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Testosterone Effects on the Breast: Implications for Testosterone Therapy for Women

WORALUK SOMBOONPORN AND SUSAN R. DAVIS

The National Health and Medical Research Council Centre of Clinical Research Excellence for the Study of Women's Health at the Jean Hailes Foundation and the Department of Obstetrics and Gynaecology, Monash University, Clayton, Victoria 3168, Australia

Androgens have important physiological effects in women. Postmenopausal androgen replacement, most commonly as testosterone therapy, is becoming increasingly widespread. This is despite the lack of clear guidelines regarding the diagnosis of androgen insufficiency, optimal therapeutic doses, and long-term safety data. With respect to the breast specifically, there is the potential for exogenous testosterone to exert either androgenic or indirect estrogenic actions, with the latter potentially increasing breast cancer risk. In experimental studies, androgens exhibit growth-inhibitory and apoptotic effects in some, but not all, breast cancer cell lines. Differing effects between cell lines appear to be due primarily to variations in concentrations of specific coregulatory proteins at the receptor level. In rodent breast cancer models, androgen action is antiproliferative and proapoptotic, and is mediated via the androgen receptor, despite the potential for testosterone and dehydroepiandrosterone to be aromatized to estrogen. The results from studies in rhesus monkeys 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. Epidemiological studies have significant methodological limitations and provide inconclusive results. The strongest data for exogenous testosterone therapy comes from primate studies. Based on such simulations, inclusion of testosterone in postmenopausal estrogen-progestin regimens has the potential to ameliorate the stimulating effects of combined estrogen-progestin on the breast. Research addressing this is warranted; however, the number of women that would be required for an adequately powered randomized controlled trial renders such a study unlikely. (*Endocrine Reviews* 25: 374–388, 2004)

I. Introduction

ANDROGENS HAVE IMPORTANT physiological effects in women. Not only are they the precursor hormones for estrogen biosynthesis in the ovaries and extragonadal tissues (1), but androgens act directly via androgen receptors (ARs) throughout the body. Androgen levels decline with increasing age in women before menopause (2, 3), and it is now accepted that many postmenopausal women experience a variety of physical symptoms secondary to androgen depletion, as well as physiological changes that affect their quality of life (4). Postmenopausal androgen replacement, most commonly as testosterone therapy, is becoming increasingly widespread. This is despite the lack of clear guidelines regarding the diagnosis of androgen insufficiency, optimal therapeutic doses, and long-term safety data. With respect to the breast specifically, there is the potential for exogenous testosterone to exert either androgenic or indirect estrogenic actions, with the latter potentially increasing breast cancer risk.

There is justifiable concern that combined oral estrogen plus progestin therapy significantly increases the risk of breast cancer in postmenopausal women (5–11). Although the underlying mechanism by which the development of breast cancer is increased in women taking combined hormone therapy is not understood, there is a considerable amount of evidence that androgens protect against estrogen's mitogenic and cancer-promoting effects on breast tissue. Labrie *et al.* (3) have recently reviewed the role of adrenal androgens in breast cancer growth with specific attention to dehydroepiandrosterone (DHEA). With the increasing in-

Abbreviations: A, Androstenedione; AR, androgen receptor; CI, confidence interval; DHEA, dehydroepiandrosterone; DHEA-S, DHEA sulfate; DHT, dihydrotestosterone; DMBA, dimethylbenz(a)antracene; ER, estrogen receptor; OR, odds ratio; PCOS, polycystic ovarian syndrome; PR, progesterone receptor; PSA, prostate-specific antigen; RR, relative risk.

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clusion of testosterone in hormonal regimens, the modulating effects of this steroid on the development of breast cancer therefore require consideration.

Thus, we have reviewed the published literature specifically pertaining to clinical studies of endogenous testosterone and testosterone therapy and breast cancer risk in premenopausal and postmenopausal women and examined the potential benefit or risk with regard to breast cancer of the administration of testosterone as part of hormone therapy.

II. Testosterone Production and Metabolism

A. Biosynthesis of testosterone

The term "androgens" refers to a group of 19-carbon steroid hormones that are associated with maleness and the induction of male secondary sexual characteristics. In women, androgens circulate in the concentration range nanomolar to micromolar, in contrast to the estrogens, the circulating concentrations of which are in the picomolar range. The major androgens in women include testosterone and dihydrotestosterone (DHT) because both have high binding affinity to the AR. Biosynthesis of the androgens takes place both in the adrenal and in the ovary and is modulated by two cytochrome P450 enzymes, P450 Scc, which catalyzes cholesterol side-chain cleavage, and P450 C_{17}, which catalyzes 17-hydroxylation and 17–20 bond cleavage (17/20 lyase), which is required for the production of DHEA and androstenedione (A) from pregnenolone and progesterone, respectively. The further metabolism of androgens involves other important enzymes including 3β-hydroxysteroid dehydrogenase, catalyzing the conversion of pregnenolone to progesterone and DHEA to A, and 17β-hydroxysteroid dehydrogenase, which catalyzes the conversion of A to testosterone. DHEA secretion is acutely stimulated by ACTH (12, 13); however, DHEA sulfate (DHEA-S), which has a long plasma half-life, does not increase acutely after ACTH administration (14).

DHEA-S, DHEA, and A are considered to be proandrogens because they require conversion to testosterone to exhibit androgenic effects. Up to 25% of testosterone may be derived from the adrenal glands, 25% is derived from the ovary, and the remaining 50% is derived from peripheral conversion of the proandrogens, with A being the main precursor (15) (Fig. 1A). Circulating testosterone can be converted to DHT by 5α-reductase, type 1 and type 2, or to estradiol by the aromatase enzyme. These conversions occur primarily in target tissues. DHT is a nonaromatizable androgen (16, 17) (Fig. 1B). Thus, androgens may exert biological effects by acting directly via the AR or indirectly after conversion to estrogen (17, 18).

B. Factors affecting circulating testosterone levels

Under normal physiological conditions, only 1–2% of total testosterone circulates free, unbound to plasma protein. The rest is bound by SHBG and albumin, with SHBG binding 66% of total circulating testosterone (19). The binding affinity for steroids bound by SHBG is DHT > testosterone > androstenediol > estradiol > estrone (20). SHBG also weakly binds

