**Distribution Agreement**

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

[Kristine Abigail Whalen] Date

Paleolithic Diet Scoring and Associations with Inflammation, Colorectal Adenomas, and Mortality

By

Kristine Abigail Whalen

Doctor of Philosophy

Epidemiology

_____

[Roberd M. Bostick]
Advisor

_____

[W. Dana Flanders]
Committee Member

_____

[Terryl J. Hartman]
Committee Member

_____

[Suzanne Judd]
Committee Member

_____

[Marjorie L. McCullough]
Committee Member

Accepted:

_____

Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

_____

Date

# Paleolithic Diet Scoring and Associations with Inflammation, Colorectal Adenomas, and Mortality

By

Kristine Abigail Whalen
A.B., Princeton University, 2008
M.P.H., Emory University, 2012

Advisor: Roberd M. Bostick, MD MPH

An abstract of
A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
Department of Epidemiology

2015

Abstract
Paleolithic Diet Scoring and Associations with Inflammation, Colorectal Adenomas, and Mortality
By Kristine Abigail Whalen

*Homo sapiens* may be more biologically adapted to the general diet that they would have eaten prior to the development of agriculture, the "Paleolithic diet". Yet few studies have examined this dietary pattern and any potential benefits it may have in reducing risk for modern chronic diseases.

In the first study, we investigated associations of two diet pattern scores, the Paleolithic and the Mediterranean, with circulating levels of two inflammation-related biomarkers, CRP and $F_2$-isoprostanes, in a pooled cross-sectional study of an elective outpatient colonoscopy population (N=646). We found statistically significant trends for decreasing mean plasma biomarker concentrations with increasing quintiles of the Paleolithic and Mediterranean diet scores.

In the second study, we assessed associations of the two diet scores with prevalent incident, sporadic colorectal adenomas in a case-control study (n=2,301) of colorectal polyps. The adjusted odds ratios comparing those in the highest to the lowest quintiles of the diet scores were, respectively, 0.71 (95% confidence interval [CI]: 0.50, 1.02; $P_{trend}$=0.02) and 0.74 (95% CI: 0.54, 1.03; $P_{trend}$=0.05) when comparing the cases to the endoscopy-negative controls, and 0.84 (95% CI: 0.56, 1.26; $P_{trend}$=0.14) and 0.77 (95% CI: 0.53, 1.11; $P_{trend}$=0.13) when comparing the cases to the community controls.

In the third study, we investigated associations of the Paleolithic and the Mediterranean diet scores with all-cause and cause-specific mortality in a longitudinal cohort of adults (REGARDS; n=21,423). During a median follow-up of 6.25 years, 2,513 participants died. The adjusted hazard ratios comparing those in the highest to those in the lowest quintiles of the diet scores were, respectively, 0.77 (95% CI 0.67, 0.89; $P_{trend}$<0.01) and 0.63 (95% CI 0.54, 0.73; $P_{trend}$<0.01).

The results of these studies suggest that more Paleolithic- or Mediterranean-like diet patterns may be associated with lower levels of systemic inflammation and oxidative stress; lower risk for incident, sporadic colorectal adenomas; and lower risk of all-cause and cause-specific mortality. Overall, this dissertation contributes to our understanding of Paleolithic diet patterns by creating the first such diet pattern score and using it to assess the diet's association with risk of chronic disease.

# Paleolithic Diet Scoring and Associations with Inflammation, Colorectal Adenomas, and Mortality

By

Kristine Abigail Whalen
A.B., Princeton University, 2008
M.P.H., Emory University, 2012

Advisor: Roberd M. Bostick, MD MPH

A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
Department of Epidemiology

2015

**Acknowledgements**

To my husband, Peter.  I love you very much.

# TABLE OF CONTENTS

# LIST OF FIGURES

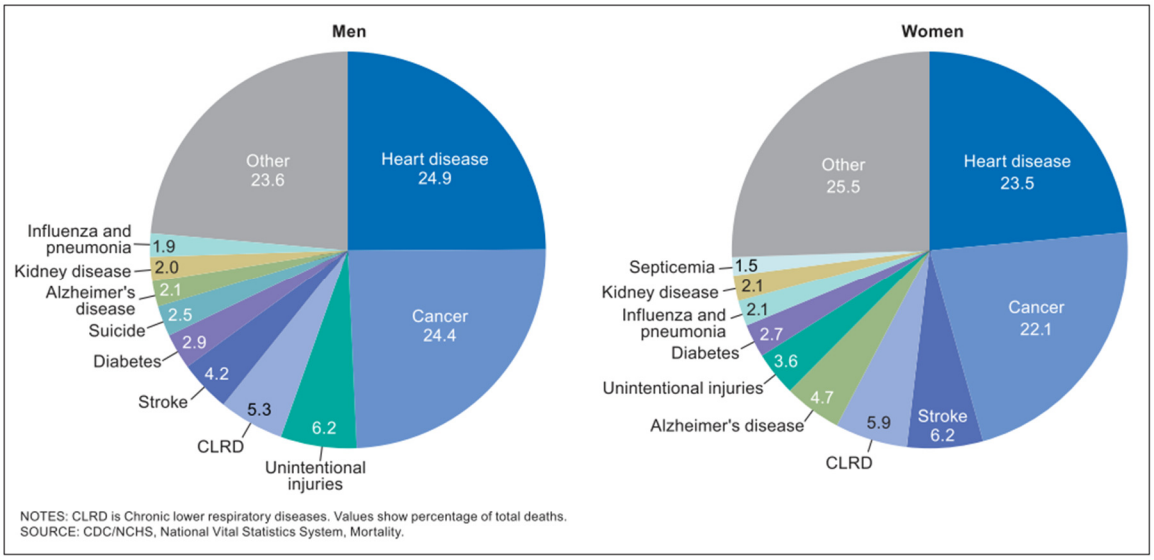
# LIST OF TABLES

# CHAPTER 1: INTRODUCTION AND BACKGROUND

## Introduction

Over the past century, improvements in sanitation, prevention and treatment of infectious diseases, and a reduction in infant mortality rates have vastly improved modern life expectancy (1). As fewer people die young, a larger proportion of the population has become at risk for developing chronic diseases (2). Today, almost half of the deaths for both men and women living in the United States can be attributed to two major chronic diseases: cardiovascular disease (CVD) and cancer (figure 1.1) (3).

*Figure 1.1: Percent distribution of the 10 leading causes of death, by sex: United States 2010 (Heron 2013). (3)*




Along with the decrease in child mortality and the increase in adult mortality due to chronic disease in the 20th century, there were dramatic cultural shifts, including in diet and lifestyle patterns, in Westernized nations. Global, large-scale industrial food production and processing largely supplanted small local farms as the basis for the global food system. The resulting ultra-processed food, while energy-dense, cheap, and quick to produce, may be responsible for drastic increases in obesity and related chronic diseases (4). Diets prior to the industrial revolution may have been less likely to lead to obesity, but agricultural societies still faced nutritional trade-offs, often favoring diets

high enough in calories to support the population over diets high in quality (5). Prior to the advent of agriculture about 10,000 years ago, humans generally lived in small hunter-gatherer groups and, in most environments, had access to a wide variety and high amount of produce, intermittent access to lean meats and fish, and very rare access to substantial amounts of salt or added sugars such as honey (6). This pre-agricultural eating pattern, referred to as the Paleolithic diet, may be associated with lower risk for chronic diseases such as CVD and cancer, yet there are few published studies of the diet, and none so far has examined risk for either of these chronic diseases.

## Background

### Nutrition, cardiovascular disease, cancer, and mortality

Diet has long been associated with CVD, cancer, and overall mortality. There are vast international differences in rates of CVD incidence that correspond to different dietary habits (7), and, when low-incidence populations adopt the diet and lifestyle of higher incidence nations, their CVD incidence rates quickly increase to match (8, 9), underlining the strong role of the environment in determining risk. Several different nutritional factors in these diets have been hypothesized to cause or prevent CVD, including dietary cholesterol, dietary fat, and dietary fat composition (7), but no single factor appears to be responsible (10). Today there is evidence that several different nutritional factors together influence CVD risk. There is strong evidence that high consumption of vegetables and nuts are associated with lower risk, and that high consumption of trans-fats, and a higher a dietary glycemic load are associated with higher risk (10).

Diet is also associated with cancer risk, although the strength of that association and its possible mechanisms varies by cancer type (11). It is estimated that with improved diet, increased physical activity, and reduced obesity, between 30-40% of cancer cases worldwide could be prevented (12). The two cancers that appear most strongly related to environmental exposures are lung cancer and colorectal cancer (CRC) (11). Lung cancer, the most common cause of cancer death in the US, is strongly related to smoking exposures. CRC, the second most common cause of cancer

death in men and women combined, shares a similar pattern of international incidence rates as that for CVD. Populations with traditionally low CRC incidence rates quickly acquire higher rates when they emigrate to Westernized, high incidence nations or adopt Westernized diet and lifestyle patterns in their home country (12, 13). While several foods and nutrients have been associated with CRC risk, these associations have been rather weak and inconsistent (12). The rather weak and inconsistent associations with single nutrients compared to the strong role of diet and lifestyle that the international rates suggest, provide evidence that examining diet as a whole rather than its separate parts may be more productive for finding ways to prevent CRC (14, 15). Other cancer types may be associated with diet, but not as strongly as is CRC, and it is often obesity, rather than individual foods, that is associated with the greatest variation in risk (12).

Diet may modulate chronic disease risk by two main mechanisms. The first mechanism by which diet is related to chronic disease is energy balance or obesity. There may also be biochemical mechanisms by which diet affects chronic disease risk, and two frequently proposed ones are inflammation and oxidative balance. Obesity, systemic inflammation, and oxidative stress have all been associated with higher risk of CVD and cancer, so identifying foods, nutrients, and diets that lead to improvements in these risk factors may lead to preventing these chronic diseases.

## Nutrition, obesity, and chronic disease

During the second half of the 20th century, not only did many chronic diseases increase in incidence, but rates of obesity increased dramatically as well. In the US today, more than a third of adults are obese (with a BMI over 30), and almost a fifth of children are classified as obese as well (16). High obesity rates are no longer only characteristic of high-income Westernized countries, but are increasingly common throughout the world. Several foods have been hypothesized to adversely impact energy balance and contribute to excess energy intake and subsequent obesity, including energy-dense foods high in sugar and/or fat and sugar-sweetened beverages (17).

Obesity is a strong risk factor for CVD and other metabolic syndrome conditions (18-20). High body fat, particularly abdominal fat, contributes to endothelial dysfunction and inflammation as

well as other harmful CVD risk factors, such as high blood pressure, dyslipidemia, and insulin resistance (20). Poor energy balance and excess body fat are also convincing risk factors for several different cancers, including colorectal, pancreatic, breast, endometrial, and kidney cancers (12). Weight loss interventions for morbid obesity, such as bariatric surgery, were associated with lower risk of cardiovascular events (OR 0.54 95% CI 0.41-0.70) and mortality (OR 0.48 95% CI 0.35-0.64) in case-control studies (21), though any possible effect of such surgeries on risk of cancer is unclear (22). Maintaining a normal weight and avoiding obesity is an important mechanism by which diet can modulate chronic disease risk.

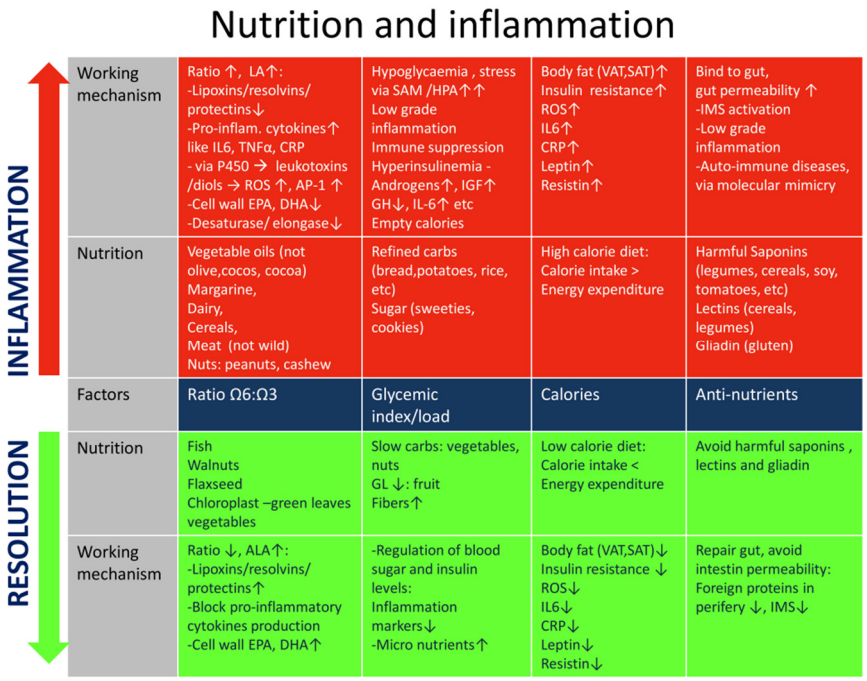
Weight loss is a $240 billion dollar-a-year industry worldwide (23). Ways to prevent or treat obesity are difficult to implement in today's food environment. The general recommendations to eat less and to move more to achieve a favorable energy balance go against the body's general need for homeostasis. Exercise interventions to increase energy expenditure generally yielded mixed results (24-34), in part because some physical activity can stimulate appetite (35). Diet changes are generally more effective for weight loss than are exercise interventions (24, 36). Yet there have been different levels of success in losing weight with different diets (37-39), especially for long-term weight loss (40, 41). Food is a fuel, but its consumption is part of a complex group of social, cultural, and psychological factors that are difficult to change. Surgery or medication interventions to reduce weight require less effort on the part of the individual than do lifestyle changes, but are not appropriate for most people, who are not excessively obese (16). Dietary guidelines for the prevention of CVD and cancer generally parallel those for weight reduction, but likely could also provide a benefit to those individuals who do not need to or who cannot lose weight on the recommended diet.

## Nutrition, inflammation, oxidative balance, and chronic disease

Inflammation and oxidative balance are two of the primary biochemical mechanisms whereby diet influences chronic disease risk (42, 43). Many foods, especially those that are highly

processed, may act as a persistent environmental trigger for systemic stress and inflammation (42). Factors (see Figure 1.2) such as the dietary polyunsaturated fatty acid (PUFA) content, the omega 3 to omega 6 fatty acid ratio, higher arachidonic acid levels, higher glycemic index, and altered insulin response to food all appear to contribute to this diet–inflammation association (42). Several specific foods have been suggested as potential triggers for a low-level systemic inflammation response, including high dietary saturated fat (44), dairy products (45), and wheat and cereal grains (46), although no single food appears to be a consistently strong inflammatory trigger across all populations. Obesity is also directly associated with systemic inflammation levels, apparently because it alters glucose oxidation: excess adipose tissue increases blood glucose levels and creates an inefficient insulin response, leading to a low-level inflammatory response to the remaining blood glucose in the circulation (47).

*Figure 1.2: Reflections of the working mechanism demonstrating how several nutritional factors could induce or inhibit inflammation. From Bosma-den Boer 2012 (42)*




Systemic low-grade inflammation is associated with diet and is a common feature of many common chronic diseases, including type II diabetes (T2D), CVD, some cancers, and many other conditions (48). Inflammation is a normal response to tissue infection or injury, but chronic

inflammation is a maladaptive response that appears to be triggered by tissue malfunction or homeostatic imbalance (49). Oxidative stress is an imbalance between the production of free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS), collectively pro-oxidants, and antioxidants leading to a cellular and intra cellular environment that is exposed to destructive free radical chemistry (50). ROS and RNS (collectively, RONS) are normal products of cellular metabolism, produced as part of the mitochondrial respiratory chain, and act as parts of certain signaling pathways. Excess levels of RONS, however, are detrimental (51). Inflammation and oxidative balance are two processes that feed into one another. Continued exposure to pro-inflammatory factors increases oxidative stress and can lead to chronic inflammation and vice versa (52, 53).

Inflammation and oxidative stress facilitate the creation of atherosclerotic lesions. Atherosclerosis is a precursor to CVD in which plaque builds in the arterial walls. Lipid and protein oxidation, especially LDL-cholesterol oxidation, are part of atherosclerotic lesion development (54, 55). If the vascular endothelium is damaged or otherwise dysfunctional, it initiates an initially protective response to assist in repairs by increasing cellular adhesion in the vascular walls. Oxidized LDL-cholesterol preferentially binds to this adhesive mix, creating a lipid-filled foam that forms the basis for a fatty streak that is the first stage in the development of an advanced atherosclerotic lesion (See Figure 1.3) (56).

*Figure 1.3: Inflammation and formation of the fatty streak, a precursor of an advanced atherosclerotic lesion. From Ridker et al. 2003 (56)*

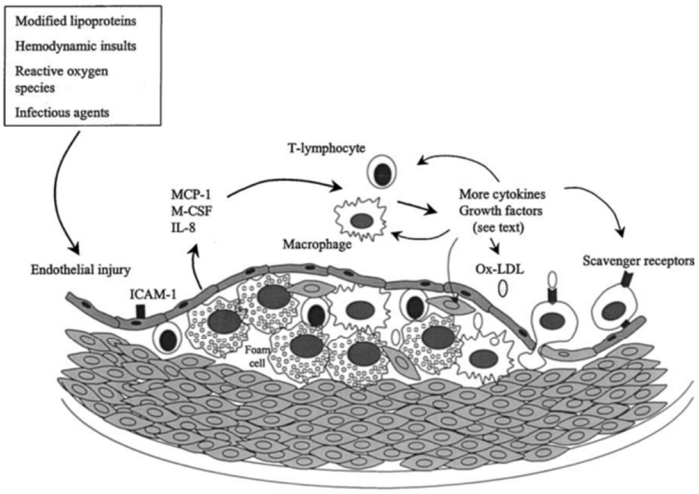
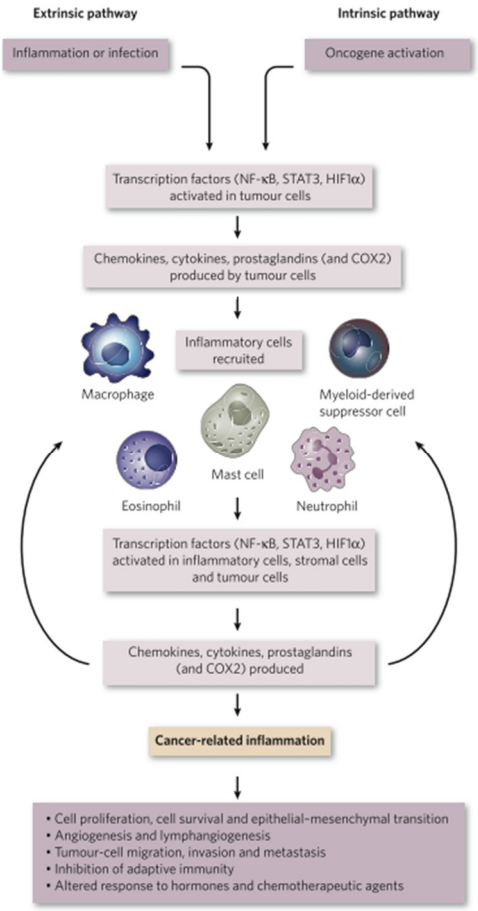



Fig. 1. Inflammation and formation of the fatty streak. ICAM-1, intercellular adhesion molecule 1; MCP-1, monocyte chemotactic protein-1; M-CSF, macrophage colony stimulating factor; IL, interleukin. Key stages in early atherogenesis (refer to text for details): (1) endothelial injury and dysfunction, (2) expression of adhesion molecules, (3) release of chemokines, (4) recruitment of inflammatory monocytes, (5) increased leukocyte adhesion and migration, (6) incorporation of ox-LDL by macrophages by way of scavenger receptors to become foam cells, (7) release of cytokines and mitogens by activated monocytes, (8) smooth muscle cell migration and proliferation. (*From* Morrow DA, Ridker PM. Inflammation in cardiovascular disease. In: Topol E, editor. Textbook of Cardiovascular Medicine Updates, vol. 2, no. 4. Cedar Knolls, NJ: Lippincott-Williams & Wilkins; 1999; with permission.)

Similarly, cancer is associated with chronic inflammation and poor oxidative balance (51-53, 57-61). Several types of chronic inflammatory conditions are associated with a higher risk of certain cancers, such as inflammatory bowel disease with colorectal cancer (CRC) and chronic hepatitis with liver cancer (61). There are some estimates that up to a quarter of all cancers worldwide are due to infection and/or chronic inflammation (52). Extrinsic inflammation can contribute to cancer, which itself can trigger additional localized inflammation (Figure 1.4) (51, 58). This local inflammation, in turn, can promote methylation which may lead to additional gene silencing (59) or can assist in tumor development by stimulating new blood vessel formation (angiogenesis) at the tumor site (57). Systemic inflammation and oxidative stress are implicated in CVD and cancer, so biomarkers of these mechanisms have potential utility as endpoints for short-term trials (62).

