

# Combined Estrogen and Testosterone Use and Risk of Breast Cancer in Postmenopausal Women

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**Background:** The role of androgens in breast cancer etiology has been unclear. Epidemiologic studies suggest that endogenous testosterone levels are positively associated with breast cancer risk in postmenopausal women. Given the increasing trend in the use of hormone therapies containing androgens, we evaluated the relation between the use of estrogen and testosterone therapies and breast cancer.

**Methods:** We conducted a prospective cohort study in the Nurses' Health Study from 1978 to 2002 to assess the risk of breast cancer associated with different types of postmenopausal hormone (PMH) formulations containing testosterone. During 24 years of follow-up (1 359 323 person-years), 4610 incident cases of invasive breast cancer were identified among postmenopausal women. Information on menopausal status, PMH use, and breast cancer diagnosis was updated every 2 years through questionnaires.

**Results:** Among women with a natural menopause, the risk of breast cancer was nearly 2.5-fold greater among current users of estrogen plus testosterone therapies (multivariate relative risk, 2.48; 95% confidence interval, 1.53-4.04) than among never users of PMHs. This analysis showed that risk of breast cancer associated with current use of estrogen and testosterone therapy was significantly greater compared with estrogen-only therapy (*P* for heterogeneity, .007) and marginally greater than estrogen and progesterone therapy (*P* for heterogeneity, .11). Women receiving PMHs with testosterone had a 17.2% (95% confidence interval, 6.7%-28.7%) increased risk of breast cancer per year of use.

**Conclusion:** Consistent with the elevation in risk for endogenous testosterone levels, women using estrogen and testosterone therapies have a significantly increased risk of invasive breast cancer.

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**T**HE ROLE OF ANDROGENS IN breast cancer etiology has been unclear. Epidemiologic studies suggest that endogenous androgen levels are positively associated with breast cancer risk. A combined reanalysis of data from 9 prospective studies investigating the association between endogenous hormone levels and risk of breast cancer reported that testosterone, androstenedione, dehydroepiandrosterone, and dehydroepiandrosterone sulfate were all associated with increased risk of breast cancer.<sup>1</sup> Additional analyses suggested that the observed association was independent of circulating estradiol.<sup>1,2</sup> Contrary to epidemiologic data, in vitro and in vivo studies have reported both proliferative<sup>3,4</sup> and antiproliferative<sup>5-7</sup> effects on cell growth.

Testosterone production takes place in the ovary, in the adrenal gland, and through peripheral conversion of androstenedione.

Circulating testosterone levels decline with increasing age but do not appear to be significantly affected by the menopausal transition.<sup>8</sup> As early as the 1940s, testosterone was reported not only to alleviate menopausal symptoms but also to restore libido.<sup>9</sup> In recent years, evidence has accumulated supporting the hypothesis that the decline in endogenous testosterone levels is associated with menopausal symptoms, including decreased libido, worse moods, and poorer quality of life.<sup>10</sup> Clinical trials have demonstrated that exogenous androgens in conjunction with estrogens can ameliorate symptoms affecting sexual function and general well-being.<sup>11,12</sup> In addition, studies have found beneficial effects of androgen therapy on bone mineral density.<sup>13-15</sup>

To date, the only orally active testosterone preparation in the United States approved by the Food and Drug Administration for the indication of menopausal symptoms in women is a fixed combination of methyltestosterone and esterified es-

tradiol.<sup>16</sup> According to the *Physicians' Desk Reference*, this combination is indicated for the treatment of "moderate to severe vasomotor symptoms associated with the menopause in those patients not improved by estrogens alone" and first became available in the United States in 1964.<sup>17,18</sup> The use of hormone therapies that include testosterone is expected to become more widespread with increased availability of hormone preparations developed specifically for women.<sup>19</sup>

It is well established that combined estrogen and progesterone (E&P) hormone therapy increases the risk of breast cancer.<sup>20,21</sup> However, there is little research evaluating the role of specific postmenopausal hormones (PMHs) containing androgens and risk of breast cancer. Given the increasing trends of use, we evaluated the relation between postmenopausal use of exogenous testosterone and risk of breast cancer using prospectively collected data.

