

Review

Testosterone and breast cancer prevention

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ABSTRACT

Testosterone (T) is the most abundant biologically active hormone in women. Androgen receptors (AR) are located throughout the body including the breast where T decreases tissue proliferation. However, T can be aromatized to estradiol (E2), which increases proliferation and hence, breast cancer (BCA) risk. Increased aromatase expression and an imbalance in the ratio of stimulatory estrogens to protective androgens impacts breast homeostasis.

Recent clinical data supports a role for T in BCA prevention. Women with symptoms of hormone deficiency treated with pharmacological doses of T alone or in combination with anastrozole (A), delivered by subcutaneous implants, had a reduced incidence of BCA. In addition, T combined with A effectively treated symptoms of hormone deficiency in BCA survivors and was not associated with recurrent disease. Most notably, T + A implants placed in breast tissue surrounding malignant tumors significantly reduced BCA tumor size, further supporting T direct antiproliferative, protective and therapeutic effect.

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1. Importance of T in women

Testosterone (T) is referred to as a 'male' hormone; however, it is the most abundant biologically active hormone in women.

It is produced in the ovaries, adrenal gland, and abundantly (i.e., over 50%) at the cellular level from androgen precursors [1]. Testosterone and its active metabolite, dihydrotestosterone, have a direct physiologic effect at the androgen receptor (AR), located in virtually every tissue and organ system including the breast [2]. T is also the precursor hormone for estradiol (E2) and has an indirect effect at the estrogen receptor (ER) via aromatization. Adequate levels of T are critical for overall mental and physical health, immune function, glycemic control and

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reducing inflammation, all of which may impact cancer occurrence [3,4].

Evidence that women become T deficient has been largely ignored. As in men, T and its precursor hormones peak in women in their twenties and decline with age [5]. Symptoms of androgen deficiency occur as early as the mid-thirties in some women and physicians are slowly recognizing the benefits of T supplementation. Subcutaneous T implants have been shown to be a safe and effective method of T delivery for over 70 years in both sexes [6,7]. Adequate levels of continuous T released from the implants also provide a major source of E2 in postmenopausal women through aromatization.

2. Effects of T on the breast and the role of aromatase

There is abundant evidence that androgens are breast protective. In vitro breast cell cultures and in vivo primates studies support that T's direct effect at the AR is antiproliferative, proapoptotic, and inhibits ER α activity and breast cancer (BCA) cell growth [8–10]. Long-term female to male transgender studies and other clinical evidence also support the protective role of androgens in the breast [11,12]. Eighty to 85% of BCA are AR positive. AR positivity is associated with more favorable tumor characteristics and better prognosis including increased disease free and overall survival [9,13]. Androgens, including T pellet implants, have been used to treat BCA in the past with success and a recent study reports a 58.5% response rate in hormone resistant metastatic BCA [14].

Correlations between sex steroid levels and BCA have been inconsistent and controversial. Some epidemiological studies show an association between T levels and BCA, while others do not, and some report lower levels of bioavailable T in women with BCA [11,15,16]. Nevertheless, association does not infer causation. The association between high T levels and BCA may reflect the correlation between high androgen levels and higher estrogen levels as evidenced by studies that adjusted for estrogens and no longer found an association between T and BCA [15]. Also, most epidemiological studies do not address the 'Obesity-insulin-testosterone' connection. Obesity and insulin increases inflammation and appear to have direct and indirect causative roles in BCA through numerous pathways including increased aromatase expression. Furthermore, insulin stimulates the production of T, accounting for 'associated' increased T levels [17–20]. Many studies also suffer from methodological limitations including the inaccuracy of T assays in women and do not address or acknowledge the 'immeasurable' paracrine, autocrine and intracrine aromatization of T to E2 [21].

