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Systemic and local effects of vaginal dehydroepiandrosterone (DHEA): NCCTG N10C1 (Alliance)

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Abstract

Background—Dehydroepiandrosterone (DHEA) is helpful for treating vaginal symptoms. This secondary analysis evaluated the impact of vaginal DHEA on hormone concentrations, bone turnover and vaginal cytology in women with a cancer history.

Methods—Postmenopausal women, diagnosed with breast or gynecologic cancer, were eligible if they reported at least moderate vaginal symptoms. Participants could be on tamoxifen or aromatase inhibitors (AI's). Women were randomized to 3.25 versus 6.5 mg/d of DHEA versus a plain moisturizer (PM) control. Sex steroid hormone levels, biomarkers of bone formation, vaginal pH and maturation index were collected at baseline and 12 weeks. Analysis included independent t-tests and Wilcoxon rank tests, comparing each DHEA arm with the control.

Results—345 women contributed evaluable blood and 46 contributed evaluable cytology and pH values. Circulating DHEA-S and testosterone levels were significantly increased in those on vaginal DHEA in a dose dependent manner compared to PM. Estradiol was significantly increased in those on 6.5 mg/d DHEA but not 3.25 mg/d ($p<.05$; $p=.05$, respectively), and not in those on

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AI's. Biomarkers of bone formation were unchanged in all arms. Maturation of vaginal cells was 100% (3.25 mg/d), 86% (6.5 mg/d) and 64% (PM); pH decreased more in DHEA arms.

Conclusion—DHEA resulted in increased hormone concentrations, though still in the lowest half or quartile of the postmenopausal range, and provided more favorable effects on vaginal cytology, compared to PM. Estrogen concentrations in women on AI's were not changed. Further research on the benefit of vaginal DHEA is warranted in hormone dependent cancers.

Background

Menopause results in reduced concentrations of circulating estradiol in the female body and can be accompanied by many symptoms, such as vaginal dryness and dyspareunia^{1,2}. Treatment for hormone sensitive breast cancer often seeks to largely deplete estradiol concentrations, sometimes by blocking aromatase, an enzyme necessary for estrogen conversion. This treatment can add to the extent and severity of menopausal symptoms, particularly vaginal symptoms such as dryness and dyspareunia^{3,4}. Vaginal symptoms due to estrogen deprivation do not spontaneously resolve, in contrast to hot flashes, as there is a cumulative detrimental effect on the vaginal tissues over time^{5,2}.

The relationship between estrogen deprivation and worsening vaginal symptoms over time is due to the major role that estrogen and estrogen receptors play in vaginal architecture^{6,7}. The vaginal wall contains squamous epithelium, a lamina propria, a smooth muscle layer and a covering membrane, all of which are influenced by estrogen. The role of estradiol is multifaceted and includes maintaining the fluid film that lines the vaginal walls, keeping the epithelium dense with more mature cells, keeping vaginal smooth muscle functional, and contributing to tissue elasticity⁶. Additionally, estrogen is responsible for vasodilatation in the lamina propria and promotes the expression of various neurotransmitters that ultimately result in increased blood flow^{6,7}.

Without estrogen, the vagina undergoes changes that can negatively impact sexual function and satisfaction. The more mature epithelial cells decrease while less mature parabasal cells become the major cytology. Collagen, blood flow, and lubrication decrease, resulting in an inflexible network of dry cells with a higher pH; this leads to an increased susceptibility to infection and itching, and an uncomfortable sensation of dryness⁸. The smooth muscle becomes less functional, challenging orgasm. Intercourse can become painful, with friable tissues subject to bleeding and trauma. Upon visual inspection, an atrophic vagina appears thin and pale, with a parched epithelium. The vagina appears shorter, with a loss of rugae, elasticity and decreased secretions^{8,9}.

Based on the important role of estrogen in the vagina, the gold standard treatment for vaginal symptoms has been localized estrogen¹⁰. However, vaginal estrogen may not be a desirable option for some women, including women who are concerned about cancer risk and those with estrogen-sensitive tumors. Even low doses of vaginal estrogen have resulted in significant increases in systemic concentrations^{11–13} or effects outside of the vagina; after 6–12 months of 7.5 micrograms of vaginal estrogen there are improvements in lipid profiles¹⁴ and/or reductions in biomarkers of bone turnover¹⁵. These “off target”, outside of the vagina effects (such as effects on lipids, bones), may occur in the absence of detectable

or clinically important increases in serum concentrations and are a theoretical risk for women with hormone sensitive cancer as estrogen can bind to receptors in any tissue, including the breast^{14,15}. Consequently, effective treatments for vaginal symptoms that do not result in estrogenic systemic activity would be a welcome option for many women, particularly those on aromatase inhibitors, as safe levels of estradiol concentration increases have not been established¹⁶.

