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Effect of Brassica Vegetable Intake on the 2:16α Estrogen Metabolite Ratio in Postmenopausal European American and African American Women

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EFFECT OF *BRASSICA* VEGETABLE INTAKE ON 2:16α ESTROGEN METABOLITE RATIO IN POSTMENOPAUSAL EUROPEAN AMERICAN AND AFRICAN AMERICAN WOMEN

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DEDICATION

First and foremost I would like to dedicate this to both of my parents, Ken and Deanne Knight, for always encouraging me to dream big and shoot for the stars. As a young child I knew that pursuing a career in cancer research was my true passion in life and they both never let me forget that, even when life threw me some curve balls. Thank you!

To my brother and sister, Cla and Kenzie. You've both always been there to encourage and cheer me on from the sidelines. You both give my life reason and meaning to want to excel in everything I do. Thank you for always believing in me and allowing me to be a great role model for you both to look up to.

To my extended family, friends, and all of the women who have battled this devastating disease, your struggles and determination to fight give us all the continued hope we need for the future.

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ABSTRACT

Background: It is well-established that many risk factors for breast cancer are related to estrogen or reproductive factors. Two estrogen metabolites of particular interest are 2-hydroxyestrone (2-OHE) and 16α-hydroxyestrone (16α-OHE) which are often reported together as a ratio of 2:16α-hydroxyestrone (2:16α –OHE). It has been demonstrated in several studies that postmenopausal women who are at a higher risk of breast cancer tend to have increased levels of 16α-OHE or decreased levels of 2-OHE levels in the body. Recent studies have shown that the phytochemical indole-3-carbinol (I3C), found in *Brassica* vegetables, impacts the conversion of 2-OHE, known as "good estrogen", by inducing the enzyme CYP1A1; however, research on the effects of *Brassica* vegetable consumption on the 2:16α-OHE ratio in randomized clinical settings is still sparse. The objective of this study is to examine whether an increase in *Brassica* vegetable consumption would alter the 2:16α-OHE estrogen metabolite ratio among postmenopausal women, by increasing 2-OHE and decreasing 16α-OHE.

Methods: Postmenopausal African American and European American women who resided in the standard metropolitan statistical area (SMSA) around the Columbia, South Carolina area were recruited between April 2002 and September 2003 for a nine-week dietary intervention. The women were randomized into one of two arms, intervention group or non-intervention (control group), during the first clinic. The women randomized to the intervention group were offered nine classes that were led by trained study dietitians over a three-week dietary intervention geared at increasing the

consumption of *Brassica* vegetables daily at home; whereas, those in the control group were asked to maintain their usual dietary habits for the duration of the study period. The study population that was used for analysis consisted of 64 women who attended both clinic visits with complete urine, dietary data, and baseline information.

Results: Compared to baseline levels, *Brassica* vegetable intake significantly increased in the intervention group by 6-fold when compared to the control group during the intervention; whereas, post intervention, *Brassica* intake only increased in the intervention about 3.5-fold when compared to the control group. At post intervention, there were no statistically significant differences between the intervention and control groups in 2-OHE (10.6 ng/ml vs. 8.7 ng/ml, respectively, p=0.4), 16α-OHE (9.6 ng/ml vs. 8.6 ng/ml, respectively, p=0.6) and 2:16α-OHE (1.3 ng/ml vs. 1.3 ng/ml, respectively, p=0.7) levels. The effect of the intervention was determined separately for high and low *Brassica* vegetable consumers at baseline, stratified by race, and stratified by breast cancer survivorship status but these did not appear to modify the effect of the intervention on 2-OHE, 16α-OHE, or 2:16α-OHE levels after adjustment for covariates.

Conslusion: These findings suggest that *Brassica* vegetables do not play a role in the modification of 2-OHE and 2:16α-OHE metabolite levels among postmenopausal women in a short, three-week intervention. These findings highlight the need for future research to further understand the biological mechanism between estrogen metabolites and *Brassica* vegetable consumption. Future research is needed to determine whether a larger increase in *Brassica* vegetables could affect estrogen metabolites and to examine whether genes may modify the effect of *Brassica* vegetables on estrogen metabolites.

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CHAPTER I

INTRODUCTION

Statement of the Problem

With the exclusion of skin cancer, breast cancer is the most commonly diagnosed cancer among women in the United States, accounting for approximately 1 in 3 cancers (DeSantis et al. 2013). The lifetime risk for a woman born today to develop breast cancer is 1 in 8 women (SEER, Stat Fact Sheets: Breast Cancer 2006-2010). In 2013, it is estimated that 232, 340 women will be diagnosed with invasive breast cancer (SEER, Stat Fact Sheets: Breast Cancer 2006-2010). In 2012, it was estimated that approximately 2.9 million women alive in the United States had a history of breast cancer (American Cancer Society, Breast Cancer Facts & Figures 2013-2014). Beginning in the 1980's, there was a sharp increase in breast cancer incidence rates which was likely due to the greater use of mammography screening leading to increased early detection of cases (American Cancer Society, Breast Cancer Facts & Figures 2013-2014). Between 2002 and 2003, rates drastically decreased; furthermore, since then, the rate of breast cancer incidence has remained relatively stable (American Cancer Society, Breast Cancer Facts & Figures 2013-2014). In 2013, it is estimated that 39,620 women will die of invasive breast cancer (DeSantis et al. 2013; SEER, Stat Fact Sheets: Breast Cancer 2006-2010). Some of the known risk factors for breast cancer include age, family history of breast

cancer, early age at first menarche, late age at menopause, nulliparity, late age at first birth, and hormone replacement therapy (American Cancer Society, Breast Cancer Facts & Figures 2013-2014). The five-year relative survival rate of breast cancer is highly correlated with stage of disease at diagnosis; meaning that as stage increases, survival rate decreases (American Cancer Society, Breast Cancer Facts & Figures 2013-2014). The breast cancer five-year relative survival rate has been increasing since 1975 in both European American (EA) and African American (AA) women; however, there seems to be a widening health disparity gap that exists between EA and AA women on breast cancer mortality (American Cancer Society, Breast Cancer Facts & Figures 2013-2014).

AA women are less likely to be diagnosed with breast cancer than their EA counterparts; however, AA women are more likely to die of breast cancer than EA when they are diagnosed (DeSantis et al. 2013; SEER, Stat Fact Sheets: Breast Cancer 2006-2010; Adams et al. 2006; Menashe et al. 2009). Furthermore, AA's are burdened with more aggressive forms of breast cancer (DeSantis et al. 2013; SEER, Stat Fact Sheets: Breast Cancer 2006-2010; Adams et al. 2006; Menashe et al. 2009). According to the SEER data from 2006-2010, the incidence rate for EA and AA women were 127.4 and 121.4 cases per 100,000 women, respectively. The age-adjusted mortality rate for EA and AA women respectively were 22.1 and 30.8 deaths per 100,000 women; moreover, the overall 5-year relative survival rate 2006-2010 was 92% for EA and only 79% for AA women (SEER, Stat Fact Sheets: Breast Cancer 2006-2010; American Cancer Society, Breast Cancer Facts & Figures 2013-2014). Therefore, not only is it important to focus on breast cancer reduction among all women in general in the United States, but to also

determine why AA women tend to present with more advanced stages of the disease and are more likely to die from breast cancer compared to other racial/ethnic groups.

Both environmental and biological factors affect the risk of breast cancer (Maizes 2005). Similar to other cancers, breast cancer risk has been linked to poverty, limited access to health care, lower education, and lower income (Leepeak et al. 2011; Tian et al. 2011; Merkin et al. 2002; Tian et al. 2010; Komenaka et al. 2010; Simon et al. 2006). Diet has also become a recognizable environmental risk factor associated with the risk of a number of cancers, including breast cancer (Ramirez et al. 2009; Higdon et al. 2007; Kim et al. 2009; World Cancer Research Fund/American Institute for Cancer Research 2007). The link between vegetables in general and the risk of breast cancer has been well-established over the last decade through numerous studies (Willet 2001; La Vecchia et al. 2001; Riboli et al. 2003; Gandini et al. 2001; Cottet et al. 2009). The combined study analysis of the Italian breast cancer studies by La Vecchia et al. (2001) and a meta-analysis by Gandini et al. (2001) of 23 studies concluded that increased vegetable consumption reduced breast cancer risk by 20 to 25%. Furthermore, Cottet et al. (2009) utilized the EPIC cohort to investigate the association between dietary pattern and risk of postmenopausal invasive breast cancer (n=2, 381). This study compared the Mediterranean diet of the Italians (consists mostly of fruits, raw vegetables, cooked vegetables, crustaceans, fish, olive oil and sunflower oil) to a more westernized diet (consists of alcohol, meat and processed foods). Two dietary patterns were observed to be associated with postmenopausal breast cancer risk: (1) higher risk of breast cancer was associated with western-type foods and alcohol; and (2) reduced risk of breast cancer was associated with Mediterranean pattern diets that were high in fruits, vegetables, fish, and

olive and sunflower oils (Cottet et al. 2009). Overall, this study suggests that the risk of postmenopausal breast cancer in women may be impacted by dietary habits, particularly following a Mediterranean-type diet may reduce risk (Cottet et al. 2009). These studies have helped garner interest on the role of specific types of vegetables that may be even more influential on breast cancer than just vegetables as a whole, particularly *Brassica* vegetables.

Brassica vegetables, like broccoli, cabbage, Brussels sprouts, cauliflower, kale, turnips and collards, contain a number of nutrients and phytochemicals that have cancer chemopreventive properties such as glucosinolates (which are converted to isothiocyanates and indoles), folate, fiber, carotenoids, vitamin C and chlorophyll (Higdon et al. 2007; Kim et al. 2009). An extensive review of epidemiologic studies published prior to 1996 by Higdon et al. (2007) reported that 67% of a total of 87 case-control studies found an inverse association between cancer risk and some type of *Brassica* vegetable; in particular, results were consistent among lung and stomach cancer. For breast cancer, the Higdon et al. paper reported that three studies found inverse associations between *Brassica* vegetables and breast cancer, while a pooled analysis of seven large prospective cohort studies found no association. Therefore, researching the link between *Brassica* vegetables and breast cancer risk is critical (Kim et al. 2009).

It is well-established that many risk factors for breast cancer are related to estrogen or reproductive factors. Two estrogen metabolites of particular interest are 2-hydroxyestrone (2-OHE) and 16α-hydroxyestrone (16α-OHE) which are often reported together as a ratio of 2:16α-hydroxyestrone ratio (2:16α-OHE) (Bradlow et al. 1994; Lord et al. 2002; Nguyen et al. 2010; Laidlaw et al. 2010). A higher incidence of disease is

associated with high levels of 16α-OHE and low levels of 2-OHE in the body in some studies (Matthews et al. 2004; Musey et al. 1987), but not all (Obi et al. 2011; Lissowska et al. 2008; Ursin et al. 1999; Cauley et al. 2003; Wellejus et al. 2005). The 16α-OHE estrogen metabolite has high estrogenic activity, which has been found to enhance cell proliferation in estrogen sensitive breast cancers; whereas 2-OHE has antiestrogenic properties (Obi et al. 2011; Higdon et al. 2009; Lord et al. 2002). It has been demonstrated in several studies that postmenopausal women who are at a higher risk of breast cancer tend to have significantly increased levels of 16α-OHE or decreased levels of 2-OHE levels in the body (Kabat et al. 1997; Lord et al. 2002; Coker et al. 1997; Ho et al. 1998). The hydroxylation of one of these metabolites has been suggested to lower the concentration of the other one because the precursor is the same between them (Bradlow et al. 1996; Falk et al. 2000). In general, studies have shown that the estrogen metabolism pathways that favor 2-OHE over 16α-OHE are associated with reduced risk of invasive breast cancer (Coker et al. 1997; Muti et al. 2000; Taioli et al. 1996; Sowers et al. 2006; Im et al. 2009; Kabat et al. 2006; Fowke et al. 2003; Meilahn et al. 1998).

The enzyme CYP1A1 catalyzes the conversion of estrone to 2-OHE; while the conversion of estrone to 16α-OHE is triggered by the enzyme CYP1B1 (Lord et al. 2002) (Figure 1.1). Studies have shown that the phytochemical indole-3-carbinol (I3C), found in *Brassica* vegetables (e.g., cabbage, broccoli, kale, turnips, collards, cauliflower, and Brussels sprouts), impacts the conversion of 2-OHE, known as "good estrogen", by inducing CYP1A1 (Lord et al. 2002; Nguyen et al. 2010; Laidlaw et al. 2010). The major in vivo product of I3C is 3, 3' diindolylmethane (DIM; 1,2); it is thought that DIM is the major mediator of chemopreventive and chemotherapeutic effects of I3C (Dalessandri et

al. 2004). Both I3C and DIM can induce the enzyme which converts estrone to 2-OHE at the expense of 16α-OHE (Dalessandri et al. 2004). A few studies have significantly shown an increase in 2-OHE urinary metabolite levels after consumption of I3C (Nguyen et al. 2010; Laidlaw el al. 2010; Marconett et al. 2010). Other studies have demonstrated that I3C is an inhibitor of human breast cancer cell proliferation (Nguyen et al. 2010; Laidlaw el al. 2010; Marconett et al. 2010).

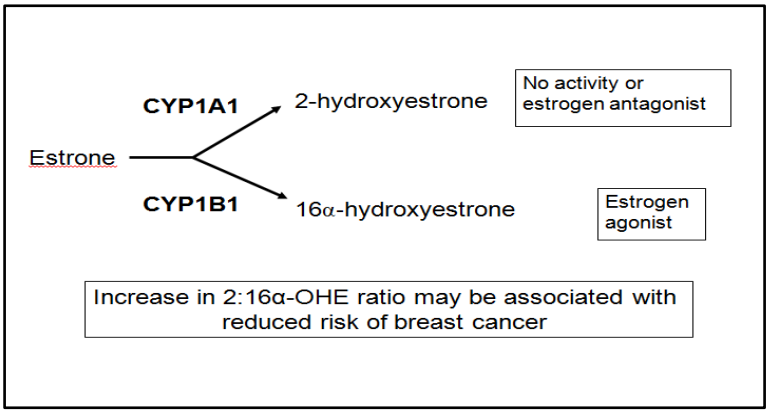

Figure 1.1 Estrone Metabolism Pathway

Research on the effects of *Brassica* vegetable consumption on the 2:16α-OHE ratio in randomized clinical settings is still sparse. Kall et al. (1997) found that the average 2:16α-OHE ratio significantly increased by 29.5% ($p<0.05$) after a group of 18 volunteers increased their consumption of broccoli to 500g/day for 12 days. Though this was a very short experimental study with no control group, the results support the idea that *Brassica* vegetables may potentially increase the 2:16α-OHE ratio. Furthermore, a randomized controlled trial by Fowke et al. (2000) found that a 20-fold increase in grams of *Brassica* vegetables consumed among 34 healthy postmenopausal women significantly increased their urinary 2:16α-OHE ratios. However, the effects of *Brassica* vegetables

on the 2:16α-OHE ratio among AA women and breast cancer survivors has not been thoroughly examined.

