



# Postmenopausal testosterone therapy and breast cancer risk

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## Abstract

**Background:** Testosterone therapy is being increasingly used in the management of postmenopausal women. However, as clinical trials have demonstrated a significantly increased risk of breast cancer with oral combined estrogen–progestin therapy, there is a need to ascertain the risk of including testosterone in such regimens. **Objective:** Evaluation of experimental and epidemiological studies pertaining to the role of testosterone in breast cancer. **Design:** Literature review. **Setting:** The Jean Hailes Foundation, Research Unit. **Main Outcome Measures:** Mammary epithelial proliferation, apoptosis and breast cancer. **Results:** In experimental studies, testosterone action is anti-proliferative and pro-apoptotic, and mediated via the AR, despite the potential for testosterone to be aromatized to estrogen. Animal studies suggest that testosterone may serve as a natural, endogenous protector of the breast and limit mitogenic and cancer promoting effects of estrogen on mammary epithelium. In premenopausal women, elevated testosterone is not associated with greater breast cancer risk. The risk of breast cancer is also not increased in women with polycystic ovary syndrome who have chronic estrogen exposure and androgen excess. However, in postmenopausal women, who are oestrogen deplete and have increased adipose aromatase activity, higher testosterone has been associated with greater breast cancer risk. **Conclusion:** Available data indicate the inclusion of testosterone in estrogen–progestin regimens has the potential to ameliorate the stimulating effects of hormones on the breast. However, testosterone therapy alone cannot be recommended for estrogen deplete women because of the potential risk of enhanced aromatisation to estrogen in this setting.

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## 1. Introduction

Testosterone exhibits important physiological effects in women, being both a precursor hormone for ovarian and extragonadal estrogen biosynthesis [1], and acting directly via androgen receptors (AR)

throughout the body. Some postmenopausal women may develop symptomatic androgen insufficiency and may benefit from testosterone therapy in terms of improved libido, sexual satisfaction, quality of life and bone mineralization [2–9]. However, as the most recent large randomized placebo-controlled clinical trials have demonstrated a significantly increased risk of breast cancer in postmenopausal women taking oral

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combined hormone therapy [10,11] there is a need to establish whether adding testosterone to hormone therapy regimens will influence this risk.

## 2. Testosterone aromatization after menopause

Testosterone may exert biological effects by acting directly via the AR or indirectly through conversion to estrogen by the aromatase enzyme and dihydrotestosterone (DHT), which is a non-aromatizable androgen, by 5 $\alpha$ -reductase type 1 and 2 [12,13]. Following menopause, the former conversion occurs primarily in adipose and target tissues such as breast, brain, bone, skin, vascular endothelium and vascular smooth muscle [13]. Increased aromatase activity and p450 aromatase gene expression has been demonstrated in adipose tissue with increasing age [14,15]. Plasma cytokines, known to be aromatase stimulating factors, such as interleukin-6, increase with age [16,17] and this may partially account for the increase in peripheral aromatase activity that is detected in older women.

Although this change is not influenced by menopause status [18], it is also possible that estrogen itself may inhibit its own bioformation in both human breast cancer cell lines [19] and animal models [20]. Consistent with these findings, intra-tumoral aromatase activity has been shown to be higher in woman with lower circulating estrogen [21,22] and relative low activity of aromatase was found in women taking hormone therapy [19,23]. These data indirectly suggest that after menopause, when circulating estrogen levels are low, an increase in aromatase levels in the breast may maintain tissue concentrations of estrogen. Thus, aromatase may control the local production of estrogen through an autocrine loop. During the process of transformation to malignancy, locally produced estrogen may stimulate the proliferation of tumor cells and vascular endothelial growth factor production. These effects are also likely to enhance tumor progression, development of angiogenesis, and, ultimately, metastasis of cancer. This has negative implications for the use of testosterone therapy in women who are postmenopausal and not receiving estrogen therapy.

With regard to 5 $\alpha$ -reductase activity, a number of studies have substantiated 5 $\alpha$ -reductase modulation by sex steroids [24–29]. Evidence for the relationship be-

tween 5 $\alpha$ -reductase activity and menopausal status is lacking.

