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Review

Testosterone and risk of breast cancer: appraisal of existing evidence

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Abstract

The objective of this review was to examine data from preclinical, clinical and epidemiological studies to evaluate if testosterone (T) poses increased risk of breast cancer in women. Appraisal of the existing literature produced several lines of evidence arguing against increased breast cancer risk with T. These include: (i) Data from breast tumor cell lines treated with androgens did not corroborate the notion that T increases breast cancer risk. On the contrary, androgens appear to be protective, as they inhibit tumor cell growth. (ii) Many of the epidemiological studies claiming an association between T and breast cancer did not adjust for estrogen levels. Studies adjusted for estrogen levels reported no association between T and breast cancer. (iii) Data from clinical studies with exogenous androgen treatment of women with endocrine and sexual disorders did not show any increase in incidence of breast cancer. (iv) Women afflicted with polycystic ovary disease, who exhibit high levels of androgens do not show increased risk of breast cancer compared to the general population. (v) Female to male transsexuals, who receive supraphysiological doses of T for long time periods prior to surgical procedures, do not report increased risk of breast cancer. (vi) Finally, women with hormone responsive primary breast cancer are treated with aromatase inhibitors, which block conversion of androgens to estrogens, thus elevating androgen levels. These women do not experience increased incidence of contralateral breast cancer nor do they experience increased tumor growth. In conclusion, the evidence available strongly suggests that T does not increase breast cancer risk in women.

Keywords: androgen excess; androgen therapy; aromatase inhibitors; breast cancer; estradiol; hormone replacement therapy; menopause; polycystic ovary disease; sex hormone binding globulin; testosterone.

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Introduction

Testosterone (T) therapy in women has been utilized since 1938 to treat various gynecological and sexual disorders (1). However, T therapy in women remains controversial, even with the plethora of information that exists in the literature. A glance at the randomized clinical studies reported to date on T therapy in women (Table 1) provides evidence for improved overall general and sexual health in women with surgical or natural menopause and with minimal side effects. T safety in women treated for sexual dysfunction has been reviewed recently and deemed use of T to be safe based on clinical data over a 3-year period (2–5). However, some argued that T therapy in women is associated with increased risk of breast cancer based on epidemiological studies (6). It should be noted that many epidemiological studies did not adjust for estrogen levels and therefore result in serious drawbacks of T measurements in women (7). To date, there are no studies which unequivocally demonstrated that T directly or indirectly causes initiation, promotion or growth of breast tumors in women. Therefore, claims that T is associated with increased breast cancer risk are unsubstantiated.

Estrogens are critical for normal development, differentiation and growth of the mammary gland and normal breast tissue expresses estrogen receptors (ERs), which regulate estrogen action in the breast (8). Approximately 65% of human breast tumors retain expression of ERα, which allows tumor cells to grow in response to circulating estrogens (9). Indeed, tumors that are ER positive and progesterone receptor (PR) positive (ER+/PR+) are considered hormone sensitive. This concept is utilized clinically to treat patients with hormone responsive breast cancer with anti-estrogens, such as tamoxifen, to inhibit tumor growth and as a prognostic factor for management of women with breast cancer (10, 11).

Androgens serve as precursors of estrogen biosynthesis and estrogens can promote tumor growth (8). Aromatase inhibitors were introduced to treat patients with ER+ tumors because it deprives the tumor of circulating estrogens (12–17). Although this treatment increases intracellular T levels, it does not increase tumor growth in response to androgens. In this review, we discuss data from preclinical, clinical and epidemiological studies, which provided evi-

Table 1 Clinical trials of testosterone treatment in women.

Author, year	Study numbers	Treatment time	Study result
Buster 2005	533	24 weeks	Increase in frequency of satisfying sexual activity, desire
Braunstein 2005	447	24 weeks	Increase in frequency of satisfying sexual activity, desire
Davis 1995	34	12 months	Increase in bone density, sexual activity, pleasure, satisfaction and orgasm
Davis 2006	77	24 weeks	Increase sexual desire, arousal, orgasm, responsiveness, self image; decrease in sexual concerns, distress
Dobs 2002	36	16 weeks	Increase in sexual activity and pleasure, lean body mass; decrease in fat mass
Lobo 2003	218	16 weeks	Increase in sexual desire and responsiveness
Miller 1998	53	12 weeks	Increase in sense of well-being; decrease in body mass index
Myers 1990	40	10 weeks	Increase in masturbatory pleasure
Raisz 1996	28	9 weeks	Increase in bone formation; decrease in triglycerides and HDL cholesterol
Shah 2006	61	16 weeks	Increase in concentration
Shifren 2006	549	24 weeks	Increase in satisfying sexual activity, desire; decrease in personal distress
Simon 2005	562	24 weeks	Increase in frequency of satisfying sexual activity, desire
Watts 1995	66	24 months	Increase in bone density; decrease in triglycerides and HDL cholesterol
Warnock 2005	107	8 weeks	Increase in sexual desire, interest

HDL, high-density lipoprotein.

dence suggesting that T does not directly increase breast cancer risk in women.

Anti-proliferative effects of androgens in human breast cancer cells in vitro and in vivo

Several preclinical studies using human breast tumor cell lines have investigated the effects of androgens on breast cancer cell growth and proliferation (Table 2). In the majority of studies (13 out of 16) androgens inhibited cellular growth and proliferation and only few studies showed mixed results. The inhibitory effect of 5α-dihydrotestosterone (5α-DHT), T and Δ4-androstenedione (Δ4-Adione) was demonstrated on the growth of the estrogen-sensitive human breast cancer cell line ZR-75-1 (18). The antiproliferative effect of androgens was competitively reversed by the antiandrogen, hydroxyflutamide, indicating an androgen receptor (AR)-mediated mechanism. Similarly, the effects of T and 5α-DHT on cell growth were examined in four different breast cancer cell lines (MCF-7, T47D, MDA MB 435S and BT-20) and demonstrated that T and 5α-DHT inhibited cell growth of all tumor cell lines investigated (19). These observations were corroborated by other studies, demonstrating that androgens inhibit the proliferation of MCF-7 breast cancer cells (20). In human ZR-75-1 breast cancer cells, 5α-DHT alone inhibited basal cell proliferation without a significant influence on cell cycle distribution. Moreover, pretreatment with 5α-DHT for 8 days, while decreasing ZR-75-1 cell number, did not result in loss of estrogen sensitivity. These observations suggest that 5α-DHT which is non-aromatizable to estrogen, is a potent inhibitor of the stimulatory effect on estrogens on breast cancer cell proliferation (21).

Human breast cancer MCF7 cells, which were stably impregnated with functional ARs, retained their capacity to proliferate when estrogens were added, but did not proliferate when androgens were added to growth medium (Figure 1) (22). MCF-7 cells transfected with AR proliferated maximally in the presence of E_2; however, natural and synthetic androgens inhibited cellular proliferation. The inhibition of cell proliferation occurred at physiological androgen concentrations (1 nM) and the effect was reversed by AR antagonist, Casodex. These observations strongly suggest androgen-induced inhibition of cell proliferation via AR-mediated mechanisms (22).

A similar study suggested that 5α-DHT produced in tumor tissue by 5α-reductase 1 possesses an antiproliferative effect primarily in tumors expressing the AR and that aromatase inhibitors are more effective in patients with tumors expressing AR and 5α-reductase type 1 (23, 24). This was attributed to increasing local synthesis of 5α-DHT from T as a result of increased activity of 5α-reductase with concomitant reduction in the available T levels as a substrate for the aromatase thus reducing the aromatase activity and estradiol synthesis (23, 24). Both 5α-DHT and the synthetic androgen mibolerone inhibited E_2-induced proliferation of T-47D breast cancer cells. The effects of androgens were reversed by an AR antagonist, suggesting that androgen action was mediated, at least in part, by the AR (25). Androgens have been hypothesized to stimulate breast cancer simply as substrates for the aromatase acting as estrogen precursors (26).

Additional evidence for the antiproliferative effects of androgens on breast cancer cell growth were derived from studies, in which both aromatizable (Δ^4-Adione) and non-aromatizable (5α-DHT) androgens inhibited MCF-7 cell proliferation (27). This effect was mostly noted when estrogen concentrations were low or absent. This is also noted in breast cancer cells with low expression of aromatase activity. Interestingly, blockade of AR function with the antiandrogen, Casodex, or with the small interfering RNA (siRNA) to block AR protein expression, inhibited the antiproliferative effect of 5α-DHT. In addition it has been suggested that T-mediated growth effects in breast cancer cells were completely inhibited by the aromatase inhibitors letrozole and 4-hydroxy-androstenedione (28). Studies of expression of estrogen-regulated proteins confirmed that T was aromatized

Table 2 Preclinical studies on breast cancer cell growth in the presence of androgens.

