

## **The Effect of Omega 3 Fatty Acids on Atrophic Vaginitis in Breast Cancer Survivors**

### **Introduction**

Breast cancer is the most prevalent type of cancer among women in the U.S. which in turn has resulted in nearly 5 million breast cancer survivors (Jemal, Ward, Siegel, & Xu, 2010). This significant survivorship statistic supports the increased *quantity* of life that breast cancer survivors have experienced. This is contrary to *quality* of life, which is often overlooked and can negatively be affected secondary to side effects imposed by treatment

Urogenital atrophy is a process which occurs in postmenopausal breast cancer survivors in response to endogenous and exogenous estrogen withdrawal. Unpleasant symptoms are often experienced secondary to the consequences of diagnosis and the life-extending treatments of chemotherapy, hormonal therapy, hormone agonists, and ovarian ablation (Kelley, 2007; Willhite & O'Connell, 2001). Urogenital atrophy affects 95% of menopausal women; 40% of those women experience moderate to severe symptoms (Carcio, 2010). This condition remains under-reported and under-diagnosed as menopausal women typically regard their vaginal symptoms as private information and often believe this is an inevitable process of aging (Carcio, 2002). When in the oncology provider's office, breast cancer survivors often rationalize that these symptoms are part of the breast cancer trajectory, they are alive, and should not complain. If not assessed by the provider, the topic rarely is discussed (Lester & Bernhard, 2009).

### **Urogenital Changes with Menopause**

#### **Normal Vaginal Environment**

Vaginal tissue is primarily squamous cells in the epithelial cell layer which respond to the stimulation of male and female hormones. The estrogen in the vaginal mucosa promotes glycogen production; as these glycogen-rich cells shed, the growth of Doderlein's lactobacilli is stimulated. The lactobilli bacteria produce lactic acid that maintains a vaginal pH of 3.5 to 4.5 (Carcio, 2002; Castelo-Branco, et al., 2005). This acidic environment serves as a protective mechanism and provides a barrier against pathogenic bacteria. The maturation of vaginal epithelial cells is dependent upon the proliferative effects of estrogen which allow for the formation of a protective outer vaginal layer. This layer consists of cornified cells that protect the delicate underlying genital structures from injury and infection.

### **Atrophic Vaginitis**

Symptoms related to atrophic vaginitis are common in the general postmenopausal female population, but are often more prevalent and severe in women treated for breast cancer as compared to age-matched women without breast cancer (Conde et al, 2005; Lester & Bernhard, 2009; Zibecchi, Greendale, & Ganz, 2003). Atrophic vaginitis is an inflammatory condition that involves the lower genitalia and produces vaginal discomfort and pain. The vaginal mucosa becomes dry and thin due to a compromised estrogenic effect with unpleasant symptoms of vaginal dryness, burning, itching, and dyspareunia (Castelo-Branco, Cancelo, Villero, Nohales, & Juliá, 2005). Vaginal dryness is a common, distressing symptom that occurs in 67% of breast cancer survivors (Lester & Bernhard, 2009); genital irritation and itching occur in 30% of survivors (Lester & Bernhard, 2009). A high percentage of breast cancer survivors desire sexual activity (79%), although 31% report that vaginal pain is a significant deterrent (Lester & Bernhard, 2009). Therefore, safe and effective interventions must be identified to

improve these symptoms and thereby improve the overall quality of life among these survivors.

### **Theory of Unpleasant Symptoms**

The theory of unpleasant symptoms can be used to explain a cluster of symptoms that are influenced and affected by a multitude of overlapping factors. The assumption underlying the theory is that there are sufficient commonalities among symptoms to warrant a theory that is not limited to one symptom, but can explain and guide research for multiple symptoms (Lenz, Gift, Pugh & Milligan, 2008).

Symptoms are of vital importance in health care and are perceived as indicators of change. In the case of atrophic vaginitis, vaginal dryness is often accompanied by genital irritation and dyspareunia. Many qualities of the symptoms are reviewed such as intensity, duration, quality and distress which contribute to performance outcomes. In the theory of unpleasant symptoms, three categories of variables are identified as influencing the occurrence of symptoms: physiologic factors, psychologic factors, and situational factors (Lenz, et al., 2008). Physiologic antecedents are often diagnosed by the presence of unpleasant symptoms; thus, the influence of physiologic factors on the experience of symptoms is well documented. In terms of atrophic vaginal dryness, physiologic evidence of decreased vaginal secretions, thin and shiny epithelium, diminished rugae, increased pH, friability, introital stenosis, and decreased vaginal elasticity influences the severity of the symptoms experienced by breast cancer survivors.

The theory of unpleasant symptoms was used to measure the intervention of omega 3 fatty acids and its mediating effect from treatment-induced physiologic effects on the incidence of unpleasant symptoms and performance outcomes. Consistent within the theory, oral omega

3 fatty acid supplementation was identified as a mediating effect that may alter the influencing physiological factors related to atrophic vaginitis with the intent that there will be a change in unpleasant symptoms associated with atrophic vaginitis and improved performance outcomes.

### **Urologic Atrophy**

Atrophy in urethral epithelium results in decreased sensitivity in urethral smooth muscle and decreased amounts of collagen in periurethral tissue (Calleja-Agius & Brincat, 2009).

Urologic complaints from women with symptoms of urethral atrophy include frequency, nocturia, urgency, incontinence, and urinary tract infections (Calleja-Agius & Brincat, 2009; Castelo-Branco, et al., 2005).

### **External Genital Atrophy**

A decreased level of estrogen can negatively affect nearly every structure of the external urogenital tract. Common changes in the atrophic vulva include loss of labial and vulvar fullness and inflammation of mucosal surfaces. The vulva undergoes a gradual loss of collagen, adipose, and water which lead to decreased turgor and elasticity; in addition, pubic hair decreases (Calleja-Agius & Brincat, 2009). As a result of these changes, vaginal ulcerations and friability can occur due to a decreased epithelial layer. The vaginal mucosa is more prone to tears with decreased elasticity, increased friability, and a loss of protective layers. The increased friability can lead to evidence of petechiae, ulcerations, and bleeding secondary to minimal friction or trauma (Calleja-Agius & Brincat, 2009; Lester & Bernhard, 2009).

The introitus may narrow and tighten, and possibly contract with a concomitant shortening of the vagina. With the loss of estrogen, the prepuce of the clitoris can atrophy (Calleja-Agius & Brincat, 2009). A urinary caruncle can also be present which appears as a red,

berry-like protrusion at the urethral meatus (Carcio, 2002.) Women often experience decreased lubrication which may create a burning, itching, or irritating sensation (Carcio, 2002; Castelo-Branco, et al., 2005). The sebaceous glands of the vagina are still present and functional in the postmenopausal woman, but the amount of secretions diminishes and the ability to provide lubrication during sexual intercourse is often delayed (Ibe & Simon, 2010). When sexual activity is attempted under these conditions, it can be a painful and unpleasant experience for the woman, as well as the partner.