Dehydroepiandrosterone (DHEA) is a vaginal treatment that has been studied in over 500 postmenopausal women^{17–19}; it is believed to act locally to improve symptoms, without influencing systemic physiology²⁰. DHEA is made by the adrenal gland and decreases with aging, not specifically with menopause. After menopause, DHEA is a major source of sex steroid hormones²¹. The Food and Drug Administration recently approved a DHEA ovule for the treatment of dyspareunia in postmenopausal women with the genitourinary syndrome of menopause. The ovule, or any other type of vaginal DHEA, had not previously been studied in women with a history of breast or gynecologic cancer.

The purpose of this paper is to report secondary physiologic outcomes from a phase III randomized, controlled clinical trial designed to evaluate two doses of a compounded vaginal DHEA gel for the improvement of vaginal symptoms, dryness or dyspareunia, in women with a history of breast or gynecologic cancer. The paper reporting the primary clinical endpoint related to symptom improvement, overall sexual function, and side effects is being published separately. Currently in this paper, we evaluate the impact of vaginal DHEA on 1) circulating sex steroid concentrations (estradiol, estrone, free and total testosterone, and DHEA-S), 2) markers of bone formation (osteocalcin and bone alkaline phosphatase), and 3) vaginal cytology, specifically maturation index and pH.

Methods

Population and design

These data were collected as part of a phase III randomized controlled trial evaluating two doses (3.25 mg/d and 6.5 mg/d) of vaginal DHEA compared to a plain moisturizer (PM). Eligibility criteria included women who reported problems with either vaginal dryness or dyspareunia, were postmenopausal, had a history of breast or gynecologic cancer, completed curative intent treatment and had no evidence of disease. Participants could be on endocrine therapy (tamoxifen or aromatase inhibitors) but had to be on it for the past two months without planned changes. Women could not be using vaginal products other than water-based lubricants during intercourse, nor could they be using any oral or transdermal hormonal products (i.e., estrogen), including soy or compounded hormones. They could not have had prior or current radiation therapy to the pelvis nor have had any active vaginal infections. All women were asked to provide a blood sample at baseline and at 12 weeks. Additionally, a small subset of accruing sites volunteered to participate in vaginal cytology collection and vaginal pH measurement.

Intervention and control

The vaginal DHEA was developed by a compounding pharmacist under the IND number 111454. The contents of the compounded base contained carbomer, squalane, vitamin E, and glycerin and was intended to act as a bioadhesive. DHEA was added to this base to provide 3.25 mg/d and 6.5 mg/d gels, while the PM arm consisted of the compounded bioadhesive base alone. Participants were instructed to use one prefilled syringe every night, after any sexual activity, if applicable, for the entire 12 weeks.

Physiologic endpoints

The evaluated physiologic endpoints included local effects on the vagina, specifically vaginal maturation index (VMI) and vaginal pH, systemic effects on serum sex steroid hormone concentrations: estradiol, estrone, free and total testosterone, dehydroepiandrosterone-sulfate (DHEA-S), and “off target” effects, specifically biomarkers of bone formation: osteocalcin and bone alkaline phosphatase. These data were collected at baseline (before the start of the study treatment), and at the end of 12 weeks of treatment. Thirty mL (no additive) and 10 mL (EDTA) of whole blood were drawn and shipped on the same day with a cold pack to Mayo Clinic, Rochester, MN where blood was processed into serum and plasma, respectively, and frozen at -80°C until the assays were performed. All labs were performed by CLIA-approved Mayo Research Laboratories, using well validated assays. Sex steroid hormone concentrations were measured to evaluate systemic absorption through vaginal tissue and explore mechanisms of action. Bone formation biomarkers were included as a surrogate measure to evaluate activity outside of the vagina, as increases in circulating estrogens improve biomarkers of bone turnover, both formation and resorption^{22,23}.

Vaginal cytology collection kits, consisting of pH papers (Baker pHIX) and a bottle of PreservCyt (Thin Prep), were provided to the subset of institutions participating in this collection. The vaginal cytology collection process included a vaginal exam, recording of the vaginal pH, and collection of vaginal cells. To collect pH, a pH indicator strip was held to the lateral wall of the mid portion of the vagina until fully moistened and color changed (15–30 seconds). The corresponding pH value was recorded on a data sheet included in the kit. While the speculum was still open, the rounded end of a spatula was used to gently scrape cells from the lateral wall of the middle portion of the vagina, opposite from the side used for pH sampling. The spatula was inserted into the Thin Prep container and gently stirred back and forth to dislodge the cells^{24,25}. The specimen was mailed to the Mayo Clinic for central pathology review. The cytological specimens were all read by the same pathologist who was blinded to the treatment assignment.

