Relationship of dietary antioxidant intake, antioxidant serum capacity, physical activity and inflammation in breast cancer survivors and individuals without a history of cancer.

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SCIENTIFIC ABSTRACT

Background: Dietary and serum antioxidants and physical activity can effect inflammation, which is associated with breast cancer risk and recurrence. This study investigated the relationship between diet, serum antioxidant capacity, physical activity, and inflammation in breast cancer survivors and individuals without cancer.

Methods: Existing demographic, dietary intake, and physical activity data of 78 breast cancer survivors and 30 individuals without cancer from the Day and Night Study conducted at Virginia Commonwealth University were used. Participants were recruited from southern Virginia. Metabolic equivalents were calculated through type, intensity, and duration of physical activity. Dietary antioxidant intake (FRAP) was calculated from Harvard Food Frequency Questionnaire data. Serum samples were analyzed for inflammation (hsCRP,IL-6,IL-1,and TNFalpha) and serum antioxidant capacity (ORAC) at Virginia Tech.

Results: Anthropometrics and inflammation were higher, and FRAP and ORAC lower in breast cancer survivors compared to individuals without cancer, although not significant. There was a significant direct relationship between FRAP and ORAC and inverse relationship between

FRAP and hsCRP. Breast cancer survivors 6+ years since diagnosis showed significant direct FRAP and IL-1 association, and inverse ORAC and TNF-alpha association. BMI was directly associated with IL-6 and CRP. Inflammation was not associated with METs or weekly activity, although there was an increasing inverse relation between METs, IL-1 and TNF- α with increasing ORAC.

Conclusion: There is a significant relationship between dietary antioxidant intake and serum antioxidant capacity and inflammation. Increased body mass index increases inflammation. Diets high in antioxidants and maintaining a healthy weight may help reduce inflammation in breast cancer survivors.

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GENERAL ABSTRACT

Background: Dietary and serum antioxidants and physical activity can effect inflammation, which is associated with breast cancer risk and recurrence. This study investigates the relationship between diet, serum antioxidant capacity, physical activity, and inflammation in breast cancer survivors and individuals without cancer.

Methods: Demographic, dietary intake, and physical activity data of 78 breast cancer survivors and 30 individuals without cancer from the Day and Night Study conducted at Virginia Commonwealth University were used. Participants were recruited in southern Virginia. Metabolic equivalents, a measure of physical activity, were calculated from type, intensity, and duration of physical activity. Dietary antioxidant intake (FRAP) was calculated from Harvard Food Frequency Questionnaire data. Serum samples were analyzed for inflammation (hsCRP, IL-6, IL-1, and TNF-alpha) and serum antioxidant capacity (ORAC) at Virginia Tech.

Results: Anthropometrics and inflammation were higher in breast cancer survivors while FRAP and ORAC were lower. Significance existed between dietary antioxidant intake and serum antioxidant capacity as well as dietary antioxidant intake and hsCRP. Higher body mass index was associated with increased inflammation. Breast cancer survivors 6+ years since diagnosis

with higher dietary antioxidant intake had lower IL-1, and with serum antioxidant capacity and TNF-alpha.

Conclusion: In this population there is a significant relationship between dietary and serum antioxidant capacity, as well as dietary antioxidant capacity and hsCRP. In breast cancer survivors 6+ years since diagnosis there are significant associations in antioxidant capacity and inflammation. This is evidence that dietary antioxidants and maintaining a healthy weight can help reduce inflammation.

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Abbreviations

hsCRP: High sensitivity C - reactive protein

IL-1: interleukin 1 β

IL-6: interleukin 6

TNF- α: tumor necrosis factor alpha

FRAP: Ferric Reduction Antioxidant Power

ORAC: Oxygen Radical Absorption Capacity

MET: Metabolic equivalent for physical activity

I. Introduction

Breast cancer is the second leading cause of death in women in United States, after lung cancer.[1] Approximately 268,670 new cases of invasive breast cancer will be diagnosed in 2017, resulting in 41,400 deaths annually.[1] Evidence in the literature suggests that lifestyle factors, such as diet and exercise, can reduce breast cancer risk and risk of breast cancer recurrence in part by reducing inflammation.[2-4] Breast cancer patients and survivors experience increased levels of inflammation as a response to cancerous tumor development, which can increase breast cancer progression by promoting the development of cancer cells.[5,6] Inflammatory biomarkers in breast cancer patients are increased, including interleukin 1β (IL-1β), IL-6, TNF- α, and hsCRP.[79] Diet and physical activity can reduce inflammation. Higher antioxidant intake reduces inflammation by the mechanism of scavenging for free radicals which cause oxidative damage.[5,6] Moderate physical activity reduces inflammation through improving insulin sensitivity.[5,6]

This study seeks to build upon the evidence in the literature around the relationship between diet, physical activity, antioxidant capacity, and inflammation in breast cancer survivors, and to validate existing findings in a population of breast cancer survivors in Southwest Virginia. Specifically, this study: (1) Compared dietary antioxidant intake and serum antioxidant capacity and inflammatory states between breast cancer survivors and non-survivors (2) Evaluated and compared the relationship of dietary antioxidant consumption and serum antioxidant capacity in breast cancer survivors and non-survivors, (3) Evaluated the relationship of dietary antioxidant consumption and serum antioxidant capacity to inflammation, and (4) Investigated the relationship between physical activity and inflammation and the moderating effect of antioxidant consumption, antioxidant capacity, and cancer status.

II. Background: Summary of Literature

Lifestyle Behaviors and Breast Cancer Risk: Non-modifiable factors

Non-modifiable factors and modifiable factors can influence the risk of developing breast cancer. Non-modifiable factors include genetic mutations, age, family history, race, age, and gender. Cancer is more likely to develop when a mutation of the gene no longer suppresses abnormal cell growth, and genetic mutations account for 5-10% of breast cancer diagnoses.[10,11] Breast cancer can be inherited through mutations of the genes BRCA1 and BRCA2 which are tumor genes.[12] Carriers of one or both of the BRCA genes have a 45-55% increased likelihood of developing breast cancer by age 70 than people who do not.[10,13,14] The STK11, PTEN and TP53 genes also are cell growth regulating genes, so mutations in these genes are associated with an increased risk of developing breast cancer.[15-17] In fact, the lifetime risk of breast cancer in women with PTEN gene mutations is 85%.[15-17] Similarly, CHEK2 is a gene which codes for a protein which suppresses cell growth, and mutations in this gene can result in a two-fold increased risk of developing breast cancer.[17-19] TP53 is a gene which terminates tumor growth, mutations in this gene account for up to 40% of genetic causes of breast cancer diagnoses.[17,20] The ATM, MRE11A, RAD50, RAD51C, PMS2, CDH1, and BRIP1 genes are responsible for repairing DNA damage and mutations in these genes are associated with an increased risk of developing breast cancer.[15-17] Female carriers of mutated CDH1genes have a 39-52% increased risk of developing breast cancer.[17]

Between 10-15% of breast cancer patients have a family history of breast cancer. A person with a first-degree relative with breast cancer doubles the risk of breast cancer for a woman. Two first-degree relatives increases breast cancer risk by 3 times.[12] Ethnicity also affects the lifetime risk of developing breast cancer. White women in the U.S. have the highest

incidence of breast cancer at 13% and American Indian women have the lowest incidence of breast cancer at 8% compared to other races. The incidence rate for Black women and Asian women is 11% incidence, and Hispanic women have a 10% breast cancer incidence.[21] Breast cancer risk is highest in women over the age of 40.[22] Women younger than 40 only account for less than 5% of breast cancer diagnoses.[11,22] The median age for women developing breast cancer in the U.S. is 62, the median age for men is 68[22]. Older age contributes to the increased risk of breast cancer because of increased exposure to endogenous estrogen, which is discussed in more depth below.

While breast cancer is more common in women, men can also develop breast cancer, although the likelihood for a man to develop breast cancer is 1 in 1,000, which is 100 times less than the risk of a woman developing breast cancer.[23] Men with a family history of breast cancer or men that carry the BRCA 1 or 2 genes have an increased risk of developing breast cancer.[24-27] Men with Klinefelter's Syndrome, a genetic condition in which a man is born with two X chromosomes and one Y chromosome, have a 20-50% greater risk of developing breast cancer than men who do not.[24] Obese men have a higher chance of developing breast cancer, due to the excess adipose tissue increasing endogenous estrogen levels which is associated with an increased risk of breast cancer.[10,27-29] Men with gynecomastia, a condition which results in enlarged breast tissue, also have an increased risk for the same reason.[27-29]

Non-modifiable factors: Drugs, Physical activity

Modifiable risk factors for breast cancer risk include alcohol intake, tobacco use, diet, and physical activity. Women who consume 2 to 5 alcoholic beverages daily have about 1½ times the risk for breast cancer as women who don't drink alcohol.[12] A meta-analysis found that

144,000 breast cancer cases and 38,000 breast cancer deaths worldwide in 2012 were associated with alcohol use.[30] The American Cancer Society's Cancer Prevention Study II Nutrition Cohort is a prospective study of over 1.2 million participants, and was established in 1982 to investigate preventive measures against cancer.[31] A 13 year follow up found that the rate of new breast cancer cases was 24% higher in smokers than in nonsmokers and 13% higher in former smokers than in nonsmokers.[32]

Physical activity and an antioxidant rich diet are effective at reducing the risk of breast cancer in part through reducing inflammation.[33,34] A meta-analysis by the American Institute of Cancer Research including 31 studies with 63,786 cases found that 30 minutes of vigorous physical activity daily reduced risk of breast cancer by 17% in premenopausal women[35] and 10% in postmenopausal women.[36] An observational study done by the National Institute of Health found that 1 hour of moderate physical activity per day decreases breast cancer risk by 16%.[37] To reduce the risk of breast cancer, the American Cancer Society recommends at least 150 minutes of moderate intensity or 75 minutes of vigorous intensity activity each week.[12]

Non-modifiable factors: Diet, Weight

A diet rich in fruits and vegetables containing alpha and beta-carotene[38] are associated with reduced risk of breast cancer. The European Prospective Investigation into Cancer and Nutrition cohort consisting of 1502 breast cancer cases and 1502 individuals without cancer found an inverse relationship between carotene consumption and development of breast cancer tumors.[38] In a prospective cohort from the Nurses' Health Study participants, premenopausal women consuming five or more servings of fruits and vegetables per day had a 23% lower risk of breast cancer than women consuming less than two servings per day.[39] Additionally, in women with a family history of breast cancer, women consuming the highest amounts of alpha

and beta-carotene, and vitamins A and C from food and supplements, had a 53%-63% lower risk of breast cancer.[39]