## METHODS

### STUDY POPULATION

The Nurses' Health Study started in 1976, when 121 700 US registered nurses between the ages of 30 and 55 years returned an initial questionnaire. Every 2 years, information on reproductive variables, medical history, and PMH use was updated through mailed questionnaires. Follow-up for this cohort of women through 2002 has been greater than 90%. This study was approved by the Committee on Human Subjects at Brigham and Women's Hospital, Boston, Mass.

### ASCERTAINMENT OF HORMONE USE

In 1976, women were asked about current use and duration of PMH use. Beginning in 1978 and on all subsequent biennial questionnaires, women were also asked about the type of PMH they used during the preceding 2 years. In 1982, 1984, and 1986, estrogen and testosterone (E&T) was specifically listed in the possible answers to the question, "what type of hormone have you used for the longest during this period?" On questionnaires sent between 1988 and 1998, E&T therapy was not explicitly listed, although participants were given an open space to write in other types of hormones. Individual responses were coded to reflect distinct categories of hormones. In 1988, esterified estrogens and methyltestosterone (Estratest; Solvay Pharmaceuticals, Brussels, Belgium) was the most frequently (29.7%) reported type of hormone containing testosterone and was currently being used by 11 women. In 1998, Estratest accounted for 90.3% of hormones reported containing testosterone and was currently being used by 588 women. Women reporting current use of E&T therapy in 1998 were mostly past users of other types of hormones including testosterone only (54.6%), estrogen only (28.8%), E&P (19.1%), progesterone only (2.1%), and other types of hormone (7.5%). Only 2.4% of current E&T users in 1998 were never users prior to initiating E&T use.

In 2000, the biennial questionnaire included Estratest on the list of possible PMH types. Women reporting use of esterified estrogens or hormones administered through a patch were considered users of "other" PMHs. Because hormones administered locally through vaginal rings, creams, or intrauterine devices do not increase circulating hormone levels,<sup>22</sup> we did not include women reporting these types of hormones in the analysis.

We categorized PMH use as never use; past PMH use; current use of estrogen alone, E&P, E&T, progesterone alone, or testosterone alone; and other current PMH use. However, be-

cause the primary exposure of interest in this analysis was current use of testosterone containing PMH, we present the results for estrogen only, testosterone only, and E&T therapies only. Women were considered current users of therapy if they reported current use of PMH at the beginning of a 2-year follow-up cycle.

Duration of PMH use was the summation of PMH use across questionnaire cycles. The initial questionnaire asked participants to report how long they had previously used hormones. From 1978 on, respondents were asked about the number of months they used hormones since the previous 2-year cycle. In analyses examining duration of use, use of testosterone-only therapies was grouped with E&T therapies because the main effects were not significantly different from one another.

### ASCERTAINMENT OF OUTCOME

Incident invasive breast cancer cases in this analysis were identified through self-report on biennial questionnaires up to June 1, 2002. Breast cancer diagnoses were confirmed by medical record review by trained personnel according to established criteria. Over 99% of reported breast cancers were confirmed on review in the medical record.

### STATISTICAL ANALYSIS

This analysis was limited to postmenopausal women only. Women were considered postmenopausal if they reported no menstrual periods within the previous 12 months with natural menopause, bilateral oophorectomy, or hysterectomy with 1 or more ovaries retained and were 54 years or older if a smoker or 56 years or older if a nonsmoker. In 1978, 35 474 postmenopausal women were included in this study. Women were included in updated analyses if they became postmenopausal during the prior period. By 2000, 70 444 women were postmenopausal and contributed person-time to the analysis. Women who reported a diagnosis of cancer other than nonmelanoma skin cancer were excluded at baseline and from subsequent follow-up analysis. Person-time for each participant was calculated from the date of return of the 1978 questionnaire to the date of breast cancer diagnosis, date of any other cancer diagnosis, death from any cause, or June 1, 2002, whichever ever came first.

