LONGITUDINAL CHANGES IN THE INFLAMMATORY POTENTIAL OF DIET AND RISK OF CANCER IN WOMEN

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LONGITUDINAL CHANGES IN THE INFLAMMATORY POTENTIAL OF DIET AND RISK OF CANCER IN WOMEN

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DEDICATION

This dissertation is dedicated to my wife, Nicole and kids, Prince, Blaise and Brielle; who have been a great source of support and encouragement, helping me to overcome the challenges of graduate school. I am truly thankful for having all of you in my life. I also dedicate this work to my mother, Mary Bernsa, who relentlessly encouraged me to always work hard for success and not to despair when success doesn’t come soon enough.
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ABSTRACT

Introduction: The dietary inflammatory index (DII) assesses an individual’s overall diet quality with regards to its inflammatory potential on a continuum from maximally anti-inflammatory (lower or healthier DII scores) to maximally pro-inflammatory (higher or unhealthy DII scores). The DII measured at one point in time has been associated with cancer risk in previous studies; however, data are lacking regarding the change in DII over time and how these changes impact cancer risk. We assessed changes in the DII, and evaluated associations between cumulative history, and changes over time in dietary inflammatory potential, and risk of colorectal cancer (CRC) and breast cancer (BRCA). Methods: Study participants were women aged 50-79 years recruited from 1993-1998 into the Women’s Health Initiative (WHI) Observational Study (OS) and Dietary Modification Trial (DMT), and followed through September 30, 2010. The DII was calculated from repeated food frequency questionnaires (FFQ) data in the OS (n=76,671) at baseline and Year 3, and in the DMT (n=48,482) at up to 11 time points. Univariate generalized estimating equations were used to compare mean DII over time, adjusting for multiple comparisons. We calculated ten cumulative averages of DII, incrementally from baseline to Year 10, categorized each average into quintiles, and estimated hazard ratios (HR) and 95% confidence intervals (95%CI) for CRC, colon, rectal cancer and invasive BRCA incidence by DII quintiles in multivariable-adjusted
Cox regression models. We also derived patterns of changes in DII between baseline and Year 3; and calculated HR for CRC, colon, rectal, and breast cancer incidence including molecular and histologic BRCA subtypes, using multivariable-adjusted Cox regression models. **Results:** In the OS, mean DII decreased from -1.14 (±2.58) at baseline to -1.50 (±2.60) at Year 3. In the DMT, DII decreased from -0.40 (±2.54) to its lowest point of -1.70 (±2.63) at Year 3 in the intervention arm and from -0.38 (±2.55) to its lowest point of -1.04 (±2.60) at Year 3 in the control arm. These changes were influenced by BMI, education, and race/ethnicity. During an average 11.7 years, 1,240 cases of CRC and 4,242 cases of BRCA were identified. HR for the association between high DII scores and CRC were consistently significantly elevated in the first seven years of follow up, for colon cancer with multivariable-adjusted HR ranging from 1.30 to 1.58 in quintile 3 vs. 1, while no significant associations were observed for rectal cancer. Compared to participants in the anti-inflammatory stable category, risk was increased in participants with a pro-inflammatory stable diet, for CRC (HR, 1.18; 95%CI, 0.99, 1.41), and for rectal cancer (HR, 1.53; 95%CI, 1.01, 2.32). HR revealed no significant association between changes in DII and risk of invasive BRCA or its subtypes. **Conclusion:** In this large prospective study of postmenopausal women, dietary inflammatory potential was relatively stable in OS participants, but decreased significantly over time in women enrolled in the DMT. DII changes were modified by BMI, education, and race/ethnicity. Long-term pro-inflammatory diets increased the risk of colon cancer, while shorter-term stable pro-inflammatory diets increased the risk of rectal cancer but not breast cancer or its subtypes. Lowering the inflammatory potential of diet could be a means for colon cancer, and potentially rectal cancer prevention in postmenopausal women.
# TABLE OF CONTENTS

DEDICATION ........................................................................................................ iii

ACKNOWLEDGEMENTS ................................................................................... iv

ABSTRACT .......................................................................................................... v

LIST OF TABLES ................................................................................................. x

LIST OF FIGURES .............................................................................................. xi

LIST OF ABBREVIATIONS .................................................................................. xii

CHAPTER 1: INTRODUCTION ............................................................................ 1

1.1 Statement of the Problem ............................................................................. 1

1.2 Purpose and Objectives ................................................................................ 3

1.3 Significance/relevance of the dissertation research ...................................... 6

1.4 Study Outline ............................................................................................... 8

CHAPTER 2: BACKGROUND .............................................................................. 9

2.1 Chronic inflammation and cancer ............................................................... 9

2.2 Dietary patterns and chronic inflammation ................................................ 17

2.3 Statistical approaches for analyzing repeated measures of diet data .......... 28

2.4 Risk factors for colorectal cancer ............................................................... 32

2.5 Risk factors for breast cancer ..................................................................... 39

CHAPTER 3: METHODS .................................................................................... 49

3.1 Statement of research aims and hypotheses ............................................... 49

3.2 Description of the study population ........................................................... 50
CHAPTER 6: A PROSPECTIVE INVESTIGATION OF CHANGES IN THE INFLAMMATORY POTENTIAL OF DIET AND RISK OF BREAST CANCER IN POSTMENOPAUSAL WOMEN ................................................................. 111

6.1 Abstract .......................................................................................................................... 112
6.2 Introduction ..................................................................................................................... 113
6.3 Methods .......................................................................................................................... 114
6.4 Results ............................................................................................................................ 121
6.5 Discussion ....................................................................................................................... 122
6.6 Conclusion ....................................................................................................................... 125
6.7 Tables and figures .......................................................................................................... 126

CHAPTER 7: LONGITUDINAL CHANGES IN DII AND RISK OF CANCER: A DISCUSSION OF STUDY RESULTS .............................................................................................................. 135

7.1 Summary of results ........................................................................................................ 135
7.2 Potential mechanisms of action ..................................................................................... 136
7.3 Strengths and limitations ............................................................................................... 137
7.4 Public health implications .............................................................................................. 138
7.5 Suggestions for future research .................................................................................... 140
7.6 Conclusion ....................................................................................................................... 140
7.7 Acknowledgements ........................................................................................................ 141

REFERENCES ................................................................................................................... 142

APPENDIX A - SHORT LIST OF WHI INVESTIGATORS ...................................................... 172
LIST OF TABLES

Table 3.1. Estimated ranges of hazard ratios for colorectal and breast cancers based on the available incidence proportions for the two cancer sites ........................................... 63

Table 4.1. Baseline characteristics of study participants, frequency (%), Women's Health Initiative, 1993-1998 .............................................................................................................. 78

Table 4.2. Bonferroni-adjusted p-values of all pair-wise comparisons of the mean dietary inflammatory index scores across years of follow-up in the Dietary Modification Trial; Women's Health Initiative, 1993-2010 .......................................................... 81

Table 4.3. Multivariable predictive model of change in dietary inflammatory index over time in the Observational Study; Women's Health Initiative, 1993-2010 .................. 85

Table 4.4. Frequencies (%) of participant's characteristics across quintiles of cumulative average DII (Years 0-3); Women's Health Initiative, 1993-2010 .......................... 102

Table 5.1. Risk of colorectal cancer by quintiles of cumulative average dietary inflammatory index over a ten-year period of time; Women's Health Initiative, 1993-2010 ........................................................................................................... 105

Table 5.2. Risk of colorectal cancer across patterns of change in the dietary inflammatory index (DII) between baseline and year 3; Women's Health Initiative, 1993-2010 .......... 109

Table 5.3. Risk of breast cancer by subtype, across patterns of change in the dietary inflammatory index (DII) between baseline and year 3; Women's Health Initiative, 1993-2010 ........................................................................ 132
LIST OF FIGURES

Figure 3.1: Sequence of steps in creating the DII. (adapted from Shivappa, N., Steck SE, Hurley TG, Hussey JR, Hebert JR, Designing and Developing a Literature-derived, Population-based Dietary Inflammatory Index. Public Health Nutr, 2013: p. 1-8.) ........ 62

Figure 4.1. Participant flow in the administration of food frequency questionnaires in the Women’s Health Initiative (WHI) Observational Study (OS) and Dietary Modification Trial (DMT), 1993-2010 ............................................................... 77

Figure 4.2. Average dietary inflammatory index (DII) scores across years of follow-up in the Dietary Modification Trial, by study arm; Women’s Health Initiative, 1993-2010^2 . 80

Figure 4.3. Average dietary inflammatory index over time by body mass index category and Dietary Modification Trial arm; Women's Health Initiative, 1993-2010 .................. 82

Figure 4.4. Average dietary inflammatory index (DII) over time by educational level and Dietary Modification Trial arm; Women's Health Initiative, 1993-2010 ...................... 83

Figure 4.5. Average dietary inflammatory index (DII) over time by race/ethnicity and Dietary Modification Trial arm; Women's Health Initiative, 1993-2010^1 ...................... 84

Figure 5.1. Participant flow in the administration of food frequency questionnaires in the Women’s Health Initiative (WHI) Observational Study (OS) and Dietary Modification Trial (DMT), 1993-2010 ............................................................... 101

Figure 5.2. Multivariable-adjusted hazard ratios for the association between cumulative average DII (highest vs. lowest quintile) and colon cancer risk; Women's Health Initiative Dietary Modification Trial, 1993-2010 ............................................................... 108

Figure 5.3. Multivariable-adjusted hazard ratios for the association between cumulative average DII (highest vs. lowest quintile) and rectal cancer risk; Women's Health Initiative Dietary Modification Trial, 1993-2010 ............................................................... 108

Figure 6.1. Participant flow in the administration of food frequency questionnaires in the Women’s Health Initiative (WHI) Observational Study (OS) and Dietary Modification Trial (DMT), 1993-2010 ............................................................... 126
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>24HR</td>
<td>24-hour dietary recall</td>
</tr>
<tr>
<td>A/PI</td>
<td>Asian/Pacific Islander</td>
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<tr>
<td>AA</td>
<td>African American</td>
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<tr>
<td>aHEI</td>
<td>Alternate healthy eating index</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>BRCA</td>
<td>Breast cancer</td>
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<tr>
<td>CFA</td>
<td>Confirmatory factor analysis</td>
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<td>COX</td>
<td>Cyclo-oxygenases</td>
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<td>CRC</td>
<td>Colorectal cancer</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>CUP</td>
<td>Continuous update project</td>
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<td>CT</td>
<td>Clinical trial</td>
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<tr>
<td>DASH</td>
<td>Dietary approaches to stop hypertension</td>
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<tr>
<td>DGA</td>
<td>Dietary guidelines for Americans</td>
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<tr>
<td>DII</td>
<td>Dietary inflammatory index</td>
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<tr>
<td>EA</td>
<td>European American</td>
</tr>
<tr>
<td>EPIC</td>
<td>European prospective investigation into cancer</td>
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<tr>
<td>ER</td>
<td>Estrogen receptor</td>
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<td>FFQ</td>
<td>Food frequency questionnaires</td>
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<tr>
<td>GEE</td>
<td>Generalized estimating equations</td>
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<tr>
<td>HEI-2010</td>
<td>Healthy eating index-2010</td>
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HER-2/NEU...............................Human epidermal growth receptor 2, neuroblastoma
HP...............................................................Hispanic
HR..............................................................Hazard ratio
IL-6..............................................................Interleukin-6
Med-diet......................................................Mediterranean dietary pattern
MUFA..........................................................Monounsaturated fatty acids
NCI..............................................................National Cancer Institute
NDSR...........................................................Nutrient Data system for research
NIH.............................................................National Institutes of Health
NSAID.........................................................Non-steroidal anti-inflammatory drugs
OR..............................................................Odds ratio
OS..............................................................Observational Study
PA..............................................................Physical activity
PCA............................................................Principal component analysis
PH..............................................................Proportional hazards
PR..............................................................Progesterone receptor
PRO...........................................................Pooled repeated observations
PUFA..........................................................Polyunsaturated fatty acids
ROS............................................................Reactive oxygen species
RPA............................................................Recreational physical activity
RRR............................................................Reduced rank regression
SAA............................................................Serum amyloid A
SEER..........................................................Surveillance, epidemiology, and end results
SFA............................................................Saturated fatty acids
TNFα...........................................................Tumor necrosis factor alpha
USPSTF……………………………………………………..US Preventive Services Task Force
WHI………………………………………………………………..Women’s Health Initiative
WCRF/AICR……..World cancer research fund / American institute for cancer research
CHAPTER 1

INTRODUCTION

1.1 Statement of the Problem

Inflammation is a process central to carcinogenesis and other chronic diseases, and there is consistent evidence that diet modulates inflammation.\textsuperscript{1-6} Evidence from both observational and intervention studies show that chronic inflammation is associated with the development of many cancers including colorectal and breast cancers.\textsuperscript{2,7-10} Studies have shown an association between chronic inflammatory conditions and subsequent malignant transformation in the inflammed tissue with some examples being inflammatory bowel disease and subsequent development of colorectal cancer,\textsuperscript{11,12} or Helicobacter pylori-related gastritis and gastric cancer.\textsuperscript{8} The etiology of inflammation varies and can be infectious, such as viruses or bacteria, or it may be a noninfectious irritant such as certain dietary factors.

Many dietary factors are known to affect inflammation, through pro-inflammatory or anti-inflammatory mechanisms. A Western-style diet tends to be rich in pro-inflammatory foods that are high in sugar (especially desserts and soft drinks), refined grains, red and processed meats, and fried foods that increase pro-inflammatory biomarkers such as C-reactive protein (CRP), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF\(\alpha\)).\textsuperscript{1-3,7,9,13,14} By contrast, diets that are rich in fruits, vegetables, whole
grains, legumes, nuts, olive oil and fish (e.g., Mediterranean-type diet) tend to be associated with reduced chronic inflammation. \textsuperscript{3,4,15-17} Likewise, East Asian populations, whose diets contain many anti-inflammatory constituents and are absent many of the pro-inflammatory components in Western diets, have very low CRP levels. \textsuperscript{18,19} Specific components of the diet also have been shown to be associated with lower levels of inflammation; e.g., fruits and vegetables, omega-3 polyunsaturated fatty acids (PUFAs), fiber, moderate alcohol intake, vitamin E, vitamin C, β-carotene, and magnesium. \textsuperscript{20} Dietary patterns are generally known to have a much wider safety margin with prudent consumption than do pharmaceuticals. \textsuperscript{21,22} Conceptually, dietary indices or patterns represent a broader picture of food and nutrient consumption, and may thus provide an approach to examining the relationship between diet and the risk of chronic diseases that may produce more intuitively appealing results that may be more predictive of disease risk as compared to the examination of individual foods or nutrients separately. \textsuperscript{23-30}

Despite the growing use of dietary pattern analysis, relatively few studies have investigated the stability of dietary patterns over time, \textsuperscript{31-37} and to the best of our knowledge, none has done so in relation to the inflammatory potential of diet. Dietary behavior is subject to change over time, \textsuperscript{34,35} and dietary behaviors mainly influence chronic disease outcomes when they persist for a longer period of time. \textsuperscript{31} Knowledge of the stability of dietary patterns over time could aid researchers in planning follow-up times right from study outset. The cost of maintaining large cohorts could be reduced if diet is proven to be stable over time. For example, reduced frequency of diet data collection may be warranted if there is not much variation in dietary habits over time. \textsuperscript{34}
Given the evidence that many dietary factors have either anti- or pro-inflammatory properties, and the idea that no nutrient is consumed alone but in conjunction with other nutrients and non-nutrient components of food, the dietary inflammatory index (DII) was developed\textsuperscript{38} and validated.\textsuperscript{39} The goal of the DII was to assess an individual’s diet on a continuum from maximally anti-inflammatory to maximally pro-inflammatory, thus providing a tool to measure the inflammatory potential of whole diets and their associations with markers of inflammation, and with the development of chronic diseases including cancer.

1.2 Purpose and Objectives

We proposed to utilize data from the Women’s Health Initiative (WHI) to describe longitudinal changes in the inflammatory potential of diet and evaluate the association of changes in the inflammatory potential of diet and risk of colorectal cancer and breast cancer in postmenopausal women. The WHI began in 1992, and enrolled a total of 161,808 women 50 to 79 years old, in 40 sites in the United States between 1993 and 1998.\textsuperscript{40} We hypothesized that the inflammatory potential of diet changes over time and long-term pro-inflammatory diets or shorter-term changes towards pro-inflammatory diets, increase risk of colorectal cancer and of breast cancer. Our study aims were the following:

Aim I: To investigate the stability of the inflammatory potential of diet over time.

The WHI recruited a study population with a high racial and geographic diversity. We proposed to calculate the DII at all the eleven time points at which food frequency questionnaires were administered in the WHI, using data from the Observational Study
We expected that the inflammatory potential of diet would significantly change over time and be influenced by social, demographic and clinical factors. The main study questions for aim #1 included the following:

1. Are there changes in dietary inflammatory potential over time?

2. If there are any significant changes, how do demographic and lifestyle factors such as body mass index (BMI), education, and race/ethnicity impact such changes?

3. What social, demographic and clinical factors predict change in DII?

4. How does the change in the inflammatory potential of diet in an intervention setting differ from that in an observational setting?

**Aim II: To evaluate the association between changes in the inflammatory potential of diet over time and risk of colorectal cancer.**

In specific aim #2, we evaluated the association between the inflammatory potential of diet and risk of colorectal cancer with the hypothesis that a long-term pro-inflammatory diet increases colorectal cancer risk. The dietary inflammatory potential assessed at baseline only has been linked to colorectal cancer risk (Tabung FK, Steck, SE, Ma Y, et al, unpublished data, 2014), however, data is lacking on the impact of longitudinal changes in dietary inflammatory potential on colorectal cancer development. Utilizing the DII to evaluate the role of long term dietary inflammatory potential on the risk of colorectal cancer is therefore warranted. The main questions for this aim included the following:
1. How does long-term cumulative history of dietary inflammatory potential affect colorectal cancer risk?

2. How do shorter-term changes in patterns of the inflammatory potential of diet over time affect risk of colorectal cancer?

3. Do risk estimates differ by anatomic subsite (colon, rectum) both for cumulative history and patterns of change in the dietary inflammatory potential?

**Aim III: To investigate the association between changes in the inflammatory potential of diet over time and risk of breast cancer in postmenopausal women.**

In specific aim #3, we investigated the association between the inflammatory potential of diet and breast cancer risk in postmenopausal women, with the hypothesis that a long-term pro-inflammatory diet increases breast cancer risk. The association between dietary patterns and breast cancer risk is inconsistent, with findings from three large cohort studies not supporting an association between dietary patterns and breast cancer risk. Given the central role of chronic inflammation in the carcinogenesis process and the modulation of inflammation by some dietary patterns, an assessment of the dietary inflammatory potential at multiple time points may be more predictive of breast cancer risk. The main questions for this aim included the following:

1. How does long-term cumulative history of dietary inflammatory potential affect breast cancer risk?

2. How do shorter-term changes in patterns of the inflammatory potential of diet over time affect risk of breast cancer?
3. Do risk estimates differ by molecular or histologic subtype of breast cancer for patterns of change in the dietary inflammatory potential?

1.3 Significance/relevance of the dissertation research

This dissertation addressed an important area of cancer research which includes the role of total diet with respect to its inflammatory potential, in relation to risk of cancer in a large, well-characterized cohort (the WHI) with adequate number of outcomes providing ample power to detect significant associations. Given that the DII has been shown to be associated with inflammatory biomarkers (Tabung FK, Steck SE, Zhang J, Ma Y, et al., unpublished data, 2014) and with colorectal cancer incidence (Tabung FK, Steck SE, Ma Y, et al., unpublished data, 2014) in this study population, the examination of DII changes over time and cancer endpoints is a crucial next step in evaluating the DII as a tool for cancer prevention.

1.3.1 High public health and clinical impact

This study is innovative in that this is the first time that repeated measures of the DII are being used to evaluate the association between changes in the dietary inflammatory potential over time and cancer endpoints. The study will likely have a large public health impact by strengthening the evidence for a new tool assessing the long-term overall quality of diet and providing support for its use in other studies of diet and cancer.

Patients at risk of inflammation-related conditions such as osteoporosis, obesity, cardiovascular disease, and diabetes, may also be at risk of cancer.\textsuperscript{51,52} Therefore a reduction in the inflammatory potential of the diet among patients with these conditions may improve overall health and reduce their cancer risk. The diagnosis of most of these chronic diseases may be also a teachable moment during which most patients undergo
lifestyle changes including diet changes to improve their survival experience.\textsuperscript{53,54} Health professionals armed with the knowledge of the inflammatory potential of diets may be able to impart sound nutritional guidance that improves the overall health of patients with inflammation-related chronic diseases.

1.3.2 The role of the inflammatory potential of whole diets and dietary patterns on cancer risk is largely unknown

Studies of individual foods and nutrients may be inadequate to elucidate the overall role of long term diets and dietary patterns on the risk of degenerative diseases including colorectal cancer and breast cancer. The role of diet in the risk of these cancers is of great interest as a potentially modifiable risk factor given that most risk factors for breast cancer are not generally modifiable. Many epidemiological studies of the association between single dietary factors and colorectal and breast cancers have not yielded consistent conclusive evidence except for overweight/obesity (increases risk of postmenopausal breast cancer and colorectal cancer),\textsuperscript{55-62} regular alcohol consumption,\textsuperscript{55,63,64} and red meat intake.\textsuperscript{65-70}

In a dietary guidelines adherence study, Harnack and colleagues found evidence to suggest that adherence to the cluster of dietary behaviors included in the Dietary Guidelines for Americans is associated with a lower risk of cancers including colon and breast cancers,\textsuperscript{71} while McCullough and colleagues found that following cancer prevention guidelines, reduces risk of cancer, cardiovascular disease, and all-cause mortality.\textsuperscript{72} This evidence supports the idea that studies of the oncogenic role of whole diets and not individual nutrients may be more appropriate for population-based cancer prevention efforts. As further evidence, the impact of total caloric intake, energy balance, and weight gain on the risk of breast\textsuperscript{73,74} and colorectal cancer\textsuperscript{75,76} indicate a role for
overall diet and dietary patterns that may not be captured in studies of individual foods and nutrients. 

1.4 Study Outline

Chapter 1 of this dissertation introduces the problem by first establishing the link between dietary patterns and chronic inflammation, and the link between inflammation and cancer, with the ultimate aim to elucidate the role of dietary inflammatory potential in cancer development. Aims have been stated and their specific significance described, in addition to the overall significance of the dissertation. Chapter 2 presents a detailed background to the aims in an extensive review of the literature. It describes the relation between diet and inflammation and inflammation and cancer, including possible mechanisms of action. Selected theoretically-derived diet quality indices and their relation to inflammation are described, including empirical methods of evaluating dietary patterns and some statistical approaches to analyzing repeated dietary exposures with dichotomous outcomes. Chapter 2 ends with a detailed review of possible risk factors for colorectal cancer and breast cancer. In chapter 3, we describe the methods used to achieve each of the three aims, including a detailed description of the DII. Results for each of the three aims are presented separately in chapters 4, 5 and 6, as standalone publishable manuscripts. Chapter 7 provides a detailed discussion of the results and a conclusion.
CHAPTER 2

BACKGROUND

2.1 Chronic inflammation and cancer

2.1.1 Overview

In the mid nineteen century, Virchow theorized that the lymphoreticular infiltrate at sites of chronic inflammation may establish the setting in which cells grow abnormally.\textsuperscript{7,77} A more contemporary version of Virchow’s hypothesis is that the inflammatory processes induced by chronic injury contribute to the multistage development of cancer and that the inflammation, rather than the specific cause of the injury, account for subsequent carcinogenicity.\textsuperscript{78} Inflammation is a crucial function of the innate immune system with acute inflammation being a self-limiting process that protects against pathogens and initiates specific immunity, however, acute inflammation does not always resolve.\textsuperscript{79} Many of the diseases of middle and old age may be driven, at least in part, by chronic and often subclinical inflammation.\textsuperscript{79} Several lines of evidence, including general or cell-specific gene inactivation and population-based studies, are consistent with the view that inflammation plays an important role in cancer causation and/or progression. As Balkwill et al. indicated,\textsuperscript{79} the links between chronic inflammation and cancer are reinforced by several concepts including the following: i) many cancers arise at sites of chronic inflammation and chronic inflammation increases cancer risk, ii) the
immune cells that mediate chronic inflammation are found in cancers and promote tumor growth, iii) cancers produce chemical mediators that regulate inflammation, iv) experimental cancers have been inhibited by the inhibition of inflammatory mediators, v) susceptibility to, and severity of cancer is altered by the variation of inflammatory genes, and vi) the long term use of non-steroidal inflammatory drugs reduces the risk of some cancers.79

More recently, the role of inflammation in cancer development was highlighted by Brucher and Jamall when they proposed a new paradigm for the epistemology of the origin of cancer.80 They stressed that less than 10% of all cancers are hereditary, and departed from the widely held concept that cancer originates from somatic mutations and an inhibition of growth suppression, followed by cell proliferation and metastasis.80 According to their new paradigm, the origin of cancer follows a sequence of events beginning with 1) a pathogenic stimulus which can be biologic or nonbiologic (including diet), 2) followed by chronic inflammation, 3) from which fibrosis develops, with associated changes in the cellular microenvironment if the inflammation does not resolve, 4) a pre-cancerous niche then develops which triggers 5) a chronic stress escape strategy 6) that transforms a normal cell to a cancer cell if the chronic stress does not resolve.80 If this hypothesis is true, then intervening to prevent or reduce chronic inflammation that may be triggered by potentially modifiable risk factors such as diet, may present an excellent opportunity for the primary prevention of cancers.