Statistical analysis

To evaluate the impact of vaginal DHEA on estradiol, estrone, free and total testosterone, DHEA-S, and markers of bone formation (osteocalcin and bone alkaline phosphatase), changes were calculated in each of the above biomarkers from baseline to 12 weeks. Two independent sample t-tests were performed, comparing each DHEA treatment arm in turn, to the PM arm. Subset analyses were also performed, comparing biomarker concentrations between those on AI's and those not on AI's. Differences at baseline in sex steroid hormone

concentrations between those on and not on AI's were also analyzed with a Wilcoxon or an equal variance t-test, as appropriate.

To evaluate the impact of vaginal DHEA on the vaginal maturation index, due to the small numbers of women who participated in the vaginal cytology collection, we did not perform statistical analyses for VMI. Rather, we calculated the number and percent of women who had increases in the percentage of intermediate and superficial cells as opposed to parabasal cells. With regard to the pH, mean values were calculated from the recorded pH values and the change from baseline in these mean values was calculated. Descriptive data for pH are reported without performing statistical tests, due to the low number of participants in this portion of the study.

Results

Eighty institutions contributed blood specimens, representing 345 participants across the three arms who had evaluable specimens at baseline and after 12 weeks; eight institutions participated in collecting vaginal tissue/pH, representing 46 women who had evaluable data. The CONSORT diagram is shown in Figure 1. The demographic characteristics of those who contributed blood specimens for the systemic effects are identical to those reported in the manuscript about the primary outcome. A majority of women (97%) had a history of breast cancer, with 2% having a history of ovarian cancer and 1% with a history of endometrial cancer. The mean age was 57 years and 95% were white. A majority of the women were on an AIs (56%) and had been on that therapy for a mean of almost 2 years. Demographic characteristics of those who contributed vaginal cytology at both data points, baseline and 12 weeks, are listed in Table 1.

Hormone and bone formation biomarker concentrations before the intervention, at 12 weeks and changes from baseline are shown in Table 2. For the group as a whole, DHEA-S levels increased in a dose-dependent manner for those receiving DHEA and were significantly different from the PM group. The change from baseline to 12 weeks in estrone and testosterone (total and free) concentrations also significantly differed from PM for both doses of DHEA. For estradiol, only the 6.5 mg/d DHEA dose demonstrated a statistically significant increase compared with PM ($p < .05$). Bone formation biomarkers were not significantly changed, irrespective of arm (Table 2).

When hormone concentrations were evaluated by whether women were on aromatase inhibitors, there were significant differences at baseline for estradiol and estrone, both being higher in those not on aromatase inhibitors (Table 3). At baseline, there were significantly higher concentrations of DHEA-S, free testosterone, bone alkaline phosphatase and osteocalcin in women who were on AIs; total testosterone concentrations were not significantly different. After 12 weeks on their assigned treatment, there were no significant increases in either estradiol or estrone concentrations among women taking AIs compared to PM, as opposed to significant increases in these concentrations in the women who were not taking an AI compared to PM (Table 4).

Cytological effects are shown in Table 5. No woman who received the PM experienced a decrease in pH to a level less than 5, while a small percent of women receiving either dose of vaginal DHEA experienced this level of decrease. In addition, more women on DHEA experienced cell maturation.

Discussion

Laboratory data from this clinical trial are consistent with previous studies that demonstrate vaginal tissue is a permeable membrane allowing for both systemic and local dose-dependent effects. Systemic sex steroid hormone concentrations were small and similar to those described by Labrie²⁶. Systemic absorption is best illustrated by the significant increases in systemic DHEA-S concentrations that were dose dependent.

The upper limit of normal for DHEA-S values per Mayo laboratories for a woman aged 50 and 60 are 200 mcg/dL and 157 mcg/dL, respectively. Post-treatment values in both DHEA arms were significantly increased from baseline but still well within the normal range for post-menopausal women.

Post-menopausal estradiol values <10 pg/mL by high sensitivity assay are considered to be low and the post-treatment mean estradiol concentrations in this study remained <5 pg/mL in both DHEA arms. Unexpectedly, however, the estradiol concentration changes from baseline were higher for the 3.25 mg/d DHEA group, with a larger standard deviation, than for the 6.5 mg/d DHEA group, even though the 3.25 mg/d DHEA group was not statistically significantly different from PM. This finding is not consistent with a dose dependent conversion of DHEA to systemic estradiol, and the reason for this is unknown. One possible explanation is that this data may reflect normal variations in estradiol levels within and between individuals.

Estrone is a less potent estrogen than estradiol. The Mayo laboratory postmenopausal reference range for the estrone assay used is 7–40 pg/mL. Mean estrone concentrations after using DHEA increased from baseline but remained in the lower half of the normal postmenopausal range (less than 15 pg/mL).

Neither estradiol nor estrone concentrations changed in women taking AIs and were not significantly different than those on PM. However, women taking AIs who received DHEA experienced the same level of improvement in their vaginal symptoms as those not on AIs²⁷. This finding would support the hypothesis that vaginal DHEA is not working through estrogenic means.