*Figure 1.4: Pathways that connect inflammation and cancer. From Mantovani et al. 2008 (58).*




## Results from supplement trials

Biomarkers of inflammation and oxidative stress are potential endpoints for interventions. Agents that disrupt or alter inflammation or oxidative balance mechanisms have been tested for the prevention of CVD and cancer, and have included aspirin (63-68), other NSAIDs (69-72), and antioxidant supplements (73, 74). Aspirin or NSAIDs have shown some promise as preventative agents (69-72, 75), but for many people the side effects of such drugs outweigh the potential preventative benefits (69-72, 76). Generally, antioxidants have had no effect on either CVD or cancer risk (73, 74). Even so, supplement and multivitamin use is pervasive in the United States, with almost half of the adults taking supplements regularly. Almost half of supplement users take these products to prevent CVD and cancer (74). Yet the clinical trial evidence for this perceived

benefit has been lacking. For the most part, in 26 trials, no supplements (including multivitamins, supplemental beta-carotene, vitamin E, selenium, vitamin A, vitamin C, folic acid, vitamin D, vitamin D and calcium, or calcium supplements) reduced CVD incidence or mortality (TABLE 1.2), all-cancer incidence, site-specific cancer incidence, cancer mortality (TABLE 1.3), or mortality (TABLE 1.4). In some notable trials, supplements actually increased the incidence of CVD or cancer. In the ATBC and CARET trials, older men at high risk of lung cancer were randomized to high doses of beta-carotene. Both trials found that high levels of supplemental beta-carotene actually increased the risk of lung cancer (HR 1.18; 95% CI 1.03, 1.36 ATBC and HR 1.28; 95% CI 1.04, 1.57 CARET), (77-80). Overall, the clinical trial evidence indicated that is unlikely that taking vitamins reduces risk of CVD or cancer in most people (10, 74).

There are several reasons why the supplementation groups in the clinical trials did not have the expected lower incidence of CVD and cancer. For the most part, the supplements in these trials contained far greater doses of the nutrient than would be found in a normal diet, and the amount of the nutrient was out of proportion with the other nutrients that would normally be in the same foods. Many of the beta-carotene studies, including ATBC and CARET, administered daily doses that were over 100 times higher than the US median dietary intake (54). In contrast, vitamin D supplement doses may have been lower than the dose required to achieve optimum serum levels of 25-hydroxyvitamin D (81). A few trials, conversely, did find a lower incidence of CVD or cancer in the supplementation group, mostly in populations and subgroups that were likely deficient in the supplement. The Nutritional Prevention of Cancer Study (NPC), conducted in the Eastern US where selenium levels in the soil are generally low, was a randomized trial of selenium supplementation to decrease the incidence of cancer in patients with a history of basal cell or squamous cell skin carcinomas. In the NPC trial, in post-hoc analyses it was found that over six years the selenium supplementation group relative to the placebo group, had a statistically significant lower incidence of the study's secondary endpoints: all cancers combined (82, 83) and cancers of the prostate (82-84), lung (82, 83), and colorectum (82, 83). In the Women's Health Initiative (WHI)

trial, among women who were not already taking supplements at baseline, those assigned to the vitamin D and calcium combination had a lower incidence of any cancer after 7 years (HR 0.86 95% CI 0.78, 0.96) (85). Both the NPC participants and this subset of the WHI participants may have had low intakes of these vitamins and minerals prior to the trial, suggesting that certain supplements may be beneficial in reducing the risk of CVD and cancer in populations that may be deficient. Individuals who are deficient in certain nutrients may find some benefit from taking supplements of those nutrients. However, taking certain nutritional supplements, particularly beta-carotene, may be harmful in doses that are much higher and out of proportion with other nutrients found in foods.

Not only could the dose of supplemental antioxidants be responsible for not finding chemopreventive effects against CVD or cancer in clinical trials, but the true active nutrient(s) in foods may not have been correctly identified or selected for testing. There are many more antioxidants in a given whole food than are normally accounted for by the standard antioxidant profiles (54), so there are many more potential antioxidant agents than have been assessed in clinical trials. In addition, CVD and cancer develop over the course of many years or even decades, and it is unclear how long or at what age participants are given these supplements in order to observe benefits. Rather than continue to pick potential preventative agents and doses one nutrient at a time, it may be more successful and of greater value to focus on how nutrients are consumed normally, through whole diets.

## Dietary pattern scores

There are several reasons why examining whole diets may be more useful than continuing to examine single-nutrients for the prevention of CVD and cancer. First, individual nutrients and foods within a diet tend to be highly collinear, making it difficult to estimate the independent effects of the individual nutrients (14). As reviewed above, relatively few individual nutrients (as supplements) have been tested in randomized trials compared to the number of possible candidates (54). The effects of individual nutrients and foods may also be synergistic rather than merely additive (86). Certain foods and nutrients may interact to increase or decrease the bioavailability or

absorption of another nutrient (14). The possible synergistic effects of foods and diets are hard to examine using extant statistical methods (87). Randomized diet trials are one way to examine diets as a whole, but such trials are expensive, and participants may not adhere well or for very long, and are not blinded to the dietary intervention they are randomized to. While randomized diet trials are the best test for diet causality in nutrition research, less expensive research to support the potential health benefits of the diets is needed beforehand.

Diet pattern analysis is one way to examine associations of whole diets with risk for chronic disease. Researchers use commonly available dietary assessment tools to generate the dietary data needed to describe various dietary patterns and then to investigate associations of the dietary patterns with a variety of health outcomes in a variety of populations. Most dietary patterns can be described/calculated using one of three different general methodologies: cluster analysis, factor analysis, and index analysis.

In a cluster analysis, people are aggregated by diet into "clusters". Large clusters represent behaviors shared by many people in the study population, whereas small clusters represent behaviors shared by few study participants. Food choices that are common to many individuals contribute little to cluster information, since individuals in a cluster share a single exposure pattern, but individuals in small clusters whose dietary habits collectively differ from those of larger clusters provide useful information. The incidence or prevalence of the health outcome under study in the small cluster is compared to that of the largest cluster as a reference. After participants are clustered, the variation of food intake for that individual is no longer considered. Each cluster is given a label that describes the dietary pattern of individuals within that cluster, but do not quantify the relative amounts of different foods or food groups across clusters as part of the cluster analysis, which makes assessing dose-response relationships difficult (88).

The second way to construct a dietary pattern is by using factor analysis, also called principal component analysis. Factor analysis is unlike cluster analysis in that information is grouped by foods and nutrients commonly eaten together, called factors, rather than by people who eat

similarly. Based on the underlying correlation matrix between the many foods assessed, those that explain the most variation are identified as factors. A single factor represents many food items that are common to diets that include that factor. For example, pizza consumption may be highly correlated with hot dog, chip, and hamburger consumption so pizza, as a factor, can represent all of these foods. Participants then receive a score for each identified factor based on their responses to the nutrition assessment tool. By comparing across all five quintiles, from highest to lowest intake, researchers can examine the dose-response pattern for each factor's association with a particular health outcome (88). Another diet pattern related to factor analysis is reduced rank regression. In reduced rank regression, unlike with factor analysis, the focus is not on explaining the variance between foods, but rather linear functions are identified that explain as much variation as possible in intermediate response variables, such as certain nutrients or blood biomarkers of interest. Reduced rank regression is intended to create a mix of both data-driven diet score methods and hypothesis-driven methods (89), but its use has not become commonly reported in the literature.

A third way to investigate diet pattern-outcome associations is to use index patterns, which are generally described prior to examining the dietary data from a study. In index analysis, scores are created to indicate closeness to *a priori*-described or recommended dietary pattern. The individuals in a population who rank the highest in a given diet pattern score are generally those who most closely follow the dietary pattern of interest (88). The methods for creating these dietary pattern scores can differ. In addition, assigning scores can be based on the absolute or relative degree of adherence to the defined diet pattern. If a score is assigned based on the absolute degree of adherence to a dietary pattern, it may facilitate detecting the true potential of a diet pattern to affect risk; however, few individuals may be found in a study population who follow it, thus resulting in suboptimal sample sizes in the higher exposure groups. If a score is assigned based on the relative closeness of participants in a study population to following the defined diet pattern, it facilitates categorizing persons into relatively equal size exposure groups; however, participants with the highest score

rankings may not be following a diet that is particularly close to the gold standard, thus resulting in underestimating the true potential of a diet pattern to affect risk.

Cluster analysis, principal component analysis, index analysis, and other diet pattern identification methods rely on the accuracy of the underlying diet assessment tools for classifications. Diet assessment tools vary, but the most common methods are diet recall interviews, participant diet journals, and food frequency questionnaires (FFQs). Diet recall interviews generally are done either in person or over the phone by trained staff, who guide participants in providing a detailed report on what they ate for a specified time period (90). Diet journals that the participants keep are another common tool for diet assessment, but participants often have difficulties maintaining quality records (90). Both diet recall interviews and diet journals require intense review by the study staff to input the correct foods and quantities into the study records, and both assessment methods can be quite expensive to use. Diet recall interviews and journals are open-ended assessments in which participants give free-form responses that are unique to their own dietary habits (91). Food frequency questionnaires are used even more commonly than diet interviews or journals, and provide a closed-form characterization of participants' eating habits which enables the assessment of long-term dietary habits in a simple, cost effective manner (91). Unlike the open-ended assessments, this closed-form assessment is highly dependent on which food items are included on the form, and requires careful consideration of the dietary habits of the target population in order to be accurate (91). When the food items included in a FFQ are tailored to a given population, high correlations of the estimated nutrient composition of the participants' diets from these questionnaires with those based on the participants' responses on diet recall interviews or journals are found (92, 93). Limiting the food items included on the FFQ, however, inherently limits the final analysis. For example, a FFQ that is constructed to assess a low-fat diet may not make a distinction between high fat hamburger and a leaner flank steak. Researchers using data from FFQs for diet pattern analysis need to consider which food items are included on the FFQs since this can greatly influence what research questions can be addressed in the resulting FFQ data.

Several studies have examined diet patterns and their associations with biomarkers of inflammation and oxidative balance. Barbaresko et al. published a systematic review of 46 studies on dietary patterns and biomarkers of inflammation, and found that, in general, diet patterns that were characterized as "Western", or that were higher in meat were usually associated with higher inflammation biomarker levels, mainly C-reactive protein (CRP). Diet patterns characterized as "healthy" or high in fruit and vegetables were usually inversely associated with inflammation (43), which was consistent with the results of several cross-sectional studies in Japan (94, 95). Diet patterns with a high glycemic load have also been associated with higher CRP levels (96), and, in a randomized controlled trial (RCT) of either a low-fat or low-carbohydrate diet in patients with type II diabetes mellitus (T2D), there were greater reductions in biomarkers of inflammation among those on the low-carbohydrate diet (97). Several studies that investigated associations between diet patterns and biomarkers of oxidative stress found similar associations of the diet patterns with the biomarkers of oxidative stress as has been found with biomarkers of inflammation. A secondary analysis of a cohort study in women found that greater adherence to the Mediterranean diet pattern (aMED diet score) was inversely associated with lower $F_2$-isoprostane levels (98), and found a similar inverse association with another biomarker of oxidative stress, oxidized glutathione (GSH/GSSG) (99). Diet patterns that are generally high in fruits and vegetables and low in processed foods and meats are consistently inversely associated with biomarkers of inflammation and oxidative stress, potential early endpoints for CVD and cancer risk.

Consistent with the results from studies that investigated associations of diet patterns with biomarkers of inflammation and oxidative stress, diet patterns high in fruits and vegetables, but low in refined grains and meat, were inversely associated with risk for CVD, including coronary heart disease, stroke, and overall-CVD mortality (89, 100, 101). Dietary patterns such as the alternative Healthy Eating Index (aHEI) (102, 103), the Mediterranean (104-106), and low carbohydrate patterns (107), have been inversely associated with various CVD outcomes. Participants with high scores on these dietary patterns tend to have higher intakes of fruits and vegetables, and lower intakes of

refined grains and meat. Overall, there was consistent evidence that the same general dietary patterns associated with lower biomarkers of inflammation and oxidative stress were also associated with lower risk of CVD.

Similarly, the diet patterns that tend to be inversely associated with colorectal cancer and its precursor, colorectal adenomas, are also higher in fruits and vegetables and lower in refined grains and meat. In 2010, two separate reviews were published on the associations of different diet patterns with CRC or adenomas (14, 15). Randi et al. 2010 found that in 32 published articles from studies in various populations around the world, patterns termed "healthy, prudent, fruit and vegetables, reduced-fat/diet foods, healthy eating index-2005, recommended food, and Mediterranean diet scores" were inversely associated with CRC risk, with risk estimates that ranged from 0.45 to 0.90 (15). Miller et al. 2010 echoed this in their review of 16 observational studies (14). In one population-based case-control study and two prospective cohort studies, there were consistent inverse associations of dietary index patterns that featured high fruit and vegetable consumption with CRC, but only among men (risk estimates ranged from 0.71 to 0.82). In principal component analyses of eight studies in men and nine in women, a fruit and vegetable pattern was also found to be more strongly inversely associated with CRC among men than women, with risk estimates that ranged from 0.66 to 0.84 for men and 0.73 to 1.06 for women (14).

Both the Randi et al. 2010 and the Miller et al. 2010 reviews also found that diet patterns with higher intakes of red and processed meat, also called unhealthy, Western, or meat and potatoes diet patterns, were associated with higher risk of CRC (14, 15). The risk estimates associated with unhealthy or Western patterns varied from 1.18 to 11.7 among the 32 papers included in the Randi et al. review (15). In the Miller et al. review, 4 of 8 studies in men and 5 of 9 studies in women found a direct association between unhealthy or Western patterns and CRC risk. Stronger associations in men than women for many diet patterns were found in many of these studies, and could possibly be the result of biologic differences between the sexes or perhaps differential responses to the same diet assessment tools (14). More recent publications have generally supported the Randi et al. 2010 and

the Miller et al. 2010 review findings (108-113), although not in all populations (114, 115). Diet patterns have also been associated with cancer-free survival in a CRC population, with a "Western" pattern being directly associated with recurrence or death while a healthier, "prudent" pattern was not statistically significantly associated with longer cancer-free survival (116). The associations of diet patterns with risk for CRC are consistent with the patterns of associations of the diets with both biomarkers of inflammation and risk for CVD.

Associations of diet patterns with risk for other non-colorectal cancers are weaker and more inconsistent. Yet the associations between diet and other cancer types are similar to those for diet and CRC, CVD, inflammation, and oxidative stress. Diets with higher amounts of fruits and vegetables tend to be inversely associated with other, non-CRC cancer risk. The World Health Organization (WHO) diet pattern, the Healthy Diet Indicator, was not associated with overall cancer risk (n=3,007; 1st to 3rd tertile HR 0.99 [95%CI 0.96-1.02]) (117), although there were modest inverse associations between produce-heavy diets and risk of head and neck cancer (22 pooled case-control studies; 1st to 5th quintile of vegetable intake OR 0.85 [95%CI 0.60, 1.19]) (118) and oral cancer (n=306; OR 0.53 [95%CI 0.28-0.98]) (119). Associations of diet patterns in general with breast cancer have been inconsistent across studies (120), but less so for an inverse Mediterranean diet-breast cancer association (121, 122). Of the various diet patterns reported on, the Mediterranean diet pattern has been the most strongly, consistently associated (inversely) with cancer incidence and mortality (106, 123-125).

In general, diet patterns high in fruits and vegetables and low in meat tend to be inversely associated with biomarkers of inflammation, CVD, and various types of cancer, especially CRC, that contribute to mortality in the developed world. Yet comparisons between studies or diet scores are difficult given the limitations of diet pattern analysis. Despite the difficulties in comparing the results of associations of diet patterns with various outcomes across studies, the use of diet patterns allows for a great deal of flexibility for examining a variety of dietary exposures in many ways in varied populations instead of, or as an important prelude to, expensive trials.

## The Mediterranean diet

The Mediterranean diet has been extensively studied as a dietary pattern, and has been successfully implemented in full diet trials, making it an excellent example of how diet pattern analysis can inform randomized trial design. The Mediterranean diet became of interest as a result of observations of coronary artery disease incidence rates around the world in the 1960s. At that time, the nations of Southern Europe had lower CVD rates and the diets that tended to be typical for those countries were different from those of other countries (7). The Mediterranean diet at that time was characterized as having high intakes of plant foods such as fruits, vegetables, and nuts, as well as whole grain cereals and breads (126). In addition, the diet included low or moderate dairy product consumption, olive oil (a high polyunsaturated fat source) as the primary source of dietary fats, low red meat consumption, and low to moderate wine consumption (126).

There have been numerous observational studies of the Mediterranean diet in relation to a variety of outcomes. In general the Mediterranean diet has been inversely associated with plasma antioxidants and anti-inflammatory cytokines (127) and biomarkers of oxidative balance (98, 99); risk of T2D (128), CVD (104, 105, 129, 130), and certain types of cancer (generally non-breast) (111, 121-125, 131-134); and with overall mortality (135-137). In a 2014 meta-analysis (included 35 prospective cohort studies) of this diet pattern it was found that for each 2-point increase in the Mediterranean diet score there was a lower risk of CVD (RR 0.90 [95%CI 0.87, 0.92]), cancer (RR 0.95 [95%CI 0.93, 0.97]), and all-cause mortality (RR 0.91 [95%CI 0.89, 0.93]) (106). Of the various diet patterns, the Mediterranean diet pattern is commonly inversely associated with chronic disease, which has provided good evidence to support conducting subsequent, more expensive diet trials.

Several randomized trials of the Mediterranean diet were reported. Among participants assigned to the Mediterranean diet, only inconsistent and weak effects on $F_2$-isoprostanes, which are biomarkers of oxidative stress, were found. One small, 4-week feeding trial found no effect on urinary $F_2$-isoprostane levels (138), and a larger trial of a Mediterranean diet intervention (PREDIMED) found only suggestive, but not statistically significant, differences in urinary $F_2$-

isoprostane levels between the intervention and control arm after one year (MedDiet +VOO -13.71 ng/mmol; MedDiet +nuts -14.82 ng/mmol; Control diet -9.32 ng/mmol; p=0.059) (139). Clinical trials of the Mediterranean diet have been more successful at reducing levels of various CVD risk factors other than oxidative stress, including total cholesterol, LDL-cholesterol, and triglyceride levels, increased HDL-cholesterol, and reduced HbA1c levels (126, 138, 140, 141). Randomized trials of the Mediterranean diet have also found a statistically significant lower incidence of CVD in primary and secondary prevention studies (126, 142, 143). One of the first such trials was the Lyon Heart Study, in which 605 participants with a recent myocardial infarction were randomized to either usual care, which included a recommendation to follow a prudent diet, or a Mediterranean diet intervention. Participants met with study staff 8 weeks post-infarct and were contacted once a year for the following 5 years. At the end of 5 years (mean follow-up 27 months) the Mediterranean intervention group had a statistically significant reduced consumption of dietary fat than the control group, similar serum lipids, but a statistically significant reduced incidence of fatal or non-fatal cardiac events (RR 0.27 95% CI 0.12, 0.59) (144). After a longer follow-up (mean 46 months), it was again found that the Mediterranean diet group relative to the control group had a statistically significant reduced incidence of fatal and non-fatal cardiac events (RR 0.44 95% CI 0.21, 0.94) (145). The Lyon Heart Study also assessed cancer incidence after 4 years and found a suggestive but non-significant 61% reduced risk for any cancer in the Mediterranean diet group relative to the standard care group (p=0.05) (146). In the second major clinical trial of a Mediterranean diet, PREDIMED, the effect of the Mediterranean diet effect on CVD primary prevention was tested. Adults between the ages of 55 and 80 (n=7,447) with no history of CVD were randomized to one of three diets: a control diet in which participants were directed to reduce dietary fat, a Mediterranean diet with supplemental extra-virgin olive oil (EVOO), or a Mediterranean diet supplemented with nuts. Early analyses while the trial was ongoing indicated that a Mediterranean diet reduced biomarkers of inflammation/atherosclerosis (147) and improved oxidative balance (139). After an average follow-up of 4.8 years, there were statistically significant fewer numbers of cardiovascular events in the

Mediterranean diet arm with supplemental EVOO (96 events, adj. HR 0.70 95% CI 0.54, 0.92) and in the Mediterranean diet arm with supplemental nuts (83 events, adj. HR 0.72 95% CI 0.54, 0.96) than in the low-fat diet control arm (109 events) (148). The Mediterranean diet serves as an excellent example of how dietary patterns can be used to provide evidence to support conducting subsequent randomized trials. In the case of the Mediterranean diet, and the subsequent trials found a beneficial effect of the diet for preventing several chronic diseases, especially CVD. Thus, research on the Mediterranean diet is a model for how to investigate other diet patterns, and the Mediterranean diet serves as a high bar to compare other diets to.

## The Paleolithic diet

Following a Paleolithic diet pattern may be beneficial in the prevention of chronic diseases, but it may not be easily studied in modern populations. Eaton and Konner first outlined the concept of a Paleolithic diet in the scientific literature in 1985 (6), and also described the theory of evolutionary discordance, which posits that differences between our modern diet and lifestyle and that of our hunter-gatherer ancestors may be responsible for some of the marked increases in chronic diseases over the past century (6, 149). A Paleolithic diet consists almost entirely of vegetables, fruit, lean meat, and fish, minimal amounts of cereal grains or dairy products. Eaton and Konner suggested that this diet has less sodium, higher calcium, less total fat, and a higher polyunsaturated to saturated fat ratio than the average modern diet (Table 1.1) (6). To construct the Paleolithic diet pattern, Eaton and Konner used two sources of information: the hominid fossil record and surveys of the dietary habits and health of extant hunter-gatherer populations.

The hominid fossil record illustrates how limited food options during times of changing environmental conditions led to anatomical changes that allowed our ancestors to adapt to many different environments. Genetic evidence points to a last common ancestor between humans and our nearest animal relatives, chimpanzees, about 6 million years ago. That ancestor, likely a knuckle-walker that thrived on the forest fruits of Africa, more closely resembled a chimpanzee than a modern human. Between 6 million years ago and 4.3 million years ago, several early hominid species

such as *Sahelanthropus tchadensis*, *Orrorin tugenesis*, and several *Ardipithecus* species appeared to have physiological adaptations that indicated they spent more time as bipeds (5). Bipedalism allowed for more efficiency when walking and freed up hands for reaching, grabbing, and manipulating. Both of these adaptations allowed them to reach previously unavailable foods as the fruit-rich rainforests shrank while the woodlands expanded. Early hominids' longer reach and more efficient gait allowed them to access better the remaining fruit and utilize a wider variety of food sources (5). Between 4 million and 1 million years ago, the hominid diet expanded from fruits to a much wider variety of seeds, plants stems, and other, tougher foods. It was at this time that the Pliocene, or Ice Age, led to an even cooler and drier African climate. In this new climate, hominids had to rely increasingly on low-calorie, hard to eat fallback foods to make up their daily energy requirements. The teeth and jaws of some of these species (*Australopiths*) were particularly impressive: huge, flat molars akin to grindstones and huge bony crests on top of their skull allowed large chewing muscles to attach and created massive jaw strength for shredding tough, fibrous foods (5). These early hominid species were adapted to find and utilize a wide variety of plant foods.