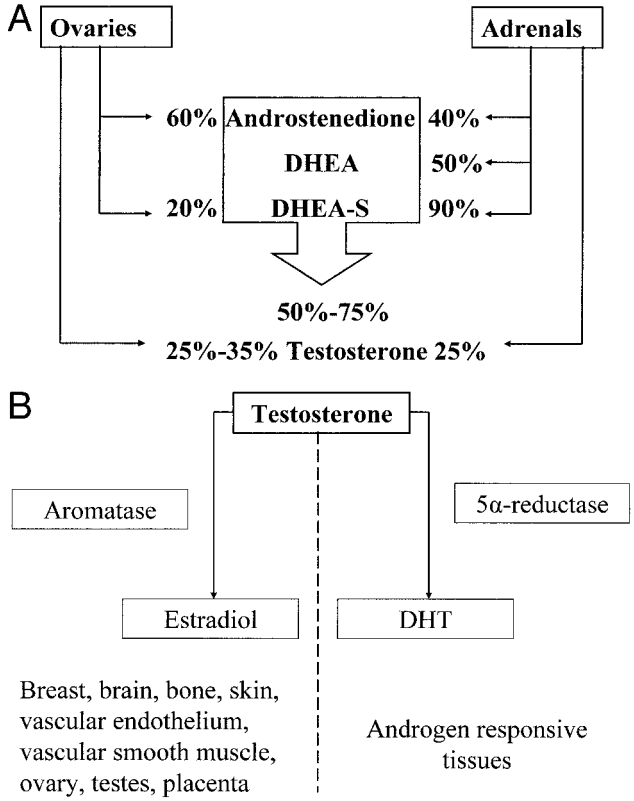

FIG. 1. Androgen production and metabolism. A, The adrenal glands produce all proandrogens, whereas the ovaries produce only DHEA, A, and testosterone. In postmenopausal women, only about 25% of circulating testosterone is directly secreted by the ovaries. The rest, 50–75%, is formed largely from circulating precursors. B, Testosterone can be converted to DHT by 5α-reductase type 1 and type 2 or to estradiol by the aromatase enzyme.

DHEA, but not DHEA-S (20). SHBG is a pivotal determinant of the bioavailability of sex steroids, and variations in the plasma levels of SHBG impact significantly on the amount of free and other bound sex steroids (20). Elevations in estradiol (as occurs during pregnancy, hyperthyroidism, and liver disease) cause a marked increase in SHBG levels, whereas hypothyroidism, obesity, and hyperinsulinemia are associated with decreased SHBG levels (21). Standard-dose oral estrogen, as used in hormone therapy, will increase SHBG with little or no effect seen with standard estradiol patch therapy (22).

In premenopausal women with regular menstrual cycles, there is a rise in testosterone and A in the late follicular phase of the menstrual cycle and in the luteal phase (23, 24). There is also a diurnal variation in testosterone in women with the peak in the morning (25). Zumoff *et al.* (2) showed lower mean 24-h values for total and calculated free testosterone among older *vs.* younger reproductive aged women (n = 33 women). Most recently, a study of 149 healthy premenopausal women with regular cycles, no exogenous hormone therapy, and no complaint of low libido showed a statistically significant decline with age for free testosterone, DHEA-S, A, and DHT, each measured after organic solvent extraction by validated methodology (26). In the late reproductive years there is failure of the midcycle rise in free

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testosterone that characterizes the menstrual cycle in young ovulating women (27). This occurs despite preservation of normal free testosterone levels at other phases of the cycle. The mean plasma concentrations of testosterone in women transiting the menopause are also significantly lower than those of younger ovulating women sampled in the early follicular phase (28). Across the perimenopausal period, neither A, DHT, or the ratio of total testosterone to SHBG (the free androgen index) appear to change acutely (19, 28). Controversy remains as to whether the postmenopausal ovary is a significant source of androgen production. Concentrations of testosterone in the ovarian vein of postmenopausal women have been shown to be higher than those in systemic venous blood, suggesting that the postmenopausal ovary continues to be an androgen-secreting organ (24). In addition, testosterone levels decrease in postmenopausal women after oophorectomy (29). However, Couzinet *et al.* (30) have proposed that the postmenopausal ovary is not a major source of androgens. Some postmenopausal women have elevated ovarian androgen production, *i.e.*, hyperthecosis, a well-established but poorly studied entity. It may well be that the androgen production of the postmenopausal ovary is variable. This variability requires further study along with associated factors.

III. Mammary Epithelial Cell Proliferation and Apoptosis

Steroids and their nuclear receptors play crucial roles in the development and maintenance of normal functions of the human mammary gland. In addition to estrogen receptor-α (ERα), estrogen receptor-β (ERβ), and progesterone receptors (PRs), ARs are present in both normal mammary tissue and breast cancer cell lines (31, 32). Hormone stimulation of mammary epithelial proliferation and apoptosis is important in tissue homeostasis. Deregulation of apoptosis can promote tumorogenesis as well as proliferation (33).

In premenopausal women, proliferation and apoptosis of normal breast epithelial tissue are higher in the luteal phase of the menstrual cycle than in the follicular phase (34, 35). In the luteal phase, both estrogen and progesterone levels are maximal (36–38). Free testosterone and A levels peak during the middle-to-late follicular phase of the menstrual cycle and remain moderately elevated up through the midluteal phase (39).

In vitro studies of normal breast tissue are important to understand physiological regulation of the mammary gland by sex steroids. However, this has been hampered by experimental difficulties (40). One problem has been that normal human breast cells lose their steroid receptors and, hence, their hormone responsiveness almost as soon as they are isolated and placed into culture (40). Only one study of hormone-responsive primary cultures of breast epithelial cells reported that estradiol, but not progesterone, stimulated proliferation (41).

In vitro studies have consistently demonstrated that estradiol is a major mitogen in breast cancer cell lines (42–44). In breast cancer cell lines, cell death has been reported to be induced by estrogen deprivation (45) or antiestrogen (46). In contrast, *in vivo* evidence that supports a role for progesterone in cell proliferation in the breast has been difficult to reproduce *in vitro* (47).

IV. Preclincial Studies of the Effects of Androgens in Breast Tissue

A. Breast cancer cell line studies

There is no *in vitro* evidence pertaining to the effects of androgens on normal human breast cells. Studies of the effects of androgens in various breast cancer cell lines predominantly support apoptotic and antiproliferative effects of androgens on the mitogenic effects of estrogens. However, divergent findings have been reported with differences according to the specific cell line, the androgen used, and its dose and estrogen status. The effects of testosterone and DHT in breast cancer cell lines are summarized in Table 1.

1. Antiproliferative effects. The antiproliferative effects depend on the following factors.

TABLE 1. Effects of testosterone and DHT on breast cancer cell lines

Study	Model	Type of androgen	Dose of androgen (M)	Main outcome measurement	Result
Boccuzzi *et al.*, 1994 (51)	MCF-7	DHT	20^{-11}–20^{-7}	Proliferation	Biphasic effect: stimulation at a very high concentration; inhibition at concentration up to 20^{-8} M
Birrell *et al.*, 1995 (52)	MCF-7, ZR75-1, TD47-D, MDA-MB-453	DHT	10^{-10}–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
Ando *et al.*, 2002 (48)	MCF-7	Testosterone	10^{-9}–10^{-6}	Proliferation	Inhibition
		DHT	10^{-9} –10^{-6}	Proliferation	Inhibition
Ortmann *et al.*, 2002 (54)	MCF-7, T47-D, BT-20, MDA-MB 435S	Testosterone	10^{-9}–10^{-7}	Proliferation	Inhibition in all four cell lines
		DHT	10^{-9}–10^{-7}	Proliferation	Inhibition in all four cell lines
Kandouz *et al.*, 1999 (58)	MCF-7, ZR75-1, T47-D	DHT	10^{-8}	Apoptosis	Proapoptotic effect
Lapointe *et al.*, 1999 (61)	ZR-75-1	DHT	10^{-9}	Bcl-2 protooncogene	Down-regulation

