FERTILITY AND STERILITY®
VOL. 77, NO. 4, SUPPL 4, APRIL 2002
Copyright ©2002 American Society for Reproductive Medicine
Published by Elsevier Science Inc.
Printed on acid-free paper in U.S.A.

Androgens and mammary growth and neoplasia

Constantine Dimitrakakis, M.D., Jian Zhou, M.D., and Carolyn A. Bondy, M.D.

Developmental Endocrinology Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland

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 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

The importance of estrogens in stimulating mammary epithelial proliferation and breast growth and in increasing the risk for breast cancer is well established. The normal ovary produces relatively larger amounts of androgen compared with estrogens (Es), however, and a variety of clinical and experimental observations suggest that androgens normally inhibit estrogenic effects on mammary growth. Both androgen and E receptors are expressed in mammary epithelium (1, 2), suggesting that the steroid hormone effects may be integrated at the level of the mammary epithelial cell.

Recent experimental data suggest that conventional E treatment regimens, both as oral contraceptives (OCs) (3) and as hormone "replacement therapy" (1), upset the normal E–androgen balance and promote unopposed estrogenic stimulation of mammary epithelial proliferation and hence potentially breast cancer risk. This is because the suppression of gonadotropins by exogenous E treatment results in globally reduced ovarian steroidogenesis, so both endogenous E and androgen production are reduced, but only Es are provided by the treatment regimens. Moreover, Es, particularly in oral form, stimulate the hepatic production of sex hormone–binding globulin (SHBG), which binds testosterone (T) with high affinity, reducing androgen bioavailability. As a result of these dual effects, both total and bioavailable T levels are significantly reduced in women taking OCs or E replacement for ovarian insufficiency (4).

This review of the literature was prompted by our concern that the iatrogenic reduction in

Received October 16, 2001; revised and accepted January 9, 2002.
Reprint requests: Carolyn A. Bondy, M.D., Developmental Endocrinology Branch, National Institute of Child Health and Human Development, National Institutes of Health, Building 10, Room 10N262, 10 Center Drive, MSC 1862, Bethesda, Maryland 20892-1862 (FAX: 301-402-0574; E-mail: bondyc@mail.nih.gov).

0015-0282/02/$22.00
PII S0015-0282(02)02979-5

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).

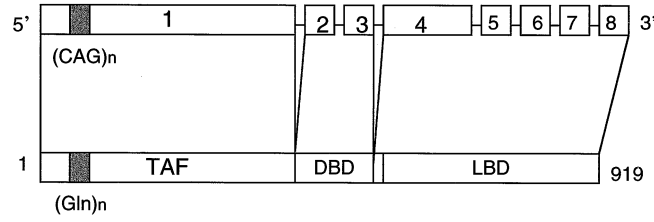
Supporting the normal inhibitory role of endogenous androgens on breast growth, androgen receptor (AR) blockade with flutamide causes gynecomastia (16), and AR deletion or inactivating mutation is associated with macromastia (and increased breast cancer). Males may also develop gynecomastia when the 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 E_2 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, E_2 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

FIGURE 1

Schematic design of the androgen receptor gene (*top*) and protein (*below*). The polymorphic trinucleotide repeat site is indicated at the *left*. Transactivating function (TAF), DNA-binding (DBD), and ligand-binding domains (LBD) are labeled.



Dimitrakakis. Androgens and the mammary gland. Fertil Steril 2002.

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 E_2 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 E_2 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).

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

TABLE 1

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

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

Dimitrakakis. Androgens and the mammary gland. Fertil Steril 2002.

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 Kleinefelter'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

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.

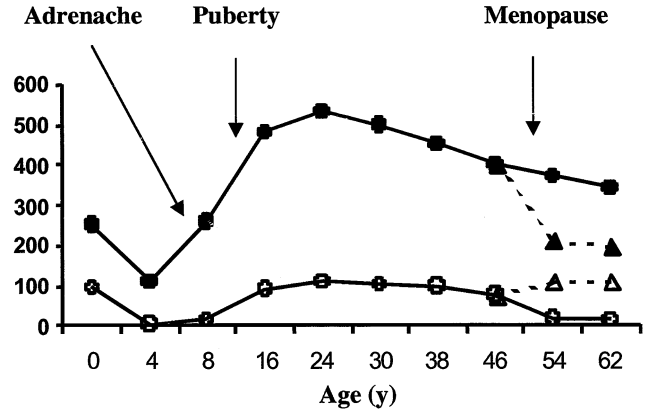
ANDROGENS AND HORMONE REPLACEMENT THERAPY

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).

