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The purpose of our study is to further evaluate the relationship between bone mineral density (BMD) and the risk of breast cancer among women age 65 years or older. All of the women are participants in the Study of Osteoporotic Fractures. We initially identified 121 cases of breast cancer over an approximate 3 years of follow-up. As part of the Department of Defense grant, we will extend the follow-up from 3 to 6 to 8 years. This extension will allow us to 1) verify our findings of a 2.5 fold increase in risk of breast cancer among women with the highest BMD compared to women with the lower BMD; 2) improve our power to look at the association between exogenous estrogen use, BMD, vertebral fractures and breast cancer; 3) improve our power to look at potentially important interactions including alcohol, estrogen, BMD and breast cancer and family history of breast cancer, BMD and breast cancer.

The scope of work involves 1) identifying potential breast cancer from the Year 6 (1992-1994) and Year 8 (1995-1997) examination of the cohort; 2) obtaining medical records; 3) adjudication of cases; 4) data analysis. The thrust of work during the first year of funding has been aimed at #1 through #3.

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Jane A Carley 9/9/98

PI - Signature Date

Table of Contents

INTRODUCTION

The focus of the current application is to further our understanding of the association between two of the most common conditions influencing a woman's health: osteoporosis and breast cancer.

We have recently reported that the relative risk of breast cancer increased with increasing BMD (1, and Appendix A). The risk of breast cancer among women in the top quartile of proximal radial BMD was 2.8 times higher than those in the lowest; the relative risks associated with top quartile BMD at the distal radius and calcaneus were 2.6 and 2.8, respectively. A test for linear trend was statistically significant for all BMD sites ($p < .01$). Results from Framingham have confirmed our findings (2). Incidence rates of breast cancer increased from 2.0 per 1000 person years among women in the lowest age specific quartile of metacarpal bone mass to 2.6, 2.7 and 7.0 among women in the second, third and highest quartile, respectively. Similar findings were reported by Meema et al (3).

We have also found that among women not taking estrogen, those with vertebral fractures had 63% decreased risk of breast cancer (relative hazard=0.37; 95% confidence interval: 0.17 to 0.80; $p=.01$) than those not taking estrogen and this association remained significant after adjustment for potential confounding factors (4). These findings suggest that the use of estrogen therapy for women with vertebral fractures should be reexamined. However, these findings are based on a small number of cases. Extension of the follow-up will allow us to confirm these initial findings of exogenous estrogen and breast cancer among women with a vertebral fracture.

BACKGROUND

The metabolism of endogenous and exogenous estrogens is important in the etiology of breast cancer. The precise mechanism and risk relationships between estrogen and breast cancer remain controversial in spite of many years of both human and animal experimental research. There are several interesting hypotheses relating estrogen to breast cancer.

The production rate or blood levels of estrogen (especially free estradiol) may be directly related to the risk of breast cancer (5) as evidenced by the reduction in the rate of increase of breast cancer with age, by the benefits of both bilateral oophorectomy and the use of an anti-estrogen (Tamoxifen and Raloxifene) (6). The recently reported, fairly consistent relationship between obesity or weight gain pre- to postmenopause (7,8) and risk of breast cancer among postmenopausal women is consistent with the higher blood estradiol and estrone levels among heavier postmenopausal women (9). The relationship between endogenous estrogen levels and breast cancer is questionable because of the lack of, or a weak relationship between, exogenous estrogen therapy and risk for breast cancer even among women who have taken estrogen therapy for a relatively long time period (10). Selection criteria, especially for long-term estrogen therapy as well as differences in metabolism between oral

estrogens and endogenous estrogens may explain (in part) the lack of excess risk associated with estrogen therapy.

In general, it is clear that steroid hormones are implicated in the risk of breast cancer although the precise underlying mechanisms remain undetermined (11). Population studies show estrogen exposure in the form of parity, age at menarche, and menopausal status to be linked to breast cancer risk. From experimental and clinical studies, it appears that estrogen can act directly on mammary tissue via estrogen receptors (12) and direct proliferative responses to physiologic doses of estrogen have been demonstrated (13).

Bone contains estrogen receptors (14) and is highly sensitive to estrogen levels in the body. Bone mineral density is positively correlated with early menarche and length of reproductive life (15). Oophorectomy (16) and prolonged amenorrhea (17,18) are associated with increased bone loss. Menopausal loss of ovarian estrogens in associated with rapid bone loss (19), eventually leading to an increased risk of fractures (20), both of which can be prevented by estrogen replacement therapy (21,22). Increased endogenous estrogen concentrations are related to increased BMD in both white and black elderly women (23,24).

If the strong relationship between bone mineral density is substantiated, then it is very likely that the association of exogenous hormone use and risk of breast cancer has been substantially underestimated because the selection of women for hormone replacement therapy would be inversely related to bone mineral density and risk of breast cancer.

BODY

Study Population

The study will utilize the women participating in the Study of Osteoporotic Fracture (SOF), a prospective study of risk factors for fracture among women aged 65+ (25). The study originally included 9,704 women recruited in four communities: Baltimore, MD, Pittsburgh area (Monongahela Valley), Minneapolis, MN, and Portland, OR. The study began in 1986 and the current round of evaluations will be concluded in July, 1996. To be eligible to participate in SOF, the women had to be at least 65 years of age, living in the community, and able to walk without the assistance of other persons, and never had a bilateral hip replacement. The women represent community-living older individuals.

The women have now had five clinical evaluations (Figure 1). In addition, women are contacted annually by questionnaire/interview. Breast cancer history was obtained at the first annual questionnaire (Year 1). Women who reported a history of breast cancer at Year 1 (approximately 500) were considered to have prevalent breast cancer and were not included in subsequent analysis of the evaluation of bone mineral density and breast cancer. The person-year at risk of incident breast cancer, therefore, begins after the Year 1 exam.

Figure 1

Baseline 1986-1988 9,704 women	Risk factors Neuromuscular tests Functional status; Appendicular BMD 12cc serum: frozen storage X-4ays: spine, hip, hand
Year 2 Exam 1988-1990	Risk factors: update New neuromuscular performance tests Functional status; Hip and spine BMD 4cc serum: frozen storage
Year 3.5 Exam 1991 7,629	Repeat X-rays of spine Back pain, disability Functional status
Year 5.0 Exam 1992-1994	Fractional calcium absorption Neuromuscular and performance measures Hip and calcaneal BMD, ultrasound Risk factors Serum and urine: frozen storage
Year 8 Exam 1995-1996	Repeat pelvis X-rays Neuromuscular and performance measures Hip and calcaneal BMD Ultrasound of calcaneal and tibia Functional status

The study sample for the DOD proposal will be the 7,894 women of the 9,704 women included in the original analysis of the relationship of bone mineral density and breast cancer in SOF. Excluded from the prior analysis were: 1) 507 prevalent breast cancer cases at Year 1, 2) 3,650 women who died before the Year 3 exam and, therefore, could not be determined whether they had incident breast cancer (of which 5 had a diagnosis of breast cancer on the death certificates) and were not identified during the 3.5 year exam, 3) 618 who had no information regarding breast cancer at the 3.5 year exam, and 4) 160 with no information regarding breast cancer at Year 1 and, therefore, could not be classified as incident or prevalent. Breast cancer information was, therefore, collected on 8,561 (92% of the 9,339) women who survived to the 3.5 year exam and to be determined whether they had incident breast cancer. The 7,894 women without breast cancer at Year 3.5 exam will be the cohort for this study.

Progress Report

1. Identification of breast cancer from the Year 5 and 8 exams (Visit 4 and 5).

One of the goals of our application was to continue follow-up of the cohort for breast cancer. We originally identified 121 cases of incident breast cancer over the first 3.2 years of follow-up. This included all breast cancers through the third clinical visit. As part of the DOD, we collected information from the 4th clinical visit (1992-94) and 5th clinic visit (1995-96). This will allow examination of our hypothesis concerning long-term prediction of breast cancer by bone mineral density. In our grant application, we expected to identify at least 250 cases. To date, we have identified and adjudicated 314 cases of breast cancer, Table 1. As shown, about 14% of cases are in-situ; 69%, localized to breast and almost 15%, invasive.

We were also interested in acquiring the Estrogen Receptor (ER)/Progestin receptor (PR) status on not only the newly identified cases but also, the original 121 cases. We have successfully obtained information on estrogen/progestin receptor status on about 80% of the cases: ER (+), 67%; ER (-), 11%; borderline (0.6%); no information, 21.0%; PR (+), 52%; PR (-), 25%; borderline, 0.6%; no information, 22%. Similar to what was reported in the Iowa Womens Health Study, about 52% of the cases with no information on receptor status are breast cancer in-situ. This implies that there is no information of ER/PR status because it is not being evaluated due to the early nature of their disease (Table 2).

We are interested in comparing risk factors for ER (+) and ER (-) breast cancer separately. Although the number of ER (-) cases is relatively small, we found relatively few differences between these 2 types of breast cancer (Table 3). The major difference we found between ER (+) and ER (-) cases was in family history of breast cancer: about 40% of women with ER (-) breast cancer reported that their mother or sister had breast cancer compared with 17% of women with ER (+) breast cancer.

2. Hormonal Aspects of Breast Cancer

One of the goals of the application was to further our understanding of the hormonal etiology of breast cancer and the potential interactions with other factors. We explored the interaction between a) bone mineral density (BMD) and family history and breast cancer; b) estrogen replacement therapy (ERT), family history, and breast cancer; c) alcohol consumption, ERT, endogenous estrogens and breast cancer.

2a. BMD and Family History of Breast Cancer

Women with a family history of breast cancer may metabolize estrogen differently than women without such a history (26). Some (27-29) but not all (30-32) previous studies have suggested that the increase in breast cancer associated with estrogen replacement therapy may be greater among women with a positive family

history of breast cancer. This, there may be a familial response to estrogen exposure as reflected in BMD. We tested the hypothesis that the BMD-breast cancer relationship differs by family history.

For these analyses, women who reported breast cancer in either a mother or sister were considered to have a positive family history. These analyses are based on our initial set of 121 cases, on whom we had family history information on 104.

Relative to women with a negative family history and proximal radius BMD in the lowest tertile, women with both a positive family history and highest tertile proximal radius BMD showed a 4.23-fold increase in breast cancer risk (95% CI 1.99-9.00), whereas highest tertile BMD in the absence of a positive family history was associated with only a 1.48-fold increase in breast cancer risk (95% CI 0.79-2.77; interaction: p=0.04) (Table 4). Among women with a negative family history, the estimated risk of breast cancer was approximately 24% higher for each standard deviation unit increase in proximal radius BMD, compared with a 97% increase in women with a positive family history (interaction p =0.07) (Table 4).

Within family history subgroups, the increase in risk associated with having a proximal radius BMD in the highest tertile compared with the lowest was 48% for women with a negative family history, while the risk was 3.41-fold higher for women with a positive family history. Similarly, for the distal radius, among women with a negative family history, those with highest tertile BMD were at twice the risk of women with lowest tertile BMD; among women with a positive family history, the increase in risk associated with highest versus lowest tertile BMD was 9.9-fold. For the calcaneus, women with a negative family history demonstrated a 1.32-fold increase for the highest tertile versus the lowest, while women with a positive family history showed a 6.5-fold increase.

2b) ERT, family history and breast cancer

Current use of ERT was associated with a relative risk (RR) of 1.33 (95% CI, 0.75 to 2.35). In univariate analyses, the association was strongest among women with a positive family history, RR=3.46 (1.20-9,97) than among women with a negative family history, RR=1.34 (0.75-2.40) but the interaction term was not significant, p=0.30.

2c) Alcohol consumption, estrogen and breast cancer

Prior research has identified alcohol consumption as a potential risk factor for breast cancer. While results of previous studies have been somewhat mixed, cohort studies have generally found an increase in breast cancer risk associated with increasing consumption of alcoholic beverages.

One possible mechanism for a breast cancer-alcohol relation is the influence of alcohol on serum hormones, specifally estrogens. Several studies have reported positive correlations between alcohol consumption and serum hormones levels, including estradiol (33) and estrone sulfate (34), as well as urinary estrogens (35) in

postmenopausal women. However, other observational studies in postmenopausal women have not demonstrated an association between serum (36,37) and urinary (38) estrogens and alcohol intake. A recent placebo controlled crossover trial in postmenopausal women reported a significant and sustained increase in estradiol levels after acute alcohol ingestion among women taking estrogen replacement therapy (ERT), but not in other women (39).

Some (40,41), but not all (42,43), previous studies have suggested that alcohol use and exogenous estrogen use may interact with respect to breast cancer risk, but to our knowledge no previous studies have examined the interrelation among alcohol consumption, endogenous hormone levels, and risk of breast cancer.

Because alcohol consumption is a potentially modifiable behavior, clarifying the breast cancer risk associated with its use may have significant clinical implications. For example, if moderate intake is associated with an increased risk of breast cancer, this risk must be weighed against the potential benefits of alcohol consumption, including a reduced risk of cardiovascular disease (44). Further, if there is an interaction between alcohol consumption and ERT, there may be implications for treatment recommendations.

We examined the association between current and lifetime alcohol consumption, measured at baseline, and subsequent development of breast cancer. Compared with non-drinkers, women who reported drinking an average 15 or more grams of alcohol (a little more than one drink) per day had a nearly three-fold increased risk of breast cancer compared to abstainers, Table 5. Multivariate adjustment for age, education, age at menarche, age at menopause, parity, smoking, use of ERT, exercise, family history, and clinic in proportional hazards regression models did not substantially change the risk (RR 3.10, 95% CI 1.55-6.21, p trend across categories=0.04). Limiting the analysis to current drinkers, heavy drinkers, those reporting consumption of three or more drinks per day during four or more of the last 30 days, had a five-fold increase in the risk of breast cancer compared to women who drank less (95% CI 2.16-14.1, p trend 0.004). Women in the top quartile of lifetime consumption (8,191 lifetime drinks or more) had an estimated 87 percent increased risk of breast cancer compared with abstainers (95% CI 1.01-3.46, p trend 0.07). None of the control variables was significantly associated with breast cancer risk.

The risk of breast cancer associated with alcohol consumption was not influenced by either use of exogenous estrogen or endogenous hormone levels. Self-reported use of ERT did not appear to modify the alcohol-breast cancer relationship (p interaction 0.85; Table 6). Similarly, there was no indication of a multiplicative interaction with respect to serum levels of estradiol, estrone, estrone sulfate, or testosterone and use of alcohol.

3) Endogenous Estrogens, and Androgens, and Breast Cancer

Endogenous estrogens may play an important role in the development of breast cancer (45). Some (46-49) but not all (50-53) prospective studies have found

significant associations between endogenous concentrations of estrogens and subsequent risk of breast cancer. Women with higher bone mineral density (BMD), a cumulative measure of endogenous estrogen have an increased risk of breast cancer (1-3). Endogenous androgens may also contribute (47,49,54). However, the relationship between serum androgens and breast cancer may not be independent of serum estrogens (55,56). The best estrogen fraction to predict risk has not been identified (45). Most studies have included measurements of total hormone levels; the concentrations of free hormone may have even stronger associations. Finally, most of the women in these studies were postmenopausal women, younger than 65 years of age.

Two randomized trials have demonstrated a reduction in primary breast cancers with tamoxifen (57) and raloxifene (58). In the Breast Cancer Prevention Trial, 4 years of tamoxifen use led to a 45% reduction in breast cancer incidence among the 13,388 women who participated in the trial (57).

Women in this study were considered "high" risk of breast cancer based on risk factors, including age $\geq$ 60 years. About 30% of women in the trial were age 60 years or older. The Multiple Outcomes of Raloxifene Evaluation (MORE), found a 70% reduction in the risk of breast cancer, especially estrogen receptor positive cancers after 33 months of treatment with raloxifene (58). About 80% of the 7,704 women in this trial, were over the age of 60 years.

Since both treatments entail costs and risk (58,59), it is important to identify women who are at the greatest risk of breast cancer and hence, most likely to benefit from anti-estrogen therapies. The current study was designed to test the hypothesis that serum concentrations of estradiol and testosterone, measured an average of 3 years before the clinical diagnosis of breast cancer are related to the risk of breast cancer in women 65 years of age or older. We hypothesized that measurements of serum hormone could be used to identify women at high risk of developing breast cancer. We used a case-cohort approach to compare serum hormone in 97 incident cases of breast cancer and a random set of controls in the Study of Osteoporotic Fractures.

The sex steroid hormones were correlated with each other and with body weight, Table 7. The magnitude of the correlation coefficients were similar in the cases and controls.

Median sex steroid hormone levels were higher in the cases compared with the random sample of the cohort, Table 8. The magnitude of the difference in median hormone concentrations ranged from 16% for total testosterone to 37% for estrone sulfate. The hormone distributions were significantly different in cases and the random sample of the cohort except for SHBG.

The association between serum hormone level and breast cancer was strongest for bioavailable estradiol: women in the highest quartile had a 5 fold (95% CI 2.0-12.0) greater risk of breast cancer compared to women in the lowest quartile, Table 9. Among the androgens, free testosterone level was strongly linked to subsequent risk of breast cancer: there was three-fold excess risk of breast cancer among women with the

highest free testosterone levels. These associations were independent of age and body weight.

Women in the highest quartile of estrone, estrone sulfate, androstenedione, DHEAS and total testosterone also had a two to 2 ½ times excess risk of breast cancer, Table 3. SHBG and the ratio of estrone sulfate to estrone were not associated with breast cancer. Results were the same when we excluded past estrogen users.

The estimated incidence rate of breast cancer was lowest (0.6 per 1000 woman years) among women with the lowest bioavailable estradiol and free testosterone, Figure 2. In contrast, the incidence of breast cancer was almost 13 times greater among women with the highest concentration of both hormones.

In a model that included levels of bioavailable estradiol, free testosterone and androstenedione, bioavailable estradiol RH=2.8; (1.3 to 5.9) and free testosterone, RH=2.2; (1.0 to 4.5) but not androstenedione RH=1.0; (0.5 to 2.0) remained significantly related to the risk of breast cancer.

The results of this study support the hypothesis that sex hormones are important in the etiology of breast cancer in older women. In particular, women with a bioavailable estradiol level above 2.0 pg/ml had a 5 fold increased risk of breast cancer compared with women with the lowest estradiol. We also found a strong relationship between the unbound portion of testosterone and the risk of breast cancer. Our results are consistent with other prospective studies of the relationship between sex steroid hormone levels and the risk of breast cancer in somewhat younger women.

The average incidence rate of breast cancer among US white women age 65 years and older is 4.6 per 1000 woman-years (25). Based on, our results we estimate that the incidence rate of breast cancer among women with the highest bioavailable estradiol and free testosterone is almost 2 fold higher than this expected rate.

The absolute concentrations of hormones, especially estradiol, were very low. Nevertheless, a gradient of risk was observed across increasing concentrations. This gradient of risk is greater than that observed between serum cholesterol concentrations and coronary heart disease especially among older women (4). These results imply that measurement of bioavailable estradiol and free testosterone could be used as a clinical measure of risk of breast cancer to identify women who may benefit from anti-estrogen treatment.

4) Bone Mineral Density and Breast Cancer: Does this reflect endogenous hormones?

Increased BMD was associated with an increased risk of breast cancer, Table 10. Inclusion of either non-SHBG bound estradiol or free testosterone in the models did not attenuate the relationship between BMD and breast cancer. In fact, the association between BMD and breast cancer was strongest in the multivariate model including both hormones as well as adjustments for amny risk factors for breast cancer. One standard deviation in BMD was associated with about at 32% increase in the risk of breast cancer (p=0.07).