Aromatase is located throughout the body including breast adipose, stromal and parenchyma cells where T can exert a direct growth inhibitory effect by binding to the AR, or an indirect growth stimulatory effect via aromatization to E2 and activation of the ER [21–23]. Multiple factors, many of which are independent risk factors for BCA, can increase aromatase activity altering the homeostatic balance of E2 to T and thereby increasing proliferation of normal and BCA cells [3]. In addition, invasive and non-invasive BCA overexpress aromatase resulting in increased local tumor production of stimulatory estrogens [21–23].

3. Subcutaneous T hormone therapy and the incidence of BCA

T supplementation in ovariectomized women is well established and in some countries T is prescribed for postmenopausal women in addition to usual hormone therapy. A retrospective, observational study following women receiving T implants in addition to conventional hormone therapy found no increase in BCA in women on T therapy compared to historical control groups, suggesting that

Table 1

Indications for aromatase inhibitor therapy including signs and symptoms of estrogen excess [6,25,26].

- BCA
- Increased risk of BCA
- ADH, LCIS, family history, BRCA positive
- Severe fibrocystic breast disease, breast pain
- Endometriosis, uterine fibroids
- Dysfunctional uterine bleeding
- Abdominal obesity, weight gain, insulin resistance
- Menstrual or migraine headaches
- Premenstrual syndrome, anxiety, irritability
- Fluid retention, bloating
- Elevated estradiol levels

BCA, breast cancer; ADH, atypical ductal hyperplasia; LCIS, lobular carcinoma in situ.

T therapy was able to reduce the estrogen/progestin therapy associated BCA risk [24].

A prospective observational study (Dayton, Ohio) was specifically designed to investigate the incidence of BCA in pre and postmenopausal women treated with T or T combined with anastrozole (T+A) subcutaneous implants for symptoms of hormone deficiency [25]. Indications for aromatase inhibitor therapy are listed in Table 1. Over 95% of women in this study did not receive concurrent estrogen therapy, as continuous T (no estrogen) was able to adequately relieve symptoms in these patients [6].

A 5-year interim analysis of the Dayton study reported a BCA incidence rate of 142 per 100,000 person-years in the intent to treat group. This represents a significant reduced incidence of BCA in women treated with T implant therapy compared to both the age-matched control group and age-specific Surveillance Epidemiology and End Results (SEER) incidence rates; the reduced incidence was even more significant in women who were adherent to T therapy (Fig. 1) [25]. An 82-month updated interim analysis continued to demonstrate a reduced incidence of BCA in women adherent to T or T+A therapy (76/100,000) compared to calculated age-matched SEER incidence rates (297/100,000), RR 0.26 [26]. Being cognizant of signs and symptoms of excess estrogen and individualizing patient therapy to selectively include A in the implant may partially account for the reduced incidence of BCA in this patient population.

4. Subcutaneous T + A therapy in breast cancer patients

BCA treatment often results in ovarian failure or hormone depletion, which can negatively affect sexual desire and cause unpleasant urogenital and vaginal symptoms. Symptoms of hormone

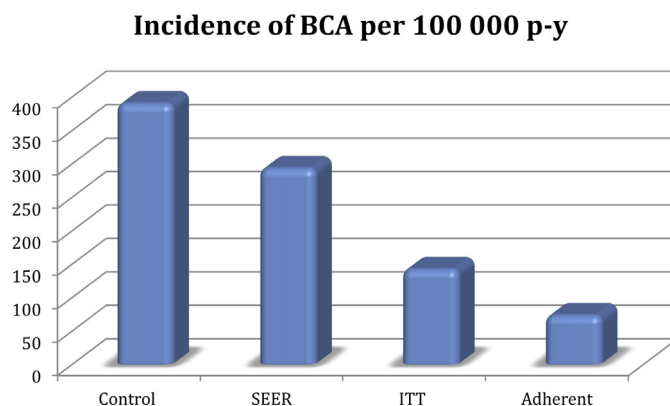


Fig. 1. Incidence of BCA per 100,000 person-years, 5-year interim analysis results: Age-matched controls (390/100,000), Age-specific SEER expected incidence rates (297/100,000), T therapy, intent to treat group (142/100,000), T therapy, patients adherent to therapy (73/100,000) [25].