Author, year	Breast cancer cell lines	Effects of androgens on cell growth	Comments
Poulin et al. 1988	ZR-75-1	↓	T, 5α-DHT and Δ^4-Adione inhibited cell growth
Ortmann et al. 2002	MCF-7; T47D; MDA MB 435S; BT-20	↓	Both T and 5α-DHT inhibited growth of all cell lines
Greeve et al. 2004	MCF-7 cells	↓	Androgens inhibited cell growth
Zhou et al. 2000	Primary mammary epithelial cells	↓	Androgens mediated down regulation of ER
de Launoit 1991	ZR-75-1	↓	Androgens inhibited cell proliferations
Szelei et al. 1997	MCF-7 cells	↓	Androgens inhibited cell growth
Cops et al. 2008	MCF-7 cells; T-47D cells	↓	Androgens reduced cell growth
Macedo et al. 2006	MCF-7 cells	↓	Androgens reduced cellular proliferation
Kampa et al. 2005	T-47D cells	↓	Inhibition of cell growth via membrane androgen receptors
Buchanan et al. 2005	T47D cells; MCF-7 cells	↓	Androgens inhibited cellular proliferation
Dimitrakakis et al. 2003	Primary mammary epithelial cells of rhesus monkeys	↓	Androgens are associated with reduced cellular proliferation
Macedo et al. 2006	MCF-7 cells	↓	Androgen inhibit cell growth
Liao and Dickson 2002	Rodent mammary epithelial cells in vitro and in vivo	↓ ↑	Androgen promote both antiproliferative and proliferative effects
Hofling et al. 2007	Human study intervention study	↓	Addition of testosterone to estrogen progesterone therapy reduced cellular proliferation
Lippman et al. 1991	MCF-7; G11; HT 39; MDA 231; Evsa E	↓ ↑	Only 1 out of 5 breast cancer cell lines did grow in response to androgens. Four other breast cancer cell lines did not grow in response to androgens in vitro
Birrell et al. 1995	MCF-7; T47D; ZR 75-1; MDA MB 453	↓ ↑	Growth of T47D and ZR 75-1 was inhibited with DHT, whereas MCF-7 cells and MDA MB 453 was stimulated by androgens

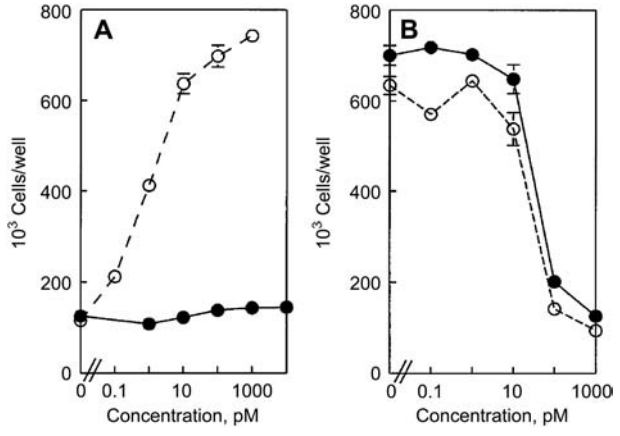

Figure 1 Androgen-induced inhibition of proliferation in human breast cancer MCF 7 cells transfected with androgen receptors. Panel (A) represents a dose-response curve to estradiol (open circles) or to the synthetic high affinity non-aromatizable androgen methyltrieneolone (R1881) (solid circles). Cells were grown for 5 days in 10% charcoal-dextran treated human serum (CDHuS). Panel (B) represents a dose-response curve to R1881. Cells were grown for 5 days in 10% CDHuS supplemented with 0.1 nM (open circles) or 1 nM (solid circles) estradiol. The y-axis shows cell number per well (mean ± SD; the x-axis represents the concentration of R1881 added in pM [Adopted with permission from Ref. (22)].

to estrogen in the MCF-7 cells. Thus, the results indicate that epithelial breast cancer cells possess the ability to aromatize circulating androgens to estrogens (28). These findings, together with data from other studies, suggest that aromatase inhibitors can exert their beneficial effects on breast cancer by increasing the local concentrations of androgens, thus inducing cell apoptosis through membrane androgen receptor activation (29). It should be noted that aromatase inhibitors are currently the treatment of choice for hormone responsive breast cancer, limiting estrogen production with concomitant increase in intracellular androgen levels (30).

Reduced mammary epithelial ERα and increased ERβ expression in response to T was reported, resulting in a marked reversal of the ERα/β ratio and reduced proliferation in the estrogen-treated monkey (31). Moreover, T treatment is associated with a significant reduction in mammary epithelial *myc* gene expression, suggesting that anti-estrogenic effects of T in the mammary gland involve changes in ERα signaling to *myc*. These changes in signaling could be responsible for reduced cellular proliferation in the presence of androgens. The antiproliferative actions of androgens are mediated via activation of the intracellular androgenic signaling (27). In a study of 88 women, addition of T counteracts breast cell proliferation as induced by estrogen and progestin therapy in postmenopausal women (32). Recently, a novel mechanism for specific and direct inhibition of ERα activity by AR in breast cancer cells was identified, adding additional evidence that androgens could be protective in

breast cancer (33). This novel mechanism involves binding of AR to consensus estrogen response elements in vitro, and to a subset of endogenous ERα regulatory sites in vivo (33). More importantly, ERα activity was inhibited by wild type AR and AR variants, with minimal intrinsic transcriptional capacity (33). These observations suggest that the effect of androgens on breast cancer cells could be mediated via inhibition of ERα signaling but not necessarily via activation of AR target gene expression. This novel hypothesis is partially supported by data demonstrating that the constitutive or truncated AR inhibited E_2-induced human breast cancer cell proliferation and reduced expression of E_2-regulated targets such as the PR. These data provide compelling arguments to binding of AR to ERα-regulated genes in vivo and permits proposing of a novel mechanism by which AR regulates ERα activity in breast cancer cells (33).

Alternative views on the role of androgens and ARs in modulating breast cancer cell growth were proposed by several investigators (8, 34, 35). Androgens were hypothesized to exert protective effects on breast tissue, ameliorating proliferative effects of estrogen alone or combined estrogen-progestin therapy.

Clinical and epidemiological studies in premenopausal women

Table 3 lists epidemiological studies, which investigated the potential association of breast cancer risk with T levels in premenopausal women. None of these studies, however, provided unequivocal data to support such contention. The European Perspective on Investigation of Cancer (EPIC) cohort with 370 breast cancer cases suggested that increased androgen levels are associated with increased breast cancer risk (36). Similarly, 65 breast cancer cases were examined and an association between f (T) and breast cancer was noted (37). Data from 197 cases were analyzed and a non-statistically significant increase in overall breast cancer risk based on T, f (T) and Δ4-Adione was found (38). Significant correlations, however, were found in invasive as well as ER+/PR+ breast cancers. Noted limitations of the studies described above include absence of matching sex steroid hormones to menstrual cycle phase and lack of an association between estrogen levels and breast cancer risk. This raises serious doubts on the validity of the data and the conclusions made, given that estrogens promote tumor growth in hormone responsive cancer.

Associations between androgen levels and risk of breast cancer in premenopausal women have been deemed non-significant (39). However, in postmenopausal women, serum T levels were shown to have a significant positive association with the odds of having breast cancer. Interestingly, the values of T in postmenopausal women were far greater than in premenopausal women and approached >155 ng/dL (5.38 nmol/L), a value that is inconsistent with physiological levels of androgens in untreated women, as reported by others (40, 41). Furthermore, the only significant association was noted at values >155 ng/dL (5.38 nmol/L), which raises suspicion

Table 3 Association of testosterone with breast cancer risk in premenopausal women.

