Androgens and mammary growth and neoplasia

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Objective: Evaluation of current clinical, experimental, genetic, and epidemiological data pertaining to the role of androgens in mammary growth and neoplasia.

Design: Literature review.

Setting: National Institutes of Health.

Subject(s): Recent, basic, clinical, and epidemiological studies.

Intervention(s): None.

Main Outcome Measure(s): Effects of androgens on mammary epithelial proliferation and/or breast cancer incidence.

Result(s): Experimental data derived from rodents and cell lines provide conflicting results that appear to be strain- and cell line–dependent. Epidemiologic studies have significant methodological limitations and provide inconclusive results. The study of molecular defects involving androgenic pathways in breast cancer is in its infancy. Clinical and nonhuman primate studies, however, suggest that androgens inhibit mammary epithelial proliferation and breast growth and that conventional estrogen treatment suppresses endogenous androgens.

Conclusion(s): Abundant clinical evidence suggests that androgens normally inhibit mammary epithelial proliferation and breast growth. Suppression of androgens by conventional estrogen treatment may thus enhance estrogenic breast stimulation and possibly breast cancer risk. Clinical trials to evaluate the impact of combined estrogen and androgen hormone replacement regimens on mammary gland homeostasis are needed to address this issue. (Fertil Steril 2002;77(Suppl 4):S26–33. ©2002 by American Society for Reproductive Medicine.)

Key Words: Breast cancer, estrogen, androgen receptor, testosterone, oral contraceptive, hormone replacement
androgens in women on E therapy might contribute to unopposed estrogenic stimulation of the breast and potentially augment breast cancer risk.

ESTROGENS, ANDROGENS, AND BREAST DEVELOPMENT

Estrogens stimulate and androgens inhibit breast development, independent of genetic sex. Pubertal rises in E levels cause breast growth in girls (5) and frequently in boys (transiently) (6). Estradiol levels are significantly higher in girls with premature thelarche than in normal prepubertal girls (7). Recently, an association between expression of a high-activity isoform of the T-metabolizing CYP3A4 and the early onset of thelarche has been documented, suggesting that decreasing T levels may also trigger early breast growth (8). Conversely, androgen excess caused by adrenal tumor or hyperplasia suppresses normal breast development in girls, despite apparently adequate E levels (9–11). In castrated male-to-female transsexuals, feminizing E therapy stimulates breast growth with full acinar and lobular formation (12), and E-treated genetically male breast tissue exhibits normal female histology. Estrogens taken to treat prostate cancer also lead to breast development in men with suppressed gonadal function and reduced T levels (13). Conversely, androgen use by female athletes and female-to-male transsexuals leads to breast atrophy (14, 15).

Supporting the normal inhibitory role of endogenous androgens on breast growth, androgen receptor (AR) blockade with flutamide causes gynecomastia (16), and AR deletion or inactivating mutation is associated with macromastia (and increased breast cancer). Males may also develop gynecomastia when the E–androgen ratio is increased because of decreased androgen production or increased aromatization (6).

It has not been possible to identify specific E–androgen ratios predictive of breast stimulation or inhibiting effects for several reasons. Estradiol and T assays have traditionally not been very sensitive in the lower ranges, and both hormones bind to SHBG, so total values may not be as informative as values of free or bioavailable hormone (4). Moreover, single-hormone measurements may not be very informative about tissue exposure over time. Both E2 and T levels vary from hour to hour in response to diurnal rhythms, diet, stress, and exercise (17, 18), so a single value may be inadequate to assess true tissue exposure.

In addition, E2 and T may be synthesized locally in peripheral tissues from circulating precursors such as DHEA or DHEAS and androstenedione (reviewed in references 19, 20). The conjugated products of steroid metabolism find their way into the circulation after peripheral action and provide evidence as to the proportion of the precursor pools of steroids used as androgen or E. Analyses of these metabolites by Labrie et al. (20) suggest that the major proportion of androgen effectors in women are derived from such an intracrine mode of action, which will not be detected by assays of circulating T or dihydrotestosterone (DHT). Interestingly, whereas circulating levels of T and DHT are 5- to 10-fold higher in men than in women, the abundance of androgen metabolites is less than twofold higher in men, suggesting that local tissue production and action of androgens in women may be more significant than previously suspected.