Consistent with our previous observation (1) and that of others (2,3), we found that higher BMD was associated with an increased risk of breast cancer. The magnitude of the association was, however, slightly weaker. This study used a design which reduced the number of women with BMD and thus reduced our power for this association. Nevertheless, we found little evidence to support the hypothesis that sex steroid hormone levels attenuate the relationship between BMD and breast cancer, suggesting that the observed relationship between BMD and breast cancer is independent of sex steroid hormone levels. The association between BMD and breast cancer may reflect other hormones we did not measure including insulin, insulin like growth factors, vitamin D or cytokines. Alternatively, BMD, is a marker of cumulative estrogen exposures including exposure to estrogens "early" in life. These "early" estrogen exposures may also contribute to the risk of breast cancer above and beyond more recent measures of endogenous hormones.

We are currently in the process of measuring IGF1, IGFBP3, IGF-BP4, $1,25(OH)^2$ vitamin D and $25(OH)^2$ vitamin D to test the hypothesis that these hormones can explain the observed relationship between BMD and breast cancer.

5) Longterm Prediction of Breast Cancer by ERT and BMD

One of the goals of the DOD grant was to examine the longerm prediction of breast cancer with respect to BMD and ERT. In our original study, the average follow-up was 3.2 years. We have now extended follow-up to 7.3 years $\pm$ 1.6 (range 0.5 to 9.8).

There was little relationship between current use of ERT at baseline and development of breast cancer over the first 7.3 years of follow-up: the RR (95% CI) of breast cancer was 1.17 (0.84-1.62) among current users and 1.03 (0.81-1.31) among ever users.

The relationship between BMD and breast cancer remained, even over 7 years of follow-up, Table 11, although the magnitude of the association was weaker. Women in the highest quartile of BMD had about a 75% increase in the risk of breast cancer than women with the lowest BMD. We found no evidence that there was an interaction between BMD and ERT use on breast cancer, Table 12. However, only about 12% of the cohort reported use of ERT at baseline limiting our statistical power to find a significant interaction.

RECOMMENDATIONS

We recommend further understanding of the relationship between BMD and breast cancer. Specifically we are pursuing these additional analyses:

a) Do IGF and its binding proteins predict breast cancer and contribute to the observation of BMD and breast cancer?

b) Do vitamin D levels help explain the association between BMD and breast cancer?

c) Do other measures of osteoporosis predict breast cancer e.g. bone loss rates, ultrasound or fracture rates?

d) Is there a relationship between breast density, BMD and sex steroid hormones?

CONCLUSIONS

The results from this study clearly support a role of estrogen in the etiology of breast cancer. This statement is supported by our observation that a) both endogenous estrogens and androgens predict breast cancer; b) BMD, as a measure of cumulative estrogen exposure, predicts breast cancer. The relationship between BMD and breast was upheld with longer term follow-up, although the magnitude of the association was weaker. The findings regarding endogenous estrogens and androgens raise the possibility that measurement of these hormones could identify women at high risk of breast cancer who may benefit from preventive therapies. Finally, the longterm prediction of breast cancer with BMD may suggest that women with very low BMD, may not need annual mammograms given their overall very low risk of breast cancer. We are currently working with Dr. Karla Kewlikowski at UCSF to model the cost effectiveness of mammograms among women age 65, incorporating measures of BMD into our cost-effectiveness model.

REFERENCES

1. Cauley JA, Lucas FL, Kuller LH, Vogt MT, Browner WS, Cummings SR. Bone mineral density and risk of breast cancer in older women. JAMA 1996;276:1404-1408.
2. Zhang Y, Kiel DP, Kreger BE, Cupples LA, Ellison RC, Dorgan JF, et al. Bone mass and the risk of breast cancer among postmenopausal women. N Engl J Med 1997;336:611-7.
3. Meema S, Meema HE. Possible estrogenic effect on bone in postmenopausal patients with mammary carcinoma. Cancer 1966;19:433-436.
4. Cummings SR, Cauley JA, Lucas FL, Browner WS, Lipschutz RC, Kuller LH. Women with vertebral fractures have a low risk of breast cancer unless they take estrogen. The Study of Osteoporotic Fractures. (Unpublished).
5. Pike MC, Spicer DV, Dalmoush L, Press MF. Estrogens, progestogens, normal breast cell proliferation, and breast cancer risk. Epidemiol Rev 1993;39:1020.
6. Richards MA, Smith IE, Dixon JM. Role of systemic treatment for primary operable breast cancer. BMJ 1994;309:1363-66.
7. Byers RE, Williamson DF. Diet, alcohol, body size and the prevention of breast cancer. In: Stoll, ed. Approaches to breast cancer prevention. Netherlands. Kluwer Academic Publishers, 113-131, 1991.
8. Chu SY, Lee NC, Wingo PA, Senie RT, Greenberg RS, Peterson HB. The relationship between body mass and breast cancer among women enrolled in the cancer and steroid hormone study. J Clin Epidemiol 1991;44:1197-1206.
9. Azziz R. Reproductive endocrinologic alterations in female asymptomatic obesity. Fertil Steril 1989;52:703-725.
10. Colditz GA. Epidemiology of breast cancer. Findings from the Nurses' Health Study. Cancer 1993;71:1480-9.
11. Clarke R, Dickson RB, Lippman ME. Hormonal aspects of breast cancer. Growth factors, drugs and stromal interactions. Crit Rev Oncol Hematol 1992;12:1-23.
12. Peterson OW, Hoyer PE, van Deurs B. Frequency and distribution of estrogen receptor-positive cells in normal, nonlactating human breast tissue. Cancer Res 1987;7:5748-52.
13. Lippman ME, Huff KK, Jakesz R, et al. Estrogens regulate production of specific growth factors in hormone-dependent human breast cancer. In Endocrinology of the Breast: Basic and Clinical Aspects. Angeli A, Bradlow HL, Dogliotti L. (Eds). Ann N Y Acad Sci, vol 464, New York, NY, 1986, pp.11-16.
14. Eriksen EF, Colvard DS, Berg NJ, et al. Evidence of estrogen receptors in normal human osteoblast-like cells. Science 1988;241:84-6.
15. Fox KM, Magazine J, Sherwin R, et al. Reproductive correlates of bone mass in elderly women. J Bone Miner Res 1993;8:901-8.
16. Bauer DC, Browner WS, Cauley JA et al. Factors associated with appendicular bone mass in older women. Ann Intern Med 1993;118:657-65.

17. Jonnavithula S, Warren MP, Fox RP, et al. Bone density is compromised in amenorrheic women despite return of menses: A 2-year study. Obstet Gynecol 1993;81:669-73.
18. Myburgh KH, Bachrach LK, Lewis B, et al. Low bone mineral density at axial and appendicular sites in amenorrheic athletes. Med Sci Sports Exer 1993;25:1197-1202.
19. Nilas L, Christiansen C. Bone mass and its relationship to age and the menopause. J Clin Endocrinol Metab 1987;65:697-702.
20. Longcope C, Baker RS, Hui SL, et al. Androgen and estrogen dynamics in women with vertebral crush fractures. Maturitas 1984;6:309-18.
21. Cauley JA, Seeley DG, Ensrud K, et al. Estrogen replacement therapy and fractures in older women. Ann Intern Med 1995;122:9-16.
22. Lindsay R. Hormone replacement therapy for prevention and treatment of osteoporosis. Am J Med 1993;95:37S-39S.
23. Cauley JA, Gutai JP, Sandler RB, et al. The relationship of endogenous estrogen to bone density and bone area in normal postmenopausal women. Am J Epidemiol 1986;124:752-61.
24. Cauley JA, Gutai JP, Kuller LH, et al. Black-white differences in serum sex hormones and bone mineral density. Am J Epidemiol 1994;139:1035-46.
25. Cummings SR, Black DM, Nevitt MC, Browner WS, Cauley JA, Genant HK, et al. Appendicular bone density and age predict hip fracture in women. JAMA 1990;263:665-668.
26. Fishman J, Bradlow HL, Fukushima DK, et al. Abnormal estrogen conjugation in women at risk for familial breast cancer at the periovulatory stage of the menstrual cycle. Cancer Res 1983;43:1884-90.
27. Newcomb PA, Longnecker MP, Storer BE, et al. Long-term hormone replacement therapy and risk of breast cancer in postmenopausal women. Am J Epidemiol 1995;142:799-95.
28. Kaufman DW, Palmer JR, de Mouzon J, et al. Estrogen replacement therapy and the risk of breast cancer: results from the Case-control Surveillance Study. Am J Epidemiol 1991;134:1375-85.
29. Hulka BS, Chambless LE, Deubner DC, et al. Breast cancer and estrogen replacement therapy. Am J Obstet Gynecol 1982;143:638-44.
30. Mills PK, Beeson WL, Phillips RL, et al. Prospective study of exogenous hormone use and breast cancer in Seventhe-day Adventists. Cancer 1989;64:591-7.
31. Schairer C, Byrne C, Keyl PM, et al. Menopausal estrogen and estrogen-progestin replacement therapy and risk of breast cancer (United States). Cancer Causes Control 1994;5:491-500.
32. Stanford JL, Weiss NS, Voigt LF, et al. Combined estrogen and progestin hormone replacement therapy in relation to risk of breast cancer in middle-aged women. JAMA 1995;274:137-42.
33. Gavaler JS, Van Thiel DH. The association between moderate alcoholic

beverage consumption and serum estradiol and testosterone levels in normal postmenopausal women: relationship to the literature. Alcohol Clin Exp Res 1992;16:87-92.

34. Hankinson SE, Willett WC, Manson JE, et al. Alcohol, height, and adiposity in relation to estrogen and prolactin levels in postmenopausal women. J Natl Cancer Inst 1995;87:1297-1302.
35. Katsouyanni K, Boyle P, Trichopoulos D. Diet and urine estrogens among postmenopausal women. Oncology 1991;48:490-4.
36. Cauley JA, Gutai JP, Kuller LH, LeDonne D, Powell JG. The epidemiology of serum sex hormones in postmenopausal women. Am J Epidemiol 1989;129:1120-31.
37. Newcomb PA, Klein R, Klein BE, et al. Association of dietary and life-style factors with sex hormones in postmenopausal women. Epidemiology 1995;6:318-21.
38. Trichopoulos D, Brown J, MacMahon B. Urine estrogens and breast cancer risk factors among post-menopausal women. Int J Cancer 1987;40:721-5.
39. Ginsburg ES, Mello NK, Mendelson JH, et al. Effects of alcohol ingestion on estrogens in postmenopausal women. JAMA 1996;276:1747-51.
40. Gapstur SM, Potter JD, Sellers TA, Folsom AR. Increased risk of breast cancer with alcohol consumption in postmenopausal women. Am J Epidemiol 1992;136:1221-31.
41. Colditz GA, Stampfer MJ, Willett WC, Hennekens CH, Rosner B, Speizer FE. Prospective study of estrogen replacement therapy and risk of breast cancer in postmenopausal women. JAMA 1990;264:2648-53.
42. Smith-Warner SA, Spiegelman D, Shiaw-Shyuan Y, et al. Alcohol and breast cancer in women. A pooled analysis of cohort studies. JAMA 1998;279:535-40.
43. Friedenreich CM. Re: "Increased risk of breast cancer with alcohol consumption in postmenopausal women." Am J Epidemiol 1994;139:541-2.
44. Rich-Edwards JW, Manson JE, Hennekens CH, Buring JE. The primary prevention of coronary heart disease in women. N Engl J Med 1995;332:1758-66.
45. Colditz GA. Relationship between estrogen levels, use of hormone replacement therapy, and breast cancer. J Cancer Inst 1998;90:814-823.
46. Toniolo PG, Levitz M, Zelenvich-Jacquotte A, Banerjee S, Koenig KL, Shore RE, et al. A prospective study of endogenous estrogens and breast cancer in post-menopausal women. J Nat Ca Institute. 1995; 87:190-7.
47. Dorgan JF, Longcope C, Stephenson HE, Falk RT, Miller R, Franz C, et al. Relation of prediagnostic serum estrogen and androgen levels to breast cancer risk. Cancer Epidemiol Biomarkers Prev 1996;5:533-539.
48. Moore JW, Clark GMG, Hoare SA, Millis RR, Hayward JL, Quinlan MK, et. al. Binding of oestradiol to blood proteins and aetiology of breast cancer. Int J Cancer 1986; 38:625-30.

49. Berrino F, Muti P, Micheli A, et al. Serum sex hormone levels after menopause and subsequent breast cancer. J Natl Cancer Inst 1996;88:291-296.
50. Helzlsouer KJ, Alberg AJ, Bush TL, Longcope C, Gordon GB, Comstock GW. A prospective study of endogenous hormones and breast cancer. Cancer Detect Prev. 1994; 18:79-85.
51. Garland CF, Friedlander NJ, Barrett-Connor E, Khaw K. Sex hormones and postmenopausal breast cancer: A prospective study in an adult community. Am J Epidemiol. 1992; 135:1220-30.
52. Wysowski DK, Comstock GW, Helsing KN, Lau HL. Sex hormone levels in serum in relation to the development of breast cancer. Am J Epidemiol. 1987; 125:791-799.
53. Bulbrook RD, Moore JW, Clark GMG, Wang DY. Relation between risk of breast cancer and biological availability of estradiol in the blood: Prospective study in Guernsey. Ann NY Acad Sci. 1986; 464:378-388.
54. Dorgan JF, Stanczyk FZ, Longcope C, Stephenson HE, Chang L, Miller R, Franz C, et al. Relationship of serum dehyroepiandrosterone (DHEA), DHEA sulfate, and 5-androstene-3, 17 -diol to risk of breast cancer in postmenopausal women. Serum hormones and breast cancer risk. 177-181.
55. Zeleniuch-Jacquotte A, Bruning PF, Bonfrer MG, Koenig KL, Shore RE, Kim MY, et al. Relation of serum levels of testosterone and dehydroepiandrosterone sulfate to risk of breast cancer in postmenopausal women. Am J Epidemiol 1997;145:1030-8.
56. Thomas HV, Key TJ, Allen DS, Moore JW, Dowsett M, Fentiman IS, Wang DY. A prospective study of endogenous serum hormone concentrations and breast cancer risk in post-menopausal women on the island of Guernsey. Brit J Cancer 1997;76:401-405.
57. National Cancer Institute. Preliminary findings of the Breast Cancer Prevention Trial. Internet website June 1, 1998: http://rex.nci.nih.gov/massmedia/pressreleases/prevtrialGRAPHS.htm.
58. Cummings SR, Eckert S, Grady D, Glusman J, Krueger K, Norton L, Powles TJ, Black D, Cauley JA, Morrow M, Bjarnason NH. Raloxifene reduces the risk of breast cancer in postmenopausal women with osteoporosis. (Submitted).
59. Evista (package insert). Indianapolis, IN: Eli Lilly and Company; 1998.

Table 1: Number of Breast Cancer Cases by Tumor Behavior (SOF) (through Year 8)

	n	(%)
Lobular in-situ	2	(0.6)
Ductal in-situ	44	(14.0)
Invasive, localized to breast	216	(69.8)
Advanced, with metastases	40	(12.7)
Other	5	(1.6)
Missing	1	(0.3)
Total	314	(100)

Number of Breast Cancer Cases by TNM Staging

	n	(%)
In-situ	45	(14)
Stage 1	175	(55.7)
Stage II_{No}	30	(9.6)
Stage II_{N1}	32	(10.2)
Stage III	8	(2.6)
Stage IV	4	(1.3)
Unknown	19	(6.1)
Missing	1	(0.3)
	314	100%

Table 2: Estrogen Receptor and Progestin Status by TNM Staging: Number of Cases

Estrogen Receptor

Stage	ER (+)	ER (-)	Borderline	No-Info/ Unknown
In-situ	8	2	1	34
Stage 1	135	19	0	21
Stage II_{No}	19	7	1	3
Stage II_{N1}	26	5	0	1
Stage III	5	1	0	2
Stage IV	1	1	0	2
Missing/Unknown	16	1	0	3

Progestin Receptor

Stage	PR (+)	PR (-)	Borderline	No-Infor/ Unknown
In-situ	7	4	0	34
Stage 1	105	45	1	24
Stage II_{No}	17	10	0	3
Stage II_{N1}	19	10	1	2
Stage III	4	2	0	2
Stage IV	0	2	0	2
Missing/Unknown	12	5	0	3

Table 3: Characteristics of Breast Cancer Cases by ER Status and Controls

	ER (+)	ER (-)	Controls
Age (yr)	70.2	70.8	71.4[a]
Weight (kg)	69.9	71.1	67.2[a]
BMI (wt/ht^2)	27.4	27.5	26.5[a]
Age, first birth (yr)	25.3	27.3	25.3
Age at menopause (yr)	48.1	49.8	48.0
Family history of breast cancer (%)	17.1%	40.9%[b]	15.1%
BMI > 30 (%)	27.7%	25.9%	20%[a]
Ever Pregnant (%)	96%	96%	84%
PR Positive (%)	76%	20%	NA
Mother fractured hip (%)	11.3%	10%	14%
BMD (g/cm^2)			
Calcaneal	0.422	0.415	0.406[a]
Distal Radius	0.382	0.363	0.363[a]

[a] $p < 0.05$ controls versus ER (+) and ER (-) cases
[a] $p < 0.05$ ER (-) versus ER (+) and controls

Table 4: Estimated Relative Risk of Breast Cancer by Family History Status and Bone Mineral Density in the Study of Osteoporotic Fractures, 1986-1993*

BMD (g/cm^2)	Negative Family History		Positive Family History		p for interaction
	RR	95% CI	RR	95% CI	
Proximal Radius					
< 0.59	1.00		1.24	0.42-3.67	0.04
0.59-0.68	1.64	0.91-2.96	1.00	0.29-3.39	
≥ 0.69	1.48	0.79-2.77	4.23	1.99-9.00	
Per 1 SD (0.10) increase	1.24	0.98-1.57	1.97	0.94-4.12	0.07
Distal Radius					
< 0.32	1.00		0.31	0.04-2.29	0.04
0.32-0.39	1.14	0.62-2.11	3.18	1.43-7.06	
≥ 0.40	1.99	1.10-3.58	3.07	1.39-6.80	
Per 1 SD (0.08) increase	1.30	1.05-1.61	1.70	0.88-3.31	0.24

* Proportional hazards regression models with interaction terms. Data were controlled for age, age at menopause, surgical menopause, parity/age at first birth, body mass index, alcohol intake, study center, and use of estrogen replacement therapy.

Lucas FL, et al. Am J Epidemiol 1998;148:22-9.