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a. Estrogen status and type of androgen used. Ando *et al.* (48) simulated the hormonal environment in pre- and postmenopausal women with an *in vitro* model utilizing the ER-positive breast cancer cell line MCF-7 exposed to DHEA, DHEA-S, androstenediol, testosterone, and DHT with or without estradiol. They found that DHEA-S and androstenediol stimulated the growth of MCF-7 cells in the absence of estradiol, but reduced cell proliferation in the presence of estradiol at 1 nmol/liter. This is consistent with the possibility of DHEA-S being converted to estrone and hence to estradiol, and androstenediol acting as a weak estrogen (49). Testosterone and DHT, at 1–100 nmol/liter, inhibited MCF-7 cell proliferation independently of the presence of estradiol. DHT alone, at 100 nmol/liter, consistently inhibited MCF-7 cell proliferation by 50% of the basal growth rate and counteracted estradiol-proliferative action by 68%. Normal circulating levels in women are approximately 0.5–2.3 nmol/liter for testosterone, and 0.2–0.8 nmol/liter for DHT. Thus, most of the concentrations for these hormones used in this and other studies are supraphysiological. Cell cycle analysis showed an enhanced number of cells in G_0/G_1 phase after 6 d of DHT treatment. Moreover, upon prolonged DHT exposure, a markedly increased AR content, mostly translocated into the nucleus, was observed. The inhibitory effect of DHT on cell proliferation was lost when the cells were treated with the AR antagonist, hydroxyflutamide (48). In cotransfection experiments, overexpression of the AR decreased estradiol-induced signaling (48). This was amplified by treatment with DHT but lost with the addition of hydroxyflutamide. When the cells were cotransfected with a mutant AR, inhibition of estradiol-induced signaling did not occur. Thus, direct androgen action appears to antagonize MCF-7 proliferation induced by estradiol, and this seems to be related to the inhibitory effects of the AR on estradiol genomic action (48). These experimental results are consistent with those of Birrell *et al.* (50) who proposed that the therapeutic action of medroxyprogesterone acetate in breast cancer may be partially mediated by the AR.

b. Androgen concentration. The effects of DHT appear to be concentration dependent. Boccuzzi *et al.* (51) reported that, at an extremely high concentration (200 nmol/liter), DHT stimulated MCF-7 cell growth through an ER-mediated mechanism, whereas lower concentrations of DHT were inhibitory.

c. Type of breast cancer cell line. The effects on proliferation *in vitro* vary according to the androgen administered and the breast cancer cell line studied (52, 53). At concentrations of 1 nmol/liter for 10 d, which is close to the normal female physiological range, Birrell *et al*. (52) reported varying stimulatory and inhibitory effects of DHT on differing breast cancer cell lines that simply could not be explained by varying hormone receptor status. The two cell lines that had no hormone receptors did not respond to treatment. DHT stimulated both the ER-positive MCF-7 cells and the ER-negative MDA-MB-453 cells. Treatment with 100-fold excess of hydroxyflutamide reversed the effects of DHT in each of the cell lines. In the same study (data not shown) the synthetic nonmetabolizable androgen mibolerone had effects similar to those of DHT, with the exception that hydroxyflutamide only partially reversed the growth-stimulatory effects of this treatment on MCF-7 and MDA-MB-453 cells. Hydroxyflutamide only partially reversed the inhibitory effects of DHT on ZR-75–1 cultures, whereas AR antisense oligonucleotides reversed the growth-inhibitory action of miberolone in this cell line.

These observations support the theory that AR expression is a necessary requirement for androgenic effects on breast cancer cell proliferation, but that the absolute levels of AR (as well as ER and PR) in cell lines are associated with neither a specific stimulatory nor inhibitory proliferative responses (52). It is likely, therefore, that additional cellular factors or the structure of the AR determine whether breast cancer cell proliferation is stimulated or inhibited in the presence of androgen (53).

In contrast to the above, Ortmann *et al*. (54) reported dose-dependent inhibition with androgens of four cell lines that each stained positively for the AR. Included among these was the BT-20 cell line, which was reported by two other groups to be AR negative (52, 55). According to proliferation kinetics, they observed a significant ($P < 0.05$) dose-dependent inhibition of cell growth by both testosterone and DHT. In the ER-negative cell lines, BT-20 and MDA-MB 4^{35}S testosterone was a more potent inhibitor of cell proliferation than DHT ($P < 0.05$), whereas in the ER-positive cells lines, MCF-7 and T47-D, stronger inhibition of proliferation was achieved with DHT than with testosterone. They proposed that partial transformation of testosterone to estrogen in ER-positive cells might be an explanation for this effect (54).

Prostate-specific antigen (PSA) is a new favorable prognostic indicator for women with breast cancer (56). Immunoreactive PSA has been reported in 30% of breast cancers and has been associated with both earlier stage disease and longer relapse-free survival (56). KLK3 (which encodes PSA) and KLK2 (encoding human kallikrein 2) are both known to be androgen regulated, but respond differentially to androgens when studied in different human breast cancer cell lines (57). Research into the various factors affecting the production of these two proteins, according to the breast cancer cell line studied, gives insight into how androgen treatment may affect different cell lines differently.

Initial experiments demonstrated that the differential androgen induction of PSA and human kallikrein 2 was not directly related to the level of AR expression or to mutations within the coding region (55). Because the action of a steroid receptor at a given promoter may be modulated by various coregulatory proteins (coactivators/corepressors), Magklara *et al*. (55) examined the expression of various known coregulatory proteins in the different breast cancer cell lines (BT-474, T-47D, ZR75–1, MCF-7, MFM-223 and BT-20). They found that the mRNA levels of steroid receptor coactivator 1, a known coactivator of the activation function 1 domain of the AR, were highest in the breast cancer cell lines with the greatest PSA production and lowest in the cell lines that secreted less PSA. This raises the possibility that the relative levels of specific coactivators/corepressors might differentially modulate AR transcriptional activity within the promoter/enhancer region of KLK2 and KLK3 of different breast cancer cell lines (55).

Thus, the ultimate effects of testosterone and DHT at the

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tissue level may not be influenced simply by absolute ligand concentrations, but by the relative concentrations of specific coregulatory proteins unique to each cell line. Hence, the effects of DHT differ between different breast cancer cell lines, despite the consistent presence of ARs. In the AR-positive human breast cancer cell lines, T47-D and ZR-75–1, DHT has been reported to be proapoptotic, with maximal effects at 10 nmol/liter (58). These findings are in line with the growth-inhibitory effects reported by Birrell *et al.* (50) in these two cell lines.