## 3. Effects of testosterone and DHT on breast: experimental studies

In experimental studies, estradiol has been consistently been shown to be a major mitogen in the breast [30–34] while testosterone and DHT have been shown to inhibit such estrogenic effects as follows.

### 3.1. Breast cancer cell lines

#### 3.1.1. Anti-proliferative effects of androgens

Normal circulating levels in women are approximately 0.2–0.8 nmol/L for DHT and 0.5–2.3 nmol/L for testosterone. At physiological doses (1 nM/L), testosterone and DHT inhibit proliferation of MCF-7 cells in both the absence of estradiol [35–37] or presence [35,38], although not all studies have found this association [39]. These inhibitory effects have also been reported in other breast cancer cell lines, ZR-75-1 [39] and T47-D [37,39]. Birrell et al. [39] reported no effect on proliferation of BT-20 cells, whereas Ortmann et al. [37] demonstrated a significant dose-dependent inhibition of cell growth by testosterone and DHT in this cell line. The contradictory result may be explained by the difference in cell proliferation assays used. Birrel et al. used a modification of a methylene blue whole cell binding assay while Ortman et al. used MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) method.

#### 3.1.2. Apoptotic effects

Bcl-2 is considered an anti-apoptotic gene [40], and Bax is a pro-apoptotic gene [41]. DHT down-regulates Bcl-2 protooncogene levels via an AR-mediated mechanism in the ZR75-1 breast cancer cell line in either the presence or absence of 17 $\beta$ -estradiol [42]. In addition, in the AR positive human breast cancer cell lines, T47-D and ZR-75-1, DHT has been reported to be pro-apoptotic with maximal effects at 10 nmol/L concentration [43]. These findings are in-line with the growth inhibitory effects reported by Birrell et al. [44] in these two cell lines. This leads us to conclude that DHT may oppose the mitogenic action of estrogen in the breast by promoting apoptosis. Table 1. summarizes the results of breast cancer cell line studies.

Table 1  
Effects of testosterone and DHT on breast cancer cell lines

Type of androgen	Model	Dose of androgen (M)	Main outcome measurement	Result	Study
Testosterone	MCF-7	$10^{-9}$ to $10^{-6}$	Proliferation	Inhibition	Ando et al. 2002 [35]
	MCF-7, T47-D, BT-20, MDA-MB 435S	$10^{-9}$ to $10^{-7}$	Proliferation	Inhibition in all four cell lines	Ortmann et al. 2002 [37]
DHT	MCF-7, ZR75-1, TD47-D, MDA-MB-453	$10^{-10}$ to $10^{-8}$	Proliferation	Stimulation in the MCF-7 and MDA-MB-453; no effect in MDA-MB-231 or BT-20, inhibition in T47-D and ZR-75-1	Birrell et al. 1995 [39]
	MCF-7	$20^{-11}$ to $20^{-7}$	Proliferation	Biphasic effect: Stimulation at a very high concentration; inhibition at concentration up to $20^{-8}$ M	Bocuzzi et al. 1994 [36]
	MCF-7	$10^{-9}$ to $10^6$	Proliferation	Inhibition	Ando et al. 2002 [35]
	MCF-7, ZR75-1, T47-D	$10^{-9}$ to $10^7$	Proliferation	Inhibition in all four cell lines	Ortmann et al. 2002 [37]
	MCF-7, ZR75-1, T47-D	$10^{-8}$	Apoptosis	Proapoptotic effect	Kandouz et al. 1999 [43]
	ZR-75-1	$10^{-9}$	Bcl-2 protein and messenger RNA	Down regulation	Lapointe et al. 1999 [42]

### 3.2. Animal studies

#### 3.2.1. Normal model

In the Noble rat model, treatment with testosterone, either alone or in combination with estrogen, gave rise to high levels of Bax expression in ‘pre-malignant’ mammary glands [41]. This supports the hypothesis that testosterone may oppose the mitogenic action of estrogen in the breast by promoting apoptosis [45].

