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Diet Quality Scores and Risk of Incident Breast Cancer in the California Teachers Study

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Diet Quality Scores and Risk of Incident Breast Cancer in the California Teachers Study

THESIS

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MASTER OF SCIENCE

in Epidemiology

by

Vikram Haridass

Thesis Committee:
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DEDICATION

To

My family and friends,
For showering me with complete love, care, and support

My Mentors,
For imparting wisdom and supporting me throughout my academic endeavors

And most of all,
My parents, Saraswathy and Shanmuganathan Haridass
For everything, I truly would not have made it this far without your unending love and support

Thank you, I am filled with so much gratitude.

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ABSTRACT

Diet Quality Scores and Risk of Incident Breast Cancer in the California Teachers Study

By

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Master of Science in Epidemiology

University of California, Irvine 2015

Professor Dr. Hoda Anton-Culver, Chair

Introduction: Diet has been implicated as a significant risk factor in terms of breast cancer progression and manifestation, but results are shaky when assessing the specific role played by diet. The Healthful Diet Score was developed using a-priori based methods that assessed the existing nutritional epidemiology evidence regarding breast cancer risk along with findings from widely-accepted standardized dietary indices: Alternate Mediterranean Diet Index, Alternative Healthy Eating Index-2010, Dietary Approach to Stop Hypertension (aMED, AHEI-2010, DASH). An increased diet quality score is indicative of greater adherence to healthy dietary patterns, which encompass all beneficial aspects of dietary consumption ranging from vitamins to polyunsaturated fat consumption. Breast cancer is the most common cancer observed amongst women in which many risk factors have been identified, including some modifiable factors such as diet. We assessed the role of overall diet quality, through our own a-priori index and standardized indices, on the risk of incident breast cancer (BRCA). **Methods:** California Teacher Study participants were women age 22-104 years old at baseline (1995-1996) who are members of California State Teachers Retirement System. Overall diet quality scores were computed for the eligible study population (n=94,404) through participants' responses to the validated-FFQ. Descriptive statistics across the various measures of overall diet quality were generated. Multivariate Cox proportional hazard regression models were constructed to provide hazard ratios and corresponding 95% confidence intervals. **Results**: In terms of overall breast cancer risk, overall diet quality as defined by AHEI-2010 exhibited the greatest reduction of risk 13% for the highest adherers vs. lowest adherers (HR_{Q5vsQ1} 0.87, 95% CI: 0.79-0.97). Similar reductions in overall breast cancer risk were also exhibited in the highest adherers of a DASH diet plan (HR_{Q5vsQ1} 0.88, 95% CI: 0.79-0.97). In terms of pre-menopausal breast cancer risk, overall diet quality was not significantly associated with breast cancer risk across all measure of diet quality. When assessing the post-menopausal subgroup in this study, the role of overall diet quality became increasingly apparent in terms of breast cancer risk in which the highest risk reduction observed was 25% for highest adherers of the AHEI-2010 diet pattern (HR_{Q5vsQ1} 0.75, 95% CI:0.70-0.91). **Conclusion:** Findings from this large prospective cohort study suggest that overall diet quality is significantly associated with invasive breast cancer risk amongst post-menopausal women, and the AHEI-2010 score displayed the strongest inverse association with breast cancer.

CHAPTER 1

INTRODUCTION

1.1 Statement of the problem:

Breast Cancer is the most common cancer in women with 1 in every 8 women born in the US, specifically where 12.4% are expected to develop breast cancer sometime in their life. In 2014, there were approximately 232,670 new cases of Invasive Breast Cancer and 62,570 In-situ Breast cancer cases [1]. Several major risk factors have been identified in this hormone-dependent cancer, including some lifestyle factors such as obesity [29]. Obesity is major problem in America, with a prevalence of more than 1 in every 3 people (~35.0%). Furthermore, obesity serves as a common morbidity worldwide where the prevalence has risen from 5% and 8% in 1980 to 11% and 15% in 2014 for men and women, respectively [1, 86]. Clinically speaking, obesity is commonly defined as having a body mass index (BMI) of ≥30 kg/m^2, where as being overweight is considered a BMI of 25- 29.9 kg/m^2. Biologically speaking, obesity is defined as the unhealthy accumulation of adipose tissue that increases one's risk for several health conditions such as myocardial infarctions and Type 2 diabetes. In terms of carcinogenesis, obesity plays a role in breast cancer, because of the endocrine nature of adipose tissue, which secretes hormones such as estrogen. Estrogen serves as the mitogen protein in this hormone-dependent cancer, which drives cellular proliferation and tumorigenesis [29]. Ultimately when adipose tissue levels reach obesity (BMI> ≥30 kg/m^2), we see elevated levels of circulating estrogen that serve to increase breast cancer risk. Secondly, obesity serves as a risk factor by inducing a state of chronic tissue inflammation [5, 37]. Past studies have identified the adipocyte cytokine, TNF-α, as the main culprit for this inflammation due the adipose tissue found near the mammary glands [3-4]. Specifically, chronic inflammation promotes a state of cellular oxidative stress characterized by harmful free radicals that cause DNA-damage, adduct formation, and mutagenesis for an aggressive tumor formation [5].

Diet plays an important role in obesity in which poor nutrition can readily promote the aggregation of adipose tissue, which has been linked to increase risk of several major health conditions by various epidemiological studies. These past findings have fueled other nutritional epidemiological studies to investigate the role of dietary habits on breast cancer risk [6-16]. These studies do not only investigate unhealthful effects, because diet also provides essential sources of nutrients such as vitamins, antioxidants, protein, fiber and carbohydrates that are necessary for key homeostatic processes. Several different methods have been utilized for assessing the role of diet on various health conditions including the following: single nutrient analysis, single food group analysis, and dietary pattern analysis.

Some of the aforementioned studies investigated the role of single nutrients/constituents on the role of breast cancer risk such as dietary fiber and carotenoid intake [8, 12]. Other studies have assessed food group intakes, which are more indicative of normal dietary habits such a salad consumption and pasta consumption in association with breast cancer risk [9, 15]. Of these food groups, twelve have been identified and commonly evaluated in these nutritional epidemiology studies, such as vegetables, fruits, nuts, legumes, dairy, red meats, etc. (Table 1.1). Past studies have found diets that are rich in nutrients, and calorically sparse-items to be associated with decreased risk of various chronic diseases [32]. Conversely, unhealthful diet patterns tend to be rich in processed, refined, and sweetened foods and red and processed meat. The method of dietary assessment accounting for the overall dietary pattern is much more informative since most individuals consume a mix of food consisting of different nutrients/constituents, rather than consuming one sole nutrient. Another major improvement on those past methods is the implementation of diet quality scores, which provides a comprehensive measure of individuals' dietary habits. Diet quality scores yield a more informative assessment of overall dietary effects on chronic disease risk [16 -23]. Dietary Approach to Stop Hypertension (DASH) index, Alternative Healthy Eating Index 2010 (AHEI-2010), and Alternate Mediterranean Diet Index (aMED) are several widely known and accepted dietary indices that have been implemented in many nutritional epidemiological studies to assess various health outcomes [16-28]. Table 1 depicts the various components and food groups characterized by these aforementioned diet indices.

Table 1.1

Components and Optimal Quantities for Scoring Standards for each food parameter of the DASH, AHEI-2010, and aMED scores by using standardized cup and ounce (oz.) equivalents from the MPED[1]

	DASH	AHEI-2010	aMED
Component	8-40 points total (8 components: 1-5 points each)	90 points total (10 components; 1-10 points each)	8 points total (8 components: 1 point each)
Vegetables	Excluding potatoes; highest quintiles	Excluding potatoes: ≥2.5 cup equivalents	Excluding potatoes: ≥ median cup equivalents
Fruit	Total fruit: Highest quintile	Whole fruit: ≥2 cup equivalents	Total fruit: ≥ median cup equivalents
Nuts/Legumes	Legumes, Nuts, Seeds: Highest quintile	Nuts : ≥1 oz. equivalents Legumes: ≥1 oz. equivalents	≥ median cup equivalents
Fish			≥ median oz. equivalents

Whole grains[2]	Highest quintile	Women: 5 oz. equivalents, Men: 6 oz. equivalents	≥ median oz. equivalents
Dairy	Low-fat dairy[3]: highest quintile		
Oils/Fats		*Trans* Fat: ≤0.5%; EPA+DHA: 250 mg; PUFA: ≥ 10%	MUFA:SFA ratio > median
Alcohol		Women: 0.5-1.5 drinks; Men: 0.5-2.0 drinks[4]	Women: 5-15 g/d, Men: 10-25 g/d
Red and Processed meats[5]	Lowest quintile	0 oz. equivalents	≤ median oz. equivalents
SSBs and fruit juice[5]	Lowest quintile[6]	0 g[7]	
Sodium	Lowest quintile	Lowest decile (mg)	

[1]Scoring standards are expressed as cup and ounce equivalents from the MyPyramid Equivalents Database whereby 1 oz = 28.3 g and 1 cup = 225 mL
DASH, Dietary Approaches to Stop Hypertension; AHEI-2010, Alternative Healthy Eating Index-2010; aMED, Alternate Mediterranean Diet Index; MPED, MyPyramid Equivalents Database; SSB, sugar-sweetened beverage
[2]Does not include popcorn, wheat germ, or wheat bran.
[3]Foods included in this definition are cottage cheese; low-fat cheese; low-fat/1% or 2% milk; nonfat/skim milk or butter milk; yogurt; and ice milk, frozen yogurt, and sherbet.
[4]Moderate drinkers (amounts in table) received maximum points, nondrinkers received 2.5 points, and heavy drinkers (more than amounts in table) received progressively lower points.
[5]Components were reverse scored such that higher intake was associated with a lower score.
[6]Foods included in this definition are other fruit juices or fruit drinks and regular sodas.
[7]Foods included in this definition are orange or grapefruit juice, other fruit juices or fruit drinks, and regular sodas.

Most of these indexes show a general overlap in efficacious food trends, but distinct differences are present amongst these three indices such as fish consumption only being assessed in the aMED score. For all the indices listed in Table 1.1, the higher the diet quality score indicates a better adherence to a healthy/efficacious diet pattern. AHEI-2010, aMED, and DASH have been utilized to investigate the role of dietary habits on breast cancer risk [24-28]. One study showed a 3% decrease in the relative risk of incident breast cancer for highest quintile of DASH diet score relative to the lowest quintile scores for post-menopausal women [28]. Another study showed a lack of significant association with DASH and AHEI-2010 and breast cancer mortality [27].

Chiuve, et al. reported a significant 7% decrease in cancer risk for women of highest AHEI-2010 quintile relative to women of the lowest quintile [25]. The inconsistencies of results in these past studies help provide evidence that a more accurate dietary index must be developed in terms breast cancer risk.

1.2 Purpose and Objectives:

We propose to assess the role of overall diet quality on Breast cancer risk through utilization of several widely accepted and validated indices (Table 1.1) in the California Teachers Study (CTS), a study population that has rich dietary data recorded for 133,479 current participants to allow us to characterize the role of overall diet quality on the risk of breast cancer. We will also develop our own index, known as the Healthful Diet Score (HDS) through a-priori methods to compare which index is more predictive of breast cancer risk. The study population consists of women 22-104 years old that are current and former public school teachers and administrators who are members of California State Teachers Retirement System. Individuals in the CTS filled out an extensive baseline questionnaire consisting of 103-item Food Frequency Questionnaire (FFQ) and questions regarding risk factors of Breast and other cancers, sent out in 1995-1996. Cancer cases are identified through annual linkage with California Cancer Registry records, which is a population-based registry of cancer incidence for California residents. Increasingly high breast cancer incidence rates have been identified in this cohort, in which invasive breast cancer incidence has been observed at a 51% higher age-standardized rate, as well as 67% higher in-situ breast cancer incidence rate than what is expected based upon race-specific state-wide rates after three years of follow-up [31].

<u>Specific Aim #1:</u> To develop our own measure of overall diet quality using a-priori methods.

Due to the aforementioned inconsistencies in nutritional epidemiology evidence regarding overall diet quality and breast cancer risk, we propose to develop our own quantitative measure of overall diet quality known as the Healthful Diet Score (HDS, HD Score). Using a-priori methods to help guide the development of this index, we will assess evidence from the current literature while building upon these widely accepted and utilized indices (Table 1.1). We expect to develop a dietary index that characterizes an individual's diet more diversely than the standardized indices, therefore not to over generalize individuals' dietary habits, but rather be all encompassing of dietary habits. We also expect the lowest quintile of the HDS to be associated with highest BMI relative to the lowest quintile of the diet scores to shed light on validity of this measure.
The main study questions of aim #1 include the following:

1. Can a more diverse and more encompassing dietary index be developed to characterize overall diet quality?

<u>Specific Aim #2:</u> To assess the role of overall diet quality via several indices on Breast Cancer risk amongst participants of The California Teacher's Study (n= 94,404).

We propose to assess the role of overall diet quality on Invasive Breast cancer risk through utilization of our a-priori index, along with the previously mentioned standardized indices (DASH, AHEI-2010, aMED). Therefore, application of these diet quality measures to the CTS study population (n=94,404) will shed light on the role of diet on breast cancer risk in this unique cohort. Utilizing our HDS in concordance with these other indices will provide evidence of associations between diet and breast cancer risk, and allow for comparison on which diet quality measure is more predictive. We hypothesize that diet does have a significant role on breast cancer risk, such that higher diet quality scores are inversely associated with breast cancer risk. Conversely, we expect individuals with a lower diet quality score to have an increased risk of breast cancer. Based off these standardize indices and current nutritional epidemiological evidence, we expect to derive an a-priori diet quality score which is more predictive of breast cancer risk.

The main study questions of aim #2 include the following:

1. Is overall diet quality associated with Breast cancer Risk?
    a. Does the association of Diet Quality differ by menopausal status or BMI?
2. Is our a-priori index, the Healthful Diet score, more predictive of breast cancer risk relative to the other widely accepted indices (aMED, DASH, AHEI-2010)?

1.3 Significance and Relevance of Thesis Research:

This thesis addresses an important matter in the field of breast cancer research in which the role of diet in breast cancer carcinogenesis is shaky and inconsistent given vastly changing landscape of dietary measures over the past couple decades. We intend to assess the role of overall diet quality, thus taking into account all fashions in which diet may have an effect on breast cancer susceptibility (i.e. inflammation, increased reproductive hormones). Given the extensive dietary data recorded on the participants and the relatively-high breast cancer incidence rates observed in CTS, this investigation into the role of overall diet quality on breast cancer risk serves as an intuitive and significant step towards advancing the field of Breast Cancer Biology. Also, comparing our Healthful Diet Score to these other a-priori based dietary measures will provide evidence of which dietary measure is more predictive of breast cancer risk thus ultimately providing better evidence for breast cancer prevention.

1.3.1 High public health and clinical impact:

This study is innovative in that it is the first time a-priori based methods have been utilized to assess the role of diet on breast cancer risk in the CTS. Also innovative for the fact that we have developed our own a-priori diet index, the Healthful Diet Score, which will be used in conjunction with these standard indices to assess breast cancer risk. This study is likely to have a large public health impact by strengthening the evidence as to which measure is more predictive of breast cancer risk to better inform future studies, while also providing a new tool (Healthful Diet Score) that characterizes the overall diet in a more diverse and all-encompassing fashion relative to these other indices.

Patients at risk for several major health conditions such as diabetes, cardiovascular disease (CVD), obesity, may also be at risk for breast cancer, therefore pinpointing efficacious dietary patterns regarding breast cancer risk, may also help improve overall survival [5, 30]. The results of this study will likely improve clinical practices by providing prevention and intervention strategies, specifically dietary changes that may decrease risk of the consulted patients.

1.3.2 The conundrum of overall diet quality and breast cancer risk:

Dietary intake clearly has a role in breast cancer, but there are more questions than answers related to the topic at this point. Evidence from past epidemiological studies leads us to believe diet may not truly play a role in breast cancer carcinogenesis, but these results may be confounded for many reasons. For instance, studies focusing on individual foods and nutrients may not be adequate to convey the overall role of long-term dietary associations on the risk of degenerative diseases such as breast cancer. Being able to characterize the role of overall diet quality on breast cancer tumor progression is of utmost importance, because majority of breast cancer risk factors are not modifiable which leaves clinicians ill-equipped when advising patients. Many studies have attempted to get around this glaring weakness, in which characterization of overall diet quality/dietary pattern has shown lower risks of cardiovascular disease, all-cause mortality and even breast cancer [33, 47]

Another reason for the lack of consistent results is often accounted to differences in breast cancer disease progression between pre-menopausal and post-menopausal women. After assessing the past evidence, breast cancer epidemiology has established that pre-menopausal disease manifestations are aggressively driven by family history of breast cancer, such as the familial inheritance of BRCA1/2 mutant genes. Whereas post-menopausal manifestations of this disease are more commonly seen in the general population in which most individuals do not genetically inherit susceptibility alleles, but are rather exposed to carcinogens during the course of their life taking a cumulative toll [1,3,5]. These findings have driven nutritional studies to assess the role of diet in breast cancer according to individual's menopausal status, which helps avoid biasing effect sizes towards the null by over-generalizing the disease.

1.4 Study Outline

Chapter one of this thesis presents the problem by first shedding light on the carcinogenic effect of dietary habits in Breast cancer, while providing brief introduction of different methods for assessing an individual's diet, with the ultimate aim to assess the association between overall diet quality and breast cancer progression. The description of specific aims, as well as overall significance of thesis research is also presented in this chapter as well. Chapter 2 will provide rich background regarding commonly investigated food groups in these nutritional epidemiological studies in terms of their overall efficacy and efficacy in breast cancer. It also describes the various dietary measures included in this analysis in detail regarding overall efficacy and efficacy in breast cancer. Chapter 3 provides a detailed description of the statistical methods utilized in this analysis. Chapter 4 contains the results from the investigational aims of this study in which descriptive tables and measure of association tables can be found. Chapter 5 serves as a discussion of findings from this study relative to other studies, and the implication of findings. This chapter also goes into detailed explanation of strengths and weaknesses along with future research steps regarding this study. Lastly, chapter 6 is the conclusion of the study in which principal findings and the implications in the public health realm can be found.

CHAPTER 2

BACKGROUND

2.1 Dietary effects in Breast Cancer:

Diet has been shown to play a role in breast cancer risk, but results regarding the type of role specifically played has not been clearly depicted due shaky and inconsistent evidence. As previously mentioned, the results of past studies have been confounded due to various inefficiencies, this makes obtaining definitive associations regarding diet's role increasingly difficult. Biologically speaking, there are many reasons to believe and elucidate pathways where dietary effects play a role in breast cancer carcinogenesis.