FIGURE 2

Average E_2 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), –○– E_2 (pg/ml), --▲-- T/ERT, --△-- E/ERT.



Dimitrakakis. Androgens and the mammary gland. Fertil Steril 2002.

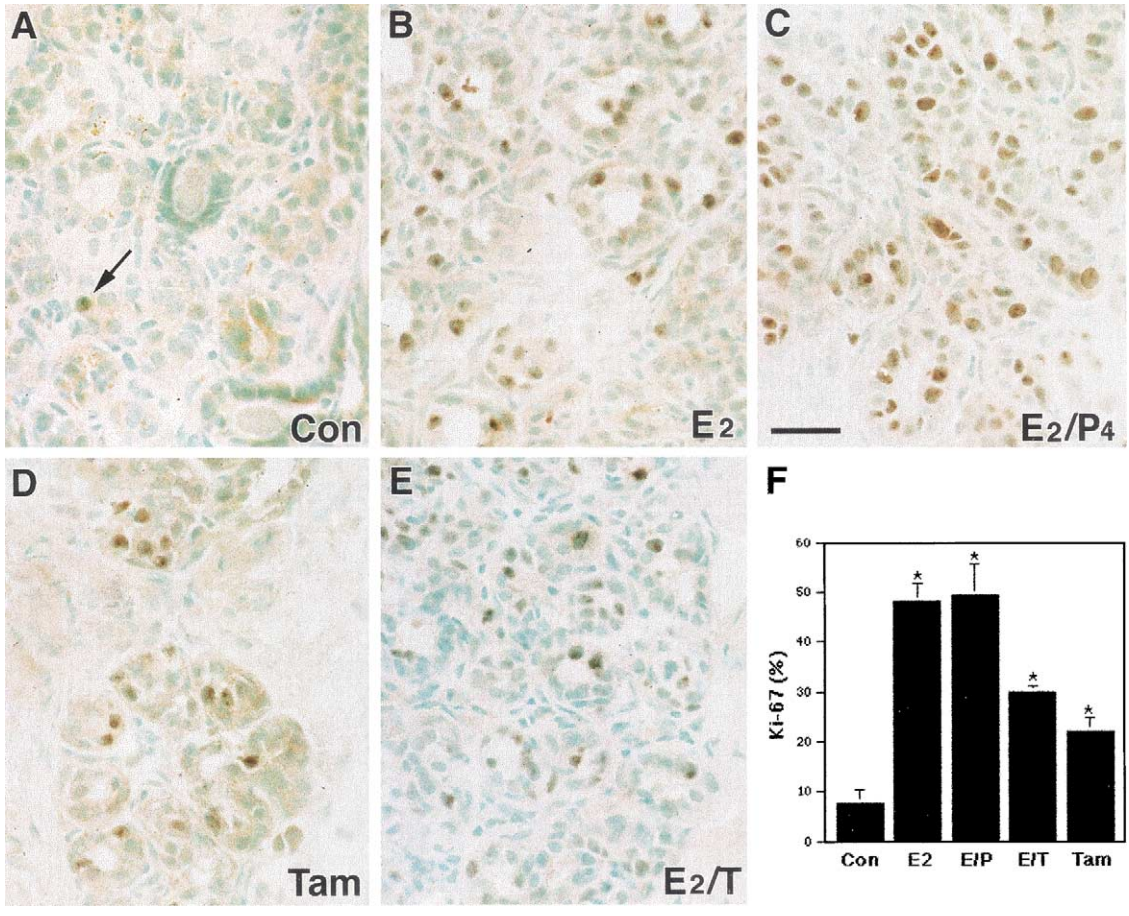
It is not yet known whether lower dose formulations are associated with a similar increase in risk. Different formulations of OCs containing different doses of Es and different progestins with more or less potent androgenic effects make it very difficult to compare and to reach some conclusions. Also, 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_2 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

FIGURE 3

Mammary epithelial proliferation shown by Ki67 immunoreactivity in ovariectomized monkeys treated with vehicle (**A**), E_2 (**B**), E_2 and P_4 (**C**), tamoxifen (**D**), and E_2 and T (**E**). Quantification of the Ki67 proliferation index is shown graphically in (**F**). Data from Zhou et al. (1).