Additional adaptations to gain access to the more calorie- and protein-dense meats came later in our evolutionary history. The earliest modern hominin fossils date to about 1.9 million years ago. While early fossils suggest that diets remained largely vegetarian, they, along with surviving tools and refuse, present increasing evidence of occasional meat eating and hunting (5). Becoming omnivores would have given hominins access to another, more energy-dense, food source. Rather than directly compete for meat with apex predators, such as lions, and scavengers, such as hyenas and vultures, hominins developed alternate methods. Hominins worked in groups with complex strategies that likely required language to coordinate, and utilized their efficient bipedalism for persistence hunting –running prey animals down until they overheated and died (5). Hunting increased the options for food as well as the calories. Hominins also made maximal use of what food they did acquire, by creating tools to butcher meat and process (e.g., grind) foods, and developing cooking to gain access to even more energy than would otherwise be possible from the raw foods (5).

These tools allowed hominin diets to be extremely flexible and adaptable and for hunter-gatherer hominin populations to boom and spread around the globe.

Although, as for current hunter-gatherers, the diets of early *Homo sapiens* differed somewhat across groups living in different locales, they shared important common characteristics. Most hunter-gatherer groups (except those that more relatively recently culturally adapted to living in high-latitude arctic areas) would have eaten a large amount and wide variety of vegetable foods; Eaton and Konner suggested that produce would have made up 65% or more of the total daily weight of food eaten (6). The vegetables and meat available would have provided very little dietary sodium (approx. 689 mg per day) relative to what is typically consumed in modern diets, with a potassium to sodium ratio close to 16:1. The calcium content of these wild plant foods was high, and yielded 1,579 mg per day as estimated from an analysis of the commonly eaten plant foods of hunter-gatherer populations (6). The fiber content of the diet would also have been very high due to this high vegetable consumption. The meat available to pre-agricultural humans was wild plant (e.g., grass) fed rather than grain-fed, leading to meat with a very different fatty acid composition. Free-living herbivore meat tends to be much leaner than that of domesticated animals, with a much higher polyunsaturated fat content per gram, as well as a small but appreciable amount of omega-3 fatty acids (6). In table 1.1, we present a comparison of the nutritional composition of this hypothesized Paleolithic diet and a current American diet. The Paleolithic diet is higher in protein, lower in carbohydrates, and lower in fat than the current American diet.

Table 1.1. Comparison of estimated nutritional characteristics of a Paleolithic diet and a current American diet, based on a Paleolithic diet composed of 65% by weight produce. Adapted from Eaton and Konner 1985 (6).

| | Paleolithic Diet | Current American Diet (2009-2010 NHANES) |
|---|---|---|
| Total Dietary Energy (%) | | |
| Protein | 34% | 16% |
| Carbohydrate | 45% | 51% |
| Fat | 21% | 34% |
| Potassium: Sodium ratio | 1.41 | 0.66 |
| Cholesterol (mg) | 591 | 261 |
| Fiber (g) | 45.7 | 16.2 |
| Sodium (mg) | 690 | 3463 |
| Calcium (mg) | 1580 | 1029 |

While it is clear that hunter-gatherer groups ate a very different diet than modern populations, it is a common misperception that individuals who survived childhood did not typically live long or healthy lives. Hunter-gatherer groups survived on a variations of the Paleolithic diet in many different regions and environments. While there is evidence that while hunter-gatherers experienced a high childhood mortality rate, the average modal age of death in adulthood (or the peak in death distribution) is estimated to have been about 72 years of age (range 68-78). In comparison, the modern US population has a modal age of death of 85 years (150), in large part because of modern quality health care. The causes of death for older hunter-gatherers also appear to be very different from those of humans in most modern societies. Heart attacks, strokes, cancer, and other degenerative diseases were apparently rare in adult hunter-gatherer societies, although these causes of death are hard to identify without modern medical equipment and training (150). An early 20th century survey of "primitive peoples" by Weston A. Price, an American dentist, found that although tribal diets were very different around the world, these diets shared similar qualities to those described by Eaton and Konner. Also, the people Price examined had almost no tooth decay, facial

deformities, crowded teeth, or birth defects, and had very low rates of infectious and chronic diseases (151). Health assessments of hunter-gatherer groups today are more contradictory, likely because there tends to be poor-quality health assessments in the remote regions in which they live (e.g., no modern medical scans) (152), and these groups often were displaced to less optimal habitat for hunter-gatherer existence.

There have been a handful of observational studies on the health of extant hunter-gatherers. The first piece of evidence that a Paleolithic diet might improve health in humans today comes from the early 1980s. Kerin O'Dea assessed 10 aborigines from Australia with Type II diabetes (T2D) both before and after a 7-week period living as traditional hunter-gatherers in the Australian bush. At the end of 7 weeks, the participants had lost a statistically significant amount of weight (average, 8 kg), and had marked improvements in glucose, insulin, and lipid biomarkers (153). Two additional cross-sectional studies of arctic peoples assessed the association between traditional diet patterns and heart disease. The traditional hunter-gatherer diet for arctic peoples tends to be meat based and high in fat—diet characteristics traditionally associated with high CVD rates (154, 155). The first study, done in an Alaskan Eskimo population, used principal components analysis to identify four distinct dietary pattern scores: 1) traditional, 2) Western, 3) purchased healthy, and 4) a beverages and sweets pattern. The associations of these diet patterns with several CVD risk factors were then investigated. The traditional diet was not associated with CRP (a biomarker of inflammation) or HDL-cholesterol, but was statistically significantly inversely association with systolic blood pressure and triglycerides (154). The second cross-sectional study, in an Inuit population, also used principal components analysis and found four similar basic dietary patterns. Again the traditional diet pattern was not associated with inflammation biomarkers or HDL-cholesterol, although there was a direct association of the traditional diet pattern with total cholesterol, mainly LDL-cholesterol levels (155). In another study, the gut microbiome of Hadza hunter-gatherers in Africa (156) was found to be statistically significantly different in respect to the composition and diversity of the gut bacteria from that of modern day Italians, although it is unclear whether these differences relate to health risks.

While the observational evidence from extant hunter-gatherer groups suggests potential health benefits of a Paleolithic type diet in modern hunter-gatherer groups, how closely such a diet can be followed in a Westernized population using foods available from typical modern sources (e.g., grocery stores), and whether it could reduce chronic disease risk in a Westernized population is unclear. Two small pilot trials, however, examined the short-term effects of the diet in healthy individuals. The first used an outpatient, controlled-feeding design for nine non-obese, sedentary volunteers. After 10 days, the participants had statistically significant reductions in blood pressure (-3.1 mmHg, $p=0.01$), plasma insulin vs. area-under-the-curve (AUC) glucose ($p=0.006$), total cholesterol (-0.8 mmol/l, $p=0.007$), LDL-cholesterol (-0.7 mmol/l, $p=0.003$), and triglycerides (-0.3 mmol/l, $p=0.01$) (157). In a second small pilot trial ($n=14$) participants were simply given dietary instruction to eat a Paleolithic diet and, at the end of three weeks, participants were found to have lower systolic blood pressures ($p=0.03$), similar to those found in the previously described trial, as well as statistically significant reductions in weight (-2.3 kg) and total daily calorie intake (-36%) (158). A third study of participants in a gym-sponsored Paleolithic diet challenge ($n=44$), had statistically significant reductions in body fat (-3.2 kg, $p<0.01$) as well as an increase in LDL-cholesterol (+12.5 mg/dL, $p<0.01$) after 10 weeks (159).

While healthy volunteers randomized to a Paleolithic diet were found to have improved lipid biomarker levels and greater weight loss, three additional small pilot trials of the diet in patients with metabolic syndrome, glucose intolerance, or T2D found even more promising results. In a small crossover trial instructed 13 participants with T2D were instructed to eat either a Paleolithic or a diabetic diet for 3 months, and then to eat the other diet for the next 3 months. Participants on the Paleolithic diet had decreased HbA1c (-0.4%, $p=0.01$), triglycerides (-0.4mmol/L, $p=0.003$), diastolic blood pressure (-4mmHg, $p=0.03$), and weight (-3 kg, $p=0.01$) and increased HDL-cholesterol (+0.08 mmol/L, $p=0.03$) compared to when they were on the diabetic diet (160). A second small pilot trial of participants with metabolic syndrome ($n=34$) who were randomized to either a Paleolithic diet or a healthy reference diet (with no intended weight loss), found that eating the

Paleolithic diet led to an average decrease in systolic and diastolic blood pressure (-9.1mmHg, p=0.015; -5.2 mmHg, p=0.038 respectively), decrease in total cholesterol (-0.52 mmol/l, p-0.037) and triglycerides (-0.89 mmol/L, p=0.001), and increase in HDL-cholesterol (+0.15 mmol/L, p=0.013). Participants on the Paleolithic intervention arm also lost weight (-1.32 kg, p=0.012), despite unsuccessful attempts to increase daily total energy intake in order to keep bodyweight stable (161). In a third small pilot trial of 29 patients with both ischemic heart disease and either glucose intolerance or T2D were randomized to follow either a Paleolithic or Mediterranean diet for 12 weeks, in the Paleolithic diet arm there was a statistically significantly greater decrease in area-under-the-curve (AUC) glucose (-26%, p<0.01) and a greater weight loss (-1.5kg p=0.03) and reduction in waist circumference (-2.7cm, p=0.03). Interestingly, after controlling for waist circumference reduction, was still a statistically significantly greater AUC glucose in the Paleolithic diet arm relative to the Mediterranean diet arm (162). The pilot trial evidence suggests that, even independent of weight loss, the Paleolithic diet may improve glucose tolerance and lipid biomarkers in populations with dysfunctional insulin responses.

The Paleolithic diet may improve biomarkers of risk and help prevent chronic disease through biochemical mechanisms and/or improved energy balance. Foods characteristic of the diet are also those that are generally associated with lower systemic inflammation and oxidative stress. There are no published reports of associations of the Paleolithic diet with biomarkers of inflammation or oxidative stress. Improved energy balance and thus percent body fat composition may be a particularly important mechanism for how the Paleolithic diet may prevent chronic diseases. A Paleolithic diet may also produce higher satiety than do other diets with the same total energy content. In a secondary analysis of one of the previously described pilot trials (160) it was found that participants on the Paleolithic diet reported a greater satiety level per calorie consumed as well as a greater satiety level per glycemic load (163). In a recent pilot trial (n=24) in which participants were provided with three individual meals given separately several weeks apart, similar effects on post-meal satiety were found. One of the meals was based on WHO nutrition recommendations, a

second was a Paleolithic-type meal with the same total energy content and macronutrient ratios as the WHO meal, and a third was a Paleolithic-type meal with a higher percentage of protein and a higher total energy content than the WHO meal. When participants were given either of the Paleolithic-type meals, in the subsequent hours, they had higher levels of appetite-suppressing gut hormones relative to when they were given the WHO meal; they also had higher perceived satiety measured on the visual analogue scale, independent of the protein or energy content of the meals (164). Not only does the Paleolithic diet appear to improve satiety in the short-term, but also there is evidence that the diet may lead to greater weight loss. There has been one larger trial (n=70) of a Paleolithic diet's effects on long-term weight loss in obese, post-menopausal women. Women were randomized to follow either a Paleolithic diet or the standard government dietary recommendations (Nordic Nutrition Recommendations (165) for two years. After six months, there was statistically significantly greater weight loss in the Paleolithic diet group (mean difference, -3.9 kg). After 24 months, however, the difference was less and no longer statistically significant (mean difference, -1.7 kg) (166). The results from several pilot studies of Paleolithic diet's effects on the possible biochemical mechanisms that influence CVD and cancer risk, suggest that it may improve energy balance and thereby mitigate chronic disease risk.

## Conclusions/gaps in the literature

There is a wealth of evidence that major chronic diseases and causes of death in the US, especially CVD and cancer, are associated with diet. Prior investigations into supplements for the primary prevention of CVD, cancer, or all-cause mortality have been unsuccessful. Rather than continue to pursue and research single-nutrient, putatively preventive agents and before conducting expensive dietary trials, dietary patterns can be a cheap and efficient way to characterize diets as a whole and assess their associations with disease risk. Evolutionary discordance could explain some of the dramatic increase in chronic disease rates over the 20$^{th}$ century. The Paleolithic diet best reflects the diet we as *Homo sapiens* ate for virtually the entirety of our evolutionary history. Yet few

populations still practice this dietary pattern, so our ability to assess whether the diet has any benefit for the prevention of chronic diseases is limited. There has been increasing interest in the Paleolithic diet in the scientific literature, but few published studies of the diet in relation to disease risk. The reported studies were small and generally short term, and none examined associations with biomarkers of inflammation or oxidative balance, any sort of neoplasm precursor, or mortality in culturally modern populations. In my dissertation I aim to address some of these gaps by developing a Paleolithic diet pattern score and assessing its associations with biomarkers of inflammation and oxidative stress, colorectal adenomas, and all-cause and cause-specific mortality in modern US populations.

Table 1.2. Overview of supplementation trials and the effect on CVD. Adapted from USPSTF Review 2013 (74).

| Trial | Population | N | Supplement | Dose | Suppl. duration | Years Follow up | CVD Outcome | Effect Measure |
|---|---|---|---|---|---|---|---|---|
| SU.VI.MAX (167, 168) | France | 13,017 | Multivitamin | 1-3 times RDA | 7.5 years | 7.5 | ↔ ischemic CVD incidence | 0.97 (0.77, 1.20) |
| | | | | | | 12.5 | ↔ ischemic CVD incidence | 0.97 (0.80, 1.17) |
| PHS II (169) | US male physicians | 14,642 | Multivitamin | Market multivitamin | 11.2 years | 11.2 | ↔ CVD incidence | 1.01 (0.91, 1.10) |
| PHS I (170, 171) | US male physicians | 22,071 | β-carotene | 50mg alternate days | 12 years | 12 | ↔ any CVD | 1.00 (0.91, 1.09) |
| | | | | | | 12.9 | ↔ any CVD | 1.00 (0.91, 1.09) |
| WHS (172) | US women health professionals | 39,876 | β-carotene | 50mg alternate days | 0-2.72 years | 4.1 | ↔ any CVD | 1.14 (0.87, 1.49) |
| SCPS (173) | Italy | 1,805 | β-carotene | 50mg daily | 5 years | 8.2 | ↔ CVD death | 1.16 (0.82, 1.64) |
| NSCPS (174) | Australia | 1,621 | β-carotene | 30mg daily | 4.5 years | 4.5 | ↔ CVD death | NR |
| CARET (80) | Smokers or asbestos exp. workers | 18,314 | β-carotene | 30mg daily | NR | 6 | ↔ CVD death | 1.02 (0.88, 1.19) |
| PHS II (175) | US male physicians | 14,642 | Vitamin E | 400 IU daily | 8 years | 8 | ↔ any CVD incidence | 1.01 (0.90, 1.13) |
| SELECT (176) | Men older than 50 with prostate cancer | 35,533 | Vitamin E | 400 IU daily | 7-12 years | 5 | ↔ any CVD incidence | 0.98 (0.88, 1.09) |
| | | | Vit. E + selenium | 400 IU/200 mcg daily | 7-12 years | 5 | ↔ any CVD incidence | 0.99 (0.89, 1.10) |
| WHS (177) | US women health professionals | 39,876 | Vitamin E | 600 IU alternate days | 8.2-10.9 years | 10.1 | ↔ any CVD incidence | 0.93 (0.82, 1.05) |
| NPC (82, 83, 178) | Eastern US with history of skin cancer | 1,312 | Selenium | 200 mcg daily | 4.5 years | 7.6 | ↔ any CVD incidence | 1.03 (0.78, 1.37) |
| SELECT (176) | Men older than 50 with prostate cancer | 35,533 | Selenium | 200 mcg daily | 7-12 years | 5 | ↔ any CVD incidence | 1.02 (0.92, 1.13) |
| CARET (79, 80, 179-181) | Smokers or asbestos exp. workers | 18,314 | Vitamin A (retinol) | 25,000 IU daily | NR | 6 | ↔ any CVD deaths | -- |
| PHS II (175, 182) | US male physicians | 14,642 | Vitamin C | 250 mg twice daily | 3 years | 8 | ↔ any CVD incidence | 0.99 (0.89, 1.11) |
| AFPPS (183, 184) | Adults with a history of colorectal adenoma | 1,021 | Folic acid | 1 mg daily | 3 years | 10.8 | ↔ MI incidence | -- |
| RECORD (185) | Participants with history of fragility fracture | 5,292 | Vitamin D | 800 IU daily | 2-5.2 years | 6.1 | ↔ any CVD incidence | 0.96 (0.82, 1.13) |
| Trivedi et al. (186) | Healthy adults | 2,686 | Vitamin D | 100,000 IU every 4 mo. | 5 years | 5 | ↔ any CVD incidence | 0.90 (0.77, 1.06) |
| WHI (187) | Women | 36,282 | Vit. D + Calcium | 200 IU twice daily | 7 years | 7 | ↔ any CVD incidence | 1.00 (0.94, 1.07) |
| ACS (188) | Post-menopausal women | 1,471 | Calcium | 1,000 mg daily | 5 years | 5 | ↔ MI incidence | 1.49 (0.86, 2.57) |
| | | | | | | 5 | ↔ CVD death | 0.51 (0.13, 2.01) |
| RECORD (185) | Participants with history of fragility fracture | 5,292 | Calcium | 1,000 mg daily | 2-5.2 years | 6.2 | ↑ possible fatal CHD | NR |

Table 1.3. Overview of supplementation trials and the effect on cancer. Adapted from USPSTF Review 2013 (74).

| Trial | Population | N | Supplement | Dose | Suppl. duration | Years Follow up | CVD Outcome | Effect Measure |
|---|---|---|---|---|---|---|---|---|
| SUVIMAX (167, 168) | France | 13,017 | Multivitamin | 1-3 times RDA | 7.5 years | 7.5 | ↔ any cancer | 0.90 (0.76, 1.06) |
| | | | | | | 12.5 | ↔ any cancer | 0.93 (0.82, 1.05) |
| PHS II (169) | US male physicians | 14,642 | Multivitamin | Market multivitamin | 11.2 years | 11.2 | ↓ any cancer | 0.92 (0.86, 0.998) |
| ATBC (77, 78) | Finland male smokers | 29,133 | β-carotene | 20mg daily | 5-8 years | 6.1 | ↑ lung cancer | 1.18 (1.03, 1.36) |
| | | | | | | 8 | ↑ lung cancer | 1.17 (1.02, 1.33) |
| | | | | | | 11 | ↔ lung cancer | 1.17 (0.98, 1.39) |
| | | | | | | 14 | ↔ lung cancer | 0.97 (0.82, 1.15) |
| PHS I (170, 171) | US male physicians | 22,071 | β-carotene | 50mg alternate days | 12 years | 12 | ↔ any cancer | 0.98 (0.91, 1.06) |
| | | | | | | 12.9 | ↔ any cancer | 1.0 (0.9, 1.0) |
| WHS (172) | US women health professionals | 39,876 | β-carotene | 50mg alternate days | 0-2.72 years | 4.1 | ↔ any cancer | 1.03 (0.89, 7.18) |
| SCPS (173) | Italy | 1,805 | β-carotene | 50mg daily | 5 years | 8.2 | ↔ cancer death | 0.83 (0.54, 1.29) |
| CARET (79, 80) | Smokers or asbestos exp. workers | 18,314 | β-carotene | 30mg daily | NR | 4 | ↑ lung cancer | 1.28 (1.04, 1.57) |
| | | | | | | 6 | ↔ lung cancer | 1.12 (0.97, 1.31) |
| ATBC (77) | Finland male smokers | 29,133 | Vitamin E | 50mg | 5-8 years | 8 | ↔ lung cancer | 0.99 (0.87, 1.12) |
| PHS II (175) | US male physicians | 14,642 | Vitamin E | 400 IU daily | 8 years | 8 | ↔ any cancer | 1.04 (0.95, 1.13) |
| SELECT (176) | Men older than 50 with prostate cancer | 35,533 | Vitamin E | 400 IU daily | 7-12 years | 5 | ↔ any cancer | 1.03 (0.91, 1.17) |
| | | | Vit. E + Selenium | 400 IU/200 mcg daily | 7-12 years | 5 | ↔ any cancer | 1.02 (0.90, 1.16) |
| WHS (177) | US women health professionals | 39,876 | Vitamin E | 600 IU alternate days | 8.2-10.9 years | 10.1 | ↔ any cancer | 1.01 (0.94, 1.06) |
| NPC (82, 83, 178) | Eastern US with history of skin cancer | 1,312 | Selenium | 200 mcg daily | 4.5 years | 7.6 | ↓ any cancer | 0.63 (0.47, 0.85) |
| SELECT (176) | Men older than 50 with prostate cancer | 35,533 | Selenium | 200 mcg daily | 7-12 years | 5 | ↔ any cancer | 1.01 (0.89, 1.15) |
| PHS II (180, 182) | US male physicians | 14,642 | Vitamin C | 250 mg twice daily | 3 years | 8 | ↔ any cancer | 1.01 (0.92, 1.10) |
| AFPPS (183, 184) | Adults with a history of colorectal adenoma | 1,021 | Folic acid | 1 mg daily | 3 years | 10.8 | ↑ non-CRCs (prostate) | -- |
| RECORD (185) | Participants with history of fragility fracture | 5,292 | Vitamin D | 800 IU daily | 2-5.2 years | 6.1 | ↔ any cancer | 1.07 (0.92, 1.25) |
| Trivedi et al. (186) | Healthy adults | 2,686 | Vitamin D | 100,000 IU every 4 months | 5 years | 5 | ↔ any cancer | 1.09 (0.86, 1.36) |
| Lappe et al. (189) | Women rural Nebraska | 1,108 | Vit. D + Calcium | 25 mcg daily | 4 years | 4 | ↓ any cancer | 0.40 (0.20, 0.82) |
| WHI (187) | Women | 36,282 | Vit. D + Calcium | 200 IU twice daily | 7 years | 7 | ↔ any cancer | 0.98 (0.91, 1.05) |
| RECORD (185) | Participants with history of fragility fracture | 5,292 | Calcium | 1,000 mg daily | 2-5.2 years | 6.2 | ↔ any cancer | 1.06 (0.91, 1.23) |
| Lappe et al. (189) | Women rural Nebraska | 1,108 | Calcium | 1,400 mg daily | 4 years | 4 | ↔ any cancer | 0.53 (0.27, 1.03) |