Proposal and Specific Aims

Using a previously-conducted randomized controlled trial in South Carolina, I hypothesized that an increase in *Brassica* vegetable consumption would alter the 2:16α-OHE estrogen metabolite ratio among postmenopausal women, by increasing 2-hydroxyestrone (2-OHE) and decreasing 16α-hydroxyestrone (16α-OHE).

The specific aims are as follows:

Aim 1: To evaluate the effect of the *Brassica* intervention on the 2:16α-OHE ratio, specifically to examine whether women in the *Brassica* intervention arm experienced an increase in 2-OHE or in the ratio of 2:16α-OHE compared to the women in the control (non-intervention) arm.

Aim 2: To evaluate the effect of the intervention on the 2:16α-OHE ratio by baseline dietary intake, examining whether diet before the *Brassica* intervention began impacted the results of the intervention.

Aim 3: To evaluate if the effect of the intervention on 2:16α-OHE ratio differs by race or breast cancer status, by comparing the mean effect of the intervention among EA and AA women and among breast cancer cases and healthy women enrolled in the study.

Significance and Research

Approximately one-third of all cancer cases are related to dietary influences (Lord et al. 2002). Numerous studies have shown that breast cancer risk increases with increasing exposure to estrogen in the body, especially by increased levels of 16α-OHE

in the presence of low levels of 2-OHE (Lord et al. 2002; Coker et al. 1997; Muti et al. 2000; Taioli et al. 1996; Sowers et al. 2006). Furthermore, the 2:16α-OHE is perhaps modifiable by diet and lifestyle interventions (Lord et al. 2002). There is growing evidence that favors the modification of the 2:16α-OHE ratio by increasing *Brassica* vegetable consumption. It is vital for epidemiologist to further understand this biological mechanism between estrogen metabolites and *Brassica* vegetable consumption because it could lead to a possible preventive measure to reduce the risk of breast cancer among women as well as the risk of other comorbidities.

It is also important to address the health disparity that exists between EA and AA women in terms of breast cancer mortality. It has been observed that AA women tend to have lower 2:16α-OHE estrogen metabolite ratio levels than EA women (Sowers et al. 2006; Lord et al. 2002; Matthews et al. 2004; Musey et al. 1987). This is likely due to both SES and biological factors; however, if *Brassica* interventions can potentially be a way to reduce breast cancer among all races, then breast cancer mortality will drop among AA women as well.

The present study utilized data from a previously-conducted randomized controlled trial in South Carolina among postmenopausal women. *Brassica* vegetables have been linked to lowering the risk of other types of cancers, like lung and colorectal cancer in some epidemiological studies; therefore, the research on this topic of *Brassica* vegetables and breast cancer risk is critical (Kim et al. 2009). The 2:16α-OHE ratio can be potentially modified through lifestyle factors like diet. A study such as this could help in understanding the biological mechanism of the link between *Brassica* vegetables and

breast cancer risk. Subsequently, this may help to inform future interventions designed to reduce the risk of breast cancer.

CHAPTER II

LITERATURE REVIEW

PART I: *Brassica* Vegetables and Breast Cancer

It has been estimated that one-third of all cancer cases are related to dietary influence (Lord et al. 2002). Over the last several decades a number of epidemiological studies have observed an inverse association between *Brassica* vegetable consumption and cancer, in particular breast cancer (Lord et al. 2002; Kim et al. 2009). *Brassica* vegetables are rich sources of glucosinolates (precursors to isothiocyanates and I3C), carotenoids, vitamin C, folate and soluble fiber, which may potentially play an important factor in cancer prevention (Kim et al. 2009); however, epidemiologic studies on *Brassica* vegetable consumption and breast cancer are inconsistent. Case-control studies have reported significant inverse associations between the risk of breast cancer and *Brassica* vegetable consumption over the last decade or so (Ambrosone et al. 2004; Terry et al. 2001; Fowke et al. 2003; Riboli et al. 2003; Gaudet et al. 2004); whereas, prospective cohorts have yielded inconsistent results with the association being weak or non-existent (Boggs et al. 2010; Masala et al. 2012; Cottet et al. 2009; Smith-Warner et al. 2001; Van Gils et al. 2005; Agurs-Collins et al. 2009).

In the Long Island Breast Cancer Study Project case-control study (1,463 cases and 1,500 controls), breast cancer risk decreased by more than one-third in the highest quintile of any vegetables [OR, 0.63; 95%CI, 0.48-0.86] or leafy vegetables [OR, 0.66;

95%CI, 0.50-0.85] intake when compared with women in the lowest quintile; furthermore, a 34% reduced risk was observed with an intake of nine or more 0.5 cup servings/week of leafy vegetables among postmenopausal women (Gaudet et al. 2004). They also found a weaker inverse association between *Brassica* vegetables and breast cancer risk in postmenopausal women [OR, 0.80; 95%CI, 0.60-1.05] (Gaudet et al. 2004).

Three other case-control studies by Ambrosone et al. (2004), Fowke et al. (2003), and Terry et al. (2001) also found an inverse association between *Brassica* vegetable intake and breast cancer risk. The Ambrosone et al. (2004) paper reported results from the Western New York Diet Study which was conducted among EA pre and postmenopausal women. They found a marginal inverse association between *Brassica* vegetable consumption, especially broccoli, and breast cancer risk among premenopausal women but only a weak or obsolete association among postmenopausal women (Ambrosone et al. 2004). Fowke et al. (2003) further demonstrated this among Chinese women in Shanghai, China by observing that greater *Brassica* vegetable consumption, measured by the urinary ITC biomarker, was associated with significantly reduced breast cancer risk. In postmenopausal Chinese women, there was a marginally significant protective trend attributed to the highest quintile of self-reported *Brassica* consumption and breast cancer (Crude OR, 0.40; 95% CI, 0.2-1.0) (Fowke et al. 2003). The Swedish Study by Terry et al. (2001) was only conducted among postmenopausal women and found that the upper decile (10%) of *Brassica* vegetable consumption was associated with a 40% lower risk of breast cancer. The relative risk (RR) among women in the highest quintile compared to the lowest quintile of *Brassica* vegetable consumption, median

serving of 1.5 per day, was an RR of 0.58 (95% CI, 0.42-0.79) (Terry et al. 2001). In contrast, a case-control study by Lissowska et al. (2008) found little or no protective effect of *Brassica* vegetables on breast cancer risk.

Until recently, prospective cohort studies have yielded inconsistent results between the association of *Brassica* vegetable consumption and breast cancer risk (Smith-Warner et al. 2001; Van Gils et al. 2005; Agurs-Collins et al. 2009). Three prospective cohorts of major interest are the Black Women's Health Study (Boggs et al. 2010), and two papers published on the European Prospective Investigation into Cancer and Nutrition (EPIC) (Masala et al. 2012; Cottet et al. 2009). The Black Women's Health Study consisted of 51,928 AA women aged 21 to 69 years of age (Boggs et al. 2010). They found that AA women who consumed more fruits or vegetables were more likely to be older, to live in the northeastern and western regions of the United States, be physically active, nonsmokers, and take multivitamins (Boggs et al. 2010). Boggs et al. (2010) also found that education level was positively associated with vegetable consumption. This study (Boggs et al. 2010) found an inverse association between breast cancer risk and *Brassica* vegetable intake; incidence ratios for 1-2, 3-5, and ≥6 servings per week compared with <1 serving per week were 0.94 (95% CI: 0.80- 1.11), 1.01 (95% CI: 0.84-1.21), and 0.80 (95% CI: 0.65-0.99), respectively. In premenopausal women, this association was stronger when comparing ≥6 servings per week compared with <1 serving per week (IRR, 0.59; 95% CI: 0.42-0.83); conversely, there was no evidence of an association among postmenopausal women (Boggs et al. 2010).

The EPIC study in Italy (Masala et al. 2012) was a large prospective cohort study of 31,000 women aged 36 to 64 years of age. A large variety of vegetables and fruit are

consumed by Mediterranean populations, so this made Italy a favorable setting to evaluate the association of these foods and breast cancer risk. Overall, an inverse association between the consumption of total vegetables and breast cancer risk emerged; in particular, they observed an inverse association in the increased intake of leafy vegetables (e.g., salad greens, chard, spinach, and other leafy greens) (highest vs. lowest quintile HR, 0.70; 95%CI, 0.57-0.86), fruiting vegetables (peppers, artichokes, aubergin, courgette, green beans, fennel, celery) and raw tomatoes (Masala et al. 2012). However, there was no evidence of an inverse association between cabbages (broccoli, brussel sprouts, cauliflower, black cabbage and savoy cabbage) and breast cancer risk when comparing the highest vs. lowest quintile (HR, 0.91; 95% CI: 0.74-1.12) (Masala et al. 2012). Thus, while generally supportive of in inverse association between *Brassica* vegetables and breast cancer, the epidemiologic literature has not always yielded strong, consistent associations. Null findings could be related to a narrow range in intake of *Brassica* vegetables in the populations that were studied (Hebert et al. 1991) or measurement error from the dietary assessment instrument used (usually food frequency questionnaires with known measurement error) (Schatzkin et al. 2003).

PART II: Breast Cancer and 2:16α-OHE

In general, studies have shown that the estrogen metabolism pathways that favor 2-OHE metabolites over 16α-OHE metabolites are associated with the reduced risk of developing invasive breast cancer, especially in postmenopausal women (Schneider et al. 1982; Coker et al. 1997; Muti et al. 2000; Taioli et al. 1996; Meilahn et al. 1998; Fowke et al. 2003; Im et al. 2009); however, there have also been a few papers that have found no association between breast cancer risk and the 2:16α-OHE ratio (Lissowska et al.

2008; Obi et al. 2011; Arslan et al. 2009; Ursin et al. 1999; Ursin et al. 1997; Cauley et al. 2003). In some studies, the ratio was found to be significantly lower in AA women than EA women (Sowers et al. 2006; Taioli et al. 1996; Coker et al. 1997). In a case-control study (74 cases, 58 controls) conducted among EA and AA pre and postmenopausal women by Coker et al. (1997), it was found that AA women had significantly lower 2-OHE (adjusted for creatinine) levels than EA; though not significant, AA women also had lower mean levels of 2:16α-OHE ratios than EA. These findings are consistent with the findings of a similar study conducted by Taioli et al. (1996), and are hypothesized to partially explain the racial disparities present in breast cancer mortality between AA and EA women.

The first study to demonstrate a link between 2:16α-OHE ratio and breast cancer risk was published in 1982 by Schneider et al. In a comparison of serum estradiol metabolites in 33 women diagnosed with breast cancer to 10 healthy women, they found that the serum 16α-OHE levels of breast cancer patients were 50% higher than the controls; 2-OHE levels were similar between the two groups (Schneider et al. 1982). Since then, other studies have gone on to demonstrate that the 2:16α-OHE ratio is significantly lower in breast cancer cases compared to controls in premenopausal women (Muti 2000), postmenopausal women (Zheng 1998; Kabat 1997; Meilahn et al. 1998) or both (Ho 1998; Kabat et al. 2006; Fowke et al. 2003).

In a nested case-control study of 676 women conducted by Muti et al. in Italy (2000), premenopausal women had a 42% decrease in breast cancer risk for the highest quintile of 2:16α-OHE ratio compared to the lowest quintile (adjusted OR, 0.58; 95% CI:

0.25-1.34). There was no association among postmenopausal women when comparing the highest vs. lowest quintile (adjusted OR, 1.29; 95% CI: 0.53-3.10) (Muti et al. 2000).

In a large cohort study of 5,104 pre and postmenopausal women by Meilahn et al. (1998), women in the highest tertile of the urinary 2:16α-OHE ratio were 30% less likely to develop breast cancer over follow-up periods of up to nine years. These results were not consistent with two case-control studies conducted by Ursin et al. (1997 and 1999) that showed no significant relationship between the 2:16α-OHE ratio and breast cancer risk among postmenopausal women; however, this could be because the sample sizes of both of these studies were not large enough to yield statistical significance (142 postmenopausal women in the 1999 study and 48 postmenopausal women in the 1997 study) (Ursin et al. 1997; Ursin et al. 1999).

Both Kabat et al. (2006) and Fowke et al. (2003) demonstrated through case-control studies that 2:16α-OHE ratios are associated with breast cancer risk in both pre and postmenopausal women. Kabat et al. (2006) used the Long Island Breast Cancer Study Project to assess the association of the urinary estrogen metabolites 2-OHE, 16α-OHE, and their ratio, among 269 invasive breast cancer cases, 158 in situ breast cancer cases and 326 controls. They found that 2:16α-OHE was inversely associated with breast cancer risk among premenopausal women and found a weaker reduction in risk among postmenopausal women; (Kabat et al. 2006). These results were similar to what Fowke et al. (2003) found in a case-control among Chinese women living in Shanghai (n=220); however, they also compared pre versus post treatment urine collection among the breast cancer patients. Subjects with higher 2:16α-OHE ratios were less likely to be diagnosed with breast cancer, but only when the urine sample was collected prior to any breast

cancer treatment (Fowke et al. 2003). Conversely, breast cancer cases among subjects whose urine was collected after treatment yielded significantly higher 2:16α-OHE ratios (Fowke et al. 2003). The cross-study differences that Fowke et al. (2003) found in the urine collection time periods may help to explain the inconsistencies that are observed in the association between breast cancer risk and 2:16α-OHE ratio.

A more recent study by Im et al. (2009) explores whether average urinary estrogen metabolites among breast cancer "high-risk women" could be a predictor of future breast cancer development; furthermore, they also addressed whether urinary 2:16α-OHE ratio may be linked to specific epidemiologic risk factors. The study population consisted of 77 high-risk women, 30 breast cancer patients and 41 controls (Im et al. 2009). The median 2:16α-OHE ratios differed significantly among the groups, with the high-risk (1.76±2.33) and breast cancer (1.29±0.80) group ratios both being significantly lower when compared to controls (2.47±1.14; P=0.0001); while no significant difference was observed between the high-risk and breast cancer group (Im et. al. 2009). The same pattern of median 2:16α-OHE ratios was observed among postmenopausal women, with the controls having higher median levels than both high-risk and breast cancer group; however, the differences were non-significant (Im et al. 2009). Moreover, there was a significant association observed between 2:16α-OHE ratio and alcohol use in the groups; there was no association observed between smoking or age and 2:16α-OHE ratio (Im et al. 2009). Overall, this study shows that 2:16α-OHE ratios have been found to be significantly lower in women at high-risk for developing breast cancer when compared to healthy women, suggesting that lower 2:16α-OHE ratios may be used as biomarkers and prognostic indicator in high-risk populations. Additionally,

further understanding of the urinary estrogen metabolite ratios may help lead to new breakthroughs in terms of risk reduction strategies.