2.1.1 Diet, inflammation, and Breast Cancer:

One pathway commonly studied in terms of diet and breast cancer involves the induced inflammatory effect on breast cancer tumor progression. Diet provides as a key source of pro- and anti-oxidizing components in which the levels of pro-oxidants must not exceed the anti-oxidant levels into order to maintain homeostasis [1,3-5]. If these levels of pro-oxidants become burdensome, a state of oxidative stress will be induced to provide optimal tissue environment for tumor progression. Oxidative stress is harmful in nature, specifically because the free radicals known as Reactive Oxygen Species (ROS) can cause direct DNA damage through adduct formation and mutagenesis to ultimately achieve genomic instability. This is beneficial for breast cancer, a disease which desires genomic instability to allow for developmental reprogramming of normal cells to tumorous growths. Past studies have shown 8-hydroxy-2'deoxyguanosine (8-OHdG) adduct formation as a common direct-DNA alteration seen in malignant breast cancer compared to normal breast tissue, and even more common among all subtypes other than Triple-negative breast cancers (P=0.036) [34].

ROS also have been shown to exhibit oxidative inactivation of enzymes such as phosphatases and kinases involved in anti-tumorigenic signaling (i.e. loss of p53 activation); as well as induce chronic inflammation through aberrant cell signaling to promote expression of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-alpha), interleukin-1ß (IL-1ß), interleukin-6 (IL-6), interleukin-8 (IL-8) and decreased expression of interleukin-4 (IL-4) and interleukin-10 (IL-10) [34-36]. TNF-alpha has been implicated as a common inflammatory cytokine found amongst adipose tissue and has been shown to be involved in breast tissue tumor initiation due to the fatty nature of breast [3-4, 36]. IL-1ß and IL-6 are derived from leukocytes such as tissue-infiltrating macrophages through chemokine recruitment to the breast epithelial cells during conditions of oxidative stress. This mass recruitment of pro-inflammatory leukocytes creates a breast tissue microenvironment with optimal conditions to promote breast cancer tumor progression [34-36]. Given the past findings, it is reasonable to believe diet induced inflammatory effects plays a role in breast cancer carcinogenesis.

2.1.2 Diet, obesity, and Breast Cancer:

Another commonly studied pathway in which diet has been shown to alter breast cancer risk is through “obesogenic-effects”, which are usually accounted to poor nutrition habits as calorically-dense and nutrient-sparse diets. Individuals who exhibit behaviors of dietary excess tend to couple in sedentary lifestyle choices (i.e. lack of physical activity), which synergistically works to promote a state of obesity. Conversely, individuals who are consuming high levels of pro-adiposity food items are lacking intake from healthful components necessary for homeostatic processes, which may further exacerbate the situation.

With regards to the carcinogenic role, obesity promotes a state of chronic tissue inflammation in which past studies have identified the adipocyte cytokine, TNF-α, as the main culprit for this inflammation [5]. Specifically, chronic inflammation serves as a major risk factor breast cancer as previously noted. Secondly, obesity serves as a major risk factor for Breast cancer due to endocrine-organ nature of adipose tissue, which secretes hormones such as estrogen, the main mitogenic compound, to promote aggressive breast tumor formation [37-38]. Given these findings, we can deduce a scenario where adipose-tissue aggregation reaches the point of obesity (BMI ≥ 30 kg/m^2) through poor nutrition thus allowing for over-secretion of endogenous to fuel carcinogenesis in Breast cancer. A study in Saudi Arabia found when comparing obese women to normal weight, the odds of breast cancer significantly increased by 129% (OR 2.29 , 95% CI: 1.68-3.13) [38]. However, diet-induce obesity is strongly confounded by menopausal status in which past studies have identified obesity as protective factor for premenopausal women, but harmful amongst post-menopausal women. These findings align with biological reasoning regarding women's health in which obesity attributed to more frequent anovulation in obese pre-menopausal women, but also attributed to elevated circulating estrogen levels in obese post-menopausal women [37].

2.2 Measures of dietary intake:

Due to the increasing inconsistency in results regarding single nutrient and food analysis as previously mentioned. Nutritional epidemiology has shied away from these past methods to focus on dietary patterns/overall diet quality to assess the oncogenic role of an individual's whole diet in breast cancer [7-16]. However, these past studies do provide the initial evidence for suspected nutrients and foods associated with breast cancer susceptibility. Two major methodologies exist and are readily used when deriving overall diet quality and dietary patterns.

2.2.1 A-posteriori based methods for dietary measures:

A-posteriori derived dietary patterns are a common practice utilized in the nutritional epidemiological world. This methodology is considered a data-driven method in which common statistical techniques used for deriving dietary patterns are observed amongst Principle Component Analyses (PCA), and cluster analyses (CA). Specifically, dietary patterns are derived using observed intake levels of the study population. A-posteriori based method also investigators to take into account many aspects of the diet rather than focusing on a few hypothesized food groups. The assessment into observed intake levels leads to less broad food groupings, which ultimately allows investigators to take into account distinctions between individual food items (i.e. all vegetables vs cruciferous vegetables vs orange-pigmented vegetables). One major drawback in the a-posteriori based methodology is its inability to build upon and utilize current scientific literature, thus appraising the current diet-disease paradigm becomes increasingly difficult. [9, 30, 40, 42]

2.2.2 A-priori based methods for dietary measures:

A-priori based measures of overall diet quality are another commonly used method in nutritional epidemiological practices. This method is considered a hypothesis-driven, because it allows investigators to utilize background evidence and scientific literature to help provide evidence for the measure. Investigators utilize existing evidence to derive a dietary measure comprised of hypothesized healthful food groups and unhealthful groups with regards to the outcome of interest [27]. This allows investigators to create dietary indices with the aim of capturing pre-defined healthy patterns. Consumption levels for these food groups of interest are then ranked accordingly to yield segmented levels of intake (i.e. quartiles, quintiles, etc.). The ranked intake levels are then scored according to their hypothesized effect on outcome of interest, which allows investigators to yield an overall diet quality score. In general, a higher score is indicative of greater adherence to a healthy dietary pattern when using any dietary measure based off an a-priori approach. One major drawback with this method pertains to the broad definition of food groups, which doesn't take into account distinct changes in the nutrients and phytochemicals of each food (i.e. Total vegetables vs. raw cruciferous vegetables vs. green-leafy vegetables). [40-42]. However, this method provides a truly indicative measure of overall diet quality due to its basis on recommended dietary guidelines or past evidence relative to a-posteriori measures that yield observed dietary patterns.

2.2.2.a Commonly used a-priori based dietary measures:

Many dietary indices exist in the nutritional epidemiological world, but the several selected for analysis in this thesis are widely accepted and implemented measures used in conjunction with several major health conditions [16-28, 30, 39]. The included indices show a general overlap in efficacious food groups but minor distinctions exist which may ultimately alter their predictive ability in terms of breast cancer. The specific components along with the beneficial levels of consumption for these various indices can be found in the earlier table (Table 1.1). The first measure is the Alternate Mediterranean Diet index (aMED), which a dietary measure used to characterize adherence to Mediterranean dietary pattern. Mediterranean dietary patterns have been reported as efficacious due its high concentration of omega-3 and omega-6 fatty acids, accompanied with high vegetable, nut, and legume consumption. Many studies have investigated the role of adherence to Mediterranean diet patterns in terms of cancer risk [43-46] Specifically, investigators found that a greater adherence (i.e. Q5 vs Q1) to a Mediterranean diet has been linked with significant 19% reduction of all cause-mortality (HR 0.81, 95% CI: 0.77-0.85) [18]. In terms of breast cancer, Mediterranean diets have shown to reduced risk of estrogen receptor negative breast cancer [26,45]. Another study showed a lack of association between breast cancer and increased adherence to Mediterranean diet, which indicates the need to further investigate this measure [39].

The next diet index included in the analysis is the Dietary Approach to Stop Hypertension (DASH) score, which is a dietary pattern that was originally developed to reduce the prevalence of hypertension and overall cardiovascular disease risk. This dietary plan was first implemented in two randomized clinical trials in which efficacious intervention effects was observed amongst participants, which ultimately drove the adoption of the DASH diet into the Dietary Guidelines for Americans (DGA). A diet higher in nutrients, low in sodium and calorically sparse in which most protein intake was obtained from plants is characterized as a greater adherence to the DASH diet plan. Due to the observed efficacy of this diet plan in other chronic-inflammation driven disease (i.e. CVD), researches have investigated the role of adherence to this dietary pattern in conjunction with cancer risk [18, 20]. Specially, individuals with the highest adherence to the DASH diet reduced their overall cancer risk by 14% when compared to individuals of lowest adherence (HR 0.86, 95% CI: 0.78- 0.95). [18, 20, 27, 30, 32, 48]

The last standardized a-priori based measure included in the analysis was the Alternative Healthy Eating Index-2010 (AHEI-2010). The main intention for developing this diet index was to characterize dietary patterns associated with lower risk of chronic disease in past clinical and epidemiological investigations [23, 25]. This index improves on its predecessors (i.e. HEI, AHEI) by predicting systemic inflammation with greater ability, which serves as a risk factor for breast cancer [18, 34]. One study found that a higher adherence to an AHEI-2010 diet was associated with a significant reduction of overall mortality [27]. In terms of cancer, women with highest adherence to this diet exhibited a significant 7% reduction in risk when compared to lowest adherers (HR 0.93, 95% CI: 0.87-0.98, $p_{trend} < 0.04$) [25]. Another study reported a significant 15% reduction in overall cancer risk for highest adherers of the AHEI-2010 diet (HR 0.85, 95% CI: 0.77-0.93) [18]. These past findings provide evidence for the necessity of this dietary measure to be implemented in the analysis.

2.2.2.b Commonly assessed food groups of a-priori based dietary measures:

- **Fruits and vegetables:**

Two integral food groups that are widely understood for their nutritious value, because these component provide a rich source of fiber and nonessential nutrients known as phytochemicals. The phytochemicals serve as our major source of antioxidants (ie. Vitamin C, Vitamin E) and other vitamins (i.e. Vitamin D, Vitamin B). Antioxidants, along with fiber, play an integral role in combating oxidative stress in which these compounds can directly neutralize ROS, and even induce expression of important detoxifying enzymes such as glutathione-s-transferase (GST) [5-6, 49-51]. These aforementioned activities play an anti-tumorigenic role in breast cancer, which has been the focus of many cancer-epidemiological studies. The results from a recent meta-analysis, comprised of fifteen prospective studies, reported a significant 11% reduction in the risk of breast cancer for highest fruit and vegetable intake relative to the lowest (RR 0.89, 95% CI: 0.80-0.99). In terms of specific consumption, increases of 200 g/day for total fruit and vegetable provided significant evidence of risk reductions of 4% for breast cancer (RR 0.96, 95% CI: 0.93-1.00) [49]. These findings help support the healthful component role that vegetable and fruit play in these dietary indices.

- **Nuts/Legumes:**

Another food group commonly assessed in diet indices as separate or combined components, because they provide similar healthful effects in terms of nutrition. Nuts and legumes provide a rich source of plant-derived protein, which protein serves as a necessary building block for such tasks as amino acid synthesis and muscle tissue homeostasis. These are highly consumed components in healthful diet patterns such as the Mediterranean diet, because it provides a more efficacious source of dietary protein (i.e. less saturated fat, less poly-aromatic hydrocarbons) than animal-derived protein, while also decreasing levels of chronic inflammation [6,11,43,45]. Specifically, legumes provide a source of phytoestrogens that have strong antioxidant properties to ultimately aid in the removal and excretion of carcinogenic compounds [55-56]. Nuts and legumes also provide a source of plant-derived fats that play a role in mitigating inflammation by providing sources of unsaturated fatty acids, which have been shown to reduce markers of systemic inflammation [6, 24,39, 50, 52]. The anti-carcinogenic properties observed amongst these components have driven many epidemiological studies to assess their role in cancer. Specifically in terms of breast cancer, individuals with highest intake of soy-rich legumes exhibited a significant reduction of risk by 24% (RR 0.76, 95%CI: 0.65-0.86). Another study found a significant reduction of breast cancer amongst all women who increased their consumption of nuts by 120 grams/day and legumes by 20 grams/day by 5% and 6%, respectively (RR 0.95, 95% CI: 0.89- 1.00; RR 0.94, 95% CI: 0.89-1.00) [39].

- **Whole grains:**

A major food group commonly seen in a-priori based dietary measures, because of the undisputed healthful effects this component provides in terms of overall health and major health conditions [11, 54]. Whole grains provide a rich source of fiber, which promotes a healthy digestive process thus beneficial for the GI tract [56]. This component also provides a source of dietary vitamins and minerals that play an integral role as co-factor in many enzymatic processes. One study utilizing surrogate measures of whole grain found that increased dietary fiber consumption was linked to significant reductions of systemic inflammation in breast cancer patients [12]. A more recent case-control study found when individuals consumed whole grains at least 7 times per week, their odds of breast cancer decreased by 51% (OR .49 , 95% CI: 0.29 -0.82) [54]. These past findings suggest whole grain consumption may have a protective effect in terms of breast cancer manifestation and progression.

- **Fish:**

Fish is another healthful food group, which is commonly disputed for its efficacious effects in terms of various health conditions. Therefore total fish consumption was only found amongst one of these standardized indices included in this analysis, the Alternate Mediterranean Diet index. However, these other indices (DASH, AHEI-2010) do not consider fish consumption as a detrimental, but rather suggest the effects are null due to inconclusive evidence. Increased fish consumption is a common practice in Mediterranean culture, and greater adherence to the aMED diet pattern has been shown to decrease the risk of several chronic diseases, including cancer [18, 30]. These findings have driven nutritional epidemiological investigations into the role of fish consumption on breast cancer risk. Studies have suggested fish provides rich sources omega-3-fatty acids, which are widely understood for their anti-inflammatory properties [39, 50, 57-59]. Fish consumption has been also examined in association with breast cancer prognosis given its role on oxidative stress [59]. A recent systematic review found that overall fish consumption was associated with a significant 49% reduction of breast cancer risk in both post- and pre-menopausal women (OR 0.51, 95% CI: 0.27-0.98) [58]. Another meta-analysis reported that for every increase of 0.1 grams/day in fish consumption, the risk of breast cancer decreased by 5% (summary RR 0.95, 95%CI: 0.9-1.0) [57]. Although not widely implemented in many standardized indices, past findings suggest the fish consumption plays a role in breast cancer susceptibility.

- **Dairy:**

Dairy provides a source of vitamin d and calcium, but also fat-containing dairy products provide sources of conjugated linoleic acid (CLA) and saturated fats. Although most of these components are beneficial in terms of overall health, the saturated fat component provides unhealthful effects creating much dispute about the efficacy of dairy consumption pertaining various health conditions (i.e. Hypertension, Diabetes) [60]. Of the included indices in this analysis, only DASH includes dairy intake in which only low-fat dairy was recognized for that reason. In terms of breast cancer, dairy has been suggested to have a protective effect due to vitamin d and calcium, and also CLA mechanisms to combat inflammation. Whereas other studies suggest breast cancer risk is increased due to higher dietary fat consumption, from dairy, to promote adipose tissue aggregation. Therefore the evidence pertaining breast cancer is also shaky, but this may be accounted to an inadequate number of past studies investigating this matter, and difference in measurement methods. One study found that an increase of 290 grams/day in total dairy consumption was significantly associated with reductions of pre-menopausal and post-menopausal breast cancer risk [39]. A meta-analysis of 11 prospective cohort studies examined the role of dairy in breast cancer and found that individuals with the highest total dairy consumption reduced their risk of breast cancer by 15% (summary RR 0.85, 95% CI: 0.76-0.95). They also found sole-milk consumption elicited a 9% reduction in breast cancer risk (summary RR 0.91, 95% CI: 0.80-1.02) [60].

- **Oils/Fats:**

Oils/fat is a common food group used to assess dietary fat quality of an individual. Some dietary measures assess the ratio of healthy fats to unhealthy fats to provide an idea of the quality of individual's dietary fat intake. Oils such as olive and vegetable oil provide rich sources of mono- and poly-unsaturated fatty acids (MUFA, PUFA), especially omega-3 and omega-6 fatty acids (i.e. alpha-linoleic acid, linoleic acid) which are known anti-inflammatory constituents [5,50,52,58]. In terms of healthy fatty acids, investigators found a significant reduction of breast cancer risk for various omega-3 constituents [58]. The harmful fats are considered saturated fatty acids (SFAs) and trans-fats (TFAs). Specifically, trans-fats exhibit resistance to metabolic processes due to their problematic trans-bonds, which allows them to linger in the body for longer periods of time and thus promote adipose aggregation. Increased consumption of these harmful fats (SFAs, TFAs) has also been linked to higher levels of BMI and circulating inflammatory cytokines, which is advantageous for degenerative diseases such as breast cancer [50,60]. In terms of breast cancer risk, high saturated fat consumption was associated with a significant 28% increase in risk of ER^+ PR^+ breast cancer (HR 1.28, 95%CI: 1.09-1.52) [13]. The investigators of this study also found that saturated fat consumption was significantly associated with the manifestation of HER2 breast cancer subtypes. The Nurses' Health Study found a significant harmful association between higher saturated fat consumption and overall breast cancer risk, which also exhibited a dose-response relationship [14]. Lastly, one cohort study found a 34% decrease in breast cancer risk for post-menopausal women who increased their unsaturated fats to saturated fats ratio by one (RR 0.66, 95% CI: 0.43-1.02) [39]. These past studies provide interesting results in terms of oil to fat ratio's role in breast cancer susceptibility, which will be further investigated in this analysis.

- **Alcohol:**

Alcohol is a commonly consumed beverage in which there is dispute regarding the efficacy of its consumption in breast cancer, due to the protective associations observed amongst other health conditions. For instance, alcohol is a staple component of the Mediterranean diet pattern that has been shown to reduce the risk of breast cancer thus suggesting alcohol consumption may be protective [26,45]. However, alcohol is a general categorization of the true Mediterranean drink of choice- wine. Wine is an alcoholic beverage that utilizes the fermentation of fruit in production, and thus exhibits inherent antioxidant properties that other forms of alcohol do not (i.e. beer, liquor) [50, 52]. Moderate alcohol consumption has also been noted as an effective method to reduce risk of hypertension and cardiovascular disease [61]. In terms of breast cancer, alcohol consumption at excessive levels is considered carcinogenic due to increased levels of alcohol's metabolite, acetaldehyde. This harmful constituent of alcohol promotes increased DNA damage through adduct formation, therefore promoting increased genomic instability to allow for successful tumor formation [62-63]. Alcohol has also been hypothesized to increase the levels of circulating oestrogen, a female hormone, in both pre-menopausal and post-menopausal women, therefore high levels of consumption induce more production of mitogenic compounds to promote agressive tumor formations [62-63]. A meta-analysis investigating the role of alcohol in breast cancer found an increased risk of breast cancer in women age 40 to 49 for those who consumed seven to thirteen drinks per week [64]. Another meta-analysis consisting of 222 studies found light alcohol consumption to be signifcantly associated with a 5% increase of female breast cancer risk (RR 1.05 , 95% CI: 1.02-1.08) [63]. Another study found that women who consumed about three to nine drinks per week had a 30% increased risk of Breast cancer (RR 1.3, 95%CI 1.1, 1.7). Given these findings, it appears that alcohol consumption does alter breast cancer risk when considering the results from past studies and the strong biological basis.