Table 5. Crude and Adjusted* Relative Risk Estimates by Alcohol Intake from Proportional Hazards Regression Models

Alcohol Use	Crude RR (95% CI)	Adjusted RR (95% CI)	p trend
Current intake (gm/day):			
None	1.00 Referent	1.00 Referent	
<1.5	0.95 (0.59-1.53)	0.89 (0.51-1.54)	
1.5-<5	1.41 (0.84-2.38)	1.13 (0.59-2.14)	
5-<15	0.97 (0.52-1.79)	1.04 (0.52-2.08)	
15+	2.66 (1.49-4.76)	3.10 (1.55-6.21)	0.04
3 or more drinks/day, past 30 days (current drinkers only):			
None	1.00 Referent	1.00 Referent	
1-3 times	1.21 (0.64-2.28)	1.35 (0.63-2.86)	
4 or more times	3.30 (1.33-8.16)	5.51 (2.16-14.1)	0.004
Lifetime consumption (# drinks):			
None	1.00 Referent	1.00 Referent	
1-813	1.05 (0.59-1.87)	0.99 (0.52-1.89)	
814-2730	1.06 (0.60-1.87)	0.94 (0.49-1.82)	
2731-8190	1.37 (0.80-2.34)	1.16 (0.61-2.20)	
8191+	1.62 (0.97-2.73)	1.87 (1.01-3.46)	0.07

*Adjusted for age, education, BMI, age at menarche, age at menopause, parity/age at first birth, family history, ERT, current smoking, physical activity, and study center

Table 6. Estimated Relative Risk* of Breast Cancer Associated with Alcohol Intake By Estrogen Replacement Therapy Use and Serum Hormone Levels

Hormone	Alcohol Intake			p interaction
	None	<5 gm/day	5+ gm/day	
Use of ERT**:				
Never	1.00 Referent	1.15 (0.60-2.21)	1.97 (0.93-4.13)	
Past	1.51 (0.73-3.12)	1.05 (0.47-2.37)	1.74 (0.67-4.49)	
Current	1.82 (0.75-4.41)	1.71 (0.70-4.19)	2.42 (0.87-6.75)	0.85
Estradiol (pg/ml):				
<=5	1.00 Referent	0.70 (0.26-1.88)	0.74 (0.19-2.80)	
>5-<9	0.82 (0.33-2.02)	0.65 (0.25-1.66)	2.20 (0.76-6.38)	
9+	1.86 (0.82-4.21)	1.31 (0.55-3.09)	2.74 (1.06-7.08)	0.65
Estrone (pg/ml):				
<17	1.00 Referent	0.87 (0.30-2.54)	1.52 (0.39-5.91)	
17-<28	2.16 (0.94-4.98)	1.10 (0.46-2.64)	1.46 (0.42-5.11)	
28+	1.95 (0.77-4.95)	1.79 (0.71-4.54)	4.10 (1.57-10.7)	0.50
Estrone Sulfate (pg/ml):				
<139	1.00 Referent	0.65 (0.26-1.65)	0.79 (0.21-3.01)	
139-<281	1.09 (0.50-2.39)	0.86 (0.37-2.00)	1.80 (0.60-5.43)	
281+	1.28 (0.56-2.96)	1.00 (0.43-2.32)	2.48 (1.02-6.06)	0.82
Testosterone (ng/dl):				
<14	1.00 Referent	1.51 (0.49-4.61)	1.78 (0.42-7.58)	
14-<24	2.89 (1.09-7.71)	2.01 (0.72-5.61)	7.60 (2.50-23.1)	
24+	4.89 (1.88-12.7)	2.45 (0.93-6.48)	4.08 (1.34-12.5)	0.21

*Adjusted for age, education, BMI, age at menarche, age at menopause, parity/age at first birth, family history, ERT**, current smoking, physical activity, and study center
**ERT, estrogen replacement therapy

Table 7: Pearson Correlation Matrix for Sex Steroid Hormones Levels, SHBG, Age and Body Weight: Controls only (n=244)

	E2	non-SHBG E2	Free E2	E1	E1S	T	Free T	A	DHEAS	SHBG	BMD +	Wt
Estradiol (E2)	1.0	0.88	0.94	0.67	0.54	0.29	0.37	0.28	0.21	-0.25	0.06	0.38
non-SHBG bound E2		1.0	0.87	0.65	0.60	0.18	0.37	0.29	0.22	-0.55	0.19	0.49
Free E2			1.0	0.59	0.49	0.23	0.32	0.24	0.16	-0.30	0.06	0.36
E1				1.0	0.92	0.21	0.23	0.33	0.27	-0.10	0.16	0.26
EIS					1.0	0.05	0.09	0.19	0.23	-0.10	0.17	0.17
Testosterone						1.0	0.94	0.46	0.20	0.11	-0.13	0.01
Free T							1.0	0.48	0.22	-0.16	-0.02	0.13
Androstenedione (A)								1.0	0.54	-0.13	-0.05	0.04
DHEAS									1.0	-0.13	0.12	0.07
SHBG										1.0	-0.27	-0.45
BMD+											1.0	0.22
Body Weight												1.0

+ Distal radius BMD
All correlations ($r \geq 0.12$), $p < 0.05$

Table 8: Median and Range of Sex Steroid Hormones in Incident Breast Cancer Cases and Random Sample of the Cohort

ESTROGENS	Case median (range)	Random Sample median (range)	p*
Estradiol (pg/ml)	8.0 (3 - 22)	6.0 (2 - 56)	0.0006
non-SHBG bound Estradiol (pg/ml)	1.31 (0.30 - 6.5)	1.0 (0.2 - 10.6)	0.001
Free Estradiol, (pg/ml)	0.14 (0 - 0.4)	0.12 (0 - 1.2)	0.0009
Estrone, (pg/ml)	24.0 (0 - 69)	20.0 (0 - 67)	0.004
Estrone sulfate (pg/ml)	221.0 (42 - 1036)	161.0 (0 - 1089)	0.004
ANDROGENS			
Androstenedione (ng/dl)	44.0 (5 - 152)	36.0 (0 - 147)	0.003
DHEAS (ng/dl)	75.0 (9 - 357)	63.5 (0 - 333)	0.04
Total Testosterone (ng/dl)	21.0 (0 - 78)	18 (0 - 76)	0.005
Free Testosterone (pg/ml)	3.0 (0 - 11.2)	2.35 (0 - 9.9)	0.003
SHBG (nmol/L)	38.0 (6 - 89)	43.0 (5 - 119)	0.20

*Wilcoxon 2 sample test

Table 9: Relative Hazard (RH) of Breast Cancer by Level of Sex Steroid Hormone Levels

ESTROGENS	No. of Subjects Cases	Random Sampl,e	RH[a]	RH (95% CI)[b]	p	p trend
Estradiol Total (pg/ml)						
Level 1 (< 5)	14	60	1.0	1.0 (referent)		
Level 2 (5- < 6)	15	39	1.4	1.4 (0.7, 3.1)	0.367	0.001
Level 3 (6- < 9)	26	85	1.3	1.4 (0.6, 3.3)	0.392	
Level 4 (≥ 9)	42	59	3.1	3.5 (1.6, 7.7)	0.002	
Bioavailable Estradiol (pg/ml)						
Quartile 1 (< 0.65)	10	70	1.0	1.0 (referent)		
Quartile 2 (0.65- < 1.10)	32	57	4.2	4.2 (1.9, 9.5)	0.0006	0.004
Quartile 3 (1.10 - < 2.00)	22	63	2.2	2.4 (1.0, 5.6)	0.054	
Quartile 4 (≥ 2.00)	33	52	4.5	5.0 (2.0, 12.4)	0.0005	
Free Estradiol (pg/ml)						
Level 1 (< 0.1)	4	25	1.0	1.0 (referent)		0.010
Level 2 (0.1)	58	170	1.7	1.8 (0.6, 5.3)	0.324	
Level 3 (0.2)	25	32	4.5	5.1 (1.5, 17.8)	0.010	
Level 4 (≥ 0.3)	10	16	2.8	3.4 (0.8, 13.8)	0.092	
Estrone (pg/ml)						
Quartile 1 (< 15)	18	64	1.0	1.0 (referent)		0.004
Quartile 2 (15 - < 21)	17	66	0.9	0.9 (0.4 - 1.9)	0.761	
Quartile 3 (21 - < 29)	30	59	1.8	1.9 (0.9 - 3.9)	0.096	
Quartile 4 (≥ 29)	32	54	2.3	2.5 (1.2 - 5.3)	0.018	

a age adjusted b adjusted for age and body weight

Table 9 Continued: Relative Hazard (RH) of Breast Cancer by Level of Sex Steroid Hormone Levels.

	No. of Subjects Cases	Random Sample	RH[a]	RH (95% CI)[b]	p	p trend
Estrone Sulfate						
Quartile 1 (< 113)	17	67	1.0	1.0 (referrent)		0.015
Quartile 2 (113- < 181)	22	64	1.4	1.4 (0.7, 3.9)	0.386	
Quartile 3 (181- < 288)	26	59	1.5	1.5 (0.7, 3.2)	0.253	
Quartil 4 (≥ 288)	32	53	2.5	2.5 (1.2, 5.3)	0.014	
Androstenedione (ng/dl)						
Quartile 1 (< 25)	16	63	1.0	1.0 (referent)		0.010
Quartile 2 (25 - < 39)	23	68	1.3	1.3 (0.6 - 2.7)	0.561	
Quartile 3 (39 - < 56)	24	61	1.3	1.2 (0.6 - 2.6)	0.590	
Quartile 4 (≥ 56)	34	52	2.6	2.6 (1.3 - 5.4)	0.010	
DHEAS (ng/dl)						
Quartile 1(< 41)	18	64	1.0	1.0 (referent)		0.039
Quartile 2(41 - < 65)	24	61	1.3	1.3 (0.6 - 2.7)	0.480	
Quartile 3 (65 - < 105)	25	64	1.6	1.6 (0.8 - 3.2)	0.233	
Quartile 4 (≥ 105)	30	55	2.1	2.1 (1.0 - 4.3)	0.045	
Total Testosterone (ng/dl)						
Quartile 1 (< 13)	14	64	1.0	1.0 (referent)		0.027
Quartile 2 (13 - < 19)	26	62	1.8	1.7 (0.8 - 3.7)	0.172	
Quartile 3 (19 - < 29)	26	60	2.5	2.5 (1.1 - 5.3)	0.022	
Quartile 4 (≥ 29)	31	58	2.3	2.3 (1.1 - 4.8)	0.030	

a age adjusted b adjusted for age and body weight

Table 9 Continued: Relative Hazard (RH) of Breast Cancer by Level of Sex Steroid Hormone Levels.

	No. of Subjects Cases	Random Sample	RH[a]	RH (95% CI)[b]	p	p trend
Free Testosterone (ng/dl)						
Quartile 1 (< 1.7)	11	66	1.0	1.0 (referrent)		0.007
Quartile 2 (1.7 - < 2.6)	26	63	2.5	2.5 (1.1, 5.6)	0.027	
Quartile 3 (2.6 - < 3.9)	29	56	3.5	3.5 (1.6, 7.9)	0.003	
Quartil 4 (≥ 3.9)	31	58	3.2	3.2 (1.4, 7.1)	0.005	
SHBG (nmol/L)						
Quartile 1 (< 29)	22	58	1.0	1.0 (referent)		0.275
Quartile 2 (29 - < 41)	33	52	1.5	1.5 (0.7 - 3.0)	0.271	
Quartile 3 (41 - < 57)	22	66	0.9	0.9 (0.4 - 1.8)	0.674	
Quartile 4 (≥ 57)	20	68	0.8	0.8 (0.3 - 1.7)	0.473	
Estrone Sulfate/Estrone						
Quartile 1 (< 6.38)	18	62	1.0	1.0 (referrent)		0.50
Quatile 2 (6.38 - < 8.95)	28	52	1.76	1.78 (0.9 - 3.6)	0.11	
Quartile 3 (8.95 - 42.68)	26	54	1.66	1.69 (0.8 - 3.4)	0.15	
Quartile 4 (≥ 42.68)	23	57	1.36	1.37 (0.7 - 2.8)	0.40	

a age adjusted
b adjusted for age and body weight

Table 10: Bone Mineral Density, non-SHBG bound estradiol, Free Testosterone, and the Risk of Breast Cancer*

Variable (unit)	Relative Hazard	(95% CI)	p
Model 1			
Distal Radius BMD (.08 g/cm^{2}2)†	1.20	(0.94 to 1.53)	0.15
Model 2			
Non-SHBG bound E2 ‡	3.70	(1.72 to 7.69)	0.0007
Model 3			
Free Testosterone‡	3.03	(1.49 to 6.25)	0.0023
Model 4			
Distal Radius BMD+ (.08 g/cm^2) †	1.21	(0.94 to 1.29)	0.14
Non SHBG bound E2 (Q2 - 4) ‡	3.57	(1.67 to 7.69)	0.001
Model 5			
Distal Radius BMD+ (.08 g/cm^2) †	1.24	(0.96 to 1.60)	0.10
Free Testosterone (Q2 - 4) ‡	3.13	(1.54 to 6.25)	0.002
Model 6			
Distal Radius BMD+ (.08 g/cm^2) †	1.25	(0.96 to 1.63)	0.10
Non SHBG bound E2 (Q2, 3 or 4) ‡	2.70	(1.23 to 5.88)	0.013
Free Testosterone (Q 2, 3 or 4) ‡	2.50	(1.18 to 5.26)	0.018
Model 7 (multivariate)¶			
Distal Radius BMD+	1.32	(0.98 to 1.78)	0.07
Non SHBG bound E2 (Q2-4) ‡	2.50	(1.09 to 5.89)	0.03
Free Testosterone (Q2-4) ‡	2.86	(1.23 to 6.67)	0.01

Table 11: Longterm Prediction of Breast Cancer Incidence Rate of Breast Cancer by Quartile of BMD: Average Follow-up 7.28 ± 1.6 years (per 1000 PYR) (includes CIS)

	# Cases	Incidence Rate	RR (95% CI)[a]
Proximal Radius (g/cm^2)			
Quartile 1 (< 0.564)	42	2.66	1.0
Quartile 2 (> 0.564- ≤ 0.636)	66	4.06	1.45 (0.98-2.14)
Quartile 3 (> 0.636- ≤ 0.707)	69	4.30	1.49 (1.0-2.21)
Quartile 4 (> 0.707)	89	5.40	1.77 (1.18-2.63)
Distal Radius (g/cm^2)			
Quartile 1 (≤ 0.302)	44	2.78	1.0
Quartile 1 (< 0.302- ≤ 0.357)	70	4.35	1.49 (1.0-2.2)
Quartile 1 (< 0.357- ≤ 0.419)	59	3.63	1.21 (0.82-1.80)
Quartile 1 (> 0.419)	92	5.74	1.83 (1.25-2.68)
Calcaneal BMD (g/cm^2)			
Quartile 1 (≤ 0.338)	42	2.82	1.0
Quartile 2 (> 0.338-≤ 0.40)	56	3.46	1.2 (0.80-1.8)
Quartile 3 (> 0.40- ≤ 0.467)	88	5.37	1.8 (1.2-2.6)
Quartile 4 (> 0.467)	82	5.01	1.6 (1.1-2.4)

a) RR adjusted for age, BMI, ERT

Table 12: Longterm Prediction of Breast Cancer: RR (95% CI) of Breast Cancer by BMD Stratified by Current Use of ERT at Baseline (includes CIS)

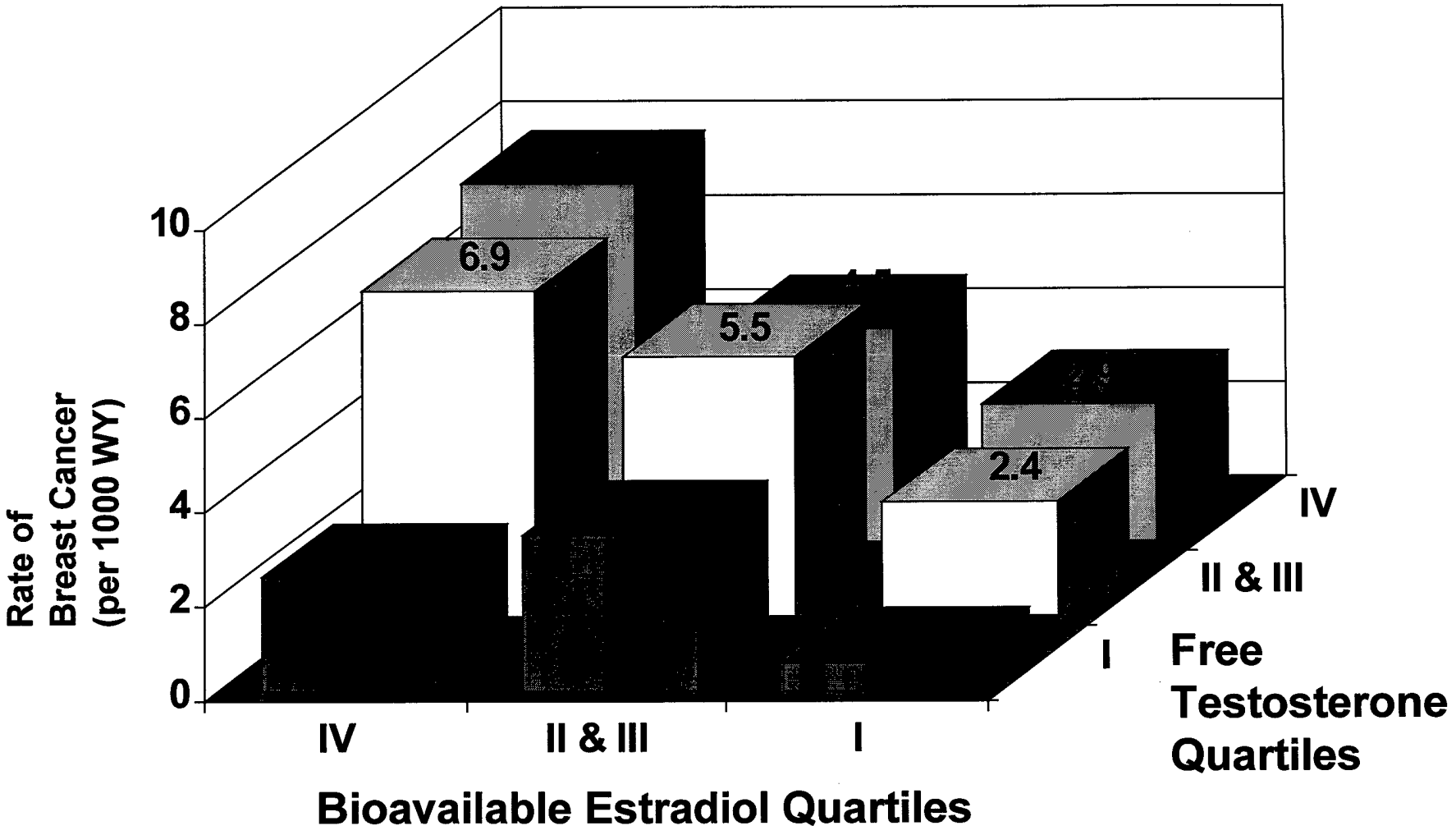
	Current ERT		Never ERT	
Proximal Radius BMD (g/cm^2)	RR	(95% CI)	RR	(95% CI)
Quartile 1	1.0	(0.36-4.3)	1.0	(0.97-2.21)
Quartile 2	1.25	(0.36-4.3)	1.46	(0.97-2.21)
Quartile 3	1.15	(0.35-3.7)	1.51	(1.0-2.30)
Quartile 4	1.64	(0.54-4.9)	1.72	(1.12-2.65)

+ adjusted for age and BMI

Figure Legend: Incidence rate of breast cancer per 1000 woman years by level of bioavailable estradiol and free testosterone.

Level 1=lowest quartile of hormone;
Level 2 = middle 2 quartiles of hormone;
Level 3= highest quartile of hormone.

* $p < 0.05$
** $p < 0.001$


Rate of
Breast Cancer
(per 1000 WY)
10
8
6
4
2
0
6.9
5.5
2.4
IV
II & III
I
Free
Testosterone
Quartiles
IV
II & III
I
Bioavailable Estradiol Quartiles

Appendix A: Manuscripts written, submitted or published

1) Lucas FL, Cauley JA, Stone RA, Cummings SR, Vogt MT, Weissfeld JL, Kuller LH. Bone mineral density and risk of breast cancer. Differences by family history of breast cancer. Am J Epidemiol 1998;148:22-9.

2) Cauley JA, Lucas FL, Kuller LH, Stone K, Browner W, Cummings SR. Elevated serum estradiol and testosterone concentrations are associated with a high risk of breast cancer. (Submitted, Ann Int Med, 7/21/98).

3) Lucas FL, Cauley JA, Jamal SA, Kuller LH. Alcohol consumption, estrogen replacement therapy, endogenous sex steroid hormones and risk of breast cancer in elderly women. (Unpublished).