PART III: *Brassica* Vegetables and 2:16α-OHE

Estrogen is metabolized along two competing pathways which are 2-OHE and 16α-OHE urinary metabolites. *Brassica* vegetables play an important role in this overall mechanism by modulating the activity of cellular enzymes that are responsible for the production of these estrogen metabolites (Maizes 2005). Indole-3-carbionol (I3C) is a dietary indole found in *Brassica* vegetables (e.g. cabbage, broccoli, kale, turnips, collards, cauliflower, and Brussels sprouts); studies have demonstrated that I3C directly induces the production of 2-OHE, known as "good estrogen" (Lord et al. 2002; Nguyen et al. 2010; Laidlaw et al. 2010; Dalessandri et al. 2004). Furthermore, the major in vivo product of I3C is 3,3'–diindolylmethane (DIM) which is thought to be the major mediator of chemopreventive and chemotherapeutic effects of I3C (Dalessandri et al. 2004; Kelloff 2000). I3C and DIM are responsible for the metabolism of estrone to form the estrogen metabolite 2-OHE (estrogen receptor antagonist) at the expense of 16α-OHE (estrogen receptor agonist) (Dalessandri et al. 2004). The enzymes responsible for the conversion of estrone to either 2-OHE or 16α-OHE are cytochrome P450 (CYPs) (Dalessandri et al. 2004). In particular, CYP1A1 favors the metabolic pathway of 2-OHE, which is considered protective (Schneider 1984; Fowke 2000; Wong 1997; Dalessandri et al. 2004); conversely, the production of 16α-OHE can lead to the stimulation of cell proliferation (Schneider et al. 1982; Kabat 1997; Dalessandri et al. 2004).

Two studies by Bradlow et al. (1994) and Laidlaw et al. (2010) examined the role of I3C on the excretion of 2-OHE and found a significant increase in the 2-OHE urinary

metabolite levels. Bradlow et al. (1994) conducted a randomized clinical trial among 60 women who were recruited from a high-risk registry of the Strang Cancer Prevention Center. The women were randomized into one of 3 arms: 400mg/day of I3C, 20g/day of α-cellulose, or placebo arm (Bradlow et al. 1994). From baseline to 3-months, there was a significant change in the mean 2:16α-OHE (0.72 to 1.19, respectively) in the arm that received 400mg/day of I3C (Bradlow et al. 1994). Within this same I3C arm, there were 3 women who had no change in these ratios over the course of the study (2 had very low metabolite ratios to begin with and one had a large ratio to begin with); these women may need larger doses of I3C to induce a response (Bradlow et al. 1994). Overall, Bradlow et al. (1994) demonstrated that I3C can directly increase the 2:16α-OHE ratio. Laidlaw et al. (2010) demonstrated similar results in a double-blind, placebo-controlled, parallel study among 98 pre and postmenopausal women who were placed into one of two arms. One arm consisted of premenopausal women not using hormonal contraceptives, with the other arm consisting of postmenopausal women not receiving hormone replacement therapy (HRT); each arm was then randomized to receive either the placebo (supplement consisting of microcrystalline cellulose) or treatment (supplements contained 200 mg I3C and 10 mg HMR lignans) for a period of 28 day (Laidlaw et al. 2010). In premenopausal women, the treatment group resulted in a significant increase in the urinary 2-OHE concentrations (6.34 to 13.29; P=0.003) and in the 2:16α-OHE ratio (0.88 to 1.66; P=0.016) (Laidlaw et al. 2010). Among postmenopausal women, the treatment group only showed a significant increase in the urinary 2-OHE concentration (9.22 to 17.37; P=0.035) but no significant change in the 2:16α-OHE ratio (4.54 to 5.40; P=.387) (Laidlaw et al. 2010). The placebo group biomarkers in both pre and postmenopausal

women exhibited no significant change in mean concentration levels (Laidlaw et al. 2010).

In contrast to dosing with I3C directly, there have been several studies that have demonstrated that *Brassica* vegetable intake can modify the 2:16α-OHE ratio (Kall et al. 1997; Fowke et al. 2000; Morrison et al. 2009). Kall et al. (1997) found that the average 2:16α-OHE ratio significantly increased by 29.5% ($p<0.05$) after a group of 18 volunteers increased their consumption of broccoli to 500g/day for 12 days. Though this was a very short experimental study with no control group, the results support the idea that *Brassica* vegetables can increase the 2:16α-OHE ratio. There have been more recent randomized clinical trials that have shown these same consistent results but over a longer period of time (Morrison et al. 2009; Fowke et al. 2000). In an intervention study by Morrison et al. (2009), 12 out of the 13 premenopausal women had a baseline 2:16α-OHE ratio that was below the recommended 2.0 cutoff; however, 11 of the 13 women showed positive increases in their ratios with three of the subjects who had the lowest baseline ratios having an average increase of 500% at the completion of the 3-month study (added 3.6 g of powdered organic Brussels sprouts and organic kale to diet) (Morrison et al. 2009). The overall average 2:16α-OHE ratio improvement was 168%, with the increase being due to increase levels of 2-OHE in 11 of the subjects and in 9 subjects due to the decrease in the 16α-OHE excretion levels (Morrison et al. 2009). A paired sample t-test analysis comparing the initial and final 2:16α-OHE ratios for all 13 subjects showed a significant increase in the mean values over the course of the study period (P=0.01) (Morrison et al. 2009).

Fowke et al. (2000) demonstrated similar results with an intensive intervention design over a 4-week period to increase *Brassica* vegetable consumption to at least 70mg/day (Fowke et al. 2000). At baseline, *Brassica* vegetable consumption on average was only about 9g/day with 2:16α-OHE ratio at 2.27 and after the intervention these, respectively, were 193g/day and 2.38, which was about a 20-fold increase in grams of *Brassica* vegetable consumption among these women (Fowke et al. 2000). In the crude analysis, there was no significant increase in the urinary 2:16α-OHE ratio associated with increased consumption of *Brassica* vegetables; however, when adjustment for other dietary parameters were applied, *Brassica* vegetable consumption was associated with a statistically significant increase in the 2:16α-OHE ratio, in that each 10g/day increase in *Brassica* vegetable consumption led to an increase of about 0.08 (95% CI, 0.02-0.15) in the 2:16α-OHE ratio (Fowke et al. 2000). It was also noted, that the 2:16α-OHE ratio shift appeared to be sensitive to the specific type of vegetables that were consumed during the intervention (Fowke et al. 2000). When multivariable model analysis was performed on the amount of broccoli, cabbage, Brussels sprouts, or other *Brassica* vegetable simultaneously, an increase of 10g of cabbage was found to be associated with an increase of 0.07 (95% CI, -0.04-0.19) in the urinary 2:16α-OHE levels, whereas an increase of 10g of broccoli was associated with only an increase in 0.01 (95% CI, -0.09-0.11) (Fowke et al. 2000). *Brassica* vegetables that were lightly cooked or raw appeared to cause an equal shift in the 2:16α-OHE ratio levels for each 10g/day of vegetables (cooked: 0.03; 95% CI, 0.03-0.10 and raw: 0.03; 95% CI, -0.08-0.13) (Fowke et al. 2000). In conclusion, a shift of 0.08 in the 2:16α-OHE ratio for every 10g/day of *Brassica* suggests that the population would need to increase *Brassica* vegetable

consumption between 12.5g/day to 75g/day in order to cause a direct, favorable shift in movement of the 2:16α-OHE ratio levels to affect the causal mechanism leading to breast cancer (Fowke et al. 2000).

CHAPTER III

MATERIALS AND METHODS

Study Population and Setting

Postmenopausal women who resided in the standard metropolitan statistical area (SMSA) around the Columbia, South Carolina area were recruited between April 2002 and September 2003. Through advertisement, a total of 138 women were recruited through a telephone screening interview from two breast care clinics under Palmetto Health (the major health care provider in the area) and from a local television and newspaper study briefing. Women were considered eligible if they were postmenopausal (defined as >1 year since last menstrual cycle), willing to be randomized, willing to provide post-intervention urine samples, were not on any special diet or weight-loss program, and had not lost over five pounds in the past year. Women were considered to be ineligible if they were not on a stable, fixed dose of medications [including hormone replacement therapy (HRT)], were diagnosed with a malignancy (other than melanoma or breast cancer) in the past five years, were on thyroid medications, had a health condition that would limit their participation, or if they consumed over 2 alcohol-containing drinks per day. Due to the older age of the sample population it was not possible to exclude women who were using diabetic medications, using antibiotics, or diuretics. Instead, women were asked to consistently ingest any medications on a stable, fixed dose during the full nine-week study period.

From the eligible sample of women, 109 women (79.0%) met with study personnel to review the study procedures, provide a signed informed consent and complete all study questionnaires, anthropometric measurements and biological samples that are described below. During the baseline clinic visit, 84 of the eligible women (77.1%) completed the baseline questionnaire. After the second clinic visit, 65 women had provided urine samples from both the first and second clinic visit. In total, 14 eligible women withdrew before ever attending the first clinic, 28 women dropped after the first clinic, 2 women had incomplete baseline questionnaires, and one woman failed to have a 24-hour dietary recall performed. Therefore, the study population that was used for analysis consisted of 64 women who attended both clinic visits with complete urine, dietary data, and baseline information (Figure 3.1)

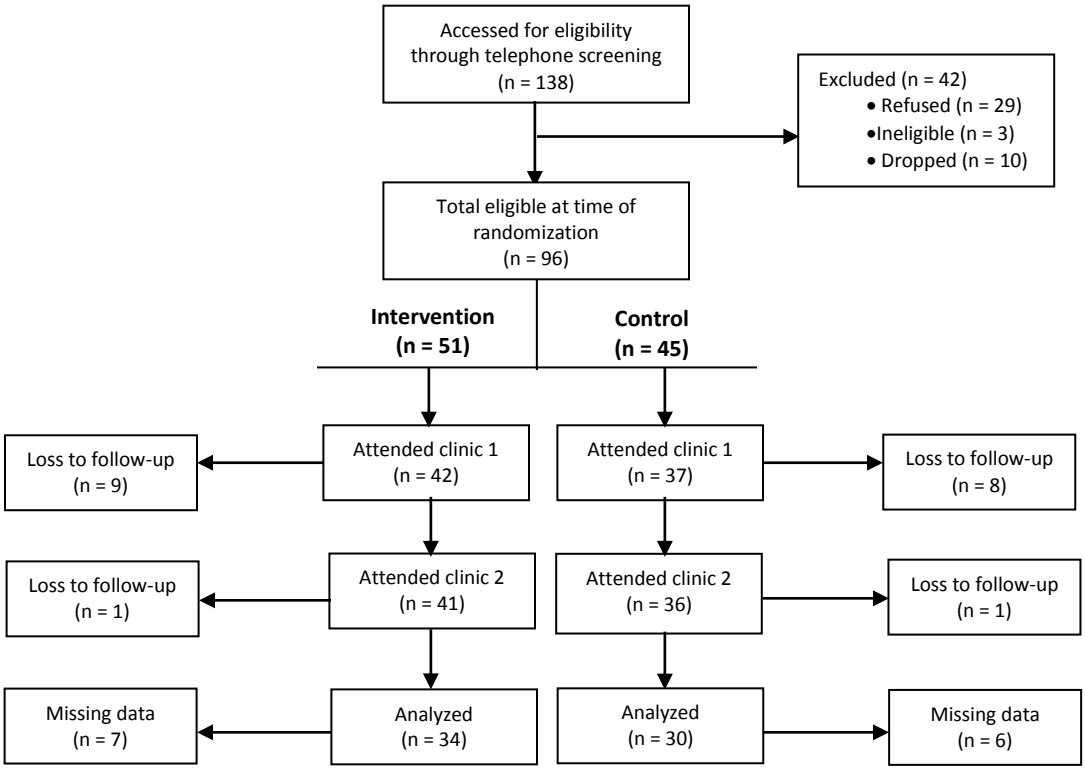

Figure 3.1: Consort Diagram of Study Participants

Study Procedures & Sample Collection

The study protocol was approved by USC and Palmetto Health institutional review boards. A written, signed informed consent was obtained during the initial screening visit from all of the women who were considered eligible to participate in the study.

Baseline, Body and Biological Sample Collection

A baseline questionnaire assessing demographics, general smoking and alcohol exposure, reproductive health history, and past week physical activity was completed at the first clinical visit. Furthermore, at both clinics anthropometric data (including height, weight, body circumference) and biological samples were collected (Figure 3.2). Waist

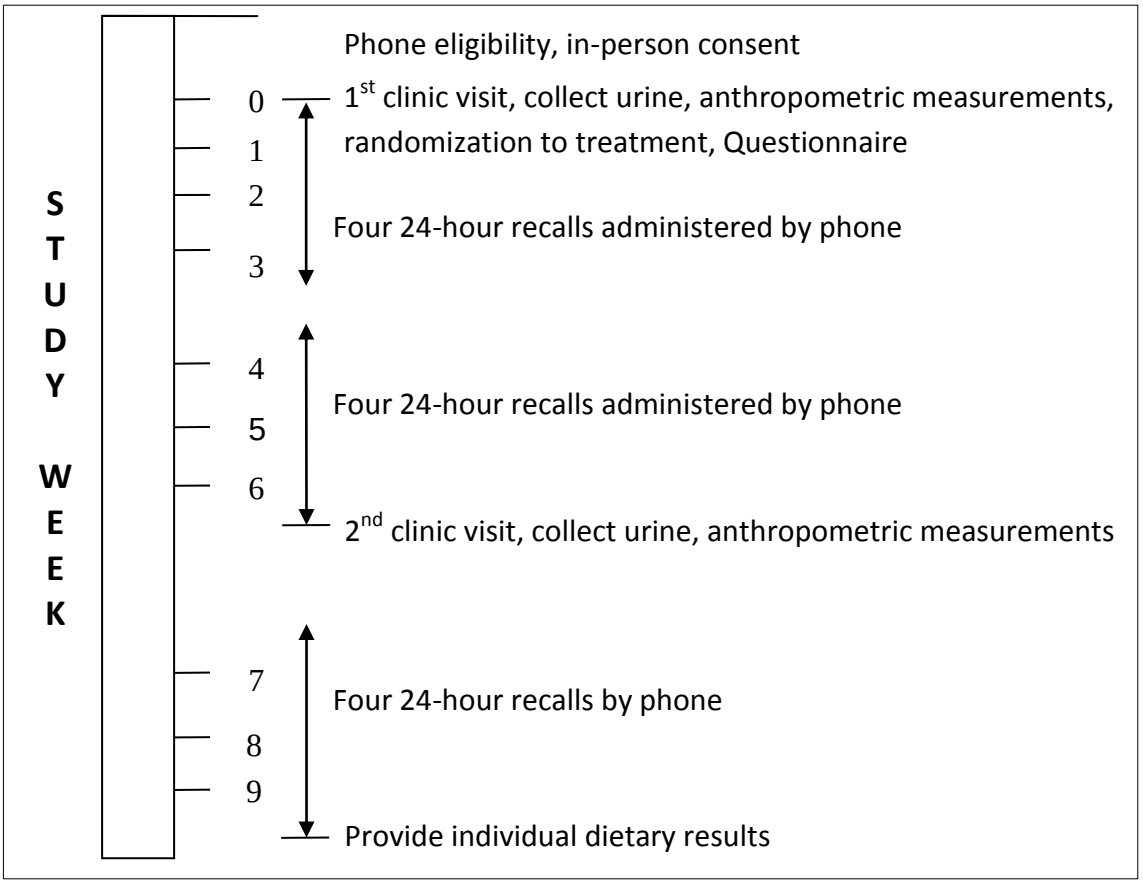

Figure 3.2: Overview of Study Design

circumference was measured by a tape measure that was held tightly in a horizontal position at the lower rib margin after the participant removed any heavy outerwear. Similarity, abdominal circumference was taken midway between the lower rib and iliac crest, and hip circumference was measured around the widest part of the hips. Percent body fat was measured using a bioelectrical impendence analyzer (Quantum II Model, RJL Systems, Clinton Twp., MI). Total body resistance and reactance were recorded to the nearest ohm by study personnel to calculate the resistance index which is defined as the height^2 divided by the total body resistance.