2. Apoptotic effects. The effects of androgens on the expression of two genes known to influence apoptosis, Bcl-2 and Bax, have been investigated. Bcl-2 is able to counteract apoptosis induced by numerous stimuli such as UV light, γ-radiation, heat shock, and chemotherapy and thus is considered antiapoptotic (59), and Bax is a proapoptotic gene (60). Lapointe *et al.* (61) reported that DHT down-regulated Bcl-2 protooncogene levels via an AR-mediated mechanism in the ZR75–1 breast cancer cell line in either the presence or absence of 17β-estradiol. This is consistent with the inhibitory effect of DHT in this cell line. Inhibition by DHT was completely prevented by coincubation with the pure antiandrogen hydroxyflutamide (61). Xie *et al.* (60) studied Bcl-2 and Bax expression in the Noble rat model, which they had established to explore the mechanisms of hormonal mammary carcinogenesis. They observed that Bcl-2 was strongly expressed in most of the mammary tumor cells, and that when animals were treated with 17β-estradiol, the mammary epithelial cells expressed higher levels of Bcl-2. Bax immunoreactivity was weak in mammary tumor cells but strongly expressed in adjacent normal or hyperplastic ductal structures. Treatment with testosterone, either alone or in combination with estrogen, gave rise to high levels of Bax expression in "premalignant" mammary glands. This supports the hypothesis that testosterone may oppose the mitogenic action of estrogen in the breast by promoting apoptosis (62).

Taken together, androgens exhibit growth-inhibitory and apoptotic effects in some, but not all, breast cancer cell lines. Differing effects between cell lines appear to be primarily due to variations in concentrations of specific coregulatory proteins at the receptor level. In rodent breast cancer models, androgen action is antiproliferative and proapoptotic, and mediated via the AR, despite the potential for testosterone and DHEA to be aromatized to estrogen.

B. Animal studies

1. DHEA. The effects of DHEA have been renewed in detail recently (3). In summary, data from *in vivo* studies support a primarily inhibitory effect of testosterone on the proliferative effects of estrogen. Dimethylbenz(a)anthracene (DMBA)-induced mammary carcinoma in the rat is a commonly used model for *in vivo* studies of the prevention and treatment of breast cancer. Labrie and associates (63–65) and Lubet *et al.* (66) have used this model extensively to study the effects of DHEA and have consistently reported an inhibitory effect of DHEA on mammary carcinoma development. Treatment with SILASTIC (Dow Corning Corp., Midland, MI) implants of DHEA leading to serum DHEA levels comparable to those observed in normal adult women (7.1 + 0.6 nmol/liter and 17.5 + 1.1 nmol/liter) progressively inhibited the development of DMBA-induced mammary carcinoma in the rat (63). Luo *et al.* (64) also demonstrated inhibition in DMBA-induced mammary carcinoma development: 279 d after DMBA administration, the incidence of mammary carcinoma had decreased from 95% in control animals to 73% ($P < 0.05$), 57% ($P < 0.01$), and 38% ($P < 0.01$) at the daily percutaneous doses of 5, 10, and 20 mg of DHEA, respectively. Moreover, the mean tumor number and the mean tumor area per tumor-bearing animal were also reduced by the same treatments. Similar outcomes have been reported in a *N*-methyl-*N*-nitrosourea-induced rat mammary tumor model with DHEA therapy (65), and a suppressive effect of DHEA has been demonstrated on the growth of estrone-stimulated sc tumor xenografts formed by AR-positive ZR-75–1 cells in ovariectomized nude mice (66).

2. Testosterone and DHT. Table 2 summarizes the effects of testosterone and DHT in animal studies. There is both direct and indirect evidence of the inhibitory effects of testosterone and DHT on DMBA-induced mammary carcinoma in ovariectomized rats (67, 68). DHT therapy reduced the number of progressing tumors from 69.2 to 29.2% in estradiol-treated animals, and the number of tumors that completely regressed increased from 11.5 to 33.3% in the same groups of animals (67). Simultaneous treatment with the antiandrogen flutamide completely prevented the inhibitory effect of DHT on tumor growth (67). DHT exhibited similar tumor-suppressing effects on ZR-75-1 breast cancer cells implanted into ovariectomized athymic mice in either the presence or absence of exogenous estradiol (69).

Zhou *et al.* (31) explored the effects of estrogen, progesterone, and testosterone on normal mammary epithelial cell proliferation and steroid receptor gene expression in the ovariectomized primate mammal. They showed that estrogen therapy alone significantly increased mammary epithelial proliferation approximately 6-fold and significantly increased the mammary epithelial level of ERα mRNA. Progesterone administration did not modify the proliferative effects of estradiol significantly. When given concurrently, testosterone reduced estradiol-induced epithelial proliferation by approximately 40% and entirely abolished the estradiol-induced augmentation of ERα gene expression. However, testosterone levels achieved in this study were supraphysiological. Zhou *et al.* (31) also investigated the effects of tamoxifen and found that it caused a 3-fold increase in mammary epithelial proliferation, measured by Ki67, but a decrease in ERα gene expression below placebo level. AR mRNA levels detected by *in situ* hybridization were not altered by estradiol alone, but were significantly reduced by estradiol plus testosterone or tamoxifen treatment. Thus, testosterone induced down-regulation of mammary epithelial proliferation and ERα gene expression. This suggests that the addition of testosterone might reduce the risk of breast cancer associated with estrogen-progestin therapy in postmenopausal women. In addition to the parallel effects of tamoxifen

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TABLE 2. Effects of testosterone and DHT in animal studies

Study	Model	Intervention	Main outcome measurement	Result
Dauvois *et al.*, 1989 (67)	DMBA-induced mammary carcinoma in rats	E *vs.* E + DHT	The number of progressing tumors	Significant decrease with E + DHT *vs.* E alone
			The number of complete responses	Significant increase with E + DHT *vs.* E alone
Dauvois *et al.*, 1991 (69)	ZR-75-1 breast cancer cells implanted into ovariectomized athymic mice	E *vs.* E + DHT	The number of progressing tumors	Significant decrease with E + DHT *vs.* E alone
			The number of complete responses	Significant decrease with E + DHT *vs.* E alone
Jayo *et al.*, 2000 (71)	Rat	Oral contraceptive *vs.* oral contraceptive + methyltestosterone	Mammary epithelial proliferation	Significant decrease with oral contraceptive + testosterone *vs.* oral contraceptive alone
Zhou *et al.*, 2000 (31)	Ovariectomized rhesus monkeys	E; E + progesterone; E + T	Mammary epithelial proliferation	Significant decrease with E + T *vs.* others
Dimitrakakis *et al.*, 2003 (70)	Normal-cycling rhesus monkeys	Flutamide *vs.* none	Mammary epithelial proliferation	Significant increase in flutamide
	Ovariectomized rhesus monkeys	E; E + progesterone; E + T; vehicle	Mammary epithelial proliferation	Significant decrease with E + T *vs.* E or E + progesterone
			ERα/ERβ	Reversal of ERα/ERβ
			MYC expression	Significant reduction

E, Estradiol; T, testosterone.

on primate mammary epithelial sex steroid receptor gene expression, Zhou *et al.* (31) also demonstrated that tamoxifen, like testosterone, reduced apolipoprotein D mRNA levels and increased IGF binding protein 5 expression in the primate mammary gland. These findings support AR-mediated effects of tamoxifen.