The addition of methyltestosterone to oral contraceptive therapy in rats causes a significant suppression of epithelial proliferation and a reduction in the number of progesterone receptor-labelled cells [46]. In rhesus monkeys either supra- or physiologic testosterone treatment reduced estradiol-induced epithelial proliferation [47,48], suggesting that testosterone can reduce breast cancer risk associated with estrogen treatment [48].

#### 3.2.2. Tumor model

DHT therapy reduced the number of progressing tumors and increased the number of tumors that completely regressed in the DMBA-induced mammary carcinoma model in ovariectomized rats [49] and on

ZR-75-1 breast cancer cells implanted into ovariectomized athymic mice in both the presence or absence of exogenous estradiol [50]. Table 2. summarizes the results from all animal studies.

## 4. Effects of testosterone on breast tissue: epidemiological studies

### 4.1. Endogenous testosterone and breast cancer

In premenopausal women, there are inconsistent results from two cross-sectional studies [51,52] and two prospective case-control studies have found no association between breast cancer risk and total testosterone levels [53,54].

Polycystic ovarian syndrome (PCOS) is a useful model of the effects of long-term exposure to hormone imbalance. Concentrations of testosterone, androstenedione, and DHEAS, and the calculated free androgen index (total testosterone nmol/L divided by SHBG nmol/L  $\times$  100) are significantly higher in women with PCOS regardless of hirsutism [55]. An increased risk of endometrial cancer has been well documented in women with this condition [56] whereas the risk of

Table 2  
Effects of testosterone and DHT in animal studies

Intervention	Model	Main outcome measurement	Result	Study
E vs. E + DHT	DMBA-induced mammary carcinoma in rats	The number of progressing tumor	Significant decrease with E + DHT vs. E alone	Dauvois et al. 1989 [49]
E vs. E + DHT	ZR-75-1 breast cancer cells implanted into ovariectomized athymic mice	The number of complete response The number of progressing tumor	Significant increase with E + DHT vs. E alone Significant decrease with E + DHT vs. E alone	Dauvois et al. 1991 [50]
Oral contraceptive vs. oral contraceptive + methyltestosterone	Rat	Mammary epithelial proliferation	Significant decrease with oral contraceptive + testosterone vs. oral contraceptive alone	Jayo et al. 2000[46]
E; E + progesterone; E + T	Ovariectomized rhesus monkeys	Mammary epithelial proliferation	Significant decrease with E + T vs. others	Zhou et al. 2000 [47]
Flutamide vs. none	Normal-cycling rhesus monkeys	Mammary epithelial proliferation	Significant increase in flutamide	Dimitrakakis et al. 2003 [48]
E; E + progesterone; E + T; vehicle	Ovariectomized rhesus monkeys	Mammary epithelial proliferation ER $\alpha$ /ER $\beta$ MYC expression	Significant decrease with E + T vs. E or E + progesterone Reversal of ER $\alpha$ /ER $\beta$ Significant reduction	

E: estradiol; DHT: dihydrotestosterone; and T: testosterone.

breast cancer is not increased [56,57] despite hyperandrogenism and long term exposure to unopposed estrogen. In fact, Gammon and Thompson [58] have reported an aged-adjusted odds ratio for breast cancer in women with this syndrome of 0.52 (95% CI 0.32–0.87). Although this risk reduction might be related to the hyperandrogenemia of this condition, cause and effect cannot be established.

In postmenopausal women, there are inconsistent results in both cross-sectional studies and prospective studies [53,59–68]. Table 3. shows the results from the prospective case-control studies. A reanalysis of prospective studies [69] reported that the relative risks associated with a doubling of total testosterone levels within the normal range were 1.37 (95% CI 1.15–1.65) for the three studies incorporating a purification step in their testosterone assay [66,67,70] and 1.44 (95% CI 1.21–1.72) for four studies that used a direct testosterone assay [62–65]. The considerable inaccuracy of direct total testosterone assays limits the interpretation of any data obtained by this methodology [71]. Fur-

thermore, a subgroup analysis was not undertaken to determine the association between breast cancer and free testosterone. This is important as free testosterone, not total testosterone, is considered the best indicator of tissue exposure to testosterone.