Dimitrakakis. Androgens and the mammary gland. Fertil Steril 2002.

ovariectomized rhesus monkeys significantly inhibits E_2-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 need for studies on the efficacy of supplementing both oral contraception and E replacement therapy with physiological replacement androgen, perhaps in a nonaromatizable form, to maintain the natural E–androgen ratios typical of normal women.

References

1. Zhou J, Ng S, Adesanya-Famuiya O, Anderson K, Bondy CA. Testosterone inhibits estrogen-induced mammary epithelial proliferation and suppresses estrogen receptor expression. FASEB J 2000;14:1725–30.
2. Hall RE, Aspinall JO, Horsfall DJ, Birrell SN, Bentel JM, Sutherland RL, et al. Expression of the androgen receptor and an androgen-responsive protein, apolipoprotein D, in human breast cancer. Br J Cancer 1996;74:1175–80.
3. Jayo MJ, Register TC, Hughes CL, Blas-Machado U, Sulistiawati E, Borgerink H, et al. Effects of an oral contraceptive combination with or without androgen on mammary tissues: a study in rats. J Soc Gynecol Investig 2000;7:257–65.
4. Lobo RA. Androgens in postmenopausal women: production, possible role, and replacement options. Obstet Gynecol Surv 2001;56:361–76.
5. Feuillan P, Merke D, Leschek EW, Cutler GB Jr. Use of aromatase inhibitors in precocious puberty. Endocr Relat Cancer 1999;6:303–6.
6. Braunstein GD. Aromatase and gynecomastia. Endocr Relat Cancer 1999;6:315–24.
7. Klein KO, Mericq V, Brown-Dawson JM, Larmore KA, Cabezas P, Cortinez A. Estrogen levels in girls with premature thelarche compared with normal prepubertal girls as determined by an ultrasensitive recombinant cell bioassay. J Pediatr 1999;134:190–2.
8. Kadlubar F, Berkowitz G, Delongchamp R, Green B, Wang C, Wolff M. The putative high activity variant, CYP3A4*1B, predicts the onset of puberty in young girls [abstract]. In: Proceedings of the American Association for Cancer Research. March 2001;vol. 42.
9. Forsbach G, Guitron-Cantu A, Vazquez-Lara J, Mota-Morales M, Diaz-Mendoza ML. Virilizing adrenal adenoma and primary amenorrhea in a girl with adrenal hyperplasia. Arch Gynecol Obstet 2000;263:134–6.
10. Sakuma T, Yamaguchi T, Abe H, Kanda F, Hanioka K, Hisano K, et al. Adrenogenital syndrome caused by an androgen-producing adrenocortical tumor. Intern Med 1994;33:790–4.
11. Summers RH, Herold DA, Seely BL. Hormonal and genetic analysis of a patient with congenital adrenal hyperplasia. Clin Chem 1996;42: 1483–7.
12. Kanhai RC, Hage JJ, van Diest PJ, Bloemena E, Mulder JW. Short-term and long-term histologic effects of castration and estrogen treatment on breast tissue of 14 male-to-female transsexuals in comparison with two chemically castrated men. Am J Surg Pathol 2000;24:74–80.
13. Hedlund PO, Henriksson P. Parenteral estrogen versus total androgen ablation in the treatment of advanced prostate carcinoma: effects on overall survival and cardiovascular mortality. The Scandinavian Prostatic Cancer Group (SPCG)-5 Trial Study. Urology 2000;55:328–33.