Pertinent to this review, the mammary gland is capable of the synthesis of both E2 and T. All the steroidogenic enzymes necessary for the formation of androgens and Es from steroid precursors—namely steroid sulfatase, 17β-hydroxysteroid dehydrogenases, 3β-hydroxysteroid dehydrogenases, 5α-reductases, and aromatase—have been reported in normal mammary tissues, breast cancer specimens, or cell lines (21–24). Breast cancer cell lines and tissue specimens express the enzymes involved in DHT as well as in E2 synthesis (21, 25–27). In a recent histochemical study, expression of 5α-reductase was significantly correlated with AR expression and 17β-hydroxysteroid dehydrogenase (HSD) (5) and 3β-HSD immunoreactivities, and the abundance of this androgenic molecular assembly was inversely correlated with tumor size, histological grade, and proliferative index (21), suggesting an inhibitory role for DHT in tumor growth.

ANDROGEN RECEPTOR

Androgen agonists such as T and DHT function by binding to the intracellular AR, which is a member of the nuclear hormone receptor superfamily comprising classic DNA-binding, hormone-binding, and activation domains (Fig. 1). Androgen receptor expression is abundant in normal mammary epithelium and in the majority of breast cancer specimens and cell lines (1, 2, 28, 29). The AR is colocalized with E and progesterone receptors in epithelial cells but is not detected in mammary stroma or myoepithelium (1, 30, 31).

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The coexpression of ER and AR in mammary epithelial cells suggests that the effects of E and androgen on mammary epithelial proliferation are integrated within the mammary epithelial cell. Interestingly, the AR gene is located on the X chromosome with no corresponding allele on the Y, so it functions solely as a single-copy gene, as shown by the complete loss of androgen effect in XY individuals with an inactivating mutation of the AR (32, 33).

The binding of T or DHT triggers a cascade of signaling events, including phosphorylation and conformational changes in the receptor, which dissociates from cytoplasmic proteins and migrates to the cell nucleus. Ligand-activated AR regulates gene expression by binding to androgen response elements (AREs) located in a gene’s enhancer or promoter region. As with other such receptors, the AR functions in transcriptional regulation in concert with a host of nuclear proteins, which may serve as coactivators or corepressors. Interestingly, the breast cancer 1 (BRCA1) gene product has recently been identified as an AR coactivator (34, 35). The BRCA1 protein binds to the AR and potentiates AR-mediated effects, suggesting that BRCA1 mutations may blunt androgen effects.

The AR has a highly polymorphic CAG repeat in exon 1 that encodes a polyglutamine stretch (Fig. 1). Longer polyglutamine repeat sequences are associated with decreased AR potency in vitro (33). The significance of the CAG repeat length for the risk of breast cancer remains unclear. One study on 304 breast cancer patients carrying a BRCA1 mutation demonstrated an earlier age of onset correlated with longer AR CAG repeat sequences (36); however, other studies have not confirmed this finding in different populations (37–40). A weak inverse association was noted between the AR trinucleotide repeat length and markers of breast tumor malignancy in another study (41). However, germline mutations in the AR gene conferring variable degrees of androgen insensitivity have been associated with the occurrence of breast cancer in men (42). It should be emphasized that none of these studies had sufficient statistical power to implicate or exclude specific AR defects in breast cancer risk.

CIRCULATING ANDROGENS AND BREAST CANCER RISK

Long-term treatment with Es increases the risk of breast cancer in both males and females (43), with estrogenic stimulation of mammary epithelial proliferation appearing to be the primary cause for this effect, although additional carcinogenic effects by E metabolites have been proposed (44). The most widely accepted risk factor for breast cancer is the cumulative dose of E that breast epithelium is exposed to over time (45). Interestingly, however, it has been difficult to correlate breast cancer risk with isolated serum E levels in epidemiological studies, probably secondary to the problems with use of single random hormone levels for the evaluation of tissue-specific exposure discussed above.

Attempts to correlate adrenal precursor steroids with breast cancer incidence have been relatively more successful or at least consistent, perhaps reflecting the importance of local tissue conversion as mentioned above. Many years ago, reduced 17-ketosteroid excretion was noted in the urine of premenopausal women with breast cancer (46) and subsequent studies have documented reduced levels of DHEA and its sulfate, DHEAS, in the serum of premenopausal breast cancer patients (47).