Cross-sectional survey data from Chinese-American and Non-Hispanic White breast cancer survivors in California, found significant associations between cruciferous vegetable intake with a decrease in menopausal symptoms ($p<0.04$).[40] Menopausal symptoms in breast cancer survivors are side effects from chemo and radiation therapy resulting in lower estrogen levels.[41] Chinese-American women that reported consuming cruciferous vegetables also had significantly reduced symptoms of hair thinning or hair loss ($p<0.006$).[40] Non-Hispanic White women that consumed cruciferous vegetables had significantly reduced symptoms of memory loss ($p<0.03$).[40] A dietary questionnaire administered in over 2000 Swedish women found an association between cruciferous vegetable consumption and lower breast cancer risk.[42]

There is limited evidence that foods containing calcium significantly reduces the risk of developing breast cancer.[36] The 2017 American Institute for Cancer Research report included a meta-analysis of 5 studies which concluded that consuming 300mg of calcium per day has the potential to decrease breast cancer significantly by 13%.[36]

Several mechanisms have been proposed for the role diet and physical activity play in breast cancer risk, one of which is its impact on weight. Excess weight is a risk factor for breast cancer, particularly in post-menopausal women. Overweight and obese post-menopausal women have a 30-60% increased risk of developing breast cancer.[12] However, premenopausal women that are slightly overweight have a reduced risk of developing breast cancer, although the mechanism for this difference is not established in the literature.[10] A BMI of 25-30 is considered overweight, and a BMI of 30 or more is considered obese and associated with excess adipose

tissue.[43] Excess adipose tissue results in an increase of endogenous estrogen in men and postmenopausal women, and increased insulin resistance. Estrogen increases the expression of insulin receptor substrate-1.[44] Insulin is a growth hormone which accelerates the cell cycle in all cells, including cell cycle progression and DNA synthesis in breast cancer cells.[45] Insulin resistance is also associated with chronic inflammation, through elevated levels of hsCRP and IL-6.[46-48] Chronic inflammation causes DNA damage which can consequently cause cancer cell initiation.[49]

Hormone and Contraceptive use

The Women's Health Initiative is a prospective study founded in 1993 dedicated to investigating prevention strategies for breast cancer and other chronic diseases in postmenopausal women.[50] A study done by the Women's Health Initiative found that women with increased blood insulin levels were twice as likely to develop breast cancer than women with normal blood insulin levels.[51] Women with early onset of menstruation[52] and late onset of menopause[53] have a longer lifetime number of menstrual cycles and exposure to estrogen, and have a higher risk of breast cancer. Pregnancy has the opposite effect, decreasing breast cancer risk since the lifetime number of menstrual cycles is reduced, which also reduces exposure to endogenous estrogen.[54] A higher number of births decreases breast cancer risk. Women who have given birth to five or more children have a 50% reduced risk of developing breast cancer compared to women who have not given birth to any children.[55] Women who have had their first pregnancy at a younger age also have a decreased risk of developing breast cancer. Women who had their first pregnancy before age 20 had a 50% lower risk of developing breast cancer compared to women who had their first pregnancy after the age of 30.[56] Breastfeeding for at least

12 months is a pregnancy related factor which reduces breast cancer due to reducing the lifetime number of menstrual cycles, as well.[52,57]

Contraceptives that use the hormones estrogen and progesterone combined increases breast cancer risk.[58] Oral contraceptive use slightly increases the risk of breast cancer due to early expression of the BRCA 1 gene in carriers of the gene, especially under the age of 25[59], putting BRCA1 carriers at a higher risk while using oral contraceptives. However, once the pills are stopped, the risk goes back to normal within 10 years.[12] Similarly, women currently using birth-control shots have an increase in breast cancer risk, although 5 years after stopping the shots, there is no increased risk. The American Cancer Society suggests that there is some evidence that birth control implants, IUD, skin patches, and vaginal rings can increase breast cancer risk due to the hormones released.[12] Hormone replacement therapy also poses a risk for breast cancer. Risk of developing breast cancer increases with increasing years of use of hormone replacement therapy containing estrogen and progesterone.[60,61] Using hormone replacement therapy for 15 years or more increases risk of developing breast cancer by 36%.[62]

Tamoxifen is a selective estrogen receptor modulating drug which blocks estrogen receptors in breast tissue.[63] Consequently, it is prescribed to breast cancer patients which have hormone-positive breast cancer, which are breast cancer cells with estrogen receptors.[63] Tamoxifen may also be prescribed to women at a high risk of developing breast cancer due to family history or carriers of genes which are associated with breast cancer.[31] Studies have found that 5 years of tamoxifen treatment reduces the risk of developing breast cancer in women with no breast cancer and also reduces recurrence of breast cancer in breast cancer patients and survivors by over 50%.[63-68]

Relationship of dietary antioxidant intake, antioxidant serum capacity and breast cancer risk

There is evidence that antioxidants, particularly vitamins C and E, may reduce risk of breast cancer in the general population as well as decrease recurrence and increase breast cancer survival. In the general population, the recommended daily allowance of vitamin C for adult women is 75 mg[69-71], and 15 mg of vitamin E.[70,72] Both vitamins are antioxidants, which reduce the levels of oxidative free radicals which damage cells. Vitamins also enhance immune function.[69-72]

Vitamin C in the diet has the potential of breast cancer risk reduction. The Nurses' Health Study is a cohort of over 80,000 women 33-60 years old followed until death, their diet and other lifestyle and health factors are monitored through a biyearly questionnaire.[73] Premenopausal women with a family history with breast cancer that had an average intake of 205 mg/day of dietary vitamin C had a 63% lower risk of developing breast cancer opposed to women consuming an average of 70 mg vitamin C per day.[74] A vitamin C intake of at least 80–110 mg/day is significantly associated with a lower breast cancer risk.[75-77]

The Mediterranean Diet is a diet which promotes the intake of minimally processed whole grains, legumes, fruits, vegetables, seafood, and unsaturated fats, and is high in vitamins including C and E.[78] The Mediterranean Diet was associated with a significant (p-value = 0.008) decreased risk of developing breast cancer in a population of over 3000 Italian and Swiss breast cancer patients and matched controls without breast cancer.[79] There were similar results in postmenopausal women in the Netherlands Cohort study of over 2000 women, a significant inverse association between Mediterranean diet score and ER-negative breast cancer (p= 0.032), and a non-significant association with ER-positive breast cancer and other breast cancer.[80] The

Swedish Women's Lifestyle and Health cohort validated these results as well, a decreased breast cancer risk was associated with a higher Mediterranean diet score.[81] The European Prospective Investigation into Cancer and nutrition cohort of over 10,000 women had similar results as well, significant inverse associations between the Mediterranean diet and overall breast cancer risk in all women ($p= 0.048$) and in postmenopausal women ($p = 0.037$).[82] In premenopausal women, a British cohort of 34,000 women established an inverse association with the Mediterranean diet and breast cancer incidence.[83] Based on a meta-analysis of these 5 cohort studies, a marginally significant 6% reduced risk of postmenopausal breast cancer was established (RR = 0.94, 95% CI, 0.88–1.01).[80]

The association between a Mediterranean diet with a reduced risk of breast cancer also exists in other ethnic groups. A case-control study of over 1000 Asian American women associated a significant inverse association with Mediterranean diet and breast cancer risk ($p = 0.009$).[84] A Spanish case-control study of 1000 cases as well had a lower breast cancer risk for women with higher Mediterranean diet scores ($p= 0.01$), and a significant 11% lower risk with every one standard deviation increase in the diet score.[85]

Dietary antioxidant measures and recurrence in breast cancer survivors

Dietary intake data can be used to calculate measures of antioxidant capacity in the diet through FRAP (Ferric Reduction Antioxidant Power). Dietary FRAP is a composite score of the individual FRAP scores of foods consumed in the diet. A dietary FRAP compendium containing the known FRAP scores of specified quantities of over 3,000 foods, and food frequency survey information is used to calculate the score.[86] There is evidence that dietary antioxidant intake can impact breast cancer risk. The Rotterdam study conducted in the Netherlands which administered food frequency questionnaires to 3200 women investigated

dietary FRAP scores and antioxidant intake (i.e., vitamin A, C, E, selenium, flavonoids and carotenoids). During a median follow up of 17 years, 199 cases with breast cancer were identified. Incident cases of breast cancer were confirmed through medical reports. High dietary FRAP score was associated with a lower risk of breast cancer.[86] Low intake of alpha and betacarotenoids and high selenium intake were associated with a higher risk of breast cancer. Individual vitamin C and E intakes did not have a significant association with breast cancer risk.[86]

A meta-analysis done in 2014 of 10 studies encompassing 17,696 breast cancer cases and 1558 breast cancer-specific deaths showed that dietary vitamin C intake was significantly associated with a reduced risk of total mortality and breast cancer-specific mortality in breast cancer patients and survivors.[87] Another retrospective cohort study done in Utah and California using the Life After Cancer Epidemiology (LACE) cohort investigated the efficacy of vitamin C and E supplementation among breast cancer survivors, along with other supplements, and the risk of breast cancer recurrence and mortality.[2] Participants who reported frequent use of vitamin C and vitamin E had a decreased risk of breast cancer recurrence. In contrast, frequent use of combination carotenoid supplements were associated with increased risk of death from breast cancer and all-cause mortality.[2] In a retrospective cohort study done in Germany, breast cancer patients receiving 7.5 g of vitamin C intravenously during chemotherapy had a significant improved performance during and after cancer treatment compared to patients not receiving the vitamin C supplementation.[88]

The Iowa Women's Health Study is a prospective cohort study of cancer survivors. 45.7% of women in the cohort have breast cancer.[89] Participants consuming a diet containing over the recommended daily allowance of vitamin E in addition to taking vitamin E supplements

and a multivitamin had a lower mortality risk.[89] A Canadian study involving over 2000 breast cancer incident cases and 2000 matched controls determined that there is a significant negative association between 10 or more years of vitamin E supplementation and breast cancer ($p<0.05$) in postmenopausal women.[90] The Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial is another prospective cohort, where participants aged 55-74 without cancer were assigned to a cancer screening intervention or standard care between 1993 and 2001.[91] A nested case-control study using the PLCO cohort involved 621 postmenopausal invasive breast cancer cases and 621 matched controls to investigate the association of vitamin E supplementation with new incident cases of breast cancer.[91] The dietary questionnaire tool assessed the usual supplement intake of vitamin E at baseline in 1993, and within the past 2 years. α-tocopherol vitamin E supplementation was associated with a significant reduced risk in developing breast cancer while δ- and γ-tocopherol vitamin E supplements were associated with a risk of developing ER+ breast cancer.[91]

