levels and breast cancer risk are similarly sparse (11, 35, 36, 37, 10, 18) and thus additional assessments are needed.

Longer term recent use of both oral contraceptives and postmenopausal hormones

has been associated with an increased risk of breast cancer (38, 39). The increase in prolactin levels observed with use of these hormones (17) could conceivably be playing a role in this effect. Other medications also are known to increase (e.g., reserpine, haldol, cimetidine, phenothiazines) or decrease (e.g., levodopa) plasma prolactin levels (17). Of these, the relationship between reserpine use, an antihypertensive medication, and breast cancer risk has been evaluated most extensively. Reserpine initially causes an acute elevation of prolactin, however, longer-term use (>5 years duration) results in about a 50% elevation in plasma levels (40). Although a positive association between reserpine use and breast cancer was noted in several previous studies (41, 42, 43), this finding was not consistent (44, 45, 46, 47, 48) and thus firm conclusions regarding the relationship could not be drawn. Reasons for this lack of consistency may include the small size of most of the studies and the exposure definition used (e.g., most investigators reported the relationship with "ever use" of reserpine only). If prolactin is a promoter rather than an initiator of breast cancer (as would seem most likely), only longer durations of use might be expected to have a discernible influence on risk, as is observed with postmenopausal hormone use (38) . Further assessment of this and other medications known to alter prolactin levels, including an evaluation of duration of medication use, are warranted.

Several indirect lines of evidence suggest prolactin could play a role in breast carcinogenesis. Although prolactin is secreted primarily by the anterior pituitary, expression in both normal (49) and malignant (50, 49, 51) breast tissue has been reported. In addition, prolactin receptors have been found on >50% of breast tumors (52,

53). Prolactin also increases DNA synthesis of breast cancer cells in vitro (5, 6, 7) and its removal inhibits the growth rate of epithelial cells from nonmalignant breast tissue (54). These findings have not been universal (8, 9), however, and might relate to the amount or type of prolactin used or the prolactin receptor status of the cells (5). Prolactin administration also is well-documented to increase mammary tumor rates in mice (3).

Several forms of prolactin circulate in human plasma: the native hormone (23 kDa), a 16-kDa fragment (55, 56), and several glycosylated forms (57, 58), among others. These different forms appear to have varying bioactivities (59, 60) and perhaps differing biologic actions (60, 61). Which isoform(s) are most related to breast cancer risk is unknown. The two laboratory methods used most commonly to assess prolactin levels are immunoassay (which we used) and bioassay. The immunoassay will identify most prolactin isoforms but to differing degrees (62); the assay is unable to distinguish between these different forms. The correlation between the two assays has generally been reported to be high (63, 64), however, this may vary by study population as a differential release of prolactin isoforms can occur (17, 65, 58).

Most research on endogenous hormones and breast cancer has focused on plasma estrogens (66, 4). The positive relationship we observe between prolactin and risk of breast cancer is similar in magnitude to that observed for plasma estrogen levels and breast cancer. Because our study provides the first detailed evaluation of this relationship, additional prospective assessments are warranted. In addition, further evaluation of medications that can alter prolactin levels and risk of breast cancer, and of other lifestyle factors that might modify prolactin levels, are needed.

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Table 1. Baseline characteristics of breast cancer cases and matched controls from the Nurses' Health Study

Characteristics	Cases	Controls	p-value*
	mean (s.d.)	mean (s.d.)	
Age (yrs)	61.5 (5.0)	61.9 (4.8)	0.32
Age at menarche (yrs)	12.5 (1.4)	12.6 (1.5)	0.30
Age at menopause (yrs)	49.1 (4.1)	49.4 (4.0)	0.39
Parity†	3.2 (1.5)	3.5 (1.5)	0.51
BMI at blood collection (kg/m^2)	25.7 (5.0)	25.7 (4.6)	0.92
Family history (%)	16.6	12.7	0.05‡
History of benign breast disease (%)	49.8	37.5	0.003‡
	median (range)	median (range)	
Prolactin ng/ml	9.0 (1.5-37.4)	7.9 (2.5-32.1)	0.01

* p-values from mixed effects regression model, controlling for the matching factors age, postmenopausal hormone use, month and time of day of blood collection and fasting status.

† among parous women only

‡ Mantel-Haenszel p-values

Table 2. Relative risk (RR) of breast cancer by category of plasma prolactin level among postmenopausal women in the Nurses' Health Study

	Quartile* of plasma prolactin level				
	1 (lowest)	2	3	4 (highest)	p-value for trend
All women (n=306 cases/448 controls)					
Cases/controls	64/121	63/112	79/112	100/103	
Simple RR †	1.0	1.08	1.44	1.87	0.02
95% CI		(0.68-1.71)	(0.93-2.23)	(1.19-2.94)	
Multivariate RR ‡	1.0	1.05	1.45	2.03	0.01
95% CI		(0.65-1.71)	(0.91-2.31)	(1.24-3.31)	
Invasive cases only (n=278 cases/ 406 controls)					
Simple RR	1.0	1.19	1.61	2.21	0.02
95% CI		(0.73-1.93)	(1.01-2.57)	(1.35-3.62)	
Multivariate RR	1.0	1.26	1.61	2.64	0.007
95% CI		(0.75-2.13)	(0.98-2.64)	(1.54-4.51)	
Excluding first two years of follow-up # (n=183 cases/ 272 control)					
Simple RR	1.0	0.73	1.31	1.99	0.008

95% CI		(0.40-1.33)	(0.75-2.29)	(1.10-3.59)	
Multivariate RR	1.0	0.69	1.34	2.39	0.004
95% CI		(0.37-1.32)	(0.72-2.51)	(1.24-4.61)	

* Quartile cutpoints for batch 1 were ≤ 6.4, 6.5-9.3, 9.4-13.7, >13.7 ng/ml and, for batch 2, ≤5.9, 6.0-7.6, 7.7-9.7 and >9.7 ng/ml.