Table 1.4. Overview of supplementation trials and the effect on all-cause mortality. Adapted from USPSTF Review 2013 (74).

| Trial | Population | N | Supplement | Dose | Suppl. duration | Years Follow up | CVD Outcome | Effect Measure |
|---|---|---|---|---|---|---|---|---|
| SU.VI.MAX (167, 168) | France | 13,017 | Multivitamin | 1-3 times RDA | 7.5 years | 7.5 | ↔ all-cause | 0.77 (0.57, 1.00) |
| | | | | | | 12.5 | ↔ all-cause | 0.87 (0.70, 1.04) |
| PHS II (169) | US male physicians | 14,642 | Multivitamin | Market multivitamin | 11.2 years | 11.2 | ↔ all-cause | 0.94 (0.88, 1.02) |
| ATBC (77, 78) | Finland male smokers | 29,133 | β-carotene | 20mg daily | 5-8 years | 6.1 | ↑ all-cause | 1.08 (1.01, 1.16) |
| | | | | | | 8 | ↑ all-cause | 1.08 (1.01, 1.16) |
| | | | | | | 11 | ↑ all-cause | 1.11 (1.03, 1.21) |
| | | | | | | 14 | ↔ all-cause | 1.01 (0.92, 1.10) |
| PHS I (170) | US male physicians | 22,071 | β-carotene | 50mg alternate days | 12 years | 12 | ↔ all-cause | 1.02 (0.93, 1.11) |
| WHS (172) | US women health professionals | 39,876 | β-carotene | 50mg alternate days | 0-2.72 years | 4.1 | ↔ all-cause | 1.07 (0.74, 1.56) |
| SCPS (173) | Italy | 1,805 | β-carotene | 50mg daily | 5 years | 8.2 | ↔ all-cause | 1.03 (0.82, 1.30) |
| CARET (79, 80) | Smokers or asbestos exp. workers | 18,314 | β-carotene | 30mg daily | NR | 4 | ↑ all-cause | 1.17 (1.03, 1.33) |
| | | | | | | 6 | ↔ all-cause | 1.08 (0.99, 1.17) |
| ATBC (77) | Finland male smokers | 29,133 | Vitamin E | 50mg | 5-8 years | 8 | ↔ all-cause | 1.02 (0.95, 1.09) |
| PHS II (175) | US male physicians | 14,642 | Vitamin E | 400 IU daily | 8 years | 8 | ↔ all-cause | 1.08 (0.98, 1.19) |
| SELECT (176) | Men older than 50 with prostate cancer | 35,533 | Vitamin E | 400 IU daily | 7-12 years | 5 | ↔ all-cause | 0.93 (0.77, 1.13) |
| | | | Vit. E + Selenium | 400 IU/200 mcg daily | 7-12 years | 5 | ↔ all-cause | 0.94 (0.77, 1.13) |
| WHS (177) | US women health professionals | 39,876 | Vitamin E | 600 IU alternate days | 8.2-10.9 years | 10.1 | ↔ all-cause | 1.04 (0.93, 1.16) |
| NPC (82, 83, 178) | Eastern US with history of skin cancer | 1,312 | Selenium | 200 mcg daily | 4.5 years | 7.6 | ↔ all-cause | 0.95 (0.73, 1.24) |
| SELECT (176) | Men older than 50 with prostate cancer | 35,533 | Selenium | 200 mcg daily | 7-12 years | 5 | ↔ all-cause | 0.99 (0.82, 1.19) |
| CARET (79, 80, 179-181) | Smokers or asbestos exp. workers | 18,314 | Vit. A + β-carotene | 25,000IU and 30mg daily | NR | 4 | ↑ all-cause | 1.17 (1.03, 1.33) |
| PHS II (171, 182) | US male physicians | 14,642 | Vitamin C | 250 mg twice daily | 3 years | 8 | ↔ all-cause | 1.07 (0.97, 1.18) |
| RECORD (185) | Participants with history of fragility fracture | 5,292 | Vitamin D | 800 IU daily | 2-5.2 years | 6.1 | ↔ all-cause | 0.93 (0.85, 1.02) |
| Trivedi et al. (186) | Healthy adults | 2,686 | Vitamin D | 100,000 IU every 4 months | 5 years | 5 | ↔ all-cause | 0.88 (0.74, 1.06) |
| WHI (187) | Women | 36,282 | Vit. D + Calcium | 200 IU twice daily | 7 years | 7 | ↔ all-cause | 0.91 (0.83, 1.01) |
| ACS (188) | Post-menopausal women | 1,471 | Calcium | 1,000 mg daily | 5 years | 5 | ↔ all-cause | 1.18 (0.73, 1.92) |
| RECORD (185) | Participants with history of fragility fracture | 5,292 | Calcium | 1,000 mg daily | 2-5.2 years | 6.2 | ↔ all-cause | 1.03 (0.94, 1.13) |

# Research Plan

## Objectives, Specific Aims, and Study Hypotheses

My primary objective for my dissertation is to investigate associations of a Paleolithic diet score with biomarkers of inflammation and oxidative balance, first diagnosed cases of prevalent colorectal adenoma, and risk of all-cause and cause-specific mortality. My secondary aim is to compare these latter findings with those from parallel analyses of a Mediterranean diet score in relation to these same outcomes. I will meet these objectives by addressing the following three specific aims.

Aim #1: Using data from a pooled elective, outpatient colonoscopy population (Markers of Adenomatous Polyps I (MAPI) and MAP II) (n=646), investigate whether higher Paleolithic and/or Mediterranean diet scores are associated with lower mean concentrations of circulating C-reactive protein and $F_2$-isoprostanes, biomarkers of inflammation and oxidative balance, respectively. I hypothesize that both diet scores will be inversely associated with these biomarkers.

Aim #2: Using data from the Minnesota Cancer Prevention Research Unit case-control study (CPRU) (564 cases; 1,202 endoscopy controls; 535 population controls), investigate whether Paleolithic and Mediterranean dietary patterns are associated with incident, sporadic colorectal adenoma. I hypothesize that both diet scores will be inversely associated with sporadic colorectal adenoma.

Aim #3: Using data from the Reasons for Geographic and Racial Differences in Stroke (REGARDS) national cohort (n=21,423), investigate whether Paleolithic or Mediterranean diet pattern scores are associated with all-cause or cause-specific mortality. I hypothesize

that both diets will be inversely associated with all-cause and chronic disease mortality, but not associated with mortality due to injury or accident.

## Methods

*Paleolithic and Mediterranean Diet Scores (Main Exposure Variables)*

For all three aims, we will use the same scoring procedures to calculate scores for both diet patterns using data from the food frequency questionnaires used in the original studies on which the aims are based. The Paleolithic and Mediterranean diet pattern scores will be constructed in a similar manner, as summarized in Table 1.5. The foods and associated point values have already been determined using published dietary guidelines for each diet (6, 132, 190). For the most part, each study participant will be assigned a quintile rank (and score from 1–5) of intake for each food category, based on the sex-specific distribution of intake. We will give more points for higher intakes of foods that we consider characteristic of the diet pattern, and for lower to no consumption of foods that we consider uncharacteristic of the diet pattern. We modified the Mediterranean diet score scheme in relation to dairy, grains and starches, and alcohol intakes as noted in Table 1.5. Although the Mediterranean diet score most often is constructed simply using two categories of intakes (high and low based on median intake), we will construct ours based on quintiles of intake to facilitate a more direct comparison of the two diet scores. For the Paleolithic diet score, we will create two unique variables. The first, a fruit and vegetable diversity score, will be created by summing the total number of responses on the food frequency questionnaire fruit and vegetable sections that indicated that the participant ate >1–3 servings of a given food item/month. More diversity is considered desirable. Second, because the Paleolithic diet had little dairy but high amounts of calcium (from wild greens) (6), to consider dietary calcium separately from dairy products we will use the residuals of a linear regression of total calcium intake on total dairy intake to represent calcium intake independent of dairy consumption. The final scores can range from 11 to 55 for the 11-component Mediterranean diet score and from 14 to 70 for the 14-component Paleolithic diet score.

Table 1.5. Paleolithic and Mediterranean Diet Pattern Score Constituents and Construction[a]

| Intake Category | Scoring | Paleolithic Diet Score[b] | Mediterranean Diet Score[c] |
|---|---|---|---|
| **Highest intake 'best'** | Points assigned to each quintile = quintile rank | Vegetables<br>Fruits<br>Fruit & vegetable diversity[d]<br>Lean meats[e]<br>Fish<br>Nuts<br>Calcium residual[f] | Vegetables<br>Fruits<br>Lean meats[e]<br>Fish<br>Nuts<br>Monounosaturated:saturated fat ratio |
| **Lowest intake 'best'** | Points assigned to each quintile = reverse quintile rank | Red and processed meats[g]<br>Sodium<br>Dairy<br>Grain and starches<br>Baked goods[h]<br>Sugar sweetened beverages<br>Alcohol (drinks/week) | Red and processed meats[g]<br>Sodium (mg) |
| **Moderate intake 'best'** | 3rd quintile +5 points, 2nd and 4th quintiles +3 points, and 1st and 5th quintiles +1 points | | Dairy<br>Grains and starches |
| **Other** | | | Alcohol:<br>Women: 5 – 15 g/day (+5 points)<br>Men: 10—25 g/day (+5 points)<br>Otherwise (+1 point) |

[a] All constituents measured in servings/week or grams/week unless otherwise indicated.
[b] Paleolithic diet score: 14 components, range of possible scores 14 – 70.
[c] Mediterranean diet score: 11 components, range of possible scores 11 – 55.
[d] Fruit & vegetable diversity calculated by summing the total number of responses on the food frequency questionnaire fruit and vegetable sections that indicated that the participant ate more than 1-3 servings of a given food item per month.
[e] Lean meats include skinless chicken or turkey, lean beef.
[f] Calcium intake from sources other than dairy; calculated as residuals from linear regression of total calcium intake (mg/day) on dairy foods intake.
[g] Nitrate processed meats and non-lean red meat consumption together.
[h] Baked goods include items such as cake, pie, and other pastry-type foods.

*Data Sources*

Aim #1: For the first aim, I will pool data from two methodologically similar case-control studies, MAPI and MAPII. Data from these studies, both cross-sectional studies of elective outpatient colonoscopy populations, conducted by the same principal investigator (RMB), will be pooled. The first study (Markers of Adenomatous Polyps I, MAPI) was conducted from 1994-1997 in Winston-Salem and Charlotte, North Carolina, and the second (MAPII) was conducted in 2002 in Columbia,

South Carolina. Participants in both studies were recruited from patients with no prior history of colorectal neoplasms who were scheduled for an elective, outpatient colonoscopy for colorectal cancer screening or gastrointestinal symptoms in several large, community-based gastroenterology practices. Initial eligibility for participation in each study required that patients be 30-74 years old, English speaking, free of known genetic syndromes associated with a predisposition to colonic neoplasia, and with no individual history of inflammatory bowel disease, adenomatous polyps, or cancers except for non-melanoma skin cancer.

Mailed questionnaires were completed at home and collected at the colonoscopy visit. Study participants provided detailed information on demographic characteristics, personal medical history, smoking history, usual physical activity (via a modified Paffenbarger questionnaire), anthropometrics, reproductive history and hormone use (women only), and family history of cancer. The frequencies of aspirin and other non-steroidal anti-inflammatory drug (NSAID) use were assessed as the number of pills taken per week. A 153-item (MAPI) or 85-item (MAPII) self-administered semi-quantitative Willett food frequency questionnaire was completed prior to colonoscopy to assess food and nutritional supplement intakes over the previous 12 months. A standard portion size and nine possible frequency-of-consumption responses, ranging from "never, or less than once per month" to "6 or more times per day" were given for each item. Total daily energy and nutrient intakes were calculated by summing energy and nutrients from all food sources using the dietary database developed by Willett (92, 191).

On the day of the colonoscopy, fasting peripheral venous blood samples were drawn into red-coated, pre-chilled Vacutainer tubes and then immediately placed on ice and shielded from light to prevent sample degradation. Blood fractions were aliquotted into amber-colored cryopreservation tubes, air was displaced with an inert gas (nitrogen in MAPI and argon in MAPII), and then the aliquots were immediately placed in a -80°C freezer until analysis. The present study was conducted after most of the stored plasma samples were exhausted from prior studies; samples for CRP were

available on 87% (n=562) of the participants, and samples for $F_2$-isoprostanes were available on 67.6% (n=437) of participants; the analyses reported herein are based on these sample sizes.

High sensitivity CRP was measured via latex-enhanced immunonephelometry on a Behring nephelometer II (BN-II) analyzer (inter-assay CV 4%; Behering Diagnostics, San Jose, CA). $F_2$-isoprostanes were measured via a highly specific and quantitative gas chromatography-mass spectrometry-based (GC-MS) method (192), by the Molecular Epidemiology and Biomarker Research Laboratory (MEBRL) at the University of Minnesota (Minneapolis, MN). This method, considered the gold standard for measuring $F_2$-isoprostanes, measures a well-defined set of $F_2$-isoprostane isomers. These were extracted from participants' samples using deuterium (4)-labeled 8-iso-prostaglandin $F_2$ alpha as an internal standard. Quality control procedures included the analysis of two control pools that had varying concentration ranges of $F_2$-isoprostanes (CV 9.5% and 11% respectively).

*Data analysis plan*

Because the distributions of CRP and $F_2$-isoprostanes tend to be right skewed, their values will be log transformed and then the adjusted geometric means and their standard errors by quintile of each dietary pattern will be computed using a general linear model (implemented using the SAS GLM procedure), controlling for the potential confounding effects of other factors. To facilitate interpretation and comparisons between the relative strengths of the diet pattern-biomarker associations, ordinal logistic regression analysis will also be used in which CRP and $F_2$-isoprostanes levels will be categorized into quintiles based on the sex-specific concentrations in the pooled study population. The multivariable unconditional ordinal logistic regression models will be used to calculate odds ratios (OR) and 95% confidence intervals (95% CI) for associations of each dietary score with a cumulative sex-specific quintile increase of each biomarker. The median of each diet score quintile will be used for calculating all tests for trend.

Aim #2: For the second aim, I will use data from the Minnesota Cancer Prevention Research Unit case-control study. CPRU study personnel collected data between April 1991 and April 1994 as part of a joint project between the University of Minnesota and a large, multi-clinic private gastroenterology practice (193, 194). The gastroenterology practice performed colonoscopies and sigmoidoscopies in 10 hospitals and endoscopy units and, at the time of the study, was responsible for approximately 60 percent of all colonoscopies in the Minneapolis metropolitan area.

The gastroenterology practice staff initiated study recruitment while scheduling elective, outpatient colonoscopies or flexible sigmoidoscopies ("endoscopies"). All 10 of the practice's endoscopy sites recruited patients. Initial eligibility for study participation required that patients be 30-74 years old, residents of the Minneapolis-St. Paul metropolitan area, English speaking, free of known genetic syndromes associated with a predisposition to colonic neoplasia, and with no individual history of inflammatory bowel disease, adenomatous polyps, or cancers except for non-melanoma skin cancer.

Participants completed mailed questionnaires prior to endoscopy, which were then collected at the endoscopy visit at which time and blood samples drawn. The endoscopists recorded polyp locations and *in vivo* sizes and shapes on standardized forms. One index study pathologist examined all removed polyps histologically using National Polyp Study diagnostic criteria (195). Based on the endoscopy and pathology findings, participants were assigned final eligibility and case/control status. To be eligible as an adenoma case or a colonoscopy-negative control, the participant must have had a complete colonoscopy reaching the cecum, had all polyps removed, not have a new diagnosis of inflammatory bowel disease, and have no polyps with invasive carcinoma (n=684). Sigmoidoscopy-negative controls had similar eligibility requirements, but completed only a flexible sigmoidoscopy (n=518). Endoscopy controls were free of both adenomatous and hyperplastic polyps at endoscopy.

In addition to the endoscopy controls, a separate group of potential community controls (n=535) was randomly selected from the 1991 Minnesota State Driver's License Registry and frequency matched to the cases on age (5-year intervals), sex, and zip code. The community control

participants were only included in the study if they met the same eligibility criteria as the colonoscopy patients except that they did not undergo colonoscopy or sigmoidoscopy to confirm their current polyp status.

Study participants provided detailed information on demographic characteristics, personal medical history, smoking history, usual physical activity, anthropometrics, reproductive history and hormone use (women only), and family history of cancer. The frequency of aspirin and non-aspirin non-steroidal anti-inflammatory drug (NSAID) use was assessed as the number of pills taken per week. A self-administered, 166-item modified semi-quantitative Willett food frequency questionnaire was used to assess food and nutritional supplement intakes over the previous 12 months. A standard portion size and nine possible frequency-of-consumption responses, ranging from "never, or less than once per month" to "6 or more times per day" were given for each food. Total energy and nutrient intakes were calculated by adding energy and nutrients from all food sources using the dietary database developed by Willett (92, 191). A total of 2,301 participants completed the study and will be included in this analysis, including 564 cases, 1,202 endoscopy controls, and 535 community controls.

*Data analysis plan*

Unconditional logistic regression models will be used to calculate odds ratios (OR) and 95% confidence intervals (95% CI) for associations of the two dietary scores with colorectal adenomas. Separate analyses will be presented for comparisons of the cases to the endoscopy controls and to the community controls. The Paleolithic and Mediterranean diet pattern scores will be analyzed as both continuous and categorical variables (quintiles) based on the distributions of the scores in the community controls. The median of each diet score quintile will be used for calculating all tests for trend.

Aim #3: For the third aim I will use data from a large national prospective cohort study, REGARDS. The study design and recruitment for REGARDS have been described previously (196-

200). Briefly, REGARDS is a longitudinal cohort study designed to investigate the causes of racial and geographic disparities in stroke. This national cohort was oversampled from the stroke belt and stroke buckle regions, with half of the study participants being white, half African American, and half of each sex within each of the three regions. From January 2003 to October 2007, 30,183 individuals older than 45 years of age enrolled and were later contacted by phone twice a year to assess incident stroke events or mortality.

An in-home visit followed the computer-assisted telephone interview 3-4 weeks later during which study staff collected blood and urine samples, measured blood pressure and body size characteristics, and conducted an ECG and a medication audit. At the in-home visit, self-administered questionnaires, including a Block 98 Food Frequency Questionnaire (FFQ) (201), were left with the participants to fill out and mail or e-mail back. Trained staff contacted cohort participants by phone every 6 months to ascertain any suspected stroke events or deaths. If a death occurred, researchers collected the death certificate and any associated medical records for the 28-day period prior to death. Any participants who could not be contacted and were considered lost to follow-up also had their information checked against the social security death index and/or the National Death Index. Cardiovascular disease mortality was defined as death from myocardial infarction, stroke, sudden death, heart failure, pulmonary embolism, other cardiac causes of death, and non-cardiac but other cardiovascular disease deaths. Cancer mortality included all deaths attributed to cancer, injury and accident deaths were recorded as a separate category, while all other deaths were categorized as "other", including deaths attributed to respiratory illness, infection, liver disease, and kidney failure.

*Data analysis plan*

We will test each variable or potential covariate for the proportional hazards assumption by several means, including Log-Log Kaplan-Meier curves, goodness of fit tests, and extended Cox models. Hazard ratios and 95% confidence intervals for all-cause and cause specific mortality will be

estimated using Cox proportional-hazards regression models, using either the participants' age as the underlying time scale or by using time on study after adjusting for age.

## Significance and Impact of the Study

There is compelling evidence that diet is associated with risk of cardiovascular disease, cancer, and mortality. Yet attempts to find a single supplemental preventative agent have not been successful. Diet is a complex suite of likely interacting exposures and should be examined as such. The macro- and micro-nutrient composition of diets can vary widely. Given the sometimes unexpected effects of diet or supplement interventions on incidence of chronic diseases, it is important to examine potential diets before testing them in large-scale trials or making public health recommendations about them. The evolutionary discordance hypothesis is that differences in the diets and lifestyles of modern populations and those of our hunter-gatherer ancestors may be responsible for some of the marked increases in the prevalence of obesity and related chronic diseases. The hypothetical ancestral diet, the Paleolithic diet, has so far been examined in only a handful of studies. Most of those studies were short-term pilot trials to test changes in lipid profiles, blood pressure, weight change, or biomarkers of glucose control.

To the best of my knowledge, the research I propose for this dissertation will be the first in in which a Paleolithic diet score is created and used to investigate associations between a Paleolithic diet pattern with various health outcomes, including biomarkers of inflammation and oxidative balance; a pre-malignant neoplasm, colorectal adenomas; and all-cause and cause-specific mortality. To better assess the strengths of the associations of the Paleolithic diet relative to those of the Mediterranean diet, the scores for the two diet patterns will be constructed using similar methods so that any differences in the observed associations between the scores would more likely be due to differences in the diets than to the score construction methods. The results of my dissertation may have important implications for further studies of the Paleolithic diet pattern in relation to chronic disease risk, as well as to understanding whether and how evolutionary discordance may be shaping

health in modern times, and what the implications for the health of individuals and the public may be.