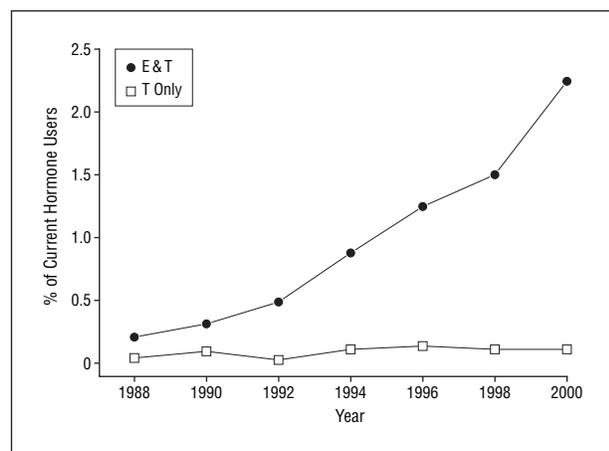
The primary analysis used incidence rates with person-months in the denominator. For each woman, person-months were allocated to a PMH use category, beginning in 1978 and updated every 2 years. We used Cox proportional hazards models to estimate relative risks (RRs) and 95% confidence intervals (CIs). Multivariate analysis included age at menopause, type of menopause, family history of breast cancer, personal history of benign breast disease, body mass index at age 18 years, weight change since age 18 years, age at menarche, parity and age at first birth, and alcohol consumption. Covariates were updated every 2 years. We conducted tests for trend by modeling the duration of use as a continuous variable and calculating a Wald statistic.<sup>23</sup> To determine whether the association between specific types of hormones and breast cancer risk were significantly different from one another, we conducted a test of heterogeneity.<sup>24</sup> All analyses were conducted with SAS version 8.2 (SAS Inc, Cary, NC).  $P < .05$  was used to determine statistical significance, and all tests of statistical significance were 2 sided.

## RESULTS

During 24 years of follow-up (1 359 323 person-years), 4610 incident cases of invasive breast cancer were identified among postmenopausal women. Overall, only a

small percentage of women in this cohort reported current use of E&T therapy. However, the increase in use over the follow-up period was substantial (**Figure**). In 1988, only 33 women included in the analysis reported current use of E&T therapy. Over a 10-year period, the proportion of current hormone users who reported use of E&T therapy increased by more than 8-fold, with 550 women reporting use in 1998. In 2000, E&T therapy accounted for 2.2% of current PMH use.

Because 47% of the person-time exposed to current use of E&T therapy occurred between 1998 and 2002, we calculated age and age-adjusted means and frequencies of breast cancer risk factors according to PMH use in 1998 (**Table 1**). Current users of E&T therapy were younger and leaner, were more likely to have had a benign breast disease, and consumed more alcohol compared with women who never used postmenopausal therapy. As expected, estrogen only



**Figure.** Percentage of current users of postmenopausal hormones containing estrogen and testosterone (E&T) and testosterone (T) only in the Nurses' Health Study (1988-2000).

and E&T users were more likely to have had a surgical menopause than never users.

The risk of developing breast cancer was 77% greater among current users of E&T therapy (multivariate RR, 1.77; 95% CI, 1.22-2.56) than among never users of PMH (**Table 2**). Current estrogen-only users had a 15% greater risk of breast cancer compared with never users (multivariate RR, 1.15; 95% CI, 1.05-1.27). The risk of breast cancer associated with E&T therapy was significantly greater than for estrogen-only therapy (*P* for heterogeneity, .03). Current use of E&P therapy was associated with a 58% increased risk of breast cancer (RR, 1.58; 95% CI, 1.44-1.73; data not presented in Table 2) compared with never users; this increase was not statistically different from current use of E&T therapy (*P* for heterogeneity, .56).

Women with current use of PMHs with testosterone for less than 5 years had an 81% increased risk of breast cancer (RR, 1.81; 95% CI, 1.21-2.70), while those using this therapy for 5 years or more were at a nonsignificant 96% increased risk (RR, 1.96; 95% CI, 0.93-4.14) compared with never users (**Table 3**). Women using PMHs with testosterone had a 9.5% increased risk of breast cancer per year of use (RR, 1.10; 95% CI, 1.03-1.16; *P* = .003).