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Bone Mineral Density and Risk of Breast Cancer

Differences by Family History of Breast Cancer

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Recent studies have suggested that bone mineral density (BMD) is related to risk of breast cancer in elderly women. This study investigated whether the level of breast cancer risk associated with BMD in women with a positive family history of breast cancer is different from that in women without a family history of breast cancer. Radial and calcaneus BMD were measured at baseline (1986–1988) in 7,250 elderly white women enrolled in the Study of Osteoporotic Fractures, and initial breast cancer status was ascertained at year 1 of follow-up. After a mean of 3.2 years of additional follow-up, 104 incident breast cancer cases, 20 of which appeared in women with a family history of breast cancer, were identified and confirmed by medical record review. Modification of the BMD effect by family history status was assessed by inclusion of interaction terms in proportional hazards regression models. Among women without a family history of breast cancer, those with a proximal radius BMD in the highest tertile were at a 1.48-fold increased risk compared with women in the lowest tertile; among women with a positive family history of breast cancer, those with highest tertile BMD were at a 3.41-fold increased risk compared with women in the lowest tertile. These results suggest that the association between BMD and breast cancer may be different in subgroups of women defined by family history. *Am J Epidemiol* 1998;148:22–9.

aged; bone density; breast neoplasms; cohort studies; estrogen replacement therapy; family characteristics

Bone and breast are both estrogen-responsive tissues. Early menarche (1, 2), late menopause (1, 3), and increased length of reproductive life (3, 4) are associated with increased risk of breast cancer. The risk may be increased with long term and/or current use (5–7) of estrogen replacement therapy (ERT).

Bone contains estrogen receptors (8). Bone mineral density (BMD) declines (9) and risk of osteoporotic fractures increases (10) after menopause, and both can be prevented by the use of ERT (11, 12). Some previous studies have suggested that BMD (13, 14) and vertebral fractures (Steven R. Cummings, University of California, San Francisco, unpublished manuscript) are associated with the risk of breast cancer in elderly women, possibly as surrogate measures of lifetime estrogen exposure.

Breast cancer in a first-degree relative is an important risk factor for the disease (15). Women with a family history of breast cancer may metabolize estrogen differently than women without such a history (16). Some (17–19) but not all (6, 7, 20) previous studies have suggested that the increase in breast cancer risk associated with ERT is greater in women with a positive family history than in other women. We hypothesized that the BMD-breast cancer relation may also differ by family history of breast cancer, and we addressed this question within the Study of Osteoporotic Fractures.

MATERIALS AND METHODS

Subjects

The Study of Osteoporotic Fractures is a multicenter prospective study of healthy elderly women recruited from population-based listings who are being followed

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Abbreviations: BMD, bone mineral density; CI, confidence interval; ERT, estrogen replacement therapy; RR, relative risk.

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for the occurrence of osteoporotic fractures. A total of 9,704 women aged 65 years and older were recruited between 1986 and 1988 from one of four areas: Baltimore, Maryland; Minneapolis, Minnesota; the Monongahela Valley in Pennsylvania; and Portland, Oregon. The Study of Osteoporotic Fractures excluded black women because of their low risk of hip fracture, as well as women who were unable to walk without the assistance of another person and women who had had bilateral hip replacements (21). One year after the baseline examination, study participants were asked to complete a questionnaire that included questions about personal and family history of breast cancer. Breast cancer status was ascertained again at a subsequent follow-up, approximately 3.2 years after the year 1 interview. The Institutional Review Board at each participating institution approved the study protocol. Each participant signed an informed consent form at entry into the study and at each clinical examination.

Ascertainment of breast cancer

This investigation of incident breast cancer included only those women in the study cohort who provided information on breast cancer status at both year 1 and the 3-year follow-up, and for whom information on family history of breast cancer was available (table 1). A total of 100 women died before completing the year 1 interview, and breast cancer status was not ascertained for 160 women. Women who reported a history of breast cancer at year 1 were considered to be prevalent cases (n = 506); prevalent cases were not verified by medical record review and were excluded from further analysis. A total of 8,938 women (92.1 percent of the study cohort) were potentially eligible for the analysis of incident breast cancer. Follow-up information allowing identification of incident breast cancer was collected at year 3. No follow-up information was available for 883 women, including 265 women who died between year 1 and the year 3 follow-up and 618 living women who either did not appear for the year 3 visit or did not answer the breast cancer questions. Death certificates were obtained for all 265 deaths; of these, one breast cancer death was identified but was not included in this analysis because the date of illness onset could not be determined.

Attempts were made to contact by telephone all 161 women who denied having breast cancer at year 1 but reported having breast cancer at year 3 (potential incident cases); these women's medical records were obtained and reviewed by a physician epidemiologist (L. H. K.). Forty women for whom self-reported breast cancer was not confirmed were excluded, for the following reasons: the medical record review showed benign disease (n = 2), the participant denied having breast cancer upon interview (n = 22), the participant refused to give an interview (n = 7), or the participant was unavailable for follow-up (n = 9). A total of 121 breast cancer cases, including four cases of carcinoma in situ, were confirmed by medical record pathology report or cancer registry record. Of the 8,015 women eligible for the breast cancer analysis (89.7 percent of those alive and free of breast cancer at year 1), 765 (9.5 percent) provided no family history information, leaving 104 cases and 7,146 controls eligible for the current analysis.

TABLE 1. Incident breast cancer and family history of breast cancer among participants in the Study of Osteoporotic Fractures, 1986–1993

	No.	%
Total cohort	9,704	100
Exclusions	766	7.9
Died prior to year 1 interview	100	1.0
No breast cancer information at year 1	160	1.7
Prevalent breast cancer reported at year 1	506	5.2
Alive and free of breast cancer at year 1	8,938	92.1
No breast cancer information available at follow-up	883	9.9
Died prior to follow-up	265	3.0
No breast cancer information at follow-up	618	6.9
Total with self-reported breast cancer information	8,055	90.1
Self-reported breast cancer not confirmed*	40	0.5
Confirmed cases	121	1.5
Controls	7,894	98.0
Missing family history information	765	9.5
Total available for family history analysis	7,250	90.0
Cases	104	1.4
Controls	7,146	98.6

* Breast cancer was not confirmed by medical record review (n = 2), participant denied having breast cancer upon interview (n = 22), participant refused interview (n = 7), or participant was unavailable for follow-up (n = 9).

Measurement of bone mass

Bone mass at entry into the study was measured in grams per square centimeter, using OsteoAnalyzers (Siemens-Osteon, Wahiwa, Hawaii). The proximal radius (forearm), distal radius (wrist), and calcaneus (heel) were scanned, with mean coefficients of variation of 2.0 percent, 1.5 percent, and 1.3 percent, respectively (21).

Other variables

At baseline, weight (measured while the participant stood in light clothing with shoes removed) was recorded with a balance beam scale (22). Self-reported height at age 25 years was used to calculate body mass index (weight (kg)/height^2 (m^2)), because women with low bone mass experience height loss secondary to vertebral fractures. Reproductive history was obtained by questionnaire and interview. Surgical menopause was defined as self-reported bilateral oophorectomy prior to natural menopause. Participants were asked about current and past use of estrogen and progestin, including dosage and duration, from age 40 years to the present (11). Reports on current use of medications were checked against the labels of medicines brought to the clinic visit. Women were categorized as never, past, or current users of ERT, as of the date of the baseline visit. We also collected information on current alcohol use (number of alcoholic drinks consumed per week, adjusted for atypical drinking, especially heavy drinking in the past 30 days). Family history of breast cancer was determined by self-report at year 1, with women reporting breast cancer in either a mother or a sister considered to have a positive family history. At year 3, participants were asked whether they had received a mammogram since study entry.

Statistical analysis

BMD was categorized into tertiles based on the distribution of values in the entire study cohort, and was also considered as a continuous variable (quantified in terms of standard deviation units). To avoid confounding by family history of breast cancer, we estimated univariate relative risks of breast cancer separately by family history subgroup, using proportional hazards regression (23). For multivariable regression models, the main effects of interest included family history of breast cancer, use of ERT, and BMD. Covariates (age, age at menopause, surgical menopause, parity/age at first birth, and body mass index) were selected a priori on the basis of their probable relation to breast cancer, BMD, ERT use, or family history. Current alcohol consumption was included as a covariate because of reported confounding of a breast cancer-ERT relation by use of alcohol (24). Study center was included to control for geographic differences. Separate models were constructed for each BMD site. Interaction terms involving family history and ERT use and BMD were constructed as cross-product terms. The statistical significance of interactions was assessed by likelihood ratio test for the addition of the set of interaction terms to the corresponding main effects model (25).

RESULTS

Twenty case-patients reported a family history of breast cancer in a mother or sister (table 2). Among women with a positive family history, cases were somewhat more likely than controls to have multiple family members affected (data not shown). None of the 20 cases and 233 (23.4 percent) of the 995 controls who provided this information had a mother or sister with breast cancer diagnosed before 50 years of age (data not shown). Only four breast cancer cases and 218 controls were current users of estrogen and progestin at baseline (data not shown).

In family history-specific univariate analysis, no consistent relation between age, age at menopause, or current alcohol consumption and risk of breast cancer was apparent (table 2). Although associations were not statistically significant at the 0.05 level, surgical menopause, current use of ERT, and late age at first birth were associated with increased risk, while higher body mass index was associated with somewhat decreased risk. Among those with a negative family history, women with proximal and distal radius BMD in the highest tertile were 1.7–2.2 times as likely to develop breast cancer as women in the lowest tertile of BMD. High calcaneus BMD was also associated with increased risk, but this increase was not statistically significant. Among women with a positive family history, being in the highest tertile of BMD for all three sites measured was significantly associated with increased risk of breast cancer.

In multivariable main effects models, women with a positive family history had an estimated 57 percent increased risk of breast cancer compared with women with a negative family history, after adjustment for age, age at menopause, surgical menopause, parity/age at first birth, body mass index, alcohol use, study center, BMD, and ERT use, although this increase in risk was not statistically significant ($p = 0.08$) (table 3). Current ERT use was associated with an increased risk of approximately 30 percent, also not statistically significant. Having proximal and distal radius BMDs in the highest tertile was associated with statistically significant increases in risk (proximal radius: relative risk (RR) = 1.78, 95 percent confidence interval (CI) 1.02–3.12; distal radius: RR = 2.39, 95 percent CI 1.37–4.19), while the increase in risk associated with highest tertile calcaneus BMD was smaller and was not statistically significant (RR = 1.53, 95 percent CI 0.87–2.70). Based on comparable main effects models, increases in radial BMD of one standard deviation were associated with an approximately 35 percent increase in risk (proximal radius: RR = 1.35, 95 percent CI 1.08–1.67; distal radius: RR = 1.37, 95 percent CI 1.12–1.66). Again, the modest increase in

TABLE 2. Data on breast cancer risk factors and univariate estimates of the relative risk of breast cancer, by family history status and case status, in the Study of Osteoporotic Fractures, 1986–1993

Risk factor	Negative family history (n = 6,200)				Positive family history (n = 1,050)			
	No. of cases	No. of controls	RR*	95% CI*	No. of cases	No. of controls	RR	95% CI
Family history of breast cancer†	84	6,116	1.00‡		20	1,030	1.46	0.90–2.37
Age (years)								
65–69	38	2,733	1.00‡		11	437	1.00‡	
70–74	31	1,911	1.21	0.75–1.94	6	334	0.71	0.26–1.91
75–79	10	952	0.78	0.39–1.57	3	165	0.65	0.18–2.34
≥80	5	520	0.79	0.31–2.02	0	94		
Age (years) at menopause								
≤40	10	947	1.00‡		3	162	1.00‡	
41–45	22	1,280	1.62	0.77–3.42	5	217	1.24	0.30–5.17
46–50	30	2,127	1.35	0.66–2.77	9	354	1.31	0.35–4.83
≥51	22	1,712	1.22	0.58–2.58	3	287	0.56	0.11–2.79
Surgical menopause								
No	70	5,165	1.00‡		15	866	1.00‡	
Yes	13	728	1.32	0.73–2.38	5	119	2.56	0.93–7.04
Estrogen replacement therapy								
Never use	46	3,595	1.00‡		8	582	1.00‡	
Past use	23	1,626	1.09	0.66–1.80	6	317	1.29	0.45–3.71
Current use	15	895	1.34	0.75–2.40	6	131	3.46	1.20–9.97
Parity/age (years) at first birth								
Nulliparous	11	1,040	1.00‡		2	187	1.00‡	
<20	6	493	1.16	0.43–3.14	3	74	3.71	0.62–22.2
20–34	58	4,115	1.32	0.69–2.51	13	694	1.87	0.42–8.27
≥35	5	255	1.83	0.64–5.26	2	37	4.61	0.65–32.8
Body mass index§								
<22.20	21	1,497	1.00‡		6	252	1.00‡	
22.20–24.75	20	1,523	0.90	0.49–1.67	3	271	0.47	0.12–1.86
24.76–27.92	21	1,544	0.92	0.50–1.68	6	270	0.97	0.31–3.01
≥27.93	22	1,552	0.95	0.53–1.74	5	236	0.88	0.27–2.88
Average no. of alcoholic drinks/week								
None	21	1,828	1.00‡		5	286	1.00‡	
<2	38	2,846	1.13	0.66–1.93	8	450	0.98	0.32–3.01
2–7	17	1,019	1.41	0.74–2.67	6	207	1.58	0.48–5.17
>7	8	423	1.70	0.75–3.84	1	87	0.71	0.08–6.09
Bone mineral density (g/cm²)								
Proximal radius								
<0.59	18	1,964	1.00‡		4	335	1.00‡	
0.59–0.68	34	2,048	1.77	1.00–3.13	3	352	0.76	0.17–3.42
≥0.69	32	2,089	1.68	0.94–2.99	13	338	3.57	1.16–11.0
Distal radius								
<0.32	19	1,964	1.00‡		1	329	1.00‡	
0.32–0.39	24	2,081	1.21	0.66–2.21	9	316	10.06	1.27–79.5
≥0.40	40	2,014	2.19	1.27–3.78	10	375	10.17	1.30–79.6
Calcaneus								
<0.36	22	1,943	1.00‡		1	332	1.00‡	
0.36–0.44	28	2,081	1.18	0.67–2.05	12	329	11.62	1.51–89.3
≥0.45	34	2,065	1.44	0.84–2.46	7	366	6.61	0.81–53.8

* RR, relative risk; CI, confidence interval.
† History of breast cancer in a mother or sister.
‡ Referent.
§ Weight (kg)/height² (m²).

TABLE 3. Estimated relative risk of breast cancer associated with bone mineral density and use of estrogen replacement therapy in the Study of Osteoporotic Fractures, 1986–1993*

Risk factor	No. of cases	RR†	95% CI†
Family history of breast cancer‡			
No	20	1.00§	
Yes	84	1.57	0.96–2.57
Estrogen replacement therapy			
Never use	54	1.00§	
Past use	29	0.98	0.60–1.59
Current use	21	1.33	0.75–2.35
Bone mineral density (g/cm²)			
Proximal radius			
<0.59	18	1.00§	
0.59–0.68	34	1.51	0.87–2.60
≥0.69	32	1.78	1.02–3.12
Per 1 SD* (0.10) increase		1.35	1.08–1.67
Distal radius			
<0.32	19	1.00§	
0.32–0.39	24	1.57	0.89–2.76
≥0.40	40	2.39	1.37–4.19
Per 1 SD (0.08) increase		1.37	1.12–1.66
Calcaneus			
<0.36	22	1.00§	
0.36–0.44	28	1.47	0.85–2.52
≥0.45	34	1.53	0.87–2.70
Per 1 SD (0.10) increase		1.18	0.93–1.48

* Summary of main effects proportional hazards regression. Data were controlled for age, age at menopause, surgical menopause, parity/age at first birth, body mass index, alcohol intake, and study center. Separate models were used for each bone mineral density site; family history and estrogen replacement therapy estimates were from the proximal radius model.

† RR, relative risk; CI, confidence interval; SD, standard deviation.

‡ History of breast cancer in a mother or sister.

§ Referent.

risk associated with an increase of one standard deviation in calcaneus BMD was not statistically significant. None of the control variables was statistically significant at the 0.05 level in multivariable models, although late age at first birth approached significance ($p = 0.08$).

Interaction terms were added to allow for separate effects of BMD and ERT within each level of family history. There was little evidence that the breast cancer-ERT association differed by family history status ($p = 0.30$ for interaction terms; data not shown), so these terms were dropped. Relative to women with a negative family history and proximal radius BMD in the lowest tertile, women with both a positive family history and highest tertile proximal radius BMD showed a 4.23-fold increase in breast cancer risk (95 percent CI 1.99–9.00), whereas highest tertile BMD in the absence of a positive family history was associated with only a 1.48-fold increase in breast cancer risk (95 percent CI 0.79–2.77; interaction: $p = 0.04$) (table 4). Among women with a negative family history, the estimated risk of breast cancer was approximately 24 percent higher for each standard deviation unit increase in proximal radius BMD, compared with a 97 percent increase in women with a positive family history (interaction $p = 0.07$). At the distal radius, highest tertile BMD was associated with a relative risk of 1.99 (95 percent CI 1.10–3.58) among women with a negative family history and a relative risk of 3.07 (95 percent CI 1.39–6.80) among women with a positive family history (relative to women with a negative family history and lowest tertile BMD). The largest increase in risk, however, was associated with second tertile BMD and a positive family history (RR = 3.18, 95 percent CI 1.43–7.06). For the calcaneus, the largest risk occurred in the women with second tertile BMD and a positive family history (RR = 3.09, 95 percent CI 1.50–6.39), while women with a negative family history and a similar BMD had essentially no increase in risk (RR = 1.04, 95 percent CI 0.57–1.87; interaction: $p = 0.01$). There was little evidence that the slope per standard deviation unit increase in distal radius or calcaneus BMD differed by family history.

Within family history subgroups, the increase in risk associated with having a proximal radius BMD in the highest tertile compared with the lowest was 48 percent for women with a negative family history, while the risk was 3.41-fold higher for women with a positive family history. Similarly, for the distal radius, among women with a negative family history, those with highest tertile BMD were at twice the risk of women with lowest tertile BMD; among women with a positive family history, the increase in risk associated with highest versus lowest tertile BMD was 9.9-fold. For the calcaneus, women with a negative family history demonstrated a 1.32-fold increase for the highest tertile versus the lowest, while women with a positive family history showed a 6.5-fold increase.

DISCUSSION

Breast cancer and osteoporosis are two of the most important health conditions affecting elderly women. We have previously shown that the two conditions may be related: Women with BMD in the highest quartile were found to have a 2- to 2.5-fold increased risk of breast cancer compared with women in the lowest quartile (13). The current report provides some evidence that the BMD-associated risk of breast cancer may differ among women by family history (mother or sister) of breast cancer. If BMD is associated with risk of breast cancer as a biologic marker of

TABLE 4. Estimated relative risk of breast cancer by family history status and bone mineral density in the Study of Osteoporotic Fractures, 1986–1993*

Bone mineral density (g/cm^2)	Negative family history		Positive family history†		*p* for interaction
	RR‡	95% CI‡	RR	95% CI	
Proximal radius					
<0.59	1.00§		1.24	0.42–3.67	0.04
0.59–0.68	1.64	0.91–2.96	1.00	0.29–3.39	
≥0.69	1.48	0.79–2.77	4.23	1.99–9.00	
Per 1 SD‡ (0.10) increase	1.24	0.98–1.57	1.97	0.94–4.12	0.07
Distal radius					
<0.32	1.00§		0.31	0.04–2.29	0.04
0.32–0.39	1.14	0.62–2.11	3.18	1.43–7.06	
≥0.40	1.99	1.10–3.58	3.07	1.39–6.80	
Per 1 SD (0.08) increase	1.30	1.05–1.61	1.70	0.88–3.31	0.24
Calcaneus					
<0.36	1.00§		0.27	0.04–2.01	0.01
0.36–0.44	1.04	0.57–1.87	3.09	1.50–6.39	
≥0.45	1.32	0.73–2.38	1.76	0.73–4.25	
Per 1 SD (0.10) increase	1.12	0.87–2.15	1.44	0.66–3.11	0.34

* Proportional hazards regression models with interaction terms. Data were controlled for age, age at menopause, surgical menopause, parity/age at first birth, body mass index, alcohol intake, study center, and use of estrogen replacement therapy.

† History of breast cancer in a mother or sister.

‡ RR, relative risk; CI, confidence interval; SD, standard deviation.