Author, year	Type of study	Study numbers	Estrogens				Androgens								Testosterone based risk	Androgen levels adjusted for estrogens
			E1	E1-S	E2	f(E2)	T	f(T)	DHT	DHEA	DHEA-S	4-Adione	Adiol			
Kaaks 2005	Prospective case-control	1096 (370 cases)	–	N/A	–	N/A	+	N/A	N/A	N/A	+	+	N/A		Positive association	No mention
Micheli 2004	Case-control	325 (65 cases)	N/A	N/A	N/A	N/A	–	+	N/A	N/A	–	–	N/A		Positive association	No
Eliassen 2006	Prospective case-control	591 (197 cases)	–	–	+	+	–	–	N/A	N/A	N/A	–	N/A		Association found only for invasive and ER+/PR+ (not overall risk)	Yes
Sturgeon 2004	Case-control	364 (169 cases)	N/A	N/A	–	N/A	–	N/A	N/A	+	N/A	–	N/A		No association	No mention
Thomas 1997	Prospective case-control	230 (62 cases)	N/A	N/A	–	N/A	–	N/A	N/A	N/A	N/A	N/A	N/A		No association	No mention

on the conclusions of this study. On the contrary, others have reported no significant association between androgen levels and breast cancer risk (42, 43). Thus, studies in premenopausal women do not provide unequivocal conclusions linking T to increased breast cancer risk.

Clinical and epidemiological studies in postmenopausal women

Table 4 lists epidemiological studies attempting to establish an association between androgens, estrogens and breast cancer risk in postmenopausal women. However, most of these studies have serious methodological limitations (44–50). Data on androgen levels in most of these studies were not adjusted for estrogen levels. Furthermore, these studies relied mainly on use of inaccurate and insensitive radioimmunoassays to measure total T or f (T) in blood samples in women. Total T was often utilized as a measure of androgen activity, without accounting for the effect of SHBG on the fraction of the active f (T) in women. Because sex steroid hormone levels in women diminish during menopause it is relevant that not only total T is measured but also f (T) and SHBG to account for androgenic activity (30).

Meta-analyses of nine studies were performed finding associations between each circulating sex steroid and breast cancer (51). However, the authors acknowledged that limitation of the androgen assays could confound the findings and stated that: ''*Given this variability it is clear that the reported hormone concentrations are not directly comparable between studies and that any pooled estimate of breast cancer risk in relation to such measures would have to take this into account*.'' Association between T, E_2 and breast cancer was found via meta-analyses of five studies, but serious flaws of the process were acknowledged (52). The authors stated that ''*However possible differences between E_2 and T assay precision, stability within women over time, and intracellular conversion of androgens to estrogens complicate the interpretation of these epidemiological analyses*.''

Contrary to the conclusions drawn from the studies cited above, several recent studies reached a different conclusion (Table 5) (53–56). Mostly, correlations between sex hormones and increased breast cancer risk were only found for E_1 and/or E_2, whereas T showed *no significant association*. It should be noted that the estrogen levels were adjusted for in the latter studies. A study which evaluated postoperative breast cancer cases came to the following conclusion ''*There was no association of T with breast cancer risk*'' (55). Associations between Δ4-Adione and breast cancer were observed, however. Sex steroid levels in patients with benign or malignant breast tumor tissue were assessed, and decreased T levels in all primary breast cancer cases were observed (57). Δ4-Adione was initially deemed to associate with increased breast cancer risk; however, after adjustment for E_1 this risk was attenuated (56). Cross-analysis of two previously completed studies measured mammary cell proliferation via fine needle aspiration (58). Assessing cellular proliferation activity utilizing fine needle aspiration breast biopsy, in response to serum sex steroid levels, demonstrated a correlation with estrogens, but no associations with androgens were found. Comparisons between healthy women and breast cancer patients confirmed antiproliferative effects of androgens on aspirated breast cells and suggest that antiproliferative properties in breast cancer patients could be mediated via downregulation of PR.

Breast hyperplasia was assessed in response to sex steroids in serum samples provided before biopsy, showing only estrogens to be associated with a significant increase in risk (59). The authors noted: ''*We found that higher serum estrogen levels – but not androgen or SHBG – were strongly associated with moderate or florid hyperplasia with or without atypia, breast cancer risk factors, and possible precursors, associated with at least 2-fold increase in breast cancer risk*.'' The assessment based on tissue proliferation marker (ki-67) demonstrated a correlation with estrogens; however, no associations with androgens were found. One might argue that directly measuring tissue activity is a better prognostic tool than depending on serum sex steroid levels, specifically as serum levels are not always representative of activity levels of androgens within the tissue.

A study utilizing the data from NSABP (National Surgical Adjuvant Breast and Bowel Project) found no associations between any sex steroids and increased breast cancer risk (10). This cohort was considered to be a high-risk population for breast cancer, yet no sex steroids were deemed to be associated with its risk. This and other studies highlight the drawbacks of utilizing endogenous sex steroid hormones to identify high-risk breast cancer patients.

In a recent large study of 646 postmenopausal women the association of plasma androgen levels with breast, ovarian and endometrial cancer risk factors among postmenopausal women was assessed (60). The authors concluded that ''*Overall breast cancer risk was not associated with any of the androgens*.'' Furthermore, a study on the effect of addition of T on combined estrogen and progesterone therapy on breast cancer cell proliferation and mammographic breast density in clinical study concluded that ''*testosterone and other androgens may have a protective influence on the breast*'' (61). In addition, T therapy over a 52-week period had no significant effect on digitally quantified absolute or percent dense mammographic area in postmenopausal women (62). These findings strongly argue against an association between T and increased breast cancer risk.

Clinical studies with estrogen and testosterone therapy

''*There is no materially increased risk of breast cancer in users of estrogen alone or esterified estrogen with methyltestosterone compared with non-users. There is an increased risk among those using conjugated estrogen plus progestin*'' (63). In contrast, the effect of adding T to estrogen replacement therapy was assessed utilizing the Nurse's Health Study (64) in which 4610 breast cancer cases were identified during the 24 years of follow-up, with updates on diagnoses occur-

Table 4 Potential positive association of testosterone with breast cancer risk in postmenopausal women.

Author, year	Type of study	Study numbers	Estrogens				Androgens							Testosterone based risk	Androgen levels adjusted for estrogens
			E_1	E_1-S	E_2	f(E_2)	T	f(T)	DHT	DHEA	DHEA-S	4-Adione	Adiol		
Missmer 2004	Prospective case-cohort	965 (322 cases)	+	+	+	+	+	+	N/A	+	+	+	N/A	Positive association	Yes, associations with T rendered non-significant after adjustment
Dorgan 1997	Prospective nested case-control	3375 (72 cases)	–	N/A	+	+	+	N/A	N/A	N/A	+	+	N/A	Positive association	No
Cummings 2006	Prospective community-cohort	574 (196 cases)	N/A	N/A	–	N/A	+	N/A	N/A	N/A	N/A	N/A	N/A	Positive association	Yes
Kahan 2006	Case-control	204 (102 cases)	–	N/A	–	N/A	+	N/A	N/A	N/A	N/A	–	N/A	Positive association	Yes
Manjer 2003	Population prospective cohort	173 cases	+	N/A	+	N/A	+	N/A	N/A	–	N/A	–	N/A	Positive association	Yes
Sieri 2009	Nested case-control	165 cases	N/A	N/A	–	N/A	+	+	N/A	N/A	N/A	N/A	N/A	Positive association	Yes
Yu 2003	Population case-control	600 (300 cases)	+	–	–	N/A	+	N/A	N/A	N/A	–	N/A	N/A	Positive association	No mention
Cauley 1999	Prospective case-cohort	341 (97 cases)	–	–	+	–	+	+	N/A	N/A	–	–	N/A	Positive association	Yes
Berrino 2005	Prospective cohort	107 (31 recurrences)	N/A	N/A	+	N/A	+	N/A	N/A	N/A	N/A	N/A	N/A	Positive association	No
Micheli 2007	Retrospective	194	N/A	N/A	N/A	N/A	+	N/A	N/A	N/A	N/A	N/A	N/A	Positive association	No
Ho 2009	Cross-sectional case-control	134 (68 cases)	N/A	N/A	–	N/A	+	N/A	N/A	N/A	N/A	N/A	N/A	Positive association	No mention
Key 2002	Reanalysis of nine prospective studies	2428 (663 cumulative cases)	+	+	+	+	+	N/A	N/A	+	+	+	N/A	Positive association	Associations with androgens rendered borderline significant after adjustment

Table 5 Potential negative association of testosterone with breast cancer risk in postmenopausal women.