Results of this laboratory analysis support the lack of “off target” effects of vaginal DHEA. Bone turnover biomarkers increase as women age, with post-menopausal values for bone alkaline phosphatase (BAP) being <22 mcg/L, and for osteocalcin being between 9–42 ng/mL. Estrogen based hormone replacement therapy at 12 weeks results in significant improvements in markers of bone formation, BAP and osteocalcin²³. BAP values for the women in this study were higher (worse) than usually seen after menopause, both at baseline and at 12 weeks and were even higher at baseline for women who were on AIs²⁸. If vaginal DHEA was having systemic effects on bone, one would expect to see significant decreases

for BAP and osteocalcin. This did not occur, with BAP increasing and osteocalcin decreasing minimally. This is consistent with a lack of “off target” bone effects. One limitation of this analysis is that data were not collected about whether women were on bisphosphonates or any other medications that could impact bone health.

Vaginal DHEA did impact androgen concentrations and the increases in androgen levels were consistent with the mild androgen side effects reported in the clinical outcomes²⁷. Total testosterone ranges normally from 8–60 ng/dL in adult women. Total testosterone levels increased statistically significantly with both doses of DHEA, compared to the PM, with larger increases seen in the higher dose arm. However, 12-week total testosterone values remained 25 ng/dL in all three arms. Total testosterone is a measure that includes bound testosterone which would generally not have physiologic activity. Free testosterone, that which is not protein bound and is available for physiologic activity, ranges normally from 3–19 ng/dL in women. In this study, mean free testosterone values were very low, <1 ng/dL with a high sensitivity assay, both before and after the study intervention, irrespective of treatment arm.

Given the small but clear increase in systemic androgen concentrations, the safety of increased androgens is at issue with regard to hormone dependent cancers such as breast cancer. Breast tissue does have enzymes required to convert DHEA into both androgens and estrogens, with a bias towards androgen formation in normal breast tissue. Data to date would suggest that the proliferation of both normal and cancerous tissue of the breast results from the balance between the stimulatory effects of estrogen and the inhibitory effects of androgens^{29,30}. There are two opposing perspectives about the safety of androgens, both informed by preclinical data which is not easy to extrapolate to the clinical setting due to complexities of cellular and tissue intracrinology³¹. In short, the current preclinical data favors a positive impact of androgens on both normal and malignant breast tissue^{30,32}.

In summary, these data support the lack of extra-vaginal physiologic effects, the absence of estradiol conversion in women on aromatase inhibitors, and improved vaginal cytological effects from DHEA. For women with severe vulvovaginal symptoms, DHEA may be helpful in providing cytological improvement which may result in more comprehensive symptom relief. Larger studies to evaluate risks versus benefits in cancer survivors, particularly those on AI's, are desired.