Antioxidant Intake and Inflammation in Breast Cancer Populations

Antioxidant supplementation has been shown to reduce inflammation in breast cancer patients. A study done in India found that in breast cancer patients receiving 500mg of vitamin C and 400 mg of vitamin E oral supplementation daily during breast cancer treatment, activities of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione were significantly increased ($P < 0.01$) while the levels of malondaldehyde and DNA damage were significantly reduced($P < 0.01$).[92] Intravenous Vitamin C during treatment in breast cancer patients was also found to reduce inflammatory cytokines including IL-1α (by 20%), IL-2 (by 20%), IL-8(by

20%), TNF-α, chemokine exotoxin (by 25%), and CRP (by 20%).[93] Vitamin E supplementation was associated with reduced levels of CRP in the Health, Eating, Activity, and Lifestyle Study, which is a multiethnic prospective cohort study of breast cancer patients.[94]

There is evidence that dietary intake can influence inflammation, evident through the Dietary Inflammatory Indices (DII) of 45 food items. A higher DII score indicates a proinflammatory diet while a lower DII score indicates an anti-inflammatory diet, which is higher in vitamins including C and E.[95] Participants from the Women's Health Initiative cohort were grouped in 5 categories based on DII score. Comparing the group with the highest score to the group with the lowest score, in the group with the highest score CRP was 2.34 times higher, TNFα was significantly higher ($p < 0.0001$), and the overall inflammatory biomarker score was significantly higher ($p < 0.0001$).[96] The overall inflammatory biomarker score included IL-1β, IL4, IL-6, IL-10, TNFα, and CRP. The relationships of dietary antioxidant intake and inflammation were explored in a cross-sectional analysis of breast cancer survivors. Participants' dietary intake were assessed by the Heathy Eating Index 2010 and their blood serum was analyzed for inflammatory cytokines. A higher Heathy Eating Index score was associated with decreased levels of IL-6 ($r=-0.46$, $P=0.002$) and TNFR-2 ($r=-0.41$; $P=0.006$).[97] In participants with a history of chemotherapy, a significant inverse association existed between Healthy Eating Index score with IL-6 ($r=-0.67$, $P=0.009$) and TNFR-2 ($r=-0.59$, $P=0.03$). In a study with a 2 year intervention of the Mediterranean diet in a population of people with metabolic syndrome, there was a reduction of CRP ($p<0.01$) and IL-6 ($p<0.02$).[98]

Dietary and Serum Antioxidant Capacity and Inflammation

In addition, there are lab measures used to calculate antioxidant capacity in foods and biological systems. Serum antioxidant level can be measured through the methods of FRAP and

ORAC (Oxygen Radical Absorption Capacity) assays. FRAP (Ferric Reduction Antioxidant Power) determines the electron-donating ability of antioxidants based on the reduction of ferric iron[99], while ORAC assesses the hydrogen-providing ability of antioxidants which is measured through the loss of a fluorescent compound reacting with hydrogen. FRAP and ORAC assays can be used to measure antioxidant capacity in foods and in serum, and yield comparable measures. A study estimating antioxidant activity in guava fruit, blueberries, and other crops found a high, positive correlation between FRAP and ORAC assays ($0.68<r<0.97$, $P< 0.01$).[100] In human serum, antioxidant testing from 45 men and women revealed a weak significant linear correlation between serum ORAC and serum FRAP ($r=0.349$, $P = 0.019$).[99] The study concluded the ORAC assay as more relevant because it utilizes a biologically relevant radical source.

There is evidence for lower serum antioxidant capacity in breast cancer survivors compared to controls. A study at the University Malaya Medical Center found that serum FRAP levels were significantly lower in breast and colorectal cancer patients compared to controls without cancer ($P <0.001$).[101] A separate study comparing newly diagnosed breast cancer patients and controls without cancer in Algeria measured serum antioxidant capacity (i.e. ORAC) and found it was significantly lower in breast cancer patients as well[102]. However, in a study done with a Caucasian breast cancer patients recently diagnosed within 1 year, serum FRAP levels were significantly higher in patients who supplemented with vitamin C compared to matched controls without cancer who did not supplement ($p = 0.006$).[103]

This thesis also seeks to explore the relationships between serum antioxidant capacity and inflammation. An observational study conducted at the University of Glasgow investigated the relationship between CRP and vitamin level in fasting blood serum of breast cancer patients, prostate cancer patients, lung cancer patients, and healthy controls without cancer.[104] Cancer

patients had significantly elevated CRP levels (P<0.001) and lower serum vitamin-antioxidant levels (P<0.001), including serum vitamin E, than the controls without cancer.[104] In the population of Korean women, lower levels of serum total antioxidant capacity were associated with significantly lower levels of IL-1 and IL-6 (P<0.0001).[105] Additionally, the serum total antioxidant capacity was significantly increased with increased dietary and supplementary vitamin A and C intake (P<0.0077).[105] There is limited evidence on the relationship between inflammation level with serum ORAC and FRAP levels, which is an objective of this thesis.

There is limited evidence for an association between dietary FRAP and serum ORAC, in studies designed with a small sample size of test subjects without breast cancer. A study in a sample of 8 elderly women explored serum FRAP and ORAC levels following ingestion of strawberries, spinach, red wine, or vitamin C.[106] Two women were assigned to ingesting each type of food, all of which are on the FRAP index. Four hours after ingesting the food, the women had increased serum FRAP ($p < 0.04$) and ORAC ($p < 0.016$) scores.[106] The significant increase in serum FRAP level was 7-12%, the significant increase in ORAC level was 7%.[106] A similar study investigated the effects of an antioxidant rich beverage in 5 German men showed an increase in serum FRAP from the mean baseline level of 1.41 mmol/L to approximately 1.50 – 1.70 mmol/L.[107] However, this increase of FRAP level was not considered significant. An objective of this thesis is to investigate the relationship of dietary FRAP and serum ORAC.

Measures of Inflammation and Normal Values

CRP, IL-6, IL-1, and TNF-α are biomarkers elevated in states of inflammation. TNF-α is created by white blood cells during acute inflammation and is responsible for apoptosis, or cell death.[108] IL-6 and IL-1 are made by leukocytes as an immune response to inflammation. IL-1 is also responsible for generating new blood cells through hematopoiesis[109] while IL-6 mediates

creation of antibodies through its role in B-cell differentiation.[109,110] CRP is an acute phase protein created by the liver during inflammation.[111] In cancer survivors, the median levels of these biomarkers in scientific studies range between 2.7-5.7 pg/mL for IL-6[112-115], 3.48 pg/mL for IL-1[116], 3.1-6.4 mg/L for CRP[94,112,117], and 0.69-23.50 pg/mL for TNF-α.[116] Median levels of inflammatory markers in generally healthy women without breast cancer or other chronic or acute diseases range between 1.79-5.80 pg/mL for IL-6[118,119], 3.1 pg/mL for IL-1[120], 0.30-0.34 pg/mL for CRP[119,121], and 2.74-7.8 pg/mL for TNF-α.[122,123]

Physical Activity and Inflammation in Breast Cancer Populations

Only about 30-35% of breast cancer survivors meet the American College of Sports Medicine guidelines of 150 minutes per week of moderate physical activity.[4,124] In fact, a study done in North Carolina found that 59% of cancer survivors decreased their physical activity after breast cancer treatment due to physical fatigue post-treatment.[124] There is evidence that physical activity can reduce inflammatory markers in breast cancer survivors. In a randomized control trial with breast cancer survivors, an intervention of 150 min/wk of moderate intensity aerobic exercise resulted in a significant reduction in the inflammatory cytokine IL-6 among exercisers who reached 80% of the intervention goal compared with the control group which did not have an exercise intervention.[125] Another randomized control trial of breast cancer survivors with an intervention of exercise on cycle ergometers three times per week for 15 weeks found that exercise decreased the inflammatory marker CRP and cholesterol levels and increased natural killer cell cytotoxic activity and unstimulated [3 H]thymidine uptake by peripheral blood lymphocytes, which is a measure of increased immune function.[126] Resistance training has also been found to reduce inflammation in breast cancer survivors. In a 16-week resistance training program, Resistance training decreased NK and NKT cell expression of TNF-α ($p = 0.005$)

indicating improvement of the inflammatory profile in breast cancer survivors.[3] A systematic review in 2012 found that increased inflammation measured by increased IL-6 and IL-1 contribute to greater fatigue in breast cancer patients undergoing radiation and chemotherapy[127], leading to the hypothesis that decreased inflammation through increased physical activity may improve fatigue.

Considering the evidence in the literature on dietary antioxidant, vitamin C, vitamin E, and physical activity associated with reducing inflammation and improving outcomes of breast cancer survivors, this study seeks to further investigate the associations between diet, physical activity, inflammation, and cancer history in a rural population of breast cancer survivors. Exploring the gap in research between dietary FRAP and serum ORAC is also an objective of this study, as well as investigating the relationship of dietary FRAP and serum ORAC with inflammatory markers.

III. Objectives, Specific Aims, Hypotheses

Based on the findings in the literature that point to increased inflammation as a factor in breast cancer development and survival outcomes, and the impact of dietary antioxidant intake and physical activity on inflammation, this study proposes the following specific aims, rationale, and hypotheses:

Specific Aim 1: Compare dietary and serum antioxidant capacity and inflammatory states between breast cancer survivors and individuals without a history of cancer.

Specific Aim 1a: Compare the dietary intake of vitamins C & E, carotenoids, flavonoids, and FRAP scores between cancer survivors and individuals without a history of cancer.

Specific Aim 1b: Compare the levels of the inflammatory biomarkers, IL-1, IL6, TNF-α, and hsCRP between cancer survivors and individuals without a history of cancer.

Rationale: Evidence in literature presented slightly lower median values of inflammatory markers in women without cancer than in cancer survivors. Based on the evidence that consuming antioxidants can prevent breast cancer, it is expected individuals without cancer would consume more antioxidants than breast cancer survivors.

Hypothesis: Breast cancer survivors have lower dietary antioxidant intake and serum antioxidant capacity, and higher levels of inflammation than individuals without a history of cancer.

Specific Aim 2: Compare the relationship of dietary antioxidant consumption and serum antioxidant capacity. Evaluate for differences in breast cancer survivors and individuals without a history of cancer.

Specific Aim 2a: Evaluate the relationship of dietary FRAP and vitamin C and E to ORAC scores of study participants.

Specific Aim 2b: Evaluate the impact of cancer status on the relationship of dietary FRAP and vitamin C and E to ORAC scores of study participants.

Rationale: There is limited evidence in literature on the relationships of dietary FRAP and serum ORAC but it is expected that dietary antioxidants from food on the FRAP score can effect serum antioxidant capacity.

Hypothesis: Dietary antioxidant consumption has a positive association with serum antioxidant capacity in both breast cancer survivors and individuals without a history of cancer.

Specific Aim 3: Evaluate the relationship of dietary antioxidant consumption and serum antioxidant capacity to inflammation.