Several studies have found, however, that adrenal androgens are increased in postmenopausal women with breast cancer (reviewed in Adams (48)). One possible explanation proposed for the divergence between premenopausal and postmenopausal findings (49) is that one adrenal “androgen,” androstenediol, also known as hermaphrodol, is a weak agonist at the E receptor. In the presence of high E levels in premenopausal women, androstenediol could have anti-estrogenic effects, whereas in the hypoestrogenic postmenopausal milieu, the agonist effect may predominate (50–52). This view remains speculative, however, and other possibilities exist. For example, DHEA suppresses the development of experimental mammary cancer in rats, apparently via local AR-mediated effects (53–55). It is possible that the high E environment in premenopausal women promotes androgenic enzyme and AR expression by mammary tissue, allowing androgenic effects by DHEA metabolites, whereas the postmenopausal, E-deficient tissue microenvironment may favor estrogenic effects.

In recent years, a number of epidemiological studies have examined the correlation between circulating androgens such as T and breast cancer risk. A major limitation of such studies is the fact that the androgen assays used in these studies were developed primarily to measure the higher levels found in men and lack reliability in the low ranges found in normal women (4). Moreover, T and androstenedione levels demonstrate substantial variability from day to day and even hour to hour, whereas most of the epidemiological data is based on a single blood sample collected at nonstandard times. Another problem in using serum T levels to gauge androgenic effects at the tissue level is that most circulating T is tightly bound to SHBG, whereas only the unbound hormone is bioactive. Sex hormone–binding globulin, and thus total T levels, vary widely based on genetic, metabolic, and endocrine influences (56), and it is now accepted that measurements of free or bioavailable T predict androgenic effects more accurately than do total T levels (4). Finally, as discussed above, most androgenic activity in women originates from the peripheral conversion of precursors such as DHEA into androgens within the cells of target tissues, and this activity will not be detected in the measurement of circulating androgens.

In studies of incident breast cancer subjects (Table 1), Secreto et al. (59, 60) found elevated T levels in premenopausal and postmenopausal cases, but a more recent study
did not find any association between T levels and postmenopausal breast cancer cases postoperatively (64). In prospective epidemiological studies, age-adjusted mean values of total and free T and E$_2$ were significantly higher prediagnostically in postmenopausal breast cancer case subjects compared with case controls (58), and E$_2$ and total T were likewise elevated in other case-control studies of postmenopausal breast cancer (57, 68).

In none of these studies, however, was it possible to dissociate the risk associated with elevated E$_2$ levels from the androgen component, and because androgens are the obligate precursors to E$_2$ synthesis, this is a major confounding factor in assessing the role of androgen, independent of the known cancer-promoting effects of E.

Several other studies have found no association between androgens and breast cancer (65–67). A recent study of 97 postmenopausal women with breast cancer found elevation of all sex steroids in cancer cases but found an association between free T and breast cancer relative risk that was statistically independent of the E-associated risk (69). Another, similar study found elevated circulating T in some cases, but after adjusting for free E$_2$, no significant independent correlation between T and breast cancer remained (70).

These observations indicate that it is very difficult to separate potential direct effects of circulating T from its potential to be aromatized into E$_2$. It would be more interesting to investigate levels of T and DHT metabolites in these studies to assess tissue exposure to androgen more directly.

As noted above, a single serum hormone determination seems unlikely to be informative about a woman’s true long-term exposure to that hormone or her specific risk of developing breast cancer. Nor does there seem to be a biologically plausible mechanism whereby androgens acting as androgens could promote breast cancer, because virtually all-clinical data suggest just the opposite.

If elevated androgen levels directly contribute to breast cancer, then women with clinically evident long-term hyperandrogenism, for instance, in the cases of polycystic ovary syndrome and congenital adrenal hyperplasia (CAH), should experience increased rates of breast cancer, but they do not (71). Moreover, androgen levels are chronically elevated in men, who have a breast cancer risk that is <1% that of women (72). This is despite the fact that E$_2$ levels over the lifespan are not very much lower in men than in women. In fact, decreased androgen levels, for instance, as present in Kleinfelter’s syndrome and other hypogonadal syndromes, increase the risk of breast cancer in males. Epidemiological studies in men indicate that low urinary androsterone and serum free-T levels are related to early onset of breast cancer, a much higher relapse rate, and a worse response to endocrine therapy (63, 73).