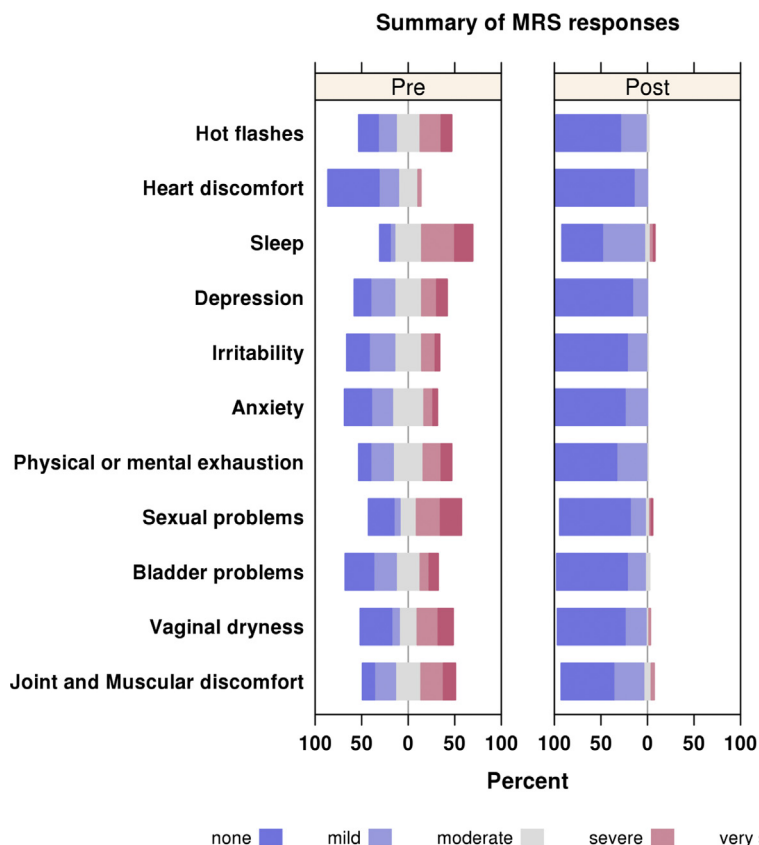


Fig. 2. Summary of distribution of severity scores in each of the 11 'Menopausal Rating Scale' categories pre- and post-therapy with T+A subcutaneous implants in BCA survivors demonstrating significant improvement in quality of life for all symptom categories [29].

deficiency including the adverse effects on sexuality may be quite severe in BCA patients in whom estrogen therapy is contraindicated. Systemic and vaginal T has been shown to relieve symptoms in BCA survivors [27]. However, there remains concern about the aromatization to E₂. We have previously reported that an 8 mg

dose of A combined with 120 mg of T in two subcutaneous implants provided therapeutic T levels without elevating estradiol [28].

The efficacy of subcutaneous implants containing T combined with A (4 or 8 mg) on menopausal symptoms has also been reported [29]. Seventy-two BCA patients, stage 0-IV, were evaluated using