### **Dyspareunia**

Many women experience pain during sexual intercourse due to decreased properties of the basal level, reduced vaginal capillary blood flow, and impaired oxygenation of the tissues. These deficits result in an inability for the vaginal structures to reach the usual level of engorgement during sexual intercourse as compared to an estrogen-primed vagina (Calleja-Agius & Brincat, 2009). In addition to dyspareunia, vulvovaginal atrophy appears to also affect sexual desire and arousal (Ibe & Simon, 2010). It remains unknown if this decrease in desire and arousal is due to physical causes or due to the woman's prior experiences of pain during foreplay and intercourse (Lester, Bernhard, & Ryan-Wenger, 2012). As the frequency of sexual intercourse decreases with their partner, vaginal lubrication further declines (Castelo-Branco, 2005; Ibe & Simon, 2010). The lack of estrogen may also cause reduced vestibular sensation, which in turn leads to a diminished intensity of orgasmic pleasure (Ibe & Simon, 2010).

### **Urogenital Atrophy in Breast Cancer Survivors**

Survival rates for women with breast cancer continue to improve; 89% of the 3.5 million women diagnosed with breast cancer reach their five-year survival mark (Jemal et al., 2009).

Due to advances in treatment and prevention, the length of life for these women has extended, often to decades of disease-free survival. Contrary to these benefits, breast cancer survivors may face side effects that are detrimental to their quality of life. Unpleasant symptoms are often experienced as a result of their diagnosis, chemotherapy, hormonal therapy, hormone agonists, and ovarian ablation (Lester & Bernhard, 2009). The diagnosis of breast cancer and multiple combinations of treatments often lead women down a rapid path to premature loss of ovarian function. The treatments they receive can either induce or exacerbate menopausal symptoms due to reduced estradiol levels that tend to be more severe than in women without breast cancer (Lester & Bernhard, 2009). The life-extended treatments that women receive for breast cancer can reduce or eradicate ovarian function which can lead to acute or chronic urogenital atrophy.

### **Effects of Chemotherapy**

Chemotherapy, specifically with alkylating agents can cause ovarian toxicity, end menstrual cycles, and cause dyspareunia and vaginal dryness (Lester & Bernhard, 2009). Women who receive high-dose chemotherapy are at increased risk for persistent vaginal dryness (MacBride, et al., 2010). Women who are younger than 40 are less likely to experience permanent amenorrhea than women aged 40 years or older following chemotherapy. Young women may still experience long-term menstrual irregularities as a result of chemotherapy and have a greater risk of premature menopause (Lester & Bernhard, 2009). These effects all lead to a greater likelihood of vaginal atrophy with adverse symptoms secondary to estrogen deficiencies. Vaginal symptoms have been reported in 23% to 61% of breast cancer survivors (Lester, et al., 2012; MacBride, et al., 2010). In a study of women treated for breast cancer,

sexual issues were reported by 60% of women, urinary issues by 55%, and vaginal dryness by 55% (Lester & Bernhard, 2009). These adverse effects can lead to decreased quality of life with negative effects on intimate relations.

### **Effects of Aromatase Inhibitors**

Aromatase inhibitors (AIs) are prescribed as systemic therapy to women with estrogen receptor positive breast cancer. In a study that compared the side effect outcomes of treatment with tamoxifen versus anastrozole, vaginal dryness was more common in the group that took anastrozole than in the group with tamoxifen (MacBride, Shuster, & Rhodes, 2010). Tamoxifen acts as an estrogen antagonist or agonist depending on the target organs and the woman's menopausal state (MacBride, et al., 2010). Tamoxifen may actually cause vulvovaginal atrophy in premenopausal women through actions as an estrogen antagonist with blockage of the naturally high levels of endogenous estrogen (MacBride, et al., 2010). However, tamoxifen may conversely act as an estrogen agonist on the urogenital tract in postmenopausal women (MacBride, et al., 2010), an ultimate benefit to women.

Today, aromatase inhibitors are more frequently prescribed in postmenopausal women with hormone receptor-positive breast cancer (Wetherby, et al., 2011) with a concomitant risk of urogenital atrophy in women with premature menopause secondary to chemotherapy, or exacerbated urogenital atrophy in menopausal women (Lester & Bernhard, 2009). Vaginal symptoms have been reported in 23% to 61% of breast cancer survivors (MacBride, et al., 2010). In a study of women treated for breast cancer, sexual issues were reported in 60% of women, urinary issues in 55%, and vaginal dryness in 55% (Lester & Bernhard, 2009). This leads to a decreased quality of life for these women who are more likely

to experience negative effects on their sex lives as a result of poor lubrication, genital pain and interference with sexual pleasure (Lester & Bernhard, 2009).

The third-generation AIs improve survival rates through a further decrease in the levels of circulating estrogens. These drugs cause a profound estrogen depletion that inhibits the aromatase enzyme and promotes the conversion of androgens to estrogens in postmenopausal women (Biglia, et al., 2010).

### **Clinical Evaluation of Urogenital Atrophy**

It is important to obtain an overall health history from the patient with complaints of urogenital atrophy to ascertain changes in overall health status. Subsequently, the history should focus on potential or recognized hormonal changes and symptoms related to urogenital atrophy. A pelvic examination with a visual exam of the external and internal surfaces of the urogenital tract is essential. A cytologic examination of a vaginal smear will establish the level of cellular maturation. The vaginal smear should be obtained from the upper one-third of the vagina in order to determine the estrogenic response in the epithelial cells and the overall level of cellular maturation (Calleja-Agius & Brincat, 2009; Carcio, 2002). A vaginal pH will provide objective evidence of the postmenopausal vaginal environment (Lester & Bernhard, 2009).

### **Cellular Maturation**

The basal cell layer of the vagina is the least mature layer and is found adjacent to the basement membrane (see Figure 2). When estrogen is present, basal cells evolve into more developed forms and accumulate as the cells mature. Parabasal cells are found above the basement membrane which is often the uppermost layer of cells that defines atrophic vaginitis (Calleja-Agius & Brincat, 2009; Carcio, 2002). These cells have large nuclei with a rounded



appearance and a smaller portion of cytoplasm (Carcio, 2002). Contiguous to this layer are the intermediate cells; the nuclei become smaller and the cytoplasm increases. The intermediate cells appear flattened and more cuboid (Carcio, 2002).