### ANDROGENS AND BREAST CANCER: EXPERIMENTAL DATA

As previously noted, steroid hormones exert most actions by binding and activating transcription factors, namely steroid hormone receptors, which in turn regulate a large number of other genes. These other gene products mediate additional events engaging additional targets and mechanisms. In vitro studies in mammary carcinoma cells have shown that androgen-induced growth factor exhibits oncogenic action (74). Another androgen-induced factor, keratinocyte growth factor (KGF), may serve as a paracrine growth factor important in the control of proliferation of normal and neoplastic mammary epithelium (75) and may be added to the increasing list of growth factors with potential roles in the progression of breast carcinomas.

Androgens stimulate or inhibit the growth of breast cancer cells in vitro depending on the cell line and clone under study (29). Androgens inhibit the proliferation of ZR-75–1 breast cancer cells via AR activation (76). Part of the growth...

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**TABLE 1**

Epidemiological studies on the association between androgen levels and breast cancer risk.

<table>
<thead>
<tr>
<th>Breast cancer risk</th>
<th>Investigators (reference no.)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased</td>
<td>Dorgan et al. (57)</td>
<td>Serum levels of T are positively associated with postmenopausal cases</td>
</tr>
<tr>
<td></td>
<td>Berrino et al. (58)</td>
<td>High serum T levels in postmenopausal women precede breast cancer occurrence</td>
</tr>
<tr>
<td></td>
<td>Secreto et al. (59)</td>
<td>Elevated levels of T in postmenopausal cases</td>
</tr>
<tr>
<td></td>
<td>Secreto et al. (60)</td>
<td>Elevated levels of T in premenopausal cases</td>
</tr>
<tr>
<td>Decreased</td>
<td>Lee et al. (61)</td>
<td>Androgen deficiency associated with premenopausal cases</td>
</tr>
<tr>
<td></td>
<td>Thomas et al. (62)</td>
<td>Increased risk in men with androgen deficiency</td>
</tr>
<tr>
<td></td>
<td>Thomas et al. (63)</td>
<td>Negative association between excretion rate of androgens and recurrence rate</td>
</tr>
<tr>
<td>No association</td>
<td>Lipworth et al. (64)</td>
<td>Serum T levels in postmenopausal women</td>
</tr>
<tr>
<td></td>
<td>Helzlsouer et al. (65)</td>
<td>Serum androstenedione levels</td>
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<td></td>
<td>Garland et al. (66)</td>
<td>Serum levels of T and androstenedione in postmenopausal women</td>
</tr>
<tr>
<td></td>
<td>Wysowski et al. (67)</td>
<td>Serum levels of T and androstenedione in premenopausal and postmenopausal cases</td>
</tr>
</tbody>
</table>

inhibitory effect is caused by down-regulation of E receptor expression, but additional inhibitory effects appear to be E independent (77). Other studies suggest that adrenal androgens stimulate the proliferation of breast cancer cells by activation of the E receptor (78). Recent data indicate that androgens can down-regulate bcl-2 proto-oncogene levels via AR-mediated mechanism, thus promoting apoptosis in human breast cancer cell lines (79, 80). Because a balance between cell proliferation and apoptosis is critical for the control of tissue growth, this finding provides a novel mechanism for the inhibitory effects of androgens on breast carcinomas.

DHEA prevents the development of mammary carcinoma induced by 7,12-dimethylbenzanthracene in the rat, and this protective effect is reversed by the anti-androgen flutamide, suggesting that DHEA’s effect is mediated by its conversion into T or DHT and activation of the AR (53, 54). A number of groups have shown that the growth of human breast cancer cell lines in nude mice and dimethylbenzanthracine-induced mammary tumors in rats are inhibited by DHT as well as by DHEA (55, 81). Indeed, androgens have been successfully used for the treatment of breast cancer in women, achieving an objective response comparable to that of other hormonal therapies (82, 83). One group, however, has found that androgens decrease the latency of E-induced breast cancer in the Noble rat (84). Although ARs are not found in the mammary stroma, this group detects increased stromal fibroblast proliferation in the androgen-treated rats, suggesting that systemic elevations of factors such as insulin-like growth factor I may play a role in this model.

ANDROGENS AND HORMONE REPLACEMENT THERAPY

Estrogens clearly induce and progestins clearly protect against endometrial cancer (85). Both endogenous and exogenous E exposure is thought to contribute to increased breast cancer risk. Since the introduction of combined OCs 40 years ago, many changes in the doses and biochemical structure have taken place, and intense research has been conducted to examine the possibility that OCs may increase the risk of breast cancer.