Author, year	Type of study	Study numbers	Estrogens				Androgens							Testosterone based risk	Androgen levels adjusted for estrogens
			E_1	E_1-S	E_2	f(E_2)	T	f(T)	DHT	DHEA	DHEA-S	4-Adione	Adiol		
Adly 2006	Case-control	331 (197 cases)	+	+	–	–	–	N/A	N/A	–	N/A	–	–	No association	Yes. Adjustment eliminated association between Adiol and risk
Rock 2008	Nested case-control	153 case-control pairs	N/A	N/A	+	+	–	–	N/A	N/A	N/A	N/A	N/A	No association	No mention
Beattie 2006	Case-cohort	410 (135 cases)	N/A	N/A	–	N/A	–	N/A	N/A	N/A	N/A	N/A	N/A	No association	No mention
Zeleniuch-Jacquotte 1997	Case-control	7054 (130 cases)	N/A	N/A	+	N/A	–	N/A	N/A	N/A	–	N/A	N/A	No association	Yes. Eliminated association between T and risk
Buist 2001	Prospective case-cohort	8203 (109 cases)	N/A	N/A	–	+ (– after BMI)	–	–	N/A	N/A	N/A	N/A	N/A	No association	No mention
Van Staa 2009	Case-cohort	8412 (21 03 Tth users)	N/A	N/A	N/A	N/A	–	N/A	N/A	N/A	N/A	N/A	N/A	No association	No mention
Mady 2000	Case-control	33	+	+	+	N/A	–	N/A	N/A	N/A	N/A	N/A	N/A	Negative association	No mention
Schairer 2005	Case-control	331 (197 cases)	+	+	+	N/A	–	N/A	N/A	–	N/A	–	–	No association	Yes
Lipworth 1996	Population based case-control	244 (122 cases)	+	N/A	N/A	N/A	–	N/A	N/A	N/A	N/A	+	N/A	No association	No mention
Tworoger 2005	Nested case-control	1989 (446 cases)	N/A	N/A	–	+	–	–	N/A	N/A	N/A	N/A	N/A	No association	No mention
Zeleniuch-Jacquotte 2004	Nested case-control	7054 (297 cases)	+	N/A	+	N/A	–	N/A	N/A	N/A	–	–	N/A	No association	Yes

BMI, body mass index.

ring every 2 years. Adding T to estrogen therapy showed a 2.5-fold greater risk of breast cancer. The risk was deemed significantly larger than estrogen alone and marginally greater than E + P. However, the study provided no evidence that T alone modulated tumor growth responses in ER + /PR + tumors. Thus, increased breast cancer risk could be explained by the increased E_2 levels rather than increased T levels.

What is perplexing in analysis of these data is that T users had an increased risk when the preparation was used for less than 5 years only. When treated for duration longer than 5 years, no significant associations were observed. This finding casts serious doubts on the outcome of this study and does not support the conclusions made by the authors that an association exits between increased breast cancer risk and T. These findings are also contrary to the conclusion made by several authors (32, 63–69). Thus, it is possible that these data are a result of confounding recruitment bias similar to that noted in the UK Million Women Study.

Non-significant associations were found between estrogen plus testosterone treatment and invasive breast cancer utilizing the WHI cohort (65). However, an increased risk for Estratest, a preparation of esterified estrogens and methyltestosterone was noted. Yet, significance was only reached in cases that had been taking the preparation for less than 1 year, with non-significant results for long-term users. This is difficult to reconcile based on the mechanism of action of sex steroid hormones.

The beneficial effects of T treatment in women's health have been proposed to warrant closely monitored and cautious use, particularly as there is only insufficient valid evidence to link T to breast cancer risk (57, 66). Addition of T to hormone replacement therapy (HRT) has been observed to decrease estrogen/progestogen (E/P) induced breast cell proliferation over a period of 6 months (32). The authors concluded: ''*Addition of T may counteract breast cell proliferation as induced by estrogen/progestogen therapy in postmenopausal women.*''

Breast cancer incidence in 508 postmenopausal women was assessed upon addition of T to HRT (69). Lowest incidence was found when only T treatment was used (238 per 100,000 women years), whereas E/P/T treatment led to an incidence of 293 per 100,000 women years. The very low incidence of breast cancer rates in response to T treatment lead to the hypothesis that T could lower the risk of conventional HRT.

Breast cancer recurrence and testosterone

Endogenous T levels have been proposed to serve as prognostic factor for breast cancer, whereas elevated levels are suggested to predict recurrent disease (70, 71). The above mentioned studies did not address the methodological limitation of reduced androgen and estrogen levels in women following menopause, and the shortcoming of T and f (T) assays. The concept of utilizing T as a prognostic factor also contradicted use of aromatase inhibitors as first line therapy in postmenopausal breast cancer. Inhibiting the conversion of androgens to estrogens increases intracellular androgen levels and limits the amount of estrogens available for biological activity. The authors made no effort to address how T increases risk of recurrence without conversion to estrogen while failing to adjust T levels for the estrogen effect (Table 6). However, multiple studies contradicted this conclusion with the following statement: ''*These data do not support the use of endogenous sex hormone levels to identify women who are at particular risk of breast cancer…*'' (10, 72). Lamenting further ''*Contrary to expectations we did not observe a relationship between serum T concentrations and risk for recurrence*'' (10).

Testosterone and mammographic density and breast cancer

Circulating levels of E_2 and T were found to be associated with breast cancer before and after adjusting for mammographic density, suggesting sex steroid levels to be associated with breast cancer independent of mammographic density (73). However, the authors failed to adjust T levels for estrogens and did not address the occurrence of higher estrogen and androgen levels in premenopausal women. In line with the authors' argument, higher sex steroid levels would lead to a more pronounced relationship between sex steroid levels and mammographic density in premenopausal women. Thus far, no such relationships have been observed, casting doubt on the conclusions made in the aforementioned study (73). In addition, studies have shown negative associations between plasma androgens and mammographic breast density in pre- and postmenopausal women (74, 75). Several other studies have shown a lack of association between T and mammographic density or independent breast cancer risk (Table 7) (73–81).

Relationship between testosterone therapy and breast cancer

A retrospective case-cohort study and a case-control study found no major increase in the risk of ischemic heart disease or breast cancer in women using T (62, 82, 83). Safety was assessed for a period of 52 weeks, resulting in four total breast cancer cases in the treatment group during that time (84). However, two of the cases showed symptoms before the start of the trial or had been treated with estrogen for 27 years in addition to a strong family history of breast cancer. Long-term safety of T treatment has not been assessed; however, evidence thus far does not indicate any association with increased breast cancer risk.

Studies with female to male transsexuals

The long-term safety use of T in female to male transsexuals showed no serious adverse events even at pharmacological doses. Safety outcomes of women using T therapy in clinical

Table 6 Testosterone association with breast cancer recurrence.

Author, year	Type of study	Study numbers	Estrogens				Androgens							Testosterone based risk	Androgen levels adjusted for estrogens
			E1	E1-S	E2	f(E2)	T	f(T)	DHT	DHEA	DHEA-S	4-Adione	Adiol		
Berrino 2005	Prospective cohort	107 (31 recurrences)	N/A	N/A	+	N/A	+	N/A	N/A	N/A	N/A	N/A	N/A	Positive association	No
Micheli 2007	Retrospective	194	N/A	N/A	N/A	N/A	+	N/A	N/A	N/A	N/A	N/A	N/A	Positive association	No
Beattie 2006	Case-cohort	410 (135 cases)	N/A	N/A	–	N/A	–	N/A	N/A	N/A	N/A	N/A	N/A	No association	No mention
Rock 2008	Nested case-control	153 case-control pairs	N/A	N/A	+	+	–	–	N/A	N/A	N/A	N/A	N/A	No association	No mention

Table 7 Association of testosterone with mammographic density in Pre-/postmenopausal women.