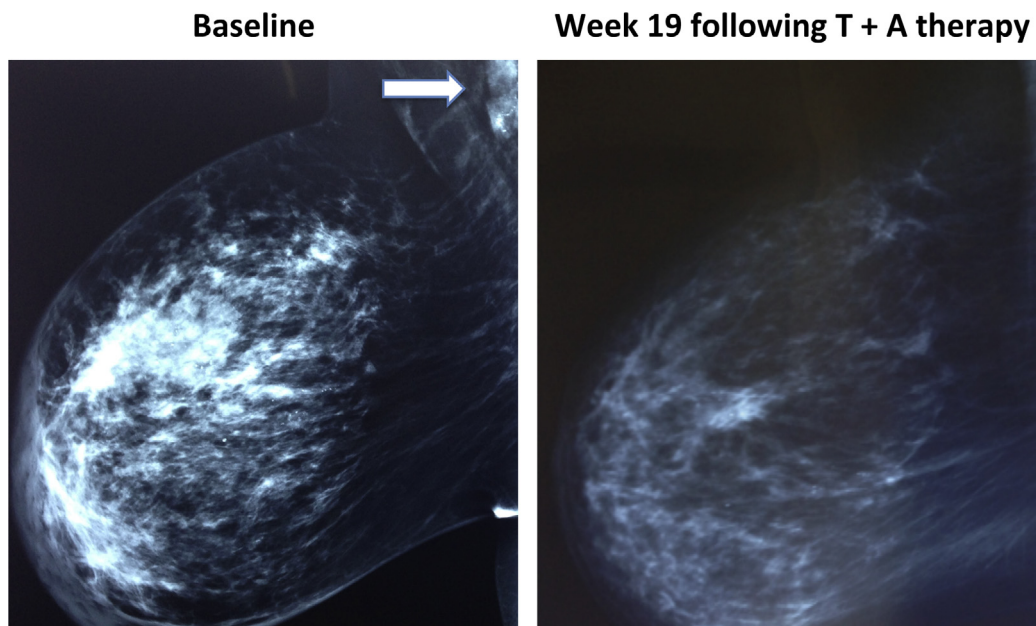


Fig. 3. Comparison mammography, right medial lateral oblique view at baseline (left) and at follow up (right), week 19 following neoadjuvant, intramammary T+A therapy of infiltrating lobular, hormone receptor positive BCA (ER+, PR+, AR+). Note the significant reduction in tumor size and absence of previously palpable axillary lymph nodes. (Previously unpublished images, Glaser, Dimitrakakis).

the validated 11-item, Health-Related Quality of Life 'Menopause Rating Scale'. Symptoms were self-rated on a scale of 0 to 4 prior to, and following T+A therapy. Therapeutic T levels were achieved without elevating E2. There was significant improvement in psychological symptoms (depressive mood, irritability, anxiety, physical and mental exhaustion), somatic symptoms (hot flashes, heart discomfort, sleep problems, pain) and urogenital symptoms including sexual desire, activity, satisfaction, vaginal dryness and bladder problems (Fig. 2). There were no cancer recurrences in up to 9.4 years of therapy. The lack of adverse events and cancer recurrence support the safety and tolerability of T+A in BCA survivors.

T+A implant therapy has also been used to treat BCA in the neoadjuvant setting using three, T (60 mg)+A (4 mg) combination implants placed in the breast tissue surrounding the malignant tumor [30]. Hormone receptor positive tumors have responded to this innovative therapy as evidenced by clinical exam, ultrasound and mammography (Fig. 3). This unique pharmacologic combination and delivery method (locally) provides continuous, simultaneous release of both T and A directly to the tumor site, allowing the beneficial effect of T at the AR without aromatization to E2. There have been no adverse drug events in these patients, further supporting the safety of pharmacologic doses of T. This direct, dose dependent response supports T therapeutic and protective actions in BCA and may prove to be a new approach to both local and systemic therapies in subgroups of patients.

Subcutaneous use of T+A implants seems promising. This is in line with other pre-clinical and clinical research, which has consistently demonstrated the protective actions of androgens in the breast. However, there is a lack of long-term prospective studies and randomized controlled trials addressing this issue. Caution must be taken in certain cell lines of BCA that may be stimulated by androgens (e.g., ER negative, Her 2 positive) or in tumors that may become resistant to therapy. Also, there is insufficient evidence to extrapolate results with subcutaneous implants to other dosages and routes of delivery including oral pills and topical gels.