Specific Aim 3a: Evaluate the relationship between dietary FRAP and serum ORAC levels to the inflammatory biomarkers IL-1, IL-6, TNF-α, and hsCRP in study participants.

Rationale: There is limited evidence that FRAP and ORAC levels effect inflammatory marker levels.

Hypothesis: Higher dietary antioxidant intake, and serum antioxidant capacity are positively associated with lower levels of inflammation.

Specific Aim 4: Investigate the relationship between physical activity to inflammation with the moderating effect of cancer status.

Specific Aim 4a: Evaluate the relationship of physical activity (METS) and BMI to the inflammatory biomarkers IL-1, IL-6, TNF-α, and hsCRP in study participants.

Specific Aim 4b: Evaluate the impact of cancer status on the relationship of physical activity (METS) to the inflammatory biomarkers IL-1, IL-6, TNF-α, and hsCRP.

Rationale: There is evidence in literature that physical activity interventions and lower BMI reduce inflammation by improving insulin sensitivity.

Hypothesis: Higher physical activity level is associated with lower levels of inflammation in both breast cancer survivors and individuals without a history of cancer, and is moderated by dietary antioxidant intake and serum antioxidant capacity.

Specific Aim 5: Investigate the relationship between physical activity and inflammation and the moderating effect of dietary antioxidant intake and serum antioxidant capacity.

Specific Aim 5a: Evaluate the impact of dietary FRAP and serum ORAC on the relationship of physical activity (METS) to the inflammatory biomarkers IL-1, IL-

6, TNF-α, and hsCRP.

Rationale: Exercise interventions and diet interventions involving antioxidant consumption in literature resulted in lower inflammation levels, so a combination of antioxidant measures and physical activity should result in lower inflammation.

Hypothesis: Higher FRAP and ORAC categories are associated with significant relationships between METs and inflammatory markers.

IV. Methods

Description of parent study

Data and biological samples collected in the Day and Night Study conducted in 20132014 at the Virginia Commonwealth University will be used for the current research project. The Day and Night study was designed to evaluate and compare diet, physical activity, and quality of life among breast cancer survivors and individuals with no cancer history. The study was approved by the Virginia Commonwealth University Institutional Review Board. Individuals were recruited from oncology hospitals in seven rural communities in the Southside and Southwest Virginia, as well as through advertising in fliers, newspaper and radio advertising. Southside Virginia refers to the region in Virginia south of the James River.

Information on demographics, cancer history, and vitamin supplementation information of participants were collected through phone interviews. Standardized surveys used included the Harvard Food Frequency Questionnaire (2007 grid)[128] and a modified physical activity questionnaire[129] which were conducted over phone interviews to collect data on usual dietary intake and physical activity of participants. The Pittsburgh Quality Sleep Index (PSQI) and Short-Form 36 Quality of Life questionnaire were also conducted through phone interviews. Self-reported anthropometrics provided by participants included height, weight, and waist circumference. Fasting blood samples were collected and processed at local laboratories contracted for that purpose. Serum, plasma, and red blood cells were separated and stored at -80 °C in cryovials.

Methods for proposed research study

The Harvard Food Frequency Questionnaire and Physical Activity Level questionnaire data from the parent study will be used for this study, as well as the demographic, anthropometrics, and medical history information. This study will analyze the stored serum samples of participants for serum ORAC, hsCRP, and inflammatory cytokine levels.

Description of surveys providing information to be used in proposed study:

Demographic, anthropometrics, and medical history

Demographic information included level of education, employment status, annual household income, health insurance, and race. Self-reported anthropometric information in the survey included participant current height, weight, and waist circumference over the phone. Information on years since diagnosis of breast cancer, treatment type for breast cancer, and last date of treatment was included on the survey. Participants were asked to report mammography results, date of diagnosis of secondary cancers, diabetes, cardiovascular disease (if applicable), and use of metformin or statins.

Dietary Intake

The Harvard Food Frequency Questionnaire is a semi-quantitative, standardized food frequency questionnaire used to evaluate usual food and nutrient intake over the past year. Questionnaires were sent to Harvard for calculation of the FRAP score and vitamin C and E score, levels of carotenoids and flavonoids, including supplementation.

Physical activity

Information of physical activity and sedentary behavior was collected through the phone interview using the modified physical activity questionnaire mentioned previously[129]. Questions

which evaluated frequency and duration of physical activity was assessed from a regular week in the participant's life. Physical activity encompassed aerobic activities, lower intensity activities, resistance exercises, walking or biking while traveling, and standing or walking at work or home. Sedentary activity questions assessed the average time per week during the past year spent sitting at work, home, or school; and sitting in a car, bus, or train.

METs, or metabolic equivalents, are calculated based on a formula, calories burned per kg per hour.[130,131] METs were calculated by multiplying the daily minutes of an activity by its corresponding MET value in the compendium of MET values.[131] The physical activities included in the total MET score were walking, running, jogging, biking, swimming, tennis, aerobic activity, yoga, other vigorous activity, arm and leg weightlifting, and standing and sitting at home and away from home. BEE is calculated according to gender, height, weight, and age.[130] The survey also queried participants' average daily hours of outdoor activity and average days of exercise per week.

Description of laboratory analyses of serum antioxidant capacity and inflammatory biomarkers

Serum samples from 78 breast cancer survivors and 30 individuals without a history of cancer that participated in the parent study were analyzed for serum antioxidant capacity (ORAC), hsCRP, IL-1, IL-6, and TNF-alpha. Samples were stored at -80 °C in cyrovials. Analyses were done at the Metabolic Phenotyping Core laboratory located at the Integrated Life Science building at Virginia Tech. Serum ORAC kit and Elisa Kits from Cell Biolabs, Inc. were used for the analyses.

ORAC analysis

The ORAC assay measures antioxidant level of blood serum through oxidation of peroxyl radicals through hydrogen atom transfer. Antioxidants in serum prevent peroxyl radical oxidation of the sample. The single value of ORAC for each sample at completion is measured through the blood antioxidant inhibition time and inhibition percentage of free radical damage.[132] 25 µL of serum samples will be added to wells along with 150 µL of the 1X Fluorescein Solution. Plate was incubated for 30 minutes at 37ºC. 25 µL of the Free Radical Initiator Solution was added to each well, then a fluorescent plate reader at 37ºC is used to read wells. The plate reader has an excitation wavelength of 480nm and an emission wavelength of 520nm. The wells were read in increments between 1 and 5 minutes for a total of 60 minutes to generate a fluorescence decay curve. The total antioxidant capacity correlates to the fluorescence decay curve, which represents the peroxyl radical antioxidant activity per sample and compared to the standard curve of vitamin E and measured in uM.[132]

Inflammatory biomarker analysis

ELISA assays supplied from Cell Biolabs Inc. were used to determine serum IL-1, IL-6, hsCRP, and TNF-alpha. ELISA procedure is as follows: 100 ul serum samples are added to the plate wells pre-coated with antibodies to the respective cytokines and will be incubated for 60 minutes at room temperature. Serum will be washed away, and Ellman's reagent, which contains conjugate antibodies and dye will be added. Plates will be incubated with the reagent for 30 minutes at room temperature, and analyzed for the degree of color change which is correlated with the amount of each cytokine in each sample. Absorbance measured on a standardized curve compared to control blanks graphed software and generated the level of each cytokine in

pg/mL.[133] The units for the inflammatory markers is as follows: pg/mL for IL-1, IL-6, and TNFalpha, and mg/L for hsCRP.

Data/Statistical Analysis

IBM SPSS was the statistical program used for all data analyses. SASS JMP was used to generate graphs in the appendix. Demographic characteristics of breast cancer survivors and individuals without a history of cancer were compared through descriptive statistics. Data on FRAP, ORAC, inflammatory markers, anthropometrics, and METs was normalized through the z-score Box Cox transformation. Anthropometrics, diet intake including calorie, macronutrient and antioxidant intake (FRAP), physical activity, and inflammatory biomarkers were compared between the two groups. The ANOVA means comparison tests were used to compare differences in dietary and serum antioxidant capacity and inflammatory states in cancer survivors and individuals without a history of cancer, while grouping for years since diagnosis. Breast cancer survivors were grouped by 1-5 years since diagnosis and 6+ years of diagnosis. This cut-off was chosen considering the effect of tamoxifen, and that tamoxifen is usually prescribed during the first 5 years post-cancer treatment.

The relationship between dietary antioxidant consumption to serum antioxidant capacity was evaluated through Spearman's nonparametric correlation analyses while controlling for diabetes, BMI (categorized as 25 or less, 25-30, and greater than 30), and years since diagnosis. Spearman correlation analyses were also used to evaluate the relationship of FRAP, vitamin C and E, and ORAC to inflammatory cytokines controlling for cancer status and BMI. The same

correlations were conducted within cancer patients grouped by years since diagnosis, 1 - 5 years and 6 or more years, as well as BMI categorization. The association of physical activity level (METs) and inflammatory markers was evaluated through Spearman correlations and grouped by years since diagnosis. The relationship of physical activity to inflammation was also evaluated with FRAP and ORAC level categories. FRAP was categorized into category 1 if the FRAP score was less than or equal to the mean of the 25th percentile, 6.36; category 2 if the score was less than or equal to the mean of the 50th percentile, 9.71; category 3 if the score was less than or equal to the mean of the 75th percentile, 16.00; and category 4 if above 16.00. ORAC was categorized into category 1 if the ORAC level was less than or equal to the mean of the 25th percentile, 102.48; category 2 if the score was less than or equal to the mean of the 50th percentile, 153.44; category 3 if the score was less than or equal to the mean of the 75th percentile, 192.08, and category 4 if above 192.08.

V. Results

Descriptives

The study consisted of 108 participants, of which 78 were breast cancer survivors and 30 individuals without breast cancer. The participant ages ranged from 22-80 years old. Overall mean age was 61. Mean age for breast cancer survivors was 60 (SD: 9.98) years old. Mean age for individuals without a history of breast cancer was 57 (SD: 12.83) years old. There was a significant difference in number of breast cancer survivors and individuals without a history of breast cancer in age ($p < 0.037$), education category 1 ($p < 0.049$) and race (African American, $p < 0.049$) (Table 1.1).