- **Red and processed meats**

Red and processed meats are a commonly assessed food group that is included in a-priori indices, because of the evidence regarding the unhealthy contributions to overall diet quality. In other words, high consumptions of this component are considered unfavorable but moderate to low consumption are not as disputed. Red and processed meats provide serve as an enriched source of dietary protein, vitamins, anti-inflammatory fatty acids, iron and other minerals that are necessary for homeostatic processes of the body. However, this food group also provides a rich source of heterocyclic amines (HCA) and polycyclic aromatic hydrocarbons (PAH) when over cooked, and dietary fats especially saturated fats [55, 56]. Processed meats have been suggested to play a carcinogenic role, because these food products are commonly preserved with smoking, salting, and curing methods that entail the usage of chemicals such as nitrates and nitrites, which can be broken down into carcinogenic metabolites (i.e. nitrosamine) [65]. PAHs, HCAs, and nitrosamines have been studied in conjunction with colon cancer, and have been noted carcinogenic compounds with mutagenic effects allowing for DNA damage through such fashions as adduct formation [16, 55]. These findings have driven epidemiological investigations into the role of red and processed meat consumption in breast cancer. Results from a recent meta-analysis depicted red meat consumption to be significantly associated with an increased risk of post-menopausal breast cancer for each 100 gram /day increase in consumption (RR 1.22, 95% CI: 1.04-1.44). In terms of processed meat, these investigators found a lack of association between high processed meat consumption and breast cancer risk, but were able to convey a significant dose-response relationship for each 30 grams/day increase in consumption and breast cancer.

- **Sugar-sweetened beverages (SSBs):**

Another unhealthful food group found amongst a-priori indices is sugar-sweetened beverages. SSBs have become increasingly popular throughout the recent decades in which this food/drink group is comprised of calorically dense beverages that provides little to no nutritional value. There has been much suggestion that the stark rise in obesity over the past decades correlates with the increasing popularity of soda and other soft drinks (i.e. fruit juice, lemonade). Developing habits of increased sugar consumption during childhood while maintaining this habit throughout adulthood could have severe implications, which may account to this rise in obesity seen in the US population. Specifically, severe in the fact that obesity serves as risk factor of chronic inflammation-related diseases such as diabetes, cardiovascular disease, and also breast cancer [25]. Not much research regarding the direct role of SSBs on breast cancer has been carried out. However, investigations have been carried out to examine the role of sugar-sweetened beverages in obesity, so we may develop a better understanding of SSBs effect on our overall health [67]. Researchers found that individuals who consumed an additional serving of SSB per day, their BMI increased by 0.24 kg/m^2 thus providing evidence of SSB's "obesogenic" role in breast cancer progression (ß= 0.24, 95% CI: .10-0.39, p-value= 0.03). They also found a significant 60% increase in the odds of obesity for each additional soft drink consumed per day, which further suggests the unhealthful role SSBs play in breast cancer manifestation (OR 1.60, 95% CI: 1.14-2.24, p-value= 0.02) [67].

- **Sodium:**

Sodium is another unhealthful component in which consumption of sodium-rich food items has been linked with several major health conditions such as hypertension, coronary heart disease, and also diabetes [25]. Given sodium intake's role in these other degenerative diseases suggests it may also play a role in breast cancer susceptibility. These findings have driven major a-priori indices, such as DASH and AHEI-2010, to implement this food group into their dietary measures. The effect of sodium consumption in breast cancer has not been commonly evaluated, but significant associations have been found in terms of colorectal carcinogenesis, which fuels our interest in this investigation [20]. One study found greater adherence to low sodium diet plans were significantly associated with reduction of estrogen receptor-negative (ER^-) breast cancer risk [68]. The Nurses' Health Study showed a greater adherence to a low sodium diet plan was associated with decreased BMI [69]. The investigators of this study also showed that greater adherence to this diet plan was associated with a 23% reduction of ER^- breast cancer risk, which falls in line with past findings (RR 0.77, 95% CI 0.61-0.97). However, this drastic risk reduction was not observed in terms of overall breast cancer for individuals with a greater adherence to this low-sodium diet plan (RR 0.95, 95% CI 0.87-1.04) [69].

2.2.2.c An overview of the Healthful Diet Score (HDS):

The development of our a-priori dietary measure (HDS) was a tremendous academic task, which involved an extensive literature search of peer-reviewed journal articles that went beyond the commonly assessed food groups found in other indices (i.e. aMED, DASH, AHEI-2010). These standardized indices are widely accepted and utilized, because they promote efficacious diet patterns shown to reduce the risk of several chronic diseases [18]. In terms of breast cancer, the evidence regarding these standard indices is shaky and inconsistent, which may account to the broader definitions of food group categories utilized in these measures [28, 39-40, 68-69]. The overgeneralization of food groups may not truly capture the distinct differences in nutrient composition of different food items that play a role in breast cancer carcinogenesis. In order to avoid overgeneralizations, the ultimate aim in developing our a-priori based dietary measure was to create an index that characterizes overall diet quality in a more comprehensive manner than these widely utilized and accepted diet indices, which primarily focus on approximately ten major food groups (Table 1.1). We attempted to achieve this aim by encompassing an array of more diverse food groups that were considered relevant in altering overall health after undergoing the aforementioned literature search. Upon assessing the peer-reviewed literature as well the other standard indices (aMED, DASH, AHEI-2010), our a-priori index the Healthful Diet Score was comprised of 23 unique items representing distinct food groups found in an individual's overall diet. Table 2.1 depicts the components of our a-priori index as well as hypothesized effect size given the current weight of evidence:

Table 2.1
Healthful Diet Score formulation

Components:	**Effect:**
Fruits	+
Vegetables	+
Nuts/Legumes	+
Fish/Seafood	+
Whole Grains	+

Coffee/Tea		+
Dairy		+
Red Meat		-
Processed Meat		-
Sugar Sweetened Beverages		-
Fried Meats		-
Sweetened Refined Grains		-
Unsweetened Refined Grains		-
Savory Snacks/Sides		-
Sweetened Foods/Desserts		-
Vegetable Based Soups/Sauces		null
Juice		null
Potatoes		null
Sauces/Dressings		null
Poultry		null
Mixed Dishes		null
Eggs		null
Alcohol:	None to moderate	+

In terms of food groups found in the Healthful Diet Score, which are not found amongst the other standardized indices included in this analysis (Table 1.1) the proceeding section serves as a presentation of the epidemiological findings regarding their role in overall health and breast cancer.

- **Coffee/Tea:**

Coffee is one of the most commonly consumed beverages around the world, in which the yearly consumption average is around 1.1 kg per capita worldwide. Coffee and tea consumption has been linked with reduced risk of several major health conditions such as type 2 diabetes and Parkinson disease [72] This may be accounted to the fact that coffee and tea serve as enriched sources of polyphenols, flavonoids and lignans [71]. In terms of cancer, these components have been shown to have strong anti-inflammatory effects thus reducing systemic oxidative stress to play an overall anti-carcinogenic role [73-74]. These components have also been suggested to play an anti-angiogenic, epigenetic, anti-apoptotic role, which are all relevant events in cancer progression [74]. Coffee and tea also provide us with our main source of caffeine in which caffeine has been suggested to alter estrogen metabolism, which plays an oncogenic role in breast cancer [71]. Considering these findings, many epidemiological investigations into role of coffee and tea consumption in breast cancer have been carried out. The Swedish study found a significant association between coffee consumption and overall breast cancer risk in which the odds of breast cancer reduced by 20% for individuals consuming more than 5 cups a day (OR .80, 95% CI: 0.64, 0.99, p-trend= 0.028). They also found coffee-consumption to be distinctly associated with reduction of risk in ER^- breast cancer manifestations [70]. A more recent meta-analyses investigating this role found a lack of association between overall coffee consumption (P=0.55) and breast cancer risk as well overall caffeine consumption (P= 0.73). However, investigators did detect a significant dose-response relationship between coffee and caffeine intake pertaining to a 2% reduction of breast cancer risk for every incremental increase of 2 cups/day (P= 0.05) [73]. Another meta-analyses comprised of 59 unique studies found a significant 6% reduction in breast cancer risk when assessing the evidence across the included studies (summary RR 0.94, 95% CI: 0.91- 0.98) [72]. Given these findings, we hypothesized that coffee and tea consumption has a protective role in terms of overall health and breast cancer and therefore implemented in the HDS.

- **Fried Meats: (fried fish and poultry)**

In order to be more diverse with our a-priori based dietary measure, we further broken down animal protein consumption into a separate category based on its unhealthful method of preparation. This preparation method involves a breading or battering process, which is then deep-fried using high heats and cooking oils of various kinds that can lead to excess oil/fat residues leftover from the frying process. The overall process increases the caloric-density of these food items that are nutrient-sparse, which only further exacerbates its unhealthiness. Excess consumption of such oily foods promote an aggregation of adiposity, specifically in which unhealthful oils and fats such as trans-fats have been show to promote a state of chronic tissue inflammation [5, 13, 50]. Individuals with increased consumption of these items have been shown to have overall diets characterized by high fat intake. Due to this extra pro-inflammatory effect of these food items as accounted to their increased oxidative capacity, we decided to characterize intake of these food items separately as their own group [76]. Also increases in consumption of such food items have been observed amongst unhealthy dietary patterns, which have been associated with increased risk of several chronic diseases [50]. One said unhealthy dietary pattern is known as the "Western" diet pattern, which has been linked to increases in breast cancer risk. Specifically, one investigation found that higher adherence to diet patterns rich in fried food consumption were associated with a 81% increase in the risk of breast cancer (OR 1.81, 95% CI: 1.32-2.50) [30]. A case-control study examining the role of high-fried food diets in breast cancer found a significant increase of 41% in overall breast cancer risk (OR 1.41, 95% CI: 1.05- 1.89) [30]. Another study found adherence to such dietary patterns increased the risk of ER^+ breast cancer by two-fold when compared to lowest adhering women [75]. Given these findings, it became evident to classify these food-items into a distinct food group with the hopes to avoid overgeneralizations that may bias the results in anyway (i.e. fried fish and fish in same group may not be truly similar).

- **Refined grains, sweetened foods and desserts:**

Refined grains characterize a food group in which grains or grain flour undergo a refining process such as bleaching or brominating that removes bran and germ and other key nutrients. For this very reason, the refined products are often enriched with vitamin B and other constituents due to the harsh refining process. An excess intake of refined grains has been frequently identified in unhealthy dietary patterns associated with increased risk of chronic disease. The increased risk may be accounted to the elevated levels of pro-inflammatory cytokines elicited in response to consumption of this food group, which provides favorable conditions for degenerative disorders such as CVD, diabetes, and breast cancer [6]. Conversely, increased intakes of components such as refined grains indicates lower intakes of healthful dietary sources, such as whole grains, due to reaching the point of satiety from these unhealthy items. Several studies have identified refined grains to be associated with increased risk of stomach, colorectal, and other cancers [46].

Sweetened desserts and foods such as pumpkin pie, cookies, and ice cream are also food sources that provide no nutritional value and promote increasing levels of adiposity when eaten excessively. Desserts and pies are often enriched with sugar, which excess carbohydrate intake has been linked to increase levels of inflammation as well [79]. Diets rich in dessert and other sweets intake was identified in starch-rich patterns along with refined grains such as bread pasta. This aforementioned dietary pattern was significantly associated with a 34% increase in breast cancer risk (OR 1.34, 95% CI: 1.10- 1.65) [80]. Another study looking at US women identified this increased intake of sweetened desserts as part of the western-dietary pattern, and was shown to increase the risk of breast cancer by 44% amongst smokers (RR 1.44, 95% CI: 1.02-2.03) [80]. This may be accounted to the increase in glycemic index and glycemic load due to the sugar enriched nature of these food items. Others have noted increased sugar consumption has been linked to increased levels of inflammation as well [50, 79].

Specifically refined grains and sugar enriched foods such as cold cereals and cakes have been shown to promote glycemic overload in individuals with high consumption rates to promote cellular growth activities in an endocrine fashion [46]. In terms of breast cancer, increased consumption of refined grains and sugars as indicated by elevated glycemic index (GI) intake has been linked with increased breast cancer risk. The Italian EPIC cohort study found that individuals with high dietary glycemic load significantly increased their risk of overall breast cancer by 45% (RR 1.45, 95% CI: 1.06-1.99) [77]. Another study investigating the role of increased bread consumption on breast cancer risk found that individuals with the highest level of consumption increased their odds significantly by 28% (OR 1.28, 95% CI: 1.03-1.58). These investigators also found that individuals with highest quintile of consumption of pasta increased their risk of overall breast cancer by 37% (OR 1.37, 95% CI: 1.00-1.88, p-trend =0.016) [78]. A recent meta-analysis consisting of ten prospective cohort studies investigating the role of glycemic load on the risk of breast cancer found a significant increase 8% for individuals with the highest GI intake relative to lowest (RR 1.08, 95% CI: 1.02-1.14). In terms of glycemic load, the investigators also found non-significant increases in the risk of breast cancer for all women [79]. Given these findings, the inclusion of refined grains and sweetened foods/desserts in the HDS seemed appropriate for our interest of overall diet quality’s role in breast cancer, as well as other health conditions.

- **Savory snacks and sides:**

This food group contains such food items as nachos and French fries, which are widely known for their nutrient-sparse and calorically dense nature. Food items commonly serve as a proxy for other items eaten concurrently, for instance fries can often serve as surrogate marker for other unhealthful food items such hamburgers and soft drinks. These assumptions fall in line with findings of dietary pattern analysis showing a high correlation in consumption between these items. Unhealthful dietary patterns like the “Western” dietary pattern have been linked to increased levels of BMI and higher incidences of obesity, which serves as a known risk factor for various chronic diseases [30]. Diets rich in savory sides have been shown to increase the risk of breast cancer, specifically through increased levels of inflammation [50]. Another study in Uruguay reported a drastic increase in breast cancer risk by approximately two-fold (OR 2.03; 95% CI: 1.11-3.72) [30]. Whereas a study in the US found similar harmful association with increased intakes of savory sides, but not as drastically shown in last study (OR 1.32, 95% CI: 1.04-1.68) [30]. This food group is also commonly referred to as salty sides and snacks, because of the sodium-rich nature of these food items. As previously noted, adherence to low sodium diet plans were shown efficacious in BMI reduction efforts as well as reduction of ER^- breast cancer [69]. The incorporation of savory foods and snacks as an unhealthful food group in our diet quality measure appeared necessary given the evidence regarding savory foods and snacks.

- **Null food groups:**

Vegetable based soups/sauces is comprised of heterogeneous food items in which many soups from cans such as tomato soup are rich in sodium, but other vegetable soups such as homemade lentil soups can be very healthful and beneficial in terms of overall diet quality. Another food item of this food group is salsa and sauces, which can be highly mixed such as ketchup and taco sauces that provide little to no nutritional value, but other condiments such as whole-tomato salsa can provide beneficial sources of nutrients. However, we believe individuals who do truly consume fresh vegetable soups/sauces will also exhibit healthful consumption levels of vegetables as seen in dietary pattern analysis papers [30]. The contribution of nutrients such as antioxidants and fiber is truly greater from sole-vegetable consumption and therefore will still be truly indicated in the overall diet quality score. Considering the prior information, we regarded this as conservative assumption to characterize vegetable based soup/sauces as a null food group in its contribution to overall diet quality.

Juice is another commonly confounded food choice that most food frequency questionnaires do not assess extensively. Juices such as grapefruit and orange juice can serve as beneficial sources of nutrients such as antioxidants necessary to combat carcinogenic components in breast cancer. However, this nutrient-full property is only exhibited of fresh fruit juices or at least juices made from whole fruit, because they consist of the original components necessary of this action. Juices that are artificially flavored and/or made from concentrates to provide the orange juice flavor are commonly misconceived as a healthful component of overall diet quality. When in fact these “juices” are not 100 percent fruit juice but are more akin to soft drinks, because of their sugar enriched nature that makes these items calorically dense. It is also not hard to believe that individuals who consume healthful juices are more likely to exhibit an increased intake of fruits in general, which we consider to be more beneficial in terms of overall diet quality. Given these considerations, we made a conservative decision to characterize juice consumption as having a nullified effect-size in the Healthful Diet Score to avoid any introduction of bias related to this food group into the analysis.

Potatoes are a common excluded food item from total vegetable category in many a-priori based dietary measures (aMED, DASH, AHEI-2010), because they provide a rich source of dietary starch and few key nutrients relative to other food items found in this category (Table 1.1). Another reason this food item is commonly excluded from vegetable food groups is due to the many unhealthful methods of preparation, which turn this healthful food item into an unhealthy one. Such as French fries that have been commonly identified in the Western dietary pattern and have been shown increase the risk of breast cancer [30, 80]. To avoid any incorporation of vegetable food-items that may not be truly healthful in terms of overall diet quality, the exclusion of potatoes from total vegetables was underwent as well as the nullification of effect size to avoid any confounding.

Sauces and Dressings is a food group consisting of such condiments and toppings as salad dressings, mayonnaise, butter/margarine on vegetable, and butter/margarine on bread. Items of this food group are highly heterogeneous and lack distinctions between the quality of each food item. For instance, salad dressing serves as good marker for increased vegetable consumption but the FFQ assessed the intake as dressing and mayonnaise consumption, which play drastically different roles in overall diet quality. Mayonnaise may not serve a proxy as for healthful food consumption, but perhaps unhealthful depending on food item being accompanied with the condiment. Butter and margarine consumption were also assessed as one distinct item, which are fundamentally different due the enriched vegetable oil nature of margarine relative to butter. However, butter and margarine on vegetables serves as good marker for vegetable consumption, but studies have linked butter and margarine consumption to increased risks of myocardial infarctions, which have been shown to share risk factors with breast cancer [41]. Given the weight of evidence, the nullification of sauces and dressings' effect size was the logical conservative approach.

Poultry serves as an efficacious source of protein, which provides less dietary fats compared to its animal protein counter parts (i.e. pork and beef). However the evidence regarding poultry consumption is inconsistent in terms of overall health. Specifically one study found a significant decrease in breast cancer risk for those adhering to diet pattern with moderate poultry consumption [26]. Another study showed that increased consumption of poultry rich processed meats increased the risk of breast cancer [66]. These findings fall in line with other studies identifying moderate levels of poultry consumption as efficacious, but increasing intake past moderate consumption leads to increased risk of several chronic diseases [51, 65]. Another pitfall of the FFQ was that poultry consumption was assessed as chicken and turkey combined, including on sandwiches. Fresh cuts of turkey and chicken can provide healthful sources of nutrients, but other sources of poultry obtained from food vendors such as grilled chicken patties undergo processes such as deboning and grinding creating a mixture of poultry meat. Given the inconsistent evidence regarding poultry consumptions role in overall health and breast cancer, nullification of effect size was warranted in the HDS.