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References

1. Gracia CR, Freeman EW. Acute consequences of the menopausal transition: the rise of common menopausal symptoms. *Endocrinology and metabolism clinics of North America*. 2004; 33(4):675–689. [PubMed: 15501640]
2. Bacon JL. The menopausal transition. *Obstet Gynecol Clin N Am*. 2017; 44:285–296. <http://dx.doi.org/10.1016/j.ogc.2017.02.008>.
3. Ganz PA, Cecchini RS, Julian TB, et al. Patient-reported outcomes with anastrozole versus tamoxifen for postmenopausal patients with ductal carcinoma in situ treated with lumpectomy plus radiotherapy (NSABP B-35): a randomised, double-blind, phase 3 clinical trial. *Lancet (London, England)*. 2016; 387(10021):857–865.
4. Cella D, Fallowfield LJ. Recognition and management of treatment-related side effects for breast cancer patients receiving adjuvant endocrine therapy. *Breast cancer research and treatment*. 2008; 107(2):167–180. [PubMed: 17876703]
5. Barton DL, Ganz PA. Symptoms: Menopause, infertility and sexual health. *Adv Exp Med Biol*. 2015; 862:115–141. [PubMed: 26059933]
6. Lara LA, Useche B, Ferriani RA, et al. The effects of hypoestrogenism on the vaginal wall: interference with the normal sexual response. *The journal of sexual medicine*. 2009; 6(1):30–39. [PubMed: 19170834]
7. Leclair DM, Ananjarajah G. Review Article: Effects of estrogen deprivation: vasomotor symptoms, urogenital atrophy, and psychobiologic effects. *Clinics in Family Practice*. 2002; 4(1):27–39.
8. Archer DF. Efficacy and tolerability of local estrogen therapy for urogenital atrophy. *Menopause (New York, NY)*. 2010; 17(1):194–203.
9. Tan O, Bradshaw K, Carr BR. Management of vulvovaginal atrophy-related sexual dysfunction in postmenopausal women: an up-to-date review. *Menopause (New York, NY)*. 2012; 19(1):109–117.
10. The role of local vaginal estrogen for treatment of vaginal atrophy in postmenopausal women: 2007 position statement of The North American Menopause Society. *Menopause (New York, NY)*. 2007; 14(3 Pt 1):355–369. quiz 370–351.
11. Santen RJ, Pinkerton JV, Conaway M, et al. Treatment of urogenital atrophy with low-dose estradiol: preliminary results. *Menopause (New York, NY)*. 2002; 9(3):179–187.
12. Kendall A, Dowsett M, Folkard E, Smith I. Caution: Vaginal estradiol appears to be contraindicated in postmenopausal women on adjuvant aromatase inhibitors. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO*. 2006; 17(4):584–587.
13. Wills S, Ravipati A, Venuturumilli P, et al. Effects of vaginal estrogens on serum estradiol levels in postmenopausal breast cancer survivors and women at risk of breast cancer taking an aromatase inhibitor or a selective estrogen receptor modulator. *Journal of oncology practice / American Society of Clinical Oncology*. 2012; 8(3):144–148.
14. Naessen T, Rodriguez-Macias K, Lithell H. Serum lipid profile improved by ultra-low doses of 17 beta-estradiol in elderly women. *The Journal of clinical endocrinology and metabolism*. 2001; 86(6):2757–2762. [PubMed: 11397883]
15. Naessen T, Berglund L, Ulmsten U. Bone loss in elderly women prevented by ultralow doses of parenteral 17beta-estradiol. *American journal of obstetrics and gynecology*. 1997; 177(1):115–119. [PubMed: 9240593]
16. Reeder-Hayes K, Muss HB. Vaginal estrogens and aromatase inhibitors: How safe is safe enough? *JAMA Oncology*. 2017; 3(3):305–306. DOI: 10.1001/jamaoncol.2016.3934 [PubMed: 27832252]
17. Labrie F, Archer D, Bouchard C, et al. Effect of intravaginal dehydroepiandrosterone (Prasterone) on libido and sexual dysfunction in postmenopausal women. *Menopause (New York, NY)*. 2009; 16(5):923–931.
18. Labrie F, Archer D, Bouchard C, et al. Intravaginal dehydroepiandrosterone (Prasterone), a physiological and highly efficient treatment of vaginal atrophy. *Menopause (New York, NY)*. 2009; 16(5):907–922.
19. Labrie F, Derogatis L, Archer DF, et al. Effect of Intravaginal Prasterone on Sexual Dysfunction in Postmenopausal Women with Vulvovaginal Atrophy. *The journal of sexual medicine*. 2015; 12(12):2401–2412. [PubMed: 26597311]

20. Labrie F, Martel C, Berube R, et al. Intravaginal prasterone (DHEA) provides local action without clinically significant changes in serum concentrations of estrogens or androgens. *The Journal of steroid biochemistry and molecular biology*. 2013; 138:359–367. [PubMed: 23954500]
21. Samaras N, Samaras D, Frangos E, Forster A, Philippe J. A review of age-related dehydroepiandrosterone decline and its association with well-known geriatric syndromes: is treatment beneficial? *Rejuvenation research*. 2013; 16(4):285–294. [PubMed: 23647054]
22. Hlaing TT, Compston JE. Biochemical markers of bone turnover – uses and limitations. *Annals of Clinical Biochemistry*. 2014; 51(2):189–202. DOI: 10.1177/0004563213515190 [PubMed: 24399365]
23. Peris P, Alvarez L, Monegal A, et al. Biochemical markers of bone turnover after surgical menopause and hormone replacement therapy. *Bone*. 1999; 2(3):349–353.
24. Tuntiviriyapun P, Panyakhamlerd K, Triratanachai S, et al. Newly developed vaginal atrophy symptoms II and vaginal pH: A better correlation in vaginal atrophy? *Climacteric*. 2015; 18(2): 246–251. DOI: 10.3109/13697137.2014.981520 [PubMed: 25374401]
25. Weber MA, Limpens J, Roovers JPWR. Assessment of vaginal atrophy: A review. *Int Urogynecol J*. 2015; 26:15–28. DOI: 10.1007/s00192-014-2464-0 [PubMed: 25047897]
26. Labrie F, Archer D, Bouchard C, et al. Serum steroid levels during 12-week intravaginal dehydroepiandrosterone administration. *Menopause (New York, NY)*. 2009; 16(5):897–906.
27. Barton DL, Sloan JA, Shuster LT, et al. Impact of vaginal dehydroepiandrosterone (DHEA) on vaginal symptoms in female cancer survivors: Trial N10C1 (Alliance). *Journal of Clinical Oncology*, 2014 ASCO Annual Meeting Abstracts. 2014; 32(15_suppl):9507. (May 20 Supplement).
28. Christiansen C, Christensen MS, Larsen NE, Transbol IB. Pathophysiological mechanisms of estrogen effect on bone metabolism. Dose-response relationships in early postmenopausal women. *The Journal of clinical endocrinology and metabolism*. 1982; 55(6):1124–1130. [PubMed: 7130340]
29. Birrell SN, Butler LM, Harris JM, Buchanan G, Tilley WD. Disruption of androgen receptor signaling by synthetic progestins may increase risk of developing breast cancer. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2007; 21(10):2285–2293. [PubMed: 17413000]
30. Traish AM, Fettes K, Miner M, Hansen ML, Guay A. Testosterone and risk of breast cancer: appraisal of existing evidence. *Hormone molecular biology and clinical investigation*. 2010; 2(1): 177–190. [PubMed: 25961191]
31. Hu DG, Selth LA, Tarulli GA, et al. Androgen and Estrogen Receptors in Breast Cancer Coregulate Human UDP-Glucuronosyltransferases 2B15 and 2B17. *Cancer research*. 2016; 76(19):5881–5893. [PubMed: 27496708]
32. Labrie F, Luu-The V, Labrie C, et al. Endocrine and intracrine sources of androgens in women: inhibition of breast cancer and other roles of androgens and their precursor dehydroepiandrosterone. *Endocrine reviews*. 2003; 24(2):152–182. [PubMed: 12700178]