An overnight fasting urine sample was provided by participants in cups that contained a preservative, ascorbic acid as 125mg to 100ml of urine, to prevent oxidation. It was unnecessary to perform 24-hour urine collections because previous data has shown that 24-hour average expressed per milligram expressed creatinine was not significantly different from first-morning expressed creatinine values. This alleviated participant burden as well. The urine was placed on ice (4°C) and then transferred to the lab to be immediately centrifuged. Within six hours of the urine collection, the samples were aliquoted into 1ml cryovials and placed into long-term storage at -80°C.

Randomization

The women were randomized into one of two arms, non-intervention (control) group or intervention group during the first clinic visit by using a fixed randomization scheme with a block size of four. We blocked on the day of clinic and the small block size was meant to reflect the average number of women who actually attended the first clinic visit on the same day. The women randomized into the control arm were asked to maintain their usual dietary habits for the duration of the study period. After completion

of the second clinic, those in control arm were invited to attend the cooking classes and receive all the intervention materials.

Intervention

Participants randomized into the dietary intervention arm were offered nine classes that were led by trained study dietitians over a three-week intervention period. The study protocol required that the participants had to attend between six to nine classes. During these classes, the registered dietitian explained study goals, scientific rationale, provided a recipe book, and administered cooking classes. The women were advised to consume an ample number of *Brassica* vegetables daily at home. A point system was put in place to assign a greater number of points to less commonly consumed *Brassica* vegetables in hopes of encouraging women to try new vegetables that belonged to the *Brassica* family. Women were asked to attain ten points. An ample amount of fresh, locally grown *Brassica* vegetables were given to study participants each week.

The primary objective of the cooking classes was to encourage women to increase their *Brassica* vegetable consumption as part of a healthy diet. In order to help participants overcome the challenge of changing their eating habits, women were shown how to incorporate more *Brassica* vegetables into their diet through cooking demonstrations that were conducted in each class and gave participants the opportunity to prepare and sample recipes that called for *Brassica* vegetables as a main ingredient. Each cooking demonstration was provided in a way to highlight a variety of cooking techniques to help participants add variety to their meals and help increase compliance to the diet. The majority of the meals prepared during the demonstrations included vegetables in the form of slaws, salads, stir-fries and dips to encourage the eating of raw

or lightly cooked vegetables, in order to provide the most nutrients to them as possible. To maintain uniformity, the dietitians were instructed in pre-class preparation, class activities, suggestions for cooking demonstrations, taste-testing, and discussion of facilitation. In order to encourage class participation and attendance, the intervention classes were structured in a way to provide instructions on increasing *Brassica* vegetable consumption as well as incorporate other healthy eating principles into the intervention. Participants were provided with written instructions as well as discussed in class how to read food labels, increase fiber, decrease fat, incorporate alternate foods (e.g., soy products), use herbs and spices, the importance of minerals and vitamins, and how to modify recipes. Each principle overall was tied into the message of increasing *Brassica* vegetable consumption. For example, participants discussed how they could modify some of their favorite recipes with inclusion of or increase in *Brassica* vegetables. The strategies used to alter food patterns included eliminating or adding foods to the baseline diet, modifying or substituting foods, and overcoming potential barriers including structural changes i.e., food availability and preparation at home, modifying recipes, portion sizes. Women were not given any advice on how to reduce total food consumption or to count calories. The dietitians also provide individual level dietary counseling to increase the consumption of *Brassica* vegetables.

Dietary Assessment

Diet was assessed on all participants through four unannounced 24-hour dietary telephone recall interviews (24HR), two weekdays and two weekend days, during each of the three study periods for a total of twelve interviews by the end of the nine-week study period. The four recalls were respectively conducted in the pre-intervention period

(weeks 1-3), intervention period (weeks 4-6), and post-intervention period (weeks 7-9). These interviews were administered by trained registered dietitians using a structured interview protocol. The recalls were administered on non-consecutive days to obtain a representative sample of a participant's actual dietary intake. Additionally, two other methods were used to track *Brassica* vegetable consumption during the intervention period. Participants randomized to the intervention arm were given a food diary to maintain during the intervention; however, these diaries were not collected for data analysis purposes. To roughly assess the average servings of commonly consumed foods containing phytoestrogens, all participants were required to complete a brief fruit and vegetable questionnaire at both clinic visits. However, this questionnaire was not analyzed in this since the 24HR provides a more accurate depiction of foods consumed during the study.

The Nutrition Data System (NDS version 34) interactive software developed by the Nutrition Coordinating Center, University of Minnesota (Minneapolis, MN) was used to collect and analyze the data for nutrient intake (NCI Food Database and Nutrient Database version 33), (Feskanich et al. 1989). A two-dimensional food proportion poster helped participants visualize and estimate food portions consumed during the previous 24-hour period.

It is well-known that longer cooking may inactivate the enzyme myrosinase which is responsible for breaking down glucosinolates into their active forms (isothiocyanates, nitriles, thiocynates, indoles) and that these active forms will be absorbed into the cooking water because they are hydrophilic (Getahun et al. 1999). Therefore, we controlled for cooking method in analysis by dichotomizing the

preparation of each vegetable into either a raw or cooked form. The NCI food database items that listed the preparation method as "raw" (and given a value of "2") for data analyses including those classified as raw, blanched, or stir-fried. All other preparation methods (i.e., cooked, soup) were categorized as "cooked" (and given a value of "1"). Additionally, since both servings of *Brassica* vegetables and cooking methods were thought to be altered by the dietary intervention and would possibly affect estrogen metabolite levels, we combined these two variables by multiplying the vegetable serving and cooking method to obtain a single variable that reflected both serving and cooking methods.

Urine Collection and Assays

The analysis of the urinary estrogen biomarker was conducted using ELISA. The estrogen assays were conducted at the University of South Carolina Cancer Center laboratory by Dr. Xie, who was blinded to the identity and assignment of the participants. The assays were performed in duplicate batches of 30 to 40 samples in a random order over a one-week period using the ESTRAMET 2/16 enzyme immunoassay kit (Immunacare, Bethlehem, PA). To ensure quality control a sample of participants who enrolled in the study and provided a urine sample at only one clinic were included in each batch of analysis. Dr. Xie used these samples in order to monitor the consistency of lab values from batch to batch. The absorbance values from six standards that were included in the ELISA kit were used to derive a standard curve. In each batch, samples that had a coefficient of variation (CV) indicating a very dilute or concentrated sample were re-assayed with a more appropriate dilution.

The total urine output was not known (i.e., 24-hour urine samples were not collected); therefore, creatinine levels also were measured in order to adjust for differences due to urine concentration. The creatinine levels were measured using the Jaffe reaction with the Cayman Chemical Creatinine Kit (Ann Arbor, MI). The urinary estrogen metabolite levels in each sample were then standardized by dividing estrogen metabolite values (ng/ml) by creatinine levels (mg/dl). The urine samples were considered unavailable if women did not provide urine samples at both clinical visits or if urine sample dilutions were not in the linear portion of the standard curve even with repeated assays. Samples that yielded values that were extreme or on either the 2-OHE or 16α-OHE assays were examined as possible outliers during statistical analysis.

Definition of Variables

The primary exposure of interest was *Brassica* vegetable consumption which was measured as a continuous variable. This was defined by computing the mean and median of each *Brassica* vegetable serving size (broccoli, green or white cabbage, Brussels Sprouts, cauliflower, kale rutabaga, red cabbage, collard greens, savoy cabbage, Chinese cabbage, turnip greens, turnip, mustard greens, and radish) separately and then computing a total mean and median of them all combined. For analysis, the four 24HRs in each three-week cycle were averaged for each participant to provide the best estimate of *Brassica* vegetable consumption for each study period. The intervention and control arms were then analyzed to see if there was a mean difference between the primary outcomes of the 2-OHE, 16α-OHE or 2:16α-OHE.

For the second aim, the primary exposure of interest was baseline *Brassica* vegetable consumption (defined as mean *Brassica* vegetable consumption pre-

intervention), and women were stratified by baseline diet to examine whether the effect of the intervention on the 2:16α-OHE ratio differed by baseline intake of *Brassica* vegetables.

For the third aim, the primary outcome was the mean 2:16α-OHE ratio, and the effect of the intervention was examined stratified by race (defined as EA or AA) or breast cancer survivorship status [breast cancer compared to women without breast cancer].

Covariates

Demographic information was obtained from the baseline questionnaire. Self-reported race was defined as European American (EA) or African American (AA). Education level was defined as three categories: less than high school, high school completed and any college. Living status was defined as two categories: living alone or not living alone. Employment history was defined by three categories: yes full time, yes part time, and no. All other variable were dichotomous (yes/no): smoking exposure (defined as being exposed in the past week to cigarette, cigar, or pipe smoke in or outside of the workplace), alcohol exposure (defined as consumption of alcohol within the past week), ever been pregnant, physical activity (defined as have you played a sport or other physically active activity during the past week), and breast cancer survivorship status; or continuous: age, , current weight (in pounds), number of pregnancies, body mass index (BMI = weight (kg) / height $(m)^2$), and percent body fat.

Statistical Analysis

All analyses were performed using SAS statistical software version 9.3. Preliminary descriptive statistics of the study population was generated, including means, medians, and standard deviations for continuous variables, and frequencies for the

categorical variables. Data transformations were applied to variables as necessary to meet the requisite normality assumptions. Tests for statistical significance was assumed as a Type I error rate of 5% and be based on a two-sided test ($p<0.05$ was considered statistically significant).

Aim 1: We determined the dietary intake of *Brassica* vegetables and other dietary factors (e.g., energy, macronutrients, fiber) by computing the mean and median serving size or gram amount within each food group or nutrient from the 24HR-derived dietary data that was collected at each of three time points (pre-, during and post-intervention). Data for individual ingredients was collected for mixed dishes so that these foods could be added into the appropriate food group. Nutrients from supplements were also added to the dietary intake estimates. For analysis, the four 24HR in each three-week cycle was averaged for each participant to provide the best estimate of dietary intake for each time point. We determined the mean, median and standard deviation of the outcome variables (2-OHE, 16α-OHE and2:16α-OHE) at each time point (baseline and post-intervention), as well as the mean change between baseline and post-intervention. Paired t-tests were used to examine whether the change in 2:16α-OHE or the individual metabolites was significantly different from zero within each intervention arm. We used the SAS GLM procedure using post-intervention estrogen metabolite levels as the outcome and the intervention status as the exposure with adjustment for baseline estrogen metabolite levels.

Aim 2: To test for intervention effect differences between high and low consumers of *Brassica* vegetables at baseline, we used the SAS GLM procedure with the

2:16α-OHE ratio as the outcome and the intervention assignment as the exposure, using a by statement to stratify by baseline dietary *Brassica* intake.

Aim 3: Similarly, the model testing differences by race and survivorship used these as predictors rather than intervention assignment. We used the SAS GLM procedure with a by statement for race or breast cancer survivorship status to stratify by these variables.

These analyses examined potential confounders, including: age, anthropometric characteristics (i.e., BMI, percent body fat), race/ethnicity, breast cancer survivorship status, reproductive factors, smoking, alcohol, physical activity, fat intake and energy intake. These potential confounders are hypothesized to be related to both *Brassica* vegetable intake and the urinary estrogen metabolites.

Potential confounding variables and effect-modifiers were chosen from the literature and entered hierarchically into the proc GLM model; those with highest p-value were removed in a stepwise procedure. . Each possible demographic and dietary confounder was examined first for normality and transformed before entering into the multiple regression analyses if high skewness (absolute value > |0.3|) or high kurtosis (absolute value >|1.0|) were evident. Variables that were found to be significant predictors and related to both the predictor (*Brassica* vegetable intake) and outcome (estrogen metabolites) were added to the final model as confounders. Analyses were also repeated excluding outliers and confounders to examine differences in the model's predictive ability.