Mixed dishes is a food group consisting of such dishes as burritos, tacos, beef stew, lasagna, and pizza which are all similar due to the heterogeneous composition observed amongst each food item. Due to a lack of qualitative accuracy in the FFQ, discerning the healthful contributions from each food item was a difficult task that would require many assumptions, which if wrong could drastically skew the results. For instance, burritos can provide beneficial sources of nutrients depending on preparation method and components used to make the burrito, but can also be calorically dense food items rich in oil and sodium. Having no knowledge of the food quality for all the mixed dishes in this food group leaves much to assumption, which is far from a conservative approach therefore the nullification of the mixed dish food group was underwent to avoid any misclassification that may bias results.

Eggs are another food group in which much confounding arises when assessing the healthful nature of this component. Eggs provide a nutritious source of protein, iron and vitamins, but are also rich with cholesterol and have therefore been suggested to increase risk of many cardiovascular outcomes. Specifically, one study noted adherence to a diet low in egg intake (i.e. 1-3 servings/week) was associated with significant decreases in levels of inflammation, which serves as known risk factor for CVD and breast cancer [81]. Unhealthful methods of preparation such as frying also exacerbate the unhealthy nature of eggs, which ultimately increases the caloric density of this food item. One common reason egg intake is not assessed in many of these a-priori based indices, because eggs often serve as proximal marker for unhealthful food items consumed as well in which eggs serve as traditional breakfast item that is often coupled with processed meats and refined grains such as sausage/bacon and toast. Providing a beneficial effect to this food group that is highly correlated with unhealthful food group consumptions will introduce bias into analysis, therefore a conservative nullification of effect size was underwent.

CHAPTER 3

METHODS

3.1 Statement of research aims and hypotheses:

The overall aim of this study was to assess the role of overall diet on the risk of breast cancer in participants of the CTS through characterization of overall diet quality derived using a-priori based approaches. Our overall hypothesis asserts that overall diet quality of an individual has an effect on breast cancer risk, such that overall diet quality has a true inverse relationship with breast cancer risk.

In specific aim #1, we created our own measure of overall diet quality using a-priori based methods to yield a dietary index known as the Healthful Diet Score (HDS). This development of the HDS was carried out with the intentions to create a more diverse measure of overall diet quality relative to the standardized measures (DASH, AHEI-2010, aMED). As an exploratory aim thus not in satisfaction of specific aim #1, we also investigated which of the several indices exhibits a stronger relationship with BMI, thus serving to validate the quality of the dietary index since diet plays a role in adiposity levels. In specific aim #2, we evaluated the relationship between overall diet quality and the risk of breast cancer across the several dietary measures. Also, the comparison across dietary measures allowed us to investigate our a-priori index's predictive ability of breast cancer risk relative to the other widely accepted indices. For aim # 2, we expect individuals with a greater adherence to a healthy overall diet plan to exhibit lower risks of breast cancer relative to less adherent individuals. Specific research questions for each aim are addressed before the statistical methods description for the two aims.

3.2 Selection of the analytical cohort:

The California Teachers Study is an ongoing investigation into the role of potential risk factors on various complex clinical manifestations amongst pre- and post-menopausal women alike. The CTS baseline study population consists of 133,479 female participants in which extensive data regarding dietary habits and possible risk factors of breast cancer and other cancers was recorded, as previously noted in section 1.2. Also to reiterate, this cohort exhibits increasingly high rates of invasive and in-situ breast cancer incidence, which provides a promising study population for investigating the role of overall diet on breast cancer risk [31].

3.2.1 Exclusion Criteria:

The initial CTS cohort could not be used to due inadequate levels of dietary data recorded for some individuals. Given that our main aim of this investigation was to assess the role of overall diet quality on breast cancer risk, several exclusions amongst the total cohort of 133,479 were underwent sequentially to yield the final analytic cohort.

Exclusion Criteria 1: The exclusion criteria regarding this concern pertained to the completeness of responses pertaining to the 103-item FFQ. A conservative approach was taken where participants who exhibit incompleteness of dietary data ≥ 25% were excluded to avoid any introduction of error due to lack of questionnaire competency. The FFQ recorded dietary habits of 103 food-items and therefore individuals missing data on greater than or equal to 26 food-items were excluded from this analysis.

Exclusion Criteria 2: Due to the role of alcohol consumption in overall diet quality and especially breast cancer, the presence of alcohol consumption data was a necessity for this analysis [18,62]. Upon the first dietary exclusion, individuals exhibiting missing data regarding alcohol consumption were then removed to properly assess the role of alcohol as a component of overall diet quality on breast cancer risk.

Exclusion Criteria 3: The next exclusion was based on participant's caloric intake (kcal/day) in which individuals found at the extreme ends of the caloric intake distribution in the CTS cohort were excluded, specifically daily caloric intake values found in the >99% and <1%. Individuals exhibiting these outlier caloric intake values may skew our assessment of overall diet quality, therefore individuals were excluded if their daily caloric intake was > 3371.4 kcal/day or < 683.5 kcal/day.

Exclusion Criteria 4: Lastly, to avoid any inclusion of individuals undergoing current lifestyle intervention plans (i.e. diet changes), participants with a history of diabetes, heart attack, stroke, and other incident cancers were excluded from the cohort. As well as the exclusion of individuals with breast cancer diagnoses prior to baseline to ensure the analytic cohort is cancer free at baseline.

3.3 Dietary Assessment:

Upon excluding women who did not meet the selection criteria for this analysis, dietary data clean up step was undergone. Specifically, the study population's dietary data was assessed to identify individuals who failed to report portion size or frequency of consumption amongst the various food-items recorded. Steps were taken such that individuals who reported "never" as the frequency response but didn't report a portion size were treated as non-consumers for that particular food. Individuals who reported frequencies greater than never, but failed to report portion size were imputed with the median portion size response for that specific food item. Lastly, individuals who failed to report frequency of consumptions were given non-consumption intake values for those particular food-items. These aforementioned steps are commonly used in nutritional epidemiology when dealing with missing dietary data, given the reasoning that these individuals answered >75% of the FFQ correctly, thus scenarios such as incomplete answers of frequency are more likely to represent non-consumption of a particular food-item. After completing the data cleanup process, the various intake levels for the foods found in this study were derived from participant's responses to the 103-item FFQ, which helped yield the exposure of interest- overall diet quality. Specifically, diet quality scores were calculated amongst each subject through utilization of several reputable dietary indices plus our own a-priori based measure (aMED, DASH, AHEI-2010, HDS).

3.3.1 Alternate Mediterranean Diet Index:

As previously noted, the Alternate Mediterranean Diet Index is an a-priori based dietary measure that characterizes an individual's adherence to a Mediterranean dietary pattern, which has been found to be significantly associated with breast cancer risk [26, 45]. The aMED is comprised of 8 components (normally 9 components but nuts and legumes were combined in this study) representing major food groups assessed in the dietary measure in which each component has a hypothesized effect in the Mediterranean diet pattern. In terms of scoring, the intake of commonly consumed components in Mediterranean diets (i.e. vegetables, nuts and legumes) was viewed as a healthful effect and therefore individuals with intake levels greater than or equal to the median intake amount received a score of +1 for that given component. Reverse scoring was carried out for foods considered unfavorable in terms of adherence to a Mediterranean diet plan (i.e. red and processed meats), specifically where individuals would receive a +1 if their intake was below than or equal to median intake amount of that given component. For alcohol consumption, women who exhibited light to moderate drinking (approximately 1 drink/day) received a score of +1. In this analysis, aMED has a possible range of 0 to 8 points in which 8 points is indicative of complete adherence to the Mediterranean diet pattern.

3.3.2 Dietary Approaches to Stop Hypertension Score:

The DASH diet plan was first shown as an efficacious intervention in a randomized clinical trial (RCT) interested in the outcome of hypertension risk reduction. The results from the RCT led to DGA's incorporation of this diet plan, which gave way to the development of this dietary index comprised of 8 major components/food groups. In the DASH index there are 5 healthful components (i.e. Dairy, Vegetables) in which intake of food groups are assessed as quintiles of consumption, therefore individuals found in the highest quintile receive a score of +5 vs. individuals found in the first quintile who receive a score of +1 for healthful components (Table 1.1). The remaining 3 components make up the unhealthful food groups (i.e. sodium, SSB) found in the DASH index where increased consumption of these foods is considered less adherent to the overall DASH diet plan. Consumption levels of these unhealthful food groups is also assessed as quintiles of intake where individuals found in the highest quintile receive a score +1 vs. intakes found in the lowest quintile that received a score +5. Given the scoring criteria of the healthful and unhealthful food groups found in DASH, the diet quality score exhibits a continuous range from 8 to 40 pts where higher scores indicate a greater adherence to the DASH diet plan.

3.3.3 Alternative Healthy Eating Index-2010:

An a-priori based measure originally developed to characterize overall dietary patterns associated with low chronic disease risk. The AHEI-2010 is a more comprehensive dietary measure compared to the other standardized indices included in this analysis, because it consists of 9 total components in which consumption levels were assessed by deciles of intake to provide ten unique consumption groups within each component excluding alcohol (Table 1.1). For components considered having a healthy effect on overall diet quality (i.e. fruits, legumes), the intake levels corresponding to the highest decile would receive a score of +10, whereas intakes corresponding to the lowest decile would receive a score of +1. Conversely, food group intakes considered to be less adherent (i.e. red meat, sodium) to the AHEI-2010 diet plan were scored unfavorably in which intakes found in the highest decile received a score of +1 vs. intakes found in the lowest decile receiving a maximum score of +10. For alcohol consumption, moderate drinkers who consumed approximately one drink per week received a score of +10 points vs. non-drinkers who received 2.5 points vs. heavy drinkers who received +5 points vs. excessive drinkers who received 0 points. Considering the scoring criteria of the Alternative Healthy Eating Index 2010, the possible scores for this dietary measure range from 8 to 90 points and like the other indices a higher score shows greater adherence to the AHEI-2010 diet plan.

3.3.4 Healthful Diet Score:

The Healthful Diet Score (HDS) was an investigational aim with the intentions of creating a more comprehensive dietary measure of overall diet quality. This goal was in partial satisfaction to investigate the role of diet on breast cancer risk due to the inconsistent evidence found in the literature regarding this topic, which may account to a lack of comprehensiveness amongst these dietary measures (i.e. AHEI-2010, DASH, aMED). A more in-depth explanation of our a-priori based dietary measure can be found in section 2.2.2.c. The HDS consists of 23 distinct food groups in which intakes of each component are assessed by quintiles of consumption and therefore yield 5 unique intake levels within each food group. Food groups that we considered having a beneficial effect in terms overall diet quality (i.e. fish, whole grains) were scored such that individuals found in the highest quintile of intake received a score of +5 vs. individuals found in the lowest quintile receiving a score of +1. Unhealthful food groups (i.e. sweetened refined grains, savory sides) of the HDS were reverse scored such that individuals with the highest quintile of intake received a score of +1 relative to individuals who were found in the lowest quintile receiving a score of +5. Also, food groups (i.e. Fruit Juice, vegetable-based soups) hypothesized to have a null effect in terms of overall health received a score of 0 due to various reasons, which can be found in section 2.2.2.c. For alcohol intake, individuals who consumed 0 to 8 ½ drinks per week received a score of +3 vs. consumption levels >8 ½ to 14 drinks per week receiving a score +2 vs. consumption levels >14 drinks per week that received a score +1. Upon yielding scores for each of 23 components, the summation of scores across all 23 groups is underwent to provide an individual's Healthful Diet Score, which characterizes adherence to an overall healthy dietary pattern given the latest evidence found in literature. The possible scores for the HDS range from 16 to 78 total points in which maximum scores indicate full adherence to our a-priori defined overall healthy diet. A low HDS is indicative of a decreased adherence to our a-priori defined healthy diet pattern, which suggests increased and decreased intakes of unfavorable and favorable components, respectively.

3.4 Statistical Analysis:

3.4.1 Statistical methods applicable to overall aim:

Confounding was assessed using a-priori based reasoning to decipher common risk factors found amongst breast cancer [29,64]. Confounders related both to the exposures and outcome must be controlled for to provide true measures of association and avoid skewness in results. The commonly adjusted for confounders were included in multivariate cox proportional hazard regression models to yield an accurate assessment of overall diet quality effects on the risk of breast cancer. Further detail regarding these specific confounders and the modeling processing can be found in section 3.4.4. Confounding was not assessed in specific aim #1 due to the fact that no statistical modeling was carried out.

Effect Modification: Due to the past evidence showing difference in breast cancer disease between post and pre-menopausal women, the presence of an effect modification relationship between overall diet quality and menopausal status was assessed to further shed light on this matter [37, 77-78]. The menopausal status in the CTS was recorded as a categorical variable with 6 distinct levels: pre-menopausal, post-menopausal no hormone therapy (ht) use, post-menopausal past ht use, post-menopausal current estrogen use, post-menopausal current estrogen and progestin use. Interaction between diet quality scores and BMI was also assessed due to the similar reasoning used for menopausal status's effect modification relationship in which this covariate was included as a continuous variable (kg/m^2) [17].

Statistical Modeling was a 3-stage process in which confounders and other independently associated covariates where included into the model with our exposure of interest (i.e dietary index) to provide measures of association pertaining to time to primary breast cancer manifestation. Given this information, multivariate cox proportional hazard regression models were utilized in which confounders' and independently associated variables' contributions to the model's overall predictive ability was assessed using Wald's chi-square statistics. This aforementioned method was utilized during the 3-stage modeling process to provide the most parsimonious model in terms of assessing the role overall diet quality on breast cancer risk. All statistical analyses were carried out in Statistical Analysis Systems software, version 9.3 (SAS, Inc., Cary, NC).

3.4.3 Statistical methods applicable to specific aim #1:

Aim #1 was to derive an a-priori based dietary measure, which was described in detail conceptually and statistically in sections 2.2.2.c and 3.3.4, respectively. After computation of each participants diet quality score for all the included scores in this analysis (HDS, aMED, DASH, AHEI-2010), validation our dietary measure was carried out through generation of macro/micronutrient intake tables and correlation analysis between theses standardized indices and the HDS. Upon validation, statistical computation of descriptive characteristic frequencies was undergone using SAS 9.3, which allowed us to summarize subjects' characteristics into frequencies and proportions exhibited within the given quintile of that specific diet quality score. Finalized tables consisted of frequencies and proportions for the confounding and independently associated variables assessed in this analysis, specifically for quintile 1, 3, and 5 to depict the changes in characteristics across the range of diet quality scores. Continuous variables were depicted as means with respective standard deviations (i.e. BMI, Physical activity). This also allowed us to decipher if our a-priori based dietary measure was more predictive of adiposity levels as indicated by BMI relative to these other widely used dietary indices in satisfaction to assess the quality of our dietary measure.

3.4.4 Statistical methods applicable to specific aim #2:

Specific aim #2 was more directly pertaining to the overall aim of the study and therefore multivariate Cox proportional hazards regression models were utilized to estimate hazard ratios (HRs) along with 95% confidence intervals (95% CIs). As previously mentioned a 3-stage modeling process was underwent in which commonly adjusted for covariates were assessed for their overall contribution to the model's fit and predictive ability in terms of breast cancer risk. Wald statistics were utilized in defining overall significance of these other covariates to provide the final predictive model used in this analysis across the several diet quality scores (aMED, HDS, DASH, AHEI-2010).

The first stage of the modeling process involved assessing the presence of significant evidence for inclusion of women's health and reproductive variables such as family history of breast cancer, and menopausal status. Specifically, the covariates assessed for their contribution to a parsimonious model were: menopausal status, family history of breast cancer, race, and age at menarche. Covariates found to significantly contribute to the model's fit when assessing the role of overall diet quality were included in stage 2 of the modeling process.

The second stage introduced confounding and independent covariates such as lifestyle factors, which make up the few modifiable risk factors observed amongst breast cancer [29,64]. These risk factors included smoking status at baseline, socioeconomic status, and moderate physical activity levels (hrs/wk). Covariates found significant through evidence of provided by the Wald's statistic ($p \leq 0.05$) were included into the model that was further built upon during stage 3.

The third stage of the model involved the introduction of dietary confounders and other commonly adjusted covariates in nutritional epidemiological assessments. Specifically, covariates found in this final stage of the modeling process were: BMI (kg/m^2), daily vitamin use (yes or no), daily caloric intake (kcal/day). Upon assessing the Wald chi-square statistics for these aforementioned covariates and corresponding p-values, inclusion of the significantly contributing variables was underwent to yield the finalized model that will be uniformly applied across the various a-priori based dietary measures.

Effect modification as previously mentioned was also assessed in which the final model, included interaction terms between menopausal status and diet quality score. Wald chi-square statistic's testing the null hypothesis of $ß = 0$ provided evidence of the interaction terms significant contribution to model's predictive ability. This was also carried out amongst BMI in which a separate model including an interaction term between dietary index score and BMI was investigated for its parsimonious contribution. Identification of susceptibility factors may further guide us in our investigation of overall diet quality and breast cancer risk.