Figure 1.
CONSORT diagram

Table 1

Demographics of those providing evaluable vaginal cytology

	3.25 DHEA (mcg/dL) (N=14)	6.5 DHEA (mcg/dL) (N=17)	Plain Moisturizer (N=15)	P-value
Type of Cancer				
Breast	14 (100%)	16 (94.17%)	13 (86.7)	0.2008 ²
Ovarian	0 (0.0%)	1 (5.9%)	0 (0.0%)	
Endometrial	0 (0.0%)	0 (0.0%)	2 (13.3%)	
Age (years)	57.7 ± 6.4	59.2 ± 4.6	61.0 ± 4.2	0.2820 ¹
Race				
White	13 (92.9%)	13 (76.5%)	13 (86.7%)	0.4044 ²
Black or African American	0 (0.0%)	3 (17.6%)	1 (6.7%)	
Not reported: patient refused, unsure	1 (7.1%)	1 (5.9%)	1 (6.7%)	
Oophorectomy				
Single	2 (14.3%)	0 (0.0%)	0 (0.0%)	0.3739 ²
Bilateral	5 (35.7%)	6 (35.3%)	5 (33.3%)	
Chemical ovarian ablation	1 (7.1%)	0 (0.0%)	1 (6.7%)	
Naturally menopausal	6 (42.9%)	11 (64.7%)	9 (60.0%)	
Current Tamoxifen Therapy (yes)	2 (14.3%)	3 (17.6%)	1 (6.7%)	0.6458 ²
Concurrent Aromatase Inhibitor Use				
Anastrozole/letrozole	7 (50.0%)	6 (35.3%)	7 (46.7%)	0.6730 ²
Exemestane	1 (7.1%)	2 (11.8%)	0 (0.0%)	
None	6 (42.9%)	9 (52.9%)	8 (53.3%)	
Current Endocrine Therapy (months)	19.4 ± 16.4	23.9 ± 14.4	21.0 ± 21.7	0.6950 ¹
Duration of Vaginal Dryness (months)	41.0 ± 40.9	57.4 ± 56.3	69.9 ± 75.3	0.5021 ¹
Previous (not current) Hormonal Therapy				
Tamoxifen	3 (21.4%)	5 (29.4%)	2 (13.3%)	0.6443 ²
Anastrozole/letrozole	3 (21.4%)	3 (17.6%)	3 (20.0%)	
Exemestane	2 (14.3%)	2 (11.8%)	0 (0.0%)	
None	6 (42.9%)	7 (41.2%)	10 (66.7%)	
Prior Endocrine Therapy (months)	41.3 ± 40.6	26.4 ± 24.0	43.3 ± 37.6	0.6613 ¹
Previous Adjuvant Chemotherapy (yes)	8 (57.1%)	9 (52.9%)	7 (46.7%)	0.8501 ²
Cigarette Smoking				
Current	0 (0.0%)	2 (11.8%)	1 (6.7%)	0.2216 ²
Past	8 (57.1%)	7 (41.2%)	3 (20.0%)	
Never	6 (42.9%)	8 (47.1%)	11 (73.3%)	

¹Kruskal Wallis

²Chi Square

Data are given as mean $\pm$ SD or n (%).