# CHAPTER 2: ASSOCIATIONS OF PALEOLITHIC AND MEDITERRANEAN DIET PATTERN SCORES WITH BIOMARKERS OF INFLAMMATION AND OXIDATIVE BALANCE

Kristine A. Whalen[1], Marjorie L. McCullough[2], W. Dana Flanders[1,3],

Terryl J. Hartman[1,4], Suzanne Judd[5], Roberd M. Bostick[1,4]

[1] Emory University Rollins School of Public Health, Department of Epidemiology

Atlanta, Georgia, United States

[1] American Cancer Society, Epidemiology Research Program, Atlanta, Georgia, United States

[1] Emory University Rollins School of Public Health, Department of Biostatistics and Bioinformatics

Atlanta, United States

[1] Emory University, Winship Cancer Institute Atlanta, Georgia, United States

[1] University of Alabama at Birmingham, Biostatistics Birmingham, Alabama, United States

## Abstract

*Background:* Chronic inflammation is associated with poor diet quality and risk for cancer and other chronic diseases. A diet-inflammation association may relate to evolutionary discordance. *Objective:* We investigated associations of two diet pattern scores, the "Paleolithic" and the Mediterranean, with circulating levels of two inflammation-related biomarkers, C-reactive protein (CRP), an acute inflammatory protein, and $F_2$-isoprostanes, a reliable marker of *in vivo* lipid peroxidation, in a pooled cross-sectional study of an elective outpatient colonoscopy population (N=646). *Methods:* We created diet scores from responses on a modified Willett food-frequency questionnaire, and measured plasma CRP and $F_2$-isoprostanes concentrations by enzyme-linked immunosorbant assay (ELISA) and gas chromatography-mass spectrometry (GCMS), respectively. Both diet scores were calculated and categorized into quintiles, and their associations with higher biomarker levels were estimated using general linear models to calculate and compare adjusted geometric means, and via unconditional ordinal logistic regression. *Results:* There were statistically significant trends for decreasing mean plasma CRP and $F_2$-isoprostanes concentrations with increasing quintiles of the Paleolithic and Mediterranean diet scores. The multivariable-adjusted odds ratios (OR) comparing those in the highest relative to those in the lowest quintiles of the Paleolithic and Mediterranean diet scores, were, respectively, 0.62 (95% confidence interval [CI] 0.37, 1.04; $P_{trend}$=0.04) and 0.55 (95% CI 0.31, 0.96; $P_{trend}$=0.01) for a higher CRP concentration, and 0.41 (95% CI 0.22, 0.75; $P_{trend}$ <0.01) and 0.48 (95% CI 0.25, 0.92; $P_{trend}$=0.01) for a higher $F_2$-isoprostanes concentration. *Conclusions:* These findings suggest that diets that are more "Paleolithic-" or Mediterranean-like may be associated with lower levels of systemic inflammation and oxidative stress.


## Introduction

Chronic inflammation is associated with many chronic diseases that have become increasingly common in the Western world (48). Reactive oxygen species (ROS) can lead to lipid peroxidation (202), a major feature of oxidative stress (203), and sustained oxidative stress can lead to increased inflammation, and vice versa (53). Both chronic inflammation and oxidative stress have been associated with cardiovascular disease (56, 204), cancer (51, 205-207), and other chronic

diseases (208). Several dietary factors influence a person's chronic inflammation level (42, 209). For example, a higher Ω-6:Ω-3 fatty acid ratio, a high intake of saturated fat, foods with a high glycemic load, and lower dietary fiber intake are associated with higher inflammation levels (42). Investigations into which foods alter systemic inflammation and oxidative balance led to several large clinical trials of nutritional supplementation to prevent cardiovascular disease (55) and cancer (210, 211), most showing limited success and even harm in some instances (77-80). While there are many reasons why these trials might not have found the expected benefits, it may be that, at least in part, the nutritional supplements used in the trials cannot sufficiently address the relevant complex and likely interacting components of diet (14, 212, 213).

To better capture the potential synergistic effects of food constituents in a complex diet, nutrition researchers have utilized dietary patterns. Dietary patterns can be entirely data driven, decided entirely *a priori*, or a combination thereof, and can be used to quantify a person's entire diet, rather than individual components. One such pattern that is of increasing interest is a "Paleolithic" diet pattern. A Paleolithic diet is roughly modeled after the diet humans ate prior to the advent of agriculture, as estimated from anthropological evidence from fossils and extant hunter-gatherers (6). The Paleolithic diet was generally composed of fruits and vegetables (large amounts and diversity) and lean meats, with very little to no grains, dairy products, or sugar. It was also high in calcium and other minerals, which are found in relatively high amounts in various wild greens (6). The discrepancy between the diets and lifestyles of *Homo sapiens* prior to the agricultural revolution and those during the modern, post-industrial revolution era, referred to as evolutionary discordance, has been proposed to account for some of the dramatic increase in chronic disease in the past century (149). There has been very limited study of this diet reported in the scientific literature, with some indications that it may improve cardiovascular and metabolic biomarkers (157-162, 166), perhaps similar to or even more so than a Mediterranean diet (162, 214). A Mediterranean diet is considered one of the healthiest diets for preventing many chronic diseases (105, 123, 148), and is associated with lower levels of biomarkers of inflammation and oxidative stress (43, 98, 139). The

Mediterranean diet is similar to the Paleolithic diet in that it emphasizes a high consumption of fruits, vegetables, and lean meats, with little added sugars; but, unlike a Paleolithic diet, is characterized by moderate intakes of dairy, grains, and alcohol (7).

We previously reported inverse associations of Paleolithic and Mediterranean diet scores with incident, sporadic colorectal adenoma (214). In this paper we present a cross-sectional analysis of data from a separate pooled elective, outpatient colonoscopy population 1) to investigate an association of a Paleolithic diet score with two circulating markers of inflammation and oxidative stress, C-reactive protein (CRP) and $F_2$-isoprostanes, respectively, and 2) to compare the latter findings with those from a parallel analysis of associations of a Mediterranean diet score with the same markers.

## Methods

### Study population and data collection

Data from two methodically similar studies, both cross-sectional studies of elective outpatient colonoscopy populations, conducted by the same principal investigator (RMB), were pooled. The first study (Markers of Adenomatous Polyps I, MAPI) was conducted from 1994-1997 in Winston-Salem and Charlotte, North Carolina, and the second (MAPII) was conducted in 2002 in Columbia, South Carolina. Participants in both studies were recruited from patients with no prior history of colorectal neoplasms who were scheduled for an elective, outpatient colonoscopy for colorectal cancer screening or gastrointestinal symptoms in several large, community-based gastroenterology practices. Initial eligibility for participation in both studies required that patients be 30-74 years old, English speaking, free of known genetic syndromes associated with a predisposition to colonic neoplasia, and with no individual history of inflammatory bowel disease, adenomatous polyps, or cancers except for non-melanoma skin cancer.

In MAPI, 669 (30%) of the 2,246 colonoscopy patients identified met these eligibility criteria; 617 (92%) were contacted, and 417 (68%) consented to participate. In MAPII, 305 (87%) of the 351 colonoscopy patients identified were eligible and 232 (76%) agreed to participate. We combined the two studies since their selection criteria, study protocols, and questionnaires were

nearly identical. Details of the study protocols for MAPI (215) and MAPII (216) were previously reported. The study protocols for both studies were approved by the respective Institutional Review Boards of the corresponding institutions, and all participants were willing to participate and able to understand and provide informed consent.

Mailed questionnaires were completed at home and collected at the colonoscopy visit. Study participants provided detailed information on demographic characteristics, personal medical history, smoking history, usual physical activity (via a modified Paffenbarger questionnaire), anthropometrics, reproductive history and hormone use (women only), and family history of cancer. The frequencies of aspirin and other non-steroidal anti-inflammatory drug (NSAID) use were assessed as the number of pills taken per week. A 153-item (MAPI) or 85-item (MAPII) self-administered semi-quantitative Willett food frequency questionnaire was completed prior to colonoscopy to assess food and nutritional supplement intakes over the previous 12 months. A standard portion size and nine possible frequency-of-consumption responses, ranging from "never, or less than once per month" to "6 or more times per day" were given for each item. Total daily energy and nutrient intakes were calculated by summing energy and nutrients from all food sources using the dietary database developed by Willett (92, 191).

On the day of the colonoscopy, fasting peripheral venous blood samples were drawn into red-coated, pre-chilled Vacutainer tubes and then immediately placed on ice and shielded from light to prevent sample degradation. Blood fractions were aliquotted into amber-colored cryopreservation tubes, air was displaced with an inert gas (nitrogen in MAPI and argon in MAPII), and then the aliquots were immediately placed in a -80°C freezer until analysis. The present study was conducted after most of the stored plasma samples were exhausted from prior studies; samples for CRP were available on 87% (n=562) of the participants, and samples for $F_2$-isoprostanes were available on 67.6% (n=437) of participants; the analyses reported herein are based on these sample sizes.

High sensitivity CRP was measured via latex-enhanced immunonephelometry on a Behring nephelometer II (BN-II) analyzer (inter-assay CV 4%; Behering Diagnostics, San Jose, CA). $F_2$-

isoprostanes were measured via a highly specific and quantitative gas chromatography-mass spectrometry-based (GC-MS) method (192), by the Molecular Epidemiology and Biomarker Research Laboratory (MEBRL) at the University of Minnesota (Minneapolis, MN). This method, considered the gold standard for measuring $F_2$-isoprostanes, measures a well-defined set of $F_2$-isoprostane isomers. These were extracted from participants' samples using deuterium (4)-labeled 8-iso-prostaglandin $F_{2\alpha}$ as an internal standard. Quality control procedures included the analysis of two control pools that had varying concentration ranges of $F_2$-isoprostanes (CV 9.5% and 11% respectively).

Dietary scores

The Paleolithic and Mediterranean diet pattern scores were constructed as described previously (214). Briefly, each study participant was assigned a quintile rank (and score from 1-5) of intake for each food group for each score, based on the sex-specific distributions in each original study population. As shown in Table 2.1, higher points were given for higher intakes of foods considered characteristic of a given score or for low to no consumption of foods considered uncharacteristic of that dietary pattern. The points for each food component were then summed to create the final diet pattern score. The final scores could range from 14-70 for the 14-component Paleolithic diet score and 11-55 for the 11-component Mediterranean diet score.

Statistical analysis

The characteristics of the study population, by quintile of each diet pattern score, were summarized and compared using chi-square tests for categorical variables and two-sample t-tests for continuous variables following a normal distribution, or the Kruskal-Wallis nonparametric test for continuous variables that did not follow a normal distribution. Because the distributions of CRP and $F_2$-isoprostanes were right skewed, their values were transformed by the natural logarithm, and then the adjusted geometric means and their standard errors by quintile of each dietary pattern were computed using a general linear model (implemented using the SAS GLM procedure), controlling for the potential confounding effects of other factors. To facilitate interpretation and comparisons

between the relative strengths of the diet pattern-biomarker associations, ordinal logistic regression analysis was also used in which CRP and $F_2$-isoprostane levels were categorized into quintiles based on the sex-specific concentrations in the pooled study population. The multivariable unconditional ordinal logistic regression models were used to calculate odds ratios (OR) and 95% confidence intervals (95% CI) for associations of each dietary score with each sex-specific quintile increase of each biomarker. The median of each diet score quintile was used for calculating all tests for trend.

Based on previous literature and biologic plausibility, potential confounding variables considered included the study (MAP1 or MAPII), regular NSAID or aspirin use (≥4 times/week), age, total energy intake, hormone replacement use (among women), sex, current smoking status, body mass index (BMI), education, physical activity, regular multivitamin use, season of the year the questionnaire was filled out, race, and family history of colon cancer in a first degree relative. Inclusion in the final models required one or more of the following criteria: biological plausibility, statistical significance, and/or whether inclusion/exclusion of the variable from the model changed the adjusted OR for the primary exposure variable by ≥10%. The final adjusted models controlled for study (MAP1 or MAPII), regular NSAID or aspirin use (≥4x/week), age, total energy intake (kcal), hormone replacement use (among women), sex, smoking (current or former/never), BMI ($kg/m^2$, categorized by WHO criteria into normal/underweight, overweight, and obese), education (no college education or some college education), physical activity (high or low based on the study population median weekly METs expenditure from moderate and vigorous activities), regular multivitamin use (≥3x/week), and the season of the year the questionnaire was filled out.

To assess potential effect measure modification, separate analyses were conducted within dichotomous categories (as defined above) of smoking (current or former/never), BMI (underweight/normal or overweight/obese, sex, NSAID and aspirin use (regular or non-regular users), multivitamin use (regular or non-regular users), physical activity (higher or lower than the pooled study population's median of 195.5 METs per week), age (younger or older than the pooled study population's median age of 56.9 years), and education (any college vs. no college).

To assess the sensitivity of the observed associations to how we defined the scores, each food component was removed from each *a priori* score one at a time to determine whether any one component substantially influenced the diet score-biomarker associations. Diet scores were also constructed using medians rather than quintiles for the food groups, and an alternative calculation of the fat ratio variable (mono + polyunsaturated fat:saturated fat) was used for the Mediterranean diet score. The presence of colon or rectal adenomas in this study population was also assessed as a potential confounder and an effect measure modifier. All analyses were conducted using SAS statistical software (SAS version 9.3, SAS Statistical Institute, Cary, NC). Two-sided tests were considered statistically significant if $p \leq 0.05$.

## Results

Selected characteristics of the participants by diet score tertile are summarized in Table 2.2. Compared to those in the lowest quintile of the Paleolithic diet score, those in the highest quintile were, on average, older; were less likely to smoke, more likely to have a bachelor's degree or higher; and consumed, on average, less total energy, more calcium (mainly via supplements), more dietary fiber and vegetables, more Ω3 fatty acids per gram of Ω6 fatty acids, and less fat and red and processed meat. The descriptive comparisons for the Mediterranean diet score were similar to those for the Paleolithic diet score. The Paleolithic diet score ranged from 24 to 61, while the Mediterranean diet score ranged from 16 to 44; the mean score for each diet pattern did not appreciably differ between the two original study populations. Also, the score ranges did not appreciably differ by sex, and the correlation between the two scores was linear, with a Pearson correlation of $\varrho$=0.70. For each quintile of the Paleolithic diet score, the percentages of participants who were in the same or different quintiles of the Mediterranean diet score are shown in Table 2.3. Among those in the lowest and highest quintiles of the Paleolithic diet score, 48.8% and 44%, respectively, were in the corresponding quintile of the Mediterranean diet score. Also, among those in each quintile of the Paleolithic diet score, there were persons who were in one of each of the quintiles of the Mediterranean diet score.

The multivariable-adjusted geometric means of serum CRP and $F_2$-isoprostanes for each quintile of each dietary pattern score are presented in Table 2.4. For each diet pattern, there was a pattern of decreasing CRP and $F_2$-isoprostane levels with increasing diet score quintile. For those in the highest relative to the lowest quintile of the Paleolithic and Mediterranean diet scores, the mean CRP levels were, proportionately, 27 and 30% lower, respectively, and the mean $F_2$-isoprostane levels were, proportionately, 15.5% and 12.6% lower, respectively. The p for trend was statistically significant for each biomarker for each diet pattern.

For additional perspective, the overall associations of the diet scores with each serum biomarker from the ordinal logistic regression analyses are also presented in Table 2.4. In the multivariable-adjusted analyses, all tests for trend were statistically significant. For those in the upper relative to those in the lowest quintile of the Paleolithic and Mediterranean diet scores, there was, respectively, an estimated 38% and 45% lower odds of having a higher plasma CRP level, and a more than 50% lower odds of having a higher plasma $F_2$-isoprostanes level.

Analyses stratified by age, sex, education, current smoking status, current NSAID/aspirin use, and physical activity are illustrated in Figure 2.1.The inverse associations of the Paleolithic diet score with CRP concentrations tended to be stronger among participants who were current smokers, overweight/obese, or less physically active (the p for a multiplicative interaction was statistically significant only for physical activity). For the Mediterranean diet score there were no statistically significant interactions across the categories of any of these variables. The inverse associations of the Paleolithic diet with $F_2$-isoprostanes concentrations tended to be stronger among those who were current smokers, younger, female, or had more than a high school education (none of the tests for multiplicative interaction was statistically significant); whereas for the Mediterranean diet score the inverse association was stronger among those who were younger or female (the p for a multiplicative interaction was statistically significant only for the latter).

In the sensitivity analyses in which each component of each dietary score was removed individually to determine its influence on the observed associations, no single component

substantially altered the associations between the diet scores and levels of either biomarker. Yet, removing some components that most clearly define the differences between the two scores (e.g., grains, dairy, monounsaturated:saturated fat ratio, calcium, sugar-sweetened beverages) did lessen the differences between the associations of the two scores with $F_2$-isoprostanes (data not shown). There were also no appreciable differences in diet–biomarker associations between those found to have colorectal adenomas at their colonoscopy and those who did not, and including adenomas as a covariate in the models did not appreciably alter the estimated associations.

## Discussion

Our results suggest that diets that are more "Paleolithic-" or Mediterranean-like may be associated with lower levels of systemic inflammation and oxidative stress. Although the two diet patterns are similar in some, but not all respects, and the overlap in how persons in this study were categorized was only moderate, the associations of the two diet patterns with the markers of inflammation and oxidative stress were quite similar. Our results also suggested that the Paleolithic diet-biomarker associations may be stronger among those who smoke, and that for either diet score the association with $F_2$-isoprostanes may be stronger among females and those who are younger. While there were other differences across subgroups, they were not consistent across diets or biomarkers.

Both the Paleolithic and Mediterranean diet patterns have elements that may reduce chronic inflammation or improve oxidative balance. Both are high in fruits, vegetables, fish, and nuts; have a more favorable Ω-6:Ω-3 fatty acid ratio and a lower glycemic load; and are less energy dense than a Western diet (likely leading to an improved energy balance (42, 209)), all of which are thought to improve systemic inflammation and oxidative balance.

There are few reported studies of a Paleolithic diet pattern in relation to biomarkers of disease risk. Six small pilot trials that examined the effects of a Paleolithic diet on cardiovascular risk and glycemic control biomarkers, such as HbA1c, plasma insulin, blood pressure, and serum triglycerides and cholesterol (157-162), generally observed improvements in these markers, although only three of these pilot trials had a control group (160-162). A slightly larger study (n=70) of the effects of a Paleolithic diet on long-term weight loss found statistically significant greater weight loss

at 6 months in the Paleolithic diet group relative to the control group, although the difference mostly dissipated after 24 months (166). Maintenance of a more normal body weight may be one way a Paleolithic diet may maintain lower levels of inflammation and oxidative stress. However, because we controlled for BMI in our models, our findings suggest that a Paleolithic diet may also reduce inflammation and oxidative stress via other mechanisms. Of further, although indirect, support of the inverse associations found in the present study of the two diet pattern scores with inflammation and oxidative stress levels, is that, in part because colorectal neoplasms are thought to be associated with inflammation and oxidative stress (217-221), we previously investigated associations of the two diet pattern scores with incident, sporadic colorectal adenoma in a case-control study in a different population than from that reported herein (214). In that study we found that both diets were similarly inversely associated with adenoma (214).

The Mediterranean diet was associated with lower circulating levels of biomarkers of inflammation and oxidative balance in several prior studies. It has generally been more strongly inversely associated with CRP than have other healthy diet patterns reported in the literature (43), and greater adherence to a Mediterranean diet pattern has also been associated with lower $F_2$-isoprostane levels as well as with other biomarkers of lipid peroxidation (98). In the PREDIMED sub-study (n=110) of participants with metabolic syndrome randomized to a Mediterranean diet supplemented with either olive oil or nuts were estimated to have an almost 50% greater reduction in mean $F_2$-isoprostane levels ($p=0.06$) relative to the low-fat diet arm after one year (139). The current study supports these previously published findings.

Unlike most diet patterns where stronger inverse associations for a wide range of outcomes are usually observed for men but not women (14), we found the Paleolithic and Mediterranean diet patterns to be more strongly inversely associated with the biomarkers—especially $F_2$-isoprostanes—among women. However, the tests for interaction were mostly not statistically significant and our sex-specific findings may be due to the sex-specific diet scoring procedure we used, which allowed for different consumption patterns across the sexes.

This study has several strengths and limitations. Strengths include that it is the first investigation of associations of a Paleolithic diet pattern with biomarkers of inflammation or oxidative stress. The Paleolithic and the Mediterranean diet patterns were similarly constructed so that differences in the findings for the two diets would be attributable to the differences in the dietary patterns rather than to the mechanics of how the scores were constructed. Limitations include that participants' diets were not perfectly consistent with the ideal of either pattern; however, this would likely result in underestimates of the inverse associations with CRP and $F_2$-isoprostanes and suggest that even moderate adherence to one of the diet patterns may be associated with lower levels of inflammation and oxidative balance. Other limitations include the study's cross-sectional design, the known limitations of assessing diet with self-reported semi-quantitative food frequency questionnaires (91, 92), and the limited number of biomarkers to characterize inflammation and oxidative balance. The two original studies that were pooled for the present analysis used different versions of the Willett FFQ; however, the diet pattern components and scores were highly correlated between the two studies. Last, individuals undergoing elective, outpatient colonoscopy may not be representative of the general United States population.

In conclusion, our findings, taken together with those from previous studies, suggest that diets that are more "Paleolithic-" or Mediterranean-like may be associated with lower levels of systemic inflammation and oxidative stress.