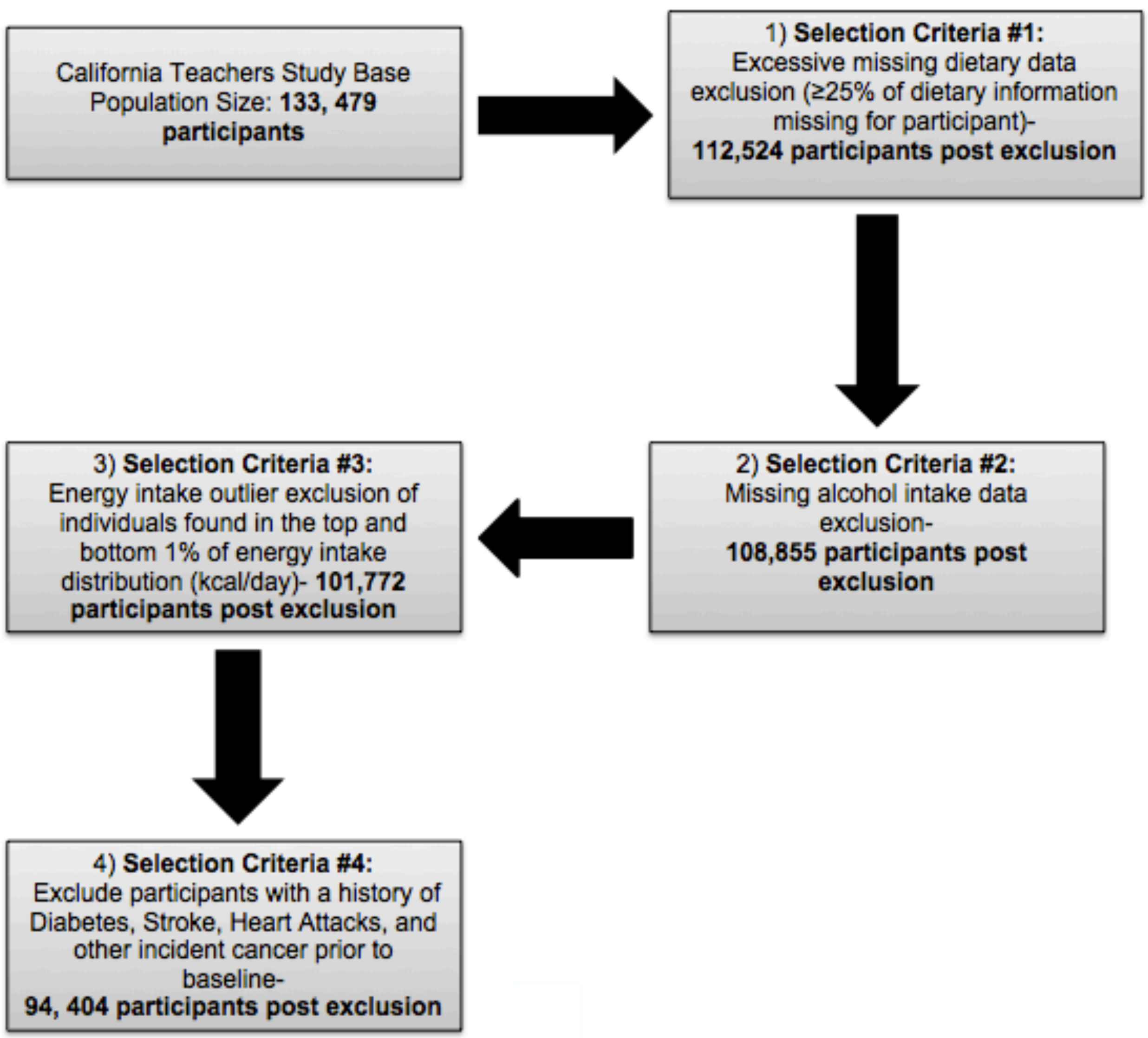
Trend Tests were carried out as well to assess dose-response relationships between breast cancer risk and adherence to the overall healthy diet patterns as indicated by these various dietary measures. This assessment was specifically carried out using the continuous measures of overall diet quality (HDS, AHEI-2010, aMED, DASH) in which Wald chi-square statistics along with corresponding p-values were used to provide evidence of dose-response relationships with breast cancer.

CHAPTER 4

Results

Upon applying selection criteria as stated in section 3.2.1, the final analytic cohort size in the CTS to be assessed in the specific aims laid out for this analysis was 94,404 subjects. See flow chart for study population selection process (Figure 4.1).

Figure 4.1: Flowchart of the study selection process



4.1 Results applicable to specific aim #1:

Specific Aim #1: Develop a more comprehensive measure of overall diet quality relative to these other standardized indices (aMED, DASH, AHEI-2010)

The efforts of this investigation to create an a-priori based dietary measure that characterized diet more comprehensively was successfully achieved. The resulting formulation of this diverse dietary measure can be found in section 2.2.2.c in which we accomplished this aim by creating a dietary index with 23 distinct food groups each with their own hypothesized effect on overall diet quality and overall health. After computing the various diet quality scores for participants, our a-priori based dietary measure was validated through generation of a correlation matrix consisting of the various diet scores to show how well our a-priori dietary index was correlating with these other standardized measures. Results from the correlation analysis show significant evidence ($p<0.05$) that our a-priori based dietary measure at least exhibited positive moderate correlation levels across the other standardized dietary measures. In terms of increasing correlation levels, the Healthful Diet Score exhibited the lowest correlation with the aMED score in which significant evidence showed moderate correlation levels (r) of .62 ($p<0.0001$). The HDS then exhibited the second highest correlation levels with the Alternative Healthy Eating Index- 2010 in which significant evidence ($p<0.0001$) conveyed a strong level of correlation ($r= 0.735$) between these two measures. Lastly, our a-priori dietary index exhibited the highest levels of correlation with the Alternate Mediterranean Diet score in which significant evidence allowed us to conclude highly strong correlation levels between these two measures ($r= 0.831$, $p<0.0001$) (See table 4.1 for correlation analysis results).

The aforementioned results of the correlation analysis with our a-priori based dietary measure provided the initial evidence that our dietary index functions in a manner similar to these widely accepted indices (aMED, DASH, AHEI-2010). However, further assessment in to validity of our measure was required thus we constructed descriptive tables consisting of macro/micronutrient intake levels across the quintiles of the Healthful Diet Score. Assessing how the healthful and unhealthful macro/micronutrients are consumed relative to an increased adherence to our a-priori defined healthy diet was a necessary step to carry out before investigating the overall aim of this analysis. Results from the macro and micronutrient tables provide interesting findings in which healthful macro- and micronutrients intake levels generally increase through higher diet score quintiles. To name a few of these components, we see such nutrients such as daily iron, daily Vitamin D, and daily carotenoids intake levels increasing with greater adherence to our a-priori defined healthy dietary pattern (Table 4.2). Conversely, micro- and macronutrients considered unhealthful were observed in a manner of decreasing intake levels as the diet score quintiles increased. Such constituents seen to decrease as our a-priori based dietary measure increased as depicted by quintiles were total daily fat, and total caloric intake, just to name a few. For instance, the mean total fat consumption for individuals with HDS found in quintile 1 was 38.6 grams/1000 kcal/day relative to individuals found in quintile 5 who exhibited mean daily total fat intake levels of 30.20 grams/1000 kcal/day for individuals with HDS scores found in quintile 1 ($\bar{x}_{Q1}$= 38.6 grams/1000 kcal/day , sd_{Q1}= 7.09 vs. $\bar{x}_{Q5}$= 30.20 grams/1000 kcal/day , sd_{Q5}= 8.45). Further depiction of micro/macronutrient intake level results across our a-priori based dietary measure can be found in Table 4.2.

An exploratory question, not linked to any specific aims, of this study was to assess how well our a-priori based diet score related to changes in adiposity levels relative to these other widely accepted indices. Frequency distribution tables were generated across quintiles of these various diet scores to yield descriptive characteristics of the analytic cohort to provide comparative tables for distinct changes in characteristics across quintiles and different diet scores. This aforementioned task allowed for fulfillment of our exploratory question of interest in this analysis with regards to healthy diet adherence and adiposity levels as measured through BMI. In terms of participant characteristics, we see a range of trends exhibited across the diet quality score quintiles for the given confounding or independently associated variablse (i.e. menopausal status, SES) (Table 4.4). Specifically when assessing the distribution of participants' characteristics across the Healthful Diet Score quintiles, the mean age at baseline for individuals found in quintile 1 was 45 years old (±12), whereas the mean age in quintile 3 was 52 years old (±13), and quintile 5 was 60 years old (±13). These findings suggest older participants tend to adhere to an overall healthier dietary pattern. In terms of race, whites were the only race to exhibit increasing proportions across the quintiles (Q_1= 81.56%, Q_3=88.60%, Q_5= 91.52%) thus suggesting whites are greater adherers to our a-priori defined healthy diet pattern relative to the other races (Table 4.3).

Menopausal status provided interesting results in which individuals with greater adherence to the Healthful Diet Score (i.e. quintile 5) tended to be post-menopausal women and especially current hormone therapy users as well. Conversely, pre-menopausal women were more likely to be found in the lower diet score quintiles thus suggesting less adherence to an overall healthy diet (Q_1= 63.83%, Q_3=40.39%, Q_5=

20.65%). The distribution of smoking status characteristics across the HDS quintiles indicated former smokers were more likely to exhibit higher adherence to a healthy diet pattern when compared to current and non-smokers of this study (Q_1= 19.53%, Q_3=29.52%, Q_5= 34.80%). Individuals with at least one 1st degree relative with breast cancer was observed in increasing proportions across the diet score quintiles whereas the other individuals were observed in decreasing proportions. In terms of SES, participants found in the highest SES category were more likely to exhibit a greater adherence to the HDS diet pattern as seen through the increasing proportions across the quintiles (Q_1= 39.58%, Q_3=46.63%, Q_5= 50.60%). Individuals who exhibited higher HDS scores (i.e. quintile 5 scores) had a higher mean physical activity (2.17 ± 2.23 hrs/wk) relative to individuals found in the lower quintiles. Vitamin use decreased across the diet score quintiles suggesting greater adherence to the HDS diet pattern requires less compensation to overall diet quality from vitamin intake.

The Alternative Healthy Eating Index 2010 provided interesting results in terms of descriptive statistics as well. Specifically, the mean age at baseline for individuals with quintile 1 diet quality scores was 45 years old (± 12) versus the mean ages in quintile 3 and 5 which was comprised of generally older individuals: 53 ± 13 years old, 58 ± 13 years old, respectively (Table 4.3). In terms of racial differences, we see that whites were the only race to exhibit a strong relationship of adherence to the AHEI-2010 diet plan, especailly seen through the increasing proportion sizes across quintiles 1, 3, and 5 (Q_1= 82.92%, Q_3=88.25%, Q_5= 90.19%). When assessing menopausal differences across the diet score quintiles, we observed post-menopausal women to be the greatest adherers especially amongst those women who used Estrogen and Progestin Therapy (EPT) (EPT(+): Q_1= 8.95%, Q_3=15.38%, Q_5= 19.14%).

As seen before with the HDS, former smokers tended to be found in increasing proportions throughout the diet score quintiles whereas individuals found in other smoking statuses were observed in a decreasing fashion (Former: Q_1= 21.96%, Q_3=30.05%, Q_5= 32.41%). Differences in breast cancer family history according to overall diet quality showed that women who have at least one 1st degree relative with breast cancer when compared to the others, were more likely to adhere to an overall healthy diet pattern (Q_1= 10.17%, Q_3=11.98%, Q_5= 13.05%). When assessing differences by socioeconomic status, we observed women who were found in the high SES category were more likely to exhibit greater adherence to this AHEI-2010 diet plan as observed through the increasing proportions throughout the quintiles (Q_1= 38.91%, Q_3=45.43%, Q_5= 50.67%). Moderate physical activity levels trended in a manner as expected in which individuals found with a more healthful overall diet quality were more likely to exercise as observed through the increasing mean exercise hours per week throughout the quintiles (Q_1= 2.18 ± 2.26, Q_3=2.3± 2.3, Q_5= 2.65 ± 2.5). Also, daily vitamin use decreased as adherence to the AHEI-2010 diet plan increased, therefore suggesting this inverse relationship may be due to dietary nutrient compensation.

The Alternative Mediterranean (aMED) Index was the next dietary measure in which descriptive statistics were yielded. The mean age across diet score quintiles went as follows: 47 ± 13, 52 ± 14, 57 ± 13 years old for quintile 1, 3, and 5 respectively. Racial differences arise amongst this dietary index in which individuals with greatest adherence to this dietary pattern tended to be white, whereas the other races where less likely to adhere (White: Q_1= 86.15%, Q_3=87.54%, Q_5=88.55%) (Table 4.3). Marginal increases of adherence were also observed amongst our Native American participants as well. Differences regarding menopausal status are present in which pre-menopausal women were more likely to be less adherent whereas post-menopausal women exhibited the greatest the adherence (Pre-menopausal: Q_1= 55.89%, Q_3=41.86%, Q_5=29.37%). Former smokers exhibited the greatest adherence to the aMED diet pattern when compared to current and non-smokers who exhibited lower adherence (Q_1= 25.28%, Q_3=29.05%, Q_5=30.44%).

Women with at least one 1st degree relative with breast cancer were more likely to adhere to an overall Mediterranean diet pattern relative to the individuals without a family history or were adopted that were observed in decreasing proportions throughout the quintiles (Q_1= 10.44%, Q_3=11.64%, Q_5=12.48%). In terms of SES, women found in the highest SES category were more likely to exhibit a greater adherence to an overall Mediterranean diet pattern as seen in the increasing proportions throughout the diet score quintiles (Q_1= 41.82%, Q_3=45.78%, Q_5=48.05%). Mean physical activity was observed in increasing levels across the quintiles of aMED in which the highest observed mean physical activity was 2.67 (±2.50) hours per week. Greater adherence to this Mediterranean diet pattern was observed to have an inverse relationship with daily vitamin use, as seen in these other dietary measures.

The final descriptive table was generated for the Dietary Approaches to Stop Hypertension score specifically across quintiles 1, 3, and 5 of the diet score. Age at baseline for individuals who were less adherent (quintile 1) to the overall DASH diet plan was 46 years old (±12) versus 59 years old (±13) for individuals who were most adherent (quintile 5) (Table 4.3). Also, Individuals who were more adherent to this dietary pattern targeting lowering cardiovascular disease risk tended to be white, but increasing levels of adherence was also observed amongst the Native American women (White: Q_1= 80.47%, Q_3=88.62%, Q_5=91.52%). When assessing menopausal difference, we observed post-menopausal women to exhibit greater adherence to the DASH diet plan relative in which increasing proportions was observed throughout the quintiles (Q_1= 7.16%, Q_3=11.06%, Q_5=15.98%). In regards to smoking status, current and non-smokers were observed in decreasing proportions throughout the quintiles versus former smokers who showed the greatest adherence to the DASH diet plan (Q_1= 21.96%, Q_3=30.05%, Q_5=32.41%).

In terms of familial aspects, women having a family history of breast cancer tended to adhere to an overall healthy dietary pattern more so than women without a family history and or those adopted (BRCAFAM1: Q_1= 9.86%, Q_3=11.86%, Q_5=12.78%). Women found in the highest SES category were also more likely to exhibit greater adherence to a more healthful dietary pattern in which increased proportions of these individuals were found throughout the DASH score quintiles (Q_1= 41.5%, Q_3=46.05%, Q_5=48.34%). The distribution of mean physical activity levels across the quintiles indicated that women with greater adherence to the DASH diet plan also tended to exercise more hours throughout the week and were less likely to take vitamins on a daily basis (Q_1= 22±4 hrs/wk, 7366 vitamin users vs Q_5= 2.72±2.59 hrs/wk, 3708 vitamin users).

With regards to the exploratory question of this thesis analysis, our a-priori based dietary measure was truly indicative of adiposity levels as detected by BMI. To reiterate, we hypothesized greater adherence to our a-priori defined dietary pattern would be associated with lower BMI levels and vice versa. When assessing the mean BMI as adherence increases to AHEI-2010 diet plan, we see a general decrease in BMI across the diet score quintiles (Q_1= 26±6 kg/m^2, Q_3= 25±5 kg/m^2, Q_5= 24±4 kg/m^2) (Table 4.3). However, these results were not similarly observed amongst individuals with increasing adherence to the aMED diet plan, specifically in which individuals with greatest adherence shared the same mean BMI as those with lowest adherence (Q_1= 25±5 kg/m^2, Q_3= 25±5 kg/m^2, Q_5= 25±5 kg/m^2). These results suggest this adherence to a Mediterranean diet pattern may not play a role in adiposity levels that serve as a risk factor for many chronic diseases [5]. Adherence to the DASH diet plan did not perform as poorly as aMED in which higher diet quality scores in DASH were associated with an decreased BMI levels for individuals in the highest quintile relative to the lower quintiles (Q_1= 25±6 kg/m^2, Q_3= 25±5 kg/m^2, Q_5= 24±4 kg/m^2). These results may suggest a threshold relationship to observe effects such as adipose tissue reduction.

In terms of our a-priori dietary measure developed in this thesis, the Healthful Diet Score, we found greater adherence to this dietary pattern to be associated with decreasing levels of BMI similar to results found with AHEI-2010 and DASH (Q_1= 25±6 kg/m^2, Q_3= 25±5 kg/m^2, Q_5= 24±5 kg/m^2). When assessing inter-index results, we see that our a-priori dietary index functioned better than aMED with regards to decreasing adiposity levels, and functioned similarly to the DASH and AHEI-2010 in which the highest adherence (quintile 5) showed the greatest decrease of BMI levels in women of the CTS. These results regarding our exploratory questions suggest the Healthful Diet Score is a successful dietary measure in terms of conditions related to overall health (i.e. BMI).