† Conditional model controlling for matching factors only.

‡ Conditional model controlling for matching factors and additionally controlling for BMI at age 18 (<21, 22-22.9, 23-24.9, ≥25 kg/m^2), history of breast cancer (no family history,mother or sister had breast cancer), age at menarche (<12 years, 12, 13, ≥ 14), age at first birth/parity (nulliparous, 1-4 children/age at first birth < 25, 1-4 child/ age at first birth 25-29, 1-4 children/ age at first birth ≥ 30, 5+ children/ age at first birth <25, 5+ children/ age at first birth ≥25), age at menopause (<45 years, 45-49, 50-55, >55, missing) and duration of postmenopausal hormone use (continuous).

Includes invasive and in situ cases.

Waist Circumference, Waist/Hip Ratio and Risk of Breast Cancer among Women

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Abstract

Background: Obesity is associated with a lower risk of breast cancer before menopause and with an increased risk of cancer after menopause. Whether body fat distribution contributes further to the risk of breast cancer remains unclear. Purpose: This study was conducted to examine prospectively the associations of waist circumference and waist/hip circumference ratio (WHR) with risk of breast cancer. Methods: A cohort of 47,382 US registered nurses reported their waist and hip circumferences along with other risk factors in 1986 and were followed every 2 years up to 1994 to identify incident cases of breast cancer. We used proportional hazards analyses to adjust simultaneously for age and other potential confounders and to compute age-adjusted and multivariate-adjusted relative risks (RR) and 95% confidence intervals (CI). All tests of statistical significance were two sided. Results: During eight years of follow-up (333,097 person-years), 197 premenopausal and 840 postmenopausal invasive breast cancers were diagnosed. After adjusting for established risk factors, waist circumference was not significantly related to risk of premenopausal breast cancer, but was positively associated with postmenopausal breast cancer (RR=1.34 for highest versus lowest quintile of waist, 95% CI, 1.05 to 1.72, p trend=0.007). The multivariate RR was 1.05 (95% CI, 1.02-1.09) for every 2 inch increase in waist circumference. When the analysis was limited to postmenopausal women who never used hormone replacement therapy, a stronger positive association was found (RR=1.88 for highest versus lowest quintile of waist, 95% CI, 1.25 to 2.85). After further controlling for body mass index, the magnitude of the positive association was only slightly attenuated (RR=1.83, 95% CI, 1.12-2.99). Among past and current postmenopausal hormone users, no significant associations were found

with waist circumference. Similar but slightly weaker associations were observed between WHR and breast cancer risk. Conclusions: These data suggest that greater waist circumference increases risk of breast cancer, especially among postmenopausal women who are otherwise at lower risk because of never having used estrogen replacement hormones.

Keywords: breast carcinoma, body fat distribution, waist circumference, waist/hip ratio.

Obesity has a complex relation to risk of breast cancer. Higher body mass index (BMI) is associated with a lower risk of breast cancer before menopause and with an increased risk of cancer after menopause, especially among postmenopausal women who never used hormone replacement therapy (1). Whether body fat distribution contributes further to the risk of breast cancer is of considerable interest. Greater upper or central body fat distribution, perhaps due to its relation with visceral adiposity, is associated with multiple hormonal and metabolic changes including insulin resistance and hyperinsulinemia, decreases in sex-hormone-binding globulin, and increases in androgen levels and the conversion of androgen to estrogen in adipose tissue (2). Therefore, women with central adiposity may have a higher risk of breast cancer compared to those having fat primarily distributed subcutaneously over the hips, buttocks, and lower extremities. In some case-control studies, central obesity measured either by waist circumference or waist/hip ratio (WHR) has been associated with increased risk of breast cancer among postmenopausal women; whereas the association has been weak or equivocal among premenopausal women (3,4). In two cohort studies, a positive relation between abdominal adiposity and postmenopausal breast cancer has been noted (5,6), but in other studies no significant association was observed (7,8). Thus, the association between body fat distribution and risk of breast cancer remains unclear.

The current analysis investigated body fat distribution measured by waist circumference and waist/hip ratio in relation to subsequent risk of premenopausal and postmenopausal breast cancer among the Nurses' Health Study cohort. We also examined whether central adiposity remained a risk factor after accounting for overall obesity, as

assessed by body mass index, and whether the association between body fat distribution and postmenopausal breast cancer was modified by postmenopausal hormone use (PMH).

Materials and Methods

The Nurses' Health Study is an ongoing follow-up study initiated in 1976 when 121,701 US female registered nurses aged 30 to 55 years responded to a mailed questionnaire on medical history and health behaviors. Follow-up questionnaires have been sent to participants every two years to identify incident cases of breast cancer and other medical events and to update information on risk factors. The follow-up rate has been 95% of potential person-years. Further details have been reported elsewhere (9).