5. Conclusion

The reduced incidence of BCA in women treated with T or T+A subcutaneous implants, the lack of recurrent disease in BCA survivors treated with T+A, and the direct clinical response of BCA tumors to intra-mammary T+A implant therapy provide strong evidence for the role of T in BCA prevention. Testosterone's impact on overall health and quality of life, immune function, glycemic control and prevention of inflammation further support T (indirect) role in cancer prevention. However, T is a major source of E2 via aromatization. Physicians prescribing T therapy should be aware of the causes and effects of increased aromatization, including stimulation of normal and cancerous breast tissue, and consider the use of aromatase inhibitors in subgroups of patients. Further research on T and T+A implants for hormone therapy, as well as for BCA prevention and therapy is warranted.

6. Practice points

- T is essential for physical health, mental health and quality of life in women including BCA survivors.
- Evidence supports that T is breast protective but is aromatized to E2.
- Diet and lifestyle are modifiable factors, which affect overall health, aromatase activity, hormone balance, and subsequently, BCA risk.
- Physicians prescribing T therapy should be aware of the causes and effects of increased aromatase activity and (local) estrogen excess.

7. Research agenda

- Continuous T and T+A should be investigated further for hormone therapy in women, including BCA survivors where estrogen therapy is contraindicated.
- T and T+A therapy should be investigated for the prevention of BCA, particularly in women at increased risk.
- T+A implants should be investigated further for BCA therapy including in the neoadjuvant setting and in premenopausal women in combination with ovarian suppression.

Contributors

None.

Competing interest

Neither author (RG, CD) has any competing interests.

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References

- [1] Longscope C. Adrenal and gonadal androgen secretion in normal female. *J Clin Endocrinol Metab* 1986;15:213–28.
- [2] Kimura N, Mizokami A, Oonuma T, Sasano H, Nagura H. Immunocytochemical localization of androgen receptor with polyclonal antibody in paraffin-embedded human tissues. *J Histochem Cytochem* 1993;41:671–8.
- [3] Williams GP. The role of oestrogen in the pathogenesis of obesity, type 2 diabetes, breast cancer and prostate disease. *Eur J Cancer Prev* 2010;19:256–71.
- [4] Saad F. The emancipation of testosterone from niche hormone to multi-system player. *Asian J Androl* 2015;17:58.
- [5] Davison SL, Bell R, Donath S, Montalto JG, Davis SR. Androgen levels in adult females: changes with age, menopause, and oophorectomy. *J Clin Endocrinol Metab* 2005;90:3847–53.
- [6] Glaser R, York AE, Dimitrakakis C. Beneficial effects of testosterone therapy in women measured by the validated Menopause Rating Scale (MRS). *Maturitas* 2011;68:355–61.
- [7] Van Staa TP, Sprafka JM. Study of adverse outcomes in women using testosterone therapy. *Maturitas* 2009;62:76–80.
- [8] Dimitrakakis C, Zhou J, Wang J, et al. A physiologic role for testosterone in limiting estrogenic stimulation of the breast. *Menopause* 2003;10:292–8.
- [9] Hickey TE, Robinson JLL, Carroll JS, Tilley WD. Minireview: the androgen receptor in breast tissues: growth inhibitor, tumor suppressor, oncogene. *Mol Endocrinol* 2012;26:1252–67.
- [10] Eigélieñé N, Elo T, Linhala M, Hurme S, Erkkola R, Härkönen P. Androgens inhibit the stimulatory action of 17 β -estradiol on normal human breast tissue in explant cultures. *J Clin Endocrinol Metab* 2012;97:E1116–27.
- [11] Dimitrakakis C. Androgens and breast cancer in men and women. *Endocrinol Metab Clin N Am* 2011;40:533–47.
- [12] Slagter MH, Gooren LJG, Scorilas A, Petraki CD, Diamandis EP. Effects of long-term androgen administration on breast tissue of female-to-male transsexuals. *J Histochem Cytochem* 2006;54:905–10.
- [13] Castellano I, Allia E, Accortanzo V, et al. Androgen receptor expression is a significant prognostic factor in estrogen receptor positive breast cancers. *Breast Cancer Res Treat* 2010;124:607–17.
- [14] Boni C, Pagano M, Panebianco M, et al. Therapeutic activity of testosterone in metastatic breast cancer. *Anticancer Res* 2014;34:1287–90.