2.1.2 Chronic inflammation and colorectal cancer

Several lines of evidence suggest that colorectal neoplasia may arise from colonic areas with chronic low grade subclinical inflammation.81,82 Chronic inflammatory bowel
diseases, such as ulcerative colitis and Crohn’s disease have been associated with increased risk of colon cancer.\textsuperscript{12} Moreover, several studies have shown a reduced risk of colon cancer with use of aspirin or other anti-inflammatory agents.\textsuperscript{79,83,84} Patients with long-standing ulcerative colitis and Crohn's disease have an increased risk of developing colorectal cancer and patients with Crohn's disease in the small intestines are at increased risk of small bowel adenocarcinoma.\textsuperscript{82,85} Inflammatory bowel disease-related colorectal cancer is the result of a process which is believed to begin from no dysplasia, progressing to indefinite dysplasia, low-grade dysplasia, high-grade dysplasia and finally to invasive adenocarcinoma.\textsuperscript{86} This is also called the adenoma-carcinoma sequence, although colorectal cancer can arise without proceeding through each of these steps.\textsuperscript{12,86,87}

Several prospective studies have supported the hypothesis that inflammation is a risk factor for the development of colon cancer.\textsuperscript{88-91} A study analyzing change in C-reactive protein (CRP) and serum amyloid A (SAA) over time in relation to risk of colorectal cancer using data from the WHI observational study, observed an increased risk of colon cancer among women in the highest quintile of CRP change compared to those in the lowest quintile (OR; 1.37, 95\%CI; 0.95, 1.97), p-trend = 0.04) but no association with SAA.\textsuperscript{89} Women with elevated concentrations of both CRP and SAA had an increased risk of colon cancer (OR; 1.50, 95\%CI; 1.12–2.00) compared to those with low concentrations.\textsuperscript{89} The study observed no positive associations with rectal cancer and weaker associations for colorectal cancer overall. Furthermore, temporal changes in biomarkers more than 3 years did not predict risk.\textsuperscript{89} An examination of the association of CRP levels with colorectal cancer incidence in a nested case-control study within the Alpha Tocopherol, Beta-Carotene Cancer Prevention Study found an increased risk of
colon cancer incidence for men with the highest concentration of CRP (OR, 2.9; 95%CI, 1.4, 6.0) compared to men with the lowest concentration, with the association being stronger among lean individuals than in heavier individuals. Another nested case-control study found that the odds of developing colorectal cancer increased with higher concentrations of CRP, such that persons in the highest quartile of CRP had a 2-fold increased risk of colorectal cancer compared with persons in the lowest quartile (OR, 2.00; 95%CI, 1.16-3.46; P_{trend} = 0.008), but the study did not conduct separate analysis for rectal cancer.

In summary, whether levels of inflammatory biomarkers are elevated before biological onset of colorectal cancer, or indeed whether inflammatory biomarkers are risk factors for the de novo development of colorectal cancer, are questions that relatively few of the prospective studies have tried to address. The presence of malignant disease may itself affect concentrations of circulating inflammatory biomarkers from retrospective case-control studies or from cohort studies where case diagnosis close in time to blood draw might reflect tumor marker status rather than true risk assessment. Whether circulating concentration of inflammatory biomarkers truly reflects colonic inflammation and/or translates into biological activity is unclear. This emphasizes the need for more research to explore the association of inflammatory biomarkers with colonic inflammation. As cancer is a relatively rare disease, small numbers of colorectal cancer cases and lack of power pose problems in many prospective epidemiologic studies.

2.1.3 Inflammation and breast cancer

Studies focusing on the tumor microenvironment have demonstrated that inflammation correlates with increased invasiveness and poor prognosis in many types of
cancer, including breast cancer. The cytokines interleukin-6 (IL-6), IL-1β and tumor necrosis factor alpha (TNFα), have been found to be associated with breast cancer progression. An analysis of data from the Health, Eating, Activity, and Lifestyle Study on the relationship between circulating markers of inflammation (CRP and SAA) and breast cancer survival found that SAA and CRP significantly predicted long-term survival in breast cancer patients, independent of race, tumor stage, and BMI. In contrast, a meta-analysis of prospective studies of the association between circulating CRP and IL-6 and the development of specific cancers, found no association with an increased breast cancer risk. In a Swedish study of 2,577,565 women to examine possible associations between mastitis and subsequent risk of breast cancer, the investigators found that breast cancer risk was slightly elevated in women with a history of mastitis (incidence rate ratio: 1.23, 95%CI, 1.02-1.49). The absence of a correlation between laterality of lesions (i.e., the breast with mastitis was not always the breast with cancer), however, did not support a causal association between inflammation (mastitis) and the development of breast cancer in the study.

An assessment of the association for use of aspirin, other non-steroidal anti-inflammatory drugs (NSAIDs), and acetaminophen with breast cancer risk among breast cancer-free (at baseline) premenopausal women in the Nurse’s Health Study II, found that regular use of aspirin (≥ 2 times/ week) was not significantly associated with breast cancer risk (RR, 1.07; 95%CI, 0.89-1.29). Additionally, non-aspirin NSAIDs or acetaminophen were not consistently associated with breast cancer risk in premenopausal women, and results did not vary by frequency (days per week), dose (tablets per week), duration of use or estrogen and progesterone receptor status of the tumor. In another
study, the use of ibuprofen or acetaminophen was not associated with breast cancer risk. In contrast, a case-control study to investigate the association of adult lifetime aspirin intake with breast cancer risk, found evidence that aspirin use throughout a woman's life may confer some benefit (adjusted OR 0.80, 95% CI: 0.68-0.94), comparing aspirin users to non-users, and a large cohort study of postmenopausal women followed for more than 6 years, found a trend of decreasing risk of incident breast cancer with increasing frequency of aspirin use (Ptrend = 0.001). The multivariate-adjusted RR of breast cancer was 0.71 (95% CI 0.58-0.87) for women who reported using aspirin six or more times per week compared with women who reported no use. No association was found between non-aspirin NSAID use and incident breast cancer.

In summary, while most of the evidence is consistent that chronic inflammation increases the risk of breast cancer recurrence or survival, the evidence has been less consistent for the association between chronic inflammation and breast cancer incidence. Most of the research on the association between chronic inflammation and breast cancer incidence has been through the intermediacy of anti-inflammatory drugs such as aspirin and non-aspirin NSAIDs and is inconsistent.

Many sites of chronic uncontrolled low grade inflammation many exist in the body at the same time and most of the biomarkers of inflammation usually employed in epidemiologic studies are non-specific. This may explain the weak associations or lack thereof, between biomarkers of inflammation and the development of breast cancer or colorectal cancer in some studies. Thus, biomarkers of inflammation are associated with breast or colorectal cancer incidence and/or progression only to the extent that these
markers correlate with breast or colorectal inflammation respectively. Moreover, CRP (used in most of the studies) is a non-specific marker of inflammation.

2.1.4 Biologic plausibility and mechanisms for inflammation and cancer

The chronic inflammatory response represents a fine balance between active inflammation, repair, and destruction that occurs in response to a persistent stimulus over a prolonged period of time. Activation of leukocytes in response to such an ongoing stimulus leads to the production of cytokines, and reactive oxygen species (ROS), resulting in accumulated tissue destruction and subsequent attempts at healing via remodeling, angiogenesis, and connective tissue replacement. A wide variety of chronic inflammatory diseases are associated with cancer. Indeed, chronic inflammation orchestrates a tumor-supporting microenvironment that is an indispensable participant in the neoplastic process. Important components in this linkage are the cytokines produced by activated innate immune cells that stimulate tumor growth and progression.

Supporting evidence for the inflammation-cancer link comes from studies showing that diverse infections and mechanistic agents trigger the inflammation associated with human cancer. These links have been confirmed especially in terms of colon cancer (colitis), gastric cancer and MALT lymphoma (Helicobacter pylori infection), liver cancer (cholangitis and hepatitis virus B and C), and Kaposi’s Sarcoma (Human Herpes Virus 8 infection). Chronic inflammation appears to predispose to the development of colon cancer in the setting of inflammatory bowel disease, following an “inflammation-dysplasia-carcinoma” model.

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1 mucosa-associated lymphoid tissue
A second line of evidence for the biologic plausibility of the association between inflammation and cancer relates to the increased expression of inflammatory mediators that occurs during tumor development. Balkwill and Mantovani demonstrated that acute inflammation triggered by the exogenous administration of inflammatory biomarkers in murine models promotes malignancy and metastasis under controlled conditions. The link between inflammation and cancer is further supported by evidence from studies showing a positive association between higher concentrations of inflammatory biomarkers and increased risk of colon cancer.\textsuperscript{8,9,112,113}

The third and complementary line of evidence is the fact that NSAIDs, which inhibit COX-2 activity and tumor development in many experimental and clinical settings, are inversely associated with certain cancers in epidemiological studies.\textsuperscript{78,114} Inflammatory cytokines induce the production of inflammatory enzymes such as the cyclo-oxygenases (COX). The expression of COX-2 and lipid mediators of inflammation increases during the multistage progression of neoplastic conditions.\textsuperscript{115} Observational studies and human intervention trials have also indicated that the regular administration of NSAIDs confers a 30–50\% reduction in colorectal cancer risk or adenoma recurrence.\textsuperscript{116,117} We previously found an association between the consumption of highly pro-inflammatory diets and higher concentrations of inflammatory biomarkers including IL-6, hs-CRP, TNFα-R2 and an overall inflammatory biomarker score derived from a combination of these biomarkers (Tabung FK, Steck SE, Zhang J, et al, unpublished data, 2014). In another study to evaluate the association between the inflammatory potential of diet and risk of colorectal cancer, we found an increased risk of colorectal cancer in
participants consuming a highly pro-inflammatory diet (Tabung FK, Steck SE, Ma Y, et al., unpublished data, 2014).

2.2 Dietary patterns and chronic inflammation

2.2.1 An overview of opportunities and challenges

The traditional approach to studying the relation between diet and disease has been to focus on the effects of specific nutrients, foods or food groups but people consume a wide variety of diets, not isolated nutrients or foods. Additionally, people eat diets in specific patterns that are influenced by the environmental conditions of living, religious opinions, personal preferences, food availability, economical status and many other cultural factors. Dietary pattern research thus offers a more comprehensive approach to the investigation of diet-disease associations. Nutritional epidemiologists cite several reasons for preferring the dietary pattern approach over the traditional nutrient-based approach, including the following: 25-27,118-121 1) nutrient-based research does not consider the complex interactions among nutrients in metabolic reactions; nutrients may interact with each other and influence their bioavailability and absorption; 2) increased consumption of one food (i.e., red meat and related products) may be associated with reduced consumption of other foods (i.e., fruit and vegetables) since the total energy intake of individuals should remain stable; 3) many nutrients are highly correlated and studying their separate effects is hampered by collinearity; 4) the effects of single nutrients may be too small to detect while the synergistic and larger effect of nutrients with similar effects may be more easily detected in dietary patterns; 5) analysis of individual nutrients may be confounded by dietary patterns and 6) the success of “whole diet” interventions including the Dietary Approaches to Stop Hypertension (DASH) trial
and the Lyon Diet Heart Study.\textsuperscript{122-124} The two main approaches for deriving dietary patterns are the \textit{a priori} or index-based approach and the \textit{a posteriori} or data-driven approach. These approaches are reviewed in more detail in sections 2.2.2 and 2.2.3.

The index-based approach is intuitively appealing, analytically simple to compute, and easily reproducible and comparable across different studies. Scores that dichotomize components do not account for the full range of foods consumed, while scores that award points for a range of intakes consider variability in food intake but not the amounts at the extremes of component intake distributions.\textsuperscript{25,28} Subjectivity may be introduced during index construction in the selection of foods for inclusion. Also, the addition of equally weighted components implies that each component is additively related to health and equally important.\textsuperscript{25}

Data-driven methods (e.g., factor analysis, cluster analysis) have shown some level of reproducibility across populations.\textsuperscript{125,126} Patterns allow for biologic interactions and can thus be the starting point for modeling different types of interactions among foods.\textsuperscript{25} While factor analysis describes the variation in food intake in the population based on correlations among dietary factors as a continuous variable, cluster analysis separates subjects into mutually exclusive groups based on dietary intake as a categorical variable.\textsuperscript{25} Generally, there is limited data on the reproducibility and validity of data-driven methods, though reproducibility in different populations can never be expected to be exact due to the data-driven nature of the approach. Subjectivity is introduced at various points including grouping of dietary items, treatment of input items (e.g., whether to use grams, servings, percent energy or standardized intake items), the choice of
analytical procedure (e.g., type of rotation to use), and deciding on the final dietary pattern solution.\textsuperscript{25}

Both index-based and data-driven approaches to dietary pattern analysis thus characterize total diet and overcome most of the limitations of single-nutrient research, and analysis results are more meaningful, interpretable and associated with health outcomes.

Regarding the relation of dietary pattern and inflammation, Western-style eating patterns are characterized by frequent intake of energy-dense food and beverage portions delivering an excess of readily available carbohydrates and fats, and few other nutrients. This eating pattern, combined with a sedentary lifestyle, results in weight gain, but it is also associated with increased production of reactive oxygen species (ROS).\textsuperscript{127} As people gain weight and become overweight and obese, CRP, along with other inflammatory mediators, also increases.\textsuperscript{128,129} ROS, also known as free radicals, lead to an acute oxidative imbalance, resulting in oxidative stress. Oxidative stress “turns on” genes that control the production of cytokines and other proteins (biomarkers) involved in inflammation.\textsuperscript{130,131} Since eating is not a one-time activity, but rather an activity that we repeat meal after meal and day after day, the diet then becomes a central point of negotiation for oxidative and inflammatory balance.

\textbf{2.2.2 Comparison of selected diet quality indices and their association with inflammation}

This section reviews the strengths and limitations of three diet quality indices [healthy eating index-2010 (HEI-2010)], dietary approaches to stop hypertension
(DASH), and the Mediterranean dietary pattern), comparing each of them to the dietary inflammatory index (DII) in terms of their ability to modulate inflammation. The discussion is undertaken from both the perspective of the theoretical underpinnings of the respective strategies for the development of each index, as well as the statistical considerations/limitations of each index.

2.2.2.a Brief overview of the dietary inflammatory index (DII)

Details of the development and validation of the DII have been described elsewhere. Briefly, an extensive literature search was performed to obtain peer-reviewed journal articles that examined the association between six inflammatory biomarkers (IL-1β, IL-4, IL-6, IL-10, TNFα, and CRP) and 45 specific foods and nutrients (components of the DII). Scores were derived and standardized to a representative global diet database constructed based on 11 datasets from diverse populations in different parts of the world. Overall DII scores for each individual represent the sum of each of the DII components in relation to the comparison database. The DII score characterizes an individual’s diet on a continuum from maximally anti-inflammatory to maximally pro-inflammatory, with a higher DII score indicating a more pro-inflammatory diet and a lower DII score indicating a more anti-inflammatory diet. A more detailed description of the DII can be found in chapter 3, section 3.5.

2.2.2.b Healthy Eating Index (HEI)

The HEI was developed based on a 10-component system of five food groups (grains, vegetables, fruits, milk and meat), four nutrients (percent energy from total fat, percent energy from saturated fat, cholesterol intake, sodium intake), and a measure of variety in food intake. Each of the 10 components has a score ranging from 0 to 10, so the
total possible index score is 100. A score of 0 indicates non-compliance with recommended amounts or ranges while a score of 10 indicates intakes closest to recommended amounts or ranges.\textsuperscript{132}

Though the HEI was developed to measure adherence to the Dietary Guidelines for Americans (DGA) and the \textit{Food Guide Pyramid}, some studies have investigated the association of the HEI and inflammation with the main finding being that the HEI does not significantly predict biomarkers of systemic inflammation such as CRP, SAA and IL-6,\textsuperscript{133-135} and has equally performed poorly in predicting chronic disease risk.\textsuperscript{136} The HEI’s low predictive ability (or lack thereof) for chronic systemic inflammation and chronic disease may be due to its inability to distinguish between the form of carbohydrate, saturated and unsaturated fats, or protein sources (e.g., processed meats versus fish). These limitations were addressed in the development of an alternate HEI (aHEI). A study comparing the disease predictive ability of the HEI and aHEI found that the aHEI significantly predicted risk of cardiovascular disease (CVD) but both indices failed to predict cancer risk.\textsuperscript{136,137} In a study to assess the association between several diet-quality indices and plasma concentrations of biomarkers of inflammation and endothelial dysfunction, higher aHEI scores were associated with lower concentrations of inflammatory biomarkers.\textsuperscript{134}

An updated version of the HEI (HEI-2010) was published in February 2013, to reflect the 2010 updated DGA (DGA-2010).\textsuperscript{138} In accordance with the DGA-2010, the HEI-2010 allows for flexibility in food choices. The advantage of this is that lack of any one commodity does not prevent anyone from having a perfect HEI-2010 score. Furthermore, the added component of seafood and plant proteins explicitly allows for
vegan diets to be scored. Similar to the aHEI, the HEI-2010 now distinguishes quality within food groups and acknowledges the health benefits of unsaturated fats. For example, whole fruit and total fruit are now separate items, in order to operationalize the recommendation to consume more whole fruit than fruit juices; and the maximum standard for fatty acids is based on the ratio of monounstaturated fatty acids (MUFA) plus polyunstaturated fatty acids (PUFA) to saturated fatty acids (SFA).  

The energy density approach adopted in the construction of the HEI-2010 adjusts for energy intake. In contrast to the food-based adequacy components of the HEI-2010 where assigning the minimum score of zero was easily determined by no intake for the specific component, arbitrary decisions had to be made for the moderation components (sodium, refined grains and empty calories), for assigning the minimum score because these components are reverse-scored and there is no scientific evidence on which to base the minimum scores. For example, no scientific evidence specifies how high a sodium intake would qualify for a score of zero. A value at approximately the 85th percentile of the 2001-2002 population distribution of 1-day intakes was used to set the minimum standards for these components.

The validity of the HEI-2010 has not yet been determined for ethnic and cultural groups, but the index would be expected to be valid for assessing the diets of subpopulations for which the DGA are appropriate because the mixed dishes and sauces that distinguish ethnic and cultural diets would be broken down into their ingredients and assigned to food groups and nutrients, which are generally culturally neutral. Also, given that the HEI-2010 has incorporated most of the limitations identified in the aHEI, its
predictive ability for both chronic systemic inflammation and inflammation-related chronic diseases will be expected to improve compared to the original HEI.

2.2.2.c Dietary Approaches to Stop Hypertension (DASH)

The DASH-style diet is typically high in fruits and vegetables, moderate in low-fat dairy products, and low in animal protein but with substantial amount of plant protein from legumes and nuts. Evidence for the usefulness of the DASH diet plan in disease prevention first came from two multicenter randomized controlled feeding trials and has been incorporated in the DGA and the National Heart Lung and Blood Institute’s DASH eating plan. These two trials demonstrated that a diet rich in fruit, vegetables, and low-fat dairy products and low in saturated and total fat (DASH diet) reduced blood pressure and that blood pressure is further reduced when the DASH diet is followed in conjunction with significant reductions in sodium intake. The DASH diet therefore includes food groups and sodium, with the food groups being grains, vegetables, fruits, dairy, lean meat, nuts/seeds/legumes, fats/oils, and sweets.

The DASH diet has been shown to be associated with reduced systemic inflammation and reduced risk of several inflammation-related chronic diseases such as diabetes, cancer, and heart disease and stroke. Several diet indices have been developed to capture the DASH diet plan and evaluate associations with health outcomes. In a recent study, Miller et al compared four established DASH indices in regards to their associations with colorectal cancer in the same population (the NIH-AARP cohort). They calculated separate indices defined by Dixon (7 food groups, saturated fat, and alcohol), Mellen (9 nutrients), Fung (7 food groups and sodium), and Guenther (8 food groups), and found that higher scores on all four indices were associated with reduced
risk of colorectal cancer in both men and women.\textsuperscript{28} Miller et al concluded that “\textit{the consistency in findings, particularly in men for both colon and rectal cancer, suggests that all indices capture an underlying construct inherent in the DASH dietary pattern, although the specific index used can affect results.”

There is no standardized methodology for calculating the DASH index and the discrepancy in the predictive ability of the four indices, where the DASH-Dixon index did not significantly predict colorectal cancer risk in women (in contrast to the other three indices) demonstrates the idea that differences in the composition of the indices and scoring algorithms can affect results.

\textbf{2.2.2.d Mediterranean Diet Score (Med-diet)}

A traditional Med-diet pattern typically has a high ratio of MUFA to SFA and omega-3 to omega-6 PUFA. It equally has a rich supply of fruits, vegetables, nuts, seeds, legumes, and grains. The Med diet has been widely studied with more than 20 different indices developed based on the Med-diet and used to evaluate health outcomes. The Med-diet has been shown to be associated with reduced chronic systemic inflammation\textsuperscript{4,134,146-150} and reduced risk of several inflammation-related chronic diseases such as diabetes,\textsuperscript{123} cancer\textsuperscript{139} and heart disease.\textsuperscript{140,151} A study to compare and evaluate the reliability of 10 of these indices showed satisfactory performance in assessing adherence to the Med-diet. However, in order to improve the reliability, and concordance between the indices, the investigators suggested further research to standardize the number and selection of components and the scoring criteria of the indices.\textsuperscript{146}

The Med-diet score as described by Trichopoulou et al.,\textsuperscript{152} has been adapted and used in many studies. Generally, the score is constructed by assigning a value of 1 to a
high intake (≥median) of each desirable component, a value of 1 to a low intake
(<median) of each undesirable component, and a value of 0 to all other intakes. Desirable
components may include vegetables, fruits, nuts, seeds, whole grains, legumes, fish,
unsaturated fats, moderate alcohol, while undesirable components may include saturated
fat, red and processed meats, and dairy products. The higher the Med diet score, the
greater the adherence to the Med-diet pattern.\textsuperscript{152}

\subsection{2.2.3 Empirical methods to derive dietary patterns}

Three statistical methods used to define dietary patterns include factor analysis,
cluster analysis, and reduced rank regression.\textsuperscript{23,125} These so-called \textit{a posteriori}
approaches build on statistical exploratory methods driven by data.\textsuperscript{153} Despite the
differences in the goal of these methods, they are similar regarding their mathematical
foundation.\textsuperscript{154} There are many opportunities for subjectivity and decisions made by the
investigators may have an impact on the number and type of patterns derived, reported,
and analyzed. Specifically, the investigator must first decide whether or not to further
collapse the primary dietary data into a smaller number of items for entry into the
analysis. If the data are collapsed, a decision must be made on how to group the data.
Next, the investigator must decide how the input variables should be treated. After the
input variables have been entered into the procedure, a decision must be made on how
many patterns (the output variables) need to be retained in the final solution, which
patterns should be reported or analyzed, and how the patterns should be
named.\textsuperscript{23,25,120,155,156}
2.2.3.a Factor analysis

Factor analysis is a multivariate statistical technique, which uses information reported in nutritional assessment methods (FFQs, 24HRs, or food records) to identify common underlying dimensions (factors or patterns) of food consumption,\textsuperscript{23} by reducing data into patterns based upon intercorrelations between dietary items.\textsuperscript{120} Factor analysis includes both principal component analysis (PCA) and confirmatory factor analysis (CFA). In nutritional epidemiology, the most commonly used method to derive dietary pattern is PCA with varimax rotation, which enhances the difference between factor loadings, and allow for easier interpretability.\textsuperscript{120} It is an appropriate modeling approach for dietary patterns that are not necessarily independent of each other (that is, correlated patterns).\textsuperscript{120} Factor analysis aggregates specific food items or food groups on the basis of the degree to which food items in the dataset are correlated with one another.\textsuperscript{23} A summary score for each pattern is then derived and can be used in either correlation or regression analysis to examine relationships between various eating patterns and outcomes of interest.\textsuperscript{23} Verasso et al., (2012) compared dietary patterns derived through PCA and CFA used as equivalent approaches in terms of stability and relevance and found that CFA may be a useful alternative to PCA in epidemiologic studies, especially when the sample size is small.\textsuperscript{154}

2.2.3.b Cluster analysis

In contrast to factor analysis, cluster analysis aggregates individuals into relatively homogeneous and mutually exclusive subgroups (clusters) with similar diets and may use several different methods to do so (e.g., K-Means or Ward’s method).\textsuperscript{23,120} Individuals can be classified into distinct clusters or groups on the basis of the frequency of food intake, the percentage of energy contributed by each food or food group, the
average grams of food intakes, standardized nutrient intakes, or a combination of dietary and biochemical measures.\textsuperscript{23} Compared to factors (continuous variables), clusters (categorical variables) may be easier to handle in the analysis since they are mutually exclusive and categorical. The idea that individuals have scores for all of the derived factors makes the concept of factor scores less intuitive than an individual belonging to a specific dietary pattern (or cluster).\textsuperscript{120}

Cluster analysis may be preferable for use in planning dietary interventions targeted to risk groups, as it allows the identification of subgroups and the association of clearly defined eating patterns with outcome measures.\textsuperscript{119} Although conceptually different, cluster analysis and factor analysis have shown similarities in grouping foods into patterns. For example, several studies have identified a healthy cluster, with important contributions from fruit, vegetables, breakfast cereals or whole grains, and low-fat dairy products, and with some including fish and nuts.\textsuperscript{119,120,157,158} One limitation of the cluster analysis approach is lower power when comparing multiple subgroups with health outcomes, relative to the linear variables generated by factor analysis (PCA). This may be one reason why PCA has been more frequently adopted in nutritional epidemiology. Nonetheless, when power is adequate, cluster analysis provides clear descriptions of existing subgroup diets.\textsuperscript{119}

2.2.3.c Reduced rank regression

Reduced rank regression (RRR) or maximum redundancy analysis determines linear functions of predictors (foods) by maximizing the explained variation in responses (disease-related nutrients). Key nutrients or biomarkers of disease (e.g., CRP, IL-6, HDL-cholesterol) function as the response variables and linear combinations of foods are
derived which maximize the explained variance in these responses. The classic PCA method selects factors that explain as much predictor variation as possible. In contrast, RRR extracts factors that explain as much response variation as possible. The results from a limited number of investigations using RRR have shown stronger relations between derived dietary patterns and cardiovascular disease than results from studies using PCA. However, Tucker argued that because the dietary patterns are “forced” to predict biological markers, the patterns are proxy variables for the biomarkers rather than independent variables, and that the biomarkers are known to be good predictors of the disease in question, therefore the dietary pattern derived is also predictive of the disease in the same or very similar populations. While this may be a valid argument, several other studies have applied RRR in different populations and settings though with mixed results that may not be necessarily related to issues of heterogeneity in study populations.

2.3 Statistical approaches for analyzing repeated measures of diet data

In longitudinal observational studies of the role of diet on health outcomes, diet can be assessed several times during follow-up. Applying these repeated diet measures in the evaluation of health outcomes is not always as straightforward as using diet data at one point in time. Standard methods of analyzing these repeated measures require that the number of measurements be constant over study participants and over time, e.g., the proportional hazards assumption in Cox regression models, and thus most analyses often ignore the repeated diet measures and use only baseline data to evaluate long term disease outcomes. The following analytical approaches make use of repeated diet data:
2.3.1 Patterns of change in diet intake over time

To create patterns of change over time in diet intake, the investigator needs to decide how many time points of diet data to use, though it becomes increasingly difficult to define patterns when using more than two time points. The data is categorized into quantiles and patterns are defined as movements between quantiles across different points in time.\textsuperscript{167} Quantiles must be chosen carefully to avoid too narrow or too wide definition of patterns. For example, tertiles provide only three categories and the investigator needs to decide whether change would be defined as movement of one tertile or two tertiles. A categorical independent variable is then created with the patterns as categories, and used to predict the outcome in an appropriate regression model (e.g., logistic regression model or Cox proportional hazards regression model).