For the current analysis, follow-up began in 1986, when waist and hip circumferences were provided by 50,828 women, and continued through May 31, 1994. We excluded women who reported any cancer other than nonmelanoma skin cancer prior to 1986 (n=3,446). If any of these conditions developed during follow-up, women were excluded from subsequent follow-up intervals. The analytic cohort thus included 47,382 women.

Measurement of exposures

In 1986, women were asked to measure their waist circumference at the level of the umbilicus and their hip at its largest circumference while standing relaxed. We asked participants to record their measurements to the nearest quarter inch. These circumferences were used to compute waist/hip ratio. We classified women into quintiles of waist, hip circumferences and WHR.

Information on age, height, current weight, parity, age at first birth, family history of breast cancer (in mother or sisters), personal history of benign breast diseases, age at menarche, menopausal status, age at menopause, physical activity, alcohol consumption and postmenopausal hormone (primarily estrogen and/or progesterone) use as well as other variables was obtained from the 1986 or prior questionnaires. Most variables were updated by biennial follow-up questionnaires. Body mass index, a measure of overall obesity, was calculated as weight in kilograms divided by the square of height in meters (kg/m^2).

Menopausal status was determined by asking women whether their menstrual periods had permanently ceased. We classified women as postmenopausal if a natural menopause or hysterectomy with bilateral oophorectomy was reported. Those with hysterectomy without bilateral oophorectomy were considered as postmenopausal at more than 54 years for current smokers or 56 years for nonsmokers (when natural menopause had occurred in 90% of the cohort). Those with hysterectomy without bilateral oophorectomy were considered as premenopausal at less than 46 years for current smokers or 48 years for nonsmokers (when natural menopause had occurred in only 10% of the cohort). Between these ages, women were classified as having uncertain menopausal status (10).

In a subsample of 140 participants living in the Boston area, the validity of self-reported waist and hip circumferences was assessed (11). Self-reported waist, hip circumferences and WHR were highly correlated with technician measurements (Pearson correlation coefficients were 0.89, 0.84 and 0.70, respectively). The self-reported waist

and hip circumferences were 0.05 and 0.54 inches smaller, and waist/hip ratio was 0.011 greater than technician measurements on the average.

Ascertainment of Cases

On biennial follow-up questionnaires, women were asked whether breast cancer had been diagnosed during the previous two years. We searched the National Death Index for nonrespondents to identify fatal breast cancers. For all cases identified, permission was requested from the subjects, or from the next of kin for decedents, to obtain hospital records and pathology reports. Hospital records were obtained for 96% of women who reported breast cancer, and self-reported breast cancers were confirmed in 99.4% of records obtained. We excluded 0.6% of records that did not confirm breast cancer. Because self-reports were highly accurate among those for whom hospital records were obtained, we included self-reported breast cancer cases from whom hospital records could not be obtained. In situ breast cancers were excluded from these analyses.

Statistical Analysis

Based on clinical judgment, reported waist circumferences >55 inches or <15 inches and hip circumferences >65 inches or <20 inches were considered as outliers and excluded from the analyses. Follow-up started in 1986 when waist and hip circumferences were first obtained. Follow-up time was accrued up to the date of breast cancer diagnosis, or date of diagnosis of any cancer other than nonmelanoma skin cancer, or the date of death, or May 31, 1994, whichever came first. Women were not included

during the intervals when their menopausal status was uncertain, but they reentered the analyses when the information became available.

Relative risk (RR) was used as the measure of association and was computed as the incidence rate in a specific category of waist, hip and WHR divided by the rate in the reference category. We used proportional hazards analyses to adjust simultaneously for age and other potential confounders and to compute age-adjusted and multivariate-adjusted RRs and 95% confidence intervals (CI) (12-13). Tests for trend were performed using the categories of waist, hip circumference or waist/hip ratio as a single continuous variable and assigning the median value to each category. All tests of statistical significance were two sided.

Results

During 333,097 person-years of follow-up from 1986 to 1994, 1037 invasive breast cancers (197 premenopausal and 840 postmenopausal) were identified. In age-adjusted analysis among premenopausal women, waist circumference and WHR were not associated with risk of breast cancer (Table 1). Hip circumference was significantly inversely associated with premenopausal breast cancer risk. The relative risks were not changed materially in the primary multivariate model which simultaneously adjusted for age, height, personal history of benign breast diseases, family history of breast cancer, age at menarche, physical activity, age at first birth and parity. The multivariate RR was 0.60 (95% CI, 0.37-0.98) for the upper versus bottom quintile of hip circumference (p trend = 0.02). To examine whether the associations with body circumferences were independent of overall obesity, BMI in 1986 was added to the primary multivariate

model. After accounting for BMI, both waist and WHR were positively, but not significantly, related to risk of premenopausal breast cancer. Women in the fifth quintile of waist circumference had a RR of 1.74 (95% CI, 0.74-4.07) compared with women in the first quintile. The RR was 1.43 (95% CI, 0.86-2.37) for the highest quintile of WHR compared to the lowest quintile. The inverse relation between hip circumference and breast cancer remained significant after accounting for BMI in 1986. Waist and hip circumferences were also simultaneously entered into the primary multivariate model. After adjusting for each other, waist tended to be positively and hip was inversely associated with premenopausal breast cancer, and the test for trend was significant for hip circumference.