CHAPTER IV

RESULTS

Descriptive Characteristics of the Study Population

Descriptive characteristics of the study population at baseline are shown in Table 4.1. At baseline, there were no significant differences between the intervention and control arms in anthropometric, demographic, or clinical measures. Women were on average 61.0 ± 8.8 years of age, tended to be classified as overweight with an average BMI of 29.0 ± 5.6 kg/m^2 and an average percent body fat of 39.5 ± 5.6. The majority of the women had not been exposed to smoke within the past week (75.0%) and exercised regularly (89.4%). More than half of the women had consumed alcohol within the past week (64.6%). Most of the women were college educated (77.3%) and married (65.2%) with 41.5% employed full-time and 43.1% unemployed. AA women comprised 28.8% of the study population (8 control and 11 intervention participants). About 90% of the women had been pregnant at least once. About one-fourth (27.3%) of the women were breast cancer survivors

Table 4.1: Baseline Demographic Characteristics of Participants by Treatment Group (n = 66)

	Control (n= 31)	**Intervention (n = 35)**	**All Participants (n = 66)**	**p-value[1]**
Mean (SD)				
Age	61.9 (9.1)	60.3 (8.7)	61.0 (8.8)	0.5
BMI, kg/m^2	28.7 (6.2)	29.3 (5.0)	29.0 (5.6)	0.6
Percent Body Fat	38.9 (6.6)	28.7 (4.6)	39.5 (5.6)	0.4
Weight, lbs	164.1 (39.3)	170.6 (33.0)	167.6 (36.0)	0.5
Number of pregnancies	2.2 (1.0)	2.6 (1.3)	2.4 (1.2)	0.3
N (%)				
Race				
European American	23 (74.2%)	24 (68.6%)	47 (71.2%)	
African-American	8 (25.8%)	11 (31.4%)	19 (28.8%)	0.6
Education				
≤ High School	8 (25.8%)	7 (20.0%)	15 (22.7%)	
>High School	23 (74.2%)	28 (80.0%)	51 (77.3%)	0.6
Employment Status				
Full Time	14 (46.7%)	13 (37.1%)	27 (41.5%)	
Part Time	4 (13.3%)	6 (17.1%)	10 (15.4%)	
Unemployed	12 (40.0%)	16 (45.7%)	28 (43.1%)	0.5
Married/Living with Partner				
Yes	18 (58.1%)	25 (71.4%)	43 (65.2%)	
No	13 (41.9%)	10 (28.6%)	23 (34.9%)	0.3
Smoking Exposure				
Yes	8 (27.6%)	8 (22.9%)	16 (25.0%)	
No	21 (72.4%)	27 (77.1%)	48 (75.0%)	0.7
Regular Alcohol Consumption				
Yes	21 (67.7%)	21 (61.8%)	42 (64.6%)	
No	10 (32.3%)	13 (38.2%)	23 (35.4%)	0.6
Physical Activity				
Regular Exercise	28 (90.3%)	31 (88.6%)	59 (89.4%)	
No Regular Exercise	3 (9.7%)	4 (11.4%)	7 (10.6%)	0.8
Ever Been Pregnant				
Yes	25 (86.2%)	31 (93.9%)	56 (90.3%)	
No	4 (13.8%)	2 (6.1%)	6 (9.7%)	0.3
Breast Cancer Survivor				
Yes	8 (25.8%)	10 (28.6%)	18 (27.3%)	
No	23 (74.2%)	25 (71.4%)	48 (72.7%)	0.8

[1]Chi-square test (p-value)

Differences in Actual Intake between Baseline and Mid-Intervention by Treatment Status

Table 4.2 shows the change in mean intake of nutrients, fruit and vegetables by treatment group from baseline to mid-intervention (median differences shown in Table 4.3). The p-value represents whether the change in the control group is different from the change in the intervention group. Both treatment groups had non-significant decreases in mean energy intake (control -47.4 ± 419.3 kcal/d; intervention -82.8 ± 308.6 kcal/d), protein intake (control -7.0 ± 25.1 g/d; intervention -3.4 ± 17.5 g/d) and fat intake (control -3.2 ± 22.2 g/d; intervention -2.3 ± 16.1 g/d), and non-significant changes in mean carbohydrate intake (control 2.2 ± 48.0 g/d; intervention -7.3 ± 44.4 g/d). Soluble fiber mean intake significantly increased only in the intervention group (control -0.1 ± 2.0 g/d; intervention 2.0 ± 2.0 g/d) with an average intake of 6.8 ± 2.1 g/d in intervention compared to 4.9 ± 1.8 g/d in controls during the intervention. Dietary fiber mean intake significantly increased in both treatment groups (control 0.3 ± 5.9 g/d; intervention 3.6 ± 5.3 g/d).

One vegetable serving was calculated as 0.5 cups of raw or cooked vegetables. Compared to baseline levels, total mean vegetable intake increased significantly only in the intervention group (control -0.5 ± 1.6 srv/d; intervention 2.4 ± 1.8 srv/d) with participants in the intervention consuming an average of 5.2 ± 1.6 srv/d compared to control participants consuming 2.8 ± 1.4 srv/d mid-intervention. Similarly, *Brassica* vegetable intake only significantly increased in the intervention group (control -0.2 ± 0.6 srv/d; intervention 2.6 ± 1.5 srv/d) with intervention participants consuming on average 3.0 ± 1.5 srv/d compared to controls only consuming on average 0.5 ± 0.6 srv/d mid-

intervention. Mean fruit intake did not change significantly in either group (control 0.1 ± 1.0 g/d; intervention -0.3 ± 1.0 g/d; p=0.14).

Table 4.2: Differences in Actual Mean Intake between Baseline and Mid-Intervention by Treatment Group (n = 66)

	Control (n= 31)			**Intervention (n = 35)**		
	Baseline	**Mid-Intervention**	**Change[2]**	**Baseline**	**Mid-Intervention**	**Change[2]**
Mean (SD)						
Energy kcal/d	1541.7 (484.7)	1494.3 (372.4)	-47.4 (419.3)	1517.2 (464.1)	1434.4 (449.2)	-82.8 (308.6)
Protein g/d	67.7 (28.7)	60.7 (15.4)	-7.0 (25.1)	61.7 (21.2)	58.3 (17.2)	-3.4 (17.5)
Fat g/d	58.3 (22.9)	55.1 (21.8)	-3.2 (22.2)	55.6 (22.3)	53.2 (24.3)	-2.3 (16.1)
Carbohydrates g/d	188.0 (58.1)	190.2 (47.4)	2.2 (48.0)	195.1 (62.8)	187.8 (58.7)	-7.3 (44.4)
Soluble Fiber g/d	5.0 (1.9)	4.9 (1.8)	***-0.1**** (2.0)	4.9 (1.7)	6.8 (2.1)	***2.0**** (2.0)
Dietary Fiber g/d	15.4 (6.6)	15.7 (7.8)	***0.3**** (5.9)	14.9 (6.0)	18.4 (6.8)	***3.6**** (5.3)
Folate mcg/d	754.6 (1090.6)	570.6 (244.9)	-184.0 (1068.8)	3320.9 (16896.7)	524.2 (176.7)	-2796.7 (16917.9)
Calcium mg/d	1107.2 (694.5)	1209.1 (595.3)	101.4 (608.4)	979.8 (576.6)	1090.7 (591.4)	111.0 (404.7)
Vitamin A IU/d	9980.3 (5580.1)	11199 (7201.6)	1218.7 (8235.7)	8024.9 (4166.7)	10971.7 (5165.7)	2946.8 (5602.0)
Vitamin D mcg/d	8.9 (5.6)	10.7 (5.8)	1.9 (5.7)	6.9 (4.3)	8.4 (5.3)	1.5 (3.9)
Vitamin C mg/d	144.0 (87.4)	165.8 (165.8)	21.8 (130.4)	190.5 (198.6)	267.0 (215.0)	76.6 (133.9)
Vegetables srv/d[1]	3.3 (1.6)	2.8 (1.4)	***-0.5**** (1.6)	2.8 (1.0)	5.2 (1.6)	***2.4**** (1.8)
Fruit srv/d[1]	1.5 (1.3)	1.5 (1.2)	0.1 (1.0)	1.7 (1.1)	1.4 (0.9)	-0.3 (1.0)
Fruit and Vegetables srv/d[1]	4.7 (2.4)	4.3 (2.2)	***-0.4**** (1.8)	4.5 (1.6)	6.6 (2.0)	***2.1**** (2.0)
Brassica Vegetables srv/d[1]	0.7 (0.7)	0.5 (0.6)	***-0.2**** (0.6)	0.4 (0.5)	3.0 (1.5)	***2.6**** (1.5)

[1]One serving is equivalent to 0.5 cups of raw or cooked vegetables

[2]Difference between the means of baseline and during intervention.

*represents significant p-values (< 0.05) which is testing whether the change in the control group is different from the change in the intervention group

Table 4.3: Differences in Actual Median Intake between Baseline and Mid-Intervention by Treatment Group (n = 66)

	Control (n= 31)			**Intervention (n = 35)**		
	Baseline	**Mid-Intervention**	**Change[2]**	**Baseline**	**Mid-Intervention**	**Change[2]**
Median (SD)						
Energy kcal/d	1494.7 (484.7)	1522.9 (372.4)	-18.4 (419.3)	1463.4 (464.1)	1301.2 (449.2)	-122.5 (308.6)
Protein g/d	62.7 (28.7)	59.2 (15.4)	-3.0 (25.1)	56.8 (21.2)	51.5 (17.2)	-1.6 (17.5)
Fat g/d	54.7 (22.9)	55.0 (21.8)	-7.7 (22.2)	52.4 (22.3)	46.0 (24.3)	-1.9 (16.1)
Carbohydrates g/d	186.6 (58.1)	186.2 (47.4)	2.3 (48.0)	188.8 (62.8)	187.5 (58.7)	-3.6 (44.4)
Soluble Fiber g/d	5.0 (1.9)	5.2 (1.8)	-0.2 ***(2.0)****	4.8 (1.7)	6.9 (2.1)	1.7 ***(2.0)****
Dietary Fiber g/d	14.2 (6.6)	14.7 (7.8)	-0.1 ***(5.9)****	14.3 (6.0)	17.3 (6.8)	3.5 ***(5.3)****
Folate mcg/d	625.0 (1090.6)	573.1 (244.9)	2.2 (1068.8)	440.5 (16896.7)	521.9 (176.7)	61.2 (16917.9)
Calcium mg/d	931.1 (694.5)	1197.3 (595.3)	117.5 (608.4)	826.6 (576.6)	950.3 (591.4)	44.7 (404.7)
Vitamin A IU/d	9985.6 (5580.1)	9493.3 (7201.6)	-881.6 (8235.7)	7747.1 (4166.7)	10296.9 (5165.7)	2563.7 (5602.0)
Vitamin D mcg/d	8.3 (5.6)	10.9 (5.8)	1.7 (5.7)	7.1 (4.3)	6.8 (5.3)	2.4 (3.9)
Vitamin C mg/d	141.2 (87.4)	120.7 (165.8)	1.4 (130.4)	124.8 (198.6)	197.0 (215.0)	61.2 (133.9)
Vegetables srv/d	3.0 (1.6)	2.8 (1.4)	-0.5 ***(1.6)****	2.8 (1.0)	5.3 (1.6)	2.4 ***(1.8)****
Fruit srv/d	1.3 (1.3)	1.4 (1.2)	0 (1.0)	1.5 (1.1)	1.3 (0.9)	-0.2 (1.0)
Fruit and Vegetables srv/d	4.7 (2.4)	4.3 (2.2)	-0.4 ***(1.8)****	4.4 (1.6)	6.6 (2.0)	2.6 ***(2.0)****
Brassica Vegetables srv/d[1]	0.5 (0.7)	0.4 (0.6)	-0.1 ***(0.6)****	0.2 (0.5)	3.1 (1.5)	2.7 ***(1.5)****

[1]One serving is equivalent to 0.5 cups of raw or cooked vegetables

[2]Difference between the median intake of baseline and during intervention.

*represents significant p-values (< 0.05) which is testing whether the change in the control group is different from the change in the intervention group

Differences in Actual Intake between Post Intervention and Baseline by Treatment Status

Table 4.4 also shows the difference in mean intake of nutrients, fruit and vegetables by treatment group between post intervention and baseline (median differences shown in Table 4.5). The p-value represents whether the change in the control group is different from the change in the intervention group. Both treatment groups non-significantly decreased mean energy intake (control -35.7 ± 438.2 kcal/d; intervention -1137.4 ± 356.9 kcal/d), protein intake (control -3.7 ± 25.9 g/d; intervention -6.1 ± 19.1 g/d), fat intake (control -1.8 ± 23.7 g/d; intervention -6.3 ± 23.0 g/d), and carbohydrate intake (control -0.5 ± 51.0 g/d; intervention -13.7 ± 59.7 g/d). Soluble fiber mean intake significantly increased in the intervention group only (control -0.3 ± 1.7 g/d; intervention 0.7 ± 2.0 g/d) with an average intake of 5.6 ± 1.5 g/d among intervention compared to 4.7 ± 1.6 g/d among controls. Dietary fiber mean intake increased only in the intervention group but was non-significant (control -1.3 ± 5.1 g/d; intervention 0.7 ± 5.0 g/d).

One vegetable serving was calculated as 0.5 cups of raw or cooked vegetables. Compared to baseline levels, total mean vegetable intake increased significantly only in the intervention group (control -0.7 ± 1.5 srv/d; intervention 0.8 ± 1.6 srv/d) with participants in intervention consuming an average of 3.6 ± 1.5 srv/d compared to controls consuming 2.6 ± 1.1 srv/d post-intervention. Similarly, *Brassica* vegetable intake only significantly increased in the intervention group, (control -0.3 ± 0.8 srv/d; intervention 1.0 ± 1.1 srv/d) with intervention participants consuming on average 1.4 ± 1.3 srv/d compared to controls only consuming on average 0.4 ± 0.6 srv/d. Fruit mean intake increased only in the control group (control 0.1 ± 1.1 srv/d; intervention -0.6 ± 1.1 srv/d)

with control participants consuming on average 1.6 ± 1.4 srv/d compared to intervention participants consuming 1.1 ± 0.9 srv/d.

Table 4.4: Differences in Actual Mean Intake between Baseline and Post Intervention by Treatment Group (n = 66)

	Control (n= 31)			**Intervention (n = 35)**		
	Baseline	**Post Intervention**	**Change[2]**	**Baseline**	**Post Intervention**	**Change[2]**
Mean (SD)						
Energy kcal/d	1541.7 (484.7)	1506.0 (372.2)	-35.7 (438.2)	1517.2 (464.1)	1379.8 (371.9)	-137.4 (356.9)
Protein g/d	67.7 (28.7)	64.0 (16.5)	-3.7 (25.9)	61.7 (21.2)	55.7 (15.5)	-6.1 (19.1)
Fat g/d	58.3 (22.9)	57.0 (24.0)	-1.8 (23.7)	55.6 (22.3)	49.3 (20.9)	-6.3 (23.0)
Carbohydrates g/d	188.0 (58.1)	187.5 (40.1)	-0.52 (51.0)	195.1 (62.8)	181.4 (63.7)	-13.7 (59.7)
Soluble Fiber g/d	5.0 (1.9)	4.7 (1.6)	***-0.31**** (1.7)	4.9 (1.7)	5.6 (1.5)	***0.7**** (2.0)
Dietary Fiber g/d	15.4 (6.6)	14.1 (5.3)	-1.3 (5.1)	14.9 (6.0)	15.6 (5.5)	0.7 (5.0)
Folate mcg/d	754.6 (1090.6)	582.7 (244.4)	-171.9 (1087.8)	3320.9 (16896.7)	491.6 (182.0)	-2829.3 (16836.1)
Calcium mg/d	1107.2 (694.5)	1289.0 (648.1)	181.8 (544.6)	979.8 (576.6)	936.8 (522.2)	-43.0 (428.6)
Vitamin A IU/d	9980.3 (5580.1)	10139.1 (5930.9)	158.8 (6526.0)	8024.9 (4166.7)	9772.9 (5597.5)	1747.9 (5111.4)
Vitamin D mcg/d	8.9 (5.6)	10.5 (6.2)	1.6 (6.2)	6.9 (4.3)	7.7 (4.6)	0.8 (3.7)
Vitamin C mg/d	144.0 (87.4)	144.4 (116.6)	0.40 (75.1)	190.5 (198.6)	241.9 (259.2)	51.3 (203.7)
Vegetables srv/d[1]	3.3 (1.6)	2.6 (1.1)	***-0.7**** (1.5)	2.8 (1.0)	3.6 (1.5)	***0.8**** (1.6)
Fruit srv/d[1]	1.5 (1.3)	1.6 (1.4)	***0.1**** (1.1)	1.7 (1.1)	1.1 (0.9)	***-0.6**** (1.1)
Fruit and Vegetables srv/d[1]	4.7 (2.4)	4.1 (2.2)	-0.6 (1.9)	4.5 (1.6)	4.7 (1.6)	0.2 (1.9)
Brassica Vegetables srv/d[1]	0.7 (0.7)	0.4 (0.6)	***-0.3**** (0.8)	0.4 (0.5)	1.4 (1.3)	***1.0**** (1.1)

[1]One serving is equivalent to 0.5 cups of raw or cooked vegetables

[2]Difference between the means of baseline and during intervention.