2.3.2 Absolute/relative changes in diet over time

This approach would typically consider one interval of time at a time. The use of absolute change may be misleading because the absolute difference between two high diet intake values or two low diet intake values across two points in time may be numerically identical and when the difference is entered in a regression model, these two participants with different diet intake levels will be classified in the same group.

The usefulness of percent change or relative change in classifying participants based on their diet intake may depend on the range of values for the specific dietary factor. For a composite factor that may include both positive and negative values within a narrow range, for example, the dietary inflammatory index, percent change may not properly classify participants. Movement from a score of -1 to -2 (1 unit change in the
anti-inflammatory direction) has a 100% change, whereas movement from 9 to 8 (another 1 unit change in the anti-inflammatory direction) only has an 11% change, even though the absolute value of the change and the direction are the same.

2.3.3 Pooled repeated observations (PRO) of diet

This approach is a generalized person-year technique which incorporates all repeated diet measurements made at equally spaced intervals of time. This is the method used in the Framingham Study Cohort. The method as originally proposed by Wu and Ware treats each time interval as a mini-follow-up study and pools observations across all intervals to examine the short-term development of disease. The outcome for this method is assumed to occur once (that is, event/no event), in contrast to other sampling designs in which the outcome is also measured repeatedly over time.

In analyses using only baseline diet data, repeated observations are ignored. These observations are time dependent and since individuals change over time, this data which could influence the outcome is lost. The PRO method uses all of this data and updates the risk factors or diet data and persons at risk at the beginning of each observation interval. For example, if 500 persons were enrolled in a study and at the end of the first interval, 30 were diagnosed with the disease of interest, while 20 were lost to follow-up or died of other causes, these 50 persons are removed from the population at risk and the remaining 450 become at risk for the next interval and so on till the last interval. The data obtained from all intervals is then pooled to yield a sample from which interval predictions for disease can be examined as opposed to one long term prediction as would be the case if only baseline diet data were used.
Three main assumptions underlie the PRO method, which include the following:
1) the time at which data is recorded is not relevant to the occurrence of an event; for example, the probability of developing disease among persons with the same risk profile in the first interval is the same as in the ninth interval, 2) the relation between risk factors (e.g., diet) and outcome is independent of time, that is, there are no secular trends, and 3) the current risk profile is all that is needed to predict risk in the next interval, meaning that a person’s past history is not important in this prediction.\textsuperscript{168}

Wu and Ware proposed a general logistic regression model to incorporate repeated measurements in predicting a dichotomous outcome.\textsuperscript{166} When the three assumptions listed above are applied to this model, it reduces to the PRO method.\textsuperscript{168} The PRO method can also be implemented using the complementary log transformation [(-log (1-p))] for the conditional probability of survival in an interval, proposed by Prentice and Gloeckler.\textsuperscript{170} These two regression models produce similar results when the intervals are short and the outcome is rare compared to number of persons at risk for the outcome.\textsuperscript{171}

\subsection*{2.3.4 Cumulative average diet}

The incidence of the outcome in an interval going forward can be related to the cumulative average of diet intake calculated from the preceding intervals. For example, the incidence of the outcome from year 3 going forward can be related to the cumulative average of diet data from baseline, years 1 to 3, while the incidence of the outcome from year 5 going forward can be related to the cumulative average of baseline, years 1-5 diet data. The averages can be calculated unweighted or weighted. For example, if previous diet history is hypothesized to influence the outcome more than current diet, more weight can be given to diet data from older intervals compared to more recent intervals. Hu and
colleagues related incidence of cardiovascular disease in two-year intervals, to cumulative average fat intake from all preceding intervals, in a study comparing approaches for modeling repeated dietary measurements. To avoid the possibility of change of diet due to subclinical disease, outcomes that developed during the period for which diet is being averaged can be excluded from analyses. Exclusion of previously diagnosed cases also ensures that only participants at risk of developing the outcome going forward are included in the models.

2.4 Risk factors for colorectal cancer

Colorectal cancer (colon and rectum cancers combined) is the third (after lung and breast cancer) most commonly diagnosed cancer and the second leading cause of cancer-related deaths in the US. The American Institute for Cancer Research (AICR) estimates that half of colorectal cancers can be prevented by healthy lifestyle habits. In total, close to 400,000 cases of colorectal cancer in the United States can be prevented each year by eating a healthy diet, undertaking regular physical activity, maintaining a healthy weight and limiting alcohol consumption. This suggests that colorectal cancer is one of the most preventable types of cancer. An analysis of colon cancer risk factors in women found reduced risk for current postmenopausal hormone use, being physically active, taking aspirin, and being screened. Women who smoked, had a consistently high relative weight, had a low physical activity level, consumed red or processed meat daily, were never screened, and consumed low daily amounts of folate, had almost a 4-fold higher cumulative risk of colon cancer by age 70 years. The study also found that for women with a high risk factor profile, adopting a healthier lifestyle could dramatically reduce colon cancer risk.
2.4.1 Dietary patterns

The 2011 Continuous Update Project report of the WCRF/AICR, on colorectal cancer found that consuming foods containing fiber and being physically active reduces colorectal cancer risk, while having excess body fat, alcohol, and intake of red and processed meats increase risk. The WCRF/AICR “Second Expert Report” indicated that there is convincing evidence linking specific dietary factors to colorectal cancer risk, but suggested the examination of broad patterns of diet as a way of understanding the causal relationship between diet and cancer development. The field of dietary patterns research has been growing rapidly, with five systematic reviews/meta-analyses examining the association between dietary patterns and colorectal cancer risk, published between 2010 and 2013. However, none of the studies included in these reviews examined associations between changes in dietary patterns and colorectal cancer risk.

Despite some differences in design, methods and population characteristics of the individual studies included in the different reviews, all five reviews produced remarkably consistent results on the associations between the dietary patterns identified and risk of colorectal cancer. Results from a posteriori patterns generally showed a reduced risk of colorectal cancer from consuming a plant-based pattern characterized by a high intake of fruits and vegetables, legumes, and some dairy, while an animal-based pattern characterized by high intake of red/processed meat, refined gains, and added sugars was associated with increased risk.

The consistency of results despite differences in the number, type and quantity of foods in the identified patterns between different populations could mean that specific differences in foods are not as important as consuming an overall plant-based or animal-
based dietary pattern to have a beneficial or detrimental effect respectively, on colorectal cancer risk.\textsuperscript{180} The reviews also included results from \textit{a priori} diet quality indices such as DASH, HEI, aHEI and Med Diet. Higher scores on the dietary indices showed a protective association with colorectal cancer risk. These indices share similar features such as the emphasis on whole grains, fruits and vegetables intakes and the penalization of excessive intake of animal products.\textsuperscript{177,180} These features broadly align \textit{a priori} dietary patterns with the patterns that have been identified using \textit{a posteriori} methods.

However, these findings are heterogeneous by gender and anatomic subsite of colorectal cancer. There has been more consistency in findings for colon cancer than for rectal cancer. In the review by Megalhaes et al., there were significant and similar findings for proximal and distal colon tumors but no significant association with rectal cancer.\textsuperscript{179} Miller et al., found more consistent results for \textit{a priori} patterns and colorectal cancer in men,\textsuperscript{177,183} while findings from \textit{a posteriori} patterns were less clear, with four of eight studies and five of nine studies observing significant associations in men and women, respectively.\textsuperscript{177}

Current data do not place emphasis on the analysis of changes over time in dietary patterns in relation to colorectal cancer risk, despite the idea that dietary behavior is not stable over time and dietary changes may impact colorectal cancer risk estimates differently than diet assessed at only one point in time. Findings of the association between dietary patterns and colorectal cancer risk have been consistent despite differences in the composition of \textit{a posteriori} and \textit{a priori} dietary patterns. The majority of studies have been conducted in North America or European populations including mostly Europeans or European Americans. Studies conducted in diverse and disparate
populations will be needed to determine the impact of sociodemographic factors (including gender) on the association between dietary patterns and risk of colorectal cancer.

2.4.2 Smoking and alcohol intake

Carcinogens from tobacco reach the colorectal mucosa through either the gut or the circulatory system and could damage or alter the expression of important cancer-related genes. Tobacco smoking has consistently been associated with colorectal adenomas (precursors of colorectal cancer) but not with colorectal cancer until recently. It has been suggested that the reason for this discrepancy may be a 35- to 40-year lag time between exposure and disease, which would not be captured by earlier studies and studies with shorter follow-up. Recent investigations, with more thorough measurement of smoking exposure and longer exposure periods, have reported a positive association between cigarette smoking and the risk of colorectal cancer. Paskett et al., investigated the associations between cigarette smoking and colorectal cancer in the Women’s Health Initiative, and found that active exposure to cigarette smoking appears to be a risk factor for rectal cancer but not colon cancer. Several systematic reviews and meta-analyses have been conducted with the consistent conclusion that cigarette smoking significantly increases colorectal cancer risk. There are however, inconsistencies relative to colorectal cancer subsite, with some studies showing positive results only for the rectum.

With the considerable evidence linking smoking to higher risk of colorectal cancer, it is also important to consider the impact of quitting smoking on risk attenuation. In a pooled analysis of eight studies to evaluate the association between cigarette
smoking history and colorectal cancer risk, researchers found that colorectal cancer risk remained increased for about 25 years after quitting smoking, and the pattern of decline in risk varied by colorectal cancer subsite. A study that examined lifetime smoking history and incidence of colorectal cancer in a large cohort of men followed for more than 12 years, also found that past and current smoking are associated with an increase in risk.

The International Agency for Research on Cancer (IARC) concluded that the burden of alcohol-associated cancer (including colorectal cancer) is substantial and needs to be considered when making public health recommendations on alcohol consumption, though unresolved issues relative to anatomical site (colon/rectum) remain. A meta-analysis of 27 cohort and 34 case-control studies found strong evidence (with dose-response) for an association between alcohol drinking of >1 drink/day and colorectal cancer risk. Several other meta-analyses have supported a positive association between alcohol intake and colorectal cancer risk. Acetaldehyde may be predominantly responsible for alcohol-associated carcinogenesis. Acetaldehyde is carcinogenic and mutagenic, binds to DNA and proteins, destroys folate and results in secondary hyperproliferation. Acetaldehyde is produced by tissue alcohol hydrogenases, cytochrome P 4502E1 and through bacterial oxidative metabolism in the upper and lower gastrointestinal tract.

2.4.3 Overweight/obesity and physical activity

In 2001, the IARC convened a panel of international experts to discuss the role of overweight, obesity and lack of physical activity in cancer prevention and control. The panel judged that there was sufficient evidence (causal) that excess body weight increases
the risk of cancers of the colon, breast (in postmenopausal women), endometrium, kidney, and adenocarcinoma of the esophagus.\textsuperscript{201} Regarding physical activity, the IARC panel judged also that there was sufficient evidence from human studies for a cancer-preventive effect of physical activity against cancers of the colon and breast.\textsuperscript{201} The WCRF/AICR 2010 CUP panel on colorectal cancer reviewed the most recent evidence on physical activity and colorectal cancer and concluded that there was convincing evidence that higher levels of physical activity, within the range studied, protect against colon cancer, with evidence of dose-response. The report further indicated that the effect is stronger for colon cancer; but with no evidence of an effect for rectal cancer. The effect was strong and consistent in men, but less so in women and there was plausible evidence for mechanisms of action in humans.\textsuperscript{174} Evidence from the European Prospective Investigation into Cancer and Nutrition (EPIC) on the association between adherence to the WCRF/AICR recommendations on weight management and physical activity, showed that risk reduction in participants in the fourth and fifth categories of the adherence score compared with those within the first category was 27\% for colorectal and 16\% for breast cancer.\textsuperscript{202}

One major class of mechanisms that may form a physiological and causal link between excess body weight, physical inactivity and cancer risk are alterations in the metabolism of endogenous hormones, including insulin, bioavailable sex steroids, insulin-like growth factor (IGF-I), IGF-binding proteins (IGFBPs)\textsuperscript{174,201,203} and chronic low-grade inflammation.\textsuperscript{204} Obesity increases insulin resistance and associated changes in blood values (high glucose, free fatty acids, insulin, and IGF-1). These circulating factors increase proliferation and decrease apoptosis of cancer cells, thus promoting tumor
growth. Sustained moderate physical activity raises the metabolic rate and increases maximal oxygen uptake. In the long term, regular periods of such activity increase the body’s metabolic efficiency and capacity, and so have a beneficial effect on body fatness. In addition, physical activity may protect against colon cancer by decreasing inflammation.

**2.4.4 Regular use of Non-steroidal anti-inflammatory drug (NSAID)**

Several studies have suggested a protective effect of aspirin and non-aspirin NSAIDs on colorectal cancer but some studies have failed to show a beneficial effect. The effect of aspirin on the risk of cancer among healthy women has been examined in the Women Health Study, a randomized controlled trial with an average follow-up time of 10.1 years. A dose of 100mg of aspirin was administered in the intervention group every other day against a placebo in the control group. The outcome was confirmed cancer of any site. This trial concluded without enough evidence that alternate day use of low-dose aspirin for an average 10 years of treatment lowers the risk of total cancer, breast, colorectal, or other site-specific cancers. Another randomized controlled trial examined the association between regular use of low-dose aspirin and incidence of invasive and noninvasive colorectal tumors. The aspirin arm was terminated after a mean follow-up of 5 years. The relative risk of developing colorectal cancer for aspirin compared with placebo was 1.15 (95%CI 0.80–1.65), with no significant trend for decreasing risk by year of follow-up.

In contrast, two large meta-analyses have demonstrated a beneficial effect of aspirin and non-aspirin NSAIDs on colorectal cancer development. In 2007, the US Preventive Services Task Force (USPSTF) commissioned the two meta-analyses: the
one examined the benefits and harms of non-aspirin NSAIDs and cyclooxygenase (COX-2) inhibitors for the prevention of colorectal cancer and adenoma. Colorectal cancer incidence was lower with non-aspirin NSAIDs in cohort studies (RR; 0.61, 95%CI; 0.48, 0.77) and case-control studies (RR; 0.70, 95%CI; 0.63, 0.78). Risk of colorectal adenoma was also reduced with non-aspirin NSAIDs use in cohort studies (RR; 0.64, 95%CI, 0.48, 0.85) and case-control studies (RR; 0.54, 95%CI, 0.40, 0.74) and by COX-2 inhibitors in randomized, controlled trials (RR; 0.72, 95%CI, 0.68 to 0.77). The other meta-analysis examined the benefits and harms of employing aspirin for the chemoprevention of colorectal cancer. In this study, regular use of aspirin reduced the incidence of colonic adenomas in randomized clinical trials (RR; 0.82, 95%CI, 0.70 to 0.95), case-control studies (RR; 0.87, 95%CI, 0.77 to 0.98), and cohort studies (RR; 0.72, 95%CI, 0.61 to 0.85). In cohort studies, regular use of aspirin was associated with reduced risk of 22% for colorectal cancer.

Despite this evidence showing that aspirin and non-aspirin NSAIDs appear to be effective at reducing the incidence of colonic adenoma and colorectal cancer, especially if used in high doses for a prolonged period of time, the USPSTF currently recommends against the use of aspirin and non-aspirin NSAIDs for the prevention of colorectal cancer in individuals at average risk for the disease (D recommendation). This is likely due to adverse side effects such as cardiovascular events and gastrointestinal harms. This recommendation may likely change as more evidence accumulates.

2.5 Risk factors for breast cancer

Breast cancer is the most commonly diagnosed cancer in women, and is the second leading cause of cancer-related deaths in American women after lung cancer.
Breast cancer in women accounts for about a third of all cancer cases and about 15% of all cancer deaths among women in the United States. Risk factors include diet, physical activity, body size, reproductive and hormonal factors, among many other factors. The role of diet in breast cancer risk is of great interest as a potentially modifiable risk factor.

2.5.1 Dietary patterns

Most of the established risk factors for breast cancer such as family history, lactation, and reproductive history are generally not modifiable. Epidemiological studies have shown that the risk of breast cancer varies with diet - a potentially modifiable factor – though the evidence is inconsistent. Dietary patterns contain a complex mix of foods, nutrients and other compounds that could influence breast cancer risk in ways not detected by studies of individual foods and nutrients. Evidence shows a positive association between the Western-style dietary pattern (rich in added sugar, refined grains, red and processed meats, and fried foods) and increased risk. Studies have also observed a decreased risk with the prudent-type dietary pattern (rich in fruits, vegetables, whole-grains, legumes, nuts, olive oil and fish), but other studies have not found significant associations with any of these dietary patterns identified by a posteriori methods except in subgroup analyses in some studies. In the Black Women’s Health Study, the prudent pattern was weakly associated with lower risk overall, but was significantly associated with lower risk in normal weight women and in women with estrogen receptor negative breast cancer. A meta-analysis of 15 prospective studies found that high intake of fruits, and fruits and vegetables combined, is associated with a weak reduction in risk of breast cancer with no dose-response. Similarly, in a pooled analysis of 8 large prospective studies, only weak and non-
significant associations were observed with increasing consumption of fruit and vegetables. Other dietary patterns investigated are the vegetarian diet. One study found no significant association between a vegetarian versus non-vegetarian diet and breast cancer risk, while there was a non-significant inverse association between vegetarianism and risk of pre- or postmenopausal breast cancer in the EPIC-Oxford cohort, United Kingdom.

Regarding a priori dietary patterns, an index derived from 23 recommended food items was not associated with breast cancer risk in one study. Similarly, in the Nurse’s Health Study, an investigation of the association of several dietary indices and postmenopausal breast cancer found no significant association with any of the indices, except when stratifying by hormone receptor status of the cancer. Women who scored high in some of the indices had a lower risk of estrogen receptor negative breast cancer.

Studies of the association between dietary patterns and breast cancer risk have been conducted in populations other than North Americans and Europeans. Some reports indicate that dietary patterns rich in vegetables and seafood are associated with a decreased breast cancer risk in Korean women, and Chinese women. Findings from one study suggest that a diet characterized by low intake of meat/starches and high intake of legumes is associated with a reduced risk of breast cancer in Asian Americans, while another study found evidence of an inverse association between a healthy dietary pattern and breast cancer risk among Iranian women. A Japanese study found that the prudent dietary pattern is negatively associated with breast cancer risk, while the high fat and Japanese patterns may increase breast cancer risk among obese Japanese
women.\textsuperscript{214} It is also important to note that none of the published studies examined the association of changes in dietary patterns and risk of breast cancer, though changes in dietary patterns may impact risk differently than dietary patterns assessed at only one point in time.

2.5.2 Overweight/obesity and physical activity

Obesity is a state of chronic systemic low-grade inflammation.\textsuperscript{229} Adipose tissue is now known to secrete a growing number of inflammatory mediators (adipokines) including CRP. The secretion of these inflammatory mediators is increased in obesity,\textsuperscript{230} and they regulate physiological and pathological processes, including immunity and inflammation.\textsuperscript{229} There is increasing epidemiologic evidence of an association between BMI and energy expenditure and the risk of breast cancer. Women who are overweight or obese, especially women who gain weight throughout adulthood, are at an increased risk for developing breast cancer after menopause.\textsuperscript{231-233} Conversely, overweight women are at reduced risk for developing breast cancer in the premenopausal years.\textsuperscript{234} A pooled analysis of cohort studies showed that BMI has significant inverse and positive associations with breast cancer among pre- and postmenopausal women, respectively. Compared with premenopausal women with a BMI of less than 21 kg/m\textsuperscript{2}, women with a BMI exceeding 31 kg/m\textsuperscript{2} had a relative risk of 0.54 (95\%CI; 0.34, 0.85). In postmenopausal women, the relative risk for these women was 1.26 (95\%CI; 1.09, 1.46).\textsuperscript{235} A meta-analysis to assess the strength of associations between BMI and different sites of cancer estimated that each 5 kg/m\textsuperscript{2} increase in BMI was associated with a 12\% increased risk of postmenopausal breast cancer (RR; 1.12; 95\%CI, 1.08, 1.16).\textsuperscript{236} In postmenopausal women, the association has been shown to be modified by hormone
replacement therapy; users are at higher risk compared to non-users, and by estrogen receptor/progesterone receptor (ER/PR) status; with women having ER+/PR+ tumors being at higher risk compared to ER-/PR- tumors.\textsuperscript{231,233,237} A meta-analysis of 9 cohorts and 22 case-control studies, further confirmed that the association between BMI and breast cancer risk is dependent on menopausal status and ER/PR status.\textsuperscript{237}

Studies have shown that physical activity increases concentrations of a number of cytokines with anti-inflammatory effects such as IL-1ra (interleukin-1 receptor antagonist) and IL-10 and inhibits the production of the pro-inflammatory cytokine TNF\textalpha.\textsuperscript{238} Physical activity also has been shown to be associated with reduce concentrations of some pro-inflammatory cytokines such as CRP and other biomarkers of inflammation.\textsuperscript{239-242} These results consistently show that physical activity reduces chronic inflammation – a crucial process in cancer development.

Physical activity has been shown to reduce breast cancer risk. A study investigating the relation between recreational physical activity (RPA) and breast cancer risk, found that RPA at any intensity level during the reproductive and postmenopausal years was associated with reduced breast cancer risk and that substantial postmenopausal weight gain may eliminate the benefits of RPA.\textsuperscript{243} Physical activity also was found to be associated with reduced breast cancer risk in the WHI, with longer duration providing the most benefit.\textsuperscript{244} The association between physical activity and postmenopausal breast cancer risk has been confirmed consistently enough that the US Department of Health and Human Services Physical Activity Guidelines Advisory Committee concluded in 2008 that “\textit{strong evidence demonstrates that, compared with less active persons, more active women have lower rates of breast cancer.”}\textsuperscript{245}
2.5.3 Hormone replacement therapy and oral contraceptive use

Evidence shows that oral contraceptive use increases a young woman's risk of breast cancer. A multisite case-control study analyzed data on women younger than 45 years of age (to maximize opportunities for extended exposure) who used oral contraceptives throughout their entire reproductive years. In this population of younger women, use of oral contraceptives for 6 months or longer was associated with an increased risk for breast cancer of 30% (OR, 1.3; 95% CI, 1.1, 1.5). There was also a significant dose-response relationship. To investigate the possibility that chance or bias, including selective screening of contraceptive users, contributed to the putative association, an evaluation of screening histories and methods of diagnosis failed to support the speculation that associations could be due to selective screening. Among women 45 years of age and older, no associations of risk with use of oral contraceptives were noted.

Increased risk of breast cancer with combined use of estrogen and progesterone has been reported in some studies. In the study by Schaier et al., the risk was greater for lean women, but there was no evidence of increased risk in heavier women, which is similar to the finding in the collaborative reanalysis. This effect modification by BMI is contrary to the study results that endogenous estrogen increases risk of breast cancer, given that overweight and obese women have relatively higher endogenous estrogen levels than lean women due to non-ovarian synthesis of estrone as a result of the peripheral conversion of androgens. Another study reported that obese postmenopausal women had a greater increase in circulating free estradiol in response to oral estrogen compared with normal weight women.
The association between exogenous estrogen use and breast cancer risk lacked clinical trial support until 1993 when the WHI began two randomized placebo-controlled trials that separately evaluated estrogen plus progestin (in women with an intact uterus) as well as estrogen alone (in women with a previous hysterectomy)\textsuperscript{251} (NB: In the absence of a uterus, estrogen treatment is the only way to relieve a woman of hot flashes or other menopausal symptoms, and in the estrogen-alone trial, there would be confounding by endogenous estrogen if the uterus is present). After a mean follow-up of 5.3 years there was a slightly increased risk of breast cancer, HR, 1.26; 95\%CI, 1.00, 1.59.\textsuperscript{251} However, women reporting prior use of estrogen plus progesterone experienced higher risk for breast cancer associated with estrogen plus progesterone use than those who never used postmenopausal hormones. Longer duration of prior use of estrogen plus progesterone appeared to have a cumulative effect of estrogen plus progestin on risk of incident breast cancer and these effects were not found to be modified by age, race/ethnicity, family history, parity, age at first birth or BMI.\textsuperscript{251}

In contrast to the substantial evidence linking exogenous hormone use (combined estrogen plus progestin) with increased breast cancer risk, the parallel WHI estrogen-alone trial showed an unanticipated potential reduction in breast cancer risk (HR, 0.77; 95\%CI, 0.59, 1.01) in the estrogen-alone group compared to the placebo group after 7.1 years of follow-up.\textsuperscript{252} Differences in breast cancer screening between the intervention and placebo groups did not explain the observed effects.\textsuperscript{40} The suggestion of a reduced risk for breast cancer motivated Stefanick et al to conduct a detailed analysis of the WHI estrogen-alone trial data focusing only on breast cancer outcome. Their main analysis results provided no evidence that the use of estrogen-alone increased risk of breast
cancer (HR, 0.80; 95%CI, 0.62, 1.04). However, in adherence-adjusted analyses that censored follow-up 6 months after a woman became nonadherent, a larger and significant reduction in the incidence of invasive breast cancer was observed in the estrogen-alone group compared with the placebo group (HR, 0.67; 95%CI, 0.47-0.97) and the risk did not differ by estrogen or progesterone receptor status of the cancer.

No significant interaction of exposure to exogenous hormone and BMI on breast cancer risk was observed in either the estrogen-alone trial or in the combined estrogen plus progestin trial. The differences in the results of the two WHI trials strongly suggest a role for progestin in increasing breast cancer risk. The biological mechanisms underlying an effect of exogenous hormones on the breast are complex. One hypothesis is that progesterone does not down-regulate estrogen and progesterone receptors in the breast may contribute to its adverse effects. It is paradoxical however, that the addition of exogenous estrogen by use of conjugated equine estrogen in the WHI trial and the reduction of endogenous estrogen by use of aromatase inhibitors (exemestane) in the MAP.3 trial both reduced risk of breast cancer incidence. The conceptual model that breast cancer growth may be stimulated or inhibited solely by the respective addition or withdrawal of estrogen thus falls apart.

In summary, the long-term effect of estrogen use on the risk of breast cancer is still an open question. Women exposed to exogenous hormones (especially combined estrogen and progestin) are at increased risk for breast cancer. The risk increases with duration of use, but also reduces after cessation of use of exogenous hormones and has largely, if not wholly disappeared after 2 to 5 years post-cessation. The increase in risk among older women exposed to exogenous hormones suggests that the trade-offs
between risks and benefits should be carefully assessed. In this assessment, it is important to consider the type of hormone as well as individual characteristics of the woman, such as BMI.