In the age-adjusted analyses among postmenopausal women, waist, hip circumference and waist/hip ratio were positively and significantly associated with risk of postmenopausal breast cancer (Table 2). The associations did not change materially after adjusting for multiple established risk factors. The positive relation between waist circumference and risk of breast cancer was monotonic (p for trend = 0.007) and the multivariate RR was 1.05 (95% CI, 1.02-1.09) for every 2 inch increase in waist circumference. Compared with women in the first quintile of waist circumference, women in the fifth quintile had a multivariate relative risk of 1.34 (95% CI, 1.05-1.72). After further accounting for BMI in 1986, this association was somewhat attenuated (RR=1.26, 95% CI, 0.88-1.81) and no longer significant. Controlling for hip circumference did not appreciably change the association with waist circumference. A similar and slightly weaker association was seen between waist/hip ratio and overall risk of postmenopausal breast cancer (p for trend = 0.005). The RR was 1.28 (95% CI, 1.02-

1.61) for the highest quintile of WHR compared to the lowest quintile in the primary multivariate model, but was attenuated slightly after further accounting for BMI in 1986 (RR=1.22, 95% CI, 0.96-1.55). Larger hip circumference was associated with a higher risk of postmenopausal breast cancer. Women in the fifth quintile of hip circumference had a multivariate relative risk of 1.29 (95% CI, 1.02-1.64) compared with women in the first quintile. This association was attenuated towards the null after accounting for BMI in 1986, or waist circumference.

Because postmenopausal hormone replacement therapy greatly elevates blood hormone levels and may modify the association between adiposity and postmenopausal breast cancer (1), we performed analysis stratified by postmenopausal hormone use (Figure 1). Among women who never used PMH, there was a stronger positive association between waist circumference and postmenopausal breast cancer. The multivariate RRs for the 4th and 5th quintiles of waist were 1.52 (95% CI, 1.02-2.27) and 1.88 (95% CI, 1.25-2.85) compared to the first quintile. The associations were only slightly attenuated after further adjusting for BMI in 1986 (relative risks were 1.50 [95% CI, 0.98-2.29] and 1.83 [95% CI, 1.12-2.99] for the 4th and 5th quintiles). Among PMH current users, no association was observed between waist and risk of postmenopausal breast cancer. This null association remained after further adjusting for BMI. Among postmenopausal women who used hormones in the past, the magnitude of the association with waist circumference was intermediate. The interaction between waist circumference and postmenopausal hormone use was of borderline significance (p=0.06, 2 degrees of freedom). For waist/hip ratio, the interaction with postmenopausal hormone use was statistically significant (p=0.03, 2 degrees of freedom) (Figure 2). Among PMH never

users, a strong positive association was observed between WHR and postmenopausal breast cancer, and the multivariate RR was 1.85 (95% CI, 1.25-2.74) for the highest quintile of WHR compared to the lowest quintile; while among PMH current and past users, there was no clear dose-response relationship between WHR and risk of breast cancer. After further accounting for BMI, the magnitude of the association with postmenopausal breast cancer became somewhat weaker in general.

We also examined the associations between waist circumference, WHR and breast cancer risk within the strata of other risk factors including age and family history of breast cancer, but did not observe any significant effect modification by these factors. We conducted additional analyses to adjust for alcohol consumption, and this did not appreciably change the associations of waist, hip circumferences and WHR with breast cancer risk in either premenopausal or postmenopausal women. We also examined BMI in 1986 and risk of breast cancer after adjusting for multiple covariates and waist circumference or WHR, and BMI was inversely and nonsignificantly associated with premenopausal breast cancer, but tended to be positively associated with postmenopausal breast cancer, especially among those who have never used postmenopausal hormones.

Discussion

In this study, waist circumference and WHR were associated with higher risk of breast cancer, particularly among postmenopausal women. Even after accounting for overall adiposity measured by BMI, larger waist circumference and WHR were still associated with an additional modest to moderate impact on breast cancer. The positive associations were much stronger among postmenopausal women who never used

hormone replacement therapy. Larger hip circumference was associated with a lower risk of premenopausal breast cancer, but was not related to postmenopausal breast cancer after accounting for overall obesity.

In a few case-control studies, WHR was found to be a risk factor for breast cancer in premenopausal women (14). However, in most case-control studies that examined regional adiposity, the associations between waist, waist/hip ratio and risk of premenopausal breast cancer were weak and equivocal (3-4,15). In a large population-based case-control study, body fat distribution measured by WHR or subscapular-to-triceps skinfold ratio was not related to breast cancer in women <45 years (16).

Central adiposity assessed by waist circumference or WHR has been associated with postmenopausal breast cancer, independent of relative weight, in several studies (14, 17-18). In two case-control studies in the Netherlands, in which breast cancer cases were detected at breast cancer screening or afterwards, waist circumference and WHR were positively associated with postmenopausal breast cancer (4). The estimated relative risks were 1.89 (95% CI, 0.80-4.48) for the upper vs lower tertile of waist/hip ratio, and 2.86 (95% CI, 1.12-7.32) for waist circumference (3). However, other studies have noted no difference in WHR between cases and controls (15). Body fat distribution was also assessed by contrasting groups of subscapular and triceps skinfold thicknesses and no relation between fat distribution and breast cancer was found among postmenopausal women (19).