14. Korkia P, Stimson GV. Indications of prevalence, practice and effects of anabolic steroid use in Great Britain. Int J Sports Med 1997;18:557–62.
15. Burgess HE, Shousha S. An immunohistochemical study of the long-term effects of androgen administration on female-to-male transsexual breast: a comparison with normal female breast and male breast showing gynaecomastia. J Pathol 1993;170:37–43.
16. Staiman VR, Lowe FC. Tamoxifen for flutamide/finasteride-induced gynecomastia. Urology 1997;50:929–33.
17. Zitzmann M, Nieschlag E. Testosterone levels in healthy men and the relation to behavioural and physical characteristics: facts and constructs. Eur J Endocrinol 2001;144:183–97.
18. Berrino F, Bellati C, Secreto G, Camerini E, Pala V, Panico S, et al. Reducing bioavailable sex hormones through a comprehensive change in diet: the diet and androgens (DIANA) randomized trial. Cancer Epidemiol Biomarkers Prev 2001;10:25–33.
19. Labrie F. Intracrinology. Mol Cell Endocrinol 1991;78:C113–8.
20. Labrie F, Luu-The V, Lin SX, Simard J, Labrie C, El-Alfy M, et al. Intracrinology: role of the family of 17 beta-hydroxysteroid dehydrogenases in human physiology and disease. J Mol Endocrinol 2000;25: 1–16.
21. Suzuki T, Darnel AD, Akahira JI, Ariga N, Ogawa S, Kaneko C, et al. 5alpha-reductases in human breast carcinoma: possible modulator of in situ androgenic actions. J Clin Endocrinol Metab 2001;86:2250–7.
22. Labrie F, Luu-The V, Lin SX, Labrie C, Simard J, Breton R, et al. The key role of 17 beta-hydroxysteroid dehydrogenases in sex steroid biology. Steroids 1997;62:148–58.
23. Labrie F, Belanger A, Simard J, Van L-T, Labrie C. DHEA and peripheral androgen and estrogen formation: intracinology. Ann NY Acad Sci 1995;774:16–28.
24. Belvedere P, Gabai G, Dalla Valle L, Accorsi P, Trivoletti M, Colombo L, et al. Occurrence of steroidogenic enzymes in the bovine mammary gland at different functional stages. J Steroid Biochem Mol Biol 1996; 59:339–47.
25. Pelletier G, Luu-The V, Tetu B, Labrie F. Immunocytochemical localization of type 5 17beta-hydroxysteroid dehydrogenase in human reproductive tissues. J Histochem Cytochem 1999;47:731–8.
26. Wiebe JP, Muzia D, Hu J, Szwajcer D, Hill SA, Seachrist JL. The 4-pregnene and 5alpha-pregnane progesterone metabolites formed in nontumorous and tumorous breast tissue have opposite effects on breast cell proliferation and adhesion. Cancer Res 2000;60:936–43.
27. Sasano H, Frost AR, Saitoh R, Harada N, Poutanen M, Vihko R, et al. Aromatase and 17 beta-hydroxysteroid dehydrogenase type 1 in human breast carcinoma. J Clin Endocrinol Metab 1996;81:4042–6.
28. Isola JJ. Immunohistochemical demonstration of androgen receptor in breast cancer and its relationship to other prognostic factors. J Pathol 1993;170:31–5.
29. Birrell SN, Bentel JM, Hickey TE, Ricciardelli C, Weger MA, Horsfall DJ, et al. Androgens induce divergent proliferative responses in human breast cancer cell lines. J Steroid Biochem Mol Biol 1995;52:459–67.