The outer superficial cells are well-differentiated and comprise the most mature layer. These are larger, flat, squared-off cells with even smaller nuclei and larger amounts of cytoplasm. It takes 7 to 10 days for vaginal epithelial cells to mature; the maturation index continually changes in response to the fluctuation of hormones. To determine therapeutic response, it is important to perform serial microscopic smears. There are no published studies that demonstrate a direct relationship between the estrogen status and maturation index. Therefore, the maturation index should not be used as the sole diagnostic criteria for atrophy (Carcio, 2002) and vice versa, the estrogen status is not a sole criterion that defines the presence or absence of vaginal atrophy.

There is evidence that the presence of parabasal cells is indicative of an estrogen deficiency (Carcio, 2002). The presence/absence of lactobacilli and white blood cells [polymorphonuclear cells (PMNs)] should also be observed. The gram-positive, aerobic non-spore-forming lactobacilli are easily visible on a wet-mount slide. An atrophic smear will show very few, if any, of these rod-shaped bacilli that usually dominate the flora of the normal estrogenized vagina. The PMNs appear as dark, granular cells with clearly segmented nuclei (Carcio, 2002). When white blood cells are prevalent, they can cause a shift of the maturation index that appears more mature as a result of the infection. Women that are sexually active tend to have a more mature vaginal epithelium than that of the abstinent woman (Carcio, 2002; Castelo-Branco, et al., 2005), as regular sexual activity improves blood circulation to the vagina.

In addition, seminal fluid contains sex steroids, prostaglandins, and essential fatty acids which serve to enrich vaginal integrity (Castelo-Branco, et al., 2005).

Vaginal pH is a reliable indicator of atrophic vaginitis and is representative of the acid or alkaline setting of the vagina. The pH is affected by the presence of lactobacilli or other organisms in the vaginal environment. In estrogen deficient women, a dramatic decrease in the amount of lactobacilli can be observed which leads to a more alkaline environment. The pH can also be affected by semen and blood which are alkaline in nature. Women should avoid sexual intercourse 3 to 4 days prior to the examination and ensure the end of menses in order to provide an accurate evaluation of the vaginal milieu.

### **Treatment of Urogenital Atrophy**

Atrophic vaginitis is most commonly treated with estrogen therapy since the sole cause is a deficiency of estrogen. The use of estrogen aids in the reversal of atrophic changes and the restoration of the vaginal mucosa (Carcio, 2002). Estrogen replacement for the relief of atrophic vaginitis is provided as a systemic estrogen, or with localized vaginal products; a combination of the two is often used. Localized vaginal estrogen products include vaginal creams, estradiol vaginal tablets, and an estradiol-releasing vaginal ring (Calleja-Aguis & Brincat, 2009; Carcio, 2002). In addition, vaginal lubricants and moisturizers can provide short-term relief of symptoms.

### **Vaginal Lubricants and Moisturizers**

Treatment for women with vaginal atrophic changes is geared toward symptom relief and reversal of the atrophic anatomic changes. The first line treatments include non-hormonal vaginal lubricants and moisturizers (Ibe & Simon, 2010) which are intended to provide relief of

symptoms. However, the non-hormonal vaginal gel products may also have positive effects on vaginal pH and maturation level (Ibe & Simon, 2010). Vaginal moisturizers provide extended relief by changes in the fluid content of the endothelium and a decrease in vaginal pH (Willhite & O'Connell, 2001). Vaginal moisturizers are safe for long-term use, but need to be used on a regular basis for optimal effect (MacBride, et al., 2010). Vaginal water or silicone-based lubricants are shorter-acting than moisturizers and are primarily intended for use at the time of sexual intercourse for relief of friction-induced discomfort. The water-based products often require repeated applications with a moderate to large amount of lubrication gel, whereas the silicone-based products typically require smaller amounts; these may interfere with erectile dysfunction in the male partner (MacBride, et al., 2010).

A recent randomized double blinded study tested the effect of a vaginal pH-balanced gel with lactic acid on vaginal atrophy in breast cancer survivors after cancer treatment (Yoo-Kyung, et al., 2011). The vaginal gel was inserted three times per week at bedtime for 12 weeks. The vaginal pH-balanced gel was shown to have a significant effect on vulvovaginal dryness and dyspareunia (Yoo-Kyung, et al., 2011). The gel also lowered vaginal pH, significantly increased the vaginal maturity index, resulted in high rates of symptom relief, and demonstrated minimal side effects (Yoo-Kyung, et al., 2011). This suggests that vaginal pH-balanced gel can be a well-tolerated and effective regimen for breast cancer survivors who may experience vaginal atrophy (Yoo-Kyung, et al., 2011).

Pilocarpine, a nonselective muscarinic receptor agonist has been found to increase vaginal mucus with a reduction in vaginal dryness (Pruthi, et al., 2011). The botanical formula has demonstrated stimulation of sensory nerve conduction and improvements in sexual

function in postmenopausal women. This formula includes ingredients such as borage seed oil, evening primrose oil, angelica extract, coleus extract, vitamin C, and vitamin E. The use of lidocaine ointment 5% and gabapentin cream 6% may also be helpful in the management of dyspareunia (Pruthi, et al., 2011).

### **Localized Vaginal Estrogen**

Localized vaginal estrogens are more effective for atrophic vaginitis than systemic estrogen replacement alone. They restore the vaginal mucosa through a binding action on the estrogen receptors within the vaginal epithelium. The initial degree of systemic absorption is low due to the atrophic vaginal epithelium but increases secondary to the improvement in vascularity through ongoing treatment (Castel-Branco, et al., 2005). These formulations also increase the release of nitric oxide, which increases blood flow to the urogenital area (Carcio, 2002).

Overall, the administration of exogenous estrogen restores vaginal pH with a positive effect on the thickening and revascularization of the epithelium (Ibe & Simon, 2010). Secondary to these anatomic improvements, local estrogen therapy increases lubrication and has a positive effect on the vaginal maturation index with an increase in the level of superficial cells. The increased vascularization of the lower urogenital tract alleviates symptoms of dryness, irritation, pruritis, urinary urgency, and dyspareunia. These changes can improve quality of life, sexual desire, arousal, and orgasmic function (Castelo-Branco, et al., 2005; Ibe & Simon, 2010).

*Systemic absorption of localized estrogen therapy*

More systemic absorption is seen with low dose estrogen creams compared to the vaginal tablets and low-dose vaginal rings (Pruthi, et al., 2011). A study that investigated low dose estrogen therapy in breast cancer survivors compared the results of a low dose vaginal cream, a vaginal tablet, and a non-hormonal moisturizer (Biglia, et al., 2010). Vaginal estrogen therapies were equally effective in relieving vaginal atrophy in postmenopausal breast cancer survivors whereas only a transient benefit was obtained with the use of a non-hormonal vaginal moisturizer on subjective and objective measures of vaginal health (Biglia, et al., 2010). After 12 weeks of vaginal estrogens, serum levels of E2 in women treated with estradiol tablets increased 2.7pg/mL whereas women that used estradiol cream had a 3.5 pg/mL change (Biglia, et al., 2010).