Subsequently, Dimitrakakis *et al.* (70) have investigated the effects of ovarian steroids in physiological doses on the mammary epithelial proliferation in ovariectomized rhesus monkeys. They studied four groups treated with placebo, estradiol alone, estradiol plus progesterone, or estradiol plus testosterone. Circulating estradiol levels were similar in the three active treatment groups, and testosterone levels were physiological. The mammary epithelial proliferation index was measured using Ki67 immunoreactivity. Estradiol alone and estradiol plus progesterone resulted in a significantly increased mammary epithelial proliferation index compared with placebo controls by approximately 3.5-fold, whereas the estradiol plus testosterone combination did not increase the proliferation index above control values (70). In addition, they found a significant reduction in mammary epithelial ERα and increase in ERβ expression in estradiol plus testosterone groups compared with estradiol alone. This effect of testosterone resulted in a reversal of the ERα/ERβ ratio, which was approximately 2.5 in the estradiol-treated group and approximately 0.7 in the estradiol-testosterone group. Moreover, testosterone treatment was associated with an approximate 50% reduction in mammary epithelial MYC expression, an estrogen-responsive gene, compared with the estradiol- and estradiol-progesterone-treated groups ($P = 0.05$), suggesting that the antiestrogenic effects of testosterone at the mammary gland involve alteration in ER signaling to MYC (70). They further investigated the importance of endogenous testosterone in intact, cycling monkeys by studying treatment with either placebo or the AR antagonist flutamide. The mammary epithelial proliferation index increased 2-fold after treatment with flutamide alone ($P = 0.03$). This suggests that testosterone may serve as a natural endogenous protector of the breast and may limit mitogenic and cancer-promoting effects of estrogen on the mammary epithelium (70). Jayo *et al.* (71) also reported that oral contraceptive therapy plus methyltestosterone in rats causes significant suppression of epithelial cell proliferation, a reduction in the number of proliferating cell nuclear antigen-labeled cells, and an increase in the number of PR-labeled cells. However, there have been no studies of the effects of testosterone plus estrogen-progestin therapy on breast epithelial cell proliferation in women.

In ovariectomized 12-month-old rats, DHT stimulated mammary gland lobulo-alveolar ductal growth (72). Medroxyprogesterone acetate induced the same effect, consistent with the findings of Birrell *et al.* (50) that androgens and medroxyprogesterone acetate share a similar mechanism of action in the breast. DHEA also stimulated lobulo-alveolar development, which was unaffected by the antiestrogen EM-800 but almost completely prevented by cotreatment with the antiandrogen flutamide (47, 72).

Taken together, available *in vivo* data indicate that in estrogen-replete normal breast tissue, androgens diminish estrogen-induced breast epithelial proliferation and abolish estrogen-induced ERα gene expression in the primate. In the absence of estrogen, androgen action mimics that of progestins in the rat mammary gland.

Thus results from the studies *in vitro* and *in vivo* 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. However,

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these are surrogate endpoints and hence the need to consider what is known from studies in women.

V. Studies in Humans of the Effects of Androgens on Breast Cancer

A. Endogenous circulating testosterone and breast cancer

1. Issues in clinical trials. Findings from case-controlled studies of the relationship between endogenous testosterone levels and breast cancer risk do not necessarily translate to women treated with exogenous testosterone. The former address endogenous androgen production, which in some women may be pathophysiological. In contrast, postmenopausal testosterone therapy is administered to women who have low testosterone levels due to low production and who are usually also treated with exogenous estrogen. Furthermore, if an association is found between endogenous circulating testosterone and breast cancer, it does not necessarily signify a causal relationship. Total testosterone, although the most common measure for clinical studies to date, does not yield specifically meaningful information about actual tissue androgen exposure. It is widely accepted that free testosterone is the strongest indicator of tissue androgen exposure and that variations in SHBG levels in women can have dramatic effects on free testosterone levels (4, 73, 74). In addition, a single value may be inadequate to assess true tissue exposure because testosterone levels vary in response to diurnal rhythms (25). Stress is also an important confounder in cross-sectional studies, because stress itself affects testosterone levels (75–78). Whether even free testosterone is a meaningful indicator of tissue androgen exposure remains controversial. Labrie *et al.* (79, 80) proposed that the major proportion of androgenic effects in women are derived from an intracrine mode of action, which will not be detected by measurement of circulating testosterone or DHT. Regarding the type of study, a clear inference of effect cannot be drawn from cross-sectional data because such research cannot provide an appropriate time sequence of exposure and outcome.

Consideration also needs to be given not only to what has been measured but also the effects of storage and the sensitivity of the assay methodology used. For example, with long-term cryopreservation, testosterone has been shown to increase by 5% per year of blood storage at −20 C (81). This may not apply to storage at much colder temperatures (82).

No rapid, simple assay of total testosterone has been shown to produce reliable results in women with low to normal testosterone levels. Direct testosterone immunoassays are limited by "noise" from assay interference and by cross-reactivity with other steroids, which become worse at low testosterone concentrations (83). Inclusion of an organic solvent extraction step when measuring total testosterone will increase the assay specificity, and if combined with chromatographic separation of testosterone from interfering steroids, a reliable result can be obtained. The gold standard methodology for measurement of free testosterone is considered to be equilibrium dialysis. Measurement of free testosterone by analog assay is notoriously unreliable, particularly at the lower end of the normal female range and is not recommended for use (83).

Finally, because estrogen is considered a strong risk factor for breast cancer, to draw any conclusion about an association between testosterone and breast cancer, a statistical method to adjust for the estrogen effect must be employed.

2. Testosterone levels and breast cancer in premenopausal women. Two cross-sectional studies have investigated the relationship between total testosterone and breast cancer risk in premenopausal women and have yielded inconsistent results (Table 3). Secreto *et al.* (84) reported an age-adjusted relative risk (RR) for high *vs.* low levels of serum total testosterone of 3.4 [95% confidence interval (CI), 1.6–7.3] and for urinary testosterone of 2.1 (95% CI, 0.9–4.8) for cases (n = 63) *vs.* controls (n = 70). Study samples were collected between cycle d 18 and d 21 irrespective of cycle length or whether ovulation had or had not occurred. The association was observed only in women whose samples were collected 5–9 d before the next menses (a period corresponding to the midluteal phase) and 10 or more days before the next menses. There was no positive association for women whose blood and urine were collected within 4 d of the next menses and who thus had cycles lasting 25 d or less. The authors' interpretation of these findings was that higher testosterone levels were detectable in cases only in the follicular or early luteal phases; however, these phases are the times of highest testosterone levels during the normal cycle (39). They also reported that high testosterone was characteristic of breast cancer patients with long menstrual cycles (>28 d) and negligible in women with short ones (<28 d) but that low SHBG levels appeared to be a protective factor for breast cancer. Thus, the high total testosterone measured corresponded to periovulatory hormone production but was not abnormally high across the cycle in premenopausal women with breast cancer, consistent with the fact that SHBG was not abnormally low in patients with breast cancer.

The most recent cross-sectional study involved 171 premenopausal women with breast cancer and 170 controls matched by age (85). No significant difference by odds ratio (OR) for breast cancer between high and low testosterone was found when data were adjusted for confounding factors. Overall, no conclusions can be made about testosterone and breast cancer in premenopausal women based on these two studies.