Table 2.1. Paleolithic and Mediterranean diet score constituents and point assignments[a]

| | Lowest intake quintile | 2nd Quintile | 3rd Quintile | 4th Quintile | Highest intake quintile |
|---|---|---|---|---|---|
| **Paleolithic diet score[b]** | | | | | |
| Vegetables | 1 | 2 | 3 | 4 | 5 |
| Fruits | 1 | 2 | 3 | 4 | 5 |
| Fruit & vegetable diversity[d] | 1 | 2 | 3 | 4 | 5 |
| Lean meats[e] | 1 | 2 | 3 | 4 | 5 |
| Fish | 1 | 2 | 3 | 4 | 5 |
| Nuts | 1 | 2 | 3 | 4 | 5 |
| Calcium[f] | 1 | 2 | 3 | 4 | 5 |
| Red and processed meats[g] | 5 | 4 | 3 | 2 | 1 |
| Sodium | 5 | 4 | 3 | 2 | 1 |
| Dairy | 5 | 4 | 3 | 2 | 1 |
| Grains and starches | 5 | 4 | 3 | 2 | 1 |
| Baked goods[h] | 5 | 4 | 3 | 2 | 1 |
| Sugar sweetened beverages | 5 | 4 | 3 | 2 | 1 |
| Alcohol (drinks/week) | 5 | 4 | 3 | 2 | 1 |
| **Mediterranean diet score[c]** | | | | | |
| Vegetables | 1 | 2 | 3 | 4 | 5 |
| Fruits | 1 | 2 | 3 | 4 | 5 |
| Lean meats[e] | 1 | 2 | 3 | 4 | 5 |
| Fish | 1 | 2 | 3 | 4 | 5 |
| Nuts | 1 | 2 | 3 | 4 | 5 |
| Monounsaturated:saturated fat ratio | 1 | 2 | 3 | 4 | 5 |
| Red and processed meats[g] | 5 | 4 | 3 | 2 | 1 |
| Sodium | 5 | 4 | 3 | 2 | 1 |
| Dairy | 1 | 3 | 5 | 3 | 1 |
| Grains and starches | 1 | 3 | 5 | 3 | 1 |
| Alcohol | Women: 5 – 15 g/day (+5 points)<br>Men: 10—25 g/day (+5 points)<br>Otherwise (+1 point) | | | | |

[a] All constituents measured in servings/week unless otherwise indicated.
[b] Paleolithic diet score: 14 components, range of possible scores 14 – 70.
[c] Mediterranean diet score: 11 components, range of possible scores 11 – 55.
[d] Fruit & vegetable diversity calculated by summing the total number of responses on the food frequency questionnaire [fruit] and vegetable sections that indicated that the participant ate more than 1-3 servings of a given food item per month.
[e] Lean meats include skinless chicken or turkey, lean beef.
[f] Calcium intake from sources other than dairy; calculated as residuals from linear regression of total calcium intake (mg/day) on dairy foods intake.
[g] Nitrate processed meats and non-lean red meat consumption together.
[h] Baked goods include items such as cake, pie, and other pastry-type food.

Table 2.2. Selected characteristics of participants (N=646); pooled MAPI and MAPII studies.

| Characteristics | Paleolithic diet score | | | | | | | Mediterranean diet score | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1st Quintile | | 3rd Quintile | | 5th Quintile | | | 1st Quintile | | 3rd Quintile | | 5th Quintile | | |
| | N=162 n or Mean | Range (24-37) % or (SD) | N=132 n or Mean | Range (41-43) % or (SD) | N=125 n or Mean | Range (48-61) % or (SD) | P value[a] | N=125 n or Mean | Range (16-24) % or (SD) | N=131 n or Mean | Range (28-30) % or (SD) | N=107 n or Mean | Range (35-44) % or (SD) | P value[a] |
| Plasma C-reactive protein (µg/mL)[b,d] | 5.9 | 6.3 | 5.4 | 6.4 | 4.6 | 6.0 | 0.65 | 6.0 | 6.6 | 5.6 | 6.4 | 4.8 | 6.1 | 0.24 |
| Plasma $F_2$-isoprostanes (ng/L)[b,d] | 92.4 | 35.4 | 88.2 | 40.3 | 73.7 | 28.2 | <0.01 | 98.7 | 42.5 | 83.1 | 44.2 | 83.7 | 45.0 | <0.01 |
| MAPI study population | 112 | 24.7 | 89 | 19.6 | 98 | 21.6 | 0.27 | 84 | 18.5 | 96 | 21.2 | 69 | 15.2 | 0.32 |
| Prevalent colorectal adenoma[d] | 56 | 24.7 | 43 | 18.9 | 46 | 20.3 | 0.50 | 46 | 20.3 | 46 | 20.3 | 32 | 14.1 | 0.19 |
| Age (yrs.)[b] | 54.3 | 9.5 | 56.0 | 8.4 | 59.8 | 8.4 | <0.01 | 54.4 | 9.3 | 58.0 | 8.6 | 58.5 | 8.4 | <0.01 |
| Male | 90 | 28.3 | 64 | 20.1 | 62 | 19.5 | 0.29 | 65 | 20.4 | 62 | 19.5 | 45 | 14.2 | 0.36 |
| White | 150 | 26.0 | 120 | 20.8 | 111 | 19.2 | 0.21 | 116 | 20.1 | 113 | 19.6 | 97 | 16.8 | 0.46 |
| Current smoker[d] | 58 | 36.9 | 31 | 19.8 | 19 | 12.1 | <0.01 | 42 | 26.8 | 27 | 17.2 | 18 | 11.5 | 0.12 |
| Physical activity (MET-hrs./wk.)[b] | 438.3 | 830.3 | 552.7 | 869.4 | 655.4 | 1030.9 | 0.25 | 463.7 | 829.3 | 430.8 | 778.8 | 553.0 | 855.3 | 0.69 |
| Body mass index (kg/m$^2$)[d] | | | | | | | | | | | | | | |
| Normal & underweight (< 25) | 54 | 25.1 | 45 | 20.9 | 46 | 21.4 | | 39 | 18.1 | 42 | 19.5 | 32 | 14.9 | |
| Overweight (25 - 29.9) | 54 | 23.6 | 45 | 19.7 | 49 | 21.4 | | 37 | 16.2 | 51 | 22.3 | 43 | 18.8 | |
| Obese (≥ 30) | 54 | 28.0 | 39 | 20.2 | 30 | 15.5 | 0.76 | 48 | 24.9 | 37 | 19.2 | 29 | 15.0 | 0.37 |
| Current ethanol intake (g/day)[d] | | | | | | | | | | | | | | |
| < 1 drink/day | 138 | 24.0 | 118 | 20.5 | 116 | 20.2 | | 115 | 20.0 | 115 | 20.0 | 98 | 17.0 | |
| 1 - 2 drinks/day | 17 | 38.6 | 9 | 20.5 | 7 | 15.9 | | 6 | 13.6 | 10 | 22.7 | 6 | 13.6 | |
| > 2 drinks/day | 6 | 23.1 | 5 | 19.2 | 2 | 7.7 | 0.33 | 4 | 15.4 | 6 | 23.1 | 3 | 11.5 | 0.81 |
| Taking NSAID or aspirin (≥ 4 per week) | 67 | 24.1 | 49 | 17.6 | 62 | 22.3 | 0.35 | 53 | 19.1 | 59 | 21.2 | 47 | 16.9 | 0.94 |
| Bachelor's degree or higher[d] | 27 | 16.3 | 39 | 23.5 | 39 | 23.5 | 0.04 | 18 | 10.8 | 34 | 20.5 | 35 | 21.1 | 0.01 |
| Hormone replacement therapy (% of women) | 33 | 19.5 | 32 | 18.9 | 42 | 24.9 | 0.07 | 27 | 16.0 | 44 | 26.0 | 35 | 20.7 | 0.11 |
| Total energy intake (kcal/day) | 2077.6 | 736.5 | 1799.7 | 732.3 | 1766.5 | 603.0 | <0.01 | 1800.6 | 694.7 | 1957.8 | 857.7 | 1988.9 | 621.9 | 0.14 |
| Total[c] calcium intake (mg/day) | 789.5 | 416.9 | 762.4 | 440.2 | 901.9 | 447.9 | <0.01 | 736.1 | 382.6 | 859.9 | 473.4 | 928.3 | 460.7 | 0.02 |
| Dietary calcium (mg/day) | 677.8 | 310.8 | 597.7 | 310.7 | 679.7 | 311.5 | 0.06 | 608.1 | 296.2 | 662.8 | 324.0 | 702.4 | 287.4 | 0.24 |
| Supplemental calcium (mg/day) | 111.7 | 293.0 | 164.7 | 340.4 | 222.2 | 375.8 | <0.01 | 128.1 | 290.3 | 197.1 | 365.3 | 225.9 | 389.3 | 0.19 |
| Dietary fiber (g/day) | 18.4 | 8.0 | 19.5 | 8.7 | 25.4 | 10.8 | <0.01 | 15.7 | 6.6 | 21.4 | 9.1 | 26.9 | 11.9 | <0.01 |
| Total fat intake (g/day) | 78.8 | 33.3 | 66.4 | 29.8 | 54.2 | 20.3 | <0.01 | 69.5 | 32.2 | 70.2 | 38.8 | 65.3 | 24.2 | 0.59 |
| Ω-3:Ω-6 ratio | 0.1 | 0.1 | 0.1 | 0.1 | 0.3 | 0.2 | <0.01 | 0.1 | 0.1 | 0.2 | 0.1 | 0.2 | 0.2 | <0.01 |
| Total red and processed meat intake (servings/day) | 0.5 | 0.5 | 0.4 | 0.5 | 0.3 | 0.4 | 0.01 | 0.5 | 0.5 | 0.5 | 0.5 | 0.3 | 0.3 | 0.01 |
| Total vegetable intake (servings/day) | 2.1 | 1.9 | 2.6 | 2.6 | 4.1 | 2.9 | <0.01 | 1.9 | 1.7 | 2.7 | 2.2 | 3.7 | 3.4 | <0.01 |

Abbreviations: SD, standard deviation; MET-hrs./wk., metabolic equivalents of tasks (hours/week); NSAID, non-steroidal anti-inflammatory drug.

[a] P values calculated using chi-square tests for categorical variables and ANOVA for continuous variables unless otherwise noted.

[b] P values calculated using Kruskal-Wallis non-parametric test.

[c] Dietary + supplemental.
[d] Missing data: Plasma C-reactive protein (n = 84); Plasma F2-isoprostanes (n = 209); Prevalent colorectal adenoma (n = 48); Age (n = 57); Smoking status (n = 15); BMI (n = 9); Current ethanol intake (n = 1); Education level (n = 4).

Table 2.3. For each quintile of the Paleolithic diet score, the number and percentages of study participants who were in the same and different quintiles of the Mediterranean diet score; pooled MAPI and MAPII studies.

| | | Paleolithic diet score quintile | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| Mediterranean diet score quintile | 1 | 79 (48.8%) | 25 (22.9%) | 16 (12.1%) | 3 (2.5%) | 2 (1.6%) |
| | 2 | 48 (29.6%) | 32 (29.4%) | 28 (21.2%) | 15 (12.7%) | 7 (5.6%) |
| | 3 | 25 (15.4%) | 30 (27.5%) | 33 (25.0%) | 30 (25.4%) | 13 (10.4%) |
| | 4 | 9 (5.6%) | 19 (17.4%) | 42 (31.8%) | 35 (29.7%) | 48 (38.4%) |
| | 5 | 1 (0.6%) | 3 (2.8%) | 13 (9.9%) | 35 (29.7%) | 55 (44.0%) |
| | | 162 (100%) | 109 (100%) | 132 (100%) | 118 (100%) | 125 (100%) |

Table 2.4. Associations of Paleolithic and Mediterranean diet scores with plasma levels[a] of C-reactive protein and F2-isoprostanes; pooled MAPI and MAPII studies.

| | Paleolithic diet score | | | | | | Mediterranean diet score | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N[a] | Adjusted Geometric Mean[b] | 95% CI | Adjusted OR[c] | 95% CI | N[a] | Adjusted Geometric Mean[b] | 95% CI | Adjusted OR[c] | 95% CI |
| **C-reactive protein (µg/mL)** | | | | | | | | | | | |
| Quintiles of diet score | | | | | | | | | | | |
| | 1 | 132 | 3.7 | (3.1, 4.5) | 1.00 | | 102 | 4.0 | (3.2, 4.9) | 1.00 | |
| | 2 | 85 | 3.3 | (2.6, 4.1) | 0.97 | (0.59, 1.61) | 102 | 3.4 | (2.8, 4.1) | 0.80 | (0.48, 1.32) |
| | 3 | 95 | 3.2 | (2.6, 3.9) | 0.91 | (0.56, 1.48) | 101 | 3.1 | (2.5, 3.8) | 0.63 | (0.37, 1.06) |
| | 4 | 95 | 2.8 | (2.3, 3.5) | 0.72 | (0.43, 1.18) | 115 | 2.7 | (2.3, 3.3) | 0.57 | (0.34, 0.95) |
| | 5 | 91 | 2.7 | (2.2, 3.4) | 0.62 | (0.37, 1.04) | 78 | 2.8 | (2.2, 3.5) | 0.55 | (0.31, 0.96) |
| *P for trend*[d] | | | *0.03* | | *0.04* | | | *0.01* | | *0.01* | |
| **$F_2$-isoprostanes (ng/L)** | | | | | | | | | | | |
| Quintiles of diet score | | | | | | | | | | | |
| | 1 | 98 | 89.8 | (83.3, 96.8) | 1.00 | | 74 | 91.5 | (84, .0 99.7) | 1.00 | |
| | 2 | 72 | 89.0 | (81.9, 96.8) | 0.83 | (0.47, 1.47) | 76 | 89.0 | (82.0, 96.5) | 0.79 | (0.43, 1.43) |
| | 3 | 69 | 82.4 | (75.7, 89.7) | 0.68 | (0.38, 1.21) | 74 | 81.5 | (75.1, 88.4) | 0.47 | (0.26, 0.87) |
| | 4 | 73 | 80.5 | (74.2, 87.3) | 0.50 | (0.29, 0.89) | 98 | 78.7 | (73.2, 84.6) | 0.47 | (0.26, 0.85) |
| | 5 | 71 | 75.9 | (69.6, 82.8) | 0.41 | (0.22, 0.75) | 61 | 80.0 | (73.1, 87.5) | 0.48 | (0.25, 0.92) |
| *P for trend*[d] | | | *<0.01* | | *<0.01* | | | *<0.01* | | *0.01* | |

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval.

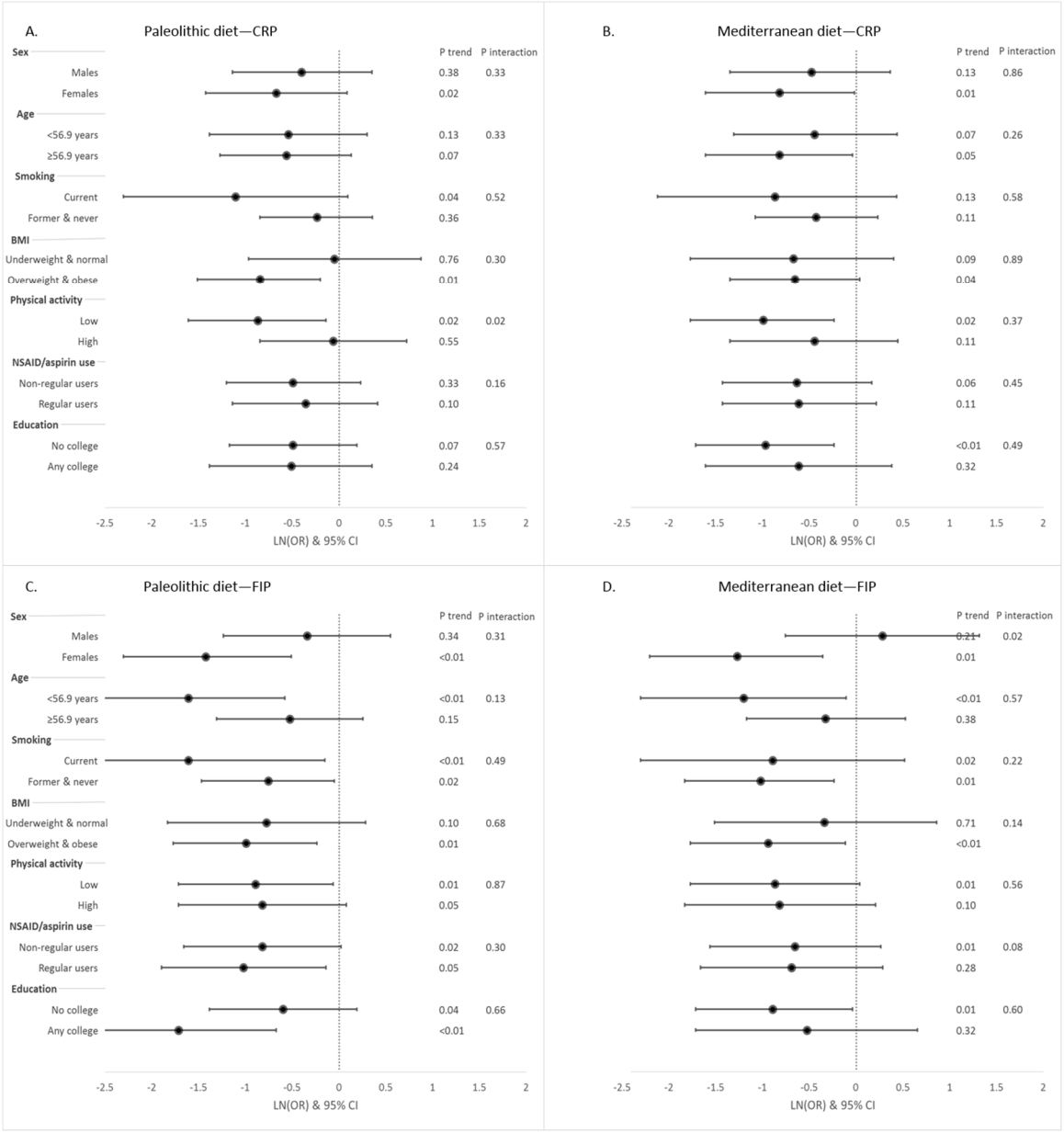
[a] Unequal sample sizes in quintiles due to ranking ties. Differences in the numbers of participants having the two biomarker assays related to serum sample availability.

[b] Adjusted geometric mean from general linear model; covariates included study (MAP1 or MAPII), regular NSAID or aspirin use (≥ 4x per week), age, total energy intake (kcal), current hormone replacement use (among women), sex, smoking (current or former and never), body mass index (kg/m$^2$, categorized by WHO criteria into normal, overweight, and obese), education level (no college education or some college education), physical activity level (high or low based on the median weekly METs expenditure in the pooled population), regular multivitamin use (≥ 3x per week), season of the year completed food frequency questionnaire.

[c] From unconditional ordinal logistic regression model; covariates adjusted for were the same as in [b]. CRP and $F_2$-isoprostanes are categorized into sex-specific quintiles from the pooled study population. For each diet score quintile, $e^{\beta}$ is the odds that the biomarker concentration is greater than the quintile cut point if the diet score is in the non-referent category relative to the odds if in the referent category.

[d] P for trend calculated by assigning the median of each diet score quintile to each quintile, and treating this quintile exposure as continuous.

*Figure 2.1. Associations of the Paleolithic and Mediterranean diet scores with plasma C-reactive protein and $F_2$-isoprostanes concentrations,[a] according to selected participant characteristics; pooled MAPI and MAPII studies.*




Abbreviations: CRP, C-reactive protein; FIP, $F_2$-isoprostanes; BMI, body mass index; OR, odds ratio; LN, natural logarithm; 95% CI, 95% confidence interval.

[a] From unconditional ordinal logistic regression model; only the comparison of the 5th relative to the 1st quintile of each diet score with the sex-specific quintiles of the biomarkers are shown; model covariates included study (MAP1 or MAPII), regular NSAID or aspirin use (≥ 4x per week), age, total energy intake (kcal), current hormone replacement use (among women), sex, smoking (current or former and never), body mass index (kg/m$^2$, categorized by WHO criteria into normal, overweight, and obese), education level (no college education or some college education), physical activity level (high or low based on the median weekly METs expenditure in the pooled population), regular multivitamin use (≥ 3x per week), season of the year completed food frequency questionnaire. CRP and $F_2$-isoprostanes are categorized into sex-specific quintiles from the pooled study population.

[b] P for trend calculated by assigning the median of each diet score quintile to each quintile, and treating this quintile exposure as continuous. CRP and $F_2$-isoprostanes are categorized into sex-specific quintiles from the pooled study population. For each quintile of each diet score, $e^{\beta}$ is the odds that the biomarker concentration is greater than the quintile cut point if the diet score is in the non-referent category compared to the odds if in the referent category.