Three studies have compared the systemic absorption of lower (10 µg twice per week) versus standard doses (25 µg twice per week) of vaginal E2 tablets in postmenopausal women. All arms demonstrated comparable results after three months of treatment, with circulating E2 levels that remained within the postmenopausal range of 3-10 pg/mL (Biglia, et al., 2010). It is rationalized that these similar increases of circulating plasma estrogens might not exert a negative effect in women with breast cancer, especially if they receive simultaneous tamoxifen due to its competitive interaction with estrogen receptors (Biglia, et al., 2010).

Nevertheless, it still cannot be concluded that even a small increase in systemic estrogens may be detrimental in women receiving AIs; an ultra-low estrogen treatment needs to be studied in the future (Biglia, et al., 2010). Future treatment for women on AIs may include a combination of vaginal estrogens with tamoxifen for a short term interval, followed by a return to their usual AI therapy (Biglia, et al., 2010)

## Vaginal Testosterone

Vaginal administration of testosterone has been evaluated for symptomatic treatment of urogenital atrophy. When taken with aromatase inhibitors, testosterone-induced proliferation of the vaginal epithelium occurs, but the AI blocks the conversion of systemic levels of testosterone to estrogen. A phase I/II pilot study to assess whether vaginal testosterone affected estradiol and testosterone levels in breast cancer patients on AIs was conducted. The intravaginal cream contained either 150 µg or 300 µg of testosterone and demonstrated no significant affect on before and after circulating estradiol levels (Witherby, et al., 2011). The study did find that circulating testosterone levels were slightly increased. The severity of dyspareunia and vaginal dryness improved significantly with treatment; a smaller degree of improvement sustained one month after completion of treatment (Witherby, et al., 2011). The study also documented that the vaginal maturation index increased, the vaginal environment became more acidic, there was a greater degree of lubrication, a color change from white to pink, and evidence of rugae formation (Witherby, et al., 2011). The two doses did not differ significantly in improvement of clinical symptoms, however the pathologic measurements did. There was a 60% higher maturation index with the 300 µg dose as compared to a 20% increase with the 150 µg dose. A significant decrease in pH as compared to baseline was also seen with the 300 µg dose whereas the 150 µg dose did not have the as significant effect (Witherby, et al., 2011).

Intravaginal administration of dehydroepiandrosterone (DHEA) has also been under investigation as a treatment for vaginal atrophy. The supplementation of DHEA allows for the biosynthesis of androgens and estrogens in specific targeted tissues such as those in the vagina

which contain the required enzymes for conversion. Very low levels of this steroid are found in the circulation because they exert their action on the same cells where they are formed. This form of treatment has been shown to increase the vaginal maturation index and decrease the pH in the vagina without increasing the level of circulating estrogen (Ibe & Simon, 2010).

A randomized double-blind placebo-controlled trial measured serum levels of dehydroepiandrosterone (DHEA) after daily intravaginal administration of one of four concentrations during a 12 week period in postmenopausal women (Pruthi, Simon, & Early, 2011). The study found that vaginal administration of DHEA was efficient in treating urogenital atrophy with no or minimal changes in serum levels of DHEA over those 12 weeks (Pruthi, et al., 2011). A recently completed trial also found intravaginal dehydroepiandrosterone (Prasterone™) to be effective on vaginal atrophy without elevation of estradiol or testosterone levels above usual postmenopausal levels (Witherby, et al., 2011).

Testosterone therapy has been associated with higher frequency of sexual activity, increased interest, desire and enjoyment, increased arousal, and increased pleasure (Castelo-Branco, et al., 2005). Data on the effects of increased levels of testosterone levels is still lacking and it is important to determine the lowest effective and least worrisome dose of testosterone in this population. The association between testosterone therapy and elevated serum testosterone levels with initial and recurrent breast cancer is still controversial and several studies suggest that a possible greater breast cancer risk associated with testosterone is indirectly mediated by a greater conversion to estrogen (Witherby, et al., 2011).

### **Complimentary Interventions**

Complementary and alternative products have been studied to determine their effects on vulvovaginal atrophy. One study noted that Vitamin E and phytoestrogen in localized gel preparation improved the symptoms of vulvovaginal atrophy (MacBride, et al., 2010). Studies document that women who did not take oral Vitamin D supplementation had significantly higher symptomatic rates of vulvovaginal atrophy and negatively affected vaginal maturation indices (Calleja-Agius & Brincat, 2009; MacBride, et al., 2010). A soy rich diet has also been investigated for the treatment of urogenital atrophy (MacBride, et al., 2010; Manonai, Songchitsomboon, Chanda, Hong, & Komindr, 2006). Soy is a phytoestrogen that has structural similarities to natural and synthetic estrogens; it binds to estrogen receptors in the vagina and the bladder. It does not exert a positive effect on the maturation of the vaginal epithelium (MacBride, et al., 2010; Manonai, et al., 2006).

### **Additional Studies**

Despite the positive effects of localized estrogen therapies on atrophic vaginitis, there remains a concern of systemic side effects from circulating levels of estrogen. Investigation of estrogen agonists/antagonists is ongoing as these non-steroidal compounds can have a proliferative effect on vaginal tissue (Ibe & Simon, 2010). Raloxifene and tamoxifen are two estrogen agonists/antagonists that are used in the chemoprophylaxis of breast cancer. Neither of these treatments has demonstrated a positive or detrimental effect on vaginal tissue in postmenopausal women. Other estrogen agonists/antagonists such as lasofoxifene and ospemifene have a positive effect on vaginal tissue in postmenopausal women. Currently in phase III trials, the selective estrogen receptor modulator (SERM) lasofoxifene has demonstrated decreased vaginal pH, increased maturational index of the vaginal epithelium,



stimulated vaginal mucous formation, and improved dyspareunia in postmenopausal women (Calleja-Agius & Brincat, 2009; Ibe & Simon, 2010). Ospemifene is under study in phase III trials for vaginal atrophy. Twelve weeks of treatment with Ospemifene was found to improve vaginal dryness and dyspareunia without any observed proliferative effects on the endometrium (Ibe & Simon, 2010).

### **Atrophic Vaginitis in Breast Cancer Survivors**

Hormonal products, whether oral or vaginal, are effective in reducing symptoms of atrophic vaginitis; however, oral hormonal products are contraindicated in women with a breast cancer history. The safety profile of vaginal estrogen products remains in question secondary to a possible mediating effect on breast cancer recurrence (Pruthi, et al., 2011). Safe and reliable interventions for atrophic vaginitis are needed to effectively reduce these disruptive symptoms and improve the quality of life in breast cancer survivors. Omega 3 fatty acids may address atrophic vaginitis through suppression of inflammation with restoration of tissue integrity and elasticity. Omega 3 fatty acids have the ability to exert these changes and provide protective healing properties (McDaniel, Massey, & Nicolaou, 2011) with effective treatment of atrophic vaginitis.