Two prospective case-control studies in which total testosterone was measured provide more consistent results (Table 3). Wysowski *et al.* (86) found no statistical associations between serum hormone levels, including total testosterone, in 17 women diagnosed with breast cancer 8–132 months after blood was drawn, each matched to four controls. Similarly, in a study of 62 premenopausal women with breast cancer and 182 controls, Thomas *et al.* (87) found no statistical difference for total testosterone between the groups

3. Polycystic ovarian syndrome (PCOS) and incidence of breast cancer. In premenopausal women, PCOS is characterized by infertility, hyperandrogenism, and obesity. Concentrations of testosterone, A, and DHEA-S, and the calculated free androgen index (total testosterone in nanomoles per liter/SHBG in nanomoles per liter × 100) are significantly higher in women with PCOS regardless of hirsutism (88). This syn-

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TABLE 3. Epidemiological studies of the association between plasma testosterone levels and risk of breast cancer in premenopausal women: study size, characteristics, and summary results

Study	Trial type	Study characteristics	Comparison made	Case/control (no. of women)	Type of T measurement	OR (95% CI)
Secreto *et al.*, 1989 (84)	Cross-sectional	Matched to age (± 6 months)	Top to bottom quartile	75/150	Total T	2.1[a] 3.4 (1.6–7.3)[b]
Yu *et al.*, 2003 (85)	Cross-sectional	Matched to age	Top to bottom tertile	171/170	Total T	1.9 (1.0–3.7)[c] 2.01 (0.96–4.2)[d]
Wysowski *et al.*, 1987 (86)	Prospective	7-yr follow-up; matched to race, age, and time since last menstrual period	Mean values for cases *vs.* controls[e]	17/68	Total T	Not applicable
Thomas *et al.*, 1997 (87)	Prospective	Matched to age, year of blood collection, and no. of years postmenopausal	1U increase in the natural log of hormone concentration	61/179	Total T	1.2 (0.6–2.4)[f]

T, Testosterone.
[a] Age adjusted.
[b] Adjusted for occupation and no. of children.
[c] Unadjusted.
[d] Adjusted for waist-hip ratio, age at first live birth, total caloric intake, fibroadenoma, and SHBG.
[e] Mean comparison resulted in no statistically significant difference of testosterone levels between cases and controls.
[f] Unadjusted OR.

TABLE 4. Epidemiological studies of the association between plasma testosterone levels and risk of breast cancer in women with PCOS: study size, characteristics, and summary results

Study	Trial type	Study characteristics	Comparison made	RR (95% CI)
Coulam *et al.*, 1983 (89)	Prospective cohort	Clinic-based study; 1270 subjects diagnosed as having chronic anovulatory syndrome were followed during 1935–1980	Observed incidence of breast cancer *vs.* expected incidence based on standard population	1.5 (0.8–2.6)
Gammon and Thompson, 1991 (91)	Case-control	Population-based study; 4730 women with breast cancer and 4688 control women aged 20–54 yr	OR	0.5 (0.3–0.9)[a]
Anderson *et al.*, 1997 (90)	Prospective cohort	Population-based study; 34,835 women at risk, age 55–69 yr, were followed during 1986–1992	Incidence of breast cancer among women with Stein-Leventhal syndrome *vs.* incidence among women without this disease in the same cohort	1.2 (0.7–2)[b] 1.0 (0.5–1.8)[c]

[a] Age-adjusted OR for age.
[b] Adjusted for age.
[c] Adjusted for age, age at menarche, age at first pregnancy, parity, oral contraceptive use, hormone replacement therapy, body mass index, waist-to-hip ratio, benign breast disease, and family history of breast carcinoma.

drome is a useful model of the effects of long-term exposure to hormone imbalance. An increased risk of endometrial cancer has been documented in women with this condition (89). Table 4 summarizes the studies of PCOS and breast cancer risk. Despite hyperandrogenism and long-term exposure to unopposed estrogen, the risk of breast cancer is not increased in women with PCOS (89, 90). In fact, Gammon and Thompson (91) have reported an age-adjusted OR 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, a cause and effect cannot be established.

4. Testosterone and breast cancer in postmenopausal women

a. Cross-sectional studies. In their cross-sectional studies, Lipworth *et al.* (92) measured hormone levels in cases 1 wk post surgery, and Secreto *et al.* (93) collected blood and urine from women after diagnosis, but before surgery. Each group felt their protocol minimized the influence of stress, but this is questionable. Both studies reported only total testosterone, not free or bioavailable testosterone. Secreto's group (93) reported that breast cancer patients with elevated urinary testosterone levels at the time of diagnosis showed a dramatic decrease in the excretion levels of this hormone after bilateral ovariectomy, and that histological examination of the excised ovaries revealed hyperplasia of interstitial cells in all hyperandrogenic patients. No such change was observed in patients with normal testosterone levels at the time of diagnosis preovariectomy (94). This implies that the hyperandrogenic postmenopausal women who developed breast cancer in Secreto's study had ovarian pathophysiology. Lipworth *et al.* (92) reported no increase for breast cancer

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with higher testosterone levels after adjustment for age, residence, and hormonal factors (OR, 0.48; 95% CI, 0.12–1.90), whereas Secreto *et al.* (93) reported a positive association after adjustment for occupation and number of children (OR, 2.7; 95% CI, 1.1–6.7). Secreto *et al.* (93) also measured DHT in postmenopausal women with breast cancer *vs.* controls and found no significant difference between the two groups.

b. Prospective case-control studies. Ten prospective case-control studies have been undertaken (81, 82, 86, 95–101). Study designs, characteristics, and results of these studies are listed in Table 5. All met the appropriate requirements for prevention of biochemical measurement bias, and case-control identification among these studies was similar. To address intraindividual variability of hormone levels with time, Berrino *et al.* (95), Hankinson *et al.* (102), and Thomas *et al.* (81) reported intraclass correlation coefficients between two blood samples. Several groups measured only total testosterone (81, 82, 86, 97, 98, 100). Overall, three of the studies did not demonstrate any significant associations without adjustment for estradiol (82, 86, 98). Dorgan *et al.* (96) reported the RR for breast cancer for women in the highest *vs.* the lowest quartile of total testosterone levels as being 6.2 (95% CI, 2.0–19.0). However, they did not control for estradiol in their calculations. Positive associations between breast cancer and total testosterone that were no longer significant after adjustment for estradiol were demonstrated by three groups (81, 97) or estrone (101). In contrast, Manjer *et al.* (100) reported an association between high total testosterone and breast cancer risk after adjustment for estradiol. Only two groups (95, 99) measured free testosterone. Cauley *et al.* (99) measured free testosterone by equilibrium dialysis, considered to be the most accurate methodology, in 97 cases and 244 controls and reported no association between free testosterone and breast cancer after adjustment for estradiol. In contrast Berrino *et al.* (95) measured free testosterone by RIA in 25 breast cancer patients and 100 controls and reported a significant association between higher free testosterone levels and breast cancer after adjustment for estradiol.