§ Referent.

cumulative estrogen exposure, then a BMD-family history interaction would imply that similar tissue-level exposure to biologically active estrogen is associated with different levels of risk depending on family history status. At high levels of BMD, the risk of breast cancer was higher among women with a positive family history than among women without such a history. Our findings are consistent with the hypothesis that there may be a subset of women—i.e., those with a positive family history—who are particularly sensitive to higher cumulative levels of estrogen, as reflected by BMD measurements.

Although the interaction between current exogenous estrogen use and family history was not statistically significant, our data are consistent with a modest increase in risk associated with current use in all subjects and a somewhat larger increase in risk with current use among women with a positive family history. Most current users in this cohort were long term users of ERT: More than 60 percent of current users had been on ERT for 10 years or more, and 30 percent had used ERT for more than 20 years.

While we are not aware of any previous studies that have addressed the question of a BMD-family history interaction, numerous studies have evaluated differential effects of ERT use in family history subgroups. At least three case-control studies have reported that the ERT-associated breast cancer risk is higher among women with a positive family history of breast cancer than among other women, although the range of risk estimates across these studies was wide (17–19). In a recent population-based case-control study, Newcomb et al. (17) reported ERT-associated relative risks of 0.93 for women without a family history and 1.39 for women with a family history among long term users, with an associated *p* value of 0.11 for the family history-ERT interaction. One meta-analysis (26) reported relative risks for any ERT use (as compared with never use) of 3.4 for women with a positive family history and 1.5 for women without a family history. On the other hand, a prospective study (7) found no family history differences for ever use of ERT, and one meta-analysis found similarly negative results (27). Nevertheless, these results suggest that the relative risk of breast cancer related to estrogen use may be higher among women with a positive family history than among women without one. Our findings are consistent with this observation.

Other studies have found an increased hormone-associated risk among women without a family history of breast cancer (6, 28, 29). However, among these, Mills et al. (6) studied Seventh-day Adventists, who have an atypically low baseline risk, while Kaufmann et al. (29) used hospital controls, who may be less likely to use ERT than the general population (30).

Fishman et al. (16) offered a possible mechanism for

a differential effect of estrogen by family history status, reporting that estrogen conjugation pathways differed by family history status, even in the absence of differences in serum estrogen levels. They postulated that a difference in the metabolic pathway in the high risk women, specifically a shift from the 2-hydroxylation pathway to the 16-α-hydroxylation pathway, may represent a shift from a more benign form of estrogen to a more biologically potent form. Yang et al. (31) recently reported different effects of estrogen metabolites on transforming growth factor-α3 promoter activity—a possible genetic mechanism for estrogen-associated risk.

Our study had several limitations. Only 20 breast cancer cases reported breast cancer in a mother or sister, which limited our power to assess interactions and to consider duration and dosage of ERT. However, the average duration of ERT use among current users was 14.9 years, suggesting that most of our women were long term users. None of our cases reported a family history with onset before age 50 in the relative, so we were unable to examine the breast cancer-BMD relation in women with early onset in a relative. We had no data regarding disease in second-degree relatives. Family history may be a more important risk factor in premenopausal disease (32); all of our subjects were postmenopausal and elderly.

Finally, all of the subjects in our cohort were elderly white women and healthy volunteers, which limits generalizability, although the incidence of breast cancer in this cohort is similar to that in the United States as a whole for this age group (33). The BMD-breast cancer association could be confounded by health-promoting activities in such a population—specifically mammographic screening, which could lead to higher breast cancer detection rates, and diet and physical activity, which are associated with higher BMD. However, only four of our breast cancer cases had carcinoma in situ, the type of lesion most likely to be detected by mammography. While we were unable to distinguish screening mammography from diagnostic mammography, we did find that women with a positive family history were more likely to have had mammograms within the past 3 years and that women with lowest tertile BMD were less likely to undergo mammography. However, our results were similar when we restricted the analysis to women who reported having mammograms.

In summary, this analysis suggests a possible familial response to estrogen exposure, as reflected in BMD among elderly women, that may account in part for the differential risk of breast cancer by family history status. These results should be replicated in a larger sample of family history-positive women, and the relation should be evaluated in younger and premenopausal women as well as in other elderly populations.

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REFERENCES

1. Kvale G, Heuch I. Menstrual factors and breast cancer risk. Cancer 1988;62:1625–31.
2. Brinton LA, Schairer C, Hoover RN, et al. Menstrual factors and risk of breast cancer. Cancer Invest 1988;6:245–54.
3. De Stavola BL, Wang DY, Allen DS, et al. The association of height, weight, menstrual and reproductive events with breast cancer: results from two prospective studies on the island of Guernsey (United Kingdom). Cancer Causes Control 1993;4:331–40.
4. Rautalahti M, Albanes D, Virtamo J, et al. Lifetime menstrual activity—indicator of breast cancer risk. Eur J Epidemiol 1993;9:17–25.

5. Colditz GA, Hankinson SE, Hunter DJ, et al. The use of estrogens and progestins and the risk of breast cancer in postmenopausal women. N Engl J Med 1995;332:1589–93.
6. Mills PK, Beeson WL, Phillips RL, et al. Prospective study of exogenous hormone use and breast cancer in Seventh-day Adventists. Cancer 1989;64:591–7.
7. Schairer C, Byrne C, Keyl PM, et al. Menopausal estrogen and estrogen-progestin replacement therapy and risk of breast cancer (United States). Cancer Causes Control 1994;5:491–500.
8. Eriksen EF, Colvard DS, Berg NJ, et al. Evidence of estrogen receptors in normal human osteoblast-like cells. Science 1988; 241:84–6.
9. Nilas L, Christiansen C. Bone mass and its relationship to age and the menopause. J Clinical Endocrinol Metab 1987;65: 697–702.
10. Longcope C, Baker RS, Hui SL, et al. Androgen and estrogen dynamics in women with vertebral crush fractures. Maturitas 1984;6:309–18.
11. Cauley JA, Seeley DG, Ensrud K, et al. Estrogen replacement therapy and fractures in older women. Study of Osteoporotic Fractures Research Group. Ann Intern Med 1995;122:9–16.
12. Lindsay R. Hormone replacement therapy for prevention and treatment of osteoporosis. Am J Med 1993;95:37S-9S.
13. Cauley JA, Lucas FL, Kuller LH, et al. Bone mineral density and risk of breast cancer in older women: The Study of Osteoporotic Fractures. Study of Osteoporotic Fractures Research Group. JAMA 1996;276:1404–8.
14. Zhang Y, Kiel DP, Kreger BE, et al. Bone mass and the risk of breast cancer among postmenopausal women. N Engl J Med 1997;336:611–17.
15. Mettlin C. Breast cancer risk factors: contributions to planning breast cancer control. Cancer 1992;69(suppl):1904–10.
16. Fishman J, Bradlow HL, Fukushima DK, et al. Abnormal estrogen conjugation in women at risk for familial breast cancer at the periovulatory stage of the menstrual cycle. Cancer Res 1983;43:1884–90.
17. Newcomb PA, Longnecker MP, Storer BE, et al. Long-term hormone replacement therapy and risk of breast cancer in postmenopausal women. Am J Epidemiol 1995;142:788–95.
18. Kaufman DW, Palmer JR, de Mouzon J, et al. Estrogen replacement therapy and the risk of breast cancer: results from the Case-Control Surveillance Study. Am J Epidemiol 1991; 134:1375–85.
19. Hulka BS, Chambless LE, Deubner DC, et al. Breast cancer and estrogen replacement therapy. Am J Obstet Gynecol 1982; 143:638–44.
20. Stanford JL, Weiss NS, Voigt LF, et al. Combined estrogen and progestin hormone replacement therapy in relation to risk of breast cancer in middle-aged women. JAMA 1995;274: 137–42.
21. Cummings SR, Black DM, Nevitt MC, et al. Appendicular bone density and age predict hip fracture in women. Study of Osteoporotic Fractures Research Group. JAMA 1990;263: 665–8.
22. Lohman TG, Martorell R. Anthropometric standardization reference manual. Champaign, IL: Human Kinetics Books, 1988.
23. Cox DR. Regression models and life tables (with discussion). J R Stat Soc B 1972;34:187–220.
24. Colditz GA, Stampfer MJ, Willett WC, et al. Prospective study of estrogen replacement therapy and risk of breast cancer in postmenopausal women. JAMA 1990;264:2648–53.
25. Kelsey JL, Whittemore AS, Evans AS, et al. Methods in observational epidemiology. 2nd ed. New York, NY: Oxford University Press, 1996.
26. Steinberg KK, Smith SJ, Thacker SB, et al. Breast cancer risk and duration of estrogen use: the role of study design in meta-analysis. Epidemiology 1994;5:415–21.
27. Colditz GA, Egan KM, Stampfer MJ. Hormone replacement therapy and risk of breast cancer: results from epidemiologic studies. Am J Obstet Gynecol 1993;168:1473–80.
28. Brinton LA, Hoover R, Fraumeni JF. Menopausal oestrogens and breast cancer risk: an expanded case-control study. Br J Cancer 1986;54:825–32.
29. Kaufman DW, Miller DR, Rosenberg L, et al. Noncontraceptive estrogen use and the risk of breast cancer. JAMA 1984; 252:63–7.
30. Barrett-Connor E. Postmenopausal estrogen and the risk of breast cancer. Ann Epidemiol 1994;4:177–80.
31. Yang NN, Venugopalan M, Hardikar S, et al. Identification of an estrogen response element activated by metabolites of 17β-estradiol and raloxifene. Science 1996;273:1222–5.
32. Brinton LA, Hoover R, Fraumeni JF Jr. Interaction of familial and hormonal risk factors for breast cancer. J Natl Cancer Inst 1982;69:817–22.
33. Kessler LG. The relationship between age and incidence of breast cancer: population and screening program data. Cancer 1992;69(suppl):1896–903.

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ELEVATED SERUM ESTRADIOL AND TESTOSTERONE CONCENTRATIONS ARE ASSOCIATED WITH A HIGH RISK OF BREAST CANCER

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ABSTRACT:

Background: There is uncertainty about the relationship between endogenous steroid hormones and the risk of breast cancer. Measurement of sex hormone levels might identify women at high risk of breast cancer who should consider preventive therapies.

Objective: To test the hypothesis that serum concentrations of estradiol and testosterone predict the risk of breast cancer.

Design: Prospective case-cohort.

Setting: 4 US clinical centers

Participants: 97 women with confirmed incident breast cancer and 244 randomly chosen controls ages 65 years or older, not taking estrogen.

Measurements: Sex steroid hormone levels were assayed using serum collected at baseline and stored at -190°C. Risk factors for breast cancer were ascertained by questionnaire. Incident breast cancers were confirmed by medical record review over an average of 3.2 years.

Results: Women with the highest ($\geq$ 2 pg/ml) bioavailable estradiol had a 5 fold (95% confidence intervals, 2.0 to 12.4) increased risk of breast cancer compared with women with the lowest concentration. The risk of breast cancer among women with the highest free testosterone compared to the lowest was 3.2 (1.4 to 7.1). The estimated incidence rate of breast cancer per 1000 woman years was 0.6 among women with the lowest bioavailable estradiol and free testosterone compared to 7.6 among women with the highest concentrations of these hormone. Traditional breast cancer risk factors were similar in the cases and random sample of the cohort.

Conclusion: Estradiol and testosterone levels may play important roles in the development

of breast cancer in older women. A single measurement of bioavailable estradiol and free testosterone is more accurate than conventional risk factors in estimating a woman's risk of breast cancer. Women identified as high risk by these hormone levels could be targeted for anti-estrogen treatment.

INTRODUCTION

One in 8 women will develop breast cancer in her lifetime and 3% will die from the disease (1,2). In 1997, over 180,000 new cases of breast cancer occurred among women in the United States (2). About one half of all breast cancers occur among women 65 years of age or over: about 1 in 14 women age 60 to 79 will develop breast cancer compared to 1 in 26 among women age 40 to 59 (2). It is estimated that there will be at least a 1% annual increase in the risk of breast cancer among women aged 60 to 79 (3).

Endogenous estrogens may play an important role in the development of breast cancer (4). Some (5-8) but not all (9-12) prospective studies have found significant associations between endogenous concentrations of estrogens and subsequent risk of breast cancer. Two recent reviews concluded that the growing body of evidence supports a relationship between estrogen levels and risk of breast cancer (4,13). Women with higher bone mineral density (BMD), a cumulative measure of endogenous estrogen have an increased risk of breast cancer (14-16). Endogenous androgens may also contribute (6,8,17). However, the relationship between serum androgens and breast cancer may not be independent of serum estrogens (18,19). The best estrogen fraction to predict risk has not been identified (4). Most studies have included measurements of total hormone levels; the concentrations of free hormone may have even stronger associations. Finally, most of the women in these studies were postmenopausal women, younger than 65 years of age.

Two randomized trials have demonstrated a reduction in primary breast cancers with tamoxifen (20) and raloxifene (21). In the Breast Cancer Prevention Trial, 4 years of tamoxifen use led to a 45% reduction in breast cancer incidence among the 13,388

women who participated in the trial (20). Women in this study were considered "high" risk of breast cancer based on risk factors, including age ≥ 60 years. About 30% of women in the trial were age 60 years or older. The Multiple Outcomes of Raloxifene Evaluation (MORE), found a 70% reduction in the risk of breast cancer, especially estrogen receptor positive cancers after 33 months of treatment with raloxifene (21). About 80% of the 7,704 women in this trial, were over the age of 60 years.

Since both treatments entail costs and risk (21,22), it is important to identify women who are at the greatest risk of breast cancer and hence, most likely to benefit from anti-estrogen therapies. The current study was designed to test the hypothesis that serum concentrations of estradiol and testosterone, measured an average of 3 years before the clinical diagnosis of breast cancer are related to the risk of breast cancer in women 65 years of age or older. We hypothesized that measurements of serum hormone could be used to identify women at high risk of developing breast cancer. We used a case-cohort approach to compare serum hormone in 97 incident cases of breast cancer and a random set of controls in the Study of Osteoporotic Fractures.

METHODS

Study Population

All subjects were participants in the Study of Osteoporotic Fractures, a prospective study of 9,704 Caucasian community dwelling women, all age $\geq$ 65, recruited at 4 clinical centers across the United States (23). Women were excluded from SOF if they reported a bilateral hip replacement or the inability to walk without the assistance of another person. During 3.2 years of follow-up, we confirmed 121 breast cancer cases including 4 cases of carcinoma in situ by review of medical record by a physician epidemiologist (LHK) (14). We excluded women reporting current estrogen replacement therapy at baseline, leaving 97 confirmed incident breast cancer cases. Using a case-cohort approach, a random sample of 247 women, who survived to the first annual visit, denied a history of breast cancer, and did not report use of estrogen at baseline were chosen as controls (24). Three of these control women who developed incident breast cancer were included in the case group.

Sex Steroid Hormones

Serum was obtained from all participants at a baseline exam in 1986 to 1988. All participants were instructed to adhere to a fat free diet overnight and the morning of the examination to minimize lipemia that might interfere with assays. Blood was drawn between 8:00 am and 2:00 pm and serum was immediately frozen to -20C. Within 2 weeks all samples were shipped to a central repository where they were stored in liquid nitrogen at -190C until assay. We measured estrogens (total estradiol), bioavailable or non sex hormone binding globulin (SHBG) bound estradiol, free estradiol, estrone and estrone sulfate, androgens (androstenedione, dehydroepiandrosterone sulfate (DHEAS), total and

free testosterone and SHBG. All assays were done blinded to breast cancer status by Corning Nichols Institute (San Juan Capistrano, Ca). The sensitivity of the assays refer to the lower limit of detection.

We determined the reproducibility of selected hormone measurements in 20 postmenopausal women by assaying levels in duplicate. Pearson correlations (all significant at $p < 0.001$) between the two measures were as follows: total testosterone ($r=0.98$); free testosterone ($r=0.97$); total estradiol ($r=0.56$); non-SHBG bound estradiol ($r=0.83$); estrone ($r=0.67$); estrone sulfate ($r=0.70$); androstenedione ($r=0.77$); DHEAS ($r=0.97$); SHBG ($r=0.97$). Initial and repeat mean values were similar.

Total estradiol was measured using liquid-liquid organic extraction, column chromatography and radioimmunoassay (RIA), (intra- and inter- assay variability, 4-12% and 6-12%, respectively; sensitivity of 2 pg/ml). Free estradiol was measured using equilibrium dialysis and is calculated using the percent dialysable estradiol and total estradiol (intra- and inter- assay variability, 3-4% and 4-6%, respectively; sensitivity of 0.1 pg/ml). Non-SHBG bound estradiol or bioavailable estradiol was estimated by the ammonium sulfate precipitation of SHBG bound steroids (intra- and inter- assay variability, 3% and 7%, respectively).

Estrone was measured using extraction, chromatography and RIA, (intra- and inter-assay variability, 8-12% and < 6-7% respectively; sensitivity of 10 pg/ml). Estrone sulfate was measured using organic extraction, enzymatic hydrolysis, celite chromatography and RIA, (intra- and inter- assay variability 6-7% and 8-10%, respectively; sensitivity of 50 pg/ml).

Androstenedione was measured using a RIA after preparation for analysis by

organic extraction and chromatography, (intra- and inter- assay variability, 6-10% and 10-20%, respectively; sensitivity of 3 ng/dl). DHEAS was measured using RIA after preparation for the analysis by serial dilution, (intra- and inter- assay variability 6-11% and 10-13%, respectively; sensitivity of 5 ng/dl). Total testosterone was measured using RIA with chromatographic purification. The free testosterone method uses equilibrium dialysis. Calculation of free testosterone adjusts for albumin concentration; (intra- and inter- assay variability, 5% and 7%, respectively; sensitivity of 1 ng/dl). SHBG is measured using RIA, (intra- and inter-assay variability of 6.9% and 4.4%, respectively; sensitivity of 5.0 nmol/).

Other Variables

Weight (in light clothes with shoes removed) was recorded with a balance beam scale. Self reported height at age 25 was used to calculate the modified body mass index (BMI; weight in kilograms divided by the square of height in meters) because women with low bone mass experience height loss secondary to vertebral fractures. A reproductive history was obtained by questionnaire and interview including information on ages at menarche, menopause, and first birth, parity and family history of breast cancer. Participants were asked about past use of estrogen replacement therapy. We asked women about whether they walked for exercise and current and lifetime cigarette and alcohol use. We calculated the average number of alcohol drinks per week with non-drinkers coded as zero intake.

Statistical Analyses

Characteristics of cases and random sample of the cohort were compared by t-test (continuous variables) or by Chi-Square (categorical variables). Sex steroid hormone levels were not normally distributed. The non parametric (Wilcoxon 2 sample) test was

used to compare the distribution of hormones in cases versus controls.

For all hormones (but total and free estradiol), the relative hazard for breast cancer was calculated (using the lowest quartile as the reference group) across quartiles of sex steroid hormone levels using a modification of the Cox proportional hazards model that accounts for the case-cohort sampling design and has been successfully applied in previous studies (24). The distribution of total and free estradiol did not allow division by quartiles; four levels of free estardiol were defined to approximate quartiles as closely as possible. Total estradiol was categorized using < 5 pg/ml as the referent group with the remaining values divided into tertiles. A test for linear trend of increasing risk of breast cancer across quartiles of hormones was carried out.

We estimated the incidence of breast cancer per 1000 woman years by both bioavailable estradiol and free testosterone. For these analyses, we combined the 2 middle quartiles of hormones and calculated incidence of breast cancer in women by level of bioavailable estradiol and free testosterone.

To test the hypothesis that the association between the precursor hormone, androstenedione, and breast cancer could be explained by levels of bioavailable estradiol and free testosterone, we calculated the relative hazard of breast cancer in multivariate models including all three hormones (androstenedione, bioavailable estradiol and free testosterone). For these analyses, we dichotomized the hormone variables and compared women in the top 3 quartiles to those in the lowest quartile.