### **Omega Three Fatty Acids**

#### **Wound Healing**

Wound healing is a complex process that involves multiple stages. In the inflammatory stage, molecular and cellular processes are initiated by a group of protein mediators known as proinflammatory cytokines (McDaniel, Belury, Ahijevych, & Blakely, 2008). These proinflammatory cytokines include interleukin-1 $\beta$ , interleukin-6, and tumor necrosis factor- $\alpha$

that are secreted by neutrophils, macrophages, mast cells, fibroblasts, and endothelial cells. This release of cytokines signals biological processes during the inflammatory stage of wound healing and binds to receptors on target cells (McDaniels, et al., 2008). This activity of the cytokines works to assist infection control and prepare tissue for further repair by increased phagocytic activity, stimulated keratinocyte migration at wound edges, proliferation, breakdown of extracellular proteins, and regulation of the release of additional cytokines and growth factor (McDaniel, et al., 2008).

The activity and synthesis of the proinflammatory cytokines are affected by the concentration of  $\omega$ -3 polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) which are primarily found in fish oil, plasma, tissue, and cellular membranes (McDaniel, 2008). The  $\omega$ -6 arachidonic acid (AA) and the  $\omega$ -3 EPA are released from the phospholipid layer of cellular membranes in response to wound healing with the EPA of preferential state (McDaniel, et al., 2008).

EPA and DHA are also thought to have an effect on the gene expression of proinflammatory cytokines at the level of transcription (McDaniel, et al., 2008). A recent study was completed on healthy individuals to test the effect of omega 3 oil on cutaneous wound healing. The dietary supplementation of  $\omega$ -3 (EPA/DHA) fatty acids was tested to observe the amount of cytokines present in the wound exudate in association with a healed wound compared to effects in the placebo group. The study found that in the intervention arm, there was a decreased plasma AA:EPA ratio from baseline, non-significant slower wound healing, and a higher production of Interleukin-1 $\beta$  24 hours after the blister wound was initiated as compared to the placebo group who took mineral oil. Even though the wound healing was

observed to be slower in the intervention group, there was still evidence to demonstrate that dietary  $\omega$ -3 PUFA affects the local production of inflammatory cytokines that regulate the wound healing process (McDaniel, et al., 2008).

Chronic wounds can result from prolonged inflammation and include excessive polymorphonuclear leukocyte (PMN) activity (McDaniel, et al., 2011). Inflammation is an essential initial stage of tissue regeneration but when prolonged, can delay advancement to subsequent healing stages. The high amounts of PMNs secrete excessive amounts of proteases that can cause tissue destruction and persistent inflammation (McDaniel, et al., 2011). The bioactive lipid mediators derived from the long-chain n-3 polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) acids have demonstrated a reduction in inflammatory responses and PMN transmigration across the endothelium (McDaniel, et al., 2011). Epidemiological studies report clinical improvements in a number of chronic inflammatory diseases with diets rich in n-3 PUFAs that contain EPA and DHA (McDaniel, et al., 2011). This has been observed by anti-inflammatory actions in disease models such as peritonitis, colitis, and periodontitis (McDaniel, et al., 2011).

In a study of n-3 PUFA oral supplements on cutaneous blister wounds, researchers noted that fish oil supplements raised the levels of EPA and DHA and reduced both the total n-6:n-3 and AA:EPA ratios from baseline values (McDaniel, et al., 2011). The study noted that the addition of EPA and DHA caused a suppression of the proinflammatory mediators at the wound site as well as increases in the anti-inflammatory mediators. EPA was found to reduce the recruitment of leukocytes and neutrophils to wound sites 12 hours after the blister wound was initiated (McDaniel, et al, 2011). The data demonstrated that EPA made it possible to

manipulate systemic levels of lipid mediators associated with inflammation reduction. This would be a beneficial supplementation in the case of heightened or prolonged inflammatory responses such as chronic wounds or trauma (McDaniel, et al., 2011).

The data from this study strengthens the hypothesis that EPA has a positive effect on the local tissue level through alteration of the anti-inflammatory pathways (McDaniel, et al., 2011). The EPA demonstrated a rise in the anti-inflammatory mediators that include resolvins and protectins with strong anti-inflammatory PMN regulatory actions (McDaniel, et al., 2011). This action promotes wound epithelialization and inflammatory resolution. The study suggested that EPA and DHA affect the levels of local and systemic lipid mediators that can be adjusted to encourage inflammation resolution, PMN down-regulation, and wound re-epithelialization (McDaniel, et al., 2011).

### **Use of Omega 3 in Atrophic Vaginitis**

The consideration of fish oil as an appropriate treatment for breast cancer survivors must include the risk of recurrent disease. Studies in animals have demonstrated that long chain n-3 fatty acids present in high levels of fish oil, have protective effects against several tumor types, including mammary tumors (Goodstine, et al., 2003). Conversely n-6 fatty acids, an unsaturated fatty acid which is very popular in American diets, enhanced mammary carcinogenesis in animal models (Goodstine, et al., 2003).

Americans generally consume relatively low amounts of n-3 fatty acids in their diets, whereas the population of Greenland Eskimos consumes considerable amounts. Even though their diets consist of a high amount of fat, the breast cancer rate is very low in this population (Bang & Dyerberg, 1980; Goodstine, et al., 2003) due to consumption of high level of n-3 vs. n-6

fatty acids. The Japanese also consume a high amount of omega-3-rich fish (n-3 PUFAs) and their breast cancer incidence rates are also very low (Goodstine, et al., 2003). A recent study in Connecticut found that the consumption of a higher n-3:n-6 PUFA intake ratio was associated with a lower risk of breast cancer in US women. In premenopausal women researchers noted that increased n-3/n-6 PUFA ratios resulted in a 41% decrease in breast cancer occurrence and an 11% decrease in odds of breast cancer in postmenopausal women (Goodstine, et al., 2003). Other studies have found even stronger inverse associations with breast cancer in postmenopausal women who consumed high amounts of n-3 PUFAs (Goodstine, et al., 2003).

### **Omega 3 Study in Breast Cancer Survivors with Atrophic Vaginitis**

A study of the effects of omega 3 fatty acid oral supplementation on signs and symptoms of atrophic vaginitis in breast cancer survivors was proposed based on the compendium of previously mentioned studies. The associations of predominantly EPA-derived benefits of wound healing of skin tissues (Figure 3) to the cascade of events in treatment-induced or exacerbated menopausal vaginal atrophy directed attention to the potential effects of omega 3 oral supplementation.