### 2.5.4 Demographic factors

Many demographic factors influence the incidence and survival rates from breast cancer. The disease is more common in older women, among women in upper rather than lower social classes, among women who never have been married, among women living in urban areas, and among European Americans than African Americans, at least among those over age 50.\(^{256}\)

The strongest risk factor for breast cancer is age. A woman’s risk of developing the disease increases as she gets older. That is because with more years of life, there are more opportunities for genetic damage (mutations) in the body, and as we age, our bodies are less capable of repairing genetic damage. According to Surveillance, Epidemiology, and End Results (SEER) program statistics from 2005-2009, the median age at diagnosis for cancer of the breast was 61 years of age. Approximately 0% were diagnosed under age 20; 2% between 20 and 34; 10% between 35 and 44; 22% between 45 and 54; 25% between 55 and 64; 20% between 65 and 74; 15% between 75 and 84; and 6% 85+ years of age.\(^{257}\)

European American women are slightly more likely to develop breast cancer (age-adjusted incidence rate: 127.3 per 100,000 women) than African American (121.2 per 100,000 women), Hispanic, and Asian women. But African American women are more likely to develop more aggressive, more advanced-stage breast cancer that is
diagnosed at younger ages. African American women are also more likely to die from breast cancer. Based on the SEER data for patients who died in 2005-2009 in the US, the breast cancer mortality rate for African American women is 31.6 per 100,000 women compared with 22.4 per 100,000 women for European American women. Some of these differences in outcomes may be due to tumor biology (e.g., higher prevalence of triple negative tumors in African Americans). Compared to European American women, women of African ancestry tend to have more aggressive breast cancers that present more frequently as estrogen receptor-negative (ER-) tumors. Triple negative comprise approximately 15% of breast cancers and have been associated with high-grade histology, aggressive clinical behavior, and poor survival. Other possible explanations for racial disparities in aggressiveness of disease include less access to mammography screening and lower quality medical care, as well as various lifestyle patterns (eating habits and weight issues for example) that are more common in some ethnic groups than in others. In a study to evaluate differences in the stage and biology of breast cancer between African American and European American women who had a screening mammogram, Grabler et al., found that African American women in the regularly screened population were less likely than irregularly screened African American women to have ER- breast cancers (26% vs. 36%, p<0.05), PR- breast cancers (35% vs. 46%, p<0.05), and poorly differentiated breast cancers (39% vs.53%, p<0.05). European American women in the irregularly screened population also had worse prognostic factors than European American women in the regularly screened population, though these were not statistically significant. Regular screening for breast cancer may thus contribute to the narrowing of racial disparities in breast cancer risk.
CHAPTER 3

METHODS

3.1 Statement of research aims and hypotheses

The overall aim of this study was to characterize longitudinal trends in the inflammatory potential of the diet and then evaluate the association of longitudinal changes in the inflammatory potential of diet and risk of colorectal cancer and breast cancer in the WHI. Our overall hypothesis is that long term changes in dietary behavior towards increased consumption of pro-inflammatory diets increases the risk of cancer over time.

In specific aim #1, we investigated the stability of the inflammatory potential of diet over time using the dietary inflammatory index (DII). In this aim we hypothesized that the inflammatory potential of diet significantly changes over time and is influenced by social, demographic and clinical factors. In specific aim #2, we examined the association between the inflammatory potential of diet and risk of colorectal cancer with the hypothesis that a sustained high level of dietary inflammatory potential over time, increases risk of colorectal cancer. In specific aim #3, we examined the association between the inflammatory potential of diet and breast cancer in postmenopausal women,
with the hypothesis that a sustained high level of dietary inflammatory potential over
time, increases risk of breast cancer in postmenopausal women. Specific research
questions have been restated in section 3.6, before the description of the statistical
methods for each of the three aims.

3.2 Description of the study population

The WHI study is a large and complex clinical investigation of strategies for the
prevention and control of some of the most common causes of morbidity and mortality
among postmenopausal women. The design of the WHI has been described in detail
States, and enrolled a total of 161,808 women between 1993 and 1998 and included full-
scale randomized controlled trials, with an average of 11.3 years of follow-up until
September 30, 2010. The WHI enrolled 93,676 women into the OS and 68,132
participants into clinical trials (CT). The WHI CT included: the DMT component,
n=48,835, the Hormone Therapy component (HT, estrogen-alone or estrogen plus
progestin, n=27,347). Participants enrolled in at least one of the clinical trial components
were screened for eligibility and invited to join the calcium and vitamin D component
(CaD, n=36,282) at their first or second annual clinic visits. For the DMT, women were
randomly assigned to a usual-diet comparison group (n = 29,294) or an intervention
group with a 20% low-fat dietary pattern with increased vegetables, fruits, and grains (n =
19,541). At baseline, the mean age was 63 years and about 18% of the women were from
ethnic minority groups including: 9.1% African-Americans (n=14,618), 4% Hispanics
(n=6,484), and 2.6% Asians (n=4,190). Women who proved to be ineligible for, or who
were unwilling to enroll in the CT components were invited to be part of the prospective cohort of women in the OS.  

Exclusion criteria for both the OS and CT included any medical condition associated with a predicted survival of less than three years, alcoholism, other drug dependency, mental illness (e.g., major depressive disorder), dementia, active participation in another intervention trial and not likely to live in the area for at least 3 years. Demographic information and dietary data were obtained by self-report using standardized questionnaires. Certified staff performed physical measurements, including blood pressure, height and weight, and blood samples at the baseline clinic visit. Women were further excluded from the DM if their diets were assessed to have <32% energy from fat. The WHI protocol was approved by the institutional review boards at the Clinical Coordinating Center at the Fred Hutchinson Cancer Research Center (Seattle, WA) and at each of the 40 Clinical Centers. 

3.3 Diet assessment

During baseline screening for the WHI, all participants completed a standardized food frequency questionnaire (FFQ) developed for the WHI to estimate average daily nutrient intake over the previous three-month period. At follow-up, the FFQ was completed at year 3 for all the observational study participants and for all participants in the DMT at year 1, and a random third of DMT participants from year 2 onwards. The three sections of the WHI FFQ included 19 adjustment questions related to type of fat intake, 122 composite and single food line items asking about frequency of consumption and portion size, and four summary questions that asked about the usual intake of fruits and vegetables and added fats for comparison to information gathered from the line
items. The nutrient database, linked to the University of Minnesota Nutrient Data System for research (NDSR), is based on the US Department of Agriculture Standard Reference Releases and manufacturer information. This FFQ has demonstrated good comparability to 24-hour dietary recall interviews and food records in the WHI. For all three study aims, we used all the FFQs in the OS and DMT and calculated the DII at eleven different time points.

3.4 Outcomes assessment

For aim #1, changes in the DII over time, calculated at 11 time points constituted our outcome of interest. A detailed description of the DII is provided in section 3.5. In aims #2 and #3, where colorectal cancer and breast cancer were the outcomes of interest, the DII was the main exposure of interest.

The WHI outcomes ascertainment and adjudication methods have been previously described. Briefly, physicians in the Clinical Centers, the Clinical Coordinating Center, and the National Institutes of Health (NIH) classified WHI outcomes. In the first stage, the local Clinical Center physician adjudicator reviewed the documents and assigned a diagnosis. All locally adjudicated primary and safety endpoint events of each trial component were then centrally reviewed. A fraction of locally adjudicated secondary endpoints were also referred for central adjudication for quality control purposes. The primary results for each clinical trial component were based on data derived from central adjudication. To minimize potential bias in the ascertainment and classification of outcomes, WHI required that local and central physician adjudicators not be exposed to any information that could result in potential unblinding, including participant contact or other aspects of the research record.
Invasive breast and colorectal cancers were documented and coded according to primary site, anatomic subsite, diagnosis date, extent of disease (stage, tumor size, and laterality), tumor morphology (behavior, grade, histology) her2neu status and estrogen and progesterone receptors status (breast cancer only). We have chosen to focus on breast and colorectal cancers in this dissertation because these cancers have been associated with diet and inflammation in previous studies and because these were primary or secondary endpoints in WHI allowing for adequate numbers of cases for analyses. Incident invasive and in situ (ductal and lobular carcinoma in situ) breast cancers, including second primaries, were ascertained and adjudicated. Incident invasive and in situ colon and rectal cancers were determined. Recurrent cancers were not included. All cancer related hospitalizations, surgeries, procedures, diagnostics or treatments for each first self-report of a malignant tumor were investigated. For the full coding of the cancer, pathology reports from diagnostic aspirations, biopsies, and surgeries, plus the discharge summary, were used.

Since the diagnosis of some early cancers and cancer precursors is dependent on whether or not screening has occurred, there was potential for over-reporting of diagnoses in some arms of the study, particularly the unblinded intervention arm of the DMT component. For this reason and for safety purposes in the HT component, all clinical trial participants had regular screening mammograms as part of study protocol. Screening for colorectal cancer was not done in WHI. At each follow-up contact (semi-annually in the clinical trial, and annually in the observational study), however, information on screening procedures for colorectal cancer was collected, including: fecal occult blood testing, flexible sigmoidoscopy, and colonoscopy.
3.5 Description of the dietary inflammatory index (DII)

The development\textsuperscript{38} and validation\textsuperscript{39} of the DII has been described elsewhere. The goal in developing the DII was to create a score for specific foods and dietary constituents thought to positively or negatively affect levels of inflammation. All research articles through the year 2010 that were identified as assessing the role of one or more of 45 different foods and dietary constituents on specific inflammatory markers were used to create the scores. Due to the large number of articles on inflammation, the literature search was limited to six well-established inflammatory markers: CRP, IL-1β, IL-4, IL-6, IL-10, and TNFα out of which CRP, IL-1β, IL-6 and TNF-α are considered pro-inflammatory biomarkers and IL-4 and IL-10 are considered anti-inflammatory cytokines. A total of 1,943 research articles were reviewed and scored in the creation of the DII.

One of three possible values was assigned to each article based on the effect of the food parameter on inflammation: “+1” was assigned if the effects were pro-inflammatory (significantly increased IL-1B, IL-6, TNFα, or CRP or decreased IL-4 or IL-10); “-1” if the effects were anti-inflammatory (significantly decreased IL-1B, IL-6, TNFα, or CRP or increased IL-4 or IL-10) and “0” if the food parameter was not significantly associated with the inflammatory marker. Full details of the scoring algorithm are described in this reference\textsuperscript{38}.

Articles were first weighted by study design, with clinical trials in humans receiving the greatest weight (i.e., 10 of possible 10) to cell culture experimental studies receiving the lowest weight (i.e., 3 of possible 10). Using these weighted values, the pro- and anti-inflammatory fractions for each food parameter were calculated. The food
parameter-specific overall inflammatory effect score was then calculated by: 1) dividing the weighted pro- and anti-inflammatory articles by total weighted number of articles and 2) subtracting the anti-inflammatory fraction from the pro-inflammatory fraction (Figure 3.1). A cut point of 236, the median of the total weighted number of articles across all the food parameters, was chosen to indicate an optimally robust pool of literature. All food parameters with a weighted number of articles ≥236 were assigned the full value of the score. Foods and constituents with a weighted number of articles <236 were adjusted as follows: 1) number of weighted articles was divided by 236; 2) the fraction was then multiplied by the food parameter-specific raw inflammatory effect score, which resulted in the food parameter-specific overall inflammatory effect score.

To avoid the arbitrariness resulting from simply using raw intake amounts (resulting in different units of measurement for various nutrients having large influences on the overall score), the DII was standardized to a representative range of dietary intake based on actual human consumption. This was accomplished by constructing a composite database representing a wide range of diets across diverse populations living in a variety of countries in different regions of the world. Authors of articles reporting on data from nutrition surveys were contacted to request access to complete datasets. A total of 11 such datasets were identified and used in developing the composite database.

Calculation of the DII in a given study is based on dietary intake data that are then linked to the global mean intake database derived from the 11 datasets. An individual’s diet is then expressed relative to the standard global mean as a z-score. This is achieved by subtracting the standard global mean from the amount reported by the individual and dividing this value by its standard deviation. To minimize the effect of “right skewing,”
this value is converted to a percentile score. To achieve a symmetrical distribution with values centered on 0 (null) and bounded between -1 (maximally anti-inflammatory) and 1 (maximally pro-inflammatory) each percentile score is doubled and then 1 is subtracted. The centered-percentile value for each food parameter is then multiplied by its respective food parameter-specific inflammatory effect score to obtain a food parameter-specific DII score. Finally, all of the food parameter-specific DII scores are summed to create the overall DII score for an individual. More positive scores represent a more pro-inflammatory diet, whereas more negative scores represent a more anti-inflammatory diet.

3.6 Statistical analysis

3.6.1 Statistical methods applicable to all three aims

Confounding in all the Cox proportional hazards (PH) regression models was assessed using the following three questions as suggested by Szklo and Nieto: 1) is the confounder related to both exposure (DII) and outcome (colorectal cancer or breast cancer)? 2) Does the exposure-outcome association seen in the age-adjusted crude model have the same magnitude and similar direction as the associations observed within strata of the potential confounder? 3) Does the exposure-outcome association seen in the crude model have the same magnitude and similar direction as that observed association in the model adjusted for the potential confounder? Confounding was not assessed in aim #1 as there was no specific exposure of interest in the prediction model for DII change.

Effect modification took precedence over confounding; that is, if a variable was assessed to be both a confounder and effect modifier, it was treated as an effect modifier.
We inserted “interaction terms” of main exposure and effect modifier in the models and considered significant effect modification at $P \leq 0.05$.

Statistical modeling consisted of variable selection and model selection. For the DII prediction model in aim #1, the automated stepwise approach was used to identify significant predictors of DII change. In the Cox PH models in aims #2 and #3, all variables assessed to be confounders or significant effect modifiers were retained in the models. Model selection was considered in specific situations to either include or exclude a covariate from the model and improve the model’s overall precision. The log-likelihood ratio test was used for model selection.

Participant characteristics were summarized using frequencies (percentage) for categorical variables and means (standard deviation) for continuous variables. All $p$-values were 2-sided, and $P < 0.05$ for aim #1 and 95% confidence intervals not including 1 for aims #2 and #3, were considered to indicate statistical significance. All statistical analyses were conducted using Statistical Analysis Systems software, version 9.3 (SAS, Inc., Cary, NC).

3.6.2 Statistical methods for specific aims #1

The statistical methods for this aim were designed to answer the following four research questions: 1) Are there changes in dietary inflammatory potential over time? 2) If there are significant changes, how do demographic and lifestyle factors impact these changes? 3) What social, demographic and clinical factors significantly predict changes in DII in an observational setting? and 4) How does the change in the inflammatory potential of diet in an intervention setting differs from that in an observational setting?
We computed mean DII scores at baseline and Year 3 in the OS and at 11 different time points between baseline and Year 10 inclusive in the DMT; and used these to describe changes over time in the OS, or plotted DII scores on graphs for a visual appraisal of the longitudinal trend, separately for the intervention and control arms of the DMT. Analyses were stratified by BMI, race/ethnicity and educational level. To determine significant differences between mean DII scores calculated at different time points, we constructed marginal linear regression models using generalized estimating equations (GEE) that adjusted for the within-subject correlation in the DII measurements, to calculate and compare all pair-wise contrast estimates between mean DII scores. The GEE model was a univariate model with time from baseline as the only independent variable and changes in the DII over time as the dependent variable, adjusted for multiple comparisons using the Bonferroni approach, and stratified in the DMT by intervention arm.

Next, we utilized stepwise linear regression to construct the most parsimonious predictive multivariable model for change in DII from baseline to Year 3 in the OS. A previous WHI study investigated predictors of dietary change and maintenance in the DMT and included intrapersonal, interpersonal, intervention characteristics and clinical center characteristics as predictors. The DMT intervention moved participants toward an anti-inflammatory diet; therefore, predictors of dietary change investigated by Tinker et al are likely to predict DII change in the DMT. We therefore focused mainly on the potential predictors of DII change in the OS. We included the following baseline variables in the stepwise regression model: baseline DII, age group, BMI (kg/m²), race/ethnicity, education, smoking status, physical activity, history of diabetes,
hypertension, arthritis, cancer, use and duration of estrogen-alone and of combined
estrogen and progesterone, use of statins, anti-depressants, and non-steroidal anti-
inflammatory drugs (NSAID) (see section 4.3 for the categories of these variables). The
entry criterion into the stepwise linear regression model was $P <0.1$, while the exit
criterion was $P >0.1$. The stepwise model identified variables that were included in a
multivariable linear regression model to calculate beta ($\beta$) coefficients, corresponding p-
values and the $R^2$ for the overall predictive model. Participants with implausible reported
energy ($<600\text{kcal/d}$ or $>5000\text{kcal/d}$), extreme BMI values ($<15\text{kg/m}^2$ or $>50\text{kg/m}^2$),
single FFQs or missing FFQs, as well as those with missing data in the predictors in the
final model were excluded from this analysis.

3.6.3 Statistical methods for specific aims #2 and #3

Statistical methods for these two aims were similar and designed to provide
answers to the following three main questions: 1) How does long-term cumulative history
of dietary inflammatory potential impact risk of colorectal cancer and risk of breast
cancer? 2) How do shorter-term changes in patterns of the inflammatory potential of diet
over time impact risk of colorectal cancer and risk of breast cancer? 3) Do risk estimates
differ by anatomic subsite (colon, rectum) of colorectal cancer and by molecular or
histologic subtype of breast cancer?

We used data from 142,511 women participating in the WHI OS and DMT.
Women with colorectal cancer or breast cancer at baseline or missing colorectal cancer or
breast status at baseline, or those who reported breast removal at baseline were excluded,
as well as women with implausible reported total energy intake values ($\leq 600 \text{kcal/day}$ or
$\geq 5000 \text{kcal/day}$) or extreme BMI values ($<15\text{kg/m}^2$ or $>50\text{kg/m}^2$).
To determine how cumulative history of dietary inflammatory potential affects risk of colorectal cancer and breast cancer, we calculated ten cumulative averages of DII incrementally starting from the average between baseline and year one DII. The cumulative average was then categorized into quintiles, and used to estimate hazards ratios for colorectal cancer or breast cancer in multivariable-adjusted Cox proportional hazards (PH) regression models, while excluding from the models colorectal cancer cases or breast cancer cases diagnosed prior to year one. This approach was repeated for the average DII of baseline, year one, and year two with cancer cases diagnosed prior to year two excluded to avoid the possibility of change in diet due to subclinical disease, and to include only participants at risk of developing cancer going forward. This approach was repeated until DII estimates at all time points were used.

For each time segment, Cox proportional hazards regression models were used to estimate hazards ratios (HR) and 95% confidence intervals (95%CI) for colorectal, colon, rectal cancer, and invasive breast cancer incidence, by quintiles of cumulative average DII, with adjustment for multiple covariates.

To determine how changes in patterns of the inflammatory potential of diet over time affect risk of colorectal cancer or breast cancer, we calculated the DII from baseline and year 3 food frequency questionnaires (FFQ) in the OS and DMT in participants with at least two FFQs at these two time points. Since diet data was assessed in the OS at baseline and Year 3 only, we selected these two time points to maximize the number of participants with at least two FFQs. We categorized the DII at both time points into quintiles (Q) and further categorized changes in the inflammatory potential of diet based on quintile differences between baseline and year 3, as follows: 1) anti-inflammatory
stable: Q1 or Q2 at both time points or change from Q3 to Q2; 2) anti-inflammatory change: changes ≤ -2Q; 3) neutral inflammation stable: changes from Q2 to Q3, Q4 to Q3 or stable at Q3 at both time points; 4) pro-inflammatory change: changes ≥ 2Q; 5) pro-inflammatory stable: Q4 or Q5 at both time points or change from Q3 to Q4. Cox proportional hazards regression models were used to estimate hazard ratios (HR) and 95% confidence intervals (95%CI) for colorectal, colon (proximal and distal), and rectal cancer incidence, by patterns of changes in DII, with adjustment for multiple covariates. Similar models were constructed for invasive breast cancer. We used AIC to determine the model with the best precision. Each covariate in the final model was tested for the proportional hazards assumption using cumulative sums of Martingale-based residuals.

All multivariable-adjusted models included the following covariates: age, race/ethnicity, educational level, smoking status, diabetes, hypertension, arthritis, NSAIDs use, category and duration of estrogen use, category and duration of estrogen & progesterone use, DMT arm, BMI, and physical activity (minutes/week) as potential confounders. Effect modification in models for both the cumulative average DII and changes in the DII and cancer incidence was investigated by included 2-way cross product terms in the models. Potential effect modifiers included age group, BMI, educational level, race/ethnicity, combined use of estrogen and progesterone. We conducted a power analyses to determine ranges of estimated HR to be obtained in the analytic models given the incidence proportions (event rate) for colorectal and breast cancers in this study, using the PASS software program (NCSS, LLC, Kaysville, Utah) (Table 3.1).
3.7 Tables and figures

1) 1943 articles on 45 food parameters identified and scored

2) Weight articles by study design and calculate pro- and anti-inflammation fractions

3) Adjust scores if total weighted articles is <236

4) Inflammatory effect score calculated from 2) and 3)

5) World composite database for 45 food parameters based on data from 11 countries
   - Calculate world mean and standard deviation for each of the 45 food parameters

6) Based on available dietary intake data calculate z-scores and centered percentiles for each of the food parameters for each individual in the study, based on the world average and standard deviation.

7) Multiply centered percentile by the inflammatory effect score to obtain "component-specific DII score"

8) Sum all of the "component specific DII score"

Figure 3.1: Sequence of steps in creating the DII. (adapted from Shivappa, N., Steck SE, Hurley TG, Hussey JR, Hebert JR, Designing and Developing a Literature-derived, Population-based Dietary Inflammatory Index. Public Health Nutr, 2013: p. 1-8.)
Table 3.1. Estimated ranges of hazard ratios for colorectal and breast cancers based on the available incidence proportions for the two cancer sites

<table>
<thead>
<tr>
<th>Cancer Site</th>
<th>Estimated range of hazard ratios (HR) based on available cancer cases</th>
<th>Cancer incidence proportion (event rate)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Models for cumulative average DII (baseline to Year 2)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorectal</td>
<td>1.06 – 1.19</td>
<td>0.0132</td>
</tr>
<tr>
<td>Colon</td>
<td>1.06 – 1.21</td>
<td>0.0107</td>
</tr>
<tr>
<td>Rectal</td>
<td>1.13 – 1.47</td>
<td>0.0026</td>
</tr>
<tr>
<td>Invasive breast cancer</td>
<td>1.03 – 1.10</td>
<td>0.0455</td>
</tr>
<tr>
<td><strong>Models for Patterns of change in DII quintiles between baseline and Year 3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorectal</td>
<td>1.04 – 1.12</td>
<td>0.0135</td>
</tr>
<tr>
<td>Colon</td>
<td>1.04 – 1.14</td>
<td>0.0113</td>
</tr>
<tr>
<td>Rectal</td>
<td>1.07 – 1.32</td>
<td>0.0024</td>
</tr>
<tr>
<td>Invasive breast cancer</td>
<td>1.02 – 1.06</td>
<td>0.0518</td>
</tr>
<tr>
<td>Triple negative BRCA</td>
<td>1.08 – 1.26</td>
<td>0.0038</td>
</tr>
<tr>
<td>HER2+/ER- subtype</td>
<td>1.12 – 1.43</td>
<td>0.0016</td>
</tr>
<tr>
<td>Luminal A BRCA</td>
<td>1.03 – 1.09</td>
<td>0.0270</td>
</tr>
<tr>
<td>Luminal B BRCA</td>
<td>1.07 – 1.25</td>
<td>0.0042</td>
</tr>
<tr>
<td>Ductal carcinoma</td>
<td>1.02 – 1.08</td>
<td>0.0338</td>
</tr>
<tr>
<td>Lobular carcinoma</td>
<td>1.06 – 1.21</td>
<td>0.0054</td>
</tr>
<tr>
<td>Mixed ductal/lobular carcinoma</td>
<td>1.05 – 1.18</td>
<td>0.0074</td>
</tr>
</tbody>
</table>

NB: power=80%, tests=2-sided, alpha=0.05, standard deviation of DII =2.30, $R^2$ varied from 0.1 to 0.9 by 0.1, and the event rate was the incidence proportion of each cancer type. (BRCA=breast cancer)
CHAPTER 4

LONGITUDINAL CHANGES IN THE DIETARY INFLAMMATORY INDEX: AN ASSESSMENT OF THE INFLAMMATORY POTENTIAL OF DIET OVER TIME IN POSTMENOPAUSAL WOMEN

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4.1 Abstract

Introduction: The dietary inflammatory index (DII) measured at one point in time has been associated with cancer risk in previous studies and repeat measures have been analyzed in relation to inflammatory biomarkers. However, data are lacking regarding the change in DII over longer periods of time. We assessed changes in the DII among women in the Women’s Health Initiative (WHI). Methods: The DII was calculated using data from repeated food frequency questionnaires in the WHI Observational Study (OS; n=76,671) at baseline and Year 3, and in the Dietary Modification Trial (DMT; n=48,482) at 11 time points. Univariate generalized estimating equations were used to compare mean DII changes over time, adjusting for multiple comparisons. Multivariable linear regression models were used to determine predictors of DII change. Results: In the OS, mean DII decreased from -1.14 at baseline to -1.50 at Year 3. In the DMT, DII decreased from -0.40 to -1.70 in the intervention arm and from -0.38 to -1.04 in the control arm from baseline to Year 3. These changes were influenced by BMI, education, and race/ethnicity. A prediction model explained ≈22% of the variance in the change in DII scores in the OS. Conclusion: In this population of postmenopausal women, dietary inflammatory potential was relatively stable in OS participants, but decreased significantly over time in women enrolled in the DMT. DII changes were modified by BMI, education, and race/ethnicity. Future research is warranted to examine whether reductions in DII over time are associated with decreased chronic disease risk.

Key words: dietary inflammatory index, Women’s Health Initiative, prediction, longitudinal trends
4.2 Introduction

Dietary index or pattern analysis can produce more intuitively appealing results that may improve prediction of disease risk as compared to examining individual foods or nutrients separately.\textsuperscript{4,23,24,26} Despite the growing use of dietary index or pattern analysis,\textsuperscript{28-30} relatively few studies have investigated the stability of dietary indices or patterns over time,\textsuperscript{31-37} or the factors influencing such stability.\textsuperscript{269-271} To the best of our knowledge, this evaluation has not been conducted in relation to the inflammatory potential of diet.

Dietary behaviors are subject to change over time,\textsuperscript{34,35} and mainly influence chronic disease outcomes when they persist over time.\textsuperscript{31} Knowledge of the longitudinal stability of dietary patterns could aid researchers in planning follow-up measurements or, as Weismayer et al. indicated,\textsuperscript{34} the cost of maintaining such cohorts could be reduced if diet is proven to be stable over time (e.g., by reducing the necessity for frequent data collection).