RESULTS

The cases and random sample of the cohort were similar with respect to age, reproductive history, family history of breast cancer, smoking and exercise, and other conventional risk factors for breast cancer, Table 1. The mean body weight tended to be higher among the cases. Cases reported more consumption of alcohol in the past year. About one-third of cases and 1/3 of the random sample of the cohort reported past use of estrogen replacement therapy. There was no significant difference in the number of years since stopping use of estrogen or duration of estrogen use between the cases and random sample of the cohort.

Sex Steroid Hormones and Breast Cancer

Median sex steroid hormone levels were higher in the cases compared with the random sample of the cohort, Table 2. The magnitude of the difference in median hormone concentrations ranged from 16% for total testosterone to 37% for estrone sulfate. The hormone distributions were significantly different in cases and the random sample of the cohort except for SHBG.

The association between serum hormone level and breast cancer was strongest for bioavailable estradiol: women in the highest quartile had a 5 fold (95% CI 2.0-12.0) greater risk of breast cancer compared to women in the lowest quartile, Table 3. Among the androgens, free testosterone level was strongly linked to subsequent risk of breast cancer: there was three-fold excess risk of breast cancer among women with the highest free testosterone levels. These associations were independent of age and body weight.

Women in the highest quartile of estrone, estrone sulfate, androstenedione, DHEAS and total testosterone also had a two to 2 ½ times excess risk of breast cancer, Table 3.

SHBG and the ratio of estrone sulfate to estrone were not associated with breast cancer. Results were the same when we excluded past estrogen users.

The estimated incidence rate of breast cancer was lowest (0.6 per 1000 woman years) among women with the lowest bioavailable estradiol and free testosterone, Figure 1. In contrast, the incidence of breast cancer was almost 13 times greater among women with the highest concentration of both hormones.

Precursor Hormone

In a model that included levels of bioavailable estradiol, free testosterone and androstenedione, bioavailable estradiol RH=2.8; (1.3 to 5.9) and free testosterone, RH=2.2; (1.0 to 4.5) but not androstenedione RH=1.0; (0.5 to 2.0) remained significantly related to the risk of breast cancer.

DISCUSSION

The results of this study support the hypothesis that sex hormones are important in the etiology of breast cancer in older women. In particular, women with a bioavailable estradiol level above 2.0 pg/ml had a 5 fold increased risk of breast cancer compared with women with the lowest estradiol. We also found a strong relationship between the unbound portion of testosterone and the risk of breast cancer. Our results are consistent with other prospective studies of the relationship between sex steroid hormone levels and the risk of breast cancer in somewhat younger women. The average incidence rate of breast cancer among US white women age 65 years and older is 4.6 per 1000 woman-years (25). Based on, our results we estimate that the incidence rate of breast cancer among women with the highest bioavailable estradiol and free testosterone is almost 2 fold higher than this expected rate.

The absolute concentrations of hormones, especially estradiol, were very low. Nevertheless, a gradient of risk was observed across increasing concentrations. This gradient of risk is greater than that observed between serum cholesterol concentrations and coronary heart disease especially among older women (4). These results imply that measurement of bioavailable estradiol and free testosterone could be used as a clinical measure of risk of breast cancer to identify women who may benefit from anti-estrogen treatment.

Sources of testosterone in postmenopausal women include direct secretion from the ovary and from the precursor, androstenedione. Testosterone could influence the risk of breast cancer directly or indirectly as a source of estradiol. Androgen receptors have been identified in human breast cancer cells although at least in vitro, activation of the androgen

receptor tends to suppress the proliferation of breast cancer cells (26). In 2 studies the association between total testosterone and breast cancer was not independent of bioavailable estradiol (18,19). However, neither of these studies measured free testosterone. In the current study, we found an association of free testosterone to breast cancer that was independent of bioavailable estradiol levels suggesting that the association may be a direct one.

The primary source of estrogens in postmenopausal women is the aromatization of androstenedione, an adrenal hormone (27). We and others (6) found an association between higher androstenedione and DHEAS and breast cancer. However, in our study, the association between androstenedione and breast cancer was no longer significant in models that included bioavailable estradiol and free testosterone, consistent with the hypothesis that increased androstenedione may contribute to increased risk of breast cancer as a precursor to estradiol and testosterone.

Local formation of androgens and estrogens within the breast may also contribute to the etiology of breast cancer. Breast fat has aromatase activity and levels of aromatase activity in adipose tissue adjacent to the malignant tumor was significantly higher than in tissues adjacent to benign lesions (28). Breast tissue also contains a sulfatase enzyme which can convert estrone sulfate to estrone which can then be converted to estradiol, thereby raising the level of estradiol in the breast (29). In our study, both estrone and estrone sulfate were directly related to the risk of breast cancer. Additional enzymatic processes, including the 17β estradiol dehydrogenase could lead to the accumulation of high levels of sex steroids within the breast tissue (30,31). It is unlikely, however, that a local increase in estrogen synthesis within the breast could account for the increased blood

levels of estradiol that we observed among women with breast cancer.

Weight gain, obesity and increased intra-abdominal fat have all been identified as possible risk factors for breast cancer (32,33), possibly due to aromatization of androstenedione to estrone in fat tissue (34). In our study, adjustment for obesity or body weight did not substantially influence the association between sex hormone level and breast cancer. However, we did not measure either thigh fat, which has been associated with greater aromatase activity and therefore higher blood estrone and estradiol levels (35) or intra-abdominal fat, which has been associated with greater concentrations of insulin, free and bioavailable estradiol and testosterone (34,35). Future studies should include these measures.

Measures of traditional risk factors for breast cancer such as age at first birth, nulliparity, early menarche, family history of breast cancer, were remarkably similar between the cases and random sample of the cohort suggesting that these conventional risk factors cannot accurately identify older women at high risk of breast cancer. Our results are consistent with other studies of older women (15). In addition, these risk factors are highly prevalent. In one study, over 98% of the population was exposed to at least one of these risk factors (36) and the majority of women with 1 or more of these risk factors do not develop breast cancer. Hence, it is unlikely that these risk factors can be used to accurately identify older women at risk of breast cancer.

There are several limitations to our study. The SOF cohort consists primarily of healthy community dwelling Caucasian elderly women; however, the overall rate of breast cancer in our cohort (4.3) per 1000 person years was similar to that observed for white women ages 65 and older in the U.S. (25). The levels of hormones in these elderly

women are relatively low and may be subject to increased laboratory variability. However, the reproducibility of sex steroid hormone levels in postmenopausal women using the same laboratory was found to be excellent (36). Hormone levels were measured only once and a single measure will have some imprecision. Although this is a one of the largest cohort studies of breast cancer, we had limited power to test for interactions among hormones and breast cancer. Current hormone levels may not reflect earlier levels. However, several studies have documented correlations of serum estrogens over several years, especially among women whose weight remains stable (36-38). Thus, it is possible that the levels of hormones measured in these women may indeed reflect exposures over a longer period of time.

In conclusion, estradiol and testosterone play important roles in the risk of breast cancer in older women. Concentrations of these hormones can estimate the risk of breast cancer and aid in decisions about treatments to decrease breast cancer risk.

REFERENCES

1. Miller B, Feuer E, Hankey B. The significance of the rising incidence of breast cancer in the United States. Oncology 1994;193-207.

2. Parker SL, Tong T, Bolden S, Wingo P. Cancer statistics. 1997 Cancer Journal of Clinicians 1997;47:5-27.

3. White E. Projected changes in breast cancer incidence due to the trend toward delayed childbearing. Am J Public Health 1987;77:495-497.

4. Colditz GA. Relationship between estrogen levels, use of hormone replacement therapy, and breast cancer. J Cancer Inst 1998;90:814-823.

5. Toniolo PG, Levitz M, Zelenvich-Jacquotte A, Banerjee S, Koenig KL, Shore RE, et al. A prospective study of endogenous estrogens and breast cancer in post-menopausal women. J Nat Ca Institute. 1995; 87:190-7.

6. Dorgan JF, Longcope C, Stephenson HE, Falk RT, Miller R, Franz C, et al. Relation of prediagnostic serum estrogen and androgen levels to breast cancer risk. Cancer Epidemiol Biomarkers Prev 1996;5:533-539.

7. Moore JW, Clark GMG, Hoare SA, Millis RR, Hayward JL, Quinlan MK, et. al. Binding of oestradiol to blood proteins and aetiology of breast cancer. Int J Cancer 1986; 38:625-30.

8. Berrino F, Muti P, Micheli A, et al. Serum sex hormone levels after menopause and subsequent breast cancer. J Natl Cancer Inst 1996;88:291-296.

9. Helzlsouer KJ, Alberg AJ, Bush TL, Longcope C, Gordon GB, Comstock GW. A prospective study of endogenous hormones and breast cancer. Cancer Detect Prev. 1994; 18:79-85.

10. Garland CF, Friedlander NJ, Barrett-Connor E, Khaw K. Sex hormones and postmenopausal breast cancer: A prospective study in an adult community. Am J Epidemiol. 1992; 135:1220-30.

11. Wysowski DK, Comstock GW, Helsing KN, Lau HL. Sex hormone levels in serum in relation to the development of breast cancer. Am J Epidemiol. 1987; 125:791-799.

12. Bulbrook RD, Moore JW, Clark GMG, Wang DY. Relation between risk of breast cancer and biological availability of estradiol in the blood: Prospective study in Guernsey. Ann NY Acad Sci. 1986; 464:378-388.

13. Thomas HV, Reeves GK, Key TJ. Endogenous estrogen and postmenopausal breast cancer: a quantitative review. Cancer Causes and Control 1997;8:922-928.

14. Cauley JA, Lucas FL, Kuller LH, Vogt MT, Browner WS, Cummings SR. Bone mineral density and risk of breast cancer in older women. JAMA 1996;276:1404-1408.

15. Zhang Y, Kiel DP, Kreger BE, Cupples LA, Ellison RC, Dorgan JF, et al. Bone mass and the risk of breast cancer among postmenopausal women. N Engl J Med 1997;336:611-7.

16. Meema S, Meema HE. Possible estrogenic effect on bone in postmenopausal patients with mammary carcinoma. Cancer 1966;19:433-436.

17. Dorgan JF, Stanczyk FZ, Longcope C, Stephenson HE, Chang L, Miller R, Franz C, et al. Relationship of serum dehyroepiandrosterone (DHEA), DHEA sulfate, and 5-androstene-3, 17 -diol to risk of breast cancer in postmenopausal women. Serum hormones and breast cancer risk. 177-181.

18. Zeleniuch-Jacquotte A, Bruning PF, Bonfrer MG, Koenig KL, Shore RE, Kim MY, et al. Relation of serum levels of testosterone and dehydroepiandrosterone sulfate to risk of breast cancer in postmenopausal women. Am J Epidemiol 1997;145:1030-8.

19. Thomas HV, Key TJ, Allen DS, Moore JW, Dowsett M, Fentiman IS, Wang DY. A prospective study of endogenous serum hormone concentrations and breast cancer risk in post-menopausal women on the island of Guernsey. Brit J Cancer 1997;76:401-405.

20. National Cancer Institute. Preliminary findings of the Breast Cancer Prevention Trial. Internet website June 1, 1998: http://rex.nci.nih.gov/massmedia/pressreleases/prevtrialGRAPHS.htm.

21. Cummings SR, Eckert S, Grady D, Glusman J, Krueger K, Norton L, Powles TJ, Black D, Cauley JA, Morrow M, Bjarnason NH. Raloxifene reduces the risk of breast cancer in postmenopausal women with osteoporosis. (Submitted).

22. Evista (package insert). Indianapolis, IN: Eli Lilly and Company; 1998.

23. Cummings SR, Black DM, Nevitt MC, Browner WS, Cauley JA, Genant HK, et al. Appendicular bone density and age predict hip fracture in women.

JAMA 1990;263:665-668.

24. Prentice RL. A case-control design for epidemiologic cohort studies and disease prevention trials. Biometrika 1986;73:1-11.

25. Miller BA, Ries LAG, Hankey BF, Kosary CL, Harras A, Devesa SS, et al. SEER Cancer Statistics Review, 1973-1990. Bethesda, Md: US Dept of Health and Human Services; 1993. National Institutes of Health publication 93-2789.

26. MacIndoe LH, Etre LA. An antiestrogenic action of androgens in human breast cancer cells. J Clin Endocrin Metab 1981;53:836-42.

27. Jaffe RB. Part II - Pathophysiology: The Menopause and Perimenopausal Period. In S.S.C. Yen and R.B. Jaffe (Eds) Reproductive Endocrinology-Physiology, Pathophysiology and Clinical Management (2nd edition). W. B. Saunders Co., 1986, pp. 406-423.

28. O'Neill JS, Miller WR. Aromatase activity in breast adipose tissue from women with benign and malignant breast diseases. Brit J Cancer 1987;56(5):601-604.

29. Santen RJ, Leszczynski D, Tilson-Mallet N, Feil PD, Wright C, Manni A, et al. Enzymatic control of estrogen production in human breast cancer: relative significance of aromatase versus sulfatase pathways. Ann NY Academy of Sciences 1986;464:117-225.

30. Thijssen JH, Blankenstein MA, Donker GH,, Daroszewski J. Endogenous steroid hormones and local aromatase activity in the breast. J Steroid Biochem Mol Biol 1991;39:799-804.

31. Perel E, Wilkins D, Killinger DW. The conversion of androstendione to estrone, estradiol, and testosterone in breast tissue. J Steroid Mol Biol 1980;13:89-94.

32. Sellers TA, Gapstur SM, Potter JD, Kushi LH, Bostick RM, Folsom AR. Association of body fat distribution and family histories of breast and ovarian cancer with risk of postmenopausal breast cancer. Am J Epidemiol 1993;138:799-803.

33. Mannisto S, Pietinen P, Pyy M, Palmgren J, Eskelinen M, Uusitupa M. Body-size indicators and risk of breast cancer according to menopause and estrogen-receptor status. Int J Cancer 1996;68:8-13.

34. Killinger DW, Perel E, Daniilescu D, Kharlip L, Lindsay WRN. The relationship between aromatase activity and body fat distribution. Steroids 1987;50:61-72.

35. Rink JD, Simpson ER, Barnard JJ, Bulun SE. Cellular characterization of adipose

tissue from various body sites of women. J Clin Endocrinol Metab 1996;81:2443-2447.

36. Rockhill B, Weinberg CR, Newman B. Population attributable fraction estimation for established breast cancer risk factors:considering the issues of high prevalence and unmodifiability. Am J Epidemiol 1998;147:826-33.

37. Hankinson SE, Manson JE, Spiegelman D, Willett WC, Longcope C, Speizer FE. Reproducibility of plasma hormone levels in postmenopausal women over a 2—3-year period. Cancer Epidemiol Biomarkers Prev 1996;4:649-654.

38. Kuller LH, Gutai JP, Meilahn E, Matthews KA, Plantinga P. Relationship of endogenous sex steroid hormones to lipids and apoproteins in postmenopausal women. Arteriosclerosis 1990;10:1058-1066.

39. Muti P, Trevisan M, Micheli A, Krogh V, Bolelli G, Sciajno R, et al. Reliability of serum hormones in premenopausal and postmenopausal women over a one-year period. Cancer Epidemiol Biomarkers Prev 1996;5:917-922.

Table 1: Descriptive Characteristics of Women with Incident Breast Cancer and Random Sample of the Cohort

	Cases n=97	Random Sample n=244	P
Age, y	70.9 ± 4.6	71.8 ± 5.0	0.14
Body weight, kg	69.9 ± 13.1	67.7 ± 11.9	0.14
BMI, kg/m^2	27.6 ± 5.4	26.5 ± 4.3	0.07
Height at age 25, cm	162.5 ± 6.2	163.2 ± 6.0	0.32
Age at menarche, y	12.8 ± 1.6	13.1 ± 1.6	0.16
Age at first birth, y	25.9 ± 5.5	25.3 ± 4.7	0.35
Age at menopause, y	46.7 ± 5.5	47.6 ± 5.6	0.21
Number of live births*	2.48 ± 1.63	2.70 ± 1.48	0.28
Surgical menopause, %	12.6	10.6	0.59
Ever Pregnant, %	84.5	79.1	0.25
Nulliparous, %	17.2	21.2	0.41
Family History of Breast Cancer, %	14.7	14.2	0.89
Take walks for exercise, %	54.6	52.5	0.72
Current Smoker, %	5.3	8.2	0.35
Drink alcohol, last 12 mos., %	74.2	70.1	0.45
Median drinks per week (range)	0.63 (0-22)	0.49 (0-21)	0.03
Past Estrogen use, %	33.7	32.0	0.76
Years since stopping estrogen+	12.4 ± 8.5	6.1 ± 8.6	0.97
Duration of Estrogen Use (years)+	5.8 ± 6.2	6.5 ± 7.1	0.42

Plus-minus values are mean ± standard deviation
*among parious women
+estrogen users only

Table 2: Median and Range of Sex Steroid Hormones in Incident Breast Cancer Cases and Random Sample of the Cohort

ESTROGENS	Case median (range)	Random Sample median (range)	p*
Estradiol (pg/ml)	8.0 (3 - 22)	6.0 (2 - 56)	0.0006
non-SHBG bound Estradiol (pg/ml)	1.31 (0.30 - 6.5)	1.0 (0.2 - 10.6)	0.001
Free Estradiol, (pg/ml)	0.14 (0 - 0.4)	0.12 (0 - 1.2)	0.0009
Estrone, (pg/ml)	24.0 (0 - 69)	20.0 (0 - 67)	0.004
Estrone sulfate (pg/ml)	221.0 (42 - 1036)	161.0 (0 - 1089)	0.004
ANDROGENS			
Androstenedione (ng/dl)	44.0 (5 - 152)	36.0 (0 - 147)	0.003
DHEAS (ng/dl)	75.0 (9 - 357)	63.5 (0 - 333)	0.04
Total Testosterone (ng/dl)	21.0 (0 - 78)	18 (0 - 76)	0.005
Free Testosterone (pg/ml)	3.0 (0 - 11.2)	2.35 (0 - 9.9)	0.003
SHBG (nmol/L)	38.0 (6 - 89)	43.0 (5 - 119)	0.20

*Wilcoxon 2 sample test

Table 3: Relative Hazard (RH) of Breast Cancer by Level of Sex Steroid Hormone Levels

ESTROGENS	No. of Subjects Cases	Random Sample	RH[a]	RH (95% CI)[b]	p	p trend
Estradiol Total (pg/ml)						
Level 1 (< 5)	14	60	1.0	1.0 (referent)		
Level 2 (5- < 6)	15	39	1.4	1.4 (0.7, 3.1)	0.367	0.001
Level 3 (6- < 9)	26	85	1.3	1.4 (0.6, 3.3)	0.392	
Level 4 (≥ 9)	42	59	3.1	3.5 (1.6, 7.7)	0.002	
Bioavailable Estradiol (pg/ml)						
Quartile 1 (< 0.65)	10	70	1.0	1.0 (referent)		
Quartile 2 (0.65- < 1.10)	32	57	4.2	4.2 (1.9, 9.5)	0.0006	0.004
Quartile 3 (1.10 - < 2.00)	22	63	2.2	2.4 (1.0, 5.6)	0.054	
Quartile 4 (≥ 2.00)	33	52	4.5	5.0 (2.0, 12.4)	0.0005	
Free Estradiol (pg/ml)						
Level 1 (< 0.1)	4	25	1.0	1.0 (referent)		0.010
Level 2 (0.1)	58	170	1.7	1.8 (0.6, 5.3)	0.324	
Level 3 (0.2)	25	32	4.5	5.1 (1.5, 17.8)	0.010	
Level 4 (≥ 0.3)	10	16	2.8	3.4 (0.8, 13.8)	0.092	
Estrone (pg/ml)						
Quartile 1 (< 15)	18	64	1.0	1.0 (referent)		0.004
Quartile 2 (15 - < 21)	17	66	0.9	0.9 (0.4 - 1.9)	0.761	
Quartile 3 (21 - < 29)	30	59	1.8	1.9 (0.9 - 3.9)	0.096	
Quartile 4 (≥ 29)	32	54	2.3	2.5 (1.2 - 5.3)	0.018	

a age adjusted b adjusted for age and body weight

Table 3 Continued: Relative Hazard (RH) of Breast Cancer by Level of Sex Steroid Hormone Levels.