There are only two prospective studies available that have utilized measures of free testosterone [62,67]. Cauley et al. [67] measured free testosterone by equilibrium dialysis, considered to be a reasonably accurate methodology, in 97 cases and 244 controls and reported that breast cancer risk was three times greater in women with the highest concentration ( $\geq 13.17$  pmol/L), but that this was no longer significant after adjustment for estradiol. In contrast Berrino et al. [62] measured free testosterone by radioimmunoassay in 25 cases and 100 controls and reported a significant association between higher free testosterone levels ( $\geq 0.86$  pg/ml) and breast cancer after adjustment for estradiol. Again, as direct free testosterone assays are considered unreliable, the value of this data is questionable [71].

Table 3  
Endogenous testosterone and risk of breast cancer in postmenopausal women

Study	Comparison made	Subjects numbers		Adjusted RR by other variables	
				Variables	RR (95%CI) <sup>a</sup>
Wysowski, 1987 [53]	Mean values of cases vs. controls <sup>b</sup>	Case	39	Not applicable	Not applicable
		Control	156	—	—
Garland, 1992 [61]	Top to bottom tertile	Case	31	Age	1 <sup>c</sup>
		Control	287	Estradiol	—
Berrino, 1996 [62]	Top to bottom tertile	Case	67	Age	5.7 (1.5–22.2)
		Control	264	Total estradiol	5.9 (1.2–29.3)
Dorgan, 1996 [63]	Top to bottom quartile	Case	71	Year since menopause, height, weight, family history of breast cancer and parity	6.2 (2.0–19.0)
		Control	133	Estradiol	—
Thomas, 1997 <sup>d</sup> [64]	Top to bottom tertile	Case	61	Age at menarche, parity, number of y postmenopausal, BMI	Not different
		Control	179	Estradiol	0.8 (0.3–2.4)
Zeleniuch-Jacquotte, 1997 <sup>d</sup> [65]	Top to bottom quartile	Case	85 <sup>e</sup>	Total estradiol, SHBG-bound estradiol	1.2 (0.4–3.5)
		Control	163	SHBG-bound estradiol	1.3 (0.5–3.7)
Hankinson, 1998 [66]	Top to bottom quartile	Case	155	BMI at age 18, family history of breast cancer, age at menarche, parity/age at first birth and past HT	1.4 (0.7–2.7)
		Control	310	Estradiol	1.1 (0.5–2.3)
Cauley, 1999 <sup>e</sup> [67]	Top to bottom quartile	Case	97	Age, BMI, age at menarche, first birth, and menopause, family history of breast cancer; physical activity, surgical menopause, and alcohol consumption	3.3 (1.1–10.3)
		Control	243	Bioavailable estradiol	2.1 (0.9–4.7)
Manjer, 2003 [68]	Top to bottom quartile	Case	173	Age, storage time, sub-cohort, parity, and oophorectomy	1.9 (1.1–3.3)
		Control	438	Estradiol, SHBG	1.9 (1.1–3.3)

RR: relative risk; OD: odds ratio; RH: relative hazard; CI: confident interval; Not different: adjusting for these variables did not affect the results.

<sup>a</sup> RR of total testosterone for breast cancer risk except for Berrino et al. and Cauley et al. RR of free testosterone for breast cancer. Reanalysis after incident case was defined as at least 2-year-interval after blood taken resulted in RR = 1.3 (0.5–3).

<sup>b</sup> No statistically significant difference of testosterone levels between cases and controls by mean comparison; — data not available in this study.

<sup>c</sup> Data about 95%CI not available.

<sup>d</sup> Data presented as OR.

<sup>e</sup> Data presented as RH.

Table 4  
Exogenous testosterone and risk of breast cancer in postmenopausal women

Study	Study characteristics	Results
Ewertz, 1988 [72]	A subgroup analysis of a case-control study  The ever use of intramuscular injections containing estradiol–testosterone (2.5 mg estradiol + 50–100 mg testosterone) given at interval of 3–7 weeks 56/1694 cases; 21/1705 controls	RR 2.3 (1.37–3.88) for estradiol plus testosterone RR 1.26 (0.58–2.74) for estradiol plus testosterone plus progestogen
Brinton, 1986 [73]	A subgroup analysis of a case-control study Oral methyltestosterone in combination with conjugated equine estrogen 25 cases; 29 controls	RR 1.05 (0.6–1.8)
Dimitrakakis, 2003 [74]	Retrospective analysis  511 Australian women treated with conventional estrogen therapy plus 50–100 mg testosterone implants A mean follow up of 5.7–2.5 years No control group	A breast cancer incidence of 240 per 100,000 women years It was equivalent to the incidence in the general population determined by the State Cancer Registry

RR: relative risk.