#### **Study Design**

A randomized, double blind Phase II screening trial was proposed to determine the effect of omega 3 fatty acids on atrophic vaginitis in postmenopausal breast cancer survivors. This institutional-approved review board (IRB) study was derived to improve our current knowledge about symptoms of atrophic vaginitis in postmenopausal breast cancer survivors, and to provide baseline data about whether oral omega-3 fatty acid oral supplementation can decrease inflammation and improve self-reported and observed outcomes. Postmenopausal

breast cancer survivors (N=52) with one or more complaint(s) of atrophic vaginal atrophy (e.g. vaginal dryness, genital irritation/itching, genital pain, or dyspareunia) were randomized to oral omega-3 supplementation or a matched placebo. Interim analyses of completed cases were planned for purposes of this undergraduate thesis.

### **Purpose and Specific Aims**

The purpose of this study was to explore whether omega-3 fatty acids can improve atrophic vaginitis in postmenopausal breast cancer survivors.

*Primary Aim #1:* To explore whether oral omega-3 fatty acids can improve self-reported symptoms related to atrophic vaginitis in postmenopausal breast cancer survivors.

*Primary Aim #2:* To explore whether oral omega-3 fatty acids can decrease inflammation related to atrophic vaginitis in postmenopausal breast cancer survivors.

### **Sample Size and Eligibility Criteria**

For this screening trial, the sample size was estimated based on the number of participants necessary to observe a measureable improvement in self-reported symptoms of pain or discomfort. The sample size was identified through a power analysis, considering a large effect (0.8 of a within group standard deviation) with 80% power at  $\alpha$ -level of .05. As an approximation to the sample size, we used a t-test for two independent samples with a two-sided test. To achieve the desired goals, 26 subjects were studied in each treatment group, for a total N of 52. Improvements in symptom profiles on the self-report instruments were sought as proxy measures of pain and discomfort related to atrophic vaginitis. As secondary outcome, vaginal pH and maturation index were utilized as a proxy measure of inflammation related to atrophic vaginitis. A reduction of 1.0-1.5 in vaginal pH will be considered clinically significant as

an improvement in inflammation related to atrophic vaginitis, although the potential effects of omega 3 fatty acids on markers of atrophic vaginitis were relatively unknown prior to the study start. Improvements of symptoms on one or more self-report instruments were considered indicative of a positive effect from the intervention.

The following inclusion criteria were used for study eligibility: female, between the age of 45-65 (later amended to 35-70), a personal history of Stage 0, I, II, or III breast cancer, at least 12 months from definitive surgical procedure, completion of chemotherapy, postmenopausal (e.g. defined as no menstrual cycle for 12 consecutive months, or surgical menopause), no current use of estrogen replacement therapy or estradiol-releasing vaginal ring or tablets, no evidence of disease (NED) of any cancer or breast cancer, no current use of oral omega 3 fatty acids (e.g. if recent consistent use of these products, off at least six months; if sporadic use of these products, off at least 3 consecutive months), Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, no history of a bleeding tendency, uncontrolled hypertension, heart disease or stroke, and normal hematopoietic and liver function studies (e.g. hemoglobin > 10g/dL, hematocrit > 30%, white blood count > 3.5K/uL, platelet count > 100,000/mm<sup>3</sup>, fasting serum glucose < 115mg/dL, total bilirubin < 1.6mg/dL, and liver transaminases < 1.5 x upper limits of normal). Potential participants could be prescribed with current use of oral anti-estrogens (e.g. tamoxifen or raloxifene), aromatase inhibitors (e.g. letrozole, exemestane, or anastrozole), or biologic therapy (e.g. continuation of adjuvant treatment with targeted agents).

Potential candidates were excluded from the study secondary to: metastatic malignancy of any kind, ongoing chemotherapy or radiation therapy (ongoing hormonal therapy and/or

biologic therapy were allowed), history of pelvic or genital radiation therapy, use of Coumadin or other anticoagulants, known active pelvic, vaginal, or urinary tract infections, current use of systemic or local applications of hormone replacement therapy, uncontrolled co-morbid conditions (e.g. ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, or unstable hypertension), psychiatric illness or social situation that would limit comprehension or adherence to study requirements, consistent use of omega-3 fatty acid concentrates or capsules within the 6 months prior to entry on the study, a known sensitivity or allergy to fish oil or omega 3 fish products, and subjects who could not give an informed consent.

## **Methods**

### **Study Enrollment and Signed Consent**

After explanation of the study, written informed consent, and verification of baseline study lab values, participants were randomized to one of two treatment arms: 3.5g of oral omega 3 fatty acids per day or matched placebo capsules. The participants were instructed to take 7 capsules per day in (at least) two divided doses for a 6-month period.

### **Study Measures**

Participants were required to complete a study visit at baseline, three- and six-months. At each visit the participant completed self-report symptom surveys, provided serum and vaginal samples, and underwent a vaginal exam completed by the study gynecologist (156 total exams for study). At the baseline visit participants were asked to complete a breast health history form and initial self-report questionnaires. These questionnaires included the Urogenital Atrophy Questionnaire (UAQ), The Female Sexual Function Instrument (FSFI), Menopause



Rating Scale (MRS), Center for Epidemiologic Studies in Depression Scale (CES-D), and Brief Pain Inventory (BPI).

### *Vaginal examination*

The vaginal examination included subjective and objective evaluation of external genital pain and discomfort, visual examination, and vaginal swab for biologic markers that represented vaginal state (e.g. maturation index, parabasal level) and cytology. The gynecologist observed and noted the color of the vaginal mucosa, amount and color of secretions, presence or absence of rugae, introitus elasticity, appearance of the introitus, appearance of the labia majora and the labia minora, color of the external genitalia, presence of any bleeding tendencies (e.g. petechiae, friability) of the vaginal tissue, presence or absence of pubic hair, presence or absence of urethral caruncle(s), and the participant's rating of the pain upon touch to the vestibule with a cotton swab. During the examination, the practitioner obtained a sample from the distal two-thirds of the lateral vaginal wall (e.g. avoiding the posterior fornix and cervix) for calculation of vaginal pH on phenolphthazine paper with a color-coded range of 4.5-7.5. A reading of 5.0 or greater was indicative of atrophic vaginitis. A wet mount was concurrently performed by the gynecologist to identify the presence or absence of vaginal yeast or bacterial infections that may alter the vaginal pH.

After completion of the genital examination by the gynecologist, the participant obtained a self-pH; practitioner-obtained vaginal pH and patient-obtained self-pH were compared as reliability measures in preparation for future cooperative group studies using a self-pH only.

### *Serum studies*

At the baseline visit, a fasting blood draw was obtained to evaluate a complete blood count (CBC), fasting serum glucose, total bilirubin, and liver transaminates to document normal hematopoietic and liver function. Serum ALT values were tested at subsequent 3- and 6-month visits as toxicity measures. At study end, additional serum evaluations are planned to verify serial hormonal levels, and measures of inflammation including cytokines. All labs with the exception of eligibility and toxicity measures will be completed at study end in order to batch samples and match lot numbers for specific lab tests.