Table 1.1: Characteristics of study participants and comparisons between breast cancer survivors and individuals without cancer			
	Breast Cancer status		Significant difference
	Yes	No	
Health Characteristics			
Breast cancer survivor	78	30	N/A
Diabetes	14% (N=11)	6.6% (N=2)	0.367
Cardiovascular disease	19% (N=15)	3.3% (N=1)	0.054*
1-5 years since dx	65% (N=51)		0.155
6+ years since dx	32% (N=25)		0.155
BMI (mean, SD)	(29.55, 6.85)	(27.60 6.45)	0.210
Demographic characteristics			
Age (mean, SD)	(60.47, 9.98)	(56.93, 12.42)	0.037*
Education category 1: less than college education	24% (N=19)	6.6% (N=2)	0.049*
Education category 2: some college education	43.6% (N=34)	53.3% (N=16)	0.1634
Education category 3: college degree, higher education	32% \(N = 25)	30% (N =9)	0.569
Income: 49k or less	51% (N = 40)	40% 12	0.639
Income: 50k or greater	43.6% (N = 34)	26.6% (N = 8)	0.320
Race: white	83.3% (N = 65)	60% (N = 18)	0.347
Race: African American	16.6% (N = 13)	26.6% (N = 8)	0.049*
*= significance at the 0.05 level			

Aim 1

There were no significant differences in anthropometrics or mean dietary antioxidant consumption in breast cancer survivors and individuals without cancer, even when breast cancer survivors were grouped by years since diagnosis (Table 1.2 & 1.3). There were also no

significant differences in mean serum antioxidant capacity and level of inflammatory markers in breast cancer survivors grouped by years of diagnosis and individuals without cancer (Table 1.3 & 1.4). However, the means of inflammation and anthropometrics were consistently higher in breast cancer survivors than controls (Table 1.2 & 1.4), while dietary and serum antioxidant levels was lower (Table 1.3). Level of inflammation consistently increased with years since diagnosis of breast cancer survivors (Table 1.4.).

Table 1.2: Means comparison of anthropometrics between breast cancer survivors grouped by years since diagnosis and individuals without cancer [a]

Anthropometric measure	Individuals without Cancer Mean (SD) (N=30)	Breast Cancer Survivors		
		Total Mean (SD), (N=78)	1-5 yrs cancer dx Mean (SD), (N=51)	6+ yrs cancer dx Mean (SD), (N=25)
BMI	27.60 (6.45)	29.55 (6.85), (p = 0.210)	29.65 (6.87), (p = 0.808)	31.30 (7.33), (p = 0.998)
waist	35.93 (5.12)	38.20 (6.26), (p = 0.134)	38.22 (6.06), (p = 0.765)	39.11 (6.13), (p = 0.876)
hip	41.81 (5.07)	43.52 (6.14), (p = 0.242)	43.58 (6.13), (p = 0.288)	45.27 (7.02), (p = 0.487)

[a] = ANOVA means comparison test

Table 1.3: Means comparison of dietary and serum antioxidant levels between breast cancer survivors grouped by years since diagnosis and individuals without cancer [a]

Antioxidant	Individuals without Cancer Mean (SD) (N=30)	Breast Cancer Survivors		
		Total Mean (SD), (N=78)	1-5 yrs cancer dx Mean (SD), (N=51)	6+ yrs cancer dx Mean (SD), (N=25)
Dietary Antioxidants				
vitamin C	253.06 (351.52)	247.79 (343.70), (p = 0.956)	246.53 (345.56), (p = 0.865)	235.49 (307.56), (p = 0.880)
vitamin E	45.76 (50.15)	54.24 (90.53), (p = 0.598)	54.79 (102.57), (p = 0.725)	41.12 (39.02), (p =0.570)
flavonoids	561.41 (652.84),	485.65 (566.17), (p = 0.379)	463.00 (536.29), (p = 0.717)	512.65 (539.93), (p = 0.791)
carotenoids	14687 (18631)	15011 (35060), (p = 0.855)	14890 (39911), (p = 0.767)	15151 (29014), (p = 0.683)
FRAP	14.26 (13.54)	13.09 (13.34), (p = 0.613)	12.63 (13.44), (p = 0.638)	12.82 (9.27), (p = 0.491)
Serum antioxidants				

ORAC	149.39 (40.55)	148.45 (71.26), (p = 0.969)	145.94 (79.61), (p = 0.844)	173.09 (57.85) (p = 0.461)

[a] = ANOVA means comparison test

Table 1.4: Means comparison of inflammatory marker level between breast cancer survivors grouped by years since diagnosis and individuals without cancer [a]

Inflammatory Marker	Individuals without Cancer Mean (SD), (N=30)	Cancer survivors		
		Total Mean (SD), (N=78)	1-5 yrs dx cancer Mean (SD), (N=51)	6+ yrs dx cancer Mean (SD), (N=25)
hsCRP	4.19 (4.19)	5.65 (6.46), (p = 0.419)	5.63 (6.47), (p = 0.791)	5.94 (5.86), (p = 0.812)
TNF-a	36.14 (78.06)	66.82(126.71), (p = 0.236)	68.43 (127.95), (p = 0.714)	69.03 (128.07), (p = 0.713)
IL-6	7.45 (18.13)	15.90 (54.7), (p = 0.498)	16.31 (55.43), (p = 0.797)	10.88 (26.65), (p = 0.742)
IL-1	2.43 (1.92)	4.22 (2.40), (p = 0.136)	4.23 (6.83), (p = 0.784)	5.13 (8.37), (p = 0.742)

[a] = ANOVA comparison test

Aim 2

Associations of the relationships between dietary antioxidant consumption and serum antioxidant level were analyzed in breast cancer survivors and individuals without cancer while categorizing for diabetes, and only in breast cancer survivors when categorizing by years since diagnosis. FRAP and ORAC had a significant positive association in all participants (r_s = 0.205, p<0.032) (Table 2). FRAP had a significant positive association with vitamin C and vitamin E (Table 2), which is consistent since vitamin C and E are part of the FRAP index[86]. In all participants, the correlation between ORAC and vitamin C approached significance (r_s = 0.185, p<0.055), (Table 2).

Table 2: Relationship of dietary antioxidant consumption and serum antioxidant capacity of breast cancer survivors grouped by years since diagnosis, individuals without cancer, and participants with and without diabetes [b]

Dietary to serum Antioxidant Comparison	Full cohort			Participants w/o cancer (N=30)	Breast Cancer Survivors		
	All Participants (N=108)	Participants with Diabetes (N=13)	Participants without Diabetes (N=93)		All (N=78)	1-5 yrs dx (N=51)	6+ yrs dx (N=25)
FRAP ORAC	0.205* p < 0.032	0.423 p < 0.149	0.182 p < 0.081	0.104 p < 0.606	0.247* p < 0.029	0.259* p < 0.024	0.052 p < 0.804
ORAC vitamin C	0.185 p < 0.055	0.173 p < 0.071	0.147 p < 0.160	0.148 p < 0.463	0.174 p<0.123	0.152 p < 0.190	0.078 p < 0.709
ORAC vitamin E	0.153 p < 0.113	0.149 p < 0.128	0.117 p < 0.263	0.069 p < 0.731	0.180 p<0.114	0.122 p < 0.293	0.205 p < 0.327
FRAP vitamin C	0.573** p < 0.000	0.673** p < 0.000	0.658** p < 0.000	0.620** p < 0.000	0.668** p < 0.000	0.663** p < 0.000	0.638** p < 0.001
FRAP vitamin E	0.569** p < 0.000	0.580** p < 0.000	0.566** p < 0.000	0.639** p < 0.000	0.521** p < 0.000	0.522** p < 0.000	0.561** p < 0.004

[b] Spearman's correlation *=significance at the 0.05 level

** = significance at the 0.01 level

Aim 3

Relationships between FRAP and ORAC with inflammatory markers were analyzed in breast cancer survivors and individuals without cancer while categorizing for BMI, and only in breast cancer survivors when categorizing by years since diagnosis. In the full cohort there was a significant direct association between FRAP and hsCRP (r_s = -0.187, p < 0.051), but not between FRAP with any other inflammatory markers (Table 3). When evaluated within breast cancer survivors grouped by years since diagnosis, there was a significant inverse association between ORAC with TNF-α (r_s =-0.410, p<0.042), and direct association between FRAP with IL-1 (r_s =0.436, p<0.029) in survivors diagnosed >6 years previous (Table 3).

Table 3: Relationship of dietary and serum antioxidant level with inflammatory marker level of breast cancer survivors grouped by years since diagnosis, individuals without cancer, and BMI categories [b]

Antioxidant consumption & capacity to inflammatory markers	Participants w/o cancer (N = 30)	Full cohort				Only cancer survivors		
		All Participants (N=108)	Participants BMI < 25 (N=31)	Participants BMI: 25-30 (N=32)	Participants BMI>30 (N=43)	All	1-5 yrs cancer dx (N=51)	6+ yrs cancer dx (N=25)
ORAC IL-6	0.233 p<0.242	-0.030 p<0.750	-0.012 p<0.899	-0.148 p<0.204	-0.165 p<0.291	-0.069 p<0.551	-0.063 p<0.586	-0.294 p<0.153
ORAC IL-1	-0.020 p<0.920	0.050 p<0.601	-0.045 p<0.648	-0.031 p<0.793	0.039 p<0.803	0.056 p<0.625	0.049 p<0.676	-0.062 p<0.770
ORAC TNF-α	-0.204 p<0.308	-0.084 p<0.375	-0.079 p<0.420	-0.178 p<0.108	-0.144 p<0.357	-0.072 p<0.532	-0.054 p<0.645	-0.410* p<0.042
ORAC hsCRP	0.141 p<0.145	0.122 p<0.200	0.128 p<0.191	-0.038 p<0.744	0.140 p<0.370	0.042 p<0.715	0.010 p<0.929	-0.020 p<0.924
FRAP IL-6	0.016 p<0.937	0.055 p<0.567	0.048 p<0.623	-0.001 p<0.991	0.111 p<0.477	0.044 p<0.703	0.049 p<0.675	0.139 p<0.509
FRAP IL-1	-0.178 p<0.374	0.084 p<0.379	0.123 p<0.209	0.073 p<0.533	0.129 p<0.410	0.155 p<0.175	0.151 p<0.193	0.436* p<0.029
FRAP TNF-α	-0.115 p<0.537	0.076 p < 0.430	-0.070 p<0.476	0.094 p<0.423	0.019 p<0.902	0.133 p<0.246	0.124 p<0.285	0.170 p<0.416
FRAP hsCRP	-0.222 p<0.265	-0.187* p<0.051	-0.173 p<0.076	-0.187 p<0.108	-0.075 p<0.633	-0.148 p<0.194	-0.155 p<0.182	-0.003 p<0.988

[b] = Spearman's correlation * = significance at the 0.05 level
** = significance at the 0.01 level

Aim 4

There were no significant associations between daily total METs and inflammatory markers in the full cohort and when grouping by years since diagnosis (Table 4.1). There were also no significant differences in mean inflammatory level between days per week of physical activity (Table 4.2), or mean METs in breast cancer survivors and individuals without cancer

(Table 4.3). However, a decline in inflammatory markers is observed with increasing days of physical activity. CRP and IL-6 had a significant relationship to BMI in the whole cohort.