*represents significant p-values (< 0.05) which is testing whether the change in the control group is different from the change in the intervention group

Table 4.5: Differences in Actual Median Intake between Baseline and Post Intervention by Treatment Group (n = 66)

	Control (n= 31)			**Intervention (n = 35)**		
	Baseline	**Post Intervention**	**Change[2]**	**Baseline**	**Post Intervention**	**Change[2]**
Median (SD)						
Energy kcal/d	1494.7 (484.7)	1465.2 (372.2)	-59.1 (438.2)	1463.4 (464.1)	1322.6 (371.9)	-183.1 (356.9)
Protein g/d	62.7 (28.7)	61.3 (16.5)	-1.8 (25.9)	56.8 (21.2)	57.2 (15.5)	-6.2 (19.1)
Fat g/d	54.7 (22.9)	51.9 (24.0)	-0.8 (23.7)	52.4 (22.3)	48.1 (20.9)	-5.3 (23.0)
Carbohydrates g/d	186.6 (58.1)	195.4 (40.1)	-1.8 (51.0)	188.8 (62.8)	167.2 (63.7)	-14.9 (59.7)
Soluble Fiber g/d	5.0 (1.9)	4.9 (1.6)	**0.1*** (1.7)	4.8 (1.7)	5.9 (1.5)	***0.9**** (2.0)
Dietary Fiber g/d	14.2 (6.6)	13.8 (5.3)	-0.5 (5.1)	14.3 (6.0)	16.1 (5.5)	0.9 (5.0)
Folate mcg/d	625.0 (1090.6)	576.4 (244.4)	-1.4 (1087.8)	440.5 (16896.7)	458.7) (182.0)	4.0 (16836.1)
Calcium mg/d	931.1 (694.5)	1332.4 (648.1)	193.7 (544.6)	826.6 (576.6)	888.6 (522.2)	-84.9 (428.6)
Vitamin A IU/d	9985.6 (5580.1)	10371.6 (5930.9)	-1270.4 (6526.0)	7747.1 (4166.7)	8647.2 (5597.5)	1603.7 (5111.4)
Vitamin D mcg/d	8.3 (5.6)	9.1 (6.2)	1.1 (6.2)	7.1 (4.3)	6.9 (4.6)	1.1 (3.7)
Vitamin C mg/d	141.2 (87.4)	112.9 (116.6)	-7.5 (75.1)	124.8 (198.6)	150.6 (259.2)	29.8 (203.7)
Vegetables srv/d	3.0 (1.6)	2.6 (1.1)	***-0.6**** (1.5)	2.8 (1.0)	3.7 (1.5)	***0.9**** (1.6)
Fruit srv/d	1.3 (1.3)	1.1 (1.4)	***0**** (1.1)	1.5 (1.1)	0.9 (0.9)	***-0.5**** (1.1)
Fruit and Vegetables srv/d	4.7 (2.4)	4.2 (2.2)	-0.6 (1.9)	4.4 (1.6)	4.5 (1.6)	0.3 (1.9)
Brassica Vegetable srv/d[1]	0.5 (0.7)	0.1 (0.6)	***-0.3**** (0.8)	0.2 (0.5)	1.0 (1.3)	***0.8**** (1.1)

[1]One serving is equivalent to 0.5 cups of raw or cooked vegetables

[2]Difference between the median intake of baseline and during intervention.

*represents significant p-values (< 0.05) which is testing whether the change in the control group is different from the change in the intervention group

Intervention Effects on Estrogen Metabolites by Treatment Status

Table 4.6 displays unadjusted means and medians in 2-OHE, 16α-OHE and 2:16α-OHE levels by treatment group between baseline and post intervention. The

estrogen metabolite levels (ng/ml) were standardized by dividing by creatinine levels (mg/ml). There were differences in the direction of the change in 2-OHE levels by treatment status with levels of 2-OHE increasing in both treatment groups (intervention mean change 0.2 ng/ml ± 2.6, p=0.4; control mean change -0.1 ng/ml ± 3.1, p=0.5) though these changes were not statistically significantly different between treatment groups. The levels of 16α-OHE non-significantly increased in the intervention group (mean change 0.3 ng/ml ± 2.2, p=0.5) and decreased in the control group (mean change -0.1 ng/ml ± 2.2, p=0.9). While 2:16α-OHE showed no mean change in the intervention or control group.

Table 4.6: Intervention effects on Estrogen Metabolites by Treatment Group[1]

	Control (n=31)				**Intervention (n=35)**			
	Baseline	**Post Intervention**	**Change[2]**	**p-value[3]**	**Baseline**	**Post Intervention**	**Change[2]**	**p-value[3]**
Means (SD)								
2-OHE ng/ml	11.7 (3.1)	11.8 (3.1)	0.1	0.5	12.6 (2.6)	12.8 (2.6)	0.2	0.4
16α-OHE ng/ml	9.2 (2.1)	9.1 (2.2)	-0.1	0.9	9.0 (2.1)	9.3 (2.2)	0.3	0.5
2: 16α-OHE ng/ml	1.3 (1.9)	1.3 (2.0)	0	0.5	1.4 (1.9)	1.4 (1.7)	0	0.7
Medians (SD)								
2-OHE ng/ml	8.8 (3.1)	6.9 (3.1)	-1.9	0.5	9.7 (2.6)	9.4 (2.6)	-0.3	0.4
16α-OHE ng/ml	8.3 (2.1)	8.4 (2.2)	0.1	0.9	7.1 (2.1)	7.9 (2.2)	0.8	0.5
2: 16α-OHE ng/ml	1.4 (1.9)	1.3 (2.0)	-0.1	0.5	1.5 (1.9)	1.4 (1.7)	-0.1	0.7

[1]Estrogen metabolite levels (ng/ml) were standardized by dividing by creatinine level (mg/ml).
[2]Within each arm, used Wilcoxen Signed Rank Test to see if the change was significantly different from zero.
[3]p-value represents within-group comparisons; no within-group change comparison was found to be significant.

Table 4.7 displays mean 2-OHE, 16α-OHE, and 2:16α-OHE at post intervention by treatment group. There was no statistically significant difference in 2-OHE between intervention and control group at post intervention after adjusting for baseline 2-OHE levels (12.5ng/ml vs. 12.2 ng/ml, respectively, p=0.9). Similarly, no statistically significant difference was observed in 2:16α-OHE between intervention and control group after adjusting for baseline 2:16α-OHE levels (1.3 ng/ml vs.1.3 ng/ml, respectively, p=0.7).

Table 4.7: Mean Follow-up of 2-OHE, 16α-OHE and 2:16α-OHE by Treatment Group[1,2]

Variable	Follow-up LS-Means	95% Confidence Interval	p-value[4]
2-OHE, ng/ml [3]			
Intervention	12.5	(10.0, 15.6)	-
Control	12.2	(9.6, 15.4)	0.9
16α-OHE, ng/ml [3]			
Intervention	9.4	(7.9, 11.2)	-
Control	9.0	(7.5, 10.8)	0.7
2:16α-OHE ng/ml			
Intervention	1.3	(1.1, 1.6)	-
Control	1.3	(1.1, 1.6)	0.7

[1]Estrogen metabolite levels (ng/ml) were standardized by dividing by creatinine level (mg/ml).
[2]Logged values used for analysis
[3]LS means adjusted for baseline 2-OHE, 16α-OHE, and 2:16α-OHE, respectively.
[4]p-value represents the difference between intervention groups.

Table 4.8 represents the final adjusted models for mean 2-OHE, 16α-OHE, and 2:16α-OHE at post intervention by treatment group. Potential confounders were assessed by starting with all potential confounders in the model and then removing them one-by-one based on p-values; final model only yielding those that were found to be significantly associated with each estrogen metabolite outcome. At post intervention, the differences in 2-OHE levels between intervention and control group were found to be statistically non-significant after adjusting for marital status, breast cancer survivorship status, age, and baseline percent fat mass (10.6 ng/ml vs. 8.7 ng/ml, respectively, p=0.4). There was

statistically non-significant difference in 16α-OHE levels between intervention and control group at post intervention after adjusting for baseline percent fat mass (9.6 ng/ml vs. 8.6 ng/ml, respectively, p=0.6). None of the potential confounders were found to be significantly associated with 2:16α-OHE levels, so only the unadjusted model results are shown (same as in Table 4.8).

Table 4.8: Final Adjusted Models for Mean Follow-up of 2-OHE, 16α-OHE and 2:16α-OHE by Treatment Group[1,2]

Variable	Follow-up LS-Means	95% Confidence Interval	p-value[4]
2-OHE, ng/ml [3]			
Intervention	10.6	(7.4, 14.9)	-
Control	8.7	(6.0, 12.2)	0.4
16α-OHE, ng/ml [3]			
Intervention	9.6	(7.4, 12.4)	-
Control	8.6	(6.7, 11.0)	0.6
2:16α-OHE ng/ml			
Intervention	1.3	(1.1, 1.6)	-
Control	1.3	(1.1, 1.6)	0.7

[1]Estrogen metabolite levels (ng/ml) were standardized by dividing by creatinine level (mg/ml).
[2]Logged values used for analysis
[3]LS means for 2-OHE adjusted for marital status, breast cancer survivor status, age, and percent fat mass. LS means for 16α-OHE adjusted for percent fat mass.
[4]p-value represents the difference between intervention groups.

Differences of Estrogen Metabolite Levels by Baseline Dietary Brassica Intake

For Aim 2, Table 4.9 depicts unadjusted mean 2-OHE, 16α-OHE, and 2:16α-OHE at post intervention by treatment group stratified by baseline dietary *Brassica* intake; whereas, Table 4.10 displays adjusted mean 2-OHE, 16α-OHE, and 2:16α-OHE at post intervention by treatment group stratified by baseline dietary *Brassica* intake. By using the median split of overall *Brassica* vegetable consumption at baseline, women were either categorized as High (≥0.5 srv/d) or Low (<0.5 srv/d) consumers of *Brassica* vegetables. When comparing the intervention to the controls, there was no statistically significant difference observed in 2-OHE levels, among high *Brassica* vegetable

consumers (9.2 ng/ml vs. 8.4 ng/ml, respectively, p=0.8) or among low *Brassica* vegetable consumers (11.2 ng/ml vs. 8.8 ng/ml, respectively, p=0.4) when adjusted for marital status, breast cancer survivorship status, age and baseline percent fat mass. Similarly, there was no statistically significant difference in 2:16α-OHE levels between intervention and controls, among high *Brassica* vegetable consumers (1.7 ng/ml vs.1.4 ng/ml, respectively, p=0.3) or among low *Brassica* vegetable consumers (1.2 ng/ml vs.1.2 ng/ml, respectively, p=0.9).

Table 4.9: Mean Follow-up of 2-OHE, 16α-OHE and 2:16α-OHE by Treatment Group[1,2] Stratified by Baseline Dietary *Brassica* Intake

Variable	Follow-up LS-Means	95% Confidence Interval	p-value[3]
High *Brassica* Vegetable Consumers[5] (n=29)			
2-OHE, ng/ml			
Intervention	14.6	(8.1, 26.0)	-
Control	11.0	(6.5, 18.7)	0.5
16α-OHE, ng/ml			
Intervention	8.6	(5.5, 13.3)	-
Control	8.1	(5.5, 12.1)	0.9
2:16α-OHE ng/ml			
Intervention	1.7	(1.2, 2.4)	-
Control	1.4	(1.0, 1.8)	0.3
Low *Brassica* Vegetable Consumers[5] (n=37)			
2-OHE, ng/ml			
Intervention	11.9	(7.5, 18.5)	-
Control	12.7	(7.4, 22.0)	0.9
16α-OHE, ng/ml			
Intervention	9.8	(7.0, 13.9)	-
Control	10.2	(6.8, 15.3)	0.9
2:16α-OHE ng/ml			
Intervention	1.2	(0.9, 1.6)	-
Control	1.2	(0.9, 1.7)	0.9

[1]Estrogen metabolite levels (ng/ml) were standardized by dividing by creatinine level (mg/ml).
[2]Logged values used for analysis
[3]p-value represents the difference between intervention groups.
[4] Baseline dietary *Brassica* intake and the interaction between treatment group and baseline dietary *Brassica* intake was found to be non-significant in all the estrogen metabolite models.
[5]Median split used to determine whether baseline *Brassica* consumption was High (≥ 0.5 srv/d) or Low (< 0.5 srv/d)
[6]One serving of *Brassica* is equivalent to 0.5 cups of raw or cooked vegetables

Table 4.10: Adjusted Mean Follow-up of 2-OHE, 16α-OHE and 2:16α-OHE by Treatment Group[1,2] Stratified by Baseline Dietary *Brassica* Intake[7]

Variable	Follow-up LS-Means	95% Confidence Interval	p-value[4]
High *Brassica* Vegetable Consumers[5] (n=29)			
2-OHE, ng/ml [3]			
Intervention	9.2	(5.1, 16.6)	-
Control	8.4	(5.2, 13.6)	0.8
16α-OHE, ng/ml [3]			
Intervention	8.5	(5.6, 12.9)	-
Control	8.0	(5.5, 11.6)	0.8
2:16α-OHE ng/ml			
Intervention	1.7	(1.2, 2.4)	-
Control	1.4	(1.0, 1.8)	0.3
Low *Brassica* Vegetable Consumers[5] (n=37)			
2-OHE, ng/ml [3]			
Intervention	11.2	(7.5, 18.5)	-
Control	8.8	(5.3, 14.6)	0.4
16α-OHE, ng/ml [3]			
Intervention	10.3	(7.4, 14.3)	-
Control	9.5	(6.3, 14.2)	0.7
2:16α-OHE ng/ml			
Intervention	1.2	(0.9, 1.6)	-
Control	1.2	(0.9, 1.7)	0.9

[1]Estrogen metabolite levels (ng/ml) were standardized by dividing by creatinine level (mg/ml).
[2]Logged values used for analysis
[3]LS means for 2-OHE adjusted for marital status, breast cancer survivor status, age and percent fat mass. LS means for 16α-OHE adjusted for by percent fat mass.
[4]p-value represents the difference between intervention groups.
[5]Median split used to determine whether *Brassica* consumptions was High (≥ 0.5 srv/d) or Low (< 0.5 srv/d)
[6]One serving of *Brassica* is equivalent to 0.5 cups of raw or cooked vegetables
[7] The interaction between treatment group and baseline dietary *Brassica* intake was found to be non-significant in all the estrogen metabolite models.