Author, year	Type of study	Study numbers	Estrogens				Androgens							Testosterone based risk	Androgen levels adjusted for estrogens
			E1	E1-S	E2	f(E2)	T	f(T)	DHT	DHEA	DHEA-S	4-Adione	Adiol		
Tamimi 2007	Nested case-control	773 (253 cases)	N/A	N/A	+	+	+	+	N/A	N/A	N/A	N/A	N/A	Positive association	No mention
Verheus 2007	Cross-sectional	775	–	N/A	–	–	–	–	N/A	N/A	–	–	N/A	No association	No mention
Warren 2006	Cross-sectional	1413	–	–	–	N/A	–	N/A	N/A	N/A	N/A	–	N/A	No association	No mention
Bremnes 2007	Population cross-sectional	722	+	N/A	–	–	–	–	N/A	N/A	–	–	N/A	No association	No mention
Walker 2009	Cross-sectional, premenopausal only	494	+	N/A	N/A	N/A	–	N/A	N/A	N/A	–	–	N/A	Negative association	No mention
Yong 2009	Cross-sectional, premenopausal only	191	–	–	+	N/A	–	N/A	N/A	–	–	–	N/A	No association	No
McCormack 2009	Cross-sectional	270	–	N/A	–	–	–	N/A	N/A	N/A	N/A	N/A	N/A	No association	No mention
Greendale 2005	Cross-sectional	404	+	N/A	+	N/A	–	N/A	N/A	N/A	N/A	N/A	N/A	No association	Yes

settings were explored with 2103 women treated with T and 6309 controls (85). There were no major differences in the rate of ischemic heart disease or breast cancer in subjects using T compared to controls and no difference in outcomes with or without HRT. There were no cases of clitorimegaly. However, there was an increase in acne and hirsutism. A review of data on androgen treatment of female to male transsexuals was performed and the authors also noted that there were no serious side effects and no increased incidence in breast cancer (86). These studies strongly suggest that androgens do not increase breast cancer risk.

Clinical studies with aromatase inhibitors

Aromatase inhibitors have been shown to improve disease-free survival among postmenopausal women with ER+ early breast cancer (12–17). Aromatase inhibitors block estrogen biosynthesis by inhibiting the conversion of androgens (Δ^4-Adione and T) to E_1 and E_2 (86). Inhibition of the aromatase is associated with increased levels of T and Δ4-Adione (12–17). Interestingly, women with hormone responsive breast cancer treated with aromatase inhibitors do not experience increased incidence of contralateral breast cancer and the breast tumor regresses with aromatase inhibitor treatment. If androgens, indeed, increase breast cancer risk, as has been proposed previously (6), then one would expect that women treated with aromatase inhibitors to have higher incidence of contralateral breast cancer and the existing cancer would not remit (6, 44–50). On the contrary, this has not been noted in the many studies with aromatase inhibitor treatment of women with breast cancer.

Studies in polycystic ovarian disease (PCOD)

PCOD is the most common endocrine disease in women of reproductive age occurring in 5–10% of women. Serum and urinary androgens were measured and authors suggested that breast cancer was more common in women with PCOD (87). On the contrary, other authors argued that T was protective against breast cancer in women with PCOD (88). A retrospective study from the Mayo Clinic showed no increased risk of breast cancer in women, but subgroup analysis of postmenopausal women with breast cancer showed a relationship; however, only five cases were analyzed (89).

More recent reviews examining the association of PCOD and breast cancer have not shown any increased risk (90). In fact a 50% reduction of breast cancer risk was noted in study subjects with PCOD, examining a large cohort of 4730 women with breast cancer and 4688 controls (91). A study utilizing the Iowa Women's Health Study also failed to show an increase in risk of women with a self-reported diagnosis of PCOD (92). In addition, a cohort of 786 women diagnosed with PCOD by histological examination did not find an association between breast cancer and PCOD (93).

Authors believe existing data to exclude a strong association between polycystic ovarian syndrome (PCOS) and breast cancer as opposed to the established positive association of PCOD and endometrial cancer (94). Even a recently performed meta-analysis on the association between PCOD and gynecological malignancies only deemed 8 out of 15 studies were eligible for assessment (95). Women with PCOD were more likely to develop endometrial (odds ratio, OR, 2.70) and ovarian cancer (OR 2.52), but no increased risk of breast cancer (OR 0.88).

Clinical evaluation of 273 women with PCOD and 276 controls was performed (96). A significant positive family history of breast cancer was found among the control group (4.35% vs. 1.30%, $p=0.02$). Patients with PCOS had three cases of breast cancer among relatives and the control group had 12 cases of breast cancer among relatives. Therefore, this study revealed that breast cancer in the relatives of infertile population (without PCOS) was significantly higher than PCOS patients. These results are inconsistent with those reported by others, which were attributed to small sample size, genetic differences and other environmental factors (97, 98).

Discussion

In women, androgens are synthesized in the ovaries and adrenal glands, and obviously play an important physiological role in women's health. In fact the concentrations of androgens in women are several-fold higher than that of estrogens, allowing utilization of androgens as substrates for aromatization to estrogens under physiological conditions. Androgens have a wide range of physiological activities in many tissues and AR expression has been demonstrated in such tissues. Furthermore, T therapy has been utilized in treatment of a host of endocrine and sexual disorders in women, since the late 1930s, with little or no reported serious side effects (1). Interestingly, several epidemiological studies have reported an association between T levels and increased breast cancer risk. However, considerable contradictory and often opposite associations between T levels and increased breast cancer risk were also reported. These discrepancies are attributed, in part, to lack of adjustment for estrogen levels and confounding factors associated with T assays in women, such as unreliability, imprecision and inaccuracy of T assays. It is also clear from the evidence discussed in this review that existing data do not support androgens as a risk for breast cancer in women; in fact, most data support a protective role for androgens in breast cancer.

In vitro studies with breast tumor cell lines (Figure 1 and Table 2) strongly suggest that androgens provide a protective effect, as they inhibit tumor cell growth. Closer examination of preclinical studies (Table 2) clearly highlighted the antiproliferative effects of androgens in breast cancer cell growth, despite their conversion to estrogens. Similarly, clinical and non-human primate studies suggest that androgens inhibit mammary epithelial proliferation and breast growth, whereas conventional estrogen treatment suppresses endogenous androgens (98, 99). Studies assessing tissue activity

in response to serum sex steroid levels, utilizing fine needle aspiration breast biopsy, and expression of Ki-67 demonstrated a correlation with estrogens; however, no associations with androgens were found (58, 59). These observations do not corroborate the notion that androgens increase breast cancer risk.

Recently, Raynaud et al. (100) reviewed the literature concerning the role of T and other androgens in breast cancer and provided a comprehensive discussion of biotransformation of androgens, and various biological effects of androgens in normal and breast cancer. The authors also assessed the risk of breast cancer and T levels in post- and premenopausal women based on available data (100). A host of earlier epidemiological studies in pre- and postmenopausal women (Tables 3 and 4) showed no consistencies among these studies and many of the conclusions drawn are based on data that were not adjusted for estrogen levels and based on imprecise and insensitive T assays. Recent examination of data in the literature (98, 100) led to similar conclusions in that epidemiological studies have significant methodological limitations and thus provide inconclusive results. In contrast, several recent epidemiological and clinical studies have emphatically stated that androgens are not associated with increased breast cancer risk (60, 61, 101).

More importantly, data from clinical studies with exogenous androgens utilized in treatment of women with endocrine and sexual disorders did not show increased incidence of breast cancer (62, 81, 83). No adverse effects were noted on mammographic density during T therapy (62). No increase in the rate of invasive breast cancer was noted when T was used to treat reduced sexual desire (81), even when estrogens were not added to a woman's hormonal replacement regimen (83). Furthermore, women with polycystic ovary disease and who are hyperandrogenized did not show increased incidence of breast cancer. In addition, data from studies on female to male transsexuals, who are treated with supraphysiological doses of T for a long time period prior to surgical procedures, did not report increased risk of breast cancer. Finally, women with hormone responsive primary breast cancer are treated with aromatase inhibitors, as a first line therapy. This treatment blocks conversion of androgens to estrogens, thus elevating androgen levels. These women do not experience increased incidence of contralateral breast cancer nor do they experience increased tumor growth. In conclusion, data from various studies available to date strongly suggest that no evidence exists to implicate T in increased breast cancer risk, and some data are suggestive of a protective role of androgens against breast cancer. Clearly, large prospective trials will be necessary to confirm or contradict these clinical and preclinical observations.