Because we<sup>25</sup> and others<sup>26</sup> have shown that including in analysis women with simple hysterectomies with a missing age of menopause results in a bias toward the null, we conducted an analysis limited to women with natural menopause only (**Table 4**). All of the risk estimates for hormone users were stronger when limited to women with natural menopause. We found an increased risk of breast cancer among current users of E&T therapy (RR, 2.48; 95% CI, 1.53-4.04). Risk of breast cancer associated with E&T therapy was significantly greater compared with estrogen-only therapy (*P* for heterogeneity, .007); E&P therapy was associated with a 66% increased risk of breast cancer (RR, 1.66; 95% CI, 1.49-

**Table 1. Age and Age-Standardized Characteristics of Postmenopausal Women in the Nurses' Health Study in 1998, According to Use of Postmenopausal Hormones**

Characteristic	Never Users (n = 18 754)	Past Users (n = 16 643)	Current Users*		
			Estrogen† Only (n = 15 489)	Estrogen and Testosterone (n = 550)	Testosterone Only (n = 37)
Age, mean, y	65.0	66.7	64.4	59.5	60.6
Age at menarche, mean, y	12.4	12.4	12.4	12.6	12.2
Age at first birth, mean, y‡	24.9	24.7	24.3	24.2	24.4
Age at menopause, mean, y	50.2	47.9	45.2	48.1	47.7
BMI at age 18 y, mean	21.6	21.4	21.2	21.2	21.3
Current BMI, mean	27.6	27.1	26.5	26.7	26.0
Parity, mean, No.‡	3.1	3.0	2.9	3.0	2.6
Alcohol, mean, g/d	4.8	4.9	4.7	5.5	4.2
Natural menopause, %	86.9	64.7	18.8	44.8	73.1
Surgical menopause, %§	2.9	17.6	40.3	26.1	15.7
Other menopause, %	10.2	17.7	41.0	29.1	11.2
Family history of breast cancer, %	15.6	14.7	12.4	16.3	4.3
Benign breast disease, %	34.8	44.8	50.6	55.0	57.9

Abbreviation: BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters).

\*21 130 Current users of estrogen plus progestin, progesterone only, and other types of hormone are not included in the table.

†Conjugated estrogens only.

‡Among parous women.

§Bilateral oophorectomy.

||Includes simple hysterectomy, hysterectomy with unilateral oophorectomy, and radiation.

**Table 2. Relative Risk of Invasive Breast Cancer in the Nurses' Health Study Diagnosed Through June 1, 2002, According to Postmenopausal Hormone Use (1978-2002)**

Participants	Person-Years	Cases, No.	RR (95% CI)		
			Adjusted*	Adjusted†	Trend, per Year‡
Never users	557 577	1647	1.00	1.00	1.00
Past users	316 713	936	0.91 (0.84-0.99)	0.90 (0.82-0.97)	0.89 (0.83-0.97)
Current users‡					
Estrogen only§	246 830	852	1.18 (1.08-1.30)	1.15 (1.05-1.27)	1.02 (1.01-1.03)
Estrogen and testosterone	5268	29	1.87 (1.29-2.71)	1.77 (1.22-2.56)	1.10 (1.03-1.16)
Testosterone only	360	3	2.69 (0.86-8.43)	2.52 (0.80-7.94)	NA

Abbreviations: CI, confidence interval; NA, not applicable; RR, relative risk.

\*Adjusted for age (5-year categories), age at menopause (continuous), and type of menopause (natural, bilateral oophorectomy, or other "nonnatural").

†Adjusted for age at menopause, type of menopause, family history of breast cancer (yes/no), personal history of benign breast disease (yes/no), body mass index (calculated as weight in kilograms divided by the square of height in meters) at age 18 years (<20, ≥20 to <22, ≥22 to <24, ≥24 to <27, and ≥27), weight change (kilograms) since age 18 years (<-2, ≥-2 to <2, ≥2 to <5, ≥5 to <10, ≥10 to <20, ≥20 to <25, and ≥25), age at menarche (<12, 12, 13, and >13 years), parity and age at first birth (nulliparous, 1-4 children and age at first birth ≤24 years, and 1-4 children and age at first birth >24 years), and alcohol consumption (grams per day) (none, <5.0, 5.0-9.9, 10.0-14.9, and ≥15.0).