Table 4.1: Relationship between physical activity (METS) and BMI with inflammation in breast cancer survivors grouped by years since diagnosis and individuals without cancer [b]

inflammatory markers	Full cohort (N=108)	Participants w/o cancer (N=30)	Cancer survivors		
			All cancer (N=78)	1-5 yrs cancer dx (N=51)	6+ yrs cancer dx (N=25)
METs IL-6	-0.032 p<0.768	0.290 p<0.142	0.027 p<0.786	-0.036 p<0.755	-0.150 p<0.475
METs IL-1	-0.041 p<0.673	-0.222 p<0.266	-0.073 p<0.459	0.030 p<0.794	0.054 p<0.797
METs TNF-α	-0.002 p<0.982	0.128 p<0.524	0.048 p<0.629	0.029 p<0.806	0.065 p<0.758
METs hsCRP	0.017 p<0.858	0.173 p<0.387	0.004 p<0.968	0.007 p<0.953	0.053 p<0.801
BMI IL-6	0.229* p<0.016	-0.068 p<0.737	0.239* p<0.012	0.271* p<0.018	0.303 p<0.141
BMI IL-1	-0.010 p<0.919	-0.276 p<0.164	0.001 p<0.992	0.065 p<0.579	0.080 p<0.704
BMI TNF- α	0.075 p<0.438	-0.307 p<0.120	0.057 p<0.552	0.101 p<0.386	0.023 p<0.913
BMI hsCRP	0.470** p=0.000	0.442* p<0.021	0.495** p=0000	0.477** p=0.000	0.423* p<0.035

[b] = Spearman's correlation

* = significance at the 0.05 level

** = significance at the 0.01 level

Table 4.2: Means comparison of inflammation and days per week of physical activity (PA) in all participants

	P-value	0-3 days per week of PA (N=43)	3-4 days per week of PA (N=24)	5+ days per week of PA (N=38)
IL-6	0.847	14.42 (51.59)	16.11 (57.74)	12.76 (29.77)
IL-1	0.940	3.62 (5.32)	3.60 (5.49)	3.35 (4.02)
TNF-α	0.776	56.72 (111.98)	49.92 (103.25)	49.49 (100.79)
hsCRP	0.397	5.69 (6.62)	5.42 (6.01)	4.90 (5.65)

[c] = Chi squared means comparison test

Table 4.3: Means comparison of physical activity between breast cancer survivors and individuals without cancer [c]				
	Individuals without Cancer Mean (SD) (N=30)	Breast Cancer Survivors		
		Total Mean (SD), (N=78)	1-5 yrs dx cancer Mean (SD), (N=51)	6+ yrs cancer dx Mean (SD), (N=25)
METs	1478 (815)	1641 (3956), (p = 0.668)	1745 (4592), (p = 0.924)	1222 (634.34), (p = 0.897)

[c] = Chi squared means comparison test

Aim 5

There were no significant associations between METs with inflammatory markers when grouped by levels of dietary and serum antioxidant capacity. However, a strengthening inverse association between METs with IL-1 and METS with TNF-alpha was observed with increasing ORAC categories (Table 5.1). When controlling for FRAP and ORAC in a partial correlation analysis, no significant associations existed between METs and inflammatory markers (Table 5.2).

Table 5.1: Relationship of physical activity level with inflammation of breast cancer survivors and individuals without cancer with the moderating effect of FRAP and ORAC categories								
	FRAP Categories				ORAC Categories			
Physical activity to inflammatory marker level	1 (N=26)	2 (N=26)	3 (N=26)	4 (N=25)	1 (N=27)	2 (N=28)	3 (N=27)	4 (N=27)
METs IL-6	0.070 p<0.485	0.009 p<0.938	0.010 p<0.944	0.025 p<0.907	-0.022 p<0.818	-0.097 p<0.387	0.005 p<0.973	0.041 p<0.841

METs IL-1	-0.062 p<0.538	-0.105 p<0.366	-0.157 p<0.283	-0.120 p<0.576	-0.037 p<0.705	-0.169 p<0.131	-0.247 p<0.205	-0.313 p<0.120
MET TNF-α	0.092 p<0.359	0.009 p<0.937	0.073 p<0.620	0.094 p<0.664	0.011 p<0.910	-0.086 p<0.445	-0.112 p<0.425	-0.232 p<0.255
METs hsCRP	0.020 p<0.843	0.064 p<0.579	0.168 p<0.250	0.148 p<0.491	0.023 p<0.815	0.068 p<0.545	0.039 p<0.779	0.047 p<0.819

[b] = Spearman's correlation

Table 5.2: Relationship of physical activity level with inflammation of breast cancer survivors and individuals without cancer with controlling for FRAP and ORAC[d]

Physical activity to inflammatory marker level	FRAP	ORAC
METs IL-6	-0.028 p<0.777	-0.037 p<0.702
METs IL-1	-0.032 p<0.743	-0.043 p<0.655
MET TNF-α	-0.053 p<0.588	-0.058 p<0.548
METs hsCRP	-0.073 p<0.452	-0.055 p<0.570

[d]= Partial Correlation

VI. Discussion

The associations between diet, physical activity, inflammation, and cancer history in a rural population were compared for differences in these factors between breast cancer survivors and individuals without a history of cancer, and correlations were used to determine associations between each of these factors. The means comparison of antioxidant levels, inflammation,

anthropometrics, and physical activity levels between breast cancer survivors and individuals without cancer yielded no significant differences in these factors (Tables 1.2, 1.3, 1.4, 4.3). However, antioxidant levels and physical activity levels were consistently lower in breast cancer survivors (Table 1.3 & 4.3) while inflammation and anthropometrics were higher (Table 1.2 & Table 1.4). Inflammation increased with years since diagnosis of cancer (Table 1.4).

The measures of dietary antioxidant and serum antioxidant capacity, FRAP and ORAC, had a significant positive correlation (Table 2). FRAP had a significant positive correlation with vitamin C and vitamin E (Table 2), which was expected since vitamin C and E are part of the FRAP index[86]. FRAP also had a significant relationship with hsCRP in all participants (Table 2). In cancer survivors with 6 or more years since diagnosis, there was a significant inverse relationship between ORAC and TNF-alpha, and direct relationship between FRAP with IL-1 (Table 3). There was also a significant correlation between ORAC and hsCRP in individuals without cancer (Table 3). There were no significant relationships between inflammation and METs (Table 4.1 & 4.2) or days per week of physical activity (Table 4.3), although the correlations between IL-1 and TNF-alpha with METs increased in strength and approached significance as ORAC category increased (Table 5.1).

The analysis of differences between mean dietary antioxidant consumption, serum antioxidant level, and inflammation levels in breast cancer survivors and individuals without breast cancer yielded no significant differences in any of these factors: anthropometric measures, or physical activity measures between breast cancer survivors and individuals without cancer (Tables 1.2, 1.3, 1.4, 4.3). However, the data did follow the expected trend that breast cancer survivors would have lower dietary antioxidant intake and serum antioxidant capacity, and higher levels of inflammation than individuals without a history of cancer (Table 1.3 & Table

1.4). Higher antioxidant intakes are associated with a lower risk of breast cancer[41,74-77], as is the antioxidant rich Mediterranean diet.[79,80,82,85] It might be expected that a greater proportion of women that have had breast cancer have dietary habits that contain less antioxidants. Additionally, the difference in mean BMI between breast cancer survivors and individuals without cancer approached significance, and breast cancer survivors had higher anthropometric measurements than individuals without cancer (Table 1.2), consistent with evidence that breast cancer survivors have higher BMI, which increases both risk for and recurrence of breast cancer.[12] The reason the differences were nonsignificant may be due to the large standard deviations of these means, and the relatively small sample size.

Additional factors associated with increased risk for breast cancer found within the breast cancer survivors include the difference in the number of breast cancer survivors and controls with cardiovascular disease, in education category 1 (less than a college education), age, and African American Race (Table 1.1). There were more breast cancer survivors with cardiovascular disease than individuals without cancer, which approached significance ($p=0.054$). An 18-year prospective study found an increased risk of cardiovascular disease in 10 year cancer survivors.[134] Cardiovascular disease is also associated with excess weight which is a risk factor for breast cancer through endogenous estrogen production.[135] Less of an education is associated with breast cancer risk due to access to care and screening opportunities, and cancer mortality data from the U.S. Bureau of the Census found an increased relative risk of developing breast cancer in women with less than 12 years of education, opposed to women with college or greater than 12 years of education.[136] Age was also significantly higher in breast cancer survivors, which is consistent with the risk factor of endogenous estrogen exposure which increases with age.[10] The number of African American women with breast cancer was

significantly higher than the number of African American women without breast cancer. Although white women have the highest incidence rate of breast cancer[22], the smaller sample size of African American women in this study could have contributed to the significant difference in number of breast cancer survivors versus individuals without cancer. Furthermore, a secondary analysis of data from the "Narrowing the Gaps in Adjuvant Therapy" study found that African American breast cancer patients had significantly more mistrust in their medical care and health care systems, which may have been a barrier to early breast cancer screening.[137]

It was expected dietary antioxidant consumption has a significant positive association with serum antioxidant capacity in both breast cancer survivors and individuals without a history of cancer. FRAP and ORAC had a significant positive association in all participants (r_s = 0.205, p<0.032), (Table 2). Based on the limited evidence in literature on the positive relationships between serum FRAP and serum ORAC[99] and dietary FRAP and serum ORAC, these results validate the expected positive relationship between dietary FRAP and serum ORAC.[106,107] In all participants, ORAC was significantly correlated with vitamin C (r_s = 0.185, p<0.055), (Table 2). The association between ORAC and vitamin C was consistent with the significant association between ORAC and FRAP, since vitamin C is part of the FRAP score. The lack of a similar finding in participants with diabetes may be explained by the finding of a cross-sectional observational pilot study that showed that plasma vitamin C levels were significantly lower in participants with type 2 diabetes.[138] A French observational cohort study showed an association between lower dietary intake of vitamin C and risk of developing type 2 diabetes.[139]

FRAP and hsCRP had a significant negative relationship in the whole cohort (Table 3). The significant relationship may not have existed in participants in the overweight and obese BMI categories due to the association between excess weight and inflammation that may have

obscured the relationship.[140] The significant inverse association between dietary antioxidant capacity and hsCRP (Table 3) is consistent with evidence from the study at the University of Glasgow that cancer patients had significantly elevated hsCRP levels ($p<0.001$) and lower serum vitamin-antioxidant levels ($p<0.001$) than the controls without cancer.[104] Although it was expected that FRAP and ORAC would be associated with other inflammatory markers, this relationship may not have existed since there is limited evidence for FRAP and ORAC being correlated with levels of inflammatory markers. Additionally, since the FRAP score is attributed to food eaten, self-report error on food frequency questionnaires could impact the associations of FRAP and inflammatory markers.