	No. of Subjects Cases	Random Sample	RH[a]	RH (95% CI)[b]	p	p trend
Estrone Sulfate						
Quartile 1 (< 113)	17	67	1.0	1.0 (referrent)		0.015
Quartile 2 (113- < 181)	22	64	1.4	1.4 (0.7, 3.9)	0.386	
Quartile 3 (181- < 288)	26	59	1.5	1.5 (0.7, 3.2)	0.253	
Quartil 4 (≥ 288)	32	53	2.5	2.5 (1.2, 5.3)	0.014	
Androstenedione (ng/dl)						
Quartile 1 (< 25)	16	63	1.0	1.0 (referent)		0.010
Quartile 2 (25 - < 39)	23	68	1.3	1.3 (0.6 - 2.7)	0.561	
Quartile 3 (39 - < 56)	24	61	1.3	1.2 (0.6 - 2.6)	0.590	
Quartile 4 (≥ 56)	34	52	2.6	2.6 (1.3 - 5.4)	0.010	
DHEAS (ng/dl)						
Quartile 1(< 41)	18	64	1.0	1.0 (referent)		0.039
Quartile 2(41 - < 65)	24	61	1.3	1.3 (0.6 - 2.7)	0.480	
Quartile 3 (65 - < 105)	25	64	1.6	1.6 (0.8 - 3.2)	0.233	
Quartile 4 (≥ 105)	30	55	2.1	2.1 (1.0 - 4.3)	0.045	
Total Testosterone (ng/dl)						
Quartile 1 (< 13)	14	64	1.0	1.0 (referent)		0.027
Quartile 2 (13 - < 19)	26	62	1.8	1.7 (0.8 - 3.7)	0.172	
Quartile 3 (19 - < 29)	26	60	2.5	2.5 (1.1 - 5.3)	0.022	
Quartile 4 (≥ 29)	31	58	2.3	2.3 (1.1 - 4.8)	0.030	

a-age adjusted b adjusted for age and body weight

Table 3 Continued: Relative Hazard (RH) of Breast Cancer by Level of Sex Steroid Hormone Levels.

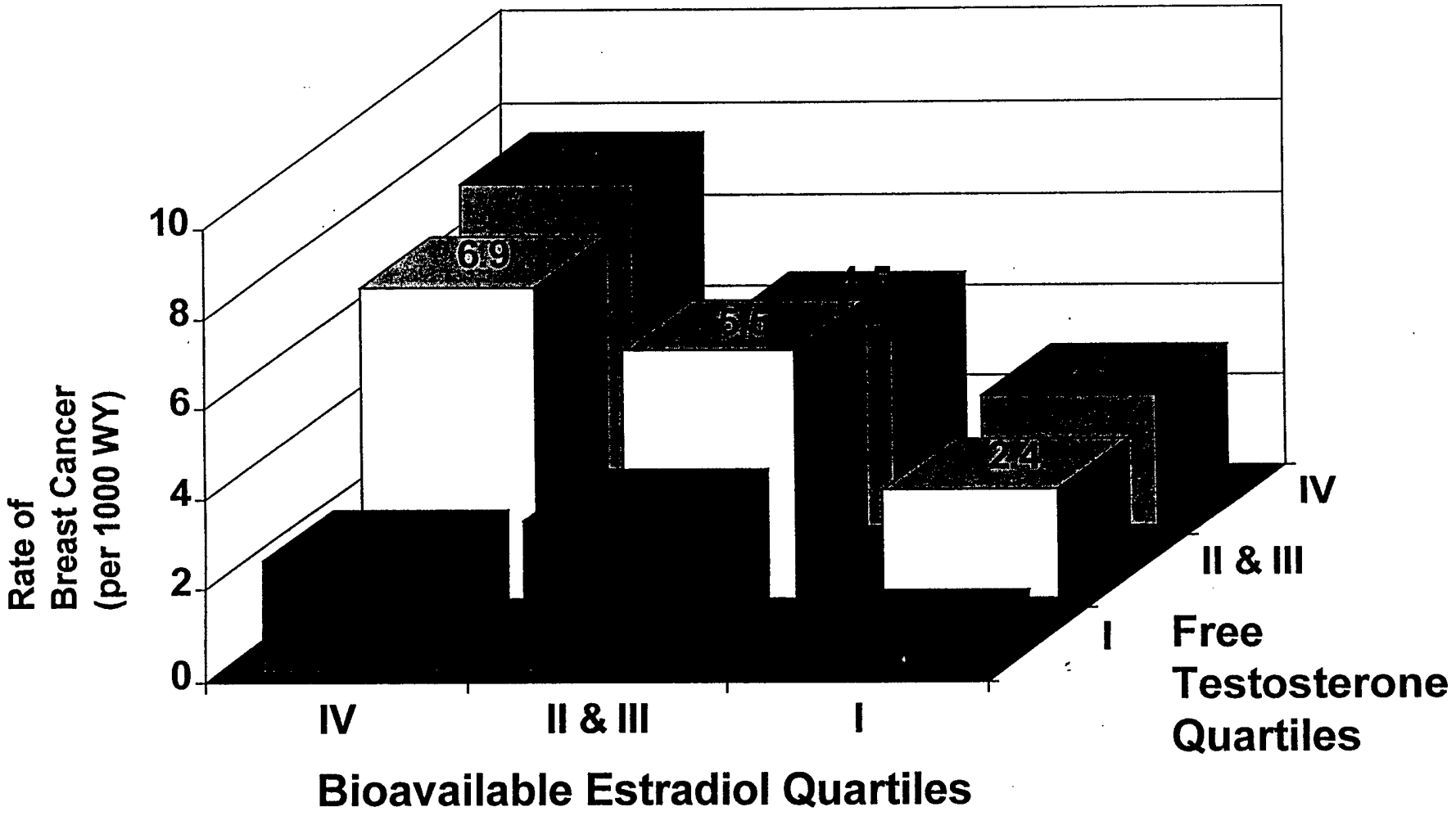
	No. of Subjects Cases	Random Sample	RH[a]	RH (95% CI)[b]	p	p trend
Free Testosterone (ng/dl)						
Quartile 1 (< 1.7)	11	66	1.0	1.0 (referrent)		0.007
Quartile 2 (1.7 - < 2.6)	26	63	2.5	2.5 (1.1, 5.6)	0.027	
Quartile 3 (2.6 - < 3.9)	29	56	3.5	3.5 (1.6, 7.9)	0.003	
Quartil 4 (≥ 3.9)	31	58	3.2	3.2 (1.4, 7.1)	0.005	
SHBG (nmol/L)						
Quartile 1 (< 29)	22	58	1.0	1.0 (referent)		0.275
Quartile 2 (29 - < 41)	33	52	1.5	1.5 (0.7 - 3.0)	0.271	
Quartile 3 (41 - < 57)	22	66	0.9	0.9 (0.4 - 1.8)	0.674	
Quartile 4 (≥ 57)	20	68	0.8	0.8 (0.3 - 1.7)	0.473	
Estrone Sulfate/Estrone						
Quartile 1 (< 6.38)	18	62	1.0	1.0 (referrent)		0.50
Quatile 2 (6.38 - < 8.95)	28	52	1.76	1.78 (0.9 - 3.6)	0.11	
Quartile 3 (8.95 - 42.68)	26	54	1.66	1.69 (0.8 - 3.4)	0.15	
Quartile 4 (≥ 42.68)	23	57	1.36	1.37 (0.7 - 2.8)	0.40	

a age adjusted
b adjusted for age and body weight

Figure Legend: Incidence rate of breast cancer per 1000 woman years by level of bioavailable estradiol and free testosterone.

Level 1=lowest quartile of hormone;
Level 2 = middle 2 quartiles of hormone;
Level 3= highest quartile of hormone.

* $p < 0.05$
** $p < 0.001$


Rate of
Breast Cancer
(per 1000 WY)
10
8
6
4
2
0
6.9
5.5
2.4
IV
II & III
I
Free
Testosterone
Quartiles
IV
II & III
I
Bioavailable Estradiol Quartiles

Alcohol Consumption, Estrogen Replacement Therapy, Endogenous Sex Steroid Hormones and Risk of Breast Cancer in Elderly Women

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ABSTRACT

Context.-- Most previous studies show an increase in breast cancer risk with increasing alcohol consumption. Previous work suggests a modification of the alcohol effect by use of estrogen replacement therapy, but the role of endogenous hormone levels in the alcohol/breast cancer relation is unknown.

Objective.-- To assess the interrelations of alcohol, estrogen replacement therapy, endogenous sex steroid hormone levels (estradiol, estrone, estrone sulfate, and testosterone), and breast cancer.

Design.-- Population-based cohort and nested case-cohort study.

Setting.-- Clinical centers located in: Baltimore, MD; Minneapolis, MN; Portland, OR; and the Monongahela Valley in PA.

Participants.-- One hundred twenty one incident breast cancer cases and 7,894 women free of breast cancer, ages 65 and older, enrolled in the Study of Osteoporotic Fractures.

Main Outcome Measure.-- Relative risk of breast cancer.

Results.-- Women who drank 15 grams or more of alcohol per day had an estimated 3-fold increase (relative risk 3.10, 95 percent confidence interval 1.55-6.21) in the risk of breast cancer compared to non-drinkers. Compared to lighter drinkers, women who consumed 3 or more drinks in a day on more than 3 occasions in a month had a 5-fold increase (relative risk 5.51, 95 percent confidence interval 2.16-14.1) in risk. The relation between alcohol and risk of breast cancer was not modified by either use of estrogen replacement therapy or endogenous hormone levels.

Conclusion.-- Alcohol consumption was associated with the risk of breast cancer in older

women. However, there was no evidence of a modification of the effect of alcohol on breast cancer risk by exogenous or endogenous hormones.

INTRODUCTION

Breast cancer is an important public health problem. In the United States, about 180,000 women develop breast cancer each year[1] and more than 40,000 die of the disease annually[2]. In addition, both the diagnosis and treatment of breast cancer have a major impact on quality of life[3,4].

Prior research has identified alcohol consumption as a potential risk factor for breast cancer. While results of previous studies have been somewhat mixed, cohort studies have generally found an increase in breast cancer risk associated with increasing consumption of alcoholic beverages[5-11]. A few prospective studies have found no effect of alcohol[12-14], while the Framingham study reported a significant negative relation between alcohol consumption and breast cancer risk[15]. A recent pooled analysis found a significant linear relation between alcohol intake and breast cancer risk[16]. Data regarding this relationship in the elderly, however, are limited.

One possible mechanism for a breast cancer-alcohol relation is the influence of alcohol on serum hormones, specifically estrogens. We have previously shown that breast cancer risk is associated with serum sex steroid hormone levels, including estradiol, estrone, estrone sulfate, and testosterone[17]. Several studies have reported positive correlations between alcohol consumption and serum hormones levels, including estradiol[18] and estrone sulfate[19], as well as urinary estrogens[20] in postmenopausal women. However, other observational studies in postmenopausal women have not demonstrated an association between serum[21,22] and urinary[23] estrogens and alcohol intake. A recent placebo controlled crossover trial in postmenopausal women reported a significant and sustained increase in estradiol levels after acute alcohol

ingestion among women taking estrogen replacement therapy (ERT), but not in other women[24].

Some[9,25], but not all[16,26], previous studies have suggested that alcohol use and exogenous estrogen use may interact with respect to breast cancer risk, but to our knowledge no previous studies have examined the interrelation among alcohol consumption, endogenous hormone levels, and risk of breast cancer.

Because alcohol consumption is a potentially modifiable behavior, clarifying the breast cancer risk associated with its use may have significant clinical implications. For example, if moderate intake is associated with an increased risk of breast cancer, this risk must be weighed against the potential benefits of alcohol consumption, including a reduced risk of cardiovascular disease[27]. Further, if there is an interaction between alcohol consumption and ERT, there may be implications for treatment recommendations.

We examined the association between current and lifetime alcohol consumption, measured at baseline, and subsequent development of breast cancer in a large population of elderly women, the Study of Osteoporotic Fractures (SOF) cohort, with special attention to modification of this relation by either use of ERT or levels of endogenous estrogen.

MATERIALS AND METHODS

Subjects

A total of 9,704 women aged 65 years and older were recruited between 1986 and 1988 from a center located in one of the following four areas: Baltimore, MD, Minneapolis, MN, the Monongahela Valley in Pennsylvania, and Portland, OR. The SOF excluded black women because of their low risk of hip fracture, women unable to walk without the assistance of another

person, and women with bilateral hip replacements[28]. The study was approved by the appropriate institutional review boards and written informed consent was obtained from all participants.

Identification of Cases and Controls

Ascertainment of breast cancer in the SOF has been previously described[29,30]. Briefly, one year after the baseline examination, SOF participants were asked to complete a questionnaire that included information about personal and family history of breast cancer. Breast cancer status was again ascertained at a subsequent follow-up, approximately 3.2 years after the year 1 interview.

This study of incident breast cancer includes only those women in the SOF cohort who provided information on breast cancer status at both year 1 and at follow-up. Of the 9,704 women enrolled in the SOF, we excluded 100 women who died before competing the year 1 interview, 160 women on whom we could not ascertain breast cancer status, and 506 women who reported a history of breast cancer at year 1 and thus were prevalent cases. In addition we excluded 265 women who died between year 1 and the end of follow-up, 618 women on whom no follow-up was available, and 40 women for whom we could not confirm a diagnosis of breast cancer. Thus, of the 9,704 women enrolled in the SOF, 8,015 (83%) were included in this analysis.

Among these 8,015 women, we confirmed 121 breast cancer cases by review of the medical record pathology report or cancer registry record. The 7,894 women who denied breast cancer at both year 1 and at follow-up served as controls.

A case-cohort sampling approach[31] was used to select serum samples for hormone assays[17]. Assays were performed for all confirmed cases with available serum samples (N = 120)

and for a random sample of women who were free of breast cancer at year 1. For purposes of this analysis, the 254 women with complete breast cancer follow-up information served as controls.

Alcohol Exposure

We collected detailed information by interview at the baseline visit regarding current and past alcohol use. Current use was defined as the average number of drinks per week adjusted for atypical drinking, especially heavy drinking over the past 30 days. For ease of comparison with other studies, drinks per week was converted to grams per day, assuming an average of 11.5 grams per drink[32]. We also collected information regarding heavy drinking (the number of times during the past month 3 or more drinks were consumed in a single day), any history of problem drinking (by self-report), the age at which drinking started, and total lifetime exposure in drinks and in years. Average number of grams per day consumed was categorized to conform with 5 categories (none, < 1.5, 1.5-<5, 5-<15, and 15 or more grams/day) typically used in other studies. Current drinking categories were also collapsed (none, <5, and 5 or more grams/day) for comparison of baseline characteristics by drinking status and for use in interaction terms. Other continuous alcohol intake variables were categorized to compare approximate quartiles with non-use.

Exogenous Estrogen

Participants were asked about current and past use of estrogen replacement therapy since age 40 years[33]. Reports of current medications were checked against the labels of drugs brought to the clinic visit. Women were categorized as never, past, or current users of ERT, as of the date of the baseline visit.

Endogenous Sex Steroid Hormones

As part of a case-cohort analysis[17], endogenous sex steroid hormones were measured on 120 cases and 254 controls. Serum was obtained from all participants at the baseline exam. All participants were instructed to adhere to a fat free diet overnight and on the morning of the examination to minimize lipemia that would interfere with assays. Blood was drawn between 8:00 am and 2:00 pm and serum was frozen at -20°C. Within two weeks all samples were shipped to a central repository where they were stored at -190°C until assay. We measured estradiol, estrone, estrone sulfate, and testosterone. All assays were done blinded to breast cancer status by Corning Nichols Institute (San Juan Capistrano, CA).

Total estradiol was measured using liquid-liquid organic extraction, column chromatography and radioimmunoassay (RIA) (intra- and inter-assay variability 5-9% and 6-12%, respectively; sensitivity of 2 pg/ml). Estrone was measured using extraction, chromatography and RIA (intra- and inter-assay variability 6-7% and < 8%, respectively; sensitivity of 10 pg/ml). Estrone sulfate was measured using organic extraction, enzymatic hydrolysis, celite chromatography and RIA (intra- and inter-assay variability 6-7% and 8-10%, respectively; sensitivity of 50 pg/ml). Total testosterone was measured using RIA with chromatographic purification. Hormone levels were categorized by tertiles.

Other variables

At baseline, weight (in light clothes with shoes removed) was recorded with a balance beam scale[34]. We calculated body mass index, defined as weight in kilograms divided by height squared in meters, using self-reported height at age 25, since elderly women may experience height loss secondary to vertebral fractures. Physical activity was defined as walking specifically

for exercise. Smoking was categorized as never, past, or current. A reproductive history was obtained by questionnaire and interview. Parity was categorized as nulliparous or age at first birth before age 20, between 20 and 34, and 35 or later. Family history of breast cancer was determined by self-report at year 1, with women reporting breast cancer in either mother or a sister considered to have a positive family history.

Statistical analysis

We compared risk factors for breast cancer among non-drinkers, light drinkers, and heavier drinkers using ttests for continuous variables and chi-square tests for categorical variables. Proportional hazards regression models were used to estimate the relative risk (RR) of breast cancer as a function of alcohol intake[35]. Covariates in multivariable models were selected based on their probable relationship to breast cancer and/or alcohol intake as reported in the literature in general, and in this cohort in particular. All models controlled for age, education, BMI, age at menarche, age at menopause, parity/age at first birth, family history, ERT, smoking, physical activity, and study center. Interaction terms involving current drinking (3 categories) with ERT (never, past, and current) use and with endogenous estrogen levels (tertiles) were added to the initial multivariable regression models to formally assess modification of risk associated with current drinking behavior by ERT use and endogenous estrogen levels, using likelihood ratio tests[36].

RESULTS

The mean age at entry into the study was 71.3 years (standard deviation 5.1 years).

Women who reported alcohol consumption were significantly younger, better educated, leaner, and more active than abstainers, table 1. Drinkers were also more likely to smoke and use estrogen replacement therapy. Drinkers and abstainers were similar with respect to age at menopause, but drinkers had slightly younger ages at menarche and were more likely to be nulliparous. Drinkers were more likely to have a history of breast cancer in a mother or sister. Drinkers were more likely to have highest tertile estradiol, estrone, estrone sulfate, and testosterone levels, although only the estrone differences were statistically significant.

Alcohol and Breast Cancer

Controls were more likely than cases to be current abstainers (44 percent versus 39 percent, respectively, table 2), and cases were more likely to drink 15 or more grams per day ($p < 0.01$). While 6 percent of cases reported more than three occasions on which they drank three or more drinks during the past month, only 2 percent of controls reported similar drinking behavior, although this difference was not statistically significant. There was little evidence of case-control differences with respect to age at onset of drinking. Lifetime exposure, both in terms of total number of years drinking and total lifetime number of drinks, was somewhat higher in cases than controls, but these differences were not statistically significant.

Compared with non-drinkers, women who reported drinking an average 15 or more grams of alcohol (a little more than one drink) per day had a nearly three-fold increased risk of breast cancer compared to abstainers, table 3. Multivariate adjustment for age, education, age at menarche, age at menopause, parity, smoking, use of ERT, exercise, family history, and clinic in proportional hazards regression models did not substantially change this risk (RR 3.10, 95% CI 1.55-6.21, p trend across categories = 0.04). Limiting the analysis to current drinkers, heavy

drinkers, those reporting consumption of three or more drinks per day during four or more of the last 30 days, had a five-fold increase in the risk of breast cancer compared to women who drank less (95% CI 2.16-14.1, p trend 0.004). Women in the top quartile of lifetime consumption (8,191 lifetime drinks or more) had an estimated 87 percent increased risk of breast cancer compared with abstainers (95% CI 1.01-3.46, p trend 0.07). None of the control variables was significantly associated with breast cancer risk.