‡Among 232 575 person-years, 1143 breast cancer cases occurred in women currently using estrogen and progesterone therapy, progesterone only, other exogenous hormones including esterified estrogens, and hormones administered through a patch.

§Conjugated estrogens only.

||Trend for estrogen and testosterone combined with testosterone only.

**Table 3. Relative Risk of Invasive Breast Cancer in the Nurses' Health Study Diagnosed Through June 1, 2002, According to Duration of PMH Use (1978-2002)**

Participants	Person-Years	Cases, No.	RR (95% CI)	
			Adjusted*	Adjusted†
Never users	557 577	1647	1.00	1.00
Past users	316 713	936	0.92 (0.85-1.00)	0.90 (0.83-0.98)
Current users, years of use‡				
Estrogen only, <5§	102 332	283	1.06 (0.93-1.21)	1.04 (0.91-1.19)
Estrogen only, ≥5§	144 498	569	1.29 (1.15-1.44)	1.25 (1.11-1.39)
Testosterone PMH, <5	4548	25	1.90 (1.27-2.83)	1.81 (1.21-2.70)
Testosterone PMH, ≥5	1079	7	2.16 (1.02-4.56)	1.96 (0.93-4.14)

Abbreviations: CI, confidence interval; PMH, postmenopausal hormone; RR, relative risk.

\*Adjusted for age (5-year categories), age at menopause (continuously), and type of menopause (natural, bilateral oophorectomy, or other "nonnatural").

†Adjusted for age at menopause, type of menopause, family history of breast cancer (yes/no), personal history of benign breast disease (yes/no), body mass index (calculated as weight in kilograms divided by the square of height in meters) at age 18 years (<20, ≥20 to <22, ≥22 to <24, ≥24 to <27, and ≥27), weight change (kilograms) since age 18 years (<-2, ≥-2 to <2, ≥2 to <5, ≥5 to <10, ≥10 to <20, ≥20 to <25, and ≥25), age at menarche (<12, 12, 13, and >13 years), parity and age at first birth (nulliparous, 1-4 children and age at first birth ≤24 years, and 1-4 children and age at first birth >24 years), alcohol consumption (grams per day) (none, <5.0, 5.0-9.9, 10.0-14.9, and ≥15.0), and current use of other hormones (estrogen and progesterone therapy, progesterone only, other exogenous hormones including esterified estrogens, and hormones administered through patch).

‡Among 232 575 person-years, 1143 breast cancer cases occurred in women currently using estrogen and progesterone therapy, progesterone only, other exogenous hormones including esterified estrogens, and hormones administered through patch.

§Conjugated estrogens only.

||Includes estrogen plus testosterone therapies and testosterone-only therapies.

1.84; data not presented in Table 4). Risk of breast cancer associated with current E&T use was marginally greater compared with E&P therapy (*P* for heterogeneity, .11).

Because the majority of E&T users were past users of other types of hormone therapy, we conducted an analysis that took into account type-specific past use of exogenous hormones and duration of prior hormone use. Although power was limited in this analysis, results were consistent in showing that current use of E&T therapy (RR, 1.45; 95% CI, 0.82-2.54) and continuous duration of use (RR, 1.07 per year of use; 95% CI, 0.96-1.20) exhibited the greatest point estimates for breast cancer risk relative to the other specific types of hormones. The small number of

women currently using E&T therapy precluded detailed analysis of risk of breast cancer according to other risk factors, tumor subtype, or receptor status.