30. Ruizeveld de Winter JA, Trapman J, Vermey M, Mulder E, Zegers ND, Van der Kwast TH. Androgen receptor expression in human tissues: an immunohistochemical study. J Histochem Cytochem 1991;39:927–36.
31. Janssen PJ, Brinkmann AO, Boersma WJ, Van der Kwast TH. Immunohistochemical detection of the androgen receptor with monoclonal antibody F39.4 in routinely processed, paraffin-embedded human tissues after microwave pre-treatment. J Histochem Cytochem 1994;42: 1169–75.
32. Chang C, Saltzman A, Yeh S, Young W, Keller E, Lee HJ, et al. Androgen receptor: an overview. Crit Rev Eukaryot Gene Expr 1995; 5:97–125.
33. Avila DM, Zoppi S, McPhaul MJ. The androgen receptor (AR) in syndromes of androgen insensitivity and in prostate cancer. J Steroid Biochem Mol Biol 2001;76:135–42.
34. Yeh S, Hu YC, Rahman M, Lin HK, Hsu CL, Ting HJ, et al. Increase of androgen-induced cell death and androgen receptor transactivation by BRCA1 in prostate cancer cells. Proc Natl Acad Sci USA 2000;97: 11256–61.
35. Park JJ, Irvine RA, Buchanan G, Koh SS, Park JM, Tilley WD, et al. Breast cancer susceptibility gene 1 (BRCAI) is a coactivator of the androgen receptor. Cancer Res 2000;60:5946–9.
36. Rebbeck TR, Kantoff PW, Krithivas K, Neuhausen S, Blackwood MA, Godwin AK, et al. Modification of BRCA1-associated breast cancer risk by the polymorphic androgen-receptor CAG repeat. Am J Hum Genet 1999;64:1371–7.
37. Given HF, Radbourne R, Oag H, Merritt S, Barclay E, Hanby AM, et al. The androgen receptor exon 1 trinucleotide repeat does not act as a modifier of the age of presentation in breast cancer. Eur J Cancer 2000;36:533–4.
38. Menin C, Banna GL, De Salvo G, Lazzarotto V, De Nicolo A, Agata S, et al. Lack of association between androgen receptor CAG polymorphism and familial breast/ovarian cancer. Cancer Lett 2001;168:31–6.
39. Dunning AM, McBride S, Gregory J, Durocher F, Foster NA, Healey CS, et al. No association between androgen or vitamin D receptor gene polymorphisms and risk of breast cancer. Carcinogenesis 1999;20: 2131–5.
40. Spurdle AB, Dite GS, Chen X, Mayne CJ, Southey MC, Batten LE, et al. Androgen receptor exon 1 CAG repeat length and breast cancer in women before age forty years. J Natl Cancer Inst 1999;91:961–6.
41. Yu H, Bharaj B, Vassilikos EJ, Giai M, Diamandis EP. Shorter CAG repeat length in the androgen receptor gene is associated with more aggressive forms of breast cancer. Breast Cancer Res Treat 2000;59: 153–61.
42. Lobaccaro JM, Lumbroso S, Belon C, Galtier-Dereure F, Bringer J, Lesimple T, et al. Male breast cancer and the androgen receptor gene. Nat Genet 1993;5(2):109–10.
43. Martin-Du Pan RC. Are the hormones of youth carcinogenic? [in French]. Ann Endocrinol (Paris) 1999;60:392–7.
44. Service RF. New role for estrogen in cancer? Science 1998;279:1631–3.
45. Henderson BE, Feigelson HS. Hormonal carcinogenesis. Carcinogenesis 2000;21:427–33.
46. Allen B. The excretion of 17-ketosteroids in the urine of patients with carcinoma of the breast. Lancet 1957;1:496–7.
47. Zumoff B, Levin J, Rosenfeld RS, Markham M, Strain GW, Fukushima DK. Abnormal 24-hr mean plasma concentrations of dehydroisoandro-