#### 4.2. Exogenous testosterone and breast cancer

The specific evidence pertaining to the use of testosterone therapy and breast cancer risk has significant methodological limitations and provides inconclusive results. Breast cancer risk was not a primary endpoint for two of these studies [72,73]. Consequently, each had only a small sample size for this subgroup analysis [72,73]. A recent retrospective study had no control group [74]. Table 4 summarized study characteristics and their results.

### 5. Clinical consideration

The term female androgen insufficiency (FAI) was proposed as a pattern of clinical symptoms in the presence of decreased bioavailable testosterone and normal estrogen status [75]. The addition of testosterone to hormone therapy has shown benefits for sexual dysfunction, mood, energy, psychological well being, bone mineral density, muscle mass and strength and adipose tissue distribution [2–7,76]. However, there are some potential risks have been reported, for example acne, excess facial and body hair, permanent deepening of voice, weight gain, emotional changes (e.g. increased anger) and adverse effects on the lipid profile [77]. As

androgens are converted to estrogens in vivo, estrogenic side-effects are also potential consequences of androgen therapy, including increased risk of breast and ovarian cancer [77].

Based on experimental studies covered in this review it is unlikely that physiological testosterone therapy in addition to estrogen therapy increases the risk of breast cancer. Alternatively, it appears that testosterone serves as a natural safe guard of the breast and limits mitogenic and cancer promoting effects of estrogen within the breast [35,37–39,42,43,45,49,50]. However, the existing evidence in women specific to exogenous testosterone therapy and the risk of breast cancer [72–74] has a significant limitations of research methodology to address this issue.

In case-control studies, high endogenous total testosterone levels have been associated with increased breast cancer risk in postmenopausal women [69], but not in premenopausal women [53,54]. It is almost impossible to be sure of a direct causal relationship between high levels of free testosterone and breast cancer although the positive association was found. Increased aromatase activity in the setting of estrogen depletion after menopause, and thus increased capacity to convert testosterone to estradiol in adipose tissue with age may be the major factors. That there is no longer an association between high levels of free testosterone and

breast cancer risk after adjustment for estradiol levels would support this [67]. Furthermore, there has been no report of an increase in breast cancer risk in women with PCOS in which hyperandrogenism coexists with elevated estrogen.

As the epidemiological studies specific to exogenous testosterone therapy and breast cancer risk have major limitations, with the available data pertaining to the effects of testosterone on the breast, the inclusion of testosterone in hormonal regimens should be limited to women symptomatic of androgen insufficiency despite adequate estrogen replacement and involve regular measurements of circulating levels of free testosterone to avoid androgen excess.

## 6. Conclusion

Breast cancer is the most common carcinoma in women [78,79]. Evidence from the most recent large randomized, placebo-controlled clinical trials shows the risk significantly increases in postmenopausal women taking estrogen plus medroxyprogesterone acetate [10,11,80] but does not increase in those taking estrogen only [81] while the clinical studies specific to exogenous testosterone therapy and breast cancer risk yield inconclusive result due to significant limitations [72–74]. However, the preclinical studies suggest that testosterone may serve as a natural, endogenous protector of the breast and limit mitogenic and cancer promoting effects of estrogen on mammary epithelium [35,37–39,42,43,46,48–50]. In contrast, in the setting of estrogen depletion, ie postmenopause, aromatase activity is increased and higher free testosterone has been associated in one study with a small increase in breast cancer risk. Thus, we propose that whereas benefits may be achieved by adding testosterone to estrogen–progesterin therapy, greater caution is required for the use of testosterone alone at this time. There is no doubt that further studies are required so that firm clinical advice can be given pertaining to the use of testosterone with or without estrogen therapy.

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