The dietary inflammatory index (DII) was developed\textsuperscript{38} and validated\textsuperscript{39} based on the evidence that many dietary factors have anti- or pro-inflammatory properties and the idea that no nutrient or food is consumed alone but in conjunction with other nutrients. In the current study, we calculated the DII based on the food frequency questionnaires (FFQ) used in the Women’s Health Initiative (WHI) Observational Study (OS) and Dietary Modification Trial (DMT). Our goal was to examine the stability of the inflammatory potential of diet, and the predictors of change in dietary inflammatory potential over time. We compared dietary behaviour change in an observational cohort of participants as well as in the DMT (i.e., intervention) population.
4.3 Methods

4.3.1 Participants

The design of the WHI has been described in detail elsewhere. The WHI began in 1992, spanning across 40 sites in the United States, and enrolled a total of 161,808 women between 1993 and 1998 and included full-scale randomized controlled trials, with ongoing follow-up. We used data up to September 30, 2010 for this investigation. The women were enrolled into the OS (n=93,676) or Clinical Trials (CT, n=68,132), with one of the CTs being the DMT (n=48,835). Other components of the CT included hormone therapy and calcium and vitamin D. The three CT components were overlapping, with some participants simultaneously recruited into more than one trial.

Exclusion criteria included any medical condition associated with a predicted survival of <3 years, alcoholism, other drug dependency, mental illness (e.g., major depressive disorder), dementia, not likely to live in the area for ≥3 years, and active participation in another intervention trial. Women were further excluded from the DMT if their diets were assessed to have <32% energy from fat. Demographic information and dietary data were obtained by self-report using standardized questionnaires, and certified staff performed physical measurements. The WHI protocol was approved by the institutional review boards at the Clinical Coordinating Center at the Fred Hutchinson Cancer Research Center (Seattle, WA) and at each of the 40 Clinical Centers.

4.3.2 Dietary Assessment

Figure 4.1 describes the administration of FFQs in the WHI OS and DMT. During screening for the WHI, all participants completed a baseline FFQ. Follow-up measures
included: an FFQ completed by all DMT participants in Year 1; an FFQ completed annually from Year 2 until study end (approximately ten years) in a random third of DMT participants; and an FFQ completed at Year 3 for ≥90% of OS participants. There was an average of two FFQs per participant in the OS and three FFQs per participant in the DMT. The 122-item WHI FFQ line item nutrient data was obtained from the University of Minnesota’s Nutrient Data system for research (NDSR) version 4.03_31 software, which is based on the US Department of Agriculture Standard Reference Releases and manufacturer information. The WHI FFQ has shown comparable results with 24-hour dietary recall interviews and food records in the WHI.266

4.3.3 Description of the DII (outcome of interest)

The main outcome of interest is longitudinal change in the DII. Details of the development38 and validation39 of the DII have been described elsewhere. Briefly, an extensive literature search was performed to obtain peer-reviewed journal articles that examined the association between six inflammatory biomarkers (IL-1β, IL-4, IL-6, IL-10, TNFα, and CRP) and 45 specific foods and nutrients (components of the DII). Scores were derived and standardized to a representative global diet database constructed based on 11 datasets from diverse populations in different parts of the world. Overall DII scores for each individual represent the sum of each of the DII components in relation to the comparison database.38 The DII score characterizes individuals’ diets on a continuum from maximally anti-inflammatory to maximally pro-inflammatory, with higher DII scores indicating more pro-inflammatory diets, while lower scores indicate more anti-inflammatory diets. In the WHI FFQ, 32 of the 45 original DII components were available for inclusion in the overall DII score. Components such as ginger, turmeric,
garlic, oregano, pepper, rosemary, eugenol, saffron, flavan-3-ol, flavones, flavonols, flavonones, anthocyanidins that are included in the original DII calculation\textsuperscript{38} were not included in the current study because they were not assessed in the WHI FFQ.

4.3.4 Statistical analysis

Participants with reported total energy intake judged to be implausible (<600kcal/d or >5000kcal/d) (n=1,796), or with extreme body mass index (BMI) (<15kg/m$^2$ or >50kg/m$^2$) (n=2,051) as well as those with only one FFQ (n=1,5479) or missing FFQ (n=32), were excluded from the current study, leaving 76,671 in the OS and 46,482 in the DMT for the final analyses (Figure 1). Frequencies and percentages were calculated to describe baseline characteristics of participants. We computed mean DII scores at baseline and Year 3 in the OS and at 11 different time points between baseline and Year 10 inclusive in the DMT; and used these to describe changes over time in the OS, or plotted DII scores on graphs for a visual appraisal of the longitudinal trend, separately for the intervention and control arms of the DMT. Analyses were stratified by BMI, education, and race/ethnicity. To determine significant differences between mean DII scores calculated at different time points, we constructed marginal linear regression models using generalized estimating equations (GEE) that adjusted for within-subject correlation in the DII measurements, in order to calculate and compare all pair-wise contrast estimates between mean DII scores. The GEE model was a univariate model with time from baseline as the only independent variable and changes in the DII over time as the dependent variable, adjusted for multiple comparisons using the Bonferroni approach, and stratified in the DMT by intervention arm.
Next, we utilized stepwise linear regression to construct the most parsimonious predictive multivariable model for change in DII from baseline to Year 3 in the OS. A previous WHI study (Tinker et al., 2007) investigated predictors of dietary change and maintenance in the DMT and included intrapersonal, interpersonal, intervention characteristics and clinical center characteristics as predictors. The DMT intervention moved participants toward an anti-inflammatory diet; therefore, predictors of dietary change investigated by Tinker et al are likely to predict DII change in the DMT. We therefore focused mainly on the potential predictors of DII change in the OS. We included the following baseline variables in the stepwise regression model: baseline DII, age group, BMI, race/ethnicity, educational level, physical activity, history of diabetes, hypertension, arthritis, cancer, use of non-steroidal anti-inflammatory drugs (NSAIDs), statins, anti-depressants, unopposed estrogen use, combined estrogen and progesterone use (Table 4.1 presents categories of potential predictors). The entry criterion into the stepwise linear regression model was \( P < 0.10 \), while the exit criterion was \( P > 0.10 \). The stepwise model identified variables that were included in a multivariable linear regression model to calculate beta (\( \beta \)) coefficients, corresponding p-values, and the \( R^2 \) for the overall predictive model. Participants with missing data in the predictors (\( n = 3,438 \)) were further excluded, leaving a final sample of 73,233 OS participants for the prediction model.

Analyses were conducted using SAS® version 9.3 (SAS Institute). All tests were 2-sided and \( p < 0.05 \) was used to assess statistical significance of parameter estimates.

### 4.4 Results

Participant characteristics were similar between OS and DMT for many covariates including race/ethnicity, educational level, smoking status, arthritis, unopposed estrogen
use, and combined estrogen and progesterone use (Table 4.1). More OS (23.8%) than DMT (16.7%) participants were ≥70 years; a higher proportion of participants in the DMT (38.4%) than OS (25.4%) were obese; and the proportion of individuals with a previous cancer diagnosis reported at baseline, was about three times higher in the OS (12.8%) than in the DMT (4.4%), likely due to cancer survivors joining the WHI but being excluded from the DMT (Table 4.1).

In the OS, the mean (±SD) overall DII decreased from -1.14 (±2.58) at baseline to -1.50 (±2.60) at Year 3. Corresponding averages for the DMT intervention arm were -0.40 (±2.54) and -1.70 (±2.63); and for the control arm, -0.38 (±2.55) and -1.04 (±2.60) (Figure 4.2) (all p-values for between-group differences across time were <0.0001). Mean DII scores at baseline were significantly different than at all other time points in both the intervention and control arms, as shown in the Bonferroni-adjusted p-values for all pairwise comparisons in Table 4.2.

There was evidence for interaction between DII change and BMI, education, and race/ethnicity; so, analyses were further stratified by these variables in the OS and DMT. In the OS, normal-weight women experienced the largest decrease in DII between baseline and Year 3 [-1.39 (±2.55) to -1.81 (±2.54)] compared to obese women [-0.78 (±2.61) to -1.04 (±2.67)]; while women with at least some college education showed the greatest change in DII [-1.39 (±2.51) to -1.77 (±2.52)] compared to women with less than a high school education, whose DII scores were more pro-inflammatory [0.26 (±2.71) to 0.06 (±2.71)]. In terms of race/ethnicity, Asians/Pacific Islanders (A/PI) experienced the largest change in DII [-1.76 (±2.53) to -2.04 (±2.51)], followed by European Americans
African Americans (AA) and Hispanics (HP) had more pro-inflammatory DII scores.

Figures 4.3, 4.4 and 4.5 present the corresponding longitudinal trends in the DMT intervention and control arms, which parallel those in the OS upon stratification by BMI, education, and race/ethnicity; though DII changes in the intervention arm were greater than in the control arm. Normal-weight women consistently experienced the largest DII decrease over time, followed by overweight women, while obese women showed the smallest decrease in DII over time (Figure 4.3). Highly educated women experienced the most anti-inflammatory changes over time (Figure 4.4). A/PI showed the largest DII decreases over time, while AA and HP showed the smallest changes over time (Figure 4.5).

The final predictive model presented in Table 4.3 explained 22% of the variance in DII changes between baseline and Year 3 in the OS. Decreases in DII over time were predicted by baseline DII (having a higher baseline DII predicted a larger decrease in DII), being A/PI or EA, having BMI<25kg/m², being more educated, being a nonsmoker, and meeting public health recommendations for physical activity.

### 4.5 Discussion

Using data from both the WHI OS and DMT, we described changes over time in the inflammatory potential of diet using the DII. The DII score in the OS remained relatively stable from baseline to Year 3, with an average change of -0.36 ± 2.35, representing about 2% of the full range of change in DII scores (-9.52 to 10.71). We demonstrated that the DII decreased substantially from baseline to Year 1 in the DMT intervention arm, achieving the lowest mean score in Year 3, and then increasing.
gradually until study end. The longitudinal trend of DII changes was similar in both arms of the DMT; however, changes in the intervention arm were almost double those observed in the control arm during the first five years of follow-up. In both the OS and DMT, participants who experienced the largest DII decrease had a normal BMI, a high educational level, and were A/PI or EA. Those who experienced the smallest decrease were obese, had less than high school education, and were HP or AA.

OS participants may have started the study consuming foods with lower mean inflammatory potential compared to DMT participants, likely due to DMT eligibility that required women to consume diets with $\geq 32\%$ energy from fat.\textsuperscript{40,266} This requirement had the effect of producing higher DII scores (i.e., more pro-inflammatory) at baseline for DMT participants because fat is a strongly pro-inflammatory component of the DII.\textsuperscript{38} It also could help explain reductions in the DII among DMT participants, who needed to meet a dietary fat entry criterion.\textsuperscript{273}

Highly educated women could be more heavily exposed to information about healthier food choices and have better financial access to a wider variety of healthier food choices than women with lower educational levels. In a study on the longitudinal trends in diet over a 20-year period, diet quality improved with higher educational attainment.\textsuperscript{274} Chaix et al. observed that poorly educated participants shopping in specific supermarket brands and in supermarkets whose catchment areas included more poorly educated residents had higher BMIs or waist circumferences.\textsuperscript{275} Additionally, Drewnowski et al. found lower levels of education and incomes, among other factors, to be consistently associated with higher obesity risk.\textsuperscript{276} These findings could partially explain our result showing that obese and less-educated participants experienced the smallest decreases in
The low DII scores in A/PI and EA compared to other race/ethnic groups may be due to different dietary patterns inherent in the cultures of the racial/ethnic groups. For example, diets of most Asian populations contain numerous anti-inflammatory constituents and lack many of the pro-inflammatory substances in Western diets.\textsuperscript{277,278} In the WHI, EA women are relatively better educated\textsuperscript{279} and may be more willing to change their diets in keeping with recommendations.\textsuperscript{276}

While the slight decrease in the dietary inflammatory potential from baseline to Year 3 in participants in the OS, provided insights into changes in dietary behavior over time, the follow-up period was insufficient to draw conclusions regarding long-term changes in dietary behavior in an observational setting. Participants in the control arm of the DMT were not asked to make dietary changes and were observed throughout the 10-year follow-up period; however, the trend in dietary behavior change over time in this group was similar, though smaller, to that observed in the intervention arm. Participants randomized to the control arm may have been motivated to change their diets prior to joining the study, and thus made personal efforts to improve their diets over time.

Some studies have examined the stability of dietary patterns over time;\textsuperscript{31-37} however, to the best of our knowledge, this is the first to study the stability of a dietary index describing the inflammatory potential over time. Previous studies reported inconsistent results on the stability of dietary behaviors over time, with some indicating stable behaviors after a short follow-up period of about 2 to 4 years,\textsuperscript{33,37} and others reporting significant changes only after a moderately long follow up (e.g., $\geq$7 years).\textsuperscript{34,36} Changes in diet over time may be due, in part, to the response to frequent updates to dietary guidelines, changes over time in the availability of different foods in some
communities, and disease diagnosis that may alter dietary intake (e.g., diabetes or hypertension). Methodologic differences between studies would include differences in duration of follow-up, frequency and method of diet assessment, and sample composition and size.

This study has several strengths including the relatively large population-based sample in the OS and DMT, good regional and racial/ethnic representation, and inclusion of large number of potential predictors of DII change. The DMT had a relatively long follow-up duration with diet assessed annually on random subsamples of the study population. Our study also had some limitations: FFQ data were not available in the OS after Year 3; thus we were not able to compare dietary behavior change between the OS and DMT beyond the first three years of follow up. The decrease in dietary inflammatory potential in the first three years may have been due to survey learning effects, in part attributed to social desirability bias, rather than a real improvement in diet quality. This limitation might have been mitigated had social desirability, an established source of bias of dietary self-report data, been measured in the WHI.\textsuperscript{280,281} In our DMT sample, not every participant had FFQ data at all 11 time points, which could have reduced the effect of survey learning as participants did not complete the FFQ every year. Sample sizes from Year 8 to 10 were very small and may not be representative of the entire DMT population. Although WHI enrolled only postmenopausal women, average DII scores in the WHI were comparable to other US populations that have been examined.\textsuperscript{39,282}

After including a comprehensive list of demographic, lifestyle and health-related factors, our final prediction model explained 22\% of the variation in DII change in the OS. This represents reasonable explanatory ability when one considers that a change
score is accompanied by large overall variance owing to the fact that the variance of a difference is the sum of the variance of the individual components\(^{283}\) (while the absolute difference can often be quite small). Other potential predictors of DII change that are outside the scope of the current study may include behavioural factors, such as those investigated by Tinker et al. in the prediction of dietary change and maintenance in the DMT.\(^{269}\)

### 4.6 Conclusion

In this population of postmenopausal women, the average DII was relatively stable in the OS from baseline to Year 3, but decreased significantly over time in a manner consistent with improved anti-inflammatory potential, achieving its lowest mean value at Year 3 in DMT intervention participants and, to a smaller extent, among control arm participants. In all three study groups, the extent of decrease was influenced by BMI, education, and race/ethnicity. Baseline DII and several demographic, lifestyle and clinical factors significantly predicted changes in the inflammatory potential of diet in the first three years of follow up in an observational setting. Future research is warranted to examine whether reductions in DII over time are associated with decreased chronic disease risk.
4.7 Tables and figures

Figure 4.1. Participant flow in the administration of food frequency questionnaires in the Women’s Health Initiative (WHI) Observational Study (OS) and Dietary Modification Trial (DMT), 1993-2010
Table 4.1. Baseline characteristics of study participants, frequency (%), Women's Health Initiative, 1993-1998

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Observational Study (n=76,671)</th>
<th>Dietary Modification Trial intervention arm (n=18,604)</th>
<th>Dietary Modification Trial control arm (n=27,878)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age groups (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50-59</td>
<td>24144 (31.5)</td>
<td>6832 (36.7)</td>
<td>10203 (36.6)</td>
</tr>
<tr>
<td>60-69</td>
<td>34293 (44.7)</td>
<td>8681 (46.7)</td>
<td>13033 (46.7)</td>
</tr>
<tr>
<td>70-79</td>
<td>18234 (23.8)</td>
<td>3091 (16.6)</td>
<td>4642 (16.7)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal ( &lt;25)</td>
<td>30577 (39.9)</td>
<td>5230 (28.1)</td>
<td>6820 (24.5)</td>
</tr>
<tr>
<td>Overweight (25.0 - &lt;30)</td>
<td>26605 (34.7)</td>
<td>6534 (35.1)</td>
<td>9940 (35.7)</td>
</tr>
<tr>
<td>Obese (≥30)</td>
<td>19489 (25.4)</td>
<td>6840 (36.8)</td>
<td>11118 (39.9)</td>
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<td>Race/ethnicity</td>
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<td></td>
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<td>Asian or Pacific Islander</td>
<td>2102 (2.7)</td>
<td>421 (2.3)</td>
<td>645 (2.3)</td>
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<tr>
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<td>Hispanic/Latino</td>
<td>2253 (3.0)</td>
<td>661 (3.6)</td>
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<td>European American</td>
<td>66331 (86.8)</td>
<td>15263 (82.2)</td>
<td>22916 (82.3)</td>
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<td>332 (1.2)</td>
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<td>5703 (30.8)</td>
<td>8609 (31.1)</td>
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<tr>
<td>Some years of college/graduate</td>
<td>54067 (71.0)</td>
<td>12604 (68.2)</td>
<td>18761 (67.7)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>38661 (50.1)</td>
<td>9502 (51.7)</td>
<td>14386 (52.1)</td>
</tr>
<tr>
<td>Former</td>
<td>32813 (43.3)</td>
<td>7715 (50.0)</td>
<td>11370 (41.2)</td>
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<tr>
<td>Current</td>
<td>4242 (5.6)</td>
<td>1169 (6.3)</td>
<td>1842 (6.7)</td>
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<td>Physical activity (PA), minutes/week</td>
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<tr>
<td>Not meeting PA recommendations</td>
<td>39636 (52.2)</td>
<td>10860 (65.2)</td>
<td>16421 (65.7)</td>
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<tr>
<td>Meeting PA recommendations</td>
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<td>5797 (34.8)</td>
<td>8567 (34.3)</td>
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<td>9875 (12.9)</td>
<td>3154 (16.9)</td>
<td>4926 (17.7)</td>
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<td>Hypertension</td>
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<td>15974 (64.5)</td>
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<td>No</td>
<td>Total</td>
</tr>
<tr>
<td>------------</td>
<td>----------</td>
<td>---------</td>
<td>-------</td>
</tr>
<tr>
<td>Arthritis</td>
<td>24147 (32.0)</td>
<td>5704 (34.5)</td>
<td>8794 (35.5)</td>
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<tr>
<td>Cancer</td>
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<td>10042 (54.5)</td>
<td>14989 (54.3)</td>
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<td>36845 (48.4)</td>
<td>8371 (45.5)</td>
<td>12596 (45.7)</td>
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<td></td>
<td>66375 (87.2)</td>
<td>17613 (95.6)</td>
<td>26382 (95.6)</td>
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<tr>
<td></td>
<td>9757 (12.8)</td>
<td>809 (4.4)</td>
<td>1210 (4.4)</td>
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</table>

Duration of estrogen use by category

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<tr>
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<th>Total</th>
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<tr>
<td>None</td>
<td>47756 (62.3)</td>
<td>11648 (62.6)</td>
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<td>&lt;5 Years</td>
<td>9785 (12.7)</td>
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<td>5 to &lt;10 Years</td>
<td>5808 (7.6)</td>
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<td>10 to &lt;15 Years</td>
<td>4609 (6.0)</td>
<td>1102 (5.9)</td>
<td>1685 (6.0)</td>
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<tr>
<td>15+ Years</td>
<td>8711 (11.4)</td>
<td>1911 (10.3)</td>
<td>2863 (10.3)</td>
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</table>

Duration of estrogen & progesterone use by category

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<tr>
<td>None</td>
<td>53804 (70.2)</td>
<td>13431 (72.2)</td>
<td>20195 (72.4)</td>
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<tr>
<td>&lt;5 Years</td>
<td>10943 (14.3)</td>
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<tr>
<td>5 to &lt;10 Years</td>
<td>6392 (8.3)</td>
<td>1388 (7.4)</td>
<td>2162 (7.8)</td>
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<tr>
<td>10 to &lt;15 Years</td>
<td>37228 (4.9)</td>
<td>749 (4.0)</td>
<td>1103 (4.0)</td>
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<tr>
<td>15+ Years</td>
<td>1808 (2.3)</td>
<td>362 (2.0)</td>
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Statin use

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<td>No</td>
<td>64049 (83.5)</td>
<td>13937 (74.9)</td>
<td>20601 (73.9)</td>
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<tr>
<td>Yes</td>
<td>12622 (16.5)</td>
<td>4667 (25.1)</td>
<td>7277 (26.1)</td>
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Antidepressant use

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</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>67557 (88.1)</td>
<td>15502 (83.3)</td>
<td>23144 (83.0)</td>
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<tr>
<td>Yes</td>
<td>9114 (11.9)</td>
<td>3102 (16.7)</td>
<td>4734 (17.0)</td>
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</table>

NSAIDs use

<table>
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<th>No</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>36819 (48.0)</td>
<td>6687 (35.9)</td>
<td>9732 (34.9)</td>
</tr>
<tr>
<td>Yes</td>
<td>39852 (52.0)</td>
<td>11917 (64.1)</td>
<td>18146 (65.1)</td>
</tr>
</tbody>
</table>

NSAIDs=Non-steroidal Anti-inflammatory Drugs
Figure 4.2. Average dietary inflammatory index (DII)\textsuperscript{1} scores across years of follow-up in the Dietary Modification Trial, by study arm; Women’s Health Initiative, 1993-2010\textsuperscript{2}

\textsuperscript{1}(P-value for the difference in DII scores between intervention and control was 0.62 at baseline, and <0.0001 for each year from year 1 onwards)

\textsuperscript{2}Numbers of participants (Intervention: 19470, 18061, 6081, 3255, 5071, 5835, 7160, 4641, 2734, 1578, and 417; Control: 29216, 26753, 8882, 4922, 7902, 9028, 10860, 7252, 4451, 2344 and 632; for Years 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, respectively)
Table 4.2. Bonferroni-adjusted p-values of all pair-wise comparisons of the mean dietary inflammatory index scores across years of follow-up in the Dietary Modification Trial; Women's Health Initiative, 1993-2010

<table>
<thead>
<tr>
<th>Time point</th>
<th>0 (18520)</th>
<th>1 (17383)</th>
<th>2 (5865)</th>
<th>3 (3131)</th>
<th>4 (4843)</th>
<th>5 (5596)</th>
<th>6 (6800)</th>
<th>7 (4404)</th>
<th>8 (2582)</th>
<th>9 (1497)</th>
<th>10 (391)</th>
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<tbody>
<tr>
<td>0 (277916)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>1 (25749)</td>
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<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.01</td>
<td>0.0004</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>2 (8524)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.11</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>3 (4717)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.23</td>
<td>0.99</td>
<td>0.99</td>
<td>0.01</td>
<td>0.001</td>
<td>0.99</td>
<td>0.99</td>
<td>0.004</td>
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<tr>
<td>4 (7553)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.68</td>
<td>0.99</td>
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<td>0.22</td>
<td>0.01</td>
<td>0.99</td>
<td>0.99</td>
<td>0.04</td>
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<tr>
<td>5 (10375)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.99</td>
<td>0.99</td>
<td>0.52</td>
<td>0.03</td>
<td>0.99</td>
<td>0.99</td>
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</tr>
<tr>
<td>6 (8634)</td>
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<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.01</td>
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<td>0.99</td>
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<tr>
<td>7 (6918)</td>
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<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
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<tr>
<td>8 (4240)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.09</td>
<td>0.003</td>
<td>0.33</td>
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<td>9 (2244)</td>
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<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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<tr>
<td>10 (600)</td>
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<td>0.99</td>
<td>0.99</td>
<td>0.24</td>
<td>0.99</td>
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</tr>
</tbody>
</table>

Blue=control arm, Green=intervention arm
Figure 4.3. Average dietary inflammatory index over time by body mass index category and Dietary Modification Trial arm; Women’s Health Initiative, 1993-2010
Figure 4.4. Average dietary inflammatory index (DII) over time by educational level and Dietary Modification Trial arm; Women’s Health Initiative, 1993-2010.
Figure 4.5. Average dietary inflammatory index (DII) over time by race/ethnicity and Dietary Modification Trial arm; Women's Health Initiative, 1993-2010.
Table 4.3. Multivariable predictive model of change in dietary inflammatory index over time in the Observational Study; Women's Health Initiative, 1993-2010

<table>
<thead>
<tr>
<th>Predictors</th>
<th>β (SE)</th>
<th>P-value (β)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline DII</td>
<td>-0.44 (0.00)</td>
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</tr>
<tr>
<td>Body mass index (kg/m²)</td>
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</tr>
<tr>
<td>Normal weight (&gt;25) referent</td>
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<td></td>
</tr>
<tr>
<td>Overweight (25 - &lt;30)</td>
<td>0.25 (0.02)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Obese (&gt;30)</td>
<td>0.10 (0.02)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Race/ethnicity</td>
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<tr>
<td>European American referent</td>
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<tr>
<td>African American</td>
<td>0.48 (0.03)</td>
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<td>Asian or Pacific Islander</td>
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<tr>
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<tr>
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<tr>
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<td>0.91</td>
</tr>
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<tr>
<td>10 to &lt;15y</td>
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<td>0.0002</td>
</tr>
<tr>
<td>≥15y</td>
<td>0.10 (0.04)</td>
<td>0.02</td>
</tr>
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CHAPTER 5

CHANGES IN THE INFLAMMATORY POTENTIAL OF DIET OVER TIME AND RISK OF COLORECTAL CANCER IN POSTMENOPAUSAL WOMEN

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5.1 Abstract

**Introduction:** To evaluate changes in the inflammatory potential of diet and subsequent risk of colorectal cancer (CRC), we used the dietary inflammatory index (DII), to predict newly incident CRC in the Women’s Health Initiative (WHI). **Methods:** Data were obtained from 110,665 postmenopausal women recruited from 1993-1998 into the WHI and followed through September 30, 2010. Food frequency questionnaires data were used to compute cumulative average DII scores that were then used in Cox proportional hazards (PH) models to estimate hazard ratios (HR) and 95% confidence intervals (95%CI) for newly incident CRC. Patterns in DII change from baseline to Year 3 were computed in a subset of 79,484 women, from which HR were calculated using Cox PH models. **Results:** HR for the association between high DII scores and CRC were consistently significantly elevated in the first seven years of follow up, for colon cancer with multivariable-adjusted HR ranging from 1.30 in Year 2 to 1.58 in Year 7, comparing the highest with the lowest quintile. No significant associations were observed between cumulative average DII and rectal cancer. Compared to participants in the anti-inflammatory stable category, risk was increased in participants with a pro-inflammatory stable diet, for CRC (HR, 1.18; 95%CI, 0.99, 1.41), and for rectal cancer (HR, 1.53; 95%CI, 1.01, 2.32). **Conclusion:** A history of long-term pro-inflammatory diets increases the risk of colon cancer while shorter-term stable pro-inflammatory diets, increase the risk of rectal cancer. Lowering the inflammatory potential of diet could be a means for colon cancer, and potentially rectal cancer prevention.