Alcohol, Hormones, and Breast Cancer

The risk of breast cancer associated with alcohol consumption was not influenced by either use of exogenous estrogen or endogenous hormone levels. Self-reported use of ERT did not appear to modify the alcohol-breast cancer relationship (p interaction 0.85; table 4). Similarly, there was no indication of a multiplicative interaction with respect to serum levels of estradiol, estrone, estrone sulfate, or testosterone and use of alcohol.

DISCUSSION

In this cohort study of elderly white women, we found that women who drank an average of slightly more than one alcoholic drink per day or more had a three-fold increase in the risk of breast cancer compared with non-drinkers. Heavier drinkers, those who had consumed three or more drinks in a day on more than 3 occasions during the past month, were at five times the breast cancer risk compared with lighter drinkers. These results are in accord with most previous cohort studies, which have demonstrated that even moderate alcohol intake is associated with increased risk of breast cancer[5-9] and that higher levels are associated with greater increases in risk of disease[8,10,16]. In contrast, one prospective study[15] found a significant decrease in breast

cancer risk with increasing alcohol consumption and a few have shown no association in either direction[12-14].

Is there a safe level of alcohol consumption at which the risk of breast cancer is not increased? Although our study found that women who consumed less than one drink per day, on average, did not develop breast cancer at a higher rate than non-drinkers, we were unable to rule out such an effect; the confidence intervals in our study included the effect sizes reported in other studies[6,7,9] and our significant trend across increasing categories was also demonstrated by a recent pooled analysis[16].

Alcohol consumption may increase breast cancer risk through its effect on serum estrogen levels. Alcohol use increased serum estrogens in postmenopausal women in some[18,19], but not all[22], studies. A placebo-controlled crossover study of the acute effects of moderate alcohol consumption suggested that the increase in serum estrogen levels with alcohol were confined to women who took ERT[24]. This finding is consistent with results from the Nurses' Health Study[25] that demonstrated an increased risk of breast cancer associated with ERT use only among current consumers of alcohol, and with those from the Iowa Women's Health Study[9]. Nevertheless, our data do not support such a relationship, consistent with the findings from the Canadian Breast Screening Study[26] and a recent pooled analysis[16]. To our knowledge, our study is the first to have examined interactions between alcohol and endogenous hormone levels. We were unable to demonstrate interactions between endogenous hormone levels and alcohol with respect to risk of breast cancer. Inconsistencies across studies may be due to differing cohort composition with respect to age, prevalence and patterns of alcohol intake, or prevalence and patterns of ERT use. Alternatively, Singletary and Meadows[37] hypothesized that interactions of alcohol with

other breast cancer risk factors such as hormones may differ according to tumor estrogen-progesterone receptor status. Alternatively, alcohol may increase the risk of breast cancer by mechanisms not involving estrogens. In animal models, ethanol results in the induction of carcinogen-activating enzymes in the liver[38,39]. Acetaldehyde, the first metabolite of ethanol, may inhibit DNA repair in animal models and in human cell culture[39].

Several limitations of this study must be considered. Alcohol consumption was assessed by self-report. Self-report is known to be fairly reliable for abstainers and light to moderate drinkers[40], but tends to underestimate intake for heavier drinkers[41]. However, because our measurement of drinking behavior occurred prior to diagnosis of breast cancer, we would expect such misclassification to be non-differential with respect to disease status, resulting in an underestimate of any effect. Because only a few women reported heavy alcohol consumption, we were unable to assess the effect of very heavy consumption. However, our assessment of the frequency of consumption of three or more drinks at one occasion may serve as an indication of the effect of heavier consumption on risk. Because drinkers were more likely to have had recent mammograms than non-drinkers, the risk associated with alcohol could represent an ascertainment bias. However, when we limited our analysis to women who had received a mammogram, our findings were unchanged. Finally, this study of elderly Caucasian women may not be generalizable to younger women or women from other racial groups.

The results of our study suggest that elderly women with moderate alcohol consumption have a three-fold increase in risk of breast cancer compared to non-drinkers. This association may have important clinical implications. Although moderate alcohol consumption has positive health effects, e.g., a reduction in risk of cardiovascular disease, these effects must be balanced

against a possible increase in breast cancer risk. Guidelines suggesting that moderate intake of alcohol is beneficial may need to be modified based on a woman's risk factor profile for breast cancer.

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REFERENCES

1. *Statistical Abstract of the United States 1997.* Washington, DC: U.S. Department of Commerce; 1997.

2. *Vital Statistics of the United States.* Hyattsville, MD: U.S. Department of Health and Human Services; 1996.

3. Trief PM, Donohue-Smith M. Counseling needs of women with breast cancer: what the women tell us. *J Psychosoc Nurs Ment Health Serv.* 1996;34:24-9.

4. Pelusi J. The lived experience of surviving breast cancer. *Oncol Nurs Forum.* 1997;24:1343-53.

5. Hiatt RA, Bawol RD. Alcoholic beverage consumption and breast cancer incidence. *Am J Epidemiol.* 1984;120:676-83.

6. Schatzkin A, Jones DY, Hoover RN, et al. Alcohol consumption and breast cancer in the epidemiologic follow-up study of the first National Health and Nutrition Examination Survey. *N Engl J Med.* 1987;316:1169-73.

7. Willett WC, Stampfer MJ, Colditz GA, Rosner BA, Hennekens CH, Speizer FE. Moderate alcohol consumption and the risk of breast cancer. *N Engl J Med.* 1987;316: 1174-80.

8. Hiatt RA, Klatsky AL, Armstrong MA. Alcohol consumption and the risk of breast cancer in a prepaid health plan. *Cancer Res.* 1988;48:2284-7.

9. Gapstur SM, Potter JD, Sellers TA, Folsom AR. Increased risk of breast cancer with alcohol consumption in postmenopausal women. *Am J Epidemiol.* 1992;136:1221-31.

10. Holmberg L, Ohlander EM, Byers T, et al. Diet and breast cancer risk. Results from a

population-based, case-control study in Sweden. *Arch Intern Med.* 1994;154:1805-11.

11. van den Brandt A, Goldbohm RA, van 't Veer P. Alcohol and breast cancer: results from the Netherlands Cohort Study. *Am J Epidemiol.* 1995;141:907-15.

12. Simon MS, Carman W, Wolfe R, Schottenfeld D. Alcohol consumption and the risk of breast cancer: a report from the Tecumseh Community Health Study. *J Clin Epidemiol.* 1991;44:755-61.

13. Graham S, Zielezny M, Marshall J, et al. Diet in the epidemiology of postmenopausal breast cancer in the New York State Cohort. *Am J Epidemiol.* 1992;136:1327-37.

14. Friedenreich CM, Howe GR, Miller AB, Jain MG. A cohort study of alcohol consumption and risk of breast cancer. *Am J Epidemiol.* 1993;137:512-20.

15. Schatzkin A, Carter CL, Green SB, et al. Is alcohol consumption related to breast cancer? Results from the Framingham Heart Study. *J Natl Cancer Inst.* 1989;81:31-35.

16. Smith-Warner SA, Spiegelman D, Shiaw-Shyuan Y, et al. Alcohol and breast cancer in women. A pooled analysis of cohort studies. *JAMA.* 1998;279:535-40.

17. Cauley JA, Lucas FL, Kuller LH, Stone K, Browner W, Cummings SR. Endogenous sex steroid hormone concentrations and bone mass predict breast cancer in older women. Submitted.

18. Gavaler JS, Van Thiel DH. The association between moderate alcoholic beverage consumption and serum estradiol and testosterone levels in normal postmenopausal women: relationship to the literature. *Alcohol Clin Exp Res.* 1992;16:87-92.

19. Hankinson SE, Willett WC, Manson JE, et al. Alcohol, height, and adiposity in relation to estrogen and prolactin levels in postmenopausal women. *J Natl Cancer Inst.* 1995;87:1297-1302.

20. Katsouyanni K, Boyle P, Trichopoulos D. Diet and urine estrogens among postmenopausal women. *Oncology.* 1991;48:490-4.

21. Cauley JA, Gutai JP, Kuller LH, LeDonne D, Powell JG. The epidemiology of serum sex hormones in postmenopausal women. *Am J Epidemiol.* 1989;129:1120-31.

22. Newcomb PA, Klein R, Klein BE, et al. Association of dietary and life-style factors with sex hormones in postmenopausal women. *Epidemiology.* 1995;6:318-21.

23. Trichopoulos D, Brown J, MacMahon B. Urine estrogens and breast cancer risk factors among post-menopausal women. *Int J Cancer.* 1987;40:721-5.

24. Ginsburg ES, Mello NK, Mendelson JH, et al. Effects of alcohol ingestion on estrogens in postmenopausal women. *JAMA.* 1996;276:1747-51.

25. Colditz GA, Stampfer MJ, Willett WC, Hennekens CH, Rosner B, Speizer FE. Prospective study of estrogen replacement therapy and risk of breast cancer in postmenopausal women. *JAMA.* 1990;264:2648-53.

26. Friedenreich CM. Re: "Increased risk of breast cancer with alcohol consumption in postmenopausal women." *Am J Epidemiol.* 1994;139:541-2.

27. Rich-Edwards JW, Manson JE, Hennekens CH, Buring JE. The primary prevention of coronary heart disease in women. *New Engl J Med.* 1995;332:1758-66.

28. Cummings SR, Black DM, Nevitt MC, et al. Appendicular bone density and age predict hip fracture in women. *JAMA.* 1990;263:665-8.

29. Cauley JA, Lucas FL, Kuller LH, Vogt MT, Browner WS, Cummings SR. Bone mineral density and risk of breast cancer in older women. *JAMA.* 1996;276:1404-8.

30. Lucas FL, Cauley JA, Stone RA, et al. Bone mineral density and risk of breast cancer:

differences by family history of breast cancer. *Am J Epidemiol.* In press.

31. Prentice RL. A case-cohort design for epidemiologic cohort studies and disease prevention trials. *Biometrika.* 1986;73:1-11.

32. Roth DH, Levy PS, Shi L, Post, E. Alcoholic beverages and breast cancer: some observations on published case-control studies. *J Clin Epidemiol.* 1994;47:207-16.

33. Cauley JA, Seeley DG, Ensrud K, Ettinger B, Black D, Cummings SR. Estrogen replacement therapy and fractures in older women. *Ann Intern Med.* 1995;122:9-16.

34. Lohman TG, Martorell R. *Anthropometric Standardization Reference Manual.* Champaign, IL: Human Kinetics Books, 1988.

35. Cox DR. Regression models and life tables (with discussion). *J R Stat Soc B.* 1972;34:187-220.

36. Kelsey JL, Whittemore AS, Evans AS, Thompson WD. *Methods in Observational Epidemiology.* 2nd ed. New York, NY: Oxford University Press, 1996.

37. Singletary KW, Meadows GG. Alcohol and breast cancer: interactions between alcohol and other risk factors. *Alcohol Clin Exp Res.* 1996;20:57A-61A.

38. Lieber CS, Seitz HK, Garro AJ, Worner TM. Alcohol-related diseases and carcinogenesis. *Cancer Res.* 1979;39:2863-86.

39. Garro AJ, Lieber CS. Alcohol and cancer. *Ann Rev Pharmacol Toxicol.* 1990;30: 219-49.

40. Giovannucci E, Colditz G, Stampfer MJ, et al. The assessment of alcohol consumption by a simple self-administered questionnaire. *Am J Epidemiol.* 1991;133:810-17.

41. Poikolainen K. Underestimation of recalled alcohol intake in relation to actual consumption.

Br J Addiction. 1985;80:215-6.

Table 1. Baseline Characteristics by Current Alcohol Consumption

Characteristic	None (N=3548)	< 5 gm/day (N=3017)	5+ gm/day (N=1450)
Age (mean,SD)	71.9 (5.4)	70.9 (4.9)	70.8 (4.6)**
Education (years)	12.1 (2.8)	12.8 (2.7)	13.3 (2.7)**
Modified BMI	26.0 (4.9)	25.4 (4.2)	24.4 (4.2)**
Age at menarche	13.1 (1.5)	13.0 (1.5)	12.9 (1.4)*
Age at menopause	46.9 (6.5)	47.1 (6.2)	47.2 (6.3)
Age at first birth (years) (N,%):			
Nulliparous	575 (16.7%)	524 (18.0%)	289 (20.9%)
<20	343 (10.0%)	213 (7.3%)	74 (5.4%)
20-34	2367 (68.9%)	2066 (70.9%)	953 (69.0%)
35+	152 (4.4%)	112 (3.8%)	66 (4.8%)**
Walks for exercise:			
No	1824 (51.4%)	1370 (45.4%)	683 (47.1%)
Yes	1723 (48.6%)	1647 (54.6%)	767 (52.9%)**
Smoking:			
Never	2541 (71.7%)	1758 (58.6%)	554 (38.3%)
Past	759 (21.4%)	963 (32.1%)	658 (45.4%)
Current	244 (6.9%)	277 (9.2%)	236 (16.3%)**
Family history of breast cancer:			
No	2816 (86.5%)	2317 (85.7%)	1067 (82.7%)
Yes	439 (13.5%)	388 (14.3%)	223 (17.3%)**
Use of ERT:			
Never	2256 (63.6%)	1639 (54.3%)	742 (51.2%)
Past	862 (24.3%)	907 (30.1%)	448 (30.9%)
Current	430 (12.1%)	471 (15.6%)	260 (17.9%)**
Estradiol (pg/ml):			
<=5	52 (32.9%)	49 (33.8%)	20 (28.2%)
>5-<9	45 (28.5%)	47 (32.4%)	16 (22.5%)
9+	61 (38.6%)	49 (33.8%)	35 (49.3%)
Estrone (pg/ml):			
<17	50 (31.7%)	40 (27.6%)	17 (23.9%)
17-<28	53 (33.5%)	64 (44.1%)	16 (22.5%)
28+	55 (34.8%)	41 (28.3%)	38 (53.5%)**
Estrone Sulfate (pg/ml):			
<139	52 (32.9%)	51 (35.2%)	18 (25.4%)
139-<281	53 (33.5%)	51 (35.2%)	18 (25.4%)
281+	53 (33.5%)	43 (29.7%)	35 (49.3%)

Testosterone (ng/dl):			
<14	49 (31.0%)	40 (27.6%)	18 (25.4%)
14-<24	55 (34.8%)	57 (39.3%)	22 (31.0%)
24+	54 (34.2%)	48 (33.1%)	31 (43.7%)

**p<0.01
*p<0.05

Last revision 4/8/98

Table 2. Alcohol Consumption in Breast Cancer Cases and Controls

Alcohol Use	Cases (N = 121)	Controls (N = 7,894)
Current use (gm/day):		
None	47 (38.8%)	3501 (44.4%)
<1.5	26 (21.5%)	1966 (24.9%)
1.5-<5	20 (16.5%)	1005 (12.7%)
5-<15	13 (10.7%)	999 (12.7%)
15+	15 (12.4%)	423 (5.4%)*
Ever drinking problem:		
No	100 (98.0%)	6355 (98.3%)
Yes	2 (2.0%)	109 (1.7%)
# times 3+ drinks/day past 30 days:		
None	74 (82.2%)	4897 (87.4%)
1-3	11 (12.2%)	599 (10.7%)
4+	5 (5.6%)	110 (2.0%)
Age started drinking (years):		
<20	29 (28.4%)	1827 (28.3%)
20-21	25 (24.5%)	1569 (24.3%)
22-26	22 (21.6%)	1488 (23.1%)
27+	26 (25.5%)	1565 (24.3%)
Total # years drinking:		
None	20 (16.5%)	1449 (18.4%)
<42	21 (17.4%)	1251 (15.9%)
42-47	27 (22.3%)	1808 (22.9%)
48-52	26 (21.5%)	1866 (23.6%)
53+	27 (22.3%)	1520 (19.3%)
Total # drinks lifetime:		
None	29 (24.0%)	2288 (29.0%)
1-813	19 (15.7%)	1387 (17.6%)
814-2730	20 (16.5%)	1441 (18.3%)
2731-8190	25 (20.7%)	1417 (18.0%)
8191+	28 (23.1%)	1361 (17.2%)

*p<0.01 Last revision 4/8/98

Table 3. Crude and Adjusted* Relative Risk Estimates from Proportional Hazards Regression Models

Alcohol Use	Crude RR (95% CI)	Adjusted RR (95% CI)	p trend
Current intake (gm/day):			
None	1.00 Referent	1.00 Referent	
<1.5	0.95 (0.59-1.53)	0.89 (0.51-1.54)	
1.5-<5	1.41 (0.84-2.38)	1.13 (0.59-2.14)	
5-<15	0.97 (0.52-1.79)	1.04 (0.52-2.08)	
15+	2.66 (1.49-4.76)	3.10 (1.55-6.21)	0.04
3 or more drinks/day, past 30 days (current drinkers only):			
None	1.00 Referent	1.00 Referent	
1-3 times	1.21 (0.64-2.28)	1.35 (0.63-2.86)	
4 or more times	3.30 (1.33-8.16)	5.51 (2.16-14.1)	0.004
Lifetime consumption (# drinks):			
None	1.00 Referent	1.00 Referent	
1-813	1.05 (0.59-1.87)	0.99 (0.52-1.89)	
814-2730	1.06 (0.60-1.87)	0.94 (0.49-1.82)	
2731-8190	1.37 (0.80-2.34)	1.16 (0.61-2.20)	
8191+	1.62 (0.97-2.73)	1.87 (1.01-3.46)	0.07

*Adjusted for age, education, BMI, age at menarche, age at menopause, parity/age at first birth, family history, ERT, current smoking, physical activity, and study center

Last revision 4/8/98

Table 4. Estimated Relative Risk* of Breast Cancer Associated with Alcohol Intake By Estrogen Replacement Therapy Use and Serum Hormone Levels

Hormone	Alcohol Intake			p interaction
	None	<5 gm/day	5+ gm/day	
Use of ERT**:				
Never	1.00 Referent	1.15 (0.60-2.21)	1.97 (0.93-4.13)	
Past	1.51 (0.73-3.12)	1.05 (0.47-2.37)	1.74 (0.67-4.49)	
Current	1.82 (0.75-4.41)	1.71 (0.70-4.19)	2.42 (0.87-6.75)	0.85
Estradiol (pg/ml):				
<=5	1.00 Referent	0.70 (0.26-1.88)	0.74 (0.19-2.80)	
>5-<9	0.82 (0.33-2.02)	0.65 (0.25-1.66)	2.20 (0.76-6.38)	
9+	1.86 (0.82-4.21)	1.31 (0.55-3.09)	2.74 (1.06-7.08)	0.65
Estrone (pg/ml):				
<17	1.00 Referent	0.87 (0.30-2.54)	1.52 (0.39-5.91)	
17-<28	2.16 (0.94-4.98)	1.10 (0.46-2.64)	1.46 (0.42-5.11)	
28+	1.95 (0.77-4.95)	1.79 (0.71-4.54)	4.10 (1.57-10.7)	0.50
Estrone Sulfate (pg/ml):				
<139	1.00 Referent	0.65 (0.26-1.65)	0.79 (0.21-3.01)	
139-<281	1.09 (0.50-2.39)	0.86 (0.37-2.00)	1.80 (0.60-5.43)	
281+	1.28 (0.56-2.96)	1.00 (0.43-2.32)	2.48 (1.02-6.06)	0.82
Testosterone (ng/dl):				
<14	1.00 Referent	1.51 (0.49-4.61)	1.78 (0.42-7.58)	
14-<24	2.89 (1.09-7.71)	2.01 (0.72-5.61)	7.60 (2.50-23.1)	
24+	4.89 (1.88-12.7)	2.45 (0.93-6.48)	4.08 (1.34-12.5)	0.21

*Adjusted for age, education, BMI, age at menarche, age at menopause, parity/age at first birth, family history, ERT**, current smoking, physical activity, and study center
**ERT, estrogen replacement therapy

Last revision 4/8/98