sterone and dehydroisoandrosterone sulfate in women with primary operable breast cancer. Cancer Res 1981;41:3360–3.
48. Adams JB. Adrenal androgens and human breast cancer: a new appraisal. Breast Cancer Res Treat 1998;51:183–8.
49. Wang DY, Allen DS, De Stavola BL, Fentiman IS, Brussen J, Bulbrook RD, et al. Urinary androgens and breast cancer risk: results from a long-term prospective study based in Guernsey. Br J Cancer 2000;82: 1577–84.
50. Spinola PG, Marchetti B, Labrie F. Adrenal steroids stimulate growth and progesterone receptor levels in rat uterus and DMBA-induced mammary tumors. Breast Cancer Res Treat 1986;8:241–8.
51. Poulin R, Labrie F. Stimulation of cell proliferation and estrogenic response by adrenal C19-delta 5-steroids in the ZR-75–1 human breast cancer cell line. Cancer Res 1986;46:4933–7.
52. Boccuzzi G, Brignardello E, Di Monaco M, Gatto V, Leonardi L, Pizzini A, et al. 5-En-androstene-3 beta,17 beta-diol inhibits the growth of MCF-7 breast cancer cells when oestrogen receptors are blocked by oestradiol. Br J Cancer 1994;70:1035–9.
53. Li S, Yan X, Belanger A, Labrie F. Prevention by dehydroepiandrosterone of the development of mammary carcinoma induced by 7,12-dimethylbenz(a)anthracene (DMBA) in the rat. Breast Cancer Res Treat 1994;29:203–17.
54. Luo S, Labrie C, Belanger A, Labrie F. Effect of dehydroepiandrosterone on bone mass, serum lipids, and dimethylbenz(a)anthracene-induced mammary carcinoma in the rat. Endocrinology 1997;138: 3387–94.
55. Gatto V, Aragno M, Gallo M, Tamagno E, Martini A, Di Monaco M, et al. Dehydroepiandrosterone inhibits the growth of DMBA-induced rat mammary carcinoma via the androgen receptor. Oncol Rep 1998; 5:241–3.
56. Tchernof A, Despres JP. Sex steroid hormones, sex hormone-binding globulin, and obesity in men and women. Horm Metab Res 2000;32: 526–36.
57. Dorgan JF, Longcope C, Stephenson HE Jr, Falk RT, Miller R, Franz C, et al. Relation of prediagnostic serum estrogen and androgen levels to breast cancer risk. Cancer Epidemiol Biomarkers Prev 1996;5:533–9.
58. Berrino F, Muti P, Micheli A, Bolelli G, Krogh V, Sciajno R, et al. Serum sex hormone levels after menopause and subsequent breast cancer. J Natl Cancer Inst 1996;88:291–6.
59. Secreto G, Toniolo P, Berrino F, Recchione C, Cavalleri A, Pisani P, et al. Serum and urinary androgens and risk of breast cancer in postmenopausal women. Cancer Res 1991;51:2572–6.
60. Secreto G, Toniolo P, Berrino F, Recchione C, Di Pietro S, Fariselli G, et al. Increased androgenic activity and breast cancer risk in premenopausal women. Cancer Res 1984;44:5902–5.
61. Lee SH, Kim SO, Kwon SW, Chung BC. Androgen imbalance in premenopausal women with benign breast disease and breast cancer. Clin Biochem 1999;32:375–80.
62. Thomas DB, Jimenez LM, McTiernan A, Rosenblatt K, Stalsberg H, Stemhagen A, et al. Breast cancer in men: risk factors with hormonal implications. Am J Epidemiol 1992;135:734–48.
63. Thomas BS, Bulbrook RD, Hayward JL, Millis RR. Urinary androgen metabolites and recurrence rates in early breast cancer. Eur J Cancer Clin Oncol 1982;18:447–51.
64. 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.
65. Helzlsouer KJ, Alberg AJ, Bush TL, Longcope C, Gordon GB, Comstock GW. A prospective study of endogenous hormones and breast cancer. Cancer Detect Prev 1994;18(2):79–85.
66. Garland CF, Friedlander NJ, Barrett-Connor E, Khaw KT. Sex hormones and postmenopausal breast cancer: a prospective study in an adult community. Am J Epidemiol 1992;135:1220–30.
67. Wysowski DK, Comstock GW, Helsing KJ, Lau HL. Sex hormone levels in serum in relation to the development of breast cancer. Am J Epidemiol 1987;125:791–9.
68. Thomas HV, Key TJ, Allen DS, Moore JW, Dowsett M, Fentiman IS, et al. A prospective study of endogenous serum hormone concentrations and breast cancer risk in post-menopausal women on the island of Guernsey. Br J Cancer 1997;76:401–5.
69. 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.
70. Zeleniuch-Jacquotte A, Bruning PF, Bonfrer JM, Koenig KL, Shore RE, Kim MY, 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.
71. Anderson KE, Sellers TA, Chen PL, Rich SS, Hong CP, Folsom AR. Association of Stein-Leventhal syndrome with the incidence of postmenopausal breast carcinoma in a large prospective study of women in Iowa. Cancer 1997;79:494–9.