A reanalysis of prospective studies (103) reported that the RRs associated with a doubling of total testosterone levels were 1.37 (95% CI, 1.15–1.65) for the three studies incorporating a purification step in their testosterone assay (98, 99, 104) and 1.44 (95% CI, 1.21–1.72) for the four studies that used a direct testosterone assay (81, 95–97). However, a subgroup analysis to determine the association between breast cancer and free testosterone levels was not undertaken. Thus, evidence from clinical studies that the free fraction of testosterone is an independent risk factor for breast cancer is lacking.

B. Exogenous testosterone therapy and breast cancer risk

Three observational studies have addressed the use of testosterone therapy and breast cancer risk (105–107). Unfortunately, the primary aim for two of these studies was not testosterone supplementation and breast cancer risk; consequently, they each had only a small sample size for this subgroup analysis. Brinton *et al.* (108) undertook a case-control study of postmenopausal estrogen use and breast cancer risk. A subgroup analysis in this study of 25 patients and 29 controls showed no significant increase in risk with oral methyltestosterone in combination with conjugated equine estrogen (RR, 1.05; 95% CI, 0.6–1.8) (108). In contrast, Ewertz (109) studied the effects of the ever-use of im injections containing estradiol-testosterone (2.5 mg estradiol plus 50 mg testosterone or 5.0 mg estradiol plus 100 mg testosterone) given at a recommended interval of 3–7 wk in a subgroup analysis. This specific therapy was used for 56 of 1694 patients and 21 of 1705 controls. An RR of 2.3 (95% CI, 1.37–3.88) was reported (109). In the same study, there was no risk for triple combination of estrogen, progestin, and testosterone (RR 1.26; 95% CI, 0.58–2.74) (109). A recent retrospective analysis of 511 Australian women treated with conventional estrogen therapy plus 50–150 mg testosterone implants with a mean follow-up of 5.7 ± 2.5 yr, but no control group, reported a breast cancer incidence of 240 per 100,000 women years (107). This was reported as equivalent to the incidence in the general population determined by the state cancer registry.

C. Implications of the detection of the AR in human breast cancer

ARs are found in more than 50% of breast tumors (110), and the significance of ARs in breast cancer has been extensively explored. With regard to nodal metastasis, Soreide *et al.* (111) reported that when the median value of AR is taken as cut-off (50.5 pmol/g), a lower AR content is an independent predictor of the likelihood of axillary metastases ($P = 0.001$). AR amount, however, did not reveal any significant prognostic information concerning relapse-free survival. There appears to be no significant association between AR expression and the degree of differentiation of ductal carcinoma *in situ* (112).

In addition to the amount of AR, correlations between the repeat length of the CAG sequence in the AR and total risk of breast cancer, age at diagnosis, recurrence after surgery, and aggressive growth have been reported. CAG repeat length is associated with a decreased ability to activate AR-responsive genes (113). More CAG repeats in the AR gene have been associated with an earlier onset of breast cancer among BRCA1 mutation carriers (114). However, Kadouri *et al.* (115) found no significant association between the number of CAG and GGC repeats in the AR and breast cancer risk in either BRCA1/2 carriers or the general population if attention was restricted to Ashkenazi Jewish carriers, or only to BRCA1 or BRCA2 carriers. One explanation for the discrepancy is sample size in that the larger number of study subjects with the BRCA1 mutation in the former study (165 with and 139 without breast cancer) could provide a statistical difference rather than smaller study subjects with BRCA1 and BRCA2 mutations (122 with and 66 without breast cancer). Therefore, an effect of the AR repeat length on BRCA1 penetrance cannot be excluded. However, this remains inconclusive for BRCA2.

The relationship between the number of CAG and GGC repeats has also been evaluated in a population-based study conducted in 524 patients and 461 controls for their relationships to breast cancer risk (116). This study suggested a

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TABLE 5. Prospective studies of the association between plasma testosterone levels and risk of breast cancer in postmenopausal women

First author, year (Ref.)	Definition of study case	Mean or median time to diagnosis (yr)	No. of cases/controls	Mean or median age (yr)	Type of T assay	Ratio of control subjects to case patients	Unadjusted effect ratios (95% CI)	Adjusted RR by other variables	Effect ratios[a] (95% CI)[b]
Wysowski, 1987 (86)	Excluded new cases diagnosed within 6 months of recruitment	2.3	39/156	61.0[c] 61.0[c]	Total T	Matched 4:1	N/A	N/A	N/A[d]
Garland, 1992 (82)	All	9.0	31/287	65.3 66.6	Total T	Full cohort	[e]	Age	1[f]
Berrino, 1996 (95)	All	3.5	67/264	59.4 54.9	Total T, free T	Matched 4:1	5.9 (1.6–21.9)	Age Estradiol	5.7 (1.5–22.2) 5.9 (1.2–29.3)
Dorgan, 1996 (96)	All	2.9	71/133	61.0 62.0	Total T	Matched 2:1	3.7 (1.4–10.0)[g]	Years since menopause, height, weight, parity, family history of breast cancer	6.2 (2.0–19.0)
Thomas, 1997 (81)	All	7.8	61/179	58.6 58.5	Total T	Matched 3:1	2.4 (1.0–5.7)	Age at menarche, parity, BMI, years postmenopausal, Estradiol	Not different 0.8 (0.3–2.4)
Zeleniuch-Jacquotte, 1997 (97)	Excluded new cases diagnosed within 6 months of recruitment	2.7	851/163	59.2 59.1	Total T	Matched 4:1	2.7 (1.1–6.8)	Total estradiol, SHBG-bound estradiol	1.2 (0.4–3.5)
Hankinson, 1998 (98)	All	2.4	155/310	62[c] 62[c]	Total T	Matched 2:1	1.34[f]	BMI at age 18, family history of breast cancer, age at menarche, parity/age at first birth, and past HT Estradiol	1.4 (0.7–2.7) 1.1 (0.5–2.3)
Cauley, 1999 (99)	All	3.2	97/243	70.9 71.8	Total T, free T	Subcohort	2.7	Age, BMI, age at menarche, first birth, menopause, family history of breast cancer, physical activity, surgical menopause, alcohol Bioavailable estradiol	3.3 (1.1–10.3) 2.1 (0.9–4.7)
Manjer, 2003 (100)	All	5.4	173/438	61.6 60.5	Total T	Matched 2:1	[e]	Age, storage time, subcohort, parity, and oophorectomy Estradiol, SHBG	1.9 (1.1–3.3) 1.9 (1.1–3.3)
Zeleniuch-Jacquotte, 2004 (101)	Excluded new cases diagnosed within 6 months of recruitment	[e,h]	297/563	60 60	Total T	Cohort	2.2 (1.3–3.6)	Age at menarche, family history of breast cancer, parity/age at first birth, history of total oophorectomy, history of breast cancer Estrone	2.4 (1.4–4.0) 1.1 (0.9–1.3)

T, Testosterone; N/A, not applicable because comparison was mean difference between cases and controls; RH, relative hazard; HT, hormone therapy; Not different, adjusting for these variables had no effect; BMI, body mass index.

[a] Effect ratios were presented as RR for Garland, Berrino, Dorgan, and Hankinson studies; as OR for Thomas and Zeleniuch-Jacquotte studies; and as RH for Cauley study.