#### *Self-report symptom profiles*

The self-report questionnaires were provided at each visit and study participants were also requested to complete them at monthly intervals; a self-addressed, stamped mailing envelope was provided for return to study personnel. Each month the participants received a monthly reminder call to document adherence based on current capsule counts, remind the participant to mail completed monthly self-report instruments, and to note any experienced side effects from the study drug. A paper daily medication diary was distributed at the beginning of the study to provide participants with a document to note daily adherence patterns and any experienced side effects (e.g. mild gastrointestinal discomfort, fishy aftertaste, belching, nausea, flatulence or loose stools, or other). A participant:study personnel pill count was also documented at 3- and 6-month visits, with participant-reported pill counts at months 1, 2, 4, & 5.

#### *Additional study measures*

Food diaries were utilized to note patterns of dietary intake of all foods and fatty acids. Measures of dietary intake included the Fred Hutchinson Food Frequency Questionnaire (FFQ)

at baseline and six months, and three-day food diaries at baseline, three-, and six-month intervals. The FFQ obtained a six-month recall of dietary patterns prior to the start, and at the end of the study period; the three-day food diaries obtained real-time records of meal and snack content that was consumed at baseline, and on or about three days before the three- and six-month visits (Newhouser, et al., 2008; Olafsdottir, Thorsdottir, Gunnarsdottir, Thorgeirdottir, & Steingrimsdottir, 2006). Measures of height, weight, body mass index (BMI), waist, and hip circumference, and waist-to-hip ratio (WHR) were obtained to note differences in body composition amongst participants and across the study period (Behan & Mbizo, 2007).

### **Data Analyses and Interpretation**

This interim study analysis was conducted to inform the study team and reader of potential outcomes after enrollment, study completion, and confidential reveal of intervention for approximately 75% of study participants. The data represented here illustrates a brief examination of isolated study parameters in association with the revealed study intervention, such as vaginal parabasal level and pH, and selected self-report symptom profiles.

Interpretations of these data were limited to current research student interest and trends; full interpretation and disclosure of the data will occur at study completion with evaluation of all measurement and study parameters.

### **Primary Aim: Self-Report Symptoms**

*Primary Aim #1:* To explore whether oral omega-3 fatty acids can improve self-reported symptoms related to atrophic vaginitis in postmenopausal breast cancer survivors.

The data obtained from the self-report questionnaires was partially analyzed and scored in the standard fashion relative to selected instruments in order to compare differences in scores

across different time points. Descriptive statistics were used. The goal was to compare differences in scores across different time points, including mean severity at baseline and the change in mean severity between baseline, midpoint (3 months) and end (6 months) of treatment.

Repeated measures ANOVA models (in future will include covariates) were fitted to the data. Separate models will be fitted for each of the scales used to measure outcomes of the study. In each case, time was used as a within-subjects variable, treatment (placebo and intervention) was used as a between-subjects variable, and subject was used as a random effect nested within treatment. Time and treatment were interacted to allow for different treatment effects between the groups over time. Through examination of appropriate contrasts, we will be able to determine whether there is a significant effect of the drug on the outcome measure(s) at each time point. This model will be augmented with covariates, such as demographic variables, systemic breast cancer treatment, body habitus measurements, dietary intake pertinent to foods rich in omega 3 fatty acids, and/or serum hormone levels.

#### *Urogenital atrophy questionnaire*

The Urogenital Atrophy Questionnaire (UAQ) is a 15-item questionnaire designed and validated as a brief, self-report instrument to assess unpleasant symptoms related to urogenital atrophy, including vaginal dryness. Responses are numerically weighted with an additive scoring system that allows assessment of function, satisfaction, pain, and urogenital quality of life in three domains (urologic, genital, and sexual) and ten factors: urinary incontinence, changes in urinary patterns, burning with urination, genital itching/odor, genital irritation,

vaginal discharge, pain with sexual activity, interest/desire in sexual activity, partner communication, and sexual activity without genital touching (Lester, et al., 2012).

#### *Female sexual function index*

The Female Sexual Function Index (FSFI) questionnaire is a 19-item questionnaire designed and validated as a brief, self-report instrument to assess female sexual function and quality of life in clinical trials. Responses are numerically weighted and easily converted to a scoring system that allows assessment of six different areas: desire, arousal, lubrication, orgasm, satisfaction, and pain (Rosen, et al., 2000).

#### *Menopause rating scale*

The Menopause Rating Scale (MRS) (Heinemann, et al., 2004) is a brief, self-report, health-related quality of life scale with eleven items. This scale was designed and validated to measure common menopausal symptoms. It is validated to measure treatment effects on quality of life using numerically weighted items with an additive scoring system. The MRS includes three subscales: psychological, somatic, and urogenital. Scores of each subscale, as well as total subscale can be evaluated (28, 29).

#### *Depression Short Scale*

The Center for Epidemiologic Studies Depression Short Scale (CES-D10) is a brief, 10-item self-report depression symptoms index. This instrument was designed to assess the presence and severity of depressive symptoms as noted in the past week. It is validated in breast cancer survivors (mean age 56.1) in a study to assess psychosocial adjustment and quality of life (Carpenter, et al., 1998). The CES-D10 used numerically weighted items with an additive scoring system (Carpenter, et al., 1998).

*Potential Pitfalls:* In the case of incomplete questionnaires, patients were approached by nursing or study personnel during clinic visits to clarify any question or concerns regarding specific items on the form. Telephone contacts were utilized to encourage completion of the monthly study questionnaires.

### **Primary Aim: Vaginal Milieu and Inflammation**

*Primary Aim #2:* To explore whether oral omega-3 fatty acids can decrease inflammation related to atrophic vaginitis in postmenopausal breast cancer survivors. The data obtained from the vaginal pH results was partially analyzed and scored in accordance with the scale and color range supplied by the manufacturer. Differences in scores between different time points were compared using descriptive statistics. Repeated measures ANOVA models were fitted to the data. Separate models will be fitted for each of the scales being used as outcomes of the study. In each case, time was used as a within-subjects variable, treatment (placebo and intervention) was used as a between-subjects variable, and subject was used as a random effect nested within treatment. Time and treatment was interacted to allow for different treatment effects between the groups over time. By examining the appropriate contrasts with the full data set, we will be able to determine whether there was a significantly different effect of the study drug on the outcome measures at each time point. This model will be augmented with covariates, such as demographic variables, systemic breast cancer treatment, body habitus measurements, dietary intake pertinent to foods rich in omega 3 fatty acids, and/or serum hormone levels.