Supplemental Table 2.1. Adjusted geometric means of Paleolithic and Mediterranean diet scores by quintiles of circulating C-reactive protein and F2-isoprostanes levels,[a] according to selected risk factors for chronic inflammation; pooled MAPI and MAPII studies.

| | CRP | | | | F2-Isoprostanes | | | |
|---|---|---|---|---|---|---|---|---|
| | Paleolithic diet score | | Mediterranean diet score | | Paleolithic diet score | | Mediterranean diet score | |
| | Adjusted geometric mean[a] | 95% CI | Adjusted geometric mean[a] | 95% CI | Adjusted geometric mean[a] | 95% CI | Adjusted geometric mean[a] | 95% CI |
| **Quintile, by smoking status** | | | | | | | | |
| Current (n = 157) | | | | | | | | |
| 1 | 4.4 | (3.2, 6.1) | 4.4 | (3.0, 6.3) | 101.1 | (89.9, 113.6) | 95.7 | (84.3, 108.7) |
| 3 | 3.8 | (2.4, 6.0) | 3.4 | (2.2, 5.3) | 80.2 | (68.1, 94.3) | 89.2 | (76.3, 104.3) |
| 5 | 3.0 | (1.7, 5.3) | 2.9 | (1.6, 5.2) | 73.6 | (61.0, 88.8) | 80.9 | (67.0, 97.7) |
| *P for trend*[b] | *0.24* | | *0.04* | | *0.23* | | *0.09* | |
| Former and never (n = 474) | | | | | | | | |
| 1 | 3.4 | (2.7, 4.2) | 3.7 | (2.8, 4.8) | 86.4 | (78.4, 95.1) | 91.7 | (81.7, 102.8) |
| 3 | 2.9 | (2.3, 3.7) | 3.0 | (2.4, 3.7) | 81.9 | (74.1, 90.6) | 80.2 | (72.8, 88.4) |
| 5 | 2.7 | (2.1, 3.4) | 2.8 | (2.1, 3.6) | 75.9 | (68.7, 83.9) | 77.1 | (69.2, 85.9) |
| *P for trend*[b] | *0.17* | | *0.14* | | *<0.01* | | *<0.01* | |
| *P for interaction* | *0.27* | | *0.07* | | *<0.01* | | *0.19* | |
| **Quintile, by BMI** | | | | | | | | |
| Normal weight & underweight (n = 215) | | | | | | | | |
| 1 | 2.1 | (1.5, 2.9) | 2.5 | (1.7, 3.7) | 75.6 | (66.5, 86.0) | 78.1 | (66.9, 91.2) |
| 3 | 1.5 | (1.0, 2.2) | 1.6 | (1.1, 2.4) | 76.1 | (65.4, 88.4) | 70.2 | (60.6, 81.3) |
| 5 | 2.2 | (1.4, 3.2) | 1.6 | (1.0, 2.6) | 68.7 | (59.0, 80.0) | 70.7 | (59.9, 83.6) |
| *P for trend*[b] | *0.85* | | *0.16* | | 0.18 | | *0.38* | |
| Overweight & obese (n = 422) | | | | | | | | |
| 1 | 5.2 | (4.2, 6.5) | 5.3 | (4.2, 6.8) | 98.3 | (89.4, 108.1) | 100.7 | (90.7, 111.8) |
| 3 | 4.5 | (3.6, 5.8) | 4.3 | (3.4, 5.4) | 87.1 | (78.4, 96.7) | 88.5 | (80.1, 97.7) |
| 5 | 3.1 | (2.4, 4.0) | 3.7 | (2.8, 4.8) | 79.9 | (71.8, 89.0) | 85.6 | (76.7, 95.5) |
| *P for trend*[b] | *<0.01* | | 0.02 | | *<0.01* | | *<0.01* | |
| *P for interaction* | *0.26* | | *0.07* | | *<0.01* | | *<0.01* | |
| **Quintile, by age** | | | | | | | | |
| < 56.9 years (n = 275) | | | | | | | | |
| 1 | 3.4 | (2.6, 4.4) | 3.6 | (2.7, 4.8) | 93.7 | (83.1, 105.6) | 97.0 | (84.8, 111.0) |
| 3 | 3.1 | (2.3, 4.2) | 2.5 | (1.8, 3.4) | 87.0 | (76.0, 99.5) | 78.6 | (68.4, 90.3) |
| 5 | 2.2 | (1.5, 3.3) | 2.5 | (1.7, 3.7) | 66.9 | (56.3, 79.5) | 82.3 | (68.6, 98.6) |
| *P for trend*[b] | *0.20* | | *0.07* | | *0.01* | | *0.01* | |
| ≥ 56.9 years (n = 371) | | | | | | | | |
| 1 | 4.0 | (3.0, 5.3) | 4.2 | (3.0, 5.8) | 86.1 | (78.2, 94.8) | 85.5 | (76.2, 95.9) |
| 3 | 3.1 | (2.3, 4.1) | 3.7 | (2.8, 4.8) | 77.6 | (69.6, 86.6) | 84.3 | (76.2, 93.2) |
| 5 | 3.0 | (2.3, 3.9) | 2.8 | (2.1, 3.7) | 78.8 | (71.5, 86.8) | 78.7 | (71.1, 87.1) |
| *P for trend*[b] | *0.02* | | *0.02* | | *0.01* | | *0.01* | |
| *P for interaction* | *0.29* | | *0.66* | | *0.18* | | *0.89* | |
| **Quintile, by sex** | | | | | | | | |
| Male (n = 318) | | | | | | | | |
| 1 | 2.8 | (2.2, 3.5) | 2.9 | (2.2, 3.7) | 73.1 | (67.2, 79.6) | 73.2 | (66.6, 80.4) |
| 3 | 2.4 | (1.8, 3.2) | 2.3 | (1.7, 3) | 66.9 | (60.1, 74.5) | 69.9 | (63.8, 76.5) |
| 5 | 2.1 | (1.5, 2.8) | 2.2 | (1.5, 3.1) | 67.7 | (61.2, 74.9) | 76.1 | (67.6, 85.6) |
| *P for trend*[b] | *0.23* | | *0.17* | | *0.04* | | *0.02* | |
| Female (n = 328) | | | | | | | | |
| 1 | 4.3 | (3.2, 5.7) | 4.7 | (3.4, 6.5) | 100.8 | (89.3, 113.7) | 103.5 | (90.1, 119.1) |
| 3 | 3.4 | (2.6, 4.5) | 3.5 | (2.6, 4.6) | 92.4 | (81.5, 104.8) | 87.8 | (77.1, 100.0) |
| 5 | 2.9 | (2.1, 4.0) | 2.9 | (2.2, 4.0) | 77.1 | (67.0, 88.7) | 80.8 | (70.8, 92.2) |
| *P for trend*[b] | *0.01* | | *0.01* | | *<0.01* | | *<0.01* | |
| *P for interaction* | *0.59* | | *0.83* | | *0.22* | | *0.26* | |

Abbreviations: 95% CI, 95% confidence interval.

[a] Adjusted geometric mean from general linear model; covariates included study (MAP1 or MAPII), regular NSAID or aspirin use (≥ 4x per week), age, total energy intake (kcal), current hormone replacement use (among women), sex, smoking (current or former and never), body mass index (kg/m$^2$, categorized by WHO criteria into normal, overweight, and obese), education level (no college education or some college education), physical activity level (high or low based on the median weekly METs expenditure in the pooled population), regular multivitamin use (≥ 3x per week), season of the year completed food frequency questionnaire.

[b] P for trend calculated by assigning the median of each diet score quintile to each quintile, and treating this quintile exposure as continuous.

# CHAPTER 3: PALEOLITHIC AND MEDITERRANEAN DIET PATTERN SCORES AND RISK FOR INCIDENT, SPORADIC COLORECTAL ADENOMAS

Kristine A. Whalen[1], Marjorie L. McCullough[2], W. Dana Flanders[1,3],

Terryl J. Hartman[1,4], Suzanne Judd[5], Roberd M. Bostick[1,4]

[1] Emory University Rollins School of Public Health, Department of Epidemiology

Atlanta, Georgia, United States

[1] American Cancer Society, Epidemiology Research Program, Atlanta, Georgia, United States

[1] Emory University Rollins School of Public Health, Department of Biostatistics and Bioinformatics

Atlanta, United States

[1] Emory University, Winship Cancer Institute Atlanta, Georgia, United States

[1] University of Alabama at Birmingham, Biostatistics Birmingham, Alabama, United States

## Abstract

A Westernized diet is associated with higher risk for colorectal neoplasms. Evolutionary discordance could explain this association. We investigated associations of scores for two proposed diet patterns, the "Paleolithic" and the Mediterranean, with incident, sporadic colorectal adenomas in a case-control study of colorectal polyps conducted in Minnesota (1991-1994). Patients with no prior history of colorectal neoplasms completed comprehensive questionnaires prior to elective, outpatient endoscopy; of these, 564 were identified as cases and 1,202 as endoscopy-negative controls. An additional group of age and sex frequency-matched community controls (n=535) was also recruited. Both diet scores were calculated and categorized into quintiles, and associations estimated using unconditional logistic regression. The multivariable-adjusted odds ratios (OR) comparing those in the highest relative to the lowest quintiles of the Paleolithic and Mediterranean diet scores were, respectively, 0.71 (95% confidence interval [CI]: 0.50, 1.02; $P_{trend}$=0.02) and 0.74 (95% CI: 0.54, 1.03; $P_{trend}$=0.05) when comparing the cases to the endoscopy-negative controls, and 0.84 (95% CI: 0.56, 1.26; $P_{trend}$=0.14) and 0.77 (95% CI: 0.53, 1.11; $P_{trend}$=0.13) when comparing the cases to the community controls. These findings suggest that higher adherence to the Paleolithic or Mediterranean diet patterns may be similarly associated with lower risk for incident, sporadic colorectal adenomas.

## Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the second leading cause of cancer mortality in the United States (222). Rapidly increasing incidence rates in previously low-incidence populations in urban China, Japan, and male Polynesians in Hawaii have coincided with those populations adopting a more westernized lifestyle (13). These changing incidence rates, along with studies of immigrant populations (13), point to a strong influence of diet and other lifestyle factors on CRC risk. Many, but not all, epidemiological studies found diets high in fruits and vegetables to be associated with lower risk of CRC (13, 223). Epidemiological studies on high fat diets and high meat consumption have generally found weak, inconsistent evidence of higher risk of CRC (12, 13), with red and processed meat consumption more convincingly associated with higher risk of CRC (12). Yet several trials testing low-fat diet, fiber supplement, or antioxidant supplement interventions were unsuccessful in reducing the recurrence of colorectal adenomatous polyps (224, 225), the precursors to most colorectal cancers (225-227).

As no single dietary constituent appears responsible for the majority of CRC risk, it may be more useful for future public health recommendations to characterize diet patterns and their relation to risk of CRC. Dietary patterns are helpful in studying the effects of total diet on health outcomes, and can be data driven and flexible enough to examine many different theoretical diets. Several reported studies used food frequency questionnaire responses to create various dietary patterns by using either purely data driven methods (e.g., principal component, factor, and cluster analyses) or by constructing index driven diet patterns in order to investigate associations of diet with CRC risk (15, 110, 111, 114, 115, 132, 220, 228, 229). A commonly examined pattern is the Mediterranean diet, which is characteristic of countries in the Mediterranean region circa 1960, when life expectancy there was among the highest in the world (7). The Mediterranean diet is characterized by high intake of fruits, vegetables, nuts, fish, and whole grains, moderate amounts of alcohol and dairy products, and low quantities of red or processed meats and sweets (7). One small observational study within a clinical trial cohort found that a Mediterranean diet pattern was associated with lower risk of colorectal adenoma recurrence, though only in women (230). While the Mediterranean diet is

considered healthier than a Westernized diet, it has also been proposed that a dietary pattern more consistent to what would have been available during late human evolution may be ideal for preventing modern chronic diseases, including cancer (6).

The evolutionary discordance hypothesis (149) is that the rapid increase in many chronic conditions and diseases over the past century stems from recent changes in diet and lifestyle patterns relative to those pursued by our evolutionary ancestors. Anthropologists have constructed a "Paleolithic Diet" that describes the general diet *Homo sapiens* would have eaten prior to the development of agriculture (6). The Paleolithic diet pattern is characterized by a wide diversity of fruits and vegetables, lean meats, eggs, and nuts; it excludes grains, dairy products, refined fats, and sugar, and is very low in salt. While there are no reported observational studies of the Paleolithic diet pattern and risk for chronic diseases, there are a few reported small pilot trials and one longer Paleolithic diet intervention (157, 160, 162, 166). In the pilot trials, the Paleolithic diet intervention compared to the Mediterranean diet appeared to provide better glucose control, increased weight loss, and reduced waist circumference after 12 weeks (162). When directed to eat a Paleolithic diet, obese post-menopausal women lost significantly more weight after six months than those on a conventional healthy diet, though this difference was mostly attenuated after two years (166).

By examining dietary patterns rather than specific food groups, we may more realistically and robustly account for the effects of multiple weak, likely interacting associations of foods and nutrients on colorectal adenoma risk. We evaluated associations of both Mediterranean and Paleolithic dietary patterns with frequency of newly-diagnosed, sporadic colorectal adenoma in a case-control study of adult men and women in the United States.

## Methods

### Study population and data collection

In the Minnesota Cancer Prevention Research Unit case-control study data were collected between April 1991 and April 1994 as part of a joint project between the University of Minnesota and a large, multi-clinic private gastroenterology practice (193, 194). The gastroenterology practice performed colonoscopies and sigmoidoscopies in 10 hospitals and endoscopy units and, at the time

of the study, was responsible for approximately 60 percent of all colonoscopies in the Minneapolis metropolitan area. The institutional review boards of the University of Minnesota and each endoscopy site approved the study. Written informed consent was obtained from each study participant.

The gastroenterology practice staff initiated study recruitment while scheduling elective, outpatient colonoscopies or flexible sigmoidoscopies ("endoscopies"). All 10 of the practices' endoscopy sites recruited patients. Initial eligibility for study participation required that patients be 30-74 years old, residents of the Minneapolis-St. Paul metropolitan area, English speaking, free of known genetic syndromes associated with a predisposition to colonic neoplasia, and with no individual history of inflammatory bowel disease, adenomatous polyps, or cancers except for non-melanoma skin cancer.

Mailed questionnaires were completed prior to endoscopy, collected at the endoscopy visit, and blood samples drawn. The endoscopists recorded polyp locations and *in vivo* sizes and shapes on standardized forms. All polyps were removed and examined histologically by a single index study pathologist using National Polyp Study diagnostic criteria (195).

Based on the endoscopy and pathology findings, participants were assigned final eligibility and case/control status. To be eligible as an adenoma case or a colonoscopy-negative control, the participant must have had a complete colonoscopy reaching the cecum, had all polyps removed, not have a new diagnosis of inflammatory bowel disease, and have no polyps with invasive carcinoma (n=684). Sigmoidoscopy-negative controls had similar eligibility requirements, but completed only a flexible sigmoidoscopy (n=518). Endoscopy controls were free of both adenomatous and hyperplastic polyps at endoscopy. The participation rate for all colonoscoped patients was 68 percent.

In addition to the endoscopy controls, a separate group of potential community controls (n=535) was randomly selected from the 1991 Minnesota State Driver's License Registry and frequency matched to the cases on age (5-year intervals), sex, and zip code. The community control

participants were only included in the study if they met the same eligibility criteria as the colonoscopy patients except that they did not undergo colonoscopy or sigmoidoscopy to confirm their current polyp status. The participation rate of the community controls was 65 percent.

Study participants provided detailed information on demographic characteristics, personal medical history, smoking history, usual physical activity, anthropometrics, reproductive history and hormone use (women only), and family history of cancer. The frequency of aspirin and non-aspirin non-steroidal anti-inflammatory drug (NSAID) use was assessed as the number of pills taken per week. A self-administered, 166-item modified semi-quantitative Willett food frequency questionnaire was used to assess food and nutritional supplement intakes over the previous 12 months. A standard portion size and nine possible frequency-of-consumption responses, ranging from "never, or less than once per month" to "6 or more times per day" were given for each food. Total energy and nutrient intakes were calculated by adding energy and nutrients from all food sources using the dietary database developed by Willett (92, 191).

A total of 2,301 participants completed the study and were included in this analysis, including 564 cases, 1,202 endoscopy controls, and 535 community controls. Participants who left >10 percent of the food frequency questionnaire questions blank (8 cases, 37 endoscopy-negative controls, and 14 community controls) or had implausible total energy intakes (<600 kcal/day or >5,000 kcal/day; 2 cases, 6 endoscopy-negative controls, and 1 community control) were excluded from the analyses.

Dietary scores

The Paleolithic and Mediterranean diet pattern scores were constructed in a similar manner, as summarized in Table 3.1. The foods and associated point values were determined before analysis using published dietary guidelines for each diet (6, 132, 190). For the most part, each study participant was assigned a quintile rank (and score from 1–5) of intake for each food category, based on the sex-specific distribution of intake in the community controls. Higher scores were given for higher intakes of foods that were considered characteristic of the diet pattern, and for lower to no

consumption of foods that were not considered characteristic of the diet pattern. For the Mediterranean diet score this scheme was modified in relation to dairy, grains and starches, and alcohol intakes as noted in Table 3.1. Although the Mediterranean diet score most often is constructed simply using two categories of intakes (high and low based on median intake), we constructed ours based on quintiles of intake to facilitate a more direct comparison of the two diet scores. For the Paleolithic diet score two unique variables were created. The first, a fruit and vegetable diversity score, was created by summing the total number of responses on the food frequency questionnaire fruit and vegetable sections that indicated that the participant ate >1–3 servings of a given food item/month. More diversity was considered desirable. Second, because the Paleolithic diet had little dairy but high amounts of calcium (from wild greens) (6), to consider dietary calcium separately from dairy products we used the residuals of a linear regression of total calcium intake on total dairy intake to represent calcium intake independent of dairy consumption. The final scores could range from 11 to 55 for the 11-component Mediterranean diet score and from 14 to 70 for the 14-component Paleolithic diet score.

Statistical analysis

The characteristics of the cases, endoscopy controls, and community controls were summarized and compared using chi-square tests for categorical variables and two-sample t-tests for continuous variables. Unconditional logistic regression models were used to calculate odds ratios (OR) and 95% confidence intervals (95% CI) for associations of the two dietary scores with colorectal adenomas. Separate analyses are presented for comparisons of the cases to the endoscopy controls and to the community controls. The Paleolithic and Mediterranean diet pattern scores were analyzed as both continuous and categorical variables (quintiles) based on the distributions of the scores in the community controls. The median of each diet score quintile was used for calculating all tests for trend.

Based on previous literature and biological plausibility, potential confounding variables considered included sex, age (continuous), race, total energy intake (kcal/day), education (in years),

body mass index (BMI kg/m$^2$), family history of colon cancer in a first-degree relative, history of diabetes, hormone replacement therapy use (women only), regular (≥ once per week) NSAID use, physical activity (Metabolic Equivalent of Task (MET)s/week), and smoking (current/former/never). Inclusion in the final models required one or more of the following criteria: biological plausibility, statistical significance, and/or whether inclusion or exclusion of the variable from the model changed the adjusted OR for the primary exposure variable by ≥10 percent. The final adjusted models controlled for age, sex, total energy intake, hormone replacement therapy use, family history of colon cancer in a first-degree relative, NSAID use, BMI, and physical activity.

To assess potential effect modification, separate analyses were conducted for each category of: age (<56 years / ≥ 56 years), sex, family history of colon cancer in a first degree relative (yes/no), smoking (ever/never), BMI (normal / overweight & obese), regular NSAID use (yes/no), and physical activity (<25 METs per week / ≥25 METs per week). In addition, separate analyses were conducted according to the cases' adenoma characteristics, including multiplicity (1 / >1) and, based on the largest adenoma, size (<1.0 cm / ≥1.0 cm), colon site (right / left), degree of atypia (mild / moderate-severe), and histologic subtype (tubular / tubulovillous and villous).

To assess the sensitivity of the associations to how we defined the scores, each food component was removed from both *a priori* scores one at a time to determine whether any one component overly influenced the diet score-adenoma associations. All analyses were conducted using SAS statistical software (SAS version 9.3, SAS Statistical Institute, Cary, NC). Two-sided tests were considered statistically significant if $P \leq 0.05$.

## Results

Selected characteristics of the cases and controls are summarized in Table 3.2. Compared to the cases, the endoscopy controls, on average, were younger, had a lower BMI, and had lower intakes of alcohol, fat, red and processed meat, and a lower total energy intake. Endoscopy controls were also more likely to be female, to have never smoked, have a higher level of education, be taking an NSAID or supplemental calcium, eat more fruits and vegetables, and, if a woman, be on hormone replacement therapy. Compared to the cases, the community controls were more likely to be female,

have never smoked, have a lower average BMI, and be less likely to have a first degree relative with a history of colorectal cancer. The mean Paleolithic diet score was slightly lower in the cases than in the community controls (41.3 vs. 42.1; $P$=0.03) and minimally lower than in the endoscopy controls. Mean Mediterranean diet scores did not substantially differ by case/control status. The Paleolithic diet score ranged from 19 to 67, while the Mediterranean diet score ranged from 13 to 46. These ranges did not appreciably differ by sex. The correlation between the two diet scores was linear and strong ($\rho$=0.76).

The overall associations of the diet scores with colorectal adenomas are presented in Table 3.3. In the multivariable-adjusted analyses, when the diet scores were treated as continuous variables, adenoma frequency was estimated to be borderline statistically significantly lower by 1-2 percent per point increase in the Paleolithic and Mediterranean diet scores. When the scores were treated as categorical variables (quintiles), the Paleolithic and Mediterranean diet scores were statistically significantly associated with adenoma frequency in the comparisons involving the endoscopy controls ($P_{trend}$=0.02 and 0.05, respectively, although the estimates for the individual quintiles were not statistically significant), but not in the comparisons involving the community controls. The odds of disease among those in the highest quintile of each score was about one fourth lower than that among those in the lowest quintile. The magnitudes of the Mediterranean diet estimated score-adenoma associations in the comparisons involving the two control groups were nearly identical to each other, but for the Paleolithic diet score they were slightly stronger in the comparison involving the endoscopy controls.

As noted in Table 3.4, the inverse associations of both diet scores with colorectal adenomas were substantially stronger among men and those who were overweight or obese. There were no consistent and clear patterns of differences in the associations of the scores with adenomas according to age (Table 3.4), family history of colorectal cancer in a first degree relative, smoking status, physical activity, or NSAID use (data not shown). As noted in Table 3.5, the inverse associations of both scores with adenomas were substantially stronger for multiple adenomas and adenomas with a

villous component, but there were no clear patterns of differences in the associations according to adenoma size, location, or degree of dysplasia (data not shown).