Differences of Estrogen Metabolite Levels by Race

Table 4.11 displays unadjusted mean 2-OHE, 16α-OHE, and 2:16α-OHE at post intervention by treatment group stratified by race; whereas, Table 4.12 shows adjusted mean 2-OHE, 16α-OHE, and 2:16α-OHE at post intervention by treatment group stratified by race. When comparing the intervention to the control group, there was no statistically significant difference observed in 2-OHE levels, among AA (8.7 ng/ml vs.

7.0 ng/ml, respectively) and EA women (11.3 ng/ml vs. 9.5 ng/ml, respectively) when adjusted for marital status, breast cancer survivorship status, age and baseline percent fat mass. Also, there was no statistically significant difference in 2:16α-OHE levels between intervention and controls, among AA (1.4 ng/ml vs.1.0 ng/ml, respectively) and EA women (1.4 ng/ml vs.1.4 ng/ml, respectively).

Table 4.11: Mean Follow-up of 2-OHE, 16α-OHE and 2:16α-OHE by Treatment Group[1,2] Stratified by Race[4]

Variable	Follow-up LS-Means	95% Confidence Interval	p-value[3]
African American Women (n=19)			
2-OHE, ng/ml			
Intervention	8.7	(4.7, 16.0)	-
Control	6.5	(3.2, 13.2)	0.5
16α-OHE, ng/ml			
Intervention	6.4	(4.0, 10.0)	-
Control	6.5	(3.8, 11.2)	0.9
2:16α-OHE ng/ml			
Intervention	1.4	(1.0, 2.0)	-
Control	1.0	(0.7, 1.5)	0.3
European American Women (n=47)			
2-OHE, ng/ml			
Intervention	15.3	(10.1, 23.1)	-
Control	14.6	(9.6, 22.2)	0.9
16α-OHE, ng/ml			
Intervention	11.1	(8.2, 15.2)	-
Control	10.2	(7.4, 14.0)	0.7
2:16α-OHE ng/ml			
Intervention	1.4	(1.1, 1.8)	-
Control	1.4	(1.1, 1.8)	0.8

[1]Estrogen metabolite levels (ng/ml) were standardized by dividing by creatinine level (mg/ml).
[2]Logged values used for analysis
[3]p-value represents the change between intervention groups.
[4]The interaction between treatment group and race was found to be non-significant in all the estrogen metabolite models

Table 4.12: Adjusted Mean Follow-up of 2-OHE, 16α-OHE and 2:16α-OHE by Treatment Group[1,2] Stratified by Race[6]

Variable	Follow-up LS-Means	95% Confidence Interval	p-value[4]
African American Women (n=19)			
2-OHE, ng/ml [3]			
Intervention	8.7	(4.7, 16.3)	-
Control	7.0	(3.6, 13.9)	0.6
16α-OHE, ng/ml [3]			
Intervention	7.1	(4.5, 11.2)	-
Control	7.3	(4.3, 12.6)	0.9
2:16α-OHE ng/ml			
Intervention	1.4	(1.0, 2.0)	-
Control	1.0	(0.7, 1.5)	0.3
European American Women (n=47)			
2-OHE, ng/ml [3]			
Intervention	11.3	(7.5, 16.9)	-
Control	9.5	(6.2, 14.4)	0.5
16α-OHE, ng/ml [3]			
Intervention	11.0	(8.1, 14.9)	-
Control	9.2	(6.7, 12.8)	0.4
2:16α-OHE ng/ml			
Intervention	1.4	(1.1, 1.8)	-
Control	1.4	(1.1, 1.8)	0.8

[1]Estrogen metabolite levels (ng/ml) were standardized by dividing by creatinine level (mg/ml).
[2]Logged values used for analysis
[3]LS means for 2-OHE adjusted for by marital status, breast cancer survivor, age and percent fat mass. LS means for 16α-OHE adjusted for by percent fat mass.
[4]p-value represents the difference between intervention groups.
[6]The interaction between treatment group and race was found to be non-significant in all the estrogen metabolite models.

Table 4.13 displays unadjusted mean 2-OHE, 16α-OHE, and 2:16α-OHE at post intervention by race and Table 4.14 displays adjusted estrogen metabolite levels at post intervention by race. In the crude analysis (Table 4.13), AA women had significantly lower 2-OHE (AA: 7.7 ng/ml; EA: 14.9 ng/ml; p=0.02) and 16α-OHE levels (AA: 6.4 ng/ml; EA: 10.7 ng/ml; p=0.02) at post intervention. However, when 2-OHE levels were adjusted for marital status, breast cancer survivorship status, age, and baseline percent fat mass, the difference among AA and EA women was found to be not statistically significant (7.9ng/ml vs. 10.4 ng/ml, respectively, p=0.3) at post intervention. Likewise, there was statistically no significant difference in 2:16α-OHE levels among AA and EA

women at post intervention (1.2 ng/ml vs. 1.4 ng/ml, respectively, p=0.3). At post intervention, 16α-OHE levels were not significantly different among AA and EA women after adjusting for baseline percent fat mass (7.2 ng/ml vs. 10.1 ng/ml, p=0.1).

Table 4.13: Mean Follow-up of 2-OHE, 16α-OHE and 2:16α-OHE by Race[1,2]

Variable	Follow-up LS-Means	95% Confidence Interval	p-value[3]
2-OHE, ng/ml			
European American	14.9	(11.1, 19.9)	-
African American	7.7	(4.9, 12.2)	0.02
16α-OHE, ng/ml			
European American	10.7	(8.5, 13.5)	-
African American	6.4	(4.5, 9.0)	0.02
2:16α-OHE ng/ml			
European American	1.4	(1.2, 1.6)	-
African American	1.2	(0.9, 1.6)	0.3

[1]Estrogen metabolite levels (ng/ml) were standardized by dividing by creatinine level (mg/ml).
[2]Logged values used for analysis
[3]p-value represents the difference between race.

Table 4.14: Adjusted Mean Follow-up of 2-OHE, 16α-OHE and 2:16α-OHE by Race[1,2]

Variable	Follow-up LS-Means	95% Confidence Interval	p-value[4]
2-OHE, ng/ml [3]			
European American	10.4	(7.6, 14.2)	-
African American	7.9	(4.8, 12.7)	0.3
16α-OHE, ng/ml [3]			
European American	10.1	(8.2, 12.6)	-
African American	7.2	(5.0, 10.2)	0.1
2:16α-OHE ng/ml			
European American	1.4	(1.2, 1.6)	-
African American	1.2	(0.9, 1.6)	0.3

[1]Estrogen metabolite levels (ng/ml) were standardized by dividing by creatinine level (mg/ml).
[2]Logged values used for analysis
[3]LS means adjusted for marital status, breast cancer survivor status, age and baseline percent fat mass for model of 2-OHE by race. LS means adjusted for baseline percent fat mass for model of 16α-OHE by race.
[4]p-value represents the difference between races.

Differences of Estrogen Metabolites by Breast Cancer Survivorship Status

Table 4.15 displays unadjusted mean 2-OHE, 16α-OHE, and 2:16α-OHE at post intervention by treatment group stratified by breast cancer survivorship status; whereas, Table 4.16 shows adjusted mean 2-OHE, 16α-OHE, and 2:16α-OHE at post intervention

by treatment group stratified by breast cancer survivorship status. When comparing the intervention to the controls at post intervention, there was no statistically significant difference detected in 2-OHE levels, among breast cancer survivors (15.9 ng/ml vs. 11.4 ng/ml, respectively, p=0.2) and disease-free women (6.5 ng/ml vs. 7.5 ng/ml, respectively, p=0.7) when adjusted for marital status, breast cancer survivorship status, age and baseline percent fat mass. Also, there was no statistically significant difference in 2:16α-OHE levels between intervention and controls, among breast cancer survivors (1.6 ng/ml vs.1.3 ng/ml, respectively, p=0.3) and disease-free women (1.0 ng/ml vs.1.2 ng/ml, respectively, p=0.4) at post intervention.

Table 4.15: Mean Follow-up of 2-OHE, 16α-OHE and 2:16α-OHE by Treatment Group[1,2] Stratified by Breast Cancer Survivorship Status[4]

Variable	Follow-up LS-Means	95% Confidence Interval	p-value[3]
Breast Cancer Survivor (n=18)			
2-OHE, ng/ml			
Intervention	16.0	(10.7, 24.0)	-
Control	13.4	(8.8, 20.5)	0.6
16α-OHE, ng/ml			
Intervention	10.3	(7.5, 14.0)	-
Control	10.1	(7.3, 14.0)	0.9
2:16α-OHE ng/ml			
Intervention	1.6	(1.2, 2.0)	-
Control	1.3	(1.0, 1.7)	0.3
Disease-Free (n=48)			
2-OHE, ng/ml			
Intervention	7.3	(3.9, 13.9)	-
Control	8.1	(4.0, 16.6)	0.8
16α-OHE, ng/ml			
Intervention	7.4	(4.5, 12.2)	-
Control	6.6	(3.8, 11.5)	0.8
2:16α-OHE ng/ml			
Intervention	1.0	(0.7, 1.4)	-
Control	1.2	(0.8, 1.9)	0.4

[1]Estrogen metabolite levels (ng/ml) were standardized by dividing by creatinine level (mg/ml).
[2]Logged values used for analysis
[3]p-value represents the difference between intervention groups.
[4]The interaction between treatment group and breast cancer survivorship was found to be non-significant in all the estrogen metabolite models.

Table 4.16: Adjusted Mean Follow-up of 2-OHE, 16α-OHE and 2:16α-OHE by Treatment Group[1,2] Stratified by Breast Cancer Survivorship Status[5]

Variable	Follow-up LS-Means	95% Confidence Interval	p-value[3]
Breast Cancer Survivor (n=18)			
2-OHE, ng/ml [3]			
Intervention	15.9	(11.0, 23.1)	-
Control	11.4	(7.8, 16.8)	0.2
16α-OHE, ng/ml [3]			
Intervention	10.3	(7.6, 14.0)	-
Control	9.2	(6.7, 12.8)	0.6
2:16α-OHE ng/ml			
Intervention	1.6	(1.2, 2.0)	-
Control	1.3	(1.0, 1.7)	0.3
Disease-Free (n=48)			
2-OHE, ng/ml [3]			
Intervention	6.5	(3.6, 11.7)	-
Control	7.5	(4.0, 14.4)	0.7
16α-OHE, ng/ml [3]			
Intervention	7.9	(4.9, 12.8)	-
Control	7.2	(4.2, 12.4)	0.8
2:16α-OHE ng/ml			
Intervention	1.0	(0.7, 1.4)	-
Control	1.2	(0.8, 1.9)	0.4

[1]Estrogen metabolite levels (ng/ml) were standardized by dividing by creatinine level (mg/ml).
[2]Logged values used for analysis
[3]LS means for 2-OHE adjusted for by age, marital status, breast cancer survivorship and percent fat mass. LS means for 16α-OHE adjusted for by percent fat mass.
[4]p-value represents the difference between intervention groups.
[5]The interaction between treatment group and breast cancer survivorship was found to be non-significant in all the estrogen metabolite models.

Table 4.17 shows unadjusted mean 2-OHE, 16α-OHE, and 2:16α-OHE at post intervention by breast cancer survivorship status and Table 4.18 displays adjusted estrogen metabolite levels at post intervention by breast cancer survivorship status. In the crude analysis (Table 4.17), disease-free women had statistically significantly lower 2-OHE levels at post intervention (breast cancer survivor: 14.7 ng/ml; disease-free: 7.7 ng/ml; p=0.02). Correspondingly, differences in 2-OHE levels remained statistically significant among breast cancer survivors and disease-free women after adjusting for marital status, age, and baseline percent fat mass (13.5 ng/ml vs. 6.8 ng/ml, respectively, p=0.01). At post intervention, 2:16α-OHE levels were observed to be nearly significant

among breast cancer survivors and disease-free women (1.4 ng/ml vs.1.1 ng/ml, respectively, p=0.09). The differences in 16α-OHE levels were observed to be higher in breast cancer survivors than disease-free women, though this was non-significant (9.8 ng/ml vs. 7.6 ng/ml, respectively).

Table 4.17: Mean Follow-up of 2-OHE, 16α-OHE and 2:16α-OHE by Breast Cancer Survivor Status[1,2]

Variable	Follow-up LS-Means	95% Confidence Interval	p-value[3]
2-OHE, ng/ml			
Breast Cancer Survivor	14.7	(11.0, 20.0)	-
Disease-Free	7.7	(5.0, 12.2)	0.02
16α-OHE, ng/ml			
Breast Cancer Survivor	10.2	(8.2, 12.2)	-
Disease-Free	7.0	(5.0, 10.0)	0.09
2:16α-OHE ng/ml			
Breast Cancer Survivor	1.4	(1.2, 1.7)	-
Disease-Free	1.1	(0.8, 1.4)	0.09

[1]Estrogen metabolite levels (ng/ml) were standardized by dividing by creatinine level (mg/ml).
[2]Logged values used for analysis
[3]p-value represents the difference between breast cancer survivorship status (yes/no).

Table 4.18: Adjusted Mean Follow-up of 2-OHE, 16α-OHE and 2:16α-OHE by Breast Cancer Survivor Status[1,2]

Variable	Follow-up LS-Means	95% Confidence Interval	p-value[4]
2-OHE, ng/ml [3]			
Breast Cancer Survivor	13.5	(10.4, 17.6)	-
Disease-Free	6.8	(4.3, 10.7)	0.01
16α-OHE, ng/ml [3]			
Breast Cancer Survivor	9.8	(7.9, 12.2)	-
Disease-Free	7.6	(5.3, 10.8)	0.2
2:16α-OHE ng/ml			
Breast Cancer Survivor	1.4	(1.2, 1.7)	-
Disease-Free	1.1	(0.8, 1.4)	0.09

[1]Estrogen metabolite levels (ng/ml) were standardized by dividing by creatinine level (mg/ml).
[2]Logged values used for analysis
[3]LS means adjusted for marital status, age and baseline percent fat mass for model of 2-OHE by breast cancer survivorship status. LS means adjusted for baseline percent fat mass for model of 16α-OHE by breast cancer survivorship status.
[4]p-value represents the difference between breast cancer survivorship status (yes/no).