The significant associations between ORAC with TNF-α, and FRAP with IL-1 in survivors diagnosed in greater than 6 years previously (Table 3) may be attributed to the change in tamoxifen exposure at this time post-treatment. A study done in mice found significantly lower levels of TNF-α ($P < 0.002$), in female mice models with lupus receiving tamoxifen treatment.[141] The positive association between IL-1 and FRAP could be attributed to the cessation of tamoxifen after 5 years post treatment, since tamoxifen use is associated with lower levels of inflammation. This is evidenced in the study above and in a clinical trial of 79 premenopausal women with breast cancer, tamoxifen treatment was associated with a significant reduction in IL-6 levels ($P = 0.001$).[142] Further research studies could collect information on dosage and frequency of tamoxifen treatment and analyze it in relation to inflammation.

There were no significant associations between physical activity as METs and inflammatory markers in the full cohort, survivors, or when grouping by years since diagnosis (Table 4.1). There were also no significant differences in mean inflammatory level between days per week of physical activity (Table 4.2). When grouping by level of antioxidant capacity, for

METs with IL-1 and METs with TNF-alpha, the negative correlation increased as the ORAC category increased, despite not reaching significance. This supports the evidence that an antioxidant rich diet which improves serum antioxidant capacity[96] and exercise may act synergistically to reduce IL-1 levels.[127]

By decreasing BMI through physical activity, inflammation can be reduced. This is supported by the significant inverse correlations between hsCRP and IL-6 with BMI (Table 4.1). hsCRP is a protein elevated during chronic inflammation,[111] and IL-6 is made by leukocytes as immune response to inflammation in order to make antibodies.[109,110] IL-1 is responsible for generating new blood cells through hematopoiesis,[109] while TNF-α is created by white blood cells to stimulate apoptosis, or cell death. [108] The functions of IL-1 and TNF-α may be a reason it is not significantly related to or effected by BMI. Insulin resistance from excess adipose tissue is associated with chronic inflammation, through elevated cytokine levels of IL-6 and evident by elevated hsCRP.[46-48] Two randomized control trials of 6-month weight loss interventions resulted in improved insulin sensitivity, or reduction of insulin resistance.[143,144] An intervention study with obese women losing 10% of their bodyweight through a calorie restricted diet resulted in the 25-75% reduction of serum IL-6, TNF-a, and other interleukin and inflammatory cytokine levels.[145] Physical activity and diet can result in reduced weight loss and inflammation. In breast cancer survivors, an intervention of 1 hour of moderate physical activity in conjunction with a lower calorie diet resulted in weight loss, and other studies involving 150-180 min/week of moderate intensity aerobic exercise or resistance training resulted in significantly lower levels of inflammation. [125,3]

Limitations and further research

There were several limitations to this research that should be taken into consideration when interpreting the findings. The insignificant findings of the relationships between dietary antioxidant intake and serum antioxidant capacity with inflammatory markers may be due to the self-report error of the food frequency questionnaire for FRAP food intakes, which is a limitation since participants may not precisely report the serving size or frequency of food consumed. A technology which could be utilized in further studies is a food tracking app, which calculates FRAP score based on serving size from a picture of the food. Participants also may be more likely to report more honest answers to the app rather than a researcher conducting the food frequency questionnaire over the phone. Additionally, the inflammatory markers analyzed, 4 inflammatory markers, are not inclusive of all those available, and limited analysis of inflammation to these only. Further studies could investigate the effect of dietary and serum antioxidant capacity on additional markers, or a composite inflammatory marker index. Further research could also investigate relationships between inflammation and other vitamins and minerals in the diet.

The physical activity questionnaires did not account for all of the physical activities that participants may engage in, which is a limitation that may be related to why there were no significant associations between physical activity level and inflammatory markers. Additionally, the amount of time and intensity for physical activity interventions in literature which showed a significant decrease in inflammatory markers may have been different than what participants did. The evidence from the literature review included interventions in breast cancer survivors doing 150 min/wk of moderate intensity aerobic exercise[125], cycle ergometers three times per week for 15 weeks found that exercise[126], and a 180 minutes of a resistance training program for 16 weeks[3], for significantly lower levels of inflammation. These are limitations to address in

following studies, to account for all physical activity of participants and investigate how many participants exercise for at least 150-180 minutes per week, perhaps through an exercise monitor since the self-report of physical activity could be subjective.

Some design limitations of this study include the low sample size of individuals without cancer which are compared to breast cancer survivors. The lack of diversity in race is also a limitation, along with the geographic location of study participants. The results from this study may not translate to people of other races or residing in different geographic areas due to different lifestyle factors. Furthermore, another design limitation was asking participants to recall their physical activity behaviors over the past year, the long time frame is subject to error since participants may not remember physical activity behaviors from earlier in the year.

VII. Conclusion

This study demonstrated that in a population of breast cancer survivors and those without a history of cancer from southern Virginia, there are no significant differences in mean dietary antioxidant consumption, anthropometrics, METs of physical activity, and level of inflammatory markers in breast cancer survivors and individuals without cancer. However, this study did demonstrate a positive association between dietary and serum antioxidant capacity, and a

significant relationship between inflammatory markers and dietary and serum antioxidant capacity in breast cancer survivors with more than 6 years since cancer diagnosis, which could be attributed to completion of tamoxifen treatment. Additionally, dietary antioxidant consumption was significantly associated with lower levels of hsCRP, a marker associated with chronic inflammation. Further research studies could be more comprehensive in collecting all data on diet and physical activity, perhaps through an app as an objective way of data collection opposed to the surveys in this study which could be subjective. Further studies could also investigate relationships between tamoxifen and inflammation and other dietary components and inflammatory markers.

VIII. References

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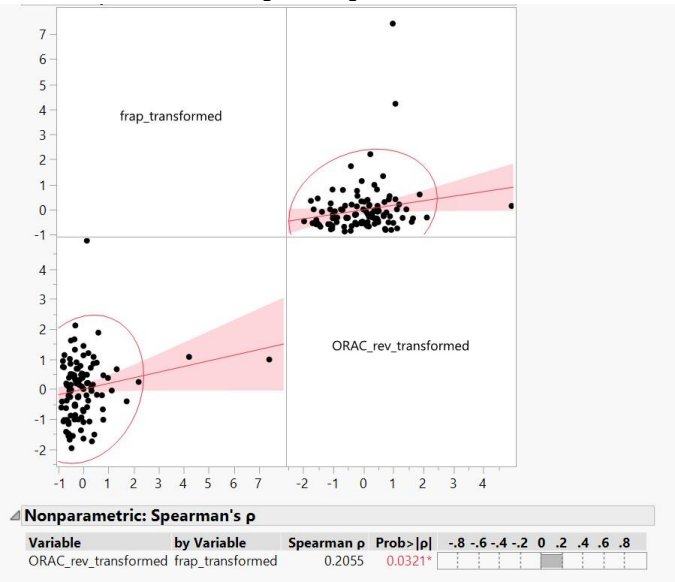
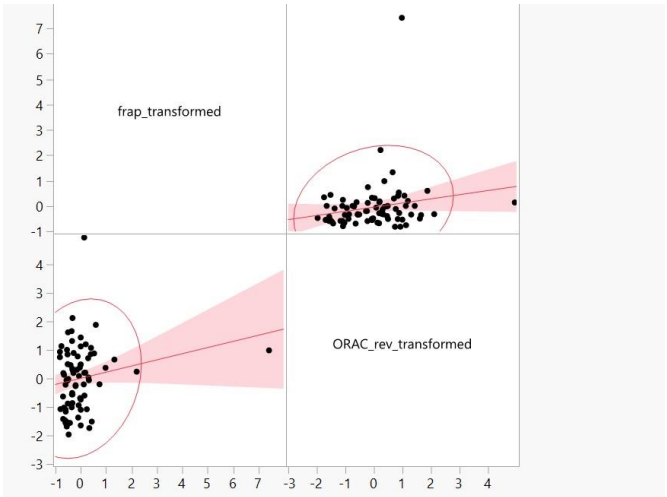
IX. Appendices

Appendix A: Graphs of significant findings

FRAP and ORAC all participants



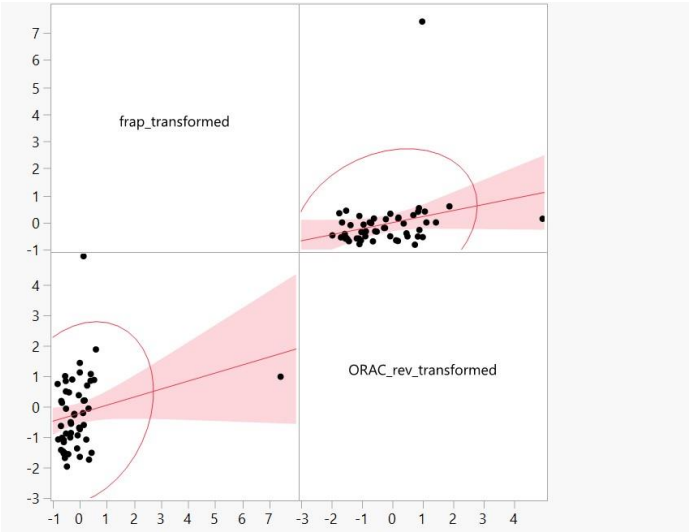
FRAP and ORAC all cancer survivors



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|
ORAC_rev_transformed	frap_transformed	0.2470	0.0292*

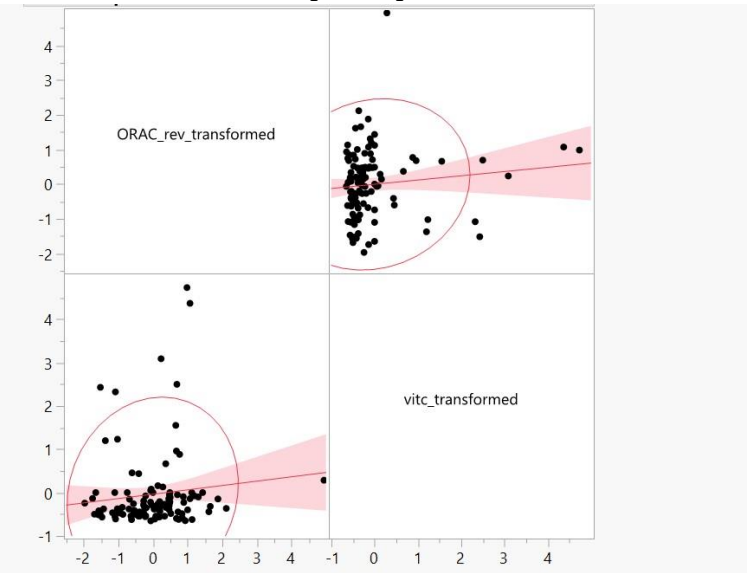
FRAP and ORAC 1-5 years cancer dx



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|
ORAC_rev_transformed	frap_transformed	0.3452	0.0131*