- [15] Traish A, Fettes K, Miner M, Hansen ML, Guay A. Testosterone and risk of breast cancer: appraisal of existing evidence. *Horm Mol Biol Clin Invest* 2010;2:177–90.
- [16] Dimitrakakis C, Zava D, Marinopoulos S, Tsigginou A, Antsaklis A, Glaser R. Low salivary testosterone levels in patients with breast cancer. *BMC Cancer* 2010;10:547.
- [17] Morris PG, Hudis CA, Giri D, et al. Inflammation and increased aromatase expression occur in the breast tissue of obese women with breast cancer. *Cancer Prev Res* 2011;4:1021–9.
- [18] Simpson ER, Brown KA. Minireview: obesity and breast cancer: a tale of inflammation and dysregulated metabolism. *Mol Endocrinol* 2013;27:715–25.
- [19] Rose DP, Vona-Davis L. The cellular and molecular mechanisms by which insulin influences breast cancer risk and progression. *Endocr Relat Cancer* 2012;19:R225–41.
- [20] Nestler JE. Insulin stimulates testosterone biosynthesis by human thecal cells from women with polycystic ovary syndrome by activating its own receptor and using inositolglycan mediators as the signal transduction system 1. *J Clin Endocrinol Metab* 1998;83.
- [21] Santen RJ, Brodie H, Simpson ER, Siiteri PK, Brodie A. History of aromatase: saga of an important biological mediator and therapeutic target. *Endocr Rev* 2009;30:343–75.
- [22] Bulun SE, Lin Z, Zhao H, et al. Regulation of aromatase expression in breast cancer tissue. *Ann N Y Acad Sci* 2009;1155:121–31.
- [23] Sasano H, Suzuki T, Miki Y, Moriya T. Intracrinology of estrogens and androgens in breast carcinoma. *J Steroid Biochem Mol Biol* 2008;108:181–5.
- [24] Dimitrakakis C, Jones RA, Liu A, Bondy CA. Breast cancer incidence in postmenopausal women using testosterone in addition to usual hormone therapy. *Menopause* 2004;11:531–5.
- [25] Glaser RL, Dimitrakakis C. Reduced breast cancer incidence in women treated with subcutaneous testosterone, or testosterone with anastrozole: a prospective, observational study. *Maturitas* 2013;76:342–9.
- [26] Glaser R, Dimitrakakis C. Reduced incidence of breast cancer in women adherent to testosterone or testosterone-anastrozole hormone therapy: updated interim analysis. In: 10th EMAS congress, May 2015. 2015 (abstract/poster P124).
- [27] Dahir M, Travers-Gustafson D. Breast cancer, aromatase inhibitor therapy, and sexual functioning: a pilot study of the effects of vaginal testosterone therapy. *Sex Med* 2014;2:8–15.
- [28] Glaser R. Subcutaneous testosterone-anastrozole implant therapy in breast cancer survivors. In: American Society of Clinical Oncology Breast Cancer Symposium. 500. 2010. p. 221.
- [29] Glaser R, York A, Dimitrakakis C. Efficacy of subcutaneous testosterone on menopausal symptoms in breast cancer survivors. 2014 Breast Cancer Symposium. *J Clin Oncol* 2014;32(Suppl. 26) (abstract 109).
- [30] Glaser RL, Dimitrakakis C. Rapid response of breast cancer to neoadjuvant intramammary testosterone-anastrozole therapy: neoadjuvant hormone therapy in breast cancer. *Menopause (New York, NY)* 2014;21:673.