Four prospective studies have examined regional adiposity and risk of breast cancer. In two studies a positive association was seen (5,6) and in the other two no relation was observed between central adiposity and risk of breast cancer (7,8). In the

Framingham cohort study, a central adiposity ratio was computed by the sum of skinfold measurements from the upper and lower trunk divided by the sum of skinfold measurements from the upper and lower extremities. Risk of breast cancer was significantly associated with truncal fat predominance; the RRs for the 4th quartile of central adiposity ratio vs the first quartile were 1.2 (95% CI, 0.6-2.4) among premenopausal women and 2.1 (95% CI, 1.0-4.6) among postmenopausal women. The positive association between central adiposity and breast cancer was not altered after adjustment for overall adiposity (5).

In the Iowa Women's Health Study, abdominal adiposity assessed by WHR was associated with an increased risk of postmenopausal breast cancer; the odd ratios for the highest versus the lowest tertile was 1.39 (95% CI, 0.99-1.96) (6). In a later report from the same cohort, the association between WHR and breast cancer risk was more pronounced among women with a positive family history of breast cancer. The age-adjusted relative risk for the fifth quintile of WHR as compared with the first quintile was 3.24 (95% CI, 2.11-4.99) in women with a family history of breast cancer (83 breast cancer cases) and 1.20 (95% CI, 0.87-1.67) in women without such a family history (382 breast cancer cases) (20). However, we did not find evidence for such an interaction in this study. The multivariate RRs for the fifth quintile vs first quintile were 1.23 (95% CI, 0.65-2.34) for waist circumference and 0.73 (95% CI, 0.40-1.33) for WHR among women with a family history of breast cancer (117 breast cancer cases); and 1.45 (95% CI, 1.10-1.92) for waist circumference and 1.40 (95% CI, 1.09-1.81) for WHR among women without a family history (693 breast cancer cases).

In a 12-year prospective study of 1462 Swedish women aged 38-60 years, there was no association between breast cancer and body fat distribution measured by waist circumference as well as by subscapular skinfold thickness, but there were only 21 incident breast cancer cases in this study (7). In a 15-year follow-up study in the Netherlands (8), body fat distribution, assessed by contrasting subscapular and triceps skinfold thicknesses, was not related to breast cancer incidence among postmenopausal women, perhaps because the measure did not include an indicator of gluteofemoral fatness, as was used in other studies.

In most previous studies a weaker association between central obesity and breast cancer has been seen among premenopausal women than among postmenopausal women, and this was true in our primary analyses. After adjusting for total obesity, the association became a little stronger among premenopausal women but did not achieve statistical significance. The weaker association between regional adiposity and breast cancer in premenopausal women may be explained by different sources of endogenous sex hormones before and after menopause (21-22).

In our study cohort, waist circumference was highly correlated with BMI (correlation coefficient = 0.8), and BMI was inversely associated with premenopausal breast cancer and positively associated with postmenopausal breast cancer (1), so that after controlling for BMI, the association between waist circumference and breast cancer became more positive among premenopausal women and attenuated among postmenopausal women. Nevertheless, our data indicated that central adiposity still had an additional association with breast cancer after accounting for overall obesity. However, neither overall obesity assessed by BMI nor central obesity assessed by waist

circumference are measured perfectly (23). Because they are highly correlated, they may in part serve as surrogates for each other. Thus, it is possible that an apparent independent effect of waist circumference on breast cancer risk after adjusting for BMI could be due to additional information conveyed by waist circumference about overall adiposity, rather than a specific effect of body fat distribution.

In our study, waist circumference was a slightly stronger predictor of breast cancer than was WHR. Waist circumference is a fairly unambiguous measure of abdominal fat, whereas the interpretation of WHR is complicated because it is the ratio of two variables, both of which could contribute to breast cancer risk (23). Also, WHR has a larger measurement error because it includes two sources of error, i.e., that from both waist and hip measurements (10). Waist circumference has been more strongly correlated with BMI and the percentage of body fat measured by bioelectric impedance analysis or dual-energy X-ray absorptiometry than has WHR (24).

The reason for the inverse association between hip circumference and risk of premenopausal breast cancer is unclear. Hip circumference reflects both muscle and fat, as well as bony structure. The persistent inverse association with hip circumference after adjusting for BMI or waist circumference may suggest that more muscle and/or larger bones are associated with lower risk of premenopausal breast cancer.

Central adiposity is associated with a decrease in sex hormone binding globulin (SHBG) concentration (25). The percentage of serum estradiol bound to SHBG closely follows SHBG concentration, so that a more central body fat distribution has been significantly related to greater bioavailability of estradiol (4,26). In addition, women with abdominal adiposity have increased levels of free fatty acids and triglycerides (27-29),

which increase the level of bioavailable estrogen by displacing estrogen from SHBG to albumin where it is less tightly bound (26). Thus, both decreases in SHBG and increases in free fatty acids and triglycerides result in increases in free estradiol levels in both premenopausal and postmenopausal women. Furthermore, it has been proposed that abdominal adiposity is associated with androgen excess and increased conversion of androgen to estrogen in adipose tissue (30). Abdominal obesity is also associated with hyperinsulinemia and insulin resistance, which also have been hypothesized to be associated with an increased risk of breast cancer (31-32).

Postmenopausal hormone use partially masked the association between central obesity and postmenopausal breast cancer, probably because exogenous hormone use elevated blood hormone levels among both lean and obese women, so that all PMH users were at increased risk of breast cancer regardless of central obesity (see Figures 1 and 2). Only among PMH never users, in whom blood hormone levels had never been influenced by exogenous hormones, was a strong positive association between abdominal obesity and postmenopausal breast cancer observed. This relationship, also seen with overall adiposity (1), strongly suggests that the increased risks of breast cancer associated with both overall and regional adiposity are primarily mediated by higher endogenous estrogen exposure.