**Key words:** changes in dietary inflammatory potential, colorectal cancer, dietary patterns, Women’s Health Initiative


5.2 Introduction

Colorectal cancer is the third most commonly diagnosed cancer in American women after lung and breast cancers. The etiology of colorectal cancer involves a complex interaction of cellular and molecular processes with environmental factors. Of these factors, dietary patterns that modulate inflammation may be important, given the central role of inflammation in the carcinogenesis process. The American Institute for Cancer Research estimates that half of colorectal cancers can be prevented by adopting healthy lifestyle behaviors including healthy dietary patterns. Dietary patterns, or dietary indices that take into account multiple dietary factors, can provide a more comprehensive assessment of diet and may be more predictive of disease processes and outcomes than single nutrients or foods.

Most dietary patterns derived through data-driven approaches or index-based methods have been shown to be associated with colorectal cancer risk. However, these findings are often heterogeneous by anatomic subsite of colorectal cancer. We previously reported that a more pro-inflammatory diet as measured by the dietary inflammatory index (DII) was associated with increased risk of colorectal cancer using baseline food frequency questionnaire data in the Women’s Health Initiative (WHI), and that the association was more pronounced for colon cancer than for rectal cancer (Tabung FK, Steck SE, Ma Y, et al., unpublished data, 2014).

Despite the growing interest in the role of dietary patterns in colorectal cancer risk, most studies have examined dietary patterns at one point in time only. However, dietary behaviors mainly influence chronic disease outcomes when they persist for a longer period of time. We have shown that DII scores decreased significantly in
women enrolled in the WHI Dietary Modification Trial. The longitudinal trend in DII scores was similar to that of percent fat reduction over time (Tabung FK, Steck SE, Zhang J, et al., unpublished data, 2014). Risk of colorectal cancer is believed to accumulate over time, thus, dietary changes over time may have a greater impact on colorectal cancer risk compared with diet assessed at only one point in time. In the current study, our objective is to evaluate the role of both the cumulative history, and the changes in the inflammatory potential of diet over time, on colorectal cancer risk.

5.3 Methods

5.3.1 Study population

The WHI study is a large and complex clinical investigation of strategies for the prevention and control of some of the most common causes of morbidity and mortality among postmenopausal women. The design of the WHI has been described in detail elsewhere. Briefly, the WHI began in 1992, implemented in 40 sites across the United States, and enrolled a total of 161,808 women between 1993 and 1998. The WHI enrolled 93,676 women into an Observational Study (OS) and 68,132 participants into Clinical Trials (CT), with an average of 11.3 years of follow-up until September 30, 2010. The CTs included three components: Hormone Therapy, Calcium and Vitamin D, and the Dietary Modification Trial (DMT). For the DMT, women were randomly assigned to a usual-diet comparison group (n=29,294) or an intervention group (n=19,541) with a 20% low-fat dietary pattern with increased vegetables, fruits, and whole grains. Women who proved to be ineligible for, or who were unwilling to enroll in the CT components were invited to be part of the prospective cohort of women in the OS.
Exclusion criteria for both the OS and CT included any medical condition associated with a predicted survival of less than three years, alcoholism, other drug dependency, mental illness (e.g., major depressive disorder), dementia, active participation in another intervention trial and not likely to live in the area for at least 3 years. Demographic information and dietary data were obtained by self-report using standardized questionnaires. Certified staff performed physical measurements, including blood pressure, height and weight, and blood samples at the baseline clinic visit. Women were further excluded from the DMT if their diets were assessed to have <32% energy from fat. The WHI protocol was approved by the institutional review boards at the Clinical Coordinating Center (CCC) at the Fred Hutchinson Cancer Research Center (Seattle, WA) and at each of the 40 Clinical Centers.  

5.3.2 Diet assessment

Figure 5.1 describes the administration of food frequency questionnaires (FFQ) in the WHI. During screening for the WHI, all participants completed a baseline FFQ. At follow-up, the FFQ was completed at Year 3 for ≈90% of OS participants. About 92% of DMT participants completed an FFQ in Year 1, and a random third of participants were invited to complete an FFQ annually from Year 2 until study end (approximately ten years) (Figure 5.1). There was an average of two FFQs per participant in the OS and three FFQs per participant in the DMT. The 122-item WHI FFQ line-item nutrient data was obtained from the University of Minnesota’s Nutrient Data system for research (NDSR) version 4.03_31 software, which is based on the US Department of Agriculture Standard Reference Releases and manufacturer information. The WHI FFQ has shown comparable results with 24-hour dietary recall interviews and food records in the WHI.
5.3.3 The dietary inflammatory index (DII)

Details of the development\textsuperscript{38} and validation\textsuperscript{39} of the DII have been described elsewhere. Briefly, an extensive literature search was performed to obtain peer-reviewed journal articles that examined the association between six well-known inflammatory biomarkers (IL-1β, IL-4, IL-6, IL-10, TNFα, and CRP) and 45 specific foods and nutrients (components of the DII). Scores were derived and standardized to a representative global diet database constructed based on 11 datasets from diverse populations in different parts of the world. Overall DII scores for each individual participant represent the sum of each of the DII components in relation to the comparison global diet database.\textsuperscript{38} The DII score characterizes an individual’s diet on a continuum from maximally anti-inflammatory to maximally pro-inflammatory, with a higher DII score indicating a more pro-inflammatory diet and a lower (i.e., more negative) DII score indicating a more anti-inflammatory diet. In the WHI FFQ, 32 of the 45 original DII components were available for inclusion in the overall DII score (see\textsuperscript{38} for list of 45 DII components). Components such as ginger, turmeric, garlic, oregano, pepper, rosemary, eugenol, saffron, flavan-3-ol, flavones, flavonols, flavonones, anthocyanidins that are included in the original DII calculation\textsuperscript{38} were not included in the current study because they were not available from the WHI FFQ.

5.3.4 Outcomes assessment

The WHI outcomes ascertainment and adjudication methods have been previously described.\textsuperscript{267} Briefly, participants (or next-of-kin) self-reported cancer diagnoses reported on questionnaires annually in the OS or semiannually in the CT through 2005 and
annually in all thereafter. Colorectal cancer events reported were verified by centrally trained physician adjudicators after review of medical records and pathology reports.

The outcome for these analyses was colorectal cancer, including cancers of the colon and rectum (including rectum and rectosigmoid). Proximal colon cancers were defined as cancers of the cecum, ascending colon, right colon, hepatic flexure of colon, and transverse colon (ICD=C18.0, C18.2-18.4), and distal colon cancers were defined as cancers of the splenic flexure of colon, descending colon, left colon and sigmoid colon (ICD=C18.5-18.7). Survival time was defined as days from enrollment or randomization until colorectal cancer diagnosis while censoring time was defined as days from enrollment or randomization until death or last contact occurring on or before September 30, 2010, in participants without colorectal cancer.

5.3.5 Statistical analysis

We utilized data from 142,511 women participating in the WHI OS and DMT. Exclusion criteria included: women with colorectal cancer at baseline or missing colorectal cancer status at baseline (n=2,272), women with reported total energy intake values judged to be implausible (≤600 kcal/day or ≥5000 kcal/day) (n=1,796) or extreme BMI values (<15kg/m^2 or > 50kg/m^2) (n=2,014), as well as women with single FFQs (n=15,122) or missing FFQs (n=32) (Figure 5.1). Additionally, we excluded participants with missing data in the covariates (n=10,610), leaving a total of 110,665 participants for these analyses (72,261 in OS and 38,404 in DMT). Frequencies and percentages were computed to describe the distribution of covariates across quintiles of cumulative average DII for the DII assessed from baseline to Year 3.
To determine the role of cumulative history of the inflammatory potential of diet in colorectal cancer risk over time, we calculated ten cumulative averages of DII incrementally starting from the average between baseline and year one DII.\textsuperscript{172} The cumulative average was then categorized into quintiles, and used to estimate hazard ratios (HR) for newly incident overall colorectal, colon, and rectal cancers, using multivariable-adjusted Cox regression models. Colorectal cancers diagnosed prior to year one were excluded from the models. This approach was repeated for the average DII of baseline, year one, and year two, with colorectal cancer cases diagnosed prior to year two excluded to avoid the possibility of change in diet due to subclinical disease and to ensure that only participants at risk of developing colorectal cancer going forward, were included in the models. This approach was repeated until DII estimates at all ten time points were used.\textsuperscript{172} We categorized the cumulative average DII into quintiles, used these to calculate HRs and then plotted the HRs on graphs for a visual appraisal of the longitudinal trend in risk, separately for colon and rectal cancers in the DMT.

To determine the role of changes in patterns of the inflammatory potential of diet over time in colorectal cancer risk, we calculated the DII from baseline and year 3 FFQs administered to 79,484 women in the OS and DMT. We categorized the DII at both time points into quintiles (Q) and further categorized changes in the inflammatory potential of diet based on quintile differences between baseline and year 3, as follows:

1. Anti-inflammatory stable: Q1 or Q2 at both time points or change from Q3 to Q2;
2. Anti-inflammatory change: changes $\leq -2Q$;
3. Neutral inflammation stable: changes from Q2 to Q3, Q4 to Q3 or stable at Q3 at both time points;
4. Pro-inflammatory change: changes ≥ 2Q;

5. Pro-inflammatory stable: Q4 or Q5 at both time points, or change from Q3 to Q4.

The names given to these categories of DII changes were meant to be qualitative only. Next, Cox proportional hazards regression models were used to estimate HR and associated 95% CI for colorectal, colon (proximal/distal), and rectal cancer incidence, by patterns of DII changes, with adjustment for multiple covariates. The anti-inflammatory stable category, considered to be the healthiest category, was the referent for all models.

Potential confounders that changed HRs by >10% were retained in the final model and included: age group (years) (50-59, 60-69, 70-79); race/ethnicity, European American (EA), African American (AA), Hispanic (HP), Asian or Pacific Islander (A/PI), and other race groups (other); educational levels (less than high school, some high school /GED, at least some college/graduate education); smoking status (current, past, never); body mass index [BMI= weight(kg)/height(m)^2] (normal weight (<25kg/m^2), overweight (25-<30 kg/m^2), and obese (≥30kg/m^2)); physical activity (PA) was categorized based on public health recommendations as meeting or not meeting PA recommendations (≥150 minutes/week of moderate intensity PA or ≥75 minutes/week of vigorous intensity PA versus<150 minutes/week of moderate intensity PA or <75 minutes/week of vigorous intensity PA, respectively); history of diabetes (yes/no), hypertension (yes/no), arthritis (yes/no); non-steroidal anti-inflammatory drug (NSAID) use (yes/no); category and duration of estrogen use and category and duration of combined estrogen and progesterone use both categorized into five groups (none, <5y, 5 to <10y, 10 to <15y, and ≥15y). Data on potential confounders were collected by self-administered questionnaires on demographics, medical history, and lifestyle factors.
Each covariate in the final models for both cumulative average DII and patterns of changes in DII was tested for proportional hazards using cumulative sums of Martingale-based residuals. Age group, and smoking status violated the PH assumption and models were therefore stratified by these two covariates. We investigated effect modification of the association between cumulative average DII, and changes in the DII and colorectal cancer incidence by age, race/ethnicity, education, BMI and NSAID use, by including two-way cross-product terms for these covariates in the models, and assessed significant effect modification at p <0.05. None of the cross-product terms were significant and therefore no subgroup analyses were conducted. Statistical significance was determined by evaluating 95%CI. Statistical analyses were conducted using SAS® version 9.3 (SAS Institute, Cary, NC), and all tests were two-sided.

5.4 Results

Table 5.1 shows the distribution of participants’ characteristics in quintiles of cumulative average DII from baseline to year 3. Proportions of most covariates differed between the quintiles. For example, there was a higher proportion of AA (15%), participants with < high school education (2%), current smokers (9%), and obese participants (37%), in the highest compared to the lowest quintile (Table 5.1). During an average 11.7 years of follow-up, 1,240 incident colorectal cancer cases (1,036 colon and 219 rectal) were identified.

Table 5.2 presents hazard ratios of the association between cumulative average DII and colorectal cancer. Comparing participants in the highest with the lowest quintile of DII, in Year 3 where OS participants had the only other diet assessment, the cumulative average DII was significantly associated with an increased risk of colorectal
cancer overall (HR, 1.33; 95%CI, 1.10, 1.61) and in subgroup analyses of OS participants (HR, 1.34; 95%CI, 1.05, 1.70) but not DMT participants (HR, 1.28; 95%CI, 0.95, 1.73). In all other years of follow-up, HR were indicative of a positive association in DMT participants but did not attain statistical significance.

Figures 5.1 and 5.2 present HR and 95% CIs for colon and rectal cancers, respectively, at the various time points of cumulative diet assessment comparing DMT participants in the highest to the lowest cumulative average DII quintile. For colon cancer, risk was not significantly increased in Year 1, but from Year 2 to Year 7, risk was consistently significantly increased, and became attenuated from Year 7 to study end in Year 10 (Figure 5.1). We found no significant results for cumulative DII and rectal cancer (Figure 5.2).

In the first 3 years of follow-up, 29.3% of participants were classified as having an anti-inflammatory stable pattern, 11.7% experienced anti-inflammatory change, 23.6% were in the neutral inflammation stable category, 12.1% experienced pro-inflammatory changes, while 23.3% were in the pro-inflammatory stable category. Table 5.3 presents the results of the associations between changes in the inflammatory potential of diet and colorectal cancer risk. Using participants in the anti-inflammatory stable category as the referent, rectal cancer risk was significantly increased in participants with a pro-inflammatory stable diet (HR, 1.53; 95%CI, 1.01, 2.32). HR for colon cancer (HR, 1.11; 95%CI, 0.91, 1.35) and overall colorectal cancer (HR, 1.18; 95%CI, 0.99, 1.41) were positive but not statistically significant (Table 5.3).
5.5 Discussion

In this large prospective study, we demonstrated that: 1) a higher cumulative average score of the DII is associated with an increased risk of colorectal cancer especially colon cancer, while 2) a stable pro-inflammatory diet from baseline to year 3 increased the risk of rectal cancer. To the best of our knowledge, this is the first study to characterize the association between the cumulative history, and changes over time in the inflammatory potential of diet, and risk of colorectal cancer. Given that FFQs were administered to OS participants at baseline and Year 3; a cumulative average DII could only be calculated for OS participants at Year 3, whereas analyses at all other time points included DMT participants. We selected these two time points for the analyses of changes in the DII over time, to include the maximum number of participants with FFQs (Figure 5.1). There was no statistically significant association between cumulative average DII and rectal cancer in the DMT, though a power calculation indicated that we could observe significant HR ranging from 1.13 to 1.47. There was however, a significantly higher risk of rectal cancer in models for changes in DII between baseline and Year 3 where analyses included subjects from both the OS and DMT.

Our results are generally similar to previous findings from studies of diet quality and colorectal cancer risk, in terms of poorer diet quality (here characterized by higher, more pro-inflammatory DII scores) being associated with increased colorectal cancer risk. These other studies assessed diet quality at only one point in time. However, in a previous study we demonstrated that diet quality with respect to its inflammatory potential improves significantly over time in an interventional setting, though it is relatively stable in an observational setting (Tabung FK, Steck SE, Zhang J et al., 28,145,202,285).
unpublished data, 2014). Risk of colorectal cancer is believed to accumulate over time; thus, dietary changes over time may have a greater impact on colorectal cancer risk compared with diet assessed at only one point in time.

However, the relative stability of the dietary inflammatory potential in an observational setting could mean that diet assessment at any point in time during the study could be equally important in determining disease risk estimates. In the current study, we obtained a HR of 1.18 (95% CI, 0.99, 1.41) for the association between changes from baseline to Year 3 in the inflammatory potential of diet and colorectal cancer risk, comparing a stable pro-inflammatory change to an anti-inflammatory stable change, and a HR of 1.34 (95% CI, 1.05, 1.70) for the association between cumulative average DII and colorectal cancer risk from baseline to Year 3, comparing the highest and lowest quintiles of cumulative average DII in the OS. These risk estimates are similar to the HR of 1.22 (95% CI, 1.05, 1.43) we obtained in a previous study, for the association between the dietary inflammatory potential at baseline only, and colorectal cancer risk (Tabung FK, Steck SE, Ma Y, et al., unpublished data, 2014).

The link between inflammation and colorectal cancer is supported by findings from several studies showing either a reduced risk of colorectal cancer with regular use of NSAIDs, or a positive association between higher concentrations of inflammatory biomarkers and increased colorectal cancer risk. Other potential mechanisms through which a pro-inflammatory diet may increase risk of colorectal cancer include components of the metabolic syndrome, especially insulin resistance or glucose intolerance, and the microbiota. A high and sustained pro-inflammatory potential of the diet may
compromise the host-microbiota mutualism favoring the proliferation of toxic bacteria that have been suggested to promote colorectal carcinogenesis.289

Strengths of the current study include accounting for changes in the inflammatory potential of diet over time in a large, well-characterized population of more than 110,000 women, a long follow-up period, the inclusion of women of diverse race/ethnic groups, and the central adjudication of colorectal cancer diagnosis. The use of a novel dietary index to score diet quality based on inflammatory potential supports the evidence linking inflammation and colorectal cancer. Limitations include known measurement error in using an FFQ for the assessment of diet and its inflammatory potential over time, potential residual or unmeasured confounding, though we adjusted for many potential confounders in the models. We assumed that the random 30% of DMT participants sampled from year 2 until study end was representative of the entire DMT study population, a plausible assumption since these random subsamples were used for intervention monitoring in the DMT, though the sample size reduced in the last two years of follow-up.

5.6 Conclusion

A history of long-term pro-inflammatory diets increases the risk of colon cancer, while shorter-term stable pro-inflammatory diets increase the risk of rectal cancer. Our findings suggest lowering the inflammatory potential of diet as a means for colon cancer, and potentially rectal cancer prevention.
5.7 Tables and figures

Figure 5.1. Participant flow in the administration of food frequency questionnaires in the Women’s Health Initiative (WHI) Observational Study (OS) and Dietary Modification Trial (DMT), 1993-2010
Table 5.1. Frequencies (%) of participant's characteristics across quintiles of cumulative average DII (Years 0-3); Women's Health Initiative, 1993-2010

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<th>Q3 (-2.103, &lt; -0.734)</th>
<th>Q4 (&lt; -0.734, 1.041)</th>
<th>Q5 (1.041, 5.315)</th>
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<td>19189 (86.7)</td>
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<td>16596 (76.8)</td>
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<td>413 (1.9)</td>
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<td>7024 (32.1)</td>
<td>8339 (35.6)</td>
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<td>Overweight (25.0 - &lt;30)</td>
<td>7728 (34.1)</td>
<td>7858 (35.1)</td>
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<td>7750 (35.4)</td>
<td>7397 (34.2)</td>
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<td>Obese (≥30)</td>
<td>5203 (23.0)</td>
<td>6227 (27.9)</td>
<td>6650 (30.0)</td>
<td>7123 (32.6)</td>
<td>8016 (37.1)</td>
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Physical activity (PA), minutes/week

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<td>Not meeting PA recommendations</td>
<td>8016 (42.2)</td>
<td>11810 (52.8)</td>
<td>12667 (57.2)</td>
<td>13521 (61.8)</td>
<td>15096 (69.8)</td>
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<tr>
<td>Meeting PA recommendations</td>
<td>13090 (5.8)</td>
<td>10560 (47.2)</td>
<td>9478 (42.8)</td>
<td>8361 (38.2)</td>
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Diabetes

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<td>18915 (85.4)</td>
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<td>Yes</td>
<td>2653 (11.7)</td>
<td>2871 (12.8)</td>
<td>3230 (14.6)</td>
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<td>3745 (17.2)</td>
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Hypertension

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<td>No</td>
<td>15986 (70.6)</td>
<td>15238 (68.1)</td>
<td>14777 (66.7)</td>
<td>1445 (66.0)</td>
<td>13799 (63.8)</td>
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<td>Yes</td>
<td>76667 (29.4)</td>
<td>7132 (31.9)</td>
<td>7368 (33.3)</td>
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Arthritis

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<td>11669 (51.5)</td>
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<td>Yes</td>
<td>10984 (48.5)</td>
<td>10945 (48.9)</td>
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NSAIDs use

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<td>9064 (40.5)</td>
<td>9387 (42.4)</td>
<td>9881 (45.2)</td>
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<td>13149 (58.0)</td>
<td>13306 (59.5)</td>
<td>12758 (57.6)</td>
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<td>11136 (51.5)</td>
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Duration of estrogen use by category

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<th>Group 5</th>
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<td>14000 (61.8)</td>
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<td>13684 (61.8)</td>
<td>13729 (62.7)</td>
<td>14198 (65.7)</td>
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<td>&lt;5 Years</td>
<td>2788 (12.3)</td>
<td>2823 (12.6)</td>
<td>2855 (12.9)</td>
<td>2900 (13.3)</td>
<td>2808 (13.0)</td>
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<tr>
<td>5 to &lt;10 Years</td>
<td>1789 (7.9)</td>
<td>1773 (7.9)</td>
<td>1752 (7.9)</td>
<td>1578 (7.2)</td>
<td>1470 (6.8)</td>
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<tr>
<td>10 to &lt;15 Years</td>
<td>1503 (6.6)</td>
<td>1464 (6.6)</td>
<td>1353 (6.1)</td>
<td>1279 (5.8)</td>
<td>1129 (5.2)</td>
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<tr>
<td>15+ Years</td>
<td>2573 (11.4)</td>
<td>2751 (12.3)</td>
<td>2501 (11.3)</td>
<td>2396 (11.0)</td>
<td>2010 (9.3)</td>
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Duration of estrogen & progesterone use by category

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<td>14746 (65.1)</td>
<td>15220 (68.0)</td>
<td>15649 (70.7)</td>
<td>15918 (72.7)</td>
<td>16585 (76.7)</td>
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<td>&lt;5 Years</td>
<td>5 to &lt;10 Years</td>
<td>10 to &lt;15 Years</td>
<td>15+ Years</td>
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<td>----------</td>
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<tr>
<td></td>
<td>3672 (16.2)</td>
<td>3316 (14.8)</td>
<td>3174 (14.3)</td>
<td>3033 (13.9)</td>
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<td>5 to &lt;10 Years</td>
<td>2293 (10.1)</td>
<td>2008 (9.0)</td>
<td>1823 (8.2)</td>
<td>1618 (7.4)</td>
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<td>10 to &lt;15 Years</td>
<td>1357 (6.0)</td>
<td>1226 (5.5)</td>
<td>1003 (4.5)</td>
<td>914 (4.2)</td>
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<tr>
<td>15+ Years</td>
<td>585 (2.6)</td>
<td>600 (2.7)</td>
<td>496 (2.3)</td>
<td>399 (1.8)</td>
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Table 5.2. Risk of colorectal cancer by quintiles of cumulative average dietary inflammatory index over a ten-year period of time; Women's Health Initiative, 1993-2010

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<th>Years of diet data assessment</th>
<th>Quintile 1 (Healthiest)</th>
<th>Quintile 2</th>
<th>Quintile 3</th>
<th>Quintile 4</th>
<th>Quintile 5 (Least healthy)</th>
<th>( P_{\text{trend}} )</th>
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<td></td>
<td>Referent</td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
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<tr>
<td>Baseline, year 1, DMT</td>
<td>108/7549</td>
<td>104/7436</td>
<td>89/7287</td>
<td>117/7251</td>
<td>119/7116</td>
<td>0.008</td>
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<td>n(cases/non cases)</td>
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<td></td>
</tr>
<tr>
<td>Age adjusted</td>
<td>1.00</td>
<td>1.06 (0.82, 1.36)</td>
<td>0.87 (0.67, 1.14)</td>
<td>1.25 (0.98, 1.60)</td>
<td>1.30 (1.02, 1.66)*</td>
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<tr>
<td>Multivariable-adjusted</td>
<td>1.00</td>
<td>0.97 (0.74, 1.27)</td>
<td>0.85 (0.64, 1.13)</td>
<td>1.11 (0.85, 1.46)</td>
<td>1.16 (0.88, 1.53)</td>
<td>0.13</td>
</tr>
<tr>
<td>Baseline, years 1,2, DMT</td>
<td>100/7734</td>
<td>98/7614</td>
<td>83/7509</td>
<td>101/7414</td>
<td>119/7304</td>
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<td>n(cases/non cases)</td>
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<tr>
<td>Age adjusted</td>
<td>1.00</td>
<td>1.03 (0.79, 1.34)</td>
<td>0.85 (0.65, 1.12)</td>
<td>1.15 (0.86, 1.48)</td>
<td>1.39 (1.08, 1.79)*</td>
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<tr>
<td>Multivariable-adjusted</td>
<td>1.00</td>
<td>0.99 (0.75, 1.31)</td>
<td>0.85 (0.63, 1.13)</td>
<td>1.05 (0.79, 1.39)</td>
<td>1.27 (0.95, 1.68)</td>
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<tr>
<td>Baseline, years 1-3, OS &amp; DMT</td>
<td>226/22427</td>
<td>257/22113</td>
<td>243/21902</td>
<td>223/21659</td>
<td>291/21324</td>
<td>&lt;0.0001</td>
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<td>n(cases/non cases)</td>
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<tr>
<td>Age adjusted</td>
<td>1.00</td>
<td>1.20 (1.01, 1.42)</td>
<td>1.14 (0.96, 1.36)</td>
<td>1.11 (0.93, 1.33)</td>
<td>1.50 (1.26, 1.77)*</td>
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<tr>
<td>Multivariable-adjusted</td>
<td>1.00</td>
<td>1.13 (0.94, 1.35)</td>
<td>1.08 (0.90, 1.30)</td>
<td>1.00 (0.83, 1.21)</td>
<td>1.33 (1.10, 1.61)*</td>
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<tr>
<td>Baseline, years 1-3: OS</td>
<td>137/14424</td>
<td>163/14370</td>
<td>159/14322</td>
<td>132/14247</td>
<td>187/14120</td>
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<td>n(cases/non cases)</td>
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<td>Age adjusted</td>
<td>1.00</td>
<td>1.20 (0.96, 1.49)</td>
<td>1.18 (0.95, 1.48)</td>
<td>1.02 (0.81, 1.29)</td>
<td>1.50 (1.21, 1.86)*</td>
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<tr>
<td>Multivariable-adjusted</td>
<td>1.00</td>
<td>1.16 (0.92, 1.46)</td>
<td>1.13 (0.89, 1.43)</td>
<td>0.94 (0.73, 1.20)</td>
<td>1.34 (1.05, 1.70)*</td>
<td>0.11</td>
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<tr>
<td>Baseline, years 1-3, DMT</td>
<td>n(cases/non cases)</td>
<td>Age adjusted</td>
<td>Multivariable-adjusted</td>
<td>Age adjusted</td>
<td>Multivariable-adjusted</td>
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<td>90/7808</td>
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<tr>
<td>Age adjusted</td>
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<td>1.05 (0.80, 1.38)</td>
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<td>1.20 (0.92, 1.57)</td>
<td>1.37 (1.06, 1.79)*</td>
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<td>1.12 (0.84, 1.51)</td>
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<td>n(cases/non cases)</td>
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<td>1.09 (0.79, 1.49)</td>
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<td>1.07 (0.79, 1.46)</td>
<td>1.17 (0.86, 1.59)</td>
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<td>Baseline, years 1-5, DMT</td>
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<td>n(cases/non cases)</td>
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<td>1.15 (0.83, 1.51)</td>
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<td>Baseline, years 1-10, DMT</td>
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<td>1.28 (0.85, 1.92)</td>
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<td>1.83 (1.10, 3.05)*</td>
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<td>1.45 (0.77, 2.74)</td>
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*Statistically significant; Hazard ratios and 95% confidence interval; All multivariable models were adjusted for age, race/ethnicity, educational level, smoking status, diabetes, hypertension, arthritis, NSAID use, category and duration of estrogen use, category and duration of estrogen & progesterone use, body mass index, physical activity and total energy intake.
Figure 5.2. Multivariable-adjusted hazard ratios for the association between cumulative average DII (highest vs. lowest quintile) and colon cancer risk; Women's Health Initiative Dietary Modification Trial, 1993-2010