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References

1. Traish AM, Feeley RJ, Guay AT. Testosterone therapy in women with gynecological and sexual disorders: a triumph of clinical endocrinology from 1938 to 2008. J Sex Med 2009;6: 334–51.
2. Braunstein GD. Safety of testosterone treatment in postmenopausal women. Fertil Steril 2007;88:1–17.
3. Braunstein GD. Management of female sexual dysfunction in postmenopausal women by testosterone administration: safety issues and controversies. J Sex Med 2007;4:859–66.
4. Braunstein GD. The Endocrine Society Clinical Practice Guideline and The North American Menopause Society position statement on androgen therapy in women: another one of Yogi's forks. J Clin Endocrinol Metab 2007;92:4091–3.
5. Shufelt CL, Braunstein GD. Safety of testosterone use in women. Maturitas 2009;63:63–6.
6. Schover LR. Androgen therapy for loss of desire in women: Is the benefit worth the breast cancer risk? Fertil Steril 2008; 90:129–40.
7. Rosner W, Auchus RJ, Azziz R, Sluss PM, Raff H. Position statement: utility, limitations, and pitfalls in measuring testosterone: an endocrine society position statement. J Clin Endocrinol Metab 2007;92:405–13.
8. Somboonporn W, Davis SR. National Health and Medical Research Council. Testosterone effects on the breast: implications for testosterone therapy for women. Endocr Rev 2004;25:374–88.
9. Birrell SN, Bentel JM, Hickey TE, Ricciardelli C, Weger MA, Horsfall DJ, Tilley WD. Androgens induce divergent proliferative responses in human breast cancer cell lines. J Steroid Biochem Mol Biol 1995;52:459–67.
10. Beattie MS, Costantino JP, Cummings SR, Wickerham DL, Vogel VG, Dowsett M, Folkerd EJ, Willett WC, Wolmark N, Hankinson SE. Endogenous sex hormones, breast cancer risk, and tamoxifen response: an ancillary study in the NSABP breast cancer prevention trial (P-1). J Natl Cancer Inst 2006; 98:110–5.
11. Anders CK, Carey LA. Biology, metastatic patterns, and treatment of patients with triple-negative breast cancer. Clin Breast Cancer 2009;9:S73–S81.
12. Smith IE, Dowsett M. Aromatase inhibitors in breast cancer. N Engl J Med 2003;348:2431–42.
13. Mouridsen H, Giobbie-Hurder A, Goldhirsch A, Thürlimann B, Paridaens R, Smith I, Mauriac L, Forbes JF, Price KN, Regan MM, Gelber RD, Coates AS. BIG 1-98 Collaborative Group, letrozole therapy alone or in sequence with tamoxifen in women with breast cancer. N Engl J Med 2009;361:766–76.
14. Goss PE, Ingle JN, Martino S, Robert NJ, Muss HB, Piccart MJ, Castiglione M, Tu D, Shepherd LE, Pritchard KI, Livingston RB, Davidson NE, Norton L, Perez EA, Abrams JS, Therasse P, Palmer MJ, Pater JL. A randomized trial of letrozole in postmenopausal women after five years of tamoxifen therapy for early-stage breast cancer. N Engl J Med 2003;349: 1793–802.
15. Coombes RC, Hall E, Gibson LJ, Paridaens R, Jassem J, Delozier T, Jones SE, Alvarez I, Bertelli G, Ortmann O, Coates AS, Bajetta E, Dodwell D, Coleman RE, Fallowfield LJ, Mickiewicz E, Andersen J, Lønning PE, Cocconi G, Stewart A, Stuart N, Snowdon CF, Carpentieri M, Massimini G, Bliss JM, van de Velde C, Intergroup Exemestane Study. A randomized trial of exemestane after two to three years of tamoxifen ther-

apy in postmenopausal women with primary breast cancer. N Engl J Med 2004;350:1081–92.
16. Thürlimann B, Keshaviah A, Coates AS, Mouridsen H, Mauriac L, Forbes JF, Paridaens R, Castiglione-Gertsch M, Gelber RD, Rabaglio M, Smith I, Wardley A, Price KN, Goldhirsch A, Breast International Group (BIG) 1-98 Collaborative Group. A comparison of letrozole and tamoxifen in postmenopausal women with early breast cancer. N Engl J Med 2005;353:2747–57.
17. Rossi E, Morabito A, Di Rella F, Esposito G, Gravina A, Labonia V, Landi G, Nuzzo F, Pacilio C, De Maio E, Di Maio M, Piccirillo MC, De Feo G, D'Aiuto G, Botti G, Chiodini P, Gallo C, Perrone F, de Matteis A. Endocrine effects of adjuvant letrozole compared with tamoxifen in hormone-responsive postmenopausal patients with early breast cancer: the HOBOE trial. J Clin Oncol 2009;27:3192–7.
18. Poulin R, Baker D, Labrie F. Androgens inhibit basal and estrogen-induced cell proliferation in the ZR-75-1 human breast cancer cell line. Breast Cancer Res Treat 1988;12: 213–25.
19. Ortmann J, Prifti S, Bohlmann MK, Rehberger-Schneider S, Strowitzki T, Rabe T. Testosterone and 5α-dihydrotestosterone inhibit in vitro growth of human breast cancer cell lines. Gynecol Endocrinol 2002;16:113–20.
20. Greeve MA, Allan RK, Harvey JM, Bentel JM. Inhibition of MCF-7 breast cancer cell proliferation by 5α-dihydrotestosterone; a role for p21(Cip1/Waf1). J Mol Endocrinol 2004;32: 793–810.
21. de Launoit Y, Dauvois S, Dufour M, Simard J, Labrie F. Inhibition of cell cycle kinetics and proliferation by the androgen 5α-dihydrotestosterone and antiestrogen N,n-butyl-N-methyl-11-[16′α-chloro-3′,17β-dihydroxy-estra-1′,3′,5′-(10')triene-7′α-yl] undecanamide in human breast cancer ZR-75-1 cells. Cancer Res 1991;11:2797–802.
22. Szelei J, Jimenez J, Soto AM, Luizzi MF, Sonnenschein C. Androgen-induced inhibition of proliferation in human breast cancer MCF7 cells transfected with androgen receptor. Endocrinology 1997;138:1406–12.
23. Suzuki T, Darnel AD, Akahira JI, Ariga N, Ogawa S, Kaneko C, Takeyama J, Moriya T, Sasano H. 5α-Reductases in human breast carcinoma: possible modulator of in situ androgenic actions. J Clin Endocrinol Metab 2001;86:2250–7.
24. Suzuki T, Miki Y, Moriya T, Akahira J, Ishida T, Hirakawa H, Yamaguchi Y, Hayashi S, Sasano H. 5α-Reductase type 1 and aromatase in breast carcinoma as regulators of in situ androgen production. Int J Cancer 2007;120:285–91.
25. Cops EJ, Bianco-Miotto T, Moore NL, Clarke CL, Birrell SN, Butler LM, Tilley WD. Antiproliferative actions of the synthetic androgen, mibolerone, in breast cancer cells are mediated by both androgen and progesterone receptors. J Steroid Biochem Mol Biol 2008;110:236–43.
26. Nicolás Díaz-Chico B, Germán Rodríguez F, González A, Ramírez R, Bilbao C, Cabrera de León A, Aguirre Jaime A, Chirino R, Navarro D, Díaz-Chico JC. Androgens and androgen receptors in breast cancer. J Steroid Biochem Mol Biol 2007;105:1–15.
27. Macedo LF, Guo Z, Tilghman SL, Sabnis GJ, Qiu Y, Brodie A. Role of androgens on MCF-7 breast cancer cell growth and on the inhibitory effect of letrozole. Cancer Res 2006;66: 7775–82.
28. Sonne-Hansen K, Lykkesfeldt AE. Endogenous aromatization of testosterone results in growth stimulation of the human MCF-7 breast cancer cell line. J Steroid Biochem Mol Biol 2005;93:25–34.
29. Kampa M, Nifli AP, Charalampopoulos I, Alexaki VI, Theodoropoulos PA, Stathopoulos EN, Gravanis A, Castanas E. Opposing effects of estradiol- and testosterone-membrane binding sites on T47D breast cancer cell apoptosis. Exp Cell Res 2005;307:41–51.
30. Macedo LF, Sabnis G, Brodie A. Aromatase inhibitors and breast cancer. Ann NY Acad Sci 2009;1155:162–73.
31. Dimitrakakis C, Zhou J, Wang J, Belanger A, LaBrie F, Cheng C, Powell D, Bondy C. A physiologic role for testosterone in limiting estrogenic stimulation of the breast. Menopause 2003; 10:292–8.
32. Hofling M, Hirschberg AL, Skoog L, Tani E, Hagerstrom T, von Schoultz B. Testosterone inhibits estrogen/progestogen-induced breast cell proliferation in postmenopausal women. Menopause 2007;14:183–90.
33. Peters AA, Buchanan G, Ricciardelli C, Bianco-Miotto T, Centenera MM, Harris JM, Jindal S, Segara D, Jia L, Moore NL, Henshall SM, Birrell SN, Coetzee GA, Sutherland RL, Butler LM, Tilley WD. Androgen receptor inhibits estrogen receptor-α activity and is prognostic in breast cancer. Cancer Res 2009;69:6131–40.
34. Birrell SN, Butler LM, Harris JM, Buchanan G, Tilley WD. Disruption of androgen receptor signaling by synthetic progestins may increase risk of developing breast cancer. FASEB J 2007;21:2285–93.
35. Labrie F. Dehydroepiandrosterone, androgens and the mammary gland. Gynecol Endocrinol 2006;22:118–30.
36. Kaaks R, Berrino F, Key T, Rinaldi S, Dossus L, Biessy C, Secreto G, Amiano P, Bingham S, Boeing H, Bueno de Mesquita HB, Chang-Claude J, Clavel-Chapelon F, Fournier A, van Gils CH, Gonzalez CA, Gurrea AB, Critselis E, Khaw KT, Krogh V, Lahmann PH, Nagel G, Olsen A, Onland-Moret NC, Overvad K, Palli D, Panico S, Peeters P, Quirós JR, Roddam A, Thiebaut A, Tjønneland A, Chirlaque MD, Trichopoulou A, Trichopoulos D, Tumino R, Vineis P, Norat T, Ferrari P, Slimani N, Riboli E. Serum sex steroids in premenopausal women and breast cancer risk within the European prospective investigation into cancer and nutrition (EPIC). J Natl Cancer Inst 2005;97:755–65.
37. Micheli A, Muti P, Secreto G, Krogh V, Meneghini E, Venturelli E, Sieri S, Pala V, Berrino F. Endogenous sex hormones and subsequent breast cancer in premenopausal women. Int J Cancer 2004;112:312–8.
38. Eliassen AH, Missmer SA, Tworoger SS, Spiegelman D, Barbieri RL, Dowsett M, Hankinson SE. Endogenous steroid hormone concentrations and risk of breast cancer among premenopausal women. J Natl Cancer Inst 2006;98:1406–15.
39. Ho CC, Rohaizak M, Zulkifli SZ, Siti-Aishah MA, Nor-Aini U, Sharifah-Noor-Akmal SH. Serum sex hormone levels in pre- and postmenopausal breast cancer patients. Singapore Med J 2009;50:513–8.
40. Davis SR, Davison SL, Donath S, Bell RJ. Circulating androgen levels and self-reported sexual function in women. J Am Med Assoc 2005;294:91–6.
41. Davison SL, Bell R, Donath S, Montalto JG, Davis SR. Androgen levels in adult females: changes with age, menopause, and oophorectomy. J Clin Endocrinol Metab 2005;90:847–53.
42. Sturgeon SR, Potischman N, Malone KE, Dorgan JF, Daling J, Schairer C, Brinton LA. Serum levels of sex hormones and breast cancer risk in premenopausal women: a case-control study. Cancer Causes Control 2004;15:45–53.
43. 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 premenopau-