In conclusion, we found waist circumference to be associated with a moderately increased risk of breast cancer, especially among postmenopausal women who never used hormone replacement therapy. This adverse impact on breast cancer appears to be in part independent of overall adiposity, and to be mediated largely through greater exposure to endogenous estrogens. Reductions in overall adiposity by exercise and dietary changes

are likely to decrease risk of postmenopausal breast cancer, possibly in part by reducing central adiposity.

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Figure legends

Figure 1. Relative risks of breast cancer by waist circumference and hormone use among postmenopausal women.

Relative risks were adjusted for age, height, personal history of benign breast disease, family history of breast cancer, physical activity, age at menarche, age at first birth, age at menopause and parity.

Figure 2. Relative risks of breast cancer by waist/hip ratio and hormone use among postmenopausal women.

Relative risks were adjusted for age, height, personal history of benign breast disease, family history of breast cancer, physical activity, age at menarche, age at first birth, age at menopause and parity.

Table 1. Relative Risk of Breast Cancer According to Waist, Hip and Waist/hip Ratio Among Premenopausal Women (1986-1994)

Variables	Cases	Person-Years of Follow-up	Age-Adjusted RR (95% CI)	Multivariate RR* (95% CI)	Adjusted for BMI** (95% CI)	Adjusted for waist or hip# (95% CI)
Waist (inch)						
15.0-27.9	56	25,796	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
28.0-29.9	47	21,804	0.96 (0.65-1.42)	0.96 (0.65-1.42)	1.07 (0.71-1.61)	1.01 (0.68-1.52)
30.0-31.9	41	14,486	1.22 (0.82-1.84)	1.22 (0.81-1.85)	1.49 (0.94-2.37)	1.35 (0.87-2.09)
32.0-35.9	35	15,481	0.97 (0.63-1.48)	0.97 (0.63-1.51)	1.38 (0.79-2.43)	1.15 (0.70-1.91)
36.0-55.0	18	8,774	0.88 (0.52-1.51)	0.90 (0.52-1.55)	1.74 (0.74-4.07)	1.23 (0.61-2.51)
P for trend			0.73	0.78	0.15	0.11
Hip (inch)						
20.0-36.9	56	19,884	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
37.0-38.4	43	21,072	0.71 (0.47-1.05)	0.69 (0.46-1.03)	0.68 (0.45-1.03)	0.65 (0.43-0.98)
38.5-40.4	48	19,614	0.83 (0.57-1.23)	0.78 (0.53-1.17)	0.76 (0.50-1.18)	0.70 (0.46-1.06)
40.5-42.9	25	12,880	0.65 (0.41-1.05)	0.60 (0.37-0.97)	0.57 (0.32-1.03)	0.48 (0.28-0.84)
43.0-65.0	25	12,891	0.65 (0.40-1.04)	0.60 (0.37-0.98)	0.56 (0.26-1.21)	0.40 (0.20-0.80)
P for trend			0.04	0.02	0.05	0.004
W/H ratio						
<0.73	58	25,962	1.00 (reference)	1.00 (reference)	1.00 (reference)	
0.73-0.75	42	18,782	0.98 (0.66-1.46)	1.00 (0.67-1.49)	1.02 (0.68-1.52)	
0.76-0.79	39	19,454	0.86 (0.57-1.29)	0.88 (0.58-1.32)	0.94 (0.62-1.42)	
0.80-0.83	32	12,210	1.11 (0.72-1.72)	1.18 (0.76-1.83)	1.32 (0.84-2.07)	
≥0.84	26	9,933	1.11 (0.70-1.77)	1.18 (0.74-1.88)	1.43 (0.86-2.37)	
P for trend			0.61	0.43	0.13	

*: Adjusted for age (continuous), height (continuous), personal history of benign breast disease (yes or no), family history of breast cancer (yes or no), age at menarche (≤10, 11-12, 13, 14, ≥15 years), physical activity (≤2.2, 2.3-4.9, 5.0-10.9, 11.0-22.9, ≥23.0 total met score), age at first birth (nulliparous, ≤24, 25-29, ≥30 years) and parity (nulliparous, 1-2, 3-4, ≥5 births).

**: Adjusted for BMI in 1986 (continuous) in addition to the above variables.

#: Relative risks for waist and hip were adjusted for each other in addition to the above variables.