Table 4.1- Correlation analysis across the various diet scores

	Peason's Correlation Coefficients (N= 94,404)			
	prob > |r| under H0: Rho=0			
Dietary Measures:	**HDS**	**AHEI-2010**	**aMED**	**DASH**
HDS	1	0.735 (<0.0001)	0.615 (<0.0001)	0.831 (<0.0001)

AHEI-2010	0.735 (<0.0001)	1	0.732(<0.0001)	0.797 (<0.0001)
aMED	0.615 (<0.0001)	0.732(<0.0001)	1	0.690 (<0.0001)
DASH	0.831 (<0.0001)	0.797 (<0.0001)	0.690 (<0.0001)	1

Table 4.2- Macro/micronutrients intake levels across varying quintiles of the Healthful Diet Score

Healthful Diet Score:		**Alcohol consumption during Past Year (g/d)**	**Daily Vitamin C (mg)**	**Daily Vitamin E (α-TE)**	**Daily Calcium (mg)**	**Daily Iron (mg)**
Quintile 1:	Mean:	6.94	74.13	9.73	687.93	11.24
	SD:	9.54	42.36	5.93	382.22	3.88
Quintile 2:	Mean:	7.84	86.06	9.22	723.59	11.40
	SD:	9.83	47.48	5.67	403.63	4.19
Quintile 3:	Mean:	8.06	94.34	8.91	755.15	11.67
	SD:	9.88	50.34	5.43	425.78	4.34
Quintile 4:	Mean:	8.05	103.71	8.54	784.03	11.90
	SD:	9.51	53.01	4.91	428.65	4.25
Quintile 5:	Mean:	7.36	120.51	8.06	864.53	12.31
	SD:	8.92	56.71	4.11	456.01	4.06

		Daily Vitamin A (IU)	**Daily Vit. B6 (mg)**	**Daily Vit. D (IU)**	**Daily Thiamin (mg)**	**Daily Folate (mcg)**
Quintile 1:	Mean:	4,316.81	1.40	146.59	1.22	317.37
	SD:	2,175.47	0.59	106.02	0.48	127.53
Quintile 2:	Mean:	5,132.35	1.45	163.26	1.21	325.88
	SD:	2,599.93	0.61	113.10	0.50	134.64
Quintile 3:	Mean:	5,780.85	1.49	176.69	1.22	332.68
	SD:	2,976.09	0.61	119.91	0.50	136.34
Quintile 4:	Mean:	6,441.54	1.53	190.14	1.22	337.80
	SD:	3,278.23	0.59	123.14	0.47	131.77
Quintile 5:	Mean:	7,718.80	1.62	224.80	1.24	348.12
	SD:	3,761.77	0.55	134.87	0.43	123.27

		Daily Carotenoids (g)	**Daily Energy Intake (kcal)**	**Daily Total Fat (g/1000 kcal)**	**Daily Sat. Fat (g/1000 kcal)**	**Daily Protein (g/1000 kcal)**
Quintile 1:	Mean:	4,440.75	1,706.46	38.59	12.72	35.86
	SD:	2,246.04	530.64	7.09	2.89	6.04
Quintile 2:	Mean:	5,317.48	1,617.45	36.56	12.00	37.66
	SD:	2,764.51	528.73	7.63	3.07	6.37

Quintile 3:	Mean:	5,981.03	1,585.41	35.09	11.48	39.00
	SD:	3,105.17	524.14	7.95	3.15	6.66
Quintile 4:	Mean:	6,678.34	1,543.77	33.33	10.82	40.45
	SD:	3,469.09	495.91	8.18	3.19	6.92
Quintile 5:	Mean:	7,936.69	1,498.42	30.20	9.74	43.60
	SD:	3,908.17	444.31	8.45	3.20	7.44

Table 4.3- Descriptive statistics across various diet quality scores quintiles

Characteristic:			**Healthful Diet Score**			
	Q1:		**Q3:**		**Q5:**	
Age at Baseline:	45 ± 12		52 ±13		60 ±13	
Race:	**Frequency (n)**	**(%)**	**Frequency (n)**	**(%)**	**Freque ncy(n)**	**(%)**
Unknown	137	0.68	138	0.73	162	0.75
White	16459	81.56	16655	88.60	19741	91.52
Black	653	3.24	393	2.09	382	1.77
Hispanic	1336	6.62	643	3.42	514	2.38
Native American	142	0.70	153	0.81	172	0.80
Asian Pacific Islander	1186	5.88	596	3.17	380	1.76
Other, mixed	267	1.32	220	1.17	218	1.01
Total:	20180		18798		21569	
Menopausal Status:						
Pre-Menopausal	12880	63.83	7592	40.39	4455	20.65
Post-meno, Never Used HT	1332	6.60	2068	11.00	3553	16.47
Post-meno, Past HT Use	640	3.17	1191	6.34	2215	10.27
Post Meno, Current ET Use	1503	7.45	2487	13.23	4027	18.67
Post-meno, Current EPT Use	1691	8.38	2838	15.10	4330	20.08
Can't Determine Menopausal Status	2134	10.57	2622	13.95	2989	13.86
Total:	20180		18798		21569	
Smoking Status:						
Never	15114	74.97	12217	65.04	13120	60.90
Former	3938	19.53	5545	29.52	7498	34.80
Current	1108	5.50	1023	5.45	926	4.30
Total:	20160		18785		21544	
Family History of Breast Cancer:						

No 1st degree relative	17445	86.45	15948	84.84	18107	83.95
At least one 1st degree relative	2008	9.95	2277	12.11	2821	13.08
Adopted, cannot determine	727	3.61	573	3.04	641	2.97
Total:	20180		18798		21569	
Socioeconomic Status:						
Low	1084	5.43	744	4.01	722	3.39
Medium-Low	3985	19.96	3074	16.58	3625	15.35
Medium-High	6997	35.04	6080	32.78	6524	30.66
High	7903	39.58	8648	46.63	10765	50.60
Total:	199969		18546		21276	
BMI (kg/m^2)[1]:	25 ± 6		25 ± 5		24 ± 5	
BMI at age 18 (kg/ m^2)[1]:	22 ± 4		21 ± 3		21 ± 3	
Moderate Physical Activity (Hrs/Wk)[1]:	2.17 ± 2.23		2.37 ± 2.33		2.62 ± 2.54	
History of Diabetes (n):	347	1.72 %	442	2.35%	911	4.22%
Daily Vitamin Use(n):	7594		5031		4010	

[1]Continuous variables depicted as Mean ± SD

Chracteristic:			**AHEI-2010 Score**			
	Q1:		**Q3:**		**Q5:**	
Age at Baseline:	45 ± 12		53 ± 13		58 ± 13	
Race:	**Frequency (n)**	**(%)**	**Frequency (n)**	**(%)**	**Freque ncy (n)**	**(%)**
Unknown	142	0.69	150	0.79	147	0.72
White	16987	82.92	16656	88.25	18299	90.19
Black	582	2.84	415	2.20	429	2.11
Hispanic	1293	6.31	655	3.47	564	2.78
Native American	147	0.72	154	0.82	165	0.81
Asian Pacific Islander	1042	5.09	638	3.38	466	2.30
Other, mixed	294	1.44	205	1.09	220	1.08
Total:	20487		18873		20290	
Menopausal Status:						
Pre-Menopausal	12444	60.74	7340	38.89	4960	24.45

Post-meno, Never Used HT	1562	7.62	2209	11.70	3025	14.91
Post-meno, Past HT Use	757	3.70	1257	6.66	1988	9.80
Post Meno, Current ET Use	1627	7.94	2603	13.79	3629	17.89
Post-meno, Current EPT Use	1834	8.95	2902	15.38	3883	19.14
Can't Determine Menopausal Status	2263	11.05	2562	13.57	2805	13.82
Total:	20487		18873		20290	
Smoking Status:						
Never	14536	71.01	12551	66.57	12690	62.63
Former	4412	21.55	5423	28.76	6958	34.34
Current	1522	7.44	880	4.67	613	3.03
Total:	20470		18854		20261	
Family History of Breast Cancer:						
No 1st degree relative	17679	86.29	16022	84.89	17055	84.06
At least one 1st degree relative	2083	10.17	2261	11.98	2647	13.05
Adopted, cannot determine	725	3.53	590	3.13	588	2.9
Total:	20487		18873		20290	
Socioeconomic Status:						
Low	1156	5.71	788	4.23	680	3.4
Medium-Low	4157	20.53	3198	17.16	3036	15.17
Medium-High	7059	34.86	6183	33.18	6154	30.76
High	7879	38.91	8467	45.43	10138	50.67
Total:	20251		18363		20008	
BMI (kg/m^2)[1]:	26 ± 6		25 ± 5		24 ± 4	
BMI at age 18 (kg/ m^2)[1]:	22 ± 4		21 ± 3		21 ± 3	
Moderate Physical Activity (Hrs/Wk)[1]:	2.18 ± 2.26		2.3 ± 2.3		2.65± 2.5	
History of Diabetes (n):	488	2.38 %	587	3.11%	534	2.63%
Daily Vitamin Use(n):	7482		5081		3800	
	[1]Continuous variables					

	depicted as mean ± SD					
Characteristic:	**aMED Score**					
	Q1:		**Q3:**		**Q5:**	
Age at Baseline:	47 ± 13		52 ± 14		57 ± 13	
Race:	**Frequency (n)**	**(%)**	**Frequency (n)**	**(%)**	**Freque ncy(n)**	**(%)**
Unknown	104	0.59	170	0.85	212	0.79
White	15198	86.15	17565	87.54	23795	88.55
Black	390	2.21	441	2.20	689	2.56
Hispanic	993	5.63	785	3.91	803	2.99
Native American	102	0.58	162	0.81	221	0.82
Asian Pacific Islander	637	3.61	706	3.52	841	3.13
Other, mixed	217	1.23	237	1.18	311	1.16
Total:	17641		20066		26872	
Menopausal Status:						
Pre-Menopausal	9860	55.89	8399	41.86	7891	29.37
Post-meno, Never Used HT	1404	7.96	2199	10.96	3797	14.13
Post-meno, Past HT Use	726	4.12	1261	6.28	2397	8.92
Post Meno, Current ET Use	1693	9.60	2614	13.03	4416	16.43
Post-meno, Current EPT Use	1899	10.76	2939	14.65	4691	17.46
Can't Determine Menopausal Status	2059	11.67	2654	13.23	3680	13.69
Total:	17641		20066		26872	
Smoking Status:						
Never	11715	66.49	13214	65.91	17940	66.83
Former	4454	25.28	5824	29.05	8170	30.44
Current	1449	8.22	1010	5.04	734	2.73
Total:	17618		20048		26844	
Family History of Breast Cancer:						
No 1st degree relative	15154	85.90	17091	85.17	22692	84.44
At least one 1st degree relative	1841	10.44	2335	11.64	3354	12.48
Adopted, cannot determine	646	3.66	640	3.19	826	3.08
Total:	17641		20066		26872	
Socioeconomic Status:						
Low	873	5.01	818	4.13	1043	3.94

Medium-Low	3337	19.15	3386	17.1	4348	16.41
Medium-High	5931	34.03	6530	32.99	8376	31.60
High	7288	41.82	9062	45.78	12736	48.05
Total:	17429		19796		26503	
BMI (kg/m^2)[1]:	25 ± 5		25 ± 5		25 ± 5	
BMI at age 18 (kg/ m^2)[1]:	21 ± 3		21 ± 3		21 ± 3	
Moderate Physical Activity (Hrs/Wk)[1]:	2.14 ± 2.22		2.33 ± 2.31		2.67 ± 2.50	
History of Diabetes (n):	310	1.76%	517	2.58%	874	3.25%
Daily Vitamin Use(n):	6319		5656		5336	
	[1]Continuous variables depicted as Mean ± SD					
Chracteristic:	**DASH diet score**					
	Q1:		**Q3:**		**Q5:**	
Age at Baseline:	46 ± 12		52 ± 13		59 ± 13	
Race:	**Frequency(n)**	**(%)**	**Frequency (n)**	**(%)**	**Frequency(n)**	**(%)**
Unknown	141	0.73	154	0.68	140	0.69
White	15589	80.47	19965	88.62	18459	91.52
Black	705	3.64	472	2.09	329	1.63
Hispanic	1277	6.59	807	3.58	490	2.43
Native American	131	0.68	188	0.83	177	0.88
Asian Pacific Islander	1262	6.51	686	3.04	357	1.77
Other, mixed	267	1.38	258	1.15	218	1.08
Total:	19372		22530		20170	
Menopausal Status:						
Pre-Menopausal	11647	60.12	9087	40.33	4846	24.03
Post-meno, Never Used HT	1387	7.16	2491	11.06	3224	15.98
Post-meno, Past HT Use	675	3.48	1411	6.26	2013	9.98
Post Meno, Current ET Use	1552	8.01	3105	13.78	3564	17.67
Post-meno, Current EPT Use	1867	9.64	3406	15.12	3782	18.75
Can't Determine Menopausal Status	2244	11.58	3030	13.45	2741	13.59
Total:	19372		22530		20170	

Smoking Status:						
Never	13679	70.69	14658	65.10	12997	64.52
Former	4250	21.96	6766	30.05	6528	32.41
Current	1423	7.35	1091	4.85	620	3.08
Total:	19352		22515		20145	
Family History of Breast Cancer:						
No 1st degree relative	16764	86.54	19152	85.01	16983	84.20
At least one 1st degree relative	1911	9.86	2671	11.86	2577	12.78
Adopted, cannot determine	697	3.60	707	3.13	610	3.02
Total:	19372		22530		20170	
Socioeconomic Status:						
Low	1017	5.31	909	4.08	756	3.8
Medium-Low	3634	18.97	3734	16.78	3288	16.53
Medium-High	6557	34.23	7365	33.09	6230	31.33
High	7950	41.5	10251	46.05	9614	48.34
Total:	19158		22259		19888	19.79
BMI (kg/m^2)[1]:	25 ± 6		25 ± 5		24 ± 4	
BMI at age 18 (kg/ m^2)[1]:	22 ± 4		21 ± 3		21 ± 3	
Moderate Physical Activity (Hrs/Wk)[1]:	2.10 ± 2.19		2.34 ± 2.27		2.72 ± 2.59	
History of Diabetes (n):	395	2.03%	590	2.62%	661	3.28%
Daily Vitamin Use(n):	7366		5949		3708	
[1]Continous variables depicted as Mean ± SD						

Table 4.4- Overall trends of Descriptive statistics across various measures of overall diet quality

	HDS	**aMED**	**DASH**	**AHEI-2010**
Chracteristic:				
Age at Baseline:	Positive	Positive	Positive	Positive
Race:				
Unknown	Positive	Null	Null	Null

White	Positive	Positive	Positive	Positive
Black	Negative	Null	Negative	Negative
Hispanic	Negative	Negative	Negative	Negative
Native American	Null	Positive	Positive	Null
Asian Pacific Islander	Negative	Negative	Negative	Negative
Other, mixed	Negative	Negative	Negative	Negative
Menopausal Status:				
Pre-Menopausal	Negative	Negative	Negative	Negative
Post-meno, Never Used HT	Positive	Positive	Positive	Positive
Post-meno, Past HT Use	Positive	Positive	Positive	Positive
Post Meno, Current ET Use	Positive	Positive	Positive	Positive
Post-meno, Current EPT Use	Positive	Positive	Positive	Positive
Can't Determine Menopausal Status	Null	Positive	Positive	Positive
Smoking Status:				
Never	Negative	Null	Negative	Negative
Former	Positive	Positive	Positive	Positive
Current	Negative	Negative	Negative	Negative
Family History of Breast Cancer:				
No 1st degree relative	Negative	Negative	Negative	Negative
At least one 1st degree relative	Positive	Positive	Positive	Positive
Adopted, cannot determine	Negative	Negative	Negative	Negative
Socioeconomic Status:				
Low	Negative	Negative	Negative	Negative
Medium-Low	Negative	Negative	Negative	Negative
Medium-High	Negative	Negative	Negative	Negative
High	Positive	Positive	Positive	Positive
BMI (kg/m^2):	Negative	Null	Negative	Negative
BMI at age 18 (kg/ m^2):	Negative	Null	Negative	Negative
Moderate Physical Activity (Hrs/Wk):	Positive	Positive	Positive	Positive

History of Diabetes (n):	Positive	Positive	Positive	Null
Daily Vitamin Use(n):	Negative	Negative	Negative	Negative

4.2 Results applicable to specific aim #2:

Specific aim #2: Examine the relationship between overall diet quality and the risk of Breast cancer.

In order to fulfill the investigational tasks brought about by specific aim #2, multivariate cox proportional hazard models were constructed in which statistically and biologically significant covariates such as confounders and other independently associated variables were included in the model. These included covariates were assessed according to their contribution to overall parsimoniousness of the prediction model, but some covariates were forced in the model regardless due to biological plausibility (i.e. Race, energy intake level). A three stage modeling process was underwent in which covariates passing the inclusion criteria for the model went on to the next stage of the modeling process. Results from this covariate selection process can be found in Table 4.5, specifically the variables that provided significant evidence for their overall contribution to models predictive ability was: menopausal status (P= 0.0704), age at menarche (P<0.001), breast cancer family history (P= 0.0047), smoking status (P<0.0001), and BMI (P=0.04). Variables included in the model due to biological reasoning were race, which is a trait that people cannot change about themselves and caloric intake that allows for key adjustment in this dietary analysis across the vast levels of caloric intake found in the CTS. Given these findings, the final cox proportional hazard model to assess role of overall diet quality on the risk of breast cancer were matched for age at a baseline, and adjusted for: race, menopausal status, age at menarche, breast cancer family history, smoking status, BMI, and energy intake.

After deducing the most parsimonious model in terms of overall diet quality and breast cancer risk, the investigation into role of diet on the risk of invasive breast cancer was carried out amongst participants of the CTS. The results across the various diet quality scores from the aforementioned investigation can be found in Table 4.6. Our a-priori based dietary measure, the Healthful Diet Score, yielded interesting results specifically in which the lowest reduction of invasive breast cancer risk in the overall study population was observed individuals in quintile 4 (50-54 pts.) (HR_{Q4vsQ1} 0.94, 95% CI: 0.85-1.04). However, no significant evidence was found when assessing the presence of a dose-response relationship between our version of an overall healthy diet and invasive breast cancer risk. When looking at the predictive quality of the aMED index in terms of breast cancer risk, we see a lack of significance across all measures of association (i.e. HR_{Q1} to HR_{Q5}) for this particular dietary measure. Women exhibiting the highest adherence to this dietary pattern showed a marginally non-significant reduction of 9% (HR 0.91, 95% CI: 0.83-1.01). Although, increasing levels of adherence to an overall Mediterranean diet pattern was significantly associated invasive breast cancer risk reduction in all women (p-trend: 0.0322). In terms of adherence to the DASH diet plan, women who exhibit the highest levels (Quintile 5: 29-40 pts.) were shown to significantly decrease their risk of invasive breast cancer by 12% (HR_{Q5vsQ1} 0.88, 95% CI: 0.79-0.97). Also, increasing levels of adherence to DASH provided highly significant evidence of a dose-response relationship, which resulted in the overall reduction of breast cancer risk (p-trend: 0.0107). The Alternative Healthy Eating index was the final a-priori based dietary measure utilized in this analysis and provided significant evidence regarding the association between overall diet quality and invasive breast cancer risk. Specifically, women showed the highest reduction of risk across all dietary measures as characterized by the significant 13% reduction for those individuals exhibiting the highest adherence to the AHEI-2010 diet plan. This measure also provided significant evidence regarding the role of increasing adherence to AHEI-2010 and the reduction of invasive breast cancer risk (p-trend: 0.0162). Given these finding, overall diet quality was shown to have a significant effect on the risk of invasive breast cancer amongst all CTS participants.

Upon investigating the role of overall diet quality on invasive breast cancer risk in the CTS, an effect modification analyses was carried out to assess the presence susceptible subgroups where diet quality may play a more significant role in breast cancer carcinogenesis. Given the strong biological basis and results from past finding as mentioned in section 2.1.2, the effect modification analysis was carried to assess if the role of overall diet quality on breast cancer risk differed by menopausal status or participant's BMI. Table 4.7 depicts the results from this analysis in which significant evidence for an effect-modification relationship between menopausal status and overall diet quality was observed, but this was relationship not shared with BMI (p=0.05, p= 0.68, respectively). Results from the effect-modification analyses drove the investigation of specific aim #2 further through assessing the role of overall diet quality by menopausal status, thus yielding two separate predictive models for the pre- and post-menopausal subgroups.

The results regarding the pre-menopausal population (n=39.445) help affirm the findings from the effect-modification analysis in which the effect of overall diet quality on invasive breast cancer risk does not mirror the results obtained from the overall study population (Table 4.8). Specifically when assessing our a-priori diet index, HDS, we observed women with the highest adherence to an overall healthy diet to exhibit the same risk as individuals with the lowest adherence (HR_{Q5vsQ1} 1.00 95%CI 0.82-1.21). Pre-menopausal women who exhibited the highest adherence to a Mediterranean diet pattern (aMED) were shown to non-significantly increase their risk of invasive breast cancer by 6% when compared to individuals with the lowest adherence (HR_{Q5vsQ1} 1.06, 95% CI: 0.89-1.28). Whereas these same individuals who exhibited the highest adherence to the Dietary Approaches to Stop Hypertension diet plan were shown to non-significantly increase there risk 1% more than individuals with the lowest adherence (HR_{Q5vsQ1} 1.01, 95% CI: 0.89-1.28). Similar results were also obtained for pre-menopausal women with the highest adherence to AHEI-2010 diet pattern in which a non-significant increase of 4% was observed (HR_{Q5vsQ1} 1.01, 95% CI: 0.89-1.28). No significant threshold effects or dose-response relationships were detected when assessing this subgroup (p-trends > 0.05). Given the results from this subgroup analysis, overall diet quality does not play a significant role in invasive breast cancer carcinogenesis amongst pre-menopausal women.