sal women on the island of Guernsey. Br J Cancer 1997; 75:1075–9.
44. Cauley JA, Lucas FL, Kuller LH, Stone K, Browner W, Cummings SR. Elevated serum estradiol and testosterone concentrations are associated with a high risk for breast cancer. Study of osteoporotic fractures research group. Ann Intern Med 1999;130:270–7.
45. Dorgan JF, Longcope C, Stephenson HE Jr, Falk RT, Miller R, Franz C, Kahle L, Campbell WS, Tangrea JA, Schatzkin A. Serum sex hormone levels are related to breast cancer risk in postmenopausal women. Environ Health Perspect 1997; 105:583–5.
46. Manjer J, Johansson R, Berglund G, et al. Postmenopausal breast cancer risk in relation to sex steroid hormones, prolactin and SHBG (Sweden). Cancer Causes Control 2003;14: 599–607.
47. Missmer SA, Eliassen AH, Barbieri RL, Hankinson SE. Endogenous estrogen, androgen, and progesterone concentrations and breast cancer risk among postmenopausal women. J Natl Cancer Inst 2004;96:1856–65.
48. Wang B, Mi M, Wang J, Wei N, Zhang Q, Zhu J, Yang S, Guo B, Xu J, Yang X. Does the increase of endogenous steroid hormone levels also affect breast cancer risk in Chinese women? A case-control study in Chongqing, China. Int J Cancer 2009;124:1892–9.
49. Yu H, Shu XO, Shi R, Dai Q, Jin F, Gao YT, Li BD, Zheng W. Plasma sex steroid hormones and breast cancer risk in Chinese women. Int J Cancer 2003;105:92–7.
50. Tworoger SS, Missmer SA, Barbieri RL, Willett WC, Colditz GA, Hankinson SE. Plasma sex hormone concentrations and subsequent risk of breast cancer among women using postmenopausal hormones. J Natl Cancer Inst 2005;97:595–602.
51. Key T, Appleby P, Barnes I, Reeves G, Endogenous Hormones and Breast Cancer Collaborative Group. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. J Natl Cancer Inst 2002;94: 606–16.
52. Hankinson SE, Eliassen AH. Endogenous estrogen, testosterone and progesterone levels in relation to breast cancer risk. J Steroid Biochem Mol Biol 2007;106:24–30.
53. Zeleniuch-Jacquotte A, Bruning PF, Bonfrer JM, 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.
54. Zeleniuch-Jacquotte A, Shore RE, Koenig KL, Akhmedkhanov A, Afanasyeva Y, Kato I, Kim MY, Rinaldi S, Kaaks R, Toniolo P. Postmenopausal levels of oestrogen, androgen, and SHBG and breast cancer: long-term results of a prospective study. Br J Cancer 2004;90:153–9.
55. Lipworth L, Adami HO, Trichopoulos D, Carlstrom K, Mantzoros C. Serum steroid hormone levels, sex hormone-binding globulin, and body mass index in the etiology of postmenopausal breast cancer. Epidemiology 1996;7:96–100.
56. Adly L, Hill D, Sherman ME, Sturgeon SR, Fears T, Mies C, Ziegler RG, Hoover RN, Schairer C. Serum concentrations of estrogens, sex hormone-binding globulin, and androgens and risk of breast cancer in postmenopausal women. Int J Cancer 2006;119:2402–7.
57. Mady EA, Ramadan EE, Ossman AA. Sex steroid hormones in serum and tissue of benign and malignant breast tumor patients. Dis Markers 2000;16:151–7.
58. Hofling M, Lofgren L, von Schoultz E, Carlstrom K, Soderqvist G. Associations between serum testosterone levels, cell proliferation and progesterone receptor content in normal and malignant breast tissue in postmenopausal women. Gynecol Endocrinol 2008;24:405–10.
59. Schairer C, Hill D, Sturgeon SR, Fears T, Mies C, Ziegler RG, Hoover RN, Sherman ME. Serum concentrations of estrogens, sex hormone binding globulin, and androgens and risk of breast hyperplasia in postmenopausal women. Cancer Epidemiol Biomarkers Prev 2005;14:1660–5.
60. Danforth KN, Eliassen AH, Tworoger SS, Missmer SA, Barbieri RL, Rosner BA, Colditz GA, Hankinson SE. The association of plasma androgen levels with breast, ovarian and endometrial cancer risk factors among postmenopausal women. Int J Cancer 2010;126:199–207.
61. Hofling M, Ma L, Sahlin L, Haglund C, Nordling S, von Schoultz B, Cline JM. Expression of the androgen receptor and syndecan-1 in breast tissue during different hormonal treatments in cynomolgus monkeys. Climacteric 2009;12:72–9.
62. Davis SR, Hirschberg AL, Wagner LK, Lodhi I, von Schoultz B. The effect of transdermal testosterone on mammographic density in postmenopausal women not receiving systemic estrogen therapy. J Clin Endocrinol Metab 2009;12:4907–13.
63. Jick SSDS, Hagberg KW, Kaye JADPH, Jick H. Postmenopausal estrogen-containing hormone therapy and the risk of breast cancer. Obstet Gynecol 2009;113:74–80.
64. Tamimi RM, Hankinson SE, Chen WY, Rosner B, Colditz GA. Combined estrogen and testosterone use and risk of breast cancer in postmenopausal women. Arch Intern Med 2006;166: 1483–9.
65. Ness RB, Albano JD, McTiernan A, Cauley JA. Influence of estrogen plus testosterone supplementation on breast cancer. Arch Intern Med 2009;169:41–6.
66. Somboonporn W. Testosterone therapy for postmenopausal women: efficacy and safety. Semin Reprod Med 2006;24:115–24.
67. Bitzer J, Kenemans P, Mueck AO, FSDeducation Group. Breast cancer risk in postmenopausal women using testosterone in combination with hormone replacement therapy. Maturitas 2008;59:209–18.
68. Dimitrakakis C, Zhou J, Bondy CA. Androgens and mammary growth and neoplasia. Fertil Steril 2002;77:26–33.
69. Dimitrakakis C, Jones RA, Liu A, Bondy CA. Breast cancer incidence in postmenopausal women using testosterone in addition to usual hormone therapy. Menopause 2004;11:531–5.
70. Berrino F, Pasanisi P, Bellati C, Venturelli E, Krogh V, Mastroianni A, Berselli E, Muti P, Secreto G. Serum testosterone levels and breast cancer recurrence. Int J Cancer 2005; 113:499–502.
71. Micheli A, Meneghini E, Secreto G, Berrino F, Venturelli E, Cavalleri A, Camerini T, Di Mauro MG, Cavadini E, De Palo G, Veronesi U, Formelli F. Plasma testosterone and prognosis of postmenopausal breast cancer patients. J Clin Oncol 2007;25:2685–90.
72. Rock CL, Flatt SW, Laughlin GA, Gold EB, Thomson CA, Natarajan L, Jones LA, Caan BJ, Stefanick ML, Hajek RA, Al-Delaimy WK, Stanczyk FZ, Pierce JP, Women's Healthy Eating and Living Study Group. Reproductive steroid hormones and recurrence-free survival in women with a history of breast cancer. Cancer Epidemiol Biomarkers Prev 2008; 17:614–20.
73. Tamimi RM, Byrne C, Colditz GA, Hankinson SE. Endogenous hormone levels, mammographic density, and subsequent risk of breast cancer in postmenopausal women. J Natl Cancer Inst 2007;99:1178–87.