*Potential Pitfalls:* In the event of a vaginal or urinary tract infection at the three- or six-month visits, the pH will be obtained one week after resolution of the problem. During the study

period, only one (resolving) yeast infection was noted, and not other vaginal infections or abnormalities were noted with the exception of vaginal warts at the six-month evaluation of one candidate. It was anticipated that vaginal pH would improve as a measure of physiologic improvement and healing of the parabasal layer, as evidenced in studies with other agents although no studies had previously measured the effect of omega 3 on vaginal pH.

### **Analyses of Data**

Preliminary analyses were conducted of 40 participants that were unblinded by the scientific study pharmacist at the end of April 2012. These participants had completed all components of the study and were no longer active participants. Examination and analyses of data were limited to physiologic biomarkers related to the vaginal examination, and limited self-reported items.

### **Vaginal Environment**

Limited analyses of the vaginal environment demonstrated few changes. Individual items from the vaginal assessment sheet were evaluated at T0, T3, and T6 time points. Using analysis of variance (ANOVA) there were no significant findings on vaginal pH, self-reported sexual problems, urinary problems, or vaginal dryness in comparison of the two study arms: placebo vs. omega 3. Figure 2 demonstrates findings:

Figure 2. Analyses of T6 items

		ANOVA				
		Sum of Squares	df	Mean Square	F	Sig.
T6 vaginal pH	Between Groups	1706.907	1	1706.907	1.128	.295
	Within Groups	57516.193	38	1513.584		
	Total	59223.100	39			
T6 MRS sexual problems	Between Groups	63.375	1	63.375	.050	.825
	Within Groups	48589.600	38	1278.674		
	Total	48652.975	39			
T6 MRS bladder problems	Between Groups	49.307	1	49.307	.038	.847
	Within Groups	49425.093	38	1300.660		
	Total	49474.400	39			
T6 MRS dryness of vagina	Between Groups	166.427	1	166.427	.116	.735
	Within Groups	54327.173	38	1429.662		
	Total	54493.600	39			

Of interest are several variables that were statistically significant. Chi-square was used to analyze uneven groups (placebo, n=15, omega 3, n=25) in this preliminary analyses. There was a statistically significant difference between the two study groups at time period '6 months' for presence/absence of rugae ( $p=0.063$ ) and presence/type of bacteria ( $p=0.021$ ) in the intervention arm with omega 3 capsules. In addition, the self-report item on the Urogenital Atrophy Questionnaire 'thought of pain with sexual activity' was statistically significant likewise in the intervention arm at time period '3 months' ( $p=0.015$ ) and '6 months' ( $p=0.016$ ). Of note, at baseline, there was no significance between the groups ( $p=0.770$ ).

These findings in these preliminary analyses of data appear promising in that perhaps a perceived decrease is present for 'anticipated pain with sexual intercourse', as well as positive correlations for two biologic markers. Detailed statistics over time between and within the groups were not conducted; perhaps additional positive findings will be noted at the time of the entire data set analyses.



## Discussion

Breast cancer is the most common cancer diagnosed among women. Therefore, it is anticipated that complaints of symptoms related to vaginal atrophy will become more frequent in the clinical setting. In addition, the number of survivors is exponentially increasing, and the use of aromatase inhibitors has significantly increased over the last decade. Therefore, the short- and long-term side effects related to atrophic vaginitis will likely be present, as indicated by previous studies (Lester, et al., 2012).

This study was designed to measure a non-hormonal intervention that would provide an optimal treatment option for those breast cancer survivors who suffer from vaginal dryness, irritation, and dyspareunia. The women on the study were very compliant with the daily drug regimen, completed monthly surveys and attend study visits at 0, 3, and 6 months. A pelvic examination with a visual exam of the external and internal surfaces was completed at each of the visits as well as the battery of self-reported symptom instruments.

### Physiological Findings

It was hypothesized that omega 3 could make a significant change in physiological determinates of atrophic vaginitis such as vaginal pH or cellular maturity. An interim analysis of the data was completed which included 40 of the participants. At that time, the placebo group had 15 participants and the omega group consisted of 25 participants. The level of cellular maturation was determined by each visit's cytologic examination of the vaginal smear from the upper one-third of the vagina. The vaginal pH, bacteria, external and internal properties were also analyzed as outlined on the vaginal examination sheet. Limited items of the participants' responses in the self-report questionnaires were analyzed to determine whether there were

any significant differences between the two treatment groups in terms of subjective symptoms. With a 95% confidence interval, the interim analysis (n=40) found a significant change in bacteria from baseline to the 6 month visit for the omega group ( $p= 0.021$ ). This indicates that the bacteria in the Omega group demonstrated a significant trend toward lactobacilli growth in the vaginal environment. The growth of lactobacilli indicates that the vaginal environment is producing the bacteria found in normal estrogenized women. The lactobacilli bacteria will produce lactic acid and facilitate a more protective acidic environment.

The omega group also had a significant change in the presence of rugae from baseline to 6 months ( $p=0.063$ ). This may indicate that the vaginal walls of the omega group are able to now form protective layers. This would lead to an increase in resilience and a decrease in irritation, friability, tearing, and ulcerations with friction or sexual intercourse.

### **Self-Reported Items**

Observation of items on the Urogenital Atrophy Questionnaire (UAQ) indicated that the omega 3 group had a significant change in concern with their anticipated worry about pain with sexual intercourse. This significant change was seen at 3 and 6 month visits ( $p=0.015$  and  $p=0.016$ , respectively). In concert with the previous findings, the omega 3 group may experience less pain with intercourse due to a more protective acidic environment and the formation of rugae which provides a protective layer. Recalculation of these findings will occur with the full data set upon completion of the study to determine if these positive findings persist.

In regard to self-reported 'vaginal dryness', a significant change was seen among the omega 3 group at the 6 month interval ( $p=0.129$  with a 90% confidence interval). This trend

toward a positive effect provides data to ponder until the entire data set is completed and ready to examine in full. But, these promising findings in the omega 3 study arm provide enough data to summarize that the omega 3 group experienced a decrease in anticipatory pain experienced with sexual intercourse.

These findings in these preliminary analyses indicate that the use of omega 3 for atrophic vaginitis in breast cancer survivors may have the potential to improve the vaginal epithelium and decrease the self-reported symptoms of dryness and dyspareunia. In the future, an analysis will be completed on the entire data set (N=52) and further potential mediated effects of the omega 3 will be determined.

### **Implications for Practice**

It is hopeful that a simple non-hormonal intervention such as omega 3 fatty acids oral supplementation will improve self-reported symptoms, decrease inflammation, and improve the vaginal environment related to atrophic vaginitis in postmenopausal breast cancer survivors. If these findings are not statistically evident, further research will be necessary to identify an optimal treatment for these unpleasant symptoms in breast cancer survivors.

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