#### COMMENT

To our knowledge, this is the first epidemiologic study to specifically address the effect of oral E&T therapy as currently used in US postmenopausal women on the risk of breast cancer. In a previous study in this cohort, Colditz et al<sup>27</sup> addressed different types of PMHs, focusing primarily on estrogen and progestin therapies. Based on 4 exposed cases in 810 person-years of follow-up, this ini-

**Table 4. Relative Risk of Invasive Breast Cancer Among Postmenopausal Women With Natural Menopause in the Nurses' Health Study Diagnosed Through June 1, 2002, According to Postmenopausal Hormone Use (1978-2002)**

Participants	Person-Years	Cases, No.	RR (95% CI)		
			Adjusted*	Adjusted†	Trend, per Year‡
Never users	381 797	1181	1.00	1.00	1.00
Past users	163 299	543	0.97 (0.87-1.08)	0.94 (0.85-1.05)	0.935 (0.844-1.037)
Current users‡					
Estrogen only§	45 993	191	1.26 (1.08-1.47)	1.23 (1.05-1.44)	1.043 (1.026-1.059)
Estrogen and testosterone	2066	17	2.65 (1.63-4.30)	2.48 (1.53-4.04)	1.172 (1.067-1.287)
Testosterone only	179	1	2.15 (0.30-15.50)	2.10 (0.29-15.18)	NA

Abbreviations: CI, confidence interval; NA, not applicable; RR, relative risk.

\*Adjusted for age at menopause (continuous).

†Adjusted for age at menopause, family history of breast cancer (yes/no), personal history of benign breast disease (yes/no), body mass index (calculated as weight in kilograms divided by the square of height in meters) at age 18 years (<20, ≥20 to <22, ≥22 to <24, ≥24 to <27, and ≥27), weight change (kilograms) since age 18 years (<-2, ≥-2 to <2, ≥2 to <5, ≥5 to <10, ≥10 to <20, ≥20 to <25, and ≥25), age at menarche (<12, 12, 13, or >13 years), parity and age at first birth (nulliparous, 1-4 children and age at first birth ≤24 years, 1-4 children and age at first birth >24 years), alcohol consumption (grams per day) (none, <5.0, 5.0-9.9, 10.0-14.9, and 15.0), and current use of other hormones (estrogen and progesterone therapy, progesterone only, other exogenous hormones including esterified estrogens, and hormones administered through a patch).

‡Among 149 682 person-years, 818 breast cancer cases occurred in women currently using estrogen and progesterone therapy, progesterone only, other exogenous hormones including esterified estrogens, and hormones administered through a patch.

§Conjugated estrogens only.

||Trend for estrogen and testosterone combined with testosterone only.

tial study reported a 60% increased risk of breast cancer among estrogen and androgen users (multivariate RR, 1.64; 95% CI, 0.53-5.09).<sup>27</sup>

The results of the current analysis suggest that E&T therapy is associated with a greater risk of breast cancer compared with estrogen-only therapy. Only a handful of population studies have examined the effects of testosterone on breast cancer risk, with inconsistent results. A study of postmenopausal women who had undergone ovariectomy and who were receiving testosterone implants in addition to estrogen-only and E&P therapies found that the observed rates of breast cancer in this population were not higher than expected rates among never users and were substantially lower than rates among E&P users.<sup>28</sup> On the basis of 7 exposed cases, the authors concluded that the addition of testosterone to combined hormone therapy may reduce the risk of breast cancer among women receiving conventional therapies.<sup>28</sup> Ewertz<sup>29</sup> reported a 2-fold (RR, 2.31; 95% CI, 1.37-3.88) increased risk of breast cancer among Danish women receiving estradiol and testosterone injections compared with nonusers. Although this study had 56 breast cancer cases exposed to E&T therapy, the treatment was an injectable containing 2.5 to 5.0 mg of estradiol and 50 to 100 mg of testosterone given at 3- to 7-week intervals and may not be comparable with oral formulations marketed in the United States. Estratest was the primary E&T therapy reported by women in the present study. The full-strength dose is an oral combination containing 1.25 mg of esterified estrogen and 2.5 mg of methyltestosterone.<sup>18</sup>

Exogenous androgens could increase the risk of breast cancer indirectly through the conversion of androgens to estrogens or more directly with effects mediated through the androgen receptor. Because breast tissue has aromatase activity,<sup>30-33</sup> testosterone may serve as a substrate for local estradiol formation in the breast. In a clinical study comparing oral estrogen alone with oral E&T therapy, E&T therapy significantly increased circulat-

ing levels of estradiol and free testosterone compared with baseline.<sup>13,34</sup> Circulating estradiol levels increased in a similar fashion to women assigned to estrogen-only therapy, while the increase in free testosterone and the reduction in luteinizing hormone and sex hormone-binding globulin levels were significantly greater among the E&T group compared with the estrogen-only group.<sup>34</sup>