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Table 2

Concentrations of sex steroid hormones and biomarkers of bone turnover at baseline and 12 weeks; change from baseline to week 12 (numbers are rounded)

Variable	Plain Moisturizer N=118	3.25 DHEA (mcg/dL) N=117	6.5 DHEA (mcg/dL) N=110
DHEA-S (mcg/dL)			
Baseline	70.3 ± 41.9	80.5 ± 47.7	74.7 ± 50.8
At 12 weeks	70.3 ± 46.6	96.2 ± 52.2 ***	103.4 ± 56.3 ***
Change from baseline	0	15.7 ± 27.2 *	28.8 ± 31 ***
Estradiol (pg/mL)			
Baseline	3.5 ± 2.3	3.6 ± 2.5	3.6 ± 2.3
At 12 weeks	3.7 ± 3.3	4.7 ± 6.4	4.0 ± 2.8
Change from baseline	0.2 ± 2.5	0.9 ± 5.0	0.6 ± 1.9 [§]
Estrone (pg/mL)			
Baseline	11 ± 10.5	10.7 ± 11.7	10.9 ± 10.4
At 12 weeks	11.2 ± 11.3	13.4 ± 16.7	13.8 ± 14.5
Change from baseline	0.2 ± 4.4	2.0 ± 6.6 [§]	3.4 ± 8.3 **
Total Testosterone (ng/dL)			
Baseline	17.8 ± 9.6	16.8 ± 8.7	16.4 ± 10.7
At 12 weeks	17.6 ± 9.1	21.1 ± 10.9 **	24.7 ± 13.1 ***
Change from baseline	-0.1 ± 5.6	4.4 ± 6.3 ***	8.3 ± 10.5 ***
Free Testosterone (ng/dL)			
Baseline	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2
At 12 weeks	0.3 ± 0.2	0.5 ± 0.3 **	0.5 ± 0.3 ***
Change from baseline	0 ± 0.2	0.1 ± 0.2 ***	0.2 ± 0.2 ***
Bone Alkaline Phosphatase (mcg/L)			
Baseline	33 ± 11.5	32.2 ± 13.5	31.6 ± 11.9
At 12 weeks	34.6 ± 12.9	35.5 ± 14.5	32.9 ± 12
Change from baseline	1.6 ± 7.4	3.3 ± 9.3	1.3 ± 8.4
Osteocalcin (ng/mL)			
Baseline	20.1 ± 8.9	20.6 ± 8.7	19.9 ± 8.6
At 12 weeks	20.5 ± 9.5	20.4 ± 8.8	19.6 ± 8.8
Change from baseline	0.4 ± 5.1	-0.2 ± 5.7	-0.1 ± 4.9

p<0.0001,

**
p<0.001,

*
p<0.01,

[§]
p<0.05

Asterisks and symbols indicating significant p-values in the 3.25 mg DHEA (mcg/dL) column refer to 3.25 DHEA (mcg/dL) vs. Plain Moisturizer.
Asterisks and symbols indicating significant p-values in the 6.5 mg DHEA (mcg/dL) column refer to 6.5 DHEA (mcg/dL) vs. Plain Moisturizer.
Data are given as mean ± SD

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Table 3

Differences in hormone and biomarker concentrations at baseline between those on aromatase inhibitors and those not on aromatase inhibitors

Hormone-biomarker	NO AI (n=159) Median concentrations	AI (N=186) Median concentrations	P-value
DHEA-S (mcg/dL)	59	69.5	0.02
Estradiol (pg/mL)	3.8	2.4	<0.0001
Estrone (pg/mL)	18	2.4	<0.0001
Free testosterone (ng/dL)	0.2	0.3	0.0038
Total testosterone (ng/dL)	14.5	15	0.5535
Bone alkaline phosphatase (mcg/L)	28.3	32.7	0.0004
Osteocalcin (ng/mL)	17.4	19.4	0.0005

Concentrations of sex steroid hormones and biomarkers of bone turnover at baseline and 12 weeks, and change from baseline to week 12 by AI use