Although many epidemiological studies in the past have not linked OC use to breast cancer risk (86, 87), a number of more recent studies have found an association, either overall or especially in subgroups of women (88–90). A large meta-analysis of the majority of previously published studies calculated a small but significant increase in relative risk (RR) of breast cancer (RR = 1.24) in current OC users (91). However, because pill users are young, this represented a very small increase in absolute risk. Also, in another recent study, women taking OCs before 1975 (high-dose formulations) who had a first-degree relative with breast cancer were at particularly high risk for breast cancer (RR = 4.6) (92).

It is not yet known whether lower dose formulations are associated with a similar increase in risk. Different formulations of OCs containing different doses of Es and different progestins with more or less potent androgenic effects make it very difficult to compare and to reach some conclusions. Also, the bulk of the currently available evidence supports a causal relationship between the use of hormone replacement therapy and breast cancer (93–96). Current, recent, and long-term users of hormone replacement therapy are associated with the highest risk. Also, the effect of concurrent progestin use appears to further increase risk above that with Es alone (95).

If androgens are protective against breast cancer, as many of the studies reviewed here suggest, then conventional hormone replacement therapy may promote breast cancer not only by increasing E exposure but also by decreasing endogenous androgen activity. Oral E therapy reduces free androgens by stimulating hepatic production of SHBG and by suppressing LH, thus inhibiting ovarian androgen production (4). Thus, institution of pharmacological E therapy at menopause may result in a drastic reduction in the T–E ratio, which is normally maintained at relatively high levels throughout a woman’s lifespan (Fig. 2).

If androgens are indeed protective against E-induced mammary proliferation, then the suppression of normal endogenous androgen may be an adverse consequence of pharmacological E therapy. Supporting this view, a recent study found that a low-dose OC induced robust mammary epithelial proliferation in rats but that addition of methyltestosterone to the therapy significantly suppressed the proliferation (3). We have shown that addition of T to E therapy in

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**FIGURE 2**

Average E, and T levels across the female lifespan, with dashed lines predicting changes in hormone levels resulting from pharmacological E therapy beginning at menopause. –T (pg/ml), –E2 (pg/ml), –T/ERT, –E2/ERT.


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**FIGURE 2**

Average E, and T levels across the female lifespan, with dashed lines predicting changes in hormone levels resulting from pharmacological E therapy beginning at menopause. –T (pg/ml), –E2 (pg/ml), –T/ERT, –E2/ERT.

ovariectomized rhesus monkeys significantly inhibits E2-induced mammary epithelial proliferation (Fig. 3) (1). In addition, T treatment significantly reduced mammary epithelial E receptor expression, suggesting a potential mechanism for the growth inhibitory effect.

In a more recent study, we found that low physiological doses of T produced serum levels in the midnormal range for women as well as rhesus monkeys (e.g., ~40 ng/dL) completely inhibits the pharmacological E therapy-induced increase in mammary epithelial proliferation. Moreover, we have recently found that treatment of intact-cycling monkeys with the AR antagonist, flutamide, resulted in a significant increase in mammary epithelial proliferation, adding to the burden of evidence that endogenous androgens normally limit mammary proliferation and hence also cancer risk.

These observations suggest that the addition of physiological doses of androgen to OC and replacement E therapy could protect the breast from “unopposed” estrogenic effects.

**SUMMARY**

This review focused on the role of androgens with respect to breast growth and neoplasia. Measurement of circulating sex steroids and their metabolites demonstrates that androgen activity is normally quite abundant in healthy women throughout the entire life cycle. Epidemiological studies investigating T levels and breast cancer risk have major theoretical and methodological limitations and do not provide any consensus. The molecular epidemiology of defects in pathways involved in androgen synthesis and activity in breast cancer holds great promise but is still in early stages. Clinical observations and experimental data indicate that androgens inhibit mammary growth and have been used with success similar to that of tamoxifen to treat breast cancer.
Given these considerations, it is of concern that current forms of E treatment in OCs and for ovarian failure result in suppression of endogenous androgen activity. Thus, there is a need for studies on the efficacy of supplementing both oral contraception and E replacement therapy with physiological replacement androgen, perhaps in a nonormatizable form, to maintain the natural E:androgen ratios typical of normal women.

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