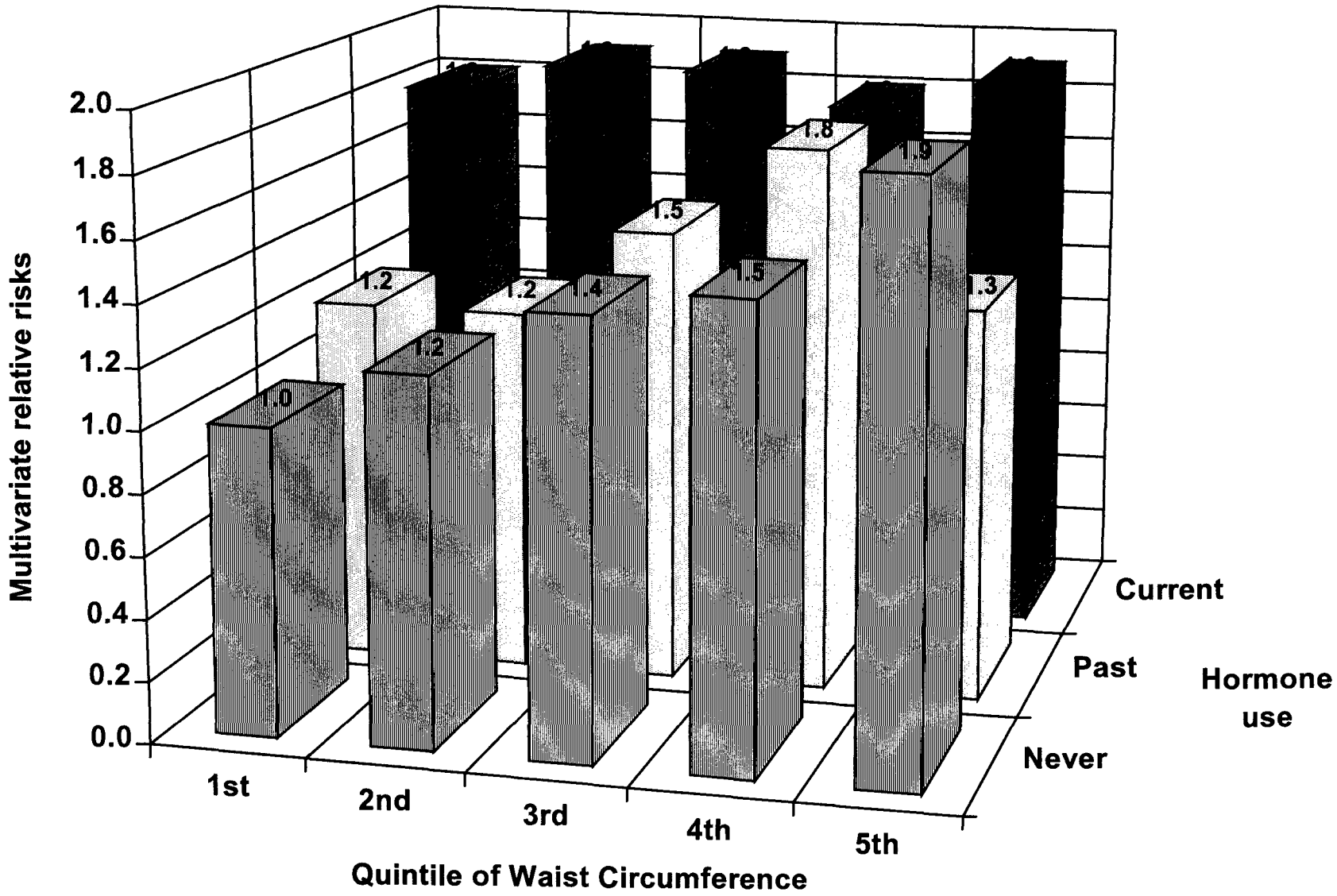
Table 2. Relative Risk of Breast Cancer According to Waist, Hip and Waist/hip Ratio Among Postmenopausal Women (1986-1994)

Variables	Cases	Person-Years of Follow-up	Age-Adjusted RR (95% CI)	Multivariate RR* (95% CI)	Adjusted for BMI** (95% CI)	Adjusted for waist or hip# (95% CI)
Waist (inch)						
15.0-27.9	121	43,875	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
28.0-29.9	160	52,377	1.07 (0.84-1.35)	1.06 (0.83-1.34)	1.05 (0.82-1.33)	1.05 (0.83-1.34)
30.0-31.9	162	46,660	1.19 (0.94-1.50)	1.15 (0.91-1.46)	1.13 (0.88-1.46)	1.15 (0.89-1.47)
32.0-35.9	243	64,474	1.25 (1.01-1.56)	1.24 (0.99-1.55)	1.20 (0.92-1.56)	1.23 (0.96-1.56)
36.0-55.0	154	39,370	1.30 (1.02-1.65)	1.34 (1.05-1.72)	1.26 (0.88-1.81)	1.32 (0.96-1.82)
P for trend			0.01	0.007	0.15	0.05
Hip (inch)						
20.0-36.9	125	44,814	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
37.0-38.4	177	51,753	1.22 (0.97-1.54)	1.15 (0.92-1.45)	1.12 (0.89-1.42)	1.11 (0.88-1.40)
38.5-40.4	204	58,456	1.22 (0.98-1.53)	1.14 (0.91-1.43)	1.07 (0.84-1.37)	1.04 (0.82-1.32)
40.5-42.9	153	45,096	1.18 (0.93-1.50)	1.08 (0.85-1.38)	0.98 (0.75-1.29)	0.93 (0.71-1.21)
43.0-65.0	181	46,637	1.35 (1.08-1.70)	1.29 (1.02-1.64)	1.07 (0.76-1.51)	0.98 (0.71-1.34)
P for trend			0.02	0.06	0.71	0.84
W/H ratio						
<0.73	132	46,332	1.00 (reference)	1.00 (reference)	1.00 (reference)	
0.73-0.75	142	44,751	1.07 (0.85-1.36)	1.08 (0.85-1.37)	1.08 (0.85-1.37)	
0.76-0.79	180	58,114	1.02 (0.82-1.28)	1.04 (0.83-1.31)	1.02 (0.81-1.28)	
0.80-0.83	198	48,118	1.33 (1.07-1.67)	1.38 (1.10-1.73)	1.34 (1.06-1.68)	
≥0.84	188	49,440	1.19 (0.95-1.50)	1.28 (1.02-1.61)	1.22 (0.96-1.55)	
P for trend			0.03	0.005	0.03	