Figure 5.3. Multivariable-adjusted hazard ratios for the association between cumulative average DII (highest vs. lowest quintile) and rectal cancer risk; Women's Health Initiative Dietary Modification Trial, 1993-2010
Table 5.3. Risk of colorectal cancer across patterns of change in the dietary inflammatory index (DII) between baseline and year 3; Women's Health Initiative, 1993-2010

<table>
<thead>
<tr>
<th>Patterns of DII changes</th>
<th>Referent</th>
<th>Anti-inflammatory stable</th>
<th>Anti-inflammatory change</th>
<th>Neutral inflammation stable</th>
<th>Pro-inflammatory change</th>
<th>Pro-inflammatory stable</th>
</tr>
</thead>
<tbody>
<tr>
<td>All participants</td>
<td>Referent</td>
<td>HR (95% CI)a</td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
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</tr>
<tr>
<td>Colorectal cancer, n(cases/non cases)</td>
<td>290/23169</td>
<td>131/9168</td>
<td>251/18575</td>
<td>122/9476</td>
<td>277/18025</td>
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</tr>
<tr>
<td>Age adjusted</td>
<td>1.00</td>
<td>1.22 (1.00, 1.49)</td>
<td>1.12 (0.95, 1.32)</td>
<td>1.12 (0.92, 1.38)</td>
<td>1.29 (1.10, 1.52)</td>
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<tr>
<td>Multivariable-adjustedb</td>
<td>1.00</td>
<td>1.13 (0.92, 1.39)</td>
<td>1.04 (0.88, 1.24)</td>
<td>1.04 (0.84, 1.29)</td>
<td>1.18 (0.9, 1.41)*</td>
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</table>

**Risk by colorectal cancer subsite**

<table>
<thead>
<tr>
<th>Colon cancer n(cases/non cases)</th>
<th>249/23210</th>
<th>107/9192</th>
<th>215/18611</th>
<th>103/9495</th>
<th>226/18076</th>
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</thead>
<tbody>
<tr>
<td>Age adjusted</td>
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<td>1.15 (0.92, 1.45)</td>
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<td>1.04 (0.86, 1.25)</td>
<td>1.01 (0.80, 1.28)</td>
<td>1.11 (0.91, 1.35)</td>
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<td>Proximalc colon n(cases/non cases)</td>
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<td>63/9192</td>
<td>131/18611</td>
<td>69/9495</td>
<td>142/18076</td>
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<tr>
<td>Age adjusted</td>
<td>1.00</td>
<td>1.19 (0.87, 1.61)</td>
<td>1.18 (0.93, 1.49)</td>
<td>1.28 (0.96, 1.70)</td>
<td>1.40 (1.11, 1.77)</td>
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<td>1.12 (0.83, 1.51)</td>
<td>1.11 (0.87, 1.42)</td>
<td>1.20 (0.90, 1.62)</td>
<td>1.23 (0.96, 1.59)</td>
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<tr>
<td>Distald colon n(cases/non cases)</td>
<td>60/23210</td>
<td>22/9191</td>
<td>55/18611</td>
<td>22/9495</td>
<td>48/18075</td>
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<tr>
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<td>0.98 (0.60, 1.59)</td>
<td>1.16 (0.80, 1.67)</td>
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<td>0.93 (0.65, 1.52)</td>
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<td>0.89 (0.54, 1.47)</td>
<td>0.99 (0.66, 1.49)</td>
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<td>Rectalcancer n(cases/non cases)</td>
<td>46/23413</td>
<td>24/9275</td>
<td>43/18783</td>
<td>20/9578</td>
<td>57/18245</td>
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</tbody>
</table>

**Notes:**
a. Adjusted for age at baseline, race, education level, body mass index, smoking status, alcohol intake, physical activity, energy intake, total fiber intake, vitamin C intake, and supplement use.
b. Adjusted for all variables in addition to focused on inflammatory status.
c. Adjusted for all variables in addition to focused on proximal colon status.
d. Adjusted for all variables in addition to focused on rectal cancer status.

*Significant at p < 0.05.
<table>
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<th>1.19 (0.79, 1.80)</th>
<th>1.13 (0.67, 1.90)</th>
<th>1.70 (1.15, 2.51)</th>
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<tr>
<td><strong>Age adjusted</strong></td>
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<td>1.37 (0.84, 2.25)</td>
<td>1.12 (0.73, 1.72)</td>
<td>1.10 (0.64, 1.88)</td>
<td>1.53 (1.01, 2.32)*</td>
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<tr>
<td><strong>Multivariable-adjusted</strong></td>
<td>1.00</td>
<td>1.24 (0.75, 2.04)</td>
<td>1.12 (0.73, 1.72)</td>
<td>1.10 (0.64, 1.88)</td>
<td>1.53 (1.01, 2.32)*</td>
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*Statistically significant in multivariable models; a Hazard ratio and 95% confidence interval; b All multivariable models were adjusted for age, race/ethnicity, educational level, smoking status, diabetes, hypertension, arthritis, NSAID use, category and duration of estrogen use, category and duration of estrogen & progesterone use, dietary modification trial arm, body mass index, physical activity; c ICD-O-2 codes used to define location of colon cancer include C18.0 (cecum), C18.2 (ascending colon, right colon), C18.3 (hepatic flexure of colon), C18.4 (transverse colon), C18.5 (splenic flexure of colon), C18.6 (descending colon, left colon) and C18.7 (sigmoid colon); d Rectal cancer included all rectum and rectosigmoid cases.
CHAPTER 6

A PROSPECTIVE INVESTIGATION OF CHANGES IN THE INFLAMMATORY POTENTIAL OF DIET AND RISK OF BREAST CANCER IN POSTMENOPAUSAL WOMEN

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1Cancer Prevention and Control Program, University of South Carolina; 2Department of Epidemiology and Biostatistics, University of South Carolina; 3Center for Research in Nutrition and Health Disparities, University of South Carolina; 4Division of Preventive and Behavioral Medicine, University of Massachusetts Medical School; 5Department of Preventive Medicine, University of Tennessee Health Science Center; 6Department of Preventive Medicine, Stony Brook University School of Medicine; 7Division of Biostatistics, University of California, Davis School of Medicine; 8Department of Epidemiology, The University of Iowa; 9Department of Epidemiology & Prevention, Wake Forest School of Medicine.

To be submitted to Breast Cancer Research and Treatment
6.1 Abstract

Introduction: We utilized the dietary inflammatory index (DII) to evaluate associations between cumulative history, and changes over time in dietary inflammatory potential, and risk of breast cancer in the Women’s Health Initiative (WHI). Methods: We included 106,644 postmenopausal women aged 50-79 years recruited from 1993-1998 into the WHI Observational Study and Dietary Modification Trial, and followed through September 30, 2010. We utilized data from food frequency questionnaires (FFQs) to calculate ten cumulative averages of DII, incrementally from baseline to Year 10, categorized each average into quintiles, and used to estimate hazards ratios (HR) and 95% confidence intervals (95% CI) for invasive breast cancer incidence in multiple Cox proportional hazards regression models. We also derived patterns of changes in DII in a subset of 76,329 women between baseline and Year 3, and used multiple Cox regression models to estimate hazard ratios (HR) and 95% CI for incidence of invasive breast cancer and its subtypes. Results: During an average 11.7 years, 4,242 cases of invasive breast cancer were identified. There was no substantial association between any of the ten averages of cumulative DII calculated between baseline and Year 10, and risk of invasive breast cancer. Also, HR revealed no substantial association between changes in DII between baseline and Year 3, and risk of invasive breast cancer or any of its subtypes. Conclusion: We did not observe a significant association between a history of long-term pro-inflammatory diets as well as shorter-term changes in the inflammatory potential of diets, and breast cancer risk in postmenopausal women. Findings imply that lowering the inflammatory potential of diet may not be a major means for breast cancer prevention.
**Key words:** changes in dietary inflammatory potential, breast cancer, dietary patterns, Women’s Health Initiative

### 6.2 Introduction

Breast cancer is the most commonly diagnosed cancer in American women\(^{173}\) and most of the risk factors, including reproductive factors,\(^{290}\) and family history of breast cancer,\(^{291}\) are generally non-modifiable. Diet, a potentially modifiable factor has been implicated in breast carcinogenesis, with specific dietary factors such as alcohol\(^{55}\) and red/processed meat\(^{66,67}\) shown to be associated with increased risk. The fact that people eat meals consisting of a wide variety of individual foods with potentially complex interactions among the foods and nutrients has led to a growing interest in the examination of broader dietary patterns in relation to breast cancer risk.

Results of previous studies examining the association between dietary patterns and breast cancer risk are inconsistent.\(^{41-47,181,182,208-211}\) Some studies have found an increased risk of breast cancer with the Western (or unhealthy) diet pattern\(^{41,42}\) or a reduced risk with the prudent (or healthy) pattern,\(^{181,182}\) while others failed to observe a significant association.\(^{43-45}\) Indeed, some studies have found results contrary to hypothesized associations; that is, higher consumption of the prudent pattern associated with increased risk\(^{42}\) and higher consumption of the Western pattern associated with reduced risk\(^{208}\) of breast cancer. Additionally, findings from three large cohort studies did not support an association between the Western or prudent patterns and breast cancer risk.\(^{44,46,47}\)

Given the central role of chronic inflammation in the carcinogenesis process\(^{110,292,293}\) dietary patterns that modulate inflammation may be more predictive of
breast cancer risk. Additionally, dietary behaviors mainly influence chronic disease outcomes, including breast cancer, when they persist for a longer period of time,\textsuperscript{31} therefore changes in diet over time or the cumulative history of diet over time may be more predictive of breast cancer risk, compared to diet assessed at one point in time. We have shown that the inflammatory potential of diet decreased significantly over time among women enrolled in the Women’s Health Initiative Dietary Modification Trial (WHI DMT) (Tabung FK, Steck SE, Zhang J, Ma Y, Liese AD, Tylavsky FA, et al., unpublished data, 2014). In the current study, we utilized the dietary inflammatory index (DII)\textsuperscript{38,39} to investigate the role of cumulative history, as well as changes in the inflammatory potential of diet, on breast cancer risk in postmenopausal women.

6.3 Methods

6.3.1 Study population

The WHI study is a large and complex clinical investigation of strategies for the prevention and control of some of the most common causes of morbidity and mortality among postmenopausal women. The design of the WHI has been described in detail elsewhere.\textsuperscript{40} Briefly, The WHI began in 1992, implemented in 40 sites across the United States, and enrolled a total of 161,808 women between 1993 and 1998. The WHI enrolled 93,676 women into an Observational Study (OS) and 68,132 participants into Clinical Trials (CT), and followed them until September 30, 2010.\textsuperscript{265} The CTs included three components: Hormone Therapy, calcium and vitamin D, and the DMT. For the DMT, women were randomly assigned to a usual-diet comparison group (n=29,294) or an intervention group (n=19,541) with a 20% low-fat dietary pattern with increased vegetables, fruits, and whole grains. Women who proved to be ineligible for, or who were
unwilling to enroll in the CT components were invited to be part of the prospective cohort of women in the OS.\textsuperscript{40}

Exclusion criteria for both the OS and CT included any medical condition associated with a predicted survival of less than three years, alcoholism, other drug dependency, mental illness (e.g., major depressive disorder), dementia, active participation in another intervention trial and not likely to live in the area for at least 3 years. Demographic information and dietary data were obtained by self-report using standardized questionnaires. Certified staff performed physical measurements, including blood pressure, height and weight, and blood samples at the baseline clinic visit. Women were further excluded from the DMT if their diets were assessed to have <32\% energy from fat.\textsuperscript{266} The WHI protocol was approved by the institutional review boards at the Clinical Coordinating Center (CCC) at the Fred Hutchinson Cancer Research Center (Seattle, WA) and at each of the 40 Clinical Centers.\textsuperscript{14}

6.3.2 Diet assessment

Figure 6.1 describes the administration of food frequency questionnaires (FFQ) in the WHI. During screening for the WHI, all participants completed a baseline FFQ. At follow-up, the FFQ was completed at Year 3 for ~90\% of OS participants. About 92\% of DMT participants completed an FFQ in Year 1, and a random third of participants were invited to complete an FFQ annually from Year 2 until study end (approximately ten years later) (Figure 6.1). There was an average of two FFQs per participant in the OS and three FFQs per participant in the DMT. The 122-item WHI FFQ line item nutrient data was obtained from the University of Minnesota’s Nutrient Data system for research (NDSR) version 4.03_31 software,\textsuperscript{272} which is based on the US Department of
Agriculture Standard Reference Releases and manufacturer information. The WHI FFQ has shown comparable results with 24-hour dietary recall interviews and food records.\textsuperscript{266}

\subsection*{6.3.3 The dietary inflammatory index (DII)}

Details of the development\textsuperscript{38} and construct validation\textsuperscript{39} of the DII have been described elsewhere. Briefly, an extensive literature search was performed to obtain peer-reviewed journal articles that examined the association between six well known inflammatory biomarkers (Interleukin (IL)-1\(\beta\), IL-4, IL-6, IL-10, tumor necrosis factor alpha, and C-reactive protein) and 45 specific foods and nutrients (components of the DII). Scores were derived and standardized to a representative global diet database constructed based on 11 datasets from diverse populations in different parts of the world. Overall DII scores for each individual participant represent the sum of each of the DII components in relation to the comparison global diet database.\textsuperscript{38} The DII score characterizes an individual’s diet on a continuum from maximally anti-inflammatory to maximally pro-inflammatory, with a higher DII score indicating a more pro-inflammatory diet and a lower (i.e., more negative) DII score indicating a more anti-inflammatory diet. In the WHI FFQ, 32 of the 45 original DII components were available for inclusion in the overall DII score (see\textsuperscript{38} for list of 45 DII components). Components such as ginger, turmeric, garlic, oregano, pepper, rosemary, eugenol, saffron, flavan-3-ol, flavones, flavonols, flavonones, anthocyanidins that are included in the original DII calculation\textsuperscript{38} were not included in the current study because they were not available from the WHI FFQ.
6.3.4 Outcomes assessment

The WHI outcomes ascertainment and adjudication process has been previously described. Briefly, participants (or next-of-kin) self-reported cancer diagnoses reported on questionnaires annually in the OS or semiannually in the CT through 2005 and annually in all thereafter. Invasive breast cancer was documented and coded according to primary site, diagnosis date, extent of disease (stage, tumor size, and laterality), tumor morphology (behavior, grade, histology) her2neu status and estrogen and progesterone receptors (ER, PR) status. Incident invasive breast cancer, including second primaries, were ascertained and adjudicated, but recurrent cancers were not included. For the full coding of the cancer, pathology reports from diagnostic aspirations, biopsies, and surgeries, plus the discharge summary, were used.

Breast cancer outcomes for the current study included invasive breast cancer, and molecular and histologic subtypes of breast cancer. Molecular subtypes were defined based on previous work by Carey et al., as follows: triple negative (HER2−, ER−, PR−), (HER2+, ER−, PR−) subtype, luminal A (ER+ and/or PR+, HER2−), and luminal B (ER+ and/or PR+, HER2+). The histological subtypes were defined based on SEER morphology codes. These included ductal carcinoma (including intraductal carcinoma, 8500/2, and infiltrating ductal carcinoma, 8500/3), lobular carcinoma (including lobular carcinoma, 8520/3, and lobular carcinoma in situ, 8520/2), and a combination of ductal and lobular carcinomas (8522/3 and 8520/2). Survival time was defined as days from enrollment or randomization until breast cancer diagnosis while censoring time was defined as days from enrollment or randomization until death or last contact occurring on or before September 30, 2010, in participants without breast cancer.
6.3.5 Statistical analysis

We utilized data from 142,511 women participating in the WHI OS (93,676) and DMT (48,835). Exclusion criteria included women with a history of breast cancer at baseline or missing breast cancer status at baseline (n=5,078), those who reported breast removal at baseline (n=277), or those with single FFQs or missing FFQs (n=14,655), as well as women with implausible reported total energy intake values (≤600 kcal/day or ≥5000 kcal/day) (n=1,796) or extreme BMI values (<15kg/m² or > 50kg/m²) (n=1,991) (Figure 6.1). Additionally, participants with missing data in the covariates listed below (n=10,797) were excluded, leaving a total of 106,644 participants for these analyses (68,319 in OS and 38,325 in DMT). Frequencies and percentages were computed to describe the distribution of covariates across quintiles of cumulative average DII for the DII assessed from baseline to Year 3.

To determine how cumulative history of the inflammatory potential of diet, affects breast cancer risk over time, we calculated cumulative averages of DII incrementally starting from the average between baseline and year one DII. The cumulative average was then categorized into quintiles, and used in multiple Cox proportional hazards (PH) models to estimate hazard ratios (HR) for the incidence of invasive breast cancer, while excluding breast cancer cases diagnosed prior to year one. This approach was repeated for the average DII of baseline, year one, and year two with breast cancer cases diagnosed prior to year two excluded to avoid the possibility of change in diet due to subclinical disease and to include only participants at risk of developing breast cancer going forward. This approach was repeated until DII estimates at all ten time points were used.
To determine how changes in patterns of the inflammatory potential of diet over time affect breast cancer risk, we calculated the DII from baseline and year 3 FFQs administered to 76,329 women in the OS and DMT. We categorized the DII at both time points into quintiles (Q) and further categorized changes in the inflammatory potential of diet based on quintile differences between baseline and year 3, as follows:

6. Anti-inflammatory stable: Q1 or Q2 at both time points or change from Q3 to Q2;
7. Anti-inflammatory change: changes ≤ -2Q;
8. Neutral inflammation stable: changes from Q2 to Q3, Q4 to Q3 or stable at Q3 at both time points;
9. Pro-inflammatory change: changes ≥ 2Q;
10. Pro-inflammatory stable: Q4 or Q5 at both time points, or change from Q3 to Q4.

The names given to these categories of DII changes were meant to be qualitative. Next, Cox regression PH models were used to estimate hazard ratios (HR) and 95% CI for the incidence of invasive breast cancer including the molecular and histological subtypes, by patterns of DII changes and with adjustment for multiple covariates. The anti-inflammatory stable category, considered to be the healthiest category, was the referent for all models.

All multivariable-adjusted models included the following covariates as potential confounders based on ≥10% change in HR between age-adjusted models with and without the potential confounder: age group (years) (50-59, 60-69, 70-79), race/ethnicity (European American (EA), African American (AA), Hispanic (HP), Asian or Pacific Islander (A/PI) and Other); educational levels (less than high school, some high school /GED, at least some college/graduate education), smoking status (current, past, and
never), body mass index [BMI= weight(kg)/height(m)^2] (normal weight (<25kg/m^2), overweight (25＜30 kg/m^2), and obese (≥30kg/m^2)); physical activity (PA), categorized based on public health recommendations, as meeting or not meeting PA recommendations (≥150 minutes/week of moderate intensity PA or ≥75 minutes/week of vigorous intensity PA versus<150 minutes/week of moderate intensity PA or <75 minutes/week of vigorous intensity PA, respectively); use of non-steroidal anti-inflammatory drug (NSAID) (yes/no); category and duration of estrogen use and category and duration of combined estrogen and progesterone use both categorized into five groups (none, <5y, 5 to <10y, 10 to <15y, and ≥15y), total energy intake (Kcal/day).

Some covariates did not change HR of the association between age-adjusted DII and breast cancer risk by ≥10% and were therefore not included in the final models. These included study participation (OS/DMT), age at menarche, age at first birth, number of live births, total duration of breastfeeding, mammography in the 2 years preceding study enrollment, oophorectomy status, and first degree relative with breast cancer. Data on potential confounders were collected by self-administered questionnaires on demographics, medical history, and lifestyle factors.

Each covariate in the final models of both the cumulative average DII and patterns of DII change was tested for proportional hazards using cumulative sums of Martingale-based residuals. Age group and combined use of estrogen and progesterone violated the PH assumption and all models were therefore stratified by these two covariates. To determine whether the association between both the cumulative average DII and changes in the DII and breast cancer incidence differed by age, race/ethnicity, education, BMI and combined use of estrogen and progesterone, we included interaction
terms for these covariates in the models and assessed significant effect modification at p <0.05. None of the interaction terms was significant. We evaluated 95% CIs to determine statistical significance. Statistical analyses were conducted using SAS version 9.3 (SAS Institute, Cary, NC), and all tests were two-sided.

6.4 Results

Table 6.1 presents the distribution of participants’ characteristics across quintiles of the cumulative average DII between baseline and Year 3. Participants with higher cumulative average DII scores (representing a more pro-inflammatory diet) consisted of a higher proportion of women who were AA or HP, overweight or obese, current smokers, not meeting physical activity guidelines, and with lower educational attainment. In contrast, participants with a more anti-inflammatory diet consisted of a higher proportion of women who were EA or A/PI, had a normal BMI, were highly educated, and adhered to physical activity guidelines. Participants were followed for an average 11.7 years, during which 4,242 cases of invasive breast cancer were identified.

Table 6.2 presents HR of the association between cumulative average DII and risk of invasive breast cancer. There was an inverse association between cumulative DII and invasive breast cancer comparing quintiles 2 and 1 in the first year of follow-up (HR, 0.81; 95%CI, 0.70, 0.93; \( P_{\text{trend}} \), 0.14), and in the second year of follow-up (HR, 0.84; 95%CI, 0.73, 0.98; \( P_{\text{trend}} \), 0.35), but the trends across quintiles of cumulative average DII were not significant for these inverse associations. No other statistically significant associations between averages of cumulative DII and risk of invasive breast cancer were observed in the multiple adjusted models.
Table 6.3 shows HR for the association between changes in DII between baseline and Year 3, and risk of invasive breast cancer and its subtypes. Overall, there was no substantial association between changes in DII over time and total breast cancer or any of its subtypes. However, there was an inverse association between changes in DII and risk of triple negative breast cancer (HR, 0.47; 95%CI, 0.28, 0.79), comparing participants in the anti-inflammatory change category to those in the anti-inflammatory stable category of changes in DII between baseline and Year 3. In participants with (HER2+, ER−, PR−) subtype of breast cancer, HRs were indicative of a positive association, comparing participants in the pro-inflammatory stable category to those in the anti-inflammatory stable category of changes in DII, but did not attain statistical significance (HR, 1.60; 95%CI, 0.91, 2.80) (Table 6.3).

6.5 Discussion

In this large prospective study of the role of cumulative history, and changes in the inflammatory potential of diet over time in breast cancer risk, we observed no significant association between either 1) the cumulative history of dietary inflammatory potential, or 2) changes in dietary inflammatory potential over time, and risk of invasive breast cancer or subtypes of breast cancer in postmenopausal women, with the exception of a reduced risk of triple negative breast cancer among women who moved toward a more anti-inflammatory diet compared to those who consumed a more stable anti-inflammatory diet from baseline to Year 3. To the best of our knowledge, this is the first study to characterize the association between the cumulative history, or changes over time in the inflammatory potential of diet, and risk of breast cancer. Given that FFQs were administered to OS participants at baseline and Year 3 only; a cumulative average
DII could be calculated for OS participants at Year 3 only and analyses at all other time points included DMT participants. We selected these two time points for the analyses of changes in the DII over time, to include the maximum number of participants with FFQs (Figure 6.1).

Our results are generally similar to many previous prospective studies that did not observe significant associations between dietary patterns and breast cancer risk, though these other studies assessed diet quality at only one point in time. Other previous studies have described heterogeneity of the association between dietary patterns and breast cancer by hormone receptor status. Cottet et al. found evidence of an increased risk of ER+/PR+ tumors with a Western dietary pattern and reduced risk of ER+/PR− tumors with a Mediterranean pattern in a French Cohort study. Fung et al found that higher consumptions of fruits and vegetables was significantly associated with decreased risk for ER- breast cancer in the Nurses Health Study, while Gaudet et al., found an inverse association between high fruit and vegetable intake and breast cancer risk among postmenopausal women with ER+ tumors. We cannot rule out that chance may account for our finding of an inverse association for invasive breast cancer comparing quintile 2 with quintile 1 of cumulative average DII in the first two years of follow-up in the DMT; and an inverse association in participants with triple negative tumors; given the number of comparisons made in this study.