Further evidence for this effect modification relationship was provided when assessing the role of overall diet quality in the post-menopausal population of the CTS (n=39,341) (Table 4.9). In general, results from this subgroup analysis mirror the results from the overall study population analysis (Table 4.6) in which similar and stronger associations are observed, unlike those found in the pre-menopausal subgroup. When assessing the role of overall diet quality as measured by the HDS, we see that post-menopausal women who exhibit the highest adherence reduce their risk of invasive breast cancer by 2% (HR_{Q5vsQ1} 0.98 95% CI: 0.86-1.05). Investigation into possible dose-response relationships provided insignificant evidence in terms of HDS and breast cancer risk. However, greater adherence to an overall Mediterranean diet pattern was shown to be significantly associated with invasive breast cancer risk (p-trend: 0.0322). Also, women with the highest adherence to this dietary pattern (aMED Quintile 5) exhibited a significant reduction in breast cancer risk of 15% when compared to the lowest adherers (HR 0.85, 95% CI: 0.74-0.96).

Adherence to the DASH diet plan was associated with post-menopausal breast cancer in which we found that individuals with the highest adherence exhibited a 12% reduction of risk relative to the lowest adhering women (HR 0.88, 95% CI: 0.77-1.01). Also, increasing adherence to this dietary pattern provided significant evidence of a dose-response relationship between overall diet quality and breast cancer risk (p-trend: 0.0107). The last measure used in our post-menopausal subgroup analysis was the AHEI-2010, which provided the strongest measures of association in terms of overall diet quality and invasive breast cancer risk when compared to the other dietary measures as was previously observed in the overall study population results. Specifically, post-menopausal women who exhibit the highest adherence to an AHEI-2010 dietary pattern exhibited significant reductions in invasive breast cancer risk of 25% relative to the lowest adherers (HR 0.75, 95% CI: 0.70-0.91). In terms of a dose-response relationship amongst AHEI-2010 and invasive breast cancer, significant evidence was found suggesting that increasing adherence to an AHEI-2010 diet plan serves as a protective factor (p-trend: 0.0162). Given the findings from the post-menopausal subgroup analysis, the evidence suggests that overall diet quality plays a significant role in invasive breast cancer carcinogenesis amongst post-menopausal women.

The performance of our a-priori based dietary measure was moderate in comparison to these other widely used dietary indices. Specifically. the greatest reduction of risk observed in the overall study population was a non-significant 5%, which was similar to aMED effect sizes but nowhere near the reduction observed by DASH and AHEI-2010 (12% and 13%, respectively). Also our a-priori based dietary index failed to exhibit a significant dose response relationship, but this relationship was observed among the other indices. The results from the pre-menopausal subgroup analysis do not shed light on the overall diet quality on breast cancer risk in which majority of associations were approximately null. In terms of HDS's performance in the post-menopausal subgroup, the highest reduction of risk was a non-signifcant 3% whereas the other dietary indices such as aMED, DASH, and especially AHEI-2010 were shown as more efficacious interventions through their respective risk reductions. These measures also provided significant evidence of dose-response relationships with post-menopausal breast cancer risk, but this trend was not exhibited with our a-priori based dietary measure.

Table 4.5- Wald Chi-Square Statistics for confounding and independent covariates from the 3-stage modeling

Variable:	Wald Chi-Square	Pr> ChiSq
Tier 1 Model		
Race	11.6456	0.0704
Menopausal status	122.85	<0.0001
Age at menarche	7.98	0.0047
Breast Cancer Family History	87.19	<0.0001
Tier 2 Model		
Smoking status	30.09	<0.0001
SES status	3.6368	0.4574
Physical Activity	0.6835	0.48084
Tier 3 Model		

BMI	4.08	0.04
Vitamin Use	0.62	0.43
Energy Intake	0.6	0.44

Table 4.6- Measures of association (HRs and 95% confidence intervals) between overall diet quality and the risk of invasive breast cancer

	All Women (n=90,244)					
Diet Score:						
	Q1	**Q2**	**Q3**	**Q4**	**Q5**	**P-trend**
Healthful Diet Score						
Score Range:	18-40	41-45	46-49	50-54	55-75	0.2054
HR (95% CI):	1.00	0.98 (0.89-1.08)	0.99 (0.90-1.1)	0.94 (0.85-1.04)	0.95 (0.86-1.05)	
Alternate Mediterranean Diet Index:						
Score Range:	0-2	3	4	5	6-9	0.0322
HR (95% CI):	1.00	0.97 (0.88-1.05)	1.00 (0.90-1.10)	0.93 (0.85-1.03)	0.91 (0.83-1.01)	
DASH Diet Score:						
Score Range:	8-19	20-22	23-25	26-28	29-40	0.0107
HR (95% CI):	1.00	0.96 (0.87- 1.06)	0.90 (0.82-0.99)	0.90 (0.82-0.99)	0.88 (0.79- 0.97)	
Alternative Healthy Eating Index-2010:						
Score Range:	10-40.5	41-47	47.5-52.5	53-59	59.5-86	0.0162
HR (95% CI):	1.00	0.98 (0.89-1.08)	0.93 (0.84-1.03)	0.94 (0.86-1.04)	0.87 (0.79-0.97)	

Table 4.7- Wald Chi-Square statistics for possible effect modification relationship with overall diet quality

Variable:	Wald Chi-Square	Pr> ChiSq
DietScore*Menopausal Status	31.17	0.0529
DierScore*BMI	6.1	0.68

Table 4.8- Measures of association (HRs and 95% confidence intervals) between overall diet quality and the risk of invasive breast cancer in Pre-menopausal women

Pre-Menopausal Women (n= 39,455)						
Diet Score:						
	Q1	**Q2**	**Q3**	**Q4**	**Q5**	**P-trend**
Healthful Diet Score						
Score Range:	18-40	41-45	46-49	50-54	55-75	0.9513
HR (95% CI):	1.00	1.02 (0.87-1.20)	1.01 (0.85- 1.20)	1.03 (0.86-1.23)	1.00 (0.82-1.21)	
Alternate Mediterranean Diet Index						
Score Range:	0-2	3	4	5	6-9	0.6039
HR (95% CI):	1.00	1.07 (0.89- 1.27)	1.07 (0.89- 1.28)	1.08 (0.90-1.30)	1.06 (0.89-1.28)	
DASH Diet Score						
Score Range:	8-19	20-22	23-25	26-28	29-40	0.7958
HR (95% CI):	1.00	0.98 (0.83-1.15)	0.91 (0.77-1.07)	0.95 (0.80-1.14)	1.01 (0.83-1.22)	
Alternative Healthy Eating Index-2010						
Score Range:	10-40.5	41-47	47.5-52.5	53-59	59.5-86	0.8274
HR (95% CI):	1.00	1.20 (1.02-1.41)	1.12 (0.94-1.34)	1.15 (0.97-1.38)	1.04 (0.85-1.27)	

Table 4.9– Measures of association (HRs and 95% confidence intervals) between overall diet quality and the risk of invasive breast cancer in Post-menopausal women

Post-Menopausal Women (n=39,341)						
Diet Score:						
	Q1	**Q2**	**Q3**	**Q4**	**Q5**	**P-trend**
Healthful Diet Score						
Score Range:	18-40	41-45	46-49	50-54	55-75	0.2054
HR (95% CI):	1.00	1.02 (0.88-1.18)	1.02 (0.89-1.18)	0.97 (0.84-1.11)	0.98 (0.86-1.13)	
Alternate Mediterranean Diet Index						
Score Range:	0-2	3	4	5	6-9	0.0322
HR (95% CI):	1	0.92 (0.79-1.06)	0.93 (0.81- 1.06)	0.86 (0.75-0.99)	0.85 (0.74-0.96)	
DASH Diet Score						
Score Range:	8-19	20-22	23-25	26-28	29-40	0.0107
HR (95% CI):	1	.95 (0.82-1.09)	0.89 (0.78-1.02)	0.91 (0.79-1.04)	0.88 (0.77-1.01)	
Alternative Healthy Eating Index-2010						
Score Range:	10-40.5	41-47	47.5-52.5	53-59	59.5-86	0.0162
HR (95% CI):	1	0.90 (0.78-1.03)	0.81 (0.71-0.93)	0.86 (0.76-0.99)	0.75 (0.70-0.91)	

CHAPTER 5

Discussion

5.1 Summary of major findings:

The purpose of this thesis was to shed light on the association between overall diet quality and the risk of breast cancer amongst participants of the California Teachers Study. Breast cancer is the most common cancer amongst women with a few known modifiable risk factors thus determining potential modifiable factors, such as overall diet quality, serves as a matter of utmost importance in this field. Given this purpose, specific aims were developed to determine participant's overall diet quality across several measures of interest (HDS, aMED, DASH, AHEI-2010), and their risk of invasive breast cancer. A summary of major findings can be seen below:

- Women who exhibited a greater adherence to healthy dietary patterns had a decreased risk of overall invasive breast cancer, relative to those individuals who exhibited weaker adherence.
- The role of overall diet quality in breast cancer risk differs by menopausal status, such that:
    - The risk of pre-menopausal breast cancer did not significantly differ between women who exhibited the greatest adherence to an overall healthy dietary pattern and those who were the least adherent.
    - Conversely, post-menopausal women who exhibited an increased adherence to overall healthy dietary patterns significantly decreased their risk of invasive breast cancer relative to the less adherent women.

5.1.1 Overall diet quality:

When assessing the role of overall diet quality in the whole study population, we observed the general relationship in which increasing adherence to an a-priori defined healthy diet pattern was associated with decreased risk of invasive breast cancer. Increased adherence in these dietary measures are commonly characterized by an overall nutrient rich and calorie-sparse diets, in which past studies have identified these as efficacious dietary patterns in terms of breast cancer [30]. Diets often rich in nutrients and sparse in empty calories ultimately provide beneficial effects such as increased antioxidant capacity, and other necessary minerals and vitamins to maintain a homeostatic tissue environment [50, 82]. Therefore, individuals who are less adherent as indicated by decreasing diet quality scores tend to exhibit higher risk levels of breast cancer relative to the more adherent individuals, as seen in this analysis [50]. These findings are concordant with those of past studies that have shown individuals who exhibit unhealthy dietary patterns such as the “Western” dietary pattern, nutrient sparse and calorically rich diets, to exhibit increased risks of breast cancer [30].

The role of overall diet quality was observed to have a differential association when conducting the subgroup analyses found in this study, such that the association of overall diet quality and breast cancer differed by menopausal status. Results from this analysis depicted a lack of evidence in regards to the association of overall diet quality and pre-menopausal breast cancer risk. Specifically pre-menopausal women who exhibited the greatest adherence to these a-priori defined healthy dietary patterns shared the same risk of invasive breast cancer as the non-adherent women, thus suggesting the presence of other important risk factors involved in premenopausal breast cancer carcinogenesis. However, the results from the post-menopausal population mirrored the results from the overall population in which majority of the measures of association became stronger when assessed solely as post-menopausal breast cancer risk. This strengthening of associations from the overall population analysis to the post-menopausal population analysis, coupled along with loss of associations in the pre-menopausal population allow us to infer that overall diet quality plays a significant role amongst post-menopausal women only. The attenuation of effect size observed in the overall study population is most likely due to the cofounding role played by menopausal status, which can introduces bias in to effect size estimates thus accounting for the underestimated effect of overall diet quality. These findings are concordant with past studies that have also assessed the role of overall diet quality amongst women in which effects appear more pronounced amongst the post-menopausal subgroup of women [37, 51]. Another study found that when pre- and post-menopausal women exhibit the similar adherence level to same healthy dietary pattern, post-menopausal women exhibit much larger reductions in breast cancer risk relative to the pre-menopausal women [82]. These findings are largely aligned with established knowledge in the oncology world in which the role of diet-induced obesity has been show play a differential role in breast cancer carcinogenesis according to menopausal status [37]. Also, majority of pre-menopausal manifestations in this disease have been accounted to familial aspects whereas post-menopausal manifestations are characterized as sporadic developments through a lifetime of carcinogenic exposures [38]. This increased susceptibility exhibited amongst post-menopausal suggest clinicians should target these individuals in their breast cancer prevention efforts.

Several dietary indices were utilized in this analysis due to the shaky and inconsistent evidence regarding the effect of diet on breast cancer risk. The results from this analysis show the greatest reduction of breast cancer risk with the Alternative Healthy Eating index 2010 in which post-menopausal women with the highest adherence were shown to significantly reduce their risk by 25%. When compared to our a-priori dietary measure, the HDS did not generate any measures of association as strongly observed with the AHEI-2010, but effect sizes and validation tables suggest that development of measure of overall diet quality was successful. However, a secondary validation study using a study population in which confounding and exposures variables are perfectly measured should be utilized to assess the true validity of our dietary measure. Specifically results from Table 4.2 convey that increases in diet quality scores corresponded with increased consumption of healthful macro- and micronutrients that have been shown to have a protective role in breast cancer, although result were not significant [8-10]. Also, we were successful in our pursuit of creating a more diverse measure of overall diet quality that is comprised of 23 distinct food groups. As previously noted, our a-priori index functioned as well as these standardized indices in predicting changes in BMI serving as a surrogate measure of adiposity, which is a known post-menopausal risk factor.

The AHEI-2010 exhibited distinct differences from the other indices included in this analysis in which sodium intake was not assessed in the aMED index and the Healthful Diet Score. Increased efficiency of this dietary measure relative to these other measures may be accounted to the inclusion of the sodium component, which has been shown to have significant effects on degenerative outcomes such as CVD, diabetes, and various cancers [25]. The AHEI-2010 also assessed alcohol and oil/fat consumption levels, which were components not found amongst the DASH diet score. These included components have been shown to play significant roles in breast cancer in which excess alcohol intake has been commonly noted as a causative factor due to increased acetaldehyde and circulating o-estrogen levels. In terms of oil/fat consumption provides an important measure assessing the role of efficacious constituents found in oils relative to the dietary fat burden that has been shown to increase oxidative stress [5, 13-14,76]. Components of oil such as omega-3 and omega-6-fatty acids have been shown to exhibit anti-inflammatory properties due to the antioxidant nature of these components, which allows for removal and neutralization of oxidative free radicals shown to promote breast cancer carcinogenesis [5,57-79]. These aforementioned components in AHEI-2010 that are not found amongst the other indices may account for these more pronounced reductions in breast cancer risk relative.

5.2 Strengths and weaknesses:

- **Strengths**

Many strengths exists within this study which can first and foremost be seen amongst the large prospective nature of this study that consists of 16 years of follow-up. This extensive follow-up period is advantageous for understanding the temporality of breast cancer manifestation relative to the present risk factors. For instance, nutritional case-control studies often have issues with temporality and exposure misclassification in which cases are more likely to report harmful exposures relative to the control subjects which can lead to drastic over- and under-reporting respectively. Another strength seen in study is observed amongst the CTS population in which invasive and in-situ breast cancer incidence rates have been noted as being exorbitantly high relative other age-standardized rates [31]. Another strength is observed amongst the extensive dietary data recorded in the CTS, specifically all 133,479 participants filled out 103-item FFQ that was validated and shown as reproducible in outside study, which serves as an integral step for this nutritional epidemiological analysis. Another strength can be seen in the ability to assess effect modification relationships and identify susceptible subgroups in terms of overall diet quality and breast cancer risk due to the large study population of the CTS. Lastly, this a-priori based analysis of associations between diet and invasive breast cancer risk was the first of its kind to be carried out in the CTS study population, to the best of our knowledge. Past nutritional studies in the CTS have assessed dietary patterns through post-hoc analysis, commonly known as a-posteriori based methods, which exhibits a data-driven nature to help characterize observed dietary patterns in the study population [85]. However, these past a-posteriori based studies do not allow investigators to build upon the latest scientific literature regarding this topic, therefore our a-priori based analysis allowed for a hypothesis-driven definition of overall diet quality and allowed for inter-index assessment to decipher the best measure with regards to breast cancer.

- **Weaknesses**

The first weakness found in this study can be accounted to a noted-strength of this study as well, the extensive nature of the dietary data recorded by the FFQ. Specifically, many individuals failed to answer various food item intake questions, which may be accounted to the sheer length of the dietary questionnaire. In order to avoid any introduction of bias through misclassification of these individuals as non-consumers, we took a conservative approach to remove individuals who exhibited missing dietary data of ≥ 25%, which led to a exclusion of 20,995 individuals in this study population, which is a substantial exclusion. However, this exclusion did not hamper the study's power (analytic cohort size, n=94,404) to detect significant effects of overall diet quality on breast cancer risk. Also, we were unable to ascertain if this exclusion was differential or not, therefore inherent exclusion biases may have been introduced into the assessment. Another weakness was in the study selection process in which the dietary exclusion were undergone before the morbidity exclusion, which is not an ideal sequence for selection, but upon re-analysis the proper order of exclusions will be carried out. A lack of repeated measures in terms of dietary habits may also be seen as a glaring weakness, because it forces the assumption that baseline reporting is truly indicative of long-term dietary habits and not a product of recall bias. However, reproducibility and validity studies have been carried out in which the CTS FFQ was shown to be reproducible and valid, thus we anticipate little recall bias [84]. Exposure misclassification may also be present in this study in which individuals who failed to report frequency of intake but answered more >75% of dietary questionnaire properly were treated as non-consumers, which may be true for majority of those participants but not guaranteed thus allowing introduction bias into this study. Confounding misclassification may also be present in this analysis in which confounders were recorded at baseline, but throughout 16 years of follow-up such confounders like menopausal status can change in which the etiology of breast cancer may as well (i.e. pre-menopausal breast cancer largely driven by familial aspects). Depending on the role of the confounder on the disease such as menopausal status where the risk has shown to be more pronounced amongst post-menopausal women, we would expect this misclassification to attenuate the role of overall diet quality in the post-menopausal population.

5.3 Implications and future research:

Given the findings of this study, overall diet quality has been shown to have a significant effect on invasive breast cancer risk amongst post-menopausal women. Further analyses need to be carried out to assess if this role is confounded by participant's hormone receptor status or hormone therapy use. However, results are very promising in which large risk reductions were noted in this study, thus providing clinicians with useful tools for breast cancer prevention when consulting at-risk post-menopausal women. With regards to our a-priori based dietary measure, the Healthful Diet Score requires further analysis to be carried out in conjunction with other outcomes where diet plays a clearly defined role such as all cause mortality or type 2 diabetes. Specifically, results yielded from our a-priori dietary measure were marginally insignificant, which indicates the presence of excess noise in our dietary measure. In hopes to decipher the source of this excess noise, we plan to carry out several sensitivity analyses to further fine-tune our dietary index in the future investigation.