74. Walker K, Fletcher O, Johnson N, Coupland B, McCormack VA, Folkerd E, Gibson L, Hillier SG, Holly JM, Moss S, Dowsett M, Peto J, dos Santos Silva I. Premenopausal mammographic density in relation to cyclic variations in endogenous sex hormone levels, prolactin, and insulin-like growth factors. Cancer Res 2009;69:6490–9.
75. Conner P, Svane G, Azavedo E, Söderqvist G, Carlström K, Gräser T, Walter F, von Schoultz B. Mammographic breast density, hormones, and growth factors during continuous combined hormone therapy. Fertil Steril 2004;6:1617–23.
76. Verheus M, Peeters PH, van Noord PA, van der Schouw YT, Grobbee DE, van Gils CH. No relationship between circulating levels of sex steroids and mammographic breast density: the prospect-EPIC cohort. Breast Cancer Res 2007;9:R53.
77. Warren R, Skinner J, Sala E, Denton E, Dowsett M, Folkerd E, Healey CS, Dunning A, Doody D, Ponder B, Luben RN, Day NE, Easton D. Associations among mammographic density, circulating sex hormones, and polymorphisms in sex hormone metabolism genes in postmenopausal women. Cancer Epidemiol Biomarkers Prev 2006;15:1502–8.
78. Bremnes Y, Ursin G, Bjurstam N, Rinaldi S, Kaaks R, Gram IT. Endogenous sex hormones, prolactin and mammographic density in postmenopausal Norwegian women. Int J Cancer 2007;121:2506–11.
79. McCormack VA, Dowsett M, Folkerd E, Johnson N, Palles C, Coupland B, Holly JM, Vinnicombe SJ, Perry NM, dos Santos Silva I. Sex steroids, growth factors and mammographic density: a cross-sectional study of UK postmenopausal Caucasian and Afro-Caribbean women. Breast Cancer Res 2009;11:R38.
80. Yong M, Atkinson C, Newton KM, Aiello Bowles EJ, Stanczyk FZ, Westerlind KC, Holt VL, Schwartz SM, Leisenring WM, Lampe JW. Associations between endogenous sex hormone levels and mammographic and bone densities in premenopausal women. Cancer Causes Control 2009;20:1039–53.
81. Greendale GA, Palla SL, Ursin G, Laughlin GA, Crandall C, Pike MC, Reboussin BA. The association of endogenous sex steroids and sex steroid binding proteins with mammographic density: results from the postmenopausal estrogen/progestin interventions mammographic density study. Am J Epidemiol 2005;162:826–34.
82. Davis SR, Wolfe R, Farrugia H, Ferdinand A, Bell RJ. The incidence of invasive breast cancer among women prescribed testosterone for low libido. J Sex Med 2009;6:1850–6.
83. van Staa TP, Sprafka JM. Study of adverse outcomes in women using testosterone therapy. Maturitas 2009;62:76–80.
84. Davis SR, Moreau M, Kroll R, Bouchard C, Panay N, Gass M. Testosterone for low libido in postmenopausal women not taking estrogen. N Engl J Med 2008;359:2005–17.
85. Jacobeit JW, Gooren LJ, Schulte HM. Safety aspects of 36 months of administration of long-acting intramuscular testosterone undecanoate for treatment of female-to-male transgender individuals. Eur J Endocrinol 2009;161:795–8.
86. Brueggemeier RW, Hackett JC, Diaz-Cruz ES. Aromatase inhibitors in the treatment of breast cancer. Endocr Rev 2005; 26:331–45.
87. Secreto G, Toniolo P, Berrino F. Serum and urinary androgens and risk of breast cancer in postmenopausal women. Cancer Res 1991;51:2572–6.
88. Gammon MD, Thompson WD. Polycystic ovaries and the risk of breast cancer. Am J Epidemiol 1991;134:818–24.
89. Coulam CB, Annegers JF, Kranz JS. Chronic anovulation syndrome and associated neoplasia. Obstet Gynecol 1983;61: 403–7.
90. Chang RJ. Polycystic ovary syndrome and hyperandrogenic states. In: Strauss JF, Barbieri RL, editors. Yen and Jaffe's reproductive endocrinology. 6th Edition. Philadelphia, PA: Elsevier, 2009:489–516.
91. Gammon MD, Thompson WD. Infertility and breast cancer: a population-based case-controlled study. Am J Epidemiol 1990; 132:708–16.
92. Anderson KE, Sellers TA, Chen PL, Rich SS, Hong CP, Folsom AR. Association of Stein-Levinthal syndrome with the incidence of postmenopausal breast carcinoma in a large prospective study of women in Iowa. Cancer 1997;79:494–9.
93. Pierpoint T, McKeigue PM, Isaacs AJ, Wild SH, Jacobs HS. Mortality of women with polycystic ovary syndrome at long-term follow-up. J Clin Epidemiol 1998;51:581–6.
94. Gadducci A, Gargini A, Palla E, Fanucchi A, Genazzani R. Polycystic ovary syndrome and gynecological cancers: is there a link? Gynecol Endocrinol 2005;20:200–8.
95. Chittenden BG, Fullerton G, Maheshwari A, Bhattacharya S. Polycystic ovary syndrome and the risk of gynaecological cancer: a systematic review. Reprod BioMed Online 2009;19: 398–404.
96. Moini A, Eslami B. Familial associations between polycystic ovarian syndrome and common diseases. J Assist Reprod Genet 2009;26:123–7.
97. Atiomo WU, El-Mahdi E, Hardiman P. Familial associations in women with polycystic ovary syndrome. Fertil Steril 2003; 80:143–5.
98. Hunter A, Vimplis S, Sharma A, Eid N, Atiomo W. To determine whether first-degree male relatives of women with polycystic ovary syndrome are at higher risk of developing cardiovascular disease and type II diabetes mellitus. J Obstet Gynaecol 2007;27:591–6.
99. Dimitrakakis C, Bondy C. Androgens and the breast. Breast Cancer Res 2009;11:212.
100. Raynaud JP, Pujos-Gautraud M, Pasqualini JR. Testosterone, other androgens and breast cancer. In: Pasqualini JP, editor. Breast cancer: prognosis, treatment, and prevention. 2nd Edition. New York: Informa Healthcare 2008:181–200.
101. Woolcott C, Shvetsov Y, Stanczyk F, Wilkens L, White K, Caberto C, Henderson B, Le Marchand L, Kolonel L, Goodman M. Plasma sex hormone concentrations and breast cancer risk in an ethnically diverse population of postmenopausal women: the Multiethnic cohort study. Endocr Relat Cancer 2010;17:125–34.