[b] Effect ratios of total testosterone, except for Berrino and Cauley studies, in which effect ratios were for free testosterone.

[c] Mean age of all participants.

[d] No statistically significant difference of testosterone levels between cases and controls by mean comparison.

[e] Data not available.

[f] 95% CI was not available.

[g] Reanalysis after incident case was defined as at least 2-yr interval after blood taken resulted in RR = 1.3 (0.5–3.4).

[h] Median age at diagnosis was 66.1 yr.

reduced risk for breast cancer in young women in whom the number of GGC repeat lengths was greater than 17. In addition, they also suggested that AR repeat length (CAG or GGC) may be partly responsible for the increased risk for early-onset breast cancer in women who use oral contraceptives, although these findings need replication in other populations (116).

VI. Aromatization and Breast Cancer Development

Estrogen biosynthesis in postmenopausal women is primarily the result of aromatization of circulating C19 steroids in extragonadal sites (1). The activity of the aromatase enzyme increases with age in fat tissue (117), and with increasing age there is greater fat tissue in the breast. Thus, hypothetically higher androgen levels in women provide increased substrate for estrogen biosynthesis within the breast. Despite this, it is also evident that estrogen itself can block its own bioformation in both human breast cancer cells and animal studies. Nakamura *et al.* (118) have shown that ovariectomy increases and estradiol treatment decreases aromatase activity in baboon mammary tissue. Subsequently, an inverse correlation between tumor aromatase activity and estrogen content has been reported in nude mice bearing xenografts of MCF-7 cells transfected with the aromatase gene (119). Moreover, an *in vitro* study in which MCF-7 cells were cultured long term in an estrogen-deprived medium and called by the acronym LTED cells found that long-term estrogen deprivation enhanced aromatase activity by 3- to 4-fold when compared with the wild-type MCF-7 cells (119). Reexposure of LTED cells to estrogen resulted in reducing aromatase activity to the levels of the wild-type MCF-7 cells (119). Consistent with these findings, intratumoral aromatase activity was higher in women with lower circulating estrogen (120, 121), and relative low activity was found in the patients taking hormone therapy (119, 122). These data 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. Therefore, it is more appropriate to use testosterone replacement only in women who have adequate estrogen replacement.

VII. Should Androgens Be Included in Postmenopausal Hormone Therapy Regimens?

Whether there is a role for the use of androgens in the management of postmenopausal women remains controversial. Clinical studies of supraphysiological testosterone therapy have shown improvements in sexual parameters in postmenopausal women (123–125). More recent studies employing more physiological doses have shown benefits in several parameters of sexual function and in mood (126–128). At present, a variety of testosterone-containing preparations are being used in clinical practice or in investigational research protocols for the treatment of sexual problems in women. Although the findings of this review indicate favorable effects of nonaromatizable androgens in the breast, in contrast to testosterone, there are few data from large well-designed randomized controlled trials to support the use of methyltestosterone in the management of sexual dysfunction in women (129). Also, data pertaining to the use of DHT in women are completely lacking. It is clear that whether or not the effects of testosterone on sexual function and mood in women are, in part, dependent on aromatization within the brain needs to be elucidated.

Combined oral estrogen-progestin postmenopausal therapy is associated with an increase in breast cancer risk (6, 7, 10). Whether this is an effect of oral estrogen or the inclusion of progestin is not known. However, the *in vitro* and *in vivo* data we have summarized indicate that in an estrogen-replete environment androgens oppose the unfavorable effects of estrogen in breast tissue. Consistent with this concept, tibolone, a synthetic steroid with estrogenic, progestogenic, and androgenic properties does not appear to have any adverse effects on breast tissue *in vitro* (130). In contrast, a large cohort study reported a RR of breast cancer of 1.45 (95% CI, 1.25–1.68) for users *vs.* nonusers of tibolone (131). However, these data should be viewed cautiously until reevaluated in a randomized controlled trial because of the inherent bias in the study population and the tendency for clinicians to prescribe tibolone rather than standard hormone therapy to women believed to be at increased breast cancer risk (132, 133). The Women's Health Initiative study reported that for the 12,304 women in the study who had never previously been treated with hormone therapy there was no increase in breast cancer risk for a mean duration of 5.2 yr of estrogen-progestin therapy (hazard ratio, 1.06; 95% CI, 0.81–1.28) (5–11). The significant increase in breast cancer risk reported in this study was related to the 4304 prior users (5–11). Thus, a large study would be required to demonstrate the risks *vs.* benefit of adding testosterone therapy to estrogen-progestin therapy for 5 yr; in addition, to explore any possibility that there may be a reduction in risk with testosterone, the study would need to be beyond 5 yr duration (133). An alternative approach would be to employ surrogate endpoints such as mammographic density and indices of breast cell proliferation and apoptosis. The latter requires breast needle biopsies in healthy women, a minor but invasive procedure. Although the findings from such research may be informative, there is still the danger of misleading results. An analogy is the substantial evidence that estrogen lowers lipids; yet in one large randomized controlled trial, estrogen-progestin therapy was associated with more cardiovascular events (5–11). Thus, 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. Testosterone therapy for women should involve regular measurements of circulating levels of free testosterone, and levels should be maintained below the upper limit of the normal physiological range for young women to avoid androgen excess (4).

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VIII. Conclusion

Breast cancer has complex etiologies; however, endogenous sex steroids clearly have a role in the progression of this disease. *In vitro* and *in vivo* studies indicate that both testosterone and DHT have a predominantly inhibitory influence on the mitogenic and cancer-promoting effects of estrogen in breast cells and promote apoptosis via the AR. There are, however, variations in these effects according to the type of breast cancer cell line studied, the androgen administered, and the dose used. These differences appear to be a consequence of differing levels of coactivator and corepressor proteins that influence AR actions in different cell types.

Unfortunately, most clinical studies have used total testosterone as a measure of androgen exposure, and these generally have shown that higher total testosterone levels are associated with increased breast cancer risk. However, these findings may reflect higher SHBG levels due to higher endogenous estrogen. There are few data pertaining to the relationship between free testosterone levels and breast cancer risk in humans using reliable assay methodology. Although studies in both premenopausal and postmenopausal women are inconclusive, there is no evidence that hyperandrogenism in women with PCOS is associated with increased breast cancer risk. Data for the use of exogenous testosterone and breast cancer risk are limited. The strongest supporting data for exogenous testosterone therapy come from primate studies. Based on such simulations, inclusion of testosterone in postmenopausal estrogen-progestin regimens has the potential to ameliorate the stimulating effects of combined estrogen-progestin on the breast. Research addressing this is warranted; however, the number of women that would be required for an adequately powered randomized controlled trial renders such a study unlikely. Unless more specific data become available, the use of testosterone should be limited to women symptomatic of androgen insufficiency despite adequate estrogen replacement.

Acknowledgments

Address all correspondence and requests for reprints to: Professor Susan R. Davis, The Jean Hailes Foundation NHMRC Centre of Clinical Research Excellence, 173 Carinish Road, Clayton, Victoria 3168, Australia. E-mail: susan.davis@jeanhailes.org.au

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