Epidemiologic studies demonstrate that circulating levels of testosterone are associated with an increased risk of breast cancer among postmenopausal women, independent of circulating estrogen levels.<sup>1</sup> In contrast, data from animal and cell studies are less clear and suggest that testosterone inhibits proliferation. The majority of in vitro studies using breast cancer cell lines report that androgens have inhibitory effects on the proliferative effects of estrogen.<sup>35</sup> However, results diverge depending on specific cell line, type and dose of androgen, and receptor status of the cell line.<sup>35</sup> A study in primates reported that administration of testosterone reduced estrogen-induced proliferation by 40%.<sup>36</sup>

The inverse association observed among past users is consistent with results from previous studies.<sup>37</sup> In a re-analysis of data from 51 studies, the Collaborative Group on Hormonal Factors in Breast Cancer found that women who had discontinued hormones for 5 or more years had a nonsignificant reduced risk of breast cancer (RR, 0.92; 95% CI, 0.72-1.12). This is likely because past users are more likely than current users to be users of shorter duration. Women who initiate PMH use tend to be leaner<sup>38,39</sup> and are at greater risk of osteoporosis<sup>39,40</sup> compared with nonusers. These data suggest that women who initiate hormone therapy for the short-term relief of menopausal symptoms<sup>41</sup> may be more likely to have lower circulating estrogen levels and therefore may be at a reduced risk of breast cancer.<sup>1</sup> A potential limitation of this study is misclassification of exposure because E&T therapies were not always explicitly listed as PMH choices on questionnaires. Because participants had to write in use

of androgen therapy during these years, it is possible that we have underestimated the number of women exposed to E&T therapy. However, information on PMH use was prospectively collected, and any misclassification would be nondifferential with respect to disease status.

Because the use of E&T therapy is a more recent clinical practice and the majority of current users were past users of other types of hormone therapy, there is the potential for confounding by past use of other therapies. Our secondary analysis, adjusting for type-specific past use and duration of prior hormone use, was limited in power but supports our findings that E&T use is associated with increased risk of breast cancer.

Women receiving PMHs may be more likely to be screened for breast cancer than those not receiving PMHs. Any detection bias that may occur, however, would be unlikely to have differential effects according to type of hormones.<sup>42</sup> The association we found between E&T therapy and breast cancer is significantly greater than the association with estrogen-only therapy, suggesting that the increased risk of breast cancer is not due to detection bias.

The increasing trend of use of testosterone-containing therapies observed in this cohort supports anecdotal evidence of an increase in the number of postmenopausal women using E&T therapy.<sup>10,43</sup> This increase is likely to be attributable to studies reporting beneficial effects on mood, libido, and bone mineral density.<sup>11,13-15</sup> Clinical trials examining these end points can be evaluated relatively soon after the onset of treatment. In contrast, the development of breast cancer can take years. Large prospective studies offer an ideal setting to examine potential long-term adverse events that are relatively uncommon in the population but may occur more frequently among those exposed to a drug.<sup>44</sup> It is important to understand the full spectrum of long-term risks associated with E&T therapies before advocating their use for short-term benefits.

Consistent with the elevation in risk with increasing endogenous testosterone level, women using E&T therapies have a significantly increased risk of invasive breast cancer. These results are also consistent with studies showing that E&P therapies with synthetic testosterone-derived progestogens are associated with a greater risk of breast cancer compared with those with micronized progesterone.<sup>45</sup> These results are especially important given the already apparent increase in trends and the expectation that more formulations containing testosterone will become specifically approved for and marketed to women. Given the substantial evidence implicating combined E&P therapy in breast cancer and the results of the present study regarding E&T therapies, women and their physicians should reconsider use and, more specifically, long-term use of these therapies.<sup>46,47</sup> Although postmenopausal therapies may provide improvement with respect to sexual functioning, general well-being, and bone health, the increased risk of breast cancer may outweigh these benefits.

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