*: Adjusted for age (continuous), height (continuous), personal history of benign breast disease (yes or no), family history of breast cancer (yes or no), physical activity (≤2.2, 2.3-4.9, 5.0-10.9, 11.0-22.9, ≥23.0 total met score), age at menarche (≤10, 11-12, 13, 14, ≥15 years), age at first birth (nulliparous, ≤24, 25-29, ≥30 years), age at menopause (≤44, 45-49, 50-54, ≥55 years), postmenopausal hormone use (never, current, or past) and parity (nulliparous, 1-2, 3-4, ≥5 births).

**: Adjusted for BMI in 1986 (continuous) in addition to the above variables.

#: Relative risks for waist and hip were adjusted for each other in addition to the above variables.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1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1.0
1.2
1.2
1.2
1.4
1.5
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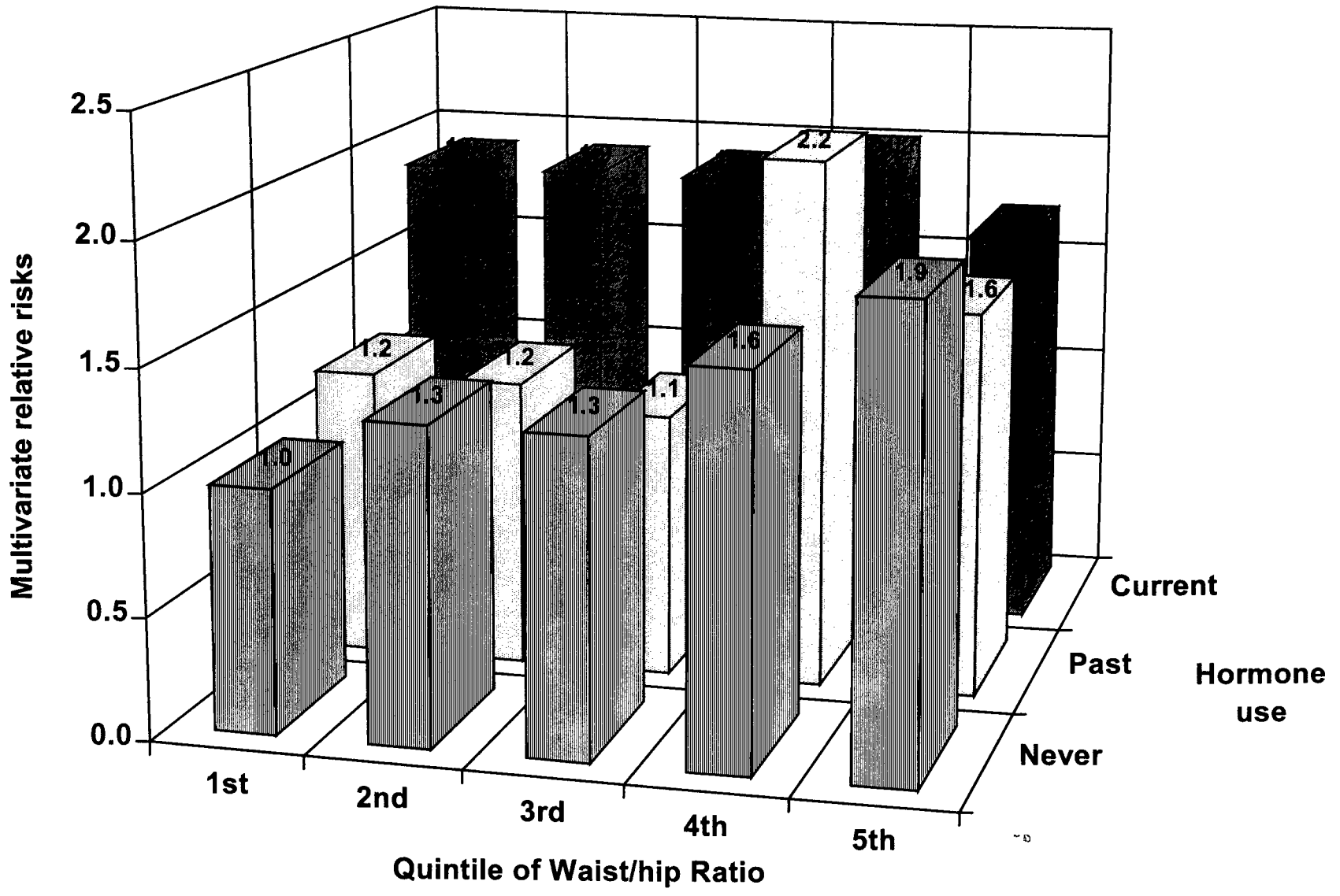
4th
5th
Quintile of Waist Circumference
Current
Past
Never
Hormone use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1.2
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1.1
1.6
2.2
1.9
1.6
1st
2nd
3rd
4th
5th
Quintile of Waist/hip Ratio
Current
Past
Never
Hormone use

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 29 May 02

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request for Change in Distribution Statements

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for Grant DAMD17-96-1-6021. Request the limited distribution statements for Accession Documents Number ADB231829 and ADB239327 be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management