72. Lopez-Otin C, Diamandis EP. Breast and prostate cancer: an analysis of common epidemiological, genetic, and biochemical features. Endocr Rev 1998;19:365–96.
73. Bulbrook RD, Thomas BS. Hormones are ambiguous risk factors for breast cancer. Acta Oncol 1989;28:841–7.
74. Kouhara H, Koga M, Kasayama S, Tanaka A, Kishimoto T, Sato B. Transforming activity of a newly cloned androgen-induced growth factor. Oncogene 1994;9:455–62.
75. Bansal GS, Cox HC, Marsh S, Gomm JJ, Yiangou C, Luqmani Y, et al. Expression of keratinocyte growth factor and its receptor in human breast cancer. Br J Cancer 1997;75:1567–74.
76. 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.
77. Poulin R, Simard J, Labrie C, Petitclerc L, Dumont M, Lagace L, et al. Down-regulation of estrogen receptors by androgens in the ZR-75–1 human breast cancer cell line. Endocrinology 1989;125:392–9.
78. Maggiolini M, Donze O, Jeannin E, Ando S, Picard D. Adrenal androgens stimulate the proliferation of breast cancer cells as direct activators of estrogen receptor alpha. Cancer Res 1999;59:4864–9.
79. Lapointe J, Fournier A, Richard V, Labrie C. Androgens down-regulate bcl-2 protooncogene expression in ZR-75-1 human breast cancer cells. Endocrinology 1999;140:416–21.
80. Kandouz M, Lombet A, Perrot JY, Jacob D, Carvajal S, Kazem A, et al. Proapoptotic effects of antiestrogens, progestins and androgen in breast cancer cells. J Steroid Biochem Mol Biol 1999;69:463–71.
81. Dauvois S, Li SM, Martel C, Labrie F. Inhibitory effect of androgens on DMBA-induced mammary carcinoma in the rat. Breast Cancer Res Treat 1989;14:299–306.
82. Tormey DC, Lippman ME, Edwards BK, Cassidy JG. Evaluation of tamoxifen doses with and without fluoxymesterone in advanced breast cancer. Ann Intern Med 1983;98(2):139–44.
83. Gordan GS, Halden A, Horn Y, Fuery JJ, Parsons RJ, Walter RM. Calusterone (7beta,17alpha-dimethyltestosterone) as primary and secondary therapy of advanced breast cancer. Oncology 1973;28(2):138–46.
84. Xie B, Tsao SW, Wong YC. Sex hormone-induced mammary carcinogenesis in female noble rats: the role of androgens. Carcinogenesis 1999;20:1597–606.
85. Horwitz KB, Tung L, Takimoto GS. Novel mechanisms of antiprogestin action. Acta Oncol 1996;35(2):129–40.
86. Centers for Disease Control. Long-term oral contraceptive use and the risk of breast cancer. The Centers for Disease Control Cancer and Steroid Hormone Study. JAMA 1983;249:1591–5.
87. Centers for Disease Control and the National Institute of Child Health and Human Development. Oral-contraceptive use and the risk of breast cancer. The Cancer and Steroid Hormone Study of the Centers for Disease Control and the National Institute of Child Health and Human Development. N Engl J Med 1986;315:405–11.
88. Van Hoften C, Burger H, Peeters PH, Grobbee DE, Van Noord PA, Leufkens HG. Long-term oral contraceptive use increases breast cancer risk in women over 55 years of age: the DOM cohort. Int J Cancer 2000;87:591–4.
89. Ursin G, Ross RK, Sullivan-Halley J, Hanisch R, Henderson B, Bernstein L. Use of oral contraceptives and risk of breast cancer in young women. Breast Cancer Res Treat 1998;50:175–84.
90. Malone KE, Daling JR, Weiss NS. Oral contraceptives in relation to breast cancer. Epidemiol Rev 1993;15:80–97.
91. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies. Lancet 1996; 347:1713–27.
92. Grabrick DM, Hartmann LC, Cerhan JR, Vierkant RA, Therneau TM, Vachon CM, et al. Risk of breast cancer with oral contraceptive use in women with a family history of breast cancer. JAMA 2000;284:1791–8.
93. Schairer C, Lubin J, Troisi R, Sturgeon S, Brinton L, Hoover R. Menopausal estrogen and estrogen-progestin replacement therapy and breast cancer risk. JAMA 2000;283:485–91.
94. Ross RK, Paganini-Hill A, Wan PC, Pike MC. Effect of hormone replacement therapy on breast cancer risk: estrogen versus estrogen plus progestin. J Natl Cancer Inst 2000;92:328–32.
95. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. Lancet 1997;350: 1047–59.
96. Colditz GA, Hankinson SE, Hunter DJ, Willett WC, Manson JE, Stampfer MJ, et al. The use of estrogens and progestins and the risk of breast cancer in postmenopausal women. N Engl J Med 1995;332:1589–93.