Two potential mechanisms by which diet may affect breast carcinogenesis include hyperinsulinemia and inflammation. Generally, dietary patterns have been shown to modulate inflammation, and inflammation exerts an important role in the carcinogenesis process. However, our findings imply that inflammation may not be a
primary mechanism through which diet substantially influences breast cancer risk. Obesity, a state of low-grade chronic inflammation\textsuperscript{229,230} and a risk factor for breast cancer in postmenopausal women,\textsuperscript{301} has been suggested to increase breast cancer risk mainly through the hormonal pathway, with increased exposure to endogenous estrogen from adipose tissue.\textsuperscript{302,303} Indeed, though concentrations of inflammatory markers have been found to be higher in obese than normal weight women,\textsuperscript{304} a meta-analysis of prospective studies did not find an association between inflammatory biomarkers and breast cancer risk.\textsuperscript{305} further indicating that inflammation may not play an important role in breast cancer development. Also, there was no data on inflammatory breast cancer in the WHI, for an assessment of the association of the inflammatory potential of diet and risk of this subtype of breast cancer.

While most of the evidence is consistent that chronic inflammation increases the risk of breast cancer recurrence or survival,\textsuperscript{94-96} the evidence has been inconsistent for the association between biomarkers of inflammation and breast cancer incidence. Studies of the association between regular use of aspirin and other NSAIDS and risk of breast cancer in postmenopausal women have found inconsistent results.\textsuperscript{300,306} However, two meta-analyses showed that regular use of aspirin and other NSAIDs is associated with reduced risk of breast cancer,\textsuperscript{307,308} though these findings were not supported by results from randomized controlled trials.\textsuperscript{206,309}

In contrast, hyperinsulinemia may play a more important role in breast carcinogenesis. Hyperinsulinemia largely explained the association between obesity and postmenopausal breast cancer in a case-cohort study,\textsuperscript{299} while glucose and insulin-like
growth factors have been found to be positively associated with breast cancer development in postmenopausal women.\textsuperscript{310}

Strengths of the current study include accounting for changes in the inflammatory potential of diet over time in a large, well-characterized population of more than 106,000 women, a long follow-up period, the inclusion of women of diverse race/ethnic groups, and the central adjudication of breast cancer diagnosis. The use of a novel dietary index to score diet quality based on inflammatory potential at multiple time points provides evidence that inflammation may not be substantially linked to breast cancer risk. Limitations include known measurement error in using an FFQ for the assessment of diet and its inflammatory potential over time, and potential residual or unmeasured confounding though we adjusted for many potential confounders in the models. We assumed that the random 30\% of DMT participants sampled from year 2 until study end was representative of the entire DMT study population, a plausible assumption since these random subsamples were used for intervention monitoring in the DMT, though the sample size reduced substantially in the last two years of follow-up.

6.6 Conclusion

In this large prospective study, we did not observe a significant association between a history of long-term pro-inflammatory diets as well as shorter-term changes in the inflammatory potential of diets, and breast cancer risk in postmenopausal women. Our findings imply that lowering the inflammatory potential of diet may not be a major means for breast cancer prevention and that if there is a role for diet in breast cancer prevention; it is likely through other mechanisms.
6.7 Tables and figures

Figure 6.1. Participant flow in the administration of food frequency questionnaires in the Women’s Health Initiative (WHI) Observational Study (OS) and Dietary Modification Trial (DMT), 1993-2010
Table 6.1. Frequencies (%) of participant's characteristics across quintiles of cumulative average DII (Years 0-3); Women's Health Initiative, 1993-2010

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Q1 (-6.586, &lt; -3.166) (healthiest)</th>
<th>Q2 (-3.166, &lt; -2.085)</th>
<th>Q3 (-2.085, &lt; -0.710)</th>
<th>Q4 (&lt; -0.710, 1.063)</th>
<th>Q5 (1.063, 5.255) (least healthy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age groups (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50-59</td>
<td>6703 (30.8)</td>
<td>6541 (30.4)</td>
<td>6940 (32.6)</td>
<td>7344 (34.8)</td>
<td>7830 (37.4)</td>
</tr>
<tr>
<td>60-69</td>
<td>10346 (47.5)</td>
<td>10103 (46.9)</td>
<td>9775 (45.9)</td>
<td>9437 (44.7)</td>
<td>9195 (43.9)</td>
</tr>
<tr>
<td>70-79</td>
<td>4715 (21.7)</td>
<td>4890 (22.7)</td>
<td>4582 (21.5)</td>
<td>4325 (20.5)</td>
<td>3918 (18.7)</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian or Pacific Islander</td>
<td>964 (4.4)</td>
<td>518 (2.4)</td>
<td>551 (2.6)</td>
<td>512 (2.4)</td>
<td>378 (1.8)</td>
</tr>
<tr>
<td>African American</td>
<td>718 (3.3)</td>
<td>1000 (4.6)</td>
<td>1380 (6.5)</td>
<td>18796 (8.9)</td>
<td>3052 (14.6)</td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>316 (1.5)</td>
<td>426 (2.0)</td>
<td>636 (3.0)</td>
<td>823 (3.9)</td>
<td>1152 (5.5)</td>
</tr>
<tr>
<td>European American</td>
<td>19473 (89.5)</td>
<td>19303 (89.6)</td>
<td>18418 (86.5)</td>
<td>17556 (83.2)</td>
<td>16007 (76.4)</td>
</tr>
<tr>
<td>Other</td>
<td>293 (1.3)</td>
<td>2879 (1.4)</td>
<td>305 (1.4)</td>
<td>336 (1.6)</td>
<td>354 (1.7)</td>
</tr>
<tr>
<td>Educational level</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; High school</td>
<td>83 (0.4)</td>
<td>143 (0.7)</td>
<td>205 (1.0)</td>
<td>257 (1.2)</td>
<td>415 (2.0)</td>
</tr>
<tr>
<td>Some high school/GED</td>
<td>4210 (19.3)</td>
<td>56482 (26.2)</td>
<td>6193 (29.1)</td>
<td>6829 (32.4)</td>
<td>8123 (38.8)</td>
</tr>
<tr>
<td>Some years of college/graduate</td>
<td>17471 (80.3)</td>
<td>157434 (73.1)</td>
<td>14899 (69.9)</td>
<td>14020 (66.4)</td>
<td>12405 (59.2)</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>10870 (49.9)</td>
<td>11198 (52.0)</td>
<td>11102 (52.1)</td>
<td>10994 (52.1)</td>
<td>10757 (51.4)</td>
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<tr>
<td>Former</td>
<td>101481 (46.6)</td>
<td>9308 (43.2)</td>
<td>9004 (42.3)</td>
<td>8724 (41.3)</td>
<td>8209 (39.2)</td>
</tr>
<tr>
<td>Current</td>
<td>746 (3.4)</td>
<td>1028 (4.8)</td>
<td>1191 (5.6)</td>
<td>1388 (6.6)</td>
<td>1977 (9.4)</td>
</tr>
<tr>
<td>Body mass index (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal weight (&lt;25)</td>
<td>9236 (42.4)</td>
<td>7897 (36.7)</td>
<td>7290 (34.2)</td>
<td>6731 (31.9)</td>
<td>6012 (28.7)</td>
</tr>
<tr>
<td>Weight Category</td>
<td>Physical Activity Recommendations</td>
<td>Physical Activity (PA), minutes/week</td>
<td></td>
<td></td>
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<td>-----------------</td>
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<tr>
<td>Overweight (25.0 - &lt;30)</td>
<td>2536 (11.4)</td>
<td>2788 (13.3)</td>
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<tr>
<td>Obese (≥30)</td>
<td>5093 (23.4)</td>
<td>6046 (28.1)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Physical activity (PA), minutes/week</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not meeting PA recommendations</td>
<td>9215 (42.3)</td>
<td>114731 (53.3)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Meeting PA recommendations</td>
<td>125497 (57.7)</td>
<td>100611 (46.7)</td>
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<tr>
<td>NSAIDs use</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>No</td>
<td>9135 (42.0)</td>
<td>8684 (40.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>12629 (58.0)</td>
<td>12850 (59.7)</td>
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<tr>
<td>Duration of estrogen use by category</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>13342 (61.3)</td>
<td>12972 (60.2)</td>
<td></td>
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<tr>
<td>&lt;5 Years</td>
<td>2682 (12.3)</td>
<td>2720 (12.6)</td>
<td></td>
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<tr>
<td>5 to &lt;10 Years</td>
<td>1746 (8.0)</td>
<td>1713 (8.0)</td>
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<tr>
<td>10 to &lt;15 Years</td>
<td>1480 (6.8)</td>
<td>1412 (6.6)</td>
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<td></td>
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<tr>
<td>15+ Years</td>
<td>2514 (11.6)</td>
<td>2717 (12.6)</td>
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<td>Duration of estrogen and progesterone use by category</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>None</td>
<td>14138 (65.0)</td>
<td>14636 (68.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5 Years</td>
<td>3551 (16.3)</td>
<td>3228 (15.0)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>5 to &lt;10 Years</td>
<td>2206 (10.1)</td>
<td>1906 (8.9)</td>
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<tr>
<td>10 to &lt;15 Years</td>
<td>1308 (6.0)</td>
<td>1177 (5.4)</td>
<td></td>
<td></td>
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<tr>
<td>15+ Years</td>
<td>561 (2.6)</td>
<td>587 (2.7)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Table 6.2. Risk of invasive breast cancer by quintiles of cumulative average dietary inflammatory index over a ten-year period of time; Women's Health Initiative, 1993-2010

<table>
<thead>
<tr>
<th>Quintile 1 (Healthiest)</th>
<th>Quintile 2</th>
<th>Quintile 3</th>
<th>Quintile 4</th>
<th>Quintile 5 (Least healthy)</th>
<th>( P_{\text{trend}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Referent</td>
<td>HR (95% CI)(^a)</td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
<td>( P_{\text{trend}} )</td>
</tr>
<tr>
<td><strong>Baseline, year 1, DMT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast cancer n(cases/non cases)(^b)</td>
<td>419/7257</td>
<td>329/7231</td>
<td>353/7058</td>
<td>399/6995</td>
<td>365/6928</td>
</tr>
<tr>
<td>Age adjusted</td>
<td>1.00</td>
<td>0.85 (0.74, 0.97)</td>
<td>0.89 (0.78, 1.01)</td>
<td>1.02 (0.89, 1.16)</td>
<td>0.96 (0.84, 1.09)</td>
</tr>
<tr>
<td>Multivariable-adjusted</td>
<td>1.00</td>
<td>0.81 (0.70, 0.93)*</td>
<td>0.90 (0.78, 1.04)</td>
<td>1.04 (0.91, 1.20)</td>
<td>0.99 (0.86, 1.15)</td>
</tr>
<tr>
<td><strong>Baseline, years 1-2, DMT</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Breast cancer n(cases/non cases)</td>
<td>387/7431</td>
<td>317/7417</td>
<td>335/7245</td>
<td>364/7165</td>
<td>332/7128</td>
</tr>
<tr>
<td>Age adjusted</td>
<td>1.00</td>
<td>0.86 (0.75, 0.99)</td>
<td>0.90 (0.78, 1.03)</td>
<td>0.99 (0.87, 1.13)</td>
<td>0.96 (0.84, 1.10)</td>
</tr>
<tr>
<td>Multivariable-adjusted</td>
<td>1.00</td>
<td>0.84 (0.73, 0.98)*</td>
<td>0.93 (0.80, 1.07)</td>
<td>1.03 (0.89, 1.19)</td>
<td>0.98 (0.84, 1.15)</td>
</tr>
<tr>
<td><strong>Baseline, years 1-3, OS and DMT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast cancer n(cases/non cases)</td>
<td>909/20855</td>
<td>885/20649</td>
<td>832/20465</td>
<td>819/20287</td>
<td>797/20146</td>
</tr>
<tr>
<td>Age adjusted</td>
<td>1.00</td>
<td>1.00 (0.92, 1.10)</td>
<td>0.97 (0.88, 1.06)</td>
<td>0.97 (0.88, 1.06)</td>
<td>0.98 (0.90, 1.08)</td>
</tr>
<tr>
<td>Multivariable-adjusted</td>
<td>1.00</td>
<td>1.00 (0.91, 1.10)</td>
<td>0.98 (0.89, 1.07)</td>
<td>0.99 (0.90, 1.10)</td>
<td>1.02 (0.92, 1.14)</td>
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<td><strong>Baseline, years 1-3: OS</strong></td>
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<td></td>
</tr>
<tr>
<td>Breast cancer n(cases/non cases)</td>
<td>561/13155</td>
<td>578/13121</td>
<td>537/13142</td>
<td>506/13102</td>
<td>476/13141</td>
</tr>
<tr>
<td>Age adjusted</td>
<td>1.00</td>
<td>1.05 (0.94, 1.18)</td>
<td>0.99 (0.88, 1.11)</td>
<td>0.96 (0.85, 1.08)</td>
<td>0.93 (0.82, 1.05)</td>
</tr>
<tr>
<td>Baseline, years 1-3, DMT</td>
<td>Breast cancer</td>
<td>n(cases/non cases)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
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<td>1.02 (0.86, 1.20)</td>
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<td>0.93 (0.78, 1.11)</td>
<td>1.02 (0.86, 1.22)</td>
<td>1.08 (0.89, 1.30)</td>
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<td>1.00 (0.84, 1.19)</td>
<td>1.02 (0.85, 1.22)</td>
<td>1.11 (0.93, 1.32)</td>
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<td>1.03 (0.84, 1.25)</td>
<td>1.07 (0.88, 1.22)</td>
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</tr>
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<td>1.00 (0.84, 1.19)</td>
<td>1.00 (0.84, 1.22)</td>
<td>1.11 (0.93, 1.32)</td>
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<tr>
<td>Multivariable-adjusted</td>
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<td>0.97 (0.80, 1.18)</td>
<td>1.03 (0.84, 1.25)</td>
<td>1.07 (0.88, 1.22)</td>
</tr>
<tr>
<td>Age adjusted</td>
<td>Multivariable-adjusted</td>
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<tr>
<td>Baseline, years 1-8, DMT</td>
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<td>149/7839</td>
<td>145/7756</td>
<td>141/7685</td>
<td>160/7450</td>
<td>137/7506</td>
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<td>1.13 (0.92, 1.39)</td>
<td>1.10 (0.89, 1.36)</td>
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<td>1.01 (0.80, 1.27)</td>
<td>1.22 (0.96, 1.53)</td>
<td>1.11 (0.86, 1.43)</td>
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<td>Breast cancer n(cases/non cases)</td>
<td>114/7836</td>
<td>112/7765</td>
<td>118/7653</td>
<td>121/7488</td>
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<td>Age adjusted</td>
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<td>0.99 (0.78, 1.26)</td>
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<td>1.15 (0.97, 1.46)</td>
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<td>1.20 (0.92, 1.56)</td>
<td>1.19 (0.89, 1.58)</td>
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<tr>
<td>Baseline, years 1-10, DMT</td>
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<tr>
<td>Breast cancer n(cases/non cases)</td>
<td>78/7848</td>
<td>80/7761</td>
<td>87/7625</td>
<td>92/7501</td>
<td>82/7526</td>
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<tr>
<td>Age adjusted</td>
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<td>1.04 (0.78, 1.38)</td>
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<td>1.19 (0.91, 1.57)</td>
<td>1.23 (0.93, 1.62)</td>
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<tr>
<td>Multivariable-adjusted</td>
<td>1.00</td>
<td>1.09 (0.80, 1.49)</td>
<td>1.21 (0.88, 1.65)</td>
<td>1.34 (0.98, 1.83)</td>
<td>1.32 (0.94, 1.84)</td>
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*Statistically significant multivariable-adjusted HR; ^HR=hazard ratio, CI=confidence interval; cases/non-cases in the multivariable models; all models were adjusted for age, race/ethnicity, education, smoking status, physical activity, body mass index, NSAID use, category and duration of estrogen use, category and duration of estrogen & progesterone use, and total energy intake.
Table 6.3. Risk of breast cancer by subtype, across patterns of change in the dietary inflammatory index (DII) between baseline and year 3; Women's Health Initiative, 1993-2010

<table>
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<tr>
<th>Patterns of DII quintile changes</th>
<th>Referent</th>
<th>Anti-inflammatory stable</th>
<th>Anti-inflammatory change</th>
<th>Neutral inflammation stable</th>
<th>Pro-inflammatory change</th>
<th>Pro-inflammatory stable</th>
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<tr>
<td>Invasive breast cancer</td>
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<td>0.89 (0.81, 0.99)</td>
<td>0.88 (0.81, 0.95)</td>
<td>0.90 (0.81, 1.00)</td>
<td>0.86 (0.79, 0.93)</td>
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<tr>
<td>Breast cancer cases/non-cases</td>
<td>1293/21015</td>
<td>456/8542</td>
<td>897/17163</td>
<td>473/8743</td>
<td>835/16912</td>
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<tr>
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<td>0.91 (0.83, 0.99)*</td>
<td>0.98 (0.88, 1.10)</td>
<td>0.94 (0.85, 1.04)</td>
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<tr>
<td>Multivariable adjusted model</td>
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<tr>
<td>Molecular subtypes of breast cancer</td>
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<tr>
<td>Triple negative (HER2−, ER−, PR−)</td>
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<td>Breast cancer cases/non-cases</td>
<td>90/21015</td>
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<td>64/17163</td>
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<td>1.07 (0.74, 1.55)</td>
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<td>1.02 (0.68, 1.52)</td>
<td>0.93 (0.66, 1.33)</td>
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<td>HER2+/ER− subtype (HER2+, ER−, PR−)</td>
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<td>Breast cancer cases/non-cases</td>
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<td>28/17163</td>
<td>15/8743</td>
<td>33/16912</td>
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<td>Breast cancer cases/non-cases</td>
<td>Age adjusted model</td>
<td>Multivariable adjusted model</td>
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<td>luminal A (ER+ and/or PR+, HER2−)</td>
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<td>257/8743</td>
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<td>404/16912</td>
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<td>luminal B (ER+ and/or PR+, HER2+)</td>
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<td>1.00</td>
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<td>0.92 (0.81, 1.05)</td>
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<td>Breast cancer cases/non-cases</td>
<td>189/21015</td>
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<td>119/17163</td>
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<td>(0.66, 1.06)</td>
<td>0.78</td>
<td>(0.57, 1.06)</td>
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</table>

*Statistically significant multivariable-adjusted HR; \(^*HR=\)hazard ratio, CI=confidence interval; \(^*\text{cases/non-cases in the multivariable models} \(^*\text{all models were adjusted for age, race/ethnicity, education, smoking status, physical activity, body mass index, NSAID use, category and duration of estrogen use, category and duration of estrogen & progesterone use, and total energy intake.}
CHAPTER 7

LONGITUDINAL CHANGES IN DII AND RISK OF CANCER: A DISCUSSION OF STUDY RESULTS

7.1 Summary of results

Our hypothesis for this dissertation was that the inflammatory potential of diet changes over time and that long-term pro-inflammatory diets or shorter-term changes towards pro-inflammatory diets increase risk of colorectal cancer and of breast cancer. Using data from both the WHI OS and DMT, we first described changes over time in the inflammatory potential of diet using the DII, and showed that the DII score in the OS remained relatively stable from baseline to Year 3, while the DII decreased substantially from baseline to Year 1 in the DMT intervention arm, achieving the lowest mean score in Year 3, and then increasing gradually until study end while still remaining lower than baseline throughout the study period. The longitudinal trend of changes in DII was similar in both arms of the DMT; however, changes in the intervention arm were almost double those observed in the control arm during the first five years of follow-up. In both the OS and DMT, participants who experienced the largest DII decrease were more likely to have a normal BMI, a high educational level, and were A/PI or EA, while those who experienced the smallest decrease were more likely to be obese, had less than high school education, and were HP or AA.
Secondly, we demonstrated that: 1) a higher cumulative average score of the DII is associated with an increased risk of colorectal cancer especially colon cancer, while 2) a stable pro-inflammatory diet over a 3-year period increases the risk of rectal cancer. We found no substantial association between either cumulative average DII or shorter-term changes in DII and breast cancer, including molecular and histologic subtypes of breast cancer. To the best of our knowledge, this is the first study to characterize longitudinal changes in the inflammatory potential of diet and the association between the cumulative history, and changes over time in the inflammatory potential of diet, and risk of colorectal cancer or breast cancer.

7.2 Potential mechanisms of action

The link between inflammation and colorectal cancer is supported by findings from several studies showing either a reduced risk of colorectal cancer with regular use of NSAIDs,\textsuperscript{83,84} or a positive association between higher concentrations of inflammatory biomarkers and colorectal cancer risk.\textsuperscript{89,113} Other potential mechanisms through which a pro-inflammatory diet may increase risk of colorectal cancer include components of the metabolic syndrome, especially insulin resistance or glucose intolerance,\textsuperscript{286-288} and the microbiota. A high and sustained pro-inflammatory potential of the diet may compromise the host-microbiota mutualism favoring the proliferation of toxic bacteria that have been suggested to promote colon carcinogenesis.\textsuperscript{289} For breast cancer, two potential mechanisms through which diet may affect breast cancer risk include hyperinsulinemia\textsuperscript{297-299} and inflammation.\textsuperscript{10,300} Generally, dietary patterns have been shown to modulate inflammation,\textsuperscript{48-50} and inflammation exerts an important role in the carcinogenesis process,\textsuperscript{10,300} but our findings imply that inflammation may not be a
substantial mechanism through which diet may influence breast cancer risk. Obesity, a state of low-grade chronic inflammation\textsuperscript{229,230} and a risk factor for breast cancer in postmenopausal women,\textsuperscript{301} has been suggested to increase breast cancer risk mainly through the hormonal pathway, with increased exposure to endogenous estrogen from adipose tissue.\textsuperscript{302,303} Indeed, though concentrations of inflammatory markers have been found to be higher in obese than normal weight women,\textsuperscript{304} a meta-analysis of prospective studies did not find an association between inflammatory biomarkers and breast cancer risk,\textsuperscript{305} further indicating that inflammation may not play an important role in breast cancer development.

### 7.3 Strengths and limitations

Major strengths of this study are the use of a large, well-characterized cohort (the WHI) with adequate number of outcomes providing ample power to detect significant associations. The DMT had a relatively long follow-up duration with diet assessed annually in random subsamples of the study population. Also, the use of a novel dietary index to score diet quality based on inflammatory potential at multiple time points provides evidence that inflammation may be substantially linked to colorectal cancer but not to breast cancer risk. Other strengths include accounting for changes in the inflammatory potential of diet over time, good regional and racial/ethnic representation, and the central adjudication of colorectal cancer and breast cancer diagnoses.

Limitations to our study included the following: FFQ data were not available in the OS after Year 3 and thus we were not able to compare dietary behavior change between the OS and DMT beyond the first three years of follow up. The decrease in dietary inflammatory potential over time may have been due to survey learning effects
rather than a real improvement in diet quality. In our DMT sample, not every participant had FFQ data at all 11 time points, which could have reduced the effect of survey learning as participants did not complete the FFQs every year. We assumed that the random 30% of DMT participants sampled from year 2 until study end was representative of the entire DMT study population, a plausible assumption since these random subsamples were used for intervention monitoring in the DMT. However, sample sizes from Year 8 to 10 were very small and may not be representative of the entire DMT population. WHI enrolled only postmenopausal women, so generalizability and interpretation of our results is restricted to this population; however, average DII scores in the WHI were comparable to other US populations that have been examined.\textsuperscript{39,282}

Other limitations include known measurement error in using an FFQ for the assessment of diet and its inflammatory potential over time, and potential residual or unmeasured confounding, though we adjusted for many potential confounders in the models.

7.4 Public health implications

This dissertation addressed an important priority area of cancer research that includes the role of dietary patterns in relation to risk of cancer. The study is highly innovative in that this is the first time that repeated measures of the DII are being used to evaluate the association between changes in the dietary inflammatory potential over time and cancer endpoints. Our findings suggest lowering the inflammatory potential of diet as a means for colon cancer, and potentially rectal cancer prevention in postmenopausal women, but we did not find enough evidence that this potential prevention strategy may apply to breast cancer. Nevertheless, striving towards a more anti-inflammatory diet may have other potential health benefits beyond cancer prevention.
Patients at risk of inflammation-related conditions such as osteoporosis, obesity, cardiovascular disease, and diabetes, may also be at risk of cancer.\textsuperscript{51,52} Therefore a reduction in the inflammatory potential of the diet among patients with these conditions may improve overall health and reduce their cancer risk. We found that changes in the DII over time in the DMT were significantly modified by BMI, education and race/ethnicity, therefore interventions to reduce the inflammatory potential of diet need to incorporate differences in these lifestyle and demographic variables, in their designs. The diagnosis of most of these chronic diseases may be also a teachable moment during which most patients undergo lifestyle changes including diet changes to improve their survival experience,\textsuperscript{53,54} therefore health professionals armed with the knowledge of changes in the inflammatory potential of diets may be able to impart sound nutritional guidance that improves the overall health of patients with inflammation-related chronic diseases.

Our finding of a relatively stable dietary inflammatory potential in an observational setting could mean that diet assessment at any point in time in a ~10 year observational study of postmenopausal women could be equally useful in determining disease risk estimates. The same conclusion would not apply for dietary intervention studies where we demonstrated that diet quality with respect to its inflammatory potential improves significantly over time in women enrolled in a low-fat, high-fiber, high-vegetable and fruit intervention. Therefore diet assessments at multiple time points in an intervention study may be necessary for a more valid association between dietary inflammatory potential and disease risk. Finally, our findings strengthen the evidence for
a new tool assessing the long term overall quality of diet and providing support for its use in other studies of diet and cancer.

7.5 Suggestions for future research

Future observational studies with multiple diet assessments beyond three years will be needed to make more adequate comparisons in dietary behavior change in an observational versus an interventional setting. Studies (both observational and interventional) with multiple diet assessments in which every participant is surveyed at all the time points of diet assessment may be expensive but necessary to avoid making assumptions that random subsamples of participants are representative of the entire study population. Finally, interventions to test reductions in the inflammatory potential of diet as a means for both colon and rectal cancer prevention are now warranted given findings in the current study.

7.6 Conclusion

In this large prospective study of postmenopausal women, the average DII was relatively stable in the OS from baseline to Year 3, but decreased significantly over time in a manner consistent with improved anti-inflammatory potential, achieving its lowest mean value at Year 3 in DMT intervention participants and, to a smaller extent, among control arm participants. In all three study groups, the extent of decrease was influenced by BMI, education, and race/ethnicity.

A history of long-term pro-inflammatory diets as well as shorter-term stable pro-inflammatory diets; increase the risk of colon cancer and possibly rectal cancer, but was not associated with breast cancer risk. Our findings suggest lowering the inflammatory
potential of diet as a means for the primary prevention of colon cancer, and potentially rectal cancer but not breast cancer or any of its subtypes in postmenopausal women.

7.7 Acknowledgements

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