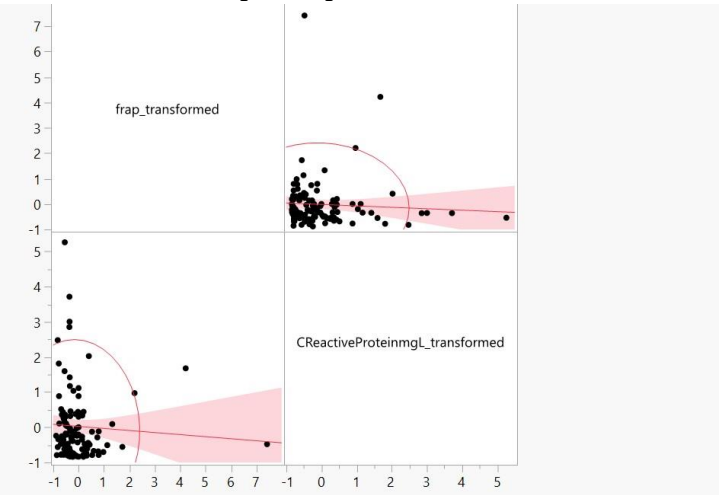
ORAC and vitamin C all participants



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|
vitc_transformed	ORAC_rev_transformed	0.1846	0.0547

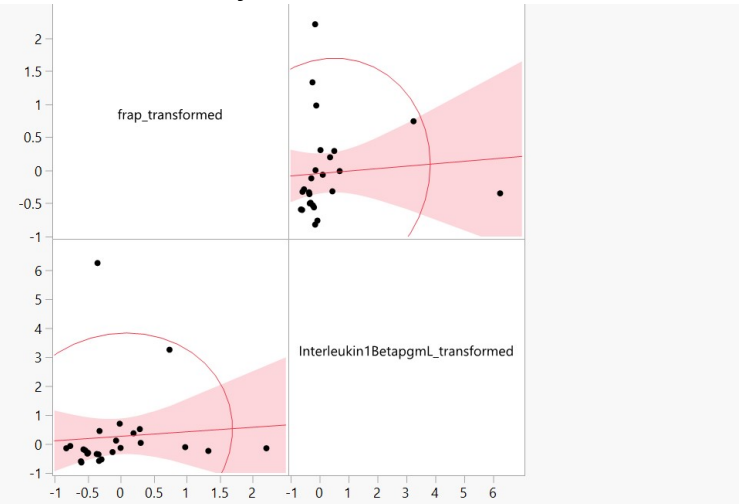
FRAP and CRP all participants



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|
CReactiveProteinmgL_transformed	frap_transformed	-0.1874	0.0510

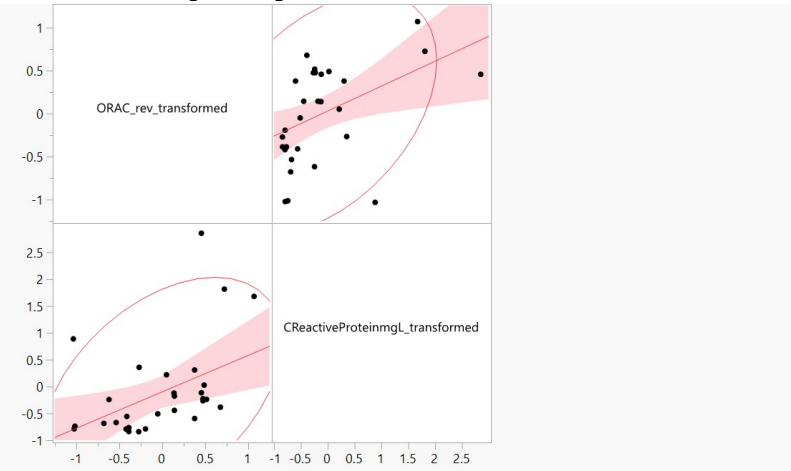
FRAP and IL-1 6+ years cancer dx



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Interleukin1BetapgmL_transformed	frap_transformed	0.4362	0.0293*	

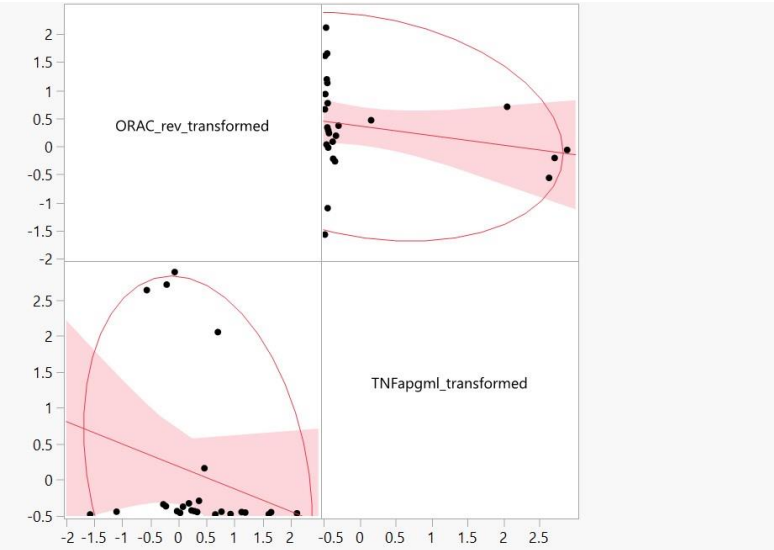
ORAC CRP all participants without cancer



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
CReactiveProteinmgL_transformed	ORAC_rev_transformed	0.5198	0.0032*	

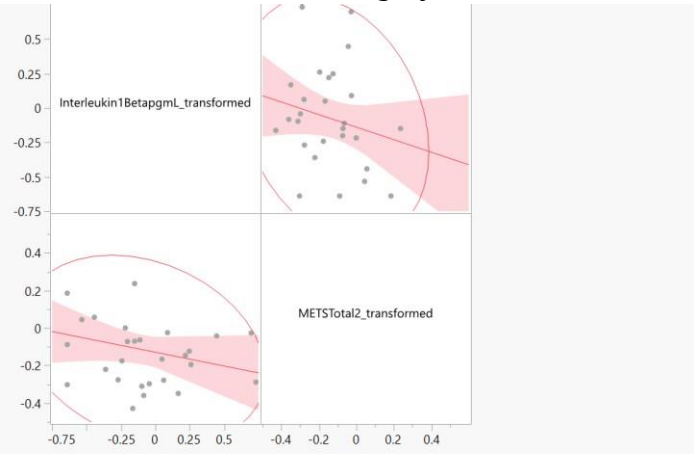
ORAC TNF-apha 6+ years diagnosis



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
TNFapgml_transformed	ORAC_rev_transformed	-0.4101	0.0418*	

METs and IL-1 in ORAC Category 3:



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
METSTotal2_transformed	Interleukin1BetapgmL_transformed	-0.2473	0.2045	

Appendix B: Questions from the validated online Physical Activity Questionnaire

What is your usual walking pace?	**MET Level**
-Easy, casual, less than 2 mph	2
-normal, average, 2-2.9 mph	2.5
-Brisk, 3-3.9 mph	3.3
-Very Brisk, over 4 mph	5

During the past year, what was your average time per week spent doing the following recreational activities?

-Walking for exercise or walking for work, use MET levels from previous question

	MET Level
-Jogging (slower than 10 min/mile)	7
-Running (10 min/mile or faster)	11
-Bicycling	8
-Tennis, squash, racquetball	7
-Lap swimming	7
-other aerobic exercise	6.5 -
lower intensity exercise (yoga, stretching)	2.5 -other
vigorous activities (lawn mowing)	5.5

-weight training or resistance exercises for legs, 3 -weight training or resistance exercises for arms 3

During the past year, on average, how many hours per week did you spend:

	MET Level
-Standing or walking around at work or away from home	2
-Standing or walking around at home	2
-Sitting at work or away from home or while driving	1.8
-Sitting at home watching TV/VCR/DVD	1.3 -Other sitting at home (reading, meals) 1.5

-In an average week, how many days do you usually exercise? (include brisk walking or more strenuous activity)

Appendix C. Further analyses of Aims Grouping by Diabetes Status

Relationship of dietary and serum antioxidant level with inflammatory marker level of participants with and without diabetes		
Antioxidant consumption & capacity to inflammatory markers	Participants with diabetes (N = 13)	Participants w/o diabetes (N=93)
ORAC IL-6	-0.012 p<0.899	0.022 p<0.836
ORAC IL-1	0.045 p<0.648	0.026 p<0.808
ORAC TNF-α	-0.079 p<0.420	-0.113 p<0.279
ORAC hsCRP	0.128 p<0.191	0.223 p<0.031
FRAP IL-6	0.048 p<0.623	0.099 p<0.343
FRAP IL-1	0.123 p<0.209	0.123 p<0.239

FRAP TNF-α	0.070 p<0.479	0.041 p<0.694
FRAP hsCRP	-0.173 p<0.076	-0.129 p<0.219
Spearman's correlations		

Aim 4: Relationship between physical activity (METS) and BMI with inflammation in participants with and without diabetes		
inflammatory markers	Participants with diabetes (N=13)	Participants w/o diabetes (N=93)
METs IL-6	0.027 p<0.786	0.081 p<0.443
METs IL-1	-0.073 p<0.459	-0.141 p<0.179
METs TNF-α	0.048 p<0.629	0.015 p<0.889
METs hsCRP	0.004 p<0.968	0.070 p<0.505
BMI IL-6	0.237* p<0.014	0.242* p<0.020
BMI IL-1	-0.004 p<0.966	-0.007 p<0.164
BMI TNF- α	0.075 p<0.447	0.045 p<0.671
BMI hsCRP	0.483** p=0.000	0.455** p<0.000
Spearman correlations * = significance at the 0.05 level **= significance at the 0.01 level		

Aim 5: Relationship of physical activity level with inflammation of participants with and without diabetes controlling for FRAP and ORAC[d]				
Physical activity to inflammatory marker level	FRAP w/ diabetes (N=13)	FRAP w/o diabetes (N=93)	ORAC w/ diabetes (N=13)	ORAC w/o diabetes (N=93)
METs IL-6	-0.026 p<0.791	-0.028 p<0.791	-0.035 p<0.720	-0.041 p<0.701
METs IL-1	-0.036 p<0.712	-0.039 p<0.710	-0.049 p<0.623	-0.053 p<0.616
MET TNF-α	-0.054 p<0.582	-0.056 p<0.593	-0.060 p<0.544	-0.068 p<0.518
METs hsCRP	-0.070 p<0.476	-0.051 p<0.633	-0.054 p<0.585	-0.034 p<0.749
[d] Partial correlation				