Table 4

Variable	Plain Moisturizer		3.25 DHEA (mcg/dL)		6.5 DHEA (mcg/dL)	
	AI N=61	No AI N=57	AI N=67	No AI N=50	AI N=58	No AI N=52
DHEA-S (mcg/dL)						
Baseline	75 ± 40.2	65.3 ± 43.4	84.8 ± 46.8	74.7 ± 48.7	77.8 ± 50.4	71.1 ± 51.5
At 12 weeks	73.4 ± 39	66.9 ± 53.7	96.1 ± 49.8	96.2 ± 55.8	101.9 ± 58.4	105.1 ± 54.5
Change from baseline	-1.6 ± 17.2	1.6 ± 37.5	11.4 ± 19.7**	21.6 ± 34.2**	24.1 ± 34.1**	34 ± 26.4**
Estradiol (pg/mL)						
Baseline	2.6 ± 0.6	4.5 ± 2.9	2.4 ± 0.1	5.2 ± 3.2	2.8 ± 1.7	4.4 ± 2.6
At 12 weeks	2.9 ± 3.2	4.6 ± 3.2	2.4 ± 0.2	7.7 ± 8.9	2.6 ± 1.3	5.8 ± 3.2
Change from baseline	0.3 ± 3.3	0.1 ± 1.4	0 ± 0.3	2.1 ± 7.6*	-0.2 ± 0.7	1.4 ± 2.4*
Estrone (pg/mL)						
Baseline	3.6 ± 3.8	18.8 ± 9.6	2.8 ± 1.6	21.5 ± 10.8	3.9 ± 6.6	18.4 ± 8.3
At 12 weeks	3.6 ± 4.0	19.6 ± 10.7	2.7 ± 1.9	27.8 ± 16.9	3.9 ± 6.6	25.6 ± 12.3
Change from baseline	0 ± 1.2	0.5 ± 6.3	0 ± 2.5	4.8 ± 9.0*	0 ± 1.4	7.2 ± 10.9**
Total Testosterone (ng/dL)						
Baseline	17.7 ± 9.7	18 ± 9.6	17 ± 8.9	16.4 ± 8.6	17.4 ± 12	15.2 ± 9.1
At 12 weeks	17.3 ± 9	18 ± 9.2	21 ± 11.1	21.1 ± 10.8	24.8 ± 13.3	24.6 ± 13.1
Change from baseline	-0.2 ± 6.4	0 ± 4.8	4.1 ± 5.5*	4.7 ± 7.3**	7.4 ± 9.0**	9.4 ± 11.9**
Free Testosterone (ng/dL)						
Baseline	0.4 ± 0.2	0.3 ± 0.2	0.4 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2
At 12 weeks	0.4 ± 0.2	0.3 ± 0.2	0.5 ± 0.3	0.4 ± 0.2	0.5 ± 0.3	0.4 ± 0.2
Change from baseline	0 ± 0.2	0 ± 0.1	0.1 ± 0.2	0.1 ± 0.1	0.2 ± 0.2	0.2 ± 0.2
Bone Alkaline Phosphatase (mcg/L)						
Baseline	35.2 ± 12.6	30.7 ± 9.8	34.1 ± 12.9	29.7 ± 14.1	34.2 ± 12.4	28.8 ± 10.7
At 12 weeks	36.8 ± 15.4	32.4 ± 9.4	38 ± 15.4	32.1 ± 12.6	33.8 ± 12.5	32 ± 11.5
Change from baseline	1.5 ± 7.8	1.7 ± 6.9	4.0 ± 10.2	2.3 ± 7.9	-0.4 ± 8.5	3.2 ± 7.8

Variable	Plain Moisturizer		3.25 DHEA (mcg/dL)		6.5 DHEA (mcg/dL)	
	AI N=61	No AI N=57	AI N=67	No AI N=50	AI N=58	No AI N=52
Osteocalcin (ng/mL)						
Baseline	21.4 ± 9.6	18.8 ± 7.9	22.1 ± 8.8	18.5 ± 8.3	21.8 ± 8.9	17.9 ± 7.9
At 12 weeks	21.9 ± 10.8	19 ± 7.8	21.7 ± 9.1	18.7 ± 8.3	21.2 ± 8.9	18 ± 8.4
Change from baseline	0.5 ± 5.8	0.2 ± 4.3	-0.5 ± 6.0	0.2 ± 5.3	-0.3 ± 6.0	0.1 ± 3.3

**
p<0.001,

*
p<0.01

Asterisks indicating significant p-values in the 3.25 mg DHEA (mcg/dL) AI column refer to 3.25 DHEA (mcg/dL) AI vs. Plain Moisturizer AI.

Asterisks indicating significant p-values in the 3.25 mg DHEA (mcg/dL) No AI column refer to 3.25 DHEA (mcg/dL) No AI vs. Plain Moisturizer No AI.

Asterisks indicating significant p-values in the 6.5 mg DHEA (mcg/dL) AI column refer to 6.5 DHEA (mcg/dL) AI vs. Plain Moisturizer No AI.

Asterisks indicating significant p-values in the 6.5 mg DHEA (mcg/dL) No AI column refer to 6.5 DHEA (mcg/dL) No AI vs. Plain Moisturizer No AI.

Data are given as mean ± SD.

Table 5

Vaginal Cytology Results

Variable	Plain Moisturizer N=15	3.25 DHEA (mcg/dL) N=14	6.5 DHEA (mcg/dL) N=17
pH at baseline	6.4	6.0	6.1
pH at week 12	5.6	5.1	5.4
change from baseline to week 12	−0.65	−0.69	−0.72
Baseline pH >5 reduced to pH ≤5 at 12 weeks	0 (0%)	4 (9%)	3 (7%)
No intermediate/superficial cells at baseline changed to some intermediate/superficial cells at 12 weeks	9 (64%)	14 (100%)	12 (86%)

Data are given as mean or n (%).