In terms of future research, studies assessing changes in overall diet quality on the risk of breast cancer should be carried out, because this approach will allow for consideration of diet quality at follow-up, which may play a more important role in breast cancer. Another, future research plan is to compute diet quality scores according menopausal status due to the different roles played by diet quality as seen in this analysis. Majority of women in this study were post-menopasual thus generating quinitles of diet scores pertaining to both pre- and post-menopausal women could allow for diet score groupings to favor post-menopausal diet quality scores, thus separate computation of diet scores must be assessed. Also studies assessing the association between overall diet quality and breast cancer prognosis would provide intriguing results as to which dietary habits may worsen or better one's course of disease. Lastly, the CTS has vast blood biomarker (i.e. serum IL-6) measurements recorded and therefore use of these measures in conjunction with reported diet quality will allow for secondary validation of dietary habits to avoid any bias through sole-questionnaire usage.

CHAPTER 6

Conclusion

In conclusion, our assessment into the role of overall diet quality on the risk of incident breast cancer in the California Teachers Study conveyed that all women who increased adherence to overall healthy diet patterns yielded significant reductions in breast cancer risk. However, the participant's menopausal status played a significant role in the true association elicited by overall diet quality on the risk of breast cancer, such that the modification of overall diet quality's association in the pre-menopausal population did not play a significant role in the outcome of incident invasive breast cancer. The findings also suggest that overall diet quality at baseline for post-menopausal women serves as a significant risk factor in terms of breast cancer, such that higher quality diets were associated with more pronounced risk reductions relative to those women with lower quality diets. Furthermore, increasing adherence to the AHEI-2010 diet plan was shown as most efficacious thus providing a specific dietary tool for breast cancer disease prevention efforts among post-menopausal women. Further research is needed pertaining the relationship between overall diet quality, hormone receptor status, and hormone therapy use, with regards to breast cancer risk.

References

1. Youlden, Danny R, et al. "The Descriptive Epidemiology of Female Breast Cancer: An International Comparison of Screening, Incidence, Survival and Mortality." *Cancer Epidemiology* 36.3 (2012): 237-48.

2. "Adult Obesity Facts." *Centers for Disease Control and Prevention*. Centers for Disease Control and Prevention, 09 Sept. 2014. Web. 14 Nov. 2014.

3. Barham, W., et al. "The Role of NF-kappaB in Mammary Tumor Initiation." *Cancer Res.* 74.19_supplement (2014): 3010.

4. Sharma, Anisha. et al. "Molecular Biomarkers & Diagnosis The Utility of Pro-inflammatory Cytokines-TNF Alpha and CRP as Indicators of Response to Chemotherapy in Patients with Breast Carcinoma." *Molecular Biomarkers & Diagnosis* 5.3 (2014): 1-5.

5. Bondia-Pons, I., Ryan, L., and Alfredo Martinez J. "Oxidative Stress and Inflammation Interactions in Human Obesity." *Journal of Physiology and Biochemistry* 68.4 (2012): 701-11.

6. Casas, R. , et al. "The Immune Protective Effect of the Mediterranean Diet against Chronic Low-grade Inflammatory Diseases." *Endocrine Metabolism Immune Disorder Drug Targets* 14.4 (2014): 245-54.

7. Shrubsole, M. J., et al. "Drinking Green Tea Modestly Reduces Breast Cancer Risk." *Journal of Nutrition* 139.2 (2008): 310-16.

8. Zhang, X., et al. "Carotenoid Intakes and Risk of Breast Cancer Defined by Estrogen Receptor and Progesterone Receptor Status: A Pooled Analysis of 18 Prospective Cohort Studies." *American Journal of Clinical Nutrition* 95.3 (2012): 713-25.

9. Baglietto, L., et al. "Dietary Patterns and Risk of Breast Cancer." *Dietary Patterns and Risk of Breast Cancer* 104.3 (2011): 524-31.

10. Jung, S., et al. "Fruit and Vegetable Intake and Risk of Breast Cancer by Hormone Receptor Status." *JNCI Journal of the National Cancer Institute* 105.3 (2013): 219-36.

11. Liu, Y., et al. "Adolescent Dietary Fiber, Vegetable Fat, Vegetable Protein, and Nut Intakes and Breast Cancer Risk." *Breast Cancer Research and Treatment* 145.2 (2014): 461-70.

12. Villaseñor, A., et al. "Dietary Fiber Is Associated with Circulating Concentrations of C-reactive Protein in Breast Cancer Survivors: The HEAL Study." *Breast Cancer Research and Treatment* 129.2 (2011): 485-94.

13. Sieri, S., et al. "Dietary Fat Intake and Development of Specific Breast Cancer Subtypes." *Journal of the National Cancer Institute* 106.5 (2014).

14. Favrid, M. "Premenopausal Dietary Fat in Relation to Pre- and Post-menopausal Breast Cancer." *Breast Cancer Research and Treatment* 145.1 (2014): 255-65.

15. Augustin, L. A., et al. "Associations of Bread and Pasta with the Risk of Cancer of the Breast and Colorectum." *Annals of Oncology* 24.12 (2013): 3094-099.

16. Reedy, J., et al. "Index-based Dietary Patterns and Risk of Colorectal Cancer: The NIH-AARP Diet and Health Study." *American Journal of Epidemiology* 168.1 (2008): 38-48.

17. Reeves, M. M., et al. "Weight loss intervention trials in women with breast cancer: a systematic review." *Obesity Reviews* 15.9 (2014): 749-768.

18. Harmon, B. E., et al. “Associations of key diet-quality indexes with mortality in the Multiethnic Cohort: the Dietary Patterns Methods Project.” *American Journal of Clinical Nutrition*, ajcn.114.090688 (2015).

19. Milà-Villarroel, R., et al. Comparison and evaluation of the reliability of indexes of adherence to the Mediterranean diet. *Public Health Nutrition*, *14* (2011): 2338–2345. [19]

20. Miller, P. E., et al. Comparison of 4 established DASH diet indexes: examining associations of index scores and colorectal cancer. *The American Journal of Clinical Nutrition*, *98*.3 (2013): 794–803.

21. Shivappa, N., et al. Designing and developing a literature-derived, population-based dietary inflammatory index. *Public Health Nutrition*, *17*.8 (2014): 1689–96.

22. Kirkpatrick, S. I., et al. Dietary assessment in food environment research: a systematic review. *American Journal of Preventive Medicine*, *46*.1 (2014).

23. Guenther, P., et al. "Update of the Healthy Eating Index: HEI-2010." *Journal of the Academy of Nutrition and Dietetics* 113.4 (2013): 569-80.

24. Kontou, Niki. "The Mediterranean Diet in Cancer Prevention: A Review." *Journal of Medicinal Food* 14.10 (2011): 1065-078.

25. Chiuve, S. E., et al. Alternative Dietary Indices Both Strongly Predict Risk of Chronic Disease. *Journal of Nutrition*, *142* (2012): 1009–1018.

26. Fung, T. T., et al. Diet quality is associated with the risk of estrogen receptor-negative breast cancer in postmenopausal women. *The Journal of Nutrition*, *136* (2006): 466–472.

27. Izano, M. A., et al. Are diet quality scores after breast cancer diagnosis associated with improved breast cancer survival? *Nutrition and Cancer*, *65*.6., (2013): 820–826.

28. Fung, T. T.,et al. Low-carbohydrate diets, dietary approaches to stop hypertension-style diets, and the risk of postmenopausal breast cancer. *American Journal of Epidemiology*, *174*.5. (2011): 652–660.

29. Yang, X. R., et al. Associations of breast cancer risk factors with tumor subtypes: A pooled analysis from the breast cancer association consortium studies. *Journal of the National Cancer Institute*, *103*.3. (2011): 250–263.

30. Albuquerque, R. C. R., Baltar, V. T., & Marchioni, D. M. L. Breast cancer and dietary patterns: A systematic review. *Nutrition Reviews*, *72*.1 (2014): 1–17.

31. Bernstein, Leslie, et al. "High breast cancer incidence rates among California teachers: results from the California Teachers Study (United States)." *Cancer Causes & Control* 13.7 (2002): 625-635.

32. Harnack L, Nicodemus K, Jacobs DR Jr, Folsom AR. An Evaluation of the Dietary Guidelines for Americans in Relation to Cancer Occurrence. *The American Journal of Clinical Nutrition* 76.4 (2002): 889-896.

33. McCullough M, Patel AV, Kushi LH, Patel R, Willett WC, Doyle C, Thun MJ, Gapstur SM. Following Cancer Prevention Guidelines Reduces Risk of Cancer, Cardiovascular Disease, and All-Cause Mortality. *Cancer Epidemiology Biomarkers & Prevention* 20.6 *(*2011): 1089-1097.

34. Jakovcevic, Danica, et al. "Breast Cancer Molecular Subtypes and Oxidative DNA Damage." *Applied Immunohistochemistry & Molecular Morphology* (2015) Web.

35. Yoshimoto, T.,Yoshimoto ,T. *Cytokine Frontiers: Regulation of Immune Responses in Health and Disease*.

36. Kolb, R., et al. "Inflammasome Activation in Obesity-associated Breast Cancer Progression." *Cancer Res.* 74.19_supplement (2014): 4098.

37. Vecchia, C. La, et al. "Overweight, Obesity, Diabetes, and Risk of Breast Cancer: Interlocking Pieces of the Puzzle." *The Oncologist* 16.6 (2011): 726-29.

38. Elkum, Naser, et al. "Obesity is a significant risk factor for breast cancer in Arab women." *BMC cancer* 14.1 (2014): 788.

39. Couto, Elisabeth, et al. "Mediterranean dietary pattern and risk of breast cancer." *PloS one* 8.2 (2013): e55374.

40. Nettleton, Jennifer A., et al. "A priori–defined dietary patterns and markers of cardiovascular disease risk in the Multi-Ethnic Study of Atherosclerosis (MESA)." *The American journal of clinical nutrition* 88.1 (2008): 185-194.

41. Sijtsma, Femke PC, et al. "Longitudinal trends in diet and effects of sex, race, and education on dietary quality score change: the Coronary Artery Risk Development in Young Adults study." *The American journal of clinical nutrition* 95.3 (2012): 580-586.

42. Kastorini, Christina-Maria, et al. "Comparative analysis of a-priori and a-posteriori dietary patterns using state-of-the-art classification algorithms: A case/case-control study." *Artificial intelligence in medicine* 59.3 (2013): 175-183.

43. Schwingshackl, Lukas, and Georg Hoffmann. "Adherence to Mediterranean diet and risk of cancer: A systematic review and meta-analysis of observational studies." *International Journal of Cancer* 135.8 (2014): 1884-1897.

44. Couto, E., et al. "Mediterranean dietary pattern and cancer risk in the EPIC cohort." *British journal of cancer* 104.9 (2011): 1493-1499.

45. Sofi, Francesco, et al. "Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis." *The American journal of clinical nutrition* 92.5 (2010): 1189-1196.

46. Giacosa, Attilio, et al. "Cancer prevention in Europe: the Mediterranean diet as a protective choice." *European Journal of Cancer Prevention* 22.1 (2013): 90-95.

47. Ward, B. W., Schiller, J. S., & Goodman, R. a..Multiple chronic conditions among US adults: a 2012 update. *Preventing Chronic Disease*. *11*.3 (2014).

48. Lichtenstein, Alice H., et al. "Diet and lifestyle recommendations revision 2006 A scientific statement from the American Heart Association nutrition committee." *Circulation* 114.1 (2006): 82-96.

49. Aune, D., et al. "Fruits, vegetables and breast cancer risk: a systematic review and meta-analysis of prospective studies." *Breast cancer research and treatment* 134.2 (2012): 479-493.

50. Galland, Leo. "Diet and inflammation." *Nutrition in Clinical Practice* 25.6 (2010): 634-640.

51. Thomson, Cynthia A. "Diet and breast cancer understanding risks and benefits." *Nutrition in Clinical Practice* 27.5 (2012): 636-650.

52. Casas, Rosa, et al. "The effects of the Mediterranean diet on biomarkers of vascular wall inflammation and plaque vulnerability in subjects with high risk for cardiovascular disease. A randomized trial." *PloS one* 9.6 (2014): e100084.

53. Dong, Jia-Yi, and Li-Qiang Qin. "Soy isoflavones consumption and risk of breast cancer incidence or recurrence: a meta-analysis of prospective studies." *Breast cancer research and treatment* 125.2 (2011): 315-323.

54. Mourouti, Niki, et al. "Whole Grain Consumption and Breast Cancer: A Case-Control Study in Women." *Journal of the American College of Nutrition* ahead-of-print (2015): 1-7.

55. Caan, Bette J., et al. "Soy food consumption and breast cancer prognosis." *Cancer Epidemiology Biomarkers & Prevention* 20.5 (2011): 854-858. [55]

56. Bahadoran, Zahra, Zeinab Karimi, and Somayeh Abedini. "Healthy dietary patterns and the risk of breast cancer: A review of current data." *American Journal of Life Sciences* 3.2-1 (2015): 1-5.

57. Zheng, Ju-Sheng, et al. "Intake of fish and marine n-3 polyunsaturated fatty acids and risk of breast cancer: meta-analysis of data from 21 independent prospective cohort studies." *Bmj* 346 (2013).

58. Gerber, Mariette. "Omega-3 fatty acids and cancers: a systematic update review of epidemiological studies." *British Journal of Nutrition* 107.S2 (2012): S228-S239.

59. Patterson, Ruth E., et al. "Marine fatty acid intake is associated with breast cancer prognosis." *The Journal of nutrition* 141.2 (2011): 201-206.

60. Gonzalez, Carlos A., and Elio Riboli. "Diet and cancer prevention: Contributions from the European Prospective Investigation into Cancer and Nutrition (EPIC) study." *European Journal of Cancer* 46.14 (2010): 2555-2562.

61. Ronksley, Paul E., et al. "Association of alcohol consumption with selected cardiovascular disease outcomes: a systematic review and meta-analysis." *Bmj* 342 (2011): d671.

62. Boyle, Peter, and Paolo Boffetta. "Alcohol consumption and breast cancer risk." *Breast Cancer Res* 11.Suppl 3 (2009): S3.

63. Bagnardi, V., et al. "Light alcohol drinking and cancer: a meta-analysis." *Annals of oncology* 24.2 (2013): 301-308.

64. Nelson, Heidi D., et al. "Risk factors for breast cancer for women aged 40 to 49 years: a systematic review and meta-analysis." *Annals of internal medicine* 156.9 (2012): 635-648.

65. Ferguson, Lynnette R. "Meat and cancer." *Meat science* 84.2 (2010): 308-313.

66. Alexander, Dominik D., et al. "A review and meta-analysis of red and processed meat consumption and breast cancer." *Nutrition research reviews* 23.02 (2010): 349-365.

67. Malik, Vasanti S., Matthias B. Schulze, and Frank B. Hu. "Intake of sugar-sweetened beverages and weight gain: a systematic review." *The American journal of clinical nutrition* 84.2 (2006): 274-288.

68. Fung, Teresa T., et al. "Diet quality is associated with the risk of estrogen receptor–negative breast cancer in postmenopausal women." *The Journal of nutrition* 136.2 (2006): 466-472.

69. Fung, Teresa T., et al. "Low-carbohydrate diets, dietary approaches to stop hypertension-style diets, and the risk of postmenopausal breast cancer." *American journal of epidemiology* (2011): kwr148.

70. Li, Jingmei, et al. "Coffee consumption modifies risk of estrogen-receptor negative breast cancer." *Breast Cancer Res* 13.3 (2011): R49. [70]

71. Boggs, Deborah A., et al. "Tea and coffee intake in relation to risk of breast cancer in the Black Women's Health Study." *Cancer Causes & Control* 21.11 (2010): 1941-1948.

72. Yu, Xiaofeng, et al. "Coffee consumption and risk of cancers: a meta-analysis of cohort studies." *BMC cancer* 11.1 (2011): 96.

73. Jiang, Wenjie, Yili Wu, and Xiubo Jiang. "Coffee and caffeine intake and breast cancer risk: An updated dose–response meta-analysis of 37 published studies." *Gynecologic oncology* 129.3 (2013): 620-629.

74. Shrubsole, Martha J., et al. "Drinking green tea modestly reduces breast cancer risk." *The Journal of nutrition* 139.2 (2009): 310-316.

75. Azim, Hamdy A., and Amal S. Ibrahim. "Breast cancer in Egypt, China and Chinese: statistics and beyond." *Journal of thoracic disease* 6.7 (2014): 864.

76. Morin, Catherine L., et al. "High Fat Diets Elevate Adipose Tissue-Derived Tumor Necrosis Factor-α Activity 1." *Endocrinology* 138.11 (1997): 4665-4671.

77. Sieri, S., et al. "High glycemic diet and breast cancer occurrence in the Italian EPIC cohort." *Nutrition, Metabolism and Cardiovascular Diseases* 23.7 (2013): 628-634.

78. Augustin, L. S. A., et al. "Associations of bread and pasta with the risk of cancer of the breast and colorectum." *Annals of oncology* (2013): mdt383.

79. Dong, Jia-Yi, and Li-Qiang Qin. "Dietary glycemic index, glycemic load, and risk of breast cancer: meta-analysis of prospective cohort studies." *Breast cancer research and treatment* 126.2 (2011): 287-294.

80. Zhang, Cai-Xia, et al. "Dietary patterns and breast cancer risk among Chinese women." *Cancer Causes & Control* 22.1 (2011): 115-124.

81. Chrysohoou, Christina, et al. "Adherence to the Mediterranean diet attenuates inflammation and coagulation process in healthy adults: The ATTICA Study." *Journal of the American College of Cardiology* 44.1 (2004): 152-158.

82. Pantavos, Athanasios, et al. "Total dietary antioxidant capacity, individual antioxidant intake and breast cancer risk: The Rotterdam study." *International Journal of Cancer* 136.9 (2015): 2178-2186.

83. Sánchez-Zamorano, Luisa María, et al. "Healthy lifestyle on the risk of breast cancer." *Cancer Epidemiology Biomarkers & Prevention* 20.5 (2011): 912-922.

84. Horn-Ross, Pamela L., et al. "Dietary assessment in the California Teachers Study: reproducibility and validity." *Cancer Causes & Control* 19.6 (2008): 595-603.

85. Link, Lilli B., et al. "Dietary patterns and breast cancer risk in the California Teachers Study cohort." *The American journal of clinical nutrition* 98.6 (2013): 1524-1532.

86. Data, Maps, and Trends." *Centers for Disease Control and Prevention*. Centers for Disease Control and Prevention, 27 Mar. 2015. Web.