CHAPTER V

DISCUSSION

Over the last few years, diet has emerged as an important environmental risk factor associated with the risk of a number of cancers, including breast cancer. The link between vegetables in general and the risk of breast cancer has been well-established through numerous studies over the last decade (Willet 2001; La Vecchia et al. 2001; Riboli et al. 2003; Gandini et al. 2001; Cottet et al. 2009); however, the link between *Brassica* vegetable consumption and the risk of breast cancer have continued to yield inconsistent results, making this present study critical in helping to understand the biological mechanism of this link. In the present study, compared to baseline levels, *Brassica* vegetable intake significantly increased in the intervention group by 6-fold when compared to control group during the intervention; whereas, post intervention, *Brassica* intake only increased in the intervention about 3.5-fold when compared to the control group. Also, during the intervention, total dietary fiber mean intake significantly increased in both treatment groups; and total fruit and vegetable mean intake, total vegetable mean intake and total soluble fiber mean intake significantly increased in the intervention group only. Post intervention showed statistically significant increases in total soluble fiber mean intake and total mean vegetable intake only in the intervention group; and total fruit mean intake increased in the control group only. Another observation noted among the intervention group only, was that the mean servings of

vegetables, fruit and vegetables, and *Brassica* vegetables during the intervention was higher than servings consumed post intervention.

Estrogen is a well-established risk factor for breast cancer. 2-OHE and 16α-OHE are two estrogen metabolites of most interest which together are often reported as 2:16α-OHE ratio. A higher incidence of disease tends to be associated with high levels of 16α-OHE and low levels of 2-OHE in the body according to some studies (Matthews et al. 2004; Musey et al. 1987), but not all (Obi et al. 2011; Lissowska et al. 2008; Ursin et al. 1999; Cauley et al. 2003; Wellejus et al. 2005). Estrogen is metabolized along two competing pathways, 2-OHE and 16α-OHE. A few studies have demonstrated that *Brassica* vegetables play a significant role in the overall mechanism in modulating the activity of cellular enzymes that are responsible for the production of these estrogen metabolites, indole-3-carbionol (I3C) in particular (Maizes 2005; Lord et al. 2002; Nguyen et al. 2010; Laidlaw et al.2010; Dalessandri et al. 2004).

In the present study, both crude and final adjusted analyses showed no significant differences in 2-OHE, 16α-OHE and 2:16α-OHE as a result of an intense three-week *Brassica* vegetable dietary intervention. Even when adjusted for baseline estrogen metabolite levels, the intervention group did have higher levels of 2-OHE levels than those in the control when adjusted for marital status, breast cancer survivorship status, age and percent fat mass; however, this was statistically non-significant. It seemed plausible that no significant change was observed post intervention because women may have already been consuming high amounts of *Brassica* vegetables at baseline; therefore, to assess whether or not diet before the *Brassica* intervention began had an overall impact on the result of the intervention, a by statement was used to stratify by baseline dietary

Brassica intake. Overall *Brassica* vegetable consumption at baseline was categorized using a median split, women were categorized as High (≥ 0.5 srv/d) or Low (< 0.5 srv/d) consumers. Women who were categorized as low *Brassica* vegetable consumers at baseline did show higher increases in their 2-OHE levels after adjusting for marital status, breast cancer survivorship status, age, and baseline percent fat mass; but, these levels were statistically non-significant. Though the difference in the 2-OHE levels was statistically non-significant, the results of the *Brassica* intervention do show that 2-OHE levels can possibly be modified through diet.

Unlike this study, there have been a few epidemiological studies that have significantly shown an increase in 2-OHE levels after consumption of I3C (Nguyen et al. 2010; Laidlaw et al. 2010; Marconett et al. 2010). In a study by Laidlaw et al. (2010), pre and postmenopausal women were randomized to receive either the placebo (supplement containing microcrystalline cellulose) or treatment (supplement containing 200 mg I3C and 10 mg HMR ligans) for a period of 28 days. Among postmenopausal women receiving a supplement of I3C and HMR ligans, a significant increase in the 2-OHE levels was observed but no significant change in the 2:16α-OHE levels (Laidlaw et al. 2010). It may be that supplements containing high levels of I3C may have a significantly greater effect on 2-OHE rather than 16α-OHE levels. Further research needs to be conducted at the genetic level on the biological mechanism behind the CYP1A1 enzyme that catalyzes the conversion of estrone to 2-OHE, which has been shown to be impacted by the phytochemical I3C found in *Brassica* vegetables.

It has been heavily documented in numerous studies that AA women are less likely to be diagnosed with breast cancer than their EA counterparts; however, AA

women are more likely to die of breast cancer than EA women when they are diagnosed (DeSantis et al. 2013; SEER, Stat Fact Sheets: Breast Cancer 2006-2010; Adams et al. 2006; Menashe et al. 2009). It is vital that the health disparity that exists between EA and AA women, in terms of breast cancer mortality, be addressed; furthermore, it has also been observed that AA women tend to have lower 2:16α-OHE levels than EA women (Soweres et al. 2006; Lord et al. 2002; Matthews et al. 2004; Musey et al. 1987). Therefore, in this present study, the effects of *Brassica* vegetable consumption and breast cancer survivors on the 2:16α-OHE among AA women was examined.

In the present study, levels of estrogen metabolites post intervention were compared between AA and EA women, regardless of whether or not they were in the intervention. Crude analysis showed that AA women compared to EA women had significantly lower levels of both 2-OHE and 16α-OHE levels; though no significant difference was seen in 2:16α-OHE levels among the two races. Conversely, after adjustment for marital status, breast cancer survivorship status, age, and baseline percent fat mass, 2-OHE levels remained lower in AA women compared to EA women but this difference was statistically non-significant. 16α-OHE levels, after adjusting for baseline percent fat mass at post intervention, showed a slight suggestion of lower levels among AA compared to EA women but this difference was also statistically non-significant. The association of race was further assessed on its impact of estrogen metabolite levels on the actual three-week intervention. In both crude and adjusted analyses, when comparing the intervention and the control groups post intervention, there was no statistically significant difference observed in 2-OHE, 16α-OHE and 2:16α-OHE levels among AA and EA women. In contrast to this study's finding, a case-control study

among EA and AA pre and postmenopausal women by Coker et al. (1997) found that AA women had significantly lower 2-OHE (adjusted for creatinine) levels than EA; additionally, though not significant, AA women also had lower mean levels of 2:16α-OHE ratios than EA.

In a similar manner, levels of estrogen metabolites post intervention were compared between breast cancer survivors and disease-free women, regardless of whether or not they were in the intervention. Crude analysis showed that breast cancer survivors compared to disease-free women had significantly higher levels of 2-OHE. Breast cancer survivors were also observed to have higher levels of 16α-OHE and 2:16α-OHE levels that showed a slight suggestion of an association but it was statistically non-significant. Similarly, 2-OHE levels remained statistically significantly higher among breast cancer survivors compared to disease- free women after adjusting for marital status, age, and baseline percent fat mass. In the final adjusted model for 16α-OHE, the difference between breast cancer survivors compared to disease-free women was found to be statistically non-significant. The association of breast cancer survivor status was further assessed on its impact of estrogen metabolite levels on the actual three-week intervention. In both crude and adjusted analyses, when comparing the intervention and the control groups post intervention, there was no statistically significant difference observed in 2-OHE, 16α-OHE and 2:16α-OHE levels among breast cancer survivors and disease-free women. Ours is the first study to examine whether effects of *Brassica* vegetables on estrogen metabolites differ by breast cancer survivor status. Other larger studies are needed to confirm these findings of no difference in effect between breast cancer survivor and disease-free postmenopausal women.

Strengths and Limitations

The present study has several strengths and some limitations. The small sample size used in this study is a major limitation which ultimately leads to low power and, concomitantly, wide confidence intervals; therefore, leading to significant associations possibly being masked. Another limitation in terms of the sample population is that only about one-fourth of the study participants were AA women, which reduced power even further in race-stratified analyses. Also, late age at first birth and dose and type of hormone replacement therapy were not available in the dataset so they could not be examined as possible confounders during analyses. It is well-documented that these variables are known risk factors for breast cancer.

Study duration and intensity of our intervention are also possible limitations to consider. Our three-week intervention may not have been long enough to observe a significant shift in 2:16α-OHE ratios; nonetheless, this seems to vary by study. A three-month study by Morrison et al. (2009) observed a significant overall mean improvement of 168% in 2:16α-OHE among 13 women with addition of 3.6 grams of powdered organic Brussels sprouts and organic kale to their diet ($p=0.01$); however, this was in premenopausal women. Fowke et al (2000) demonstrated similar results in 34 postmenopausal women but the intervention was over a 4-week period to increase *Brassica* consumption to at least 70mg/day. *Brassica* vegetable consumption increased about 20-fold from baseline to after the intervention (respectively, 9g/d to 193g/d) in which 2:16α-OHE ratio statistically significantly increased from 2.27 to 2.38 when adjusted for water-soluble fiber, protein, energy and social approval score (Fowke et al. 2000). Our study was similar in duration to Fowke et al. 2000 but *Brassica* vegetable

consumption only increased about 6-fold during our intervention, which is a smaller increase than the 20-fold increase Fowke et al. 2000 observed. Therefore, we cannot exclude the possibility that a more intense intervention would have observed a larger effect.

Other common limitations deal with different types of biasness. Attrition bias is a concern among randomized clinical trials. In this present study, 96 women were initially randomized into either the intervention or control group (51 intervention and 45 controls); however, by the time the study was completed, there were only 64 women (34 intervention and 30 controls) with complete data. Also, information bias is possible in terms of reporting dietary intake through the 24-hour recalls used in this study. One possible reason is due to the detail about the intake relying heavily upon the participant's memory. Lastly, there may be a reporting effect related to social desirability where participants may over-report the intake of healthy foods and under-report alcohol, high calorie and high fat foods in hopes of pleasing the interviewer. However, though 24-hour recalls do have these limitations, this study tried to control for some of them by having trained dietitians administering the recalls, and administering multiple recalls on non-consecutive days in order to obtain a representative sample of the participant's actual dietary intake.

One of the major strengths of this study, as well as other randomized clinical trials, is minimized allocation bias due to the randomization of participants into study groups. This allows the investigator to isolate and quantify the effect of the actual intervention that is being conducted, and decreases the chance of confounding by other factors. Furthermore, this allowed for the manipulation of our exposure, increase

Brassica vegetable consumption, by women being randomized to the intervention or control group in which dietary habits were supposed to remain unchanged. Another strength is that a number of potential confounders were assessed in order to determine which ones would be controlled for in the final estrogen metabolite models. Covariates included in the models included marital status, breast cancer survivorship status, age, and baseline percent fat mass in the 2-OHE models and baseline percent fat mass in the 16α-OHE models.

To our knowledge, this is one of few studies to examine the effects of *Brassica* vegetables on the 2:16α-OHE ratio among postmenopausal AA women and breast cancer survivors. Although the results of this present study are not generalizable to the population as a whole due to demographic differences, the information observed can be used by other studies, those involved in preventive cancer programs, and by clinicians.

In summary, these finding suggests that *Brassica* vegetables do not play a role in the modification of 2-OHE and 2:16α-OHE metabolite levels among postmenopausal women in a short intervention. These findings highlight the need for future research to further understand the biological mechanism between estrogen metabolites and *Brassica* vegetable consumption because it could lead to a possible preventive measure to not only reduce the risk of breast cancer and other comorbidities among women in general but to also reduce the health disparity that exist between AA and EA women.

Future Research

Future research is needed to examine whether 2-OHE, 16α-OHE and 2:16α-OHE metabolite levels can be modified through increased *Brassica* vegetable consumption in hopes that they could play a vital role in reducing the risk of breast cancer among all

women. To improve the current study, a larger randomized clinical trial to determine if 2-OHE, 16α-OHE and 2:16α-OHE levels can be modified by increased *Brassica* vegetable consumption is one recommendation. Increasing the sample size would help to increase power to observe significant associations. Furthermore, it is vital to close the health disparity gap that exists between AA and EA women, so increasing the percentage of AA women in a study like this could also improve the study design. Determining the amount of *Brassica* vegetables and specific types of *Brassica* vegetables needed to observe a change in estrogen metabolite levels would also be considered beneficial. Other possible confounders that were not specifically analyzed included age at first birth and medications, in particular hormone replacement therapies and supplements. Women were asked to maintain a stable, fixed dose of any medications (including hormone replacement therapy) and supplements throughout the study period but having data to adjust during analyses could be useful. Lastly, further research of these estrogen metabolite levels in different racial groups may be influenced not only by lifestyle and environmental factors but by genetic factors as well. It is highly possible that AA and EA women, as well as other races, have variations in the *CYP1A1* and *CYP1B1* genotypes that could maybe explain why the overall levels of 2-OHE and 16α-OHE metabolite levels were observed to be lower in AA women in this study.

CHAPTER VI

CONCLUSION

Research on the effects of *Brassica* vegetable consumption on the 2:16α-OHE metabolite ratio in randomized clinical settings is still sparse; in addition, the effect of *Brassica* vegetables on the estrogen metabolite ratio among AA women and breast cancer survivors has not thoroughly been examined. This study provided an opportunity to further explore the effects of *Brassica* vegetable intake on the 2:16α-OHE metabolite ratio among postmenopausal EA and AA women. *Brassica* vegetables have been linked to lowering the risk of other types of cancer, in particular lung and colorectal cancer, in some epidemiological studies; therefore, the research on this topic is critical (Kim et al. 2009). The 2:16α-OHE metabolite ratio can be potentially modified through lifestyle factors like diet. It is vital to further understand the biological mechanism of the link between *Brassica* vegetables and breast cancer risk. Subsequently, this may help to inform future interventions designed to reduce the risk of breast cancer; moreover, also help in closing the health disparity that exists between AA and EA women.

Although several studies have shown that increasing the number of servings of *Brassica* vegetables can decrease the 2:16α-OHE ratio, this study found no significant differences in either the 2-OHE or 2:16α-OHE metabolite levels after an intense three-week *Brassica* intervention. Furthermore, this lack of association was not modified by

any of the potential anthropometric, demographic, or dietary intake characteristics. When controlled for baseline estrogen metabolite levels, the intervention group was found to have higher levels of 2-OHE levels than control group, adjusted for marital status, breast cancer survivorship status, age and baseline percent fat mass; however, this difference was statistically non-significant. It was observed that AA women compared to EA women, overall had lower levels of 2-OHE and 16α-OHE throughout the study but there was no difference in the ratio of the estrogen metabolites between ethnic groups. Similarly, differences by breast cancer status were observed whereby 2-OHE levels remained significantly higher among breast cancer survivors compared to disease free women after adjusting for marital status, age, and baseline percent fat mass.

Future research is needed to determine whether a larger increase in *Brassica* vegetables could affect estrogen metabolites and to examine whether genes may modify the effect of *Brassica* vegetables on estrogen metabolites.

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