In the sensitivity analyses in which each component of each dietary score was removed from its respective score one at a time, we found no substantial differences from the associations reported in the tables (data not shown).

## Discussion

Our results suggest that more Paleolithic- and Mediterranean-like dietary patterns may be similarly inversely associated with risk for colorectal adenomas, perhaps especially for men and those who are overweight or obese, as well as for multiple adenomas or adenomas with a villous component.

The Paleolithic and Mediterranean diet patterns both have several components that could plausibly reduce adenoma risk. Both dietary patterns are high in fruits and vegetables that may help improve oxidative balance, increase dietary fiber, and reduce total energy intake, all of which are thought to reduce colorectal adenoma and cancer risk (12, 194, 231, 232). They are also both low in red, processed, and fatty meats, which are thought to increase colorectal cancer risk via several mechanisms (12, 13, 227). The two diet patterns may also reduce systemic inflammation, which is associated with lower risk of colorectal cancer (43, 233). Given that overweight and obese individuals tend to have higher levels of systemic inflammation (234), our findings of stronger inverse associations of the diets with adenomas among those who are overweight or obese provide some indirect support for the hypothesis that inflammation is a key pathway by which these diet patterns act. However, women generally have a higher level of systemic inflammation than do men, yet the associations of the diet patterns with adenoma were stronger among men. Stronger associations between dietary patterns and colorectal adenomas or colorectal cancer in men have frequently been reported, and it is unclear whether this may be related to true biological differences in diet effects (235), differences in diet patterns, or differential diet measurement (14). The stronger associations for multiple adenomas and adenomas with a villous component may be related to inflammation, though the exact mechanism is unclear (236).

The Paleolithic diet pattern was examined in three small pilot dietary intervention studies, one uncontrolled (in a healthy, non-obese population) (157) and two with comparison groups eating conventional healthy diets (in populations of Type-2 diabetes or ischemic heart disease patients) (160, 162); the results from these 12-week trials suggested that the Paleolithic diet pattern may improve blood pressure, serum cholesterol, glycemic control, and C-reactive protein independent of any decrease in weight. A longer trial of post-menopausal obese women directed to eat a Paleolithic diet or a Nordic Nutrition Recommended (low-fat, high-fiber) diet (165) found greater fat loss (-6.5 vs. -2.6 kg, $P<0.001$) and lower levels of triglycerides at 6 months in the Paleolithic diet group, though much of the fat loss was attenuated after two years (-4.6 vs. -2.9 kg, $P=0.095$) (237).

While there are no previous epidemiologic reports of a Paleolithic diet score in relation to colorectal neoplasms, six prospective cohort studies have examined the Mediterranean diet score—one in relation to incident adenomas, one in relation to adenoma recurrence, and four in relation to incident carcinomas—generally finding inverse associations. Among Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial participants, a higher Mediterranean diet score was inversely associated with adenoma (OR for the highest relative to the lowest quintile = 0.79 [95% CI: 0.68, 0.92; $P_{trend}$ <0.001]) (228). A principal components analysis conducted in the European Cancer Prevention Intervention Study identified a Mediterranean-like dietary pattern that was associated with significantly less 3-year adenoma recurrence only among women (OR=0.30 for highest tertile relative to lowest tertile [95% CI: 0.09, 0.98; $P_{trend}$ =0.04]) (230). In the four studies of incident colorectal cancer, the findings for the highest relative to the lowest quantiles of the score were 1) in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, HR=0.89 (95% CI: 0.80, 0.99; $P_{trend}$=0.02) (111); 2) in the Italian component of the EPIC cohort, HR=0.50 (95% CI: 0.35, 0.71; $P_{trend}$=0.04) (110); 3) in the NIH-AARP Diet and Health Study, RR=0.72 (95% CI: 0.63, 0.83) and 0.89 (95% CI: 0.72, 1.11) among men and women, respectively (229); and 4) in the Nurses Health Study (NHS) and Health Professionals Follow-up Study (HPFS) cohorts, the RRs were 0.88

(95% CI: 0.71, 1.09; $P_{trend}$=0.25) and 0.89 (95% CI: 0.77, 1.01; $P_{trend}$=0.06) in men and women, respectively (132).

This study had several strengths and limitations. Strengths included standardized pathologic verification of adenomas, thereby reducing outcome misclassification; the use of two control groups, each with their own strengths and limitations; assessment of exposure information prior to endoscopy, reducing opportunity for recall bias; and the detailed information collected on potentially confounding variables. Whereas there was minimal outcome misclassification among the endoscopy controls, they may have been more similar to the cases in various respects, and whereas the community controls may have been more representative of the general population, some may have been undiagnosed cases; thus, for different reasons, the estimated associations with both control groups likely were attenuated. Although age and sex are known risk factors for colorectal neoplasms and were controlled for in the analyses, the degree to which the endoscopy controls were, on average, younger and more likely to be women, raises the possibility of some selection bias. While the inverse associations between each diet and colorectal adenoma frequency were similar to each other, the point estimates for the associations of the fifth relative to the first quintiles were not statistically significant, underscoring the importance of investigating these diets in larger, preferably prospective, studies. An important limitation of our study was that for the most part the actual diets of the participants could not be considered to be strongly consistent with the Paleolithic or Mediterranean diet patterns. This suggests that our findings may substantially underestimate the potential of the diet patterns for reducing risk for colorectal adenomas. Finally, while our Paleolithic diet score was data-derived for the quintile cutoffs, and thus study specific (see reference 13 for a review of diet scores), the schema can be applied to other study populations.

In conclusion, our findings, taken in context with those from previous studies, suggest that a Paleolithic or Mediterranean diet pattern may be inversely associated with risk for incident, sporadic colorectal adenomas.

Table 3.1. Paleolithic and Mediterranean Diet Pattern Score Constituents and Construction[a]

| Intake Category | Scoring | Paleolithic Diet Score[b] | Mediterranean Diet Score[c] |
|---|---|---|---|
| **Highest intake 'best'** | Points assigned to each quintile = quintile rank<br><br>(e.g., highest and lowest quintiles scored +5 and +1 points, respectively) | Vegetables<br>Fruits<br>Fruit & vegetable diversity[d]<br>Lean meats[e]<br>Fish<br>Nuts<br>Calcium[f] | Vegetables<br>Fruits<br>Lean meats[e]<br>Fish<br>Nuts<br>Monosaturated:saturated fat ratio |
| **Lowest intake 'best'** | Points assigned to each quintile = reverse quintile rank<br><br>(e.g., highest and lowest quintiles scored +1 and +5 points, respectively) | Red and processed meats[g]<br>Sodium<br>Dairy<br>Grain and starches<br>Baked goods[h]<br>Sugar sweetened beverages<br>Alcohol (drinks/week) | Red and processed meats[g]<br>Sodium (mg) |
| **Moderate intake 'best'** | 3rd quintile scored +5,<br>2nd and 4th quintiles scored +3, and<br>1st and 5th quintiles scored +1 points | | Dairy<br>Grains and starches |
| **Other** | | | Alcohol:<br>Women: 5 – 15 g/day (+5 points)<br>Men: 10—25 g/day (+5 points)<br>Otherwise (+1 point) |

[a] All constituents measured in servings/week unless otherwise indicated.
[b] Paleolithic diet score: 14 components, range of possible scores 14 – 70.
[c] Mediterranean diet score: 11 components, range of possible scores 11 – 55.
[d] Fruit & vegetable diversity calculated by summing the total number of responses on the food frequency questionnaire fruit and vegetable sections that indicated that the participant ate more than 1-3 servings of a given food item per month.
[e] Lean meats include skinless chicken or turkey, lean beef.
[f] Calcium intake from sources other than dairy; calculated as residuals from linear regression of total calcium intake (mg/day) on dairy foods intake.
[g] Nitrate processed meats and non-lean red meat consumption together.
[h] Baked goods include items such as cake, pie, and other pastry-type foods.

Table 3.2. Selected Characteristics of Participants (N=2,301); Minnesota CPRU Case-Control Study of Incident, Sporadic Colorectal Adenomas, 1991-1994

| Characteristics | Cases (n = 564) | | Endoscopy controls[a] (n = 1,202) | | | Community controls (n = 535) | | |
|---|---|---|---|---|---|---|---|---|
| | Mean (SD) | % | Mean (SD) | % | *P value*[b] | Mean (SD) | % | *P value*[b] |
| Age (years) | 58.1 (9.7) | | 46.5 (6.4) | | < 0.01 | 57.7 (10.4) | | 0.46 |
| Male | | 61.7 | | 38.8 | < 0.01 | | 55.1 | 0.03 |
| White | | 97.7 | | 97.2 | 0.44 | | 97.2 | 0.47 |
| 1st degree relative with colon cancer | | 16.1 | | 20.0 | 0.06 | | 6.9 | < 0.01 |
| Never smoker | | 32.5 | | 46.3 | < 0.01 | | 44.1 | < 0.01 |
| Physical activity (MET-hrs./wk.) | 9.5 (9.5) | | 8.9 (8.3) | | 0.13 | 9.9 (9.7) | | 0.57 |
| Body mass index (kg/m$^2$) | 27.4 (4.7) | | 26.6 (4.9) | | < 0.01 | 26.8 (4.5) | | 0.05 |
| Current ethanol intake (g/day) | 5.2 (7.6) | | 3.6 (7.8) | | < 0.01 | 4.5 (8.8) | | 0.22 |
| Taking NSAID | | 36.4 | | 46.6 | < 0.01 | | 39.4 | 0.29 |
| Education (yrs.) | 14.0 (3.3) | | 14.5 (3.2) | | < 0.01 | 14.1 (2.9) | | 0.36 |
| Hormone replacement therapy[c] | | 14.8 | | 66.3 | < 0.01 | | 18.9 | 0.25 |
| Total energy intake (kcal/day) | 2,090.7 (775.7) | | 2,002.5 (718.3) | | 0.02 | 2,054.5 (719.2) | | 0.42 |
| Paleolithic Diet Score | 41.3 (6.7) | | 41.8 (6.7) | | 0.13 | 42.1 (6.7) | | 0.05 |
| Mediterranean Diet Score | 29.2 (5.6) | | 29.3 (5.4) | | 0.05 | 29.7 (5.4) | | 0.13 |
| Total calcium intake (mg/day) | 959.4 (531.1) | | 990.1 (518.3) | | 0.25 | 987.7 (552.4) | | 0.39 |
| Dietary | 860.4 (455.0) | | 837.9 (428.8) | | 0.31 | 882.8 (470.1) | | 0.42 |
| Supplemental | 99.0 (269.2) | | 152.2 (329.5) | | < 0.01 | 104.9 (262.5) | | 0.71 |
| Dietary fiber intake (g/day) | 21.8 (9.6) | | 22.3 (10.2) | | 0.34 | 22.2 (9.7) | | 0.46 |
| Total fat intake (g/day) | 73.1 (34.4) | | 66.8 (30.3) | | < 0.01 | 70.2 (31.3) | | 0.15 |
| Total red and processed meat intake (servings/wk.) | 7.3 (6.1) | | 6.1 (4.9) | | <0.01 | 6.9 (5.6) | | 0.16 |
| Total fruit and vegetable intake (servings/wk.) | 42.3 (23.7) | | 45.6 (26.9) | | 0.01 | 44.5 (23.5) | | 0.12 |

Abbreviations: SD, standard deviation; MET-hrs./wk., metabolic equivalents of tasks (hours/week); NSAID, non-steroidal anti-inflammatory drug.

[a] Endoscopy controls include those who had a colonoscopy and those who had only a flexible sigmoidoscopy.

[b] *P* values were calculated using chi-square tests for categorical variables and two-sample t-tests for continuous variables.

[c] In women, $n = 883$.

Table 3.3. Associations of Paleolithic and Mediterranean Diet Scores with Incident, Sporadic, Colorectal Adenomas; Minnesota CPRU Case-Control Study, 1991-1994

| | Endoscopy-negative controls[a] | | | | | | | | Community controls | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Paleolithic Diet Score | | | | Mediterranean Diet Score | | | | Paleolithic Diet Score | | | | Mediterranean Diet Score | | | |
| | Crude OR | 95% CI | Adjusted OR[b] | 95% CI | Crude OR | 95% CI | Adjusted OR[b] | 95% CI | Crude OR | 95% CI | Adjusted OR[b] | 95% CI | Crude OR | 95% CI | Adjusted OR[b] | 95% CI |
| **Continuous** | 0.99 | 0.97, 1.01 | 0.98 | 0.98, 1.01 | 0.99 | 0.98, 1.01 | 0.98 | 0.96, 1.00 | 0.98 | 0.97, 1.00 | 0.98 | 0.96, 1.00 | 0.98 | 0.96, 1.00 | 0.98 | 0.96, 1.00 |
| **Quintiles** | | | | | | | | | | | | | | | | |
| 1 | 1.00 | | 1.00 | | 1.00 | | 1.00 | | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| 2 | 0.96 | 0.71, 1.29 | 0.95 | 0.69, 1.32 | 0.79 | 0.59, 1.07 | 0.76 | 0.55, 1.05 | 1.12 | 0.78, 1.60 | 1.04 | 0.72, 1.51 | 0.69 | 0.49, 0.99 | 0.70 | 0.49, 1.02 |
| 3 | 0.85 | 0.63, 1.15 | 0.83 | 0.60, 1.15 | 0.87 | 0.65, 1.18 | 0.87 | 0.63, 1.21 | 0.91 | 0.64, 1.30 | 0.84 | 0.58, 1.22 | 0.79 | 0.55, 1.12 | 0.78 | 0.54, 1.13 |
| 4 | 0.80 | 0.58, 1.10 | 0.70 | 0.49, 0.99 | 0.77 | 0.56, 1.05 | 0.66 | 0.47, 0.94 | 0.73 | 0.50, 1.06 | 0.71 | 0.48, 1.05 | 0.71 | 0.49, 1.04 | 0.67 | 0.45, 0.99 |
| 5 | 0.83 | 0.60, 1.15 | 0.71 | 0.50, 1.02 | 0.88 | 0.65, 1.19 | 0.74 | 0.54, 1.03 | 0.85 | 0.58, 1.24 | 0.84 | 0.56, 1.26 | 0.79 | 0.55, 1.12 | 0.77 | 0.53, 1.11 |
| *P for trend* | *0.14* | | *0.02* | | *0.31* | | *0.05* | | *0.11* | | *0.14* | | *0.18* | | *0.13* | |

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; MET-hrs./wk., metabolic equivalents of tasks (hours/week).

[a] Endoscopy controls included those who had a colonoscopy and those who had only a flexible sigmoidoscopy.

[b] From unconditional regression model; covariates included age (continuous), sex, family history of colon cancer in a first-degree relative (yes/no), regular (≥ once/week) non-steroidal anti-inflammatory use (categorical), body mass index (kg/m$^2$; continuous), physical activity (MET-hrs./wk., continuous), total energy intake (kcal/day, continuous), and hormone replacement therapy use (in women, categorical).

Table 3.4. Associations of Paleolithic and Mediterranean Diet Scores With Incident, Sporadic Colorectal Adenomas According to Selected Risk Factors for Colorectal Neoplasms; Minnesota CPRU Case-Control Study, 1991-1994

| | Endoscopy-negative controls[a] | | | | Community controls | | | |
|---|---|---|---|---|---|---|---|---|
| | Paleolithic Diet Score | | Mediterranean Diet Score | | Paleolithic Diet Score | | Mediterranean Diet Score | |
| **Quintile, by Sex** | Adjusted OR[b] | 95% CI | Adjusted OR[b] | 95% CI | Adjusted OR[b] | 95% CI | Adjusted OR[b] | 95% CI |
| Males | | | | | | | | |
| 1 | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| 2 | 0.86 | 0.57, 1.32 | 0.60 | 0.39, 0.94 | 0.94 | 0.59, 1.48 | 0.75 | 0.47, 1.20 |
| 3 | 0.74 | 0.45, 1.22 | 0.64 | 0.40, 1.04 | 0.60 | 0.35, 1.01 | 0.82 | 0.49, 1.37 |
| 4 | 0.68 | 0.40, 1.14 | 0.38 | 0.23, 0.61 | 0.75 | 0.44, 1.30 | 0.54 | 0.33, 0.89 |
| 5 | 0.49 | 0.31, 0.77 | 0.58 | 0.36, 0.95 | 0.73 | 0.44, 1.20 | 0.76 | 0.45, 1.26 |
| *P for trend* | *<0.01* | | *0.01* | | *0.11* | | *0.13* | |
| Females | | | | | | | | |
| 1 | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| 2 | 1.00 | 0.60, 1.64 | 0.86 | 0.52, 1.42 | 0.98 | 0.54, 1.79 | 0.75 | 0.41, 1.35 |
| 3 | 1.08 | 0.68, 1.73 | 1.11 | 0.65, 1.92 | 1.12 | 0.64, 1.97 | 0.71 | 0.37, 1.34 |
| 4 | 0.83 | 0.46, 1.50 | 1.16 | 0.73, 1.84 | 0.86 | 0.44, 1.71 | 0.97 | 0.55, 1.71 |
| 5 | 1.20 | 0.70, 2.04 | 1.17 | 0.70, 1.96 | 0.91 | 0.49, 1.68 | 0.94 | 0.50, 1.74 |
| *P for trend* | *0.71* | | *0.36* | | *0.71* | | *0.99* | |
| *P for interaction* | *0.07* | | *0.16* | | *0.15* | | *0.66* | |
| **Quintile, by BMI** | | | | | | | | |
| Normal weight & underweight | | | | | | | | |
| 1 | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| 2 | 0.86 | 0.47, 1.56 | 0.98 | 0.56, 1.71 | 0.80 | 0.41, 1.57 | 0.94 | 0.50, 1.77 |
| 3 | 0.81 | 0.46, 1.40 | 0.96 | 0.49, 1.88 | 1.35 | 0.71, 2.58 | 0.78 | 0.36, 1.65 |
| 4 | 0.91 | 0.49, 1.70 | 0.93 | 0.54, 1.62 | 0.89 | 0.44, 1.79 | 1.07 | 0.56, 2.06 |
| 5 | 0.90 | 0.48, 1.66 | 1.35 | 0.76, 2.39 | 0.98 | 0.49, 1.96 | 1.22 | 0.63, 2.36 |
| *P for trend* | *0.83* | | *0.37* | | *0.87* | | *0.51* | |
| Overweight & Obese | | | | | | | | |
| 1 | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| 2 | 1.17 | 0.79, 1.72 | 0.72 | 0.48, 1.08 | 0.95 | 0.61, 1.47 | 0.67 | 0.43, 1.06 |
| 3 | 0.92 | 0.59, 1.41 | 0.86 | 0.57, 1.29 | 0.61 | 0.38, 0.97 | 0.75 | 0.47, 1.18 |
| 4 | 0.63 | 0.41, 0.96 | 0.54 | 0.35, 0.81 | 0.59 | 0.37, 0.95 | 0.58 | 0.37, 0.92 |
| 5 | 0.65 | 0.42, 1.01 | 0.56 | 0.36, 0.88 | 0.74 | 0.45, 1.21 | 0.60 | 0.37, 0.99 |
| *P for trend* | *0.01* | | *<0.01* | | *0.04* | | *0.02* | |
| *P for interaction* | *0.71* | | *0.38* | | *0.43* | | *0.13* | |
| **Quintile, by Age** | | | | | | | | |
| <56 years | | | | | | | | |
| 1 | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| 2 | 1.01 | 0.62, 1.63 | 0.89 | 0.54, 1.45 | 0.98 | 0.53, 1.81 | 0.87 | 0.48, 1.59 |
| 3 | 0.86 | 0.52, 1.43 | 1.20 | 0.74, 1.94 | 0.69 | 0.37, 1.29 | 1.09 | 0.60, 2.00 |
| 4 | 0.92 | 0.54, 1.58 | 0.72 | 0.41, 1.26 | 0.83 | 0.43, 1.61 | 0.57 | 0.29, 1.12 |
| 5 | 0.78 | 0.45, 1.34 | 0.66 | 0.39, 1.12 | 1.25 | 0.62, 2.53 | 1.25 | 0.63, 2.48 |
| *P for trend* | *0.35* | | *0.13* | | *0.85* | | *0.95* | |
| 56 years or older | | | | | | | | |
| 1 | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| 2 | 0.94 | 0.60, 1.48 | 0.63 | 0.41, 0.98 | 1.06 | 0.66, 1.72 | 0.59 | 0.36, 0.94 |
| 3 | 0.80 | 0.52, 1.24 | 0.68 | 0.43, 1.06 | 0.89 | 0.55, 1.42 | 0.62 | 0.38, 1.01 |
| 4 | 0.59 | 0.37, 0.95 | 0.63 | 0.40, 0.98 | 0.64 | 0.39, 1.05 | 0.68 | 0.42, 1.11 |
| 5 | 0.65 | 0.40, 1.06 | 0.74 | 0.48, 1.14 | 0.69 | 0.42, 1.15 | 0.60 | 0.38, 0.94 |
| *P for trend* | *0.02* | | *0.16* | | *0.04* | | *0.06* | |
| *P for interaction* | *0.53* | | *0.10* | | *0.50* | | *0.44* | |

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; BMI, body mass index.

[a] Endoscopy controls included those who had a colonoscopy and those who had only a flexible sigmoidoscopy.
[b] From unconditional regression model; covariates included age (continuous), sex, family history of colon cancer in a first-degree relative (yes/no), regular (≥ once/week) non-steroidal anti-inflammatory use, body mass index ($kg/m^2$), physical activity (MET-hrs./wk.), total energy intake (kcal/day), and hormone replacement therapy use (in women).

Table 3.5. Associations of Paleolithic and Mediterranean Diet Scores with Incident, Sporadic Colorectal Adenomas According to Selected Adenoma Characteristics; Minnesota CPRU Case-Control Study, 1991-1994

| | Endoscopy-negative controls[a] | | | | Community controls | | | |
|---|---|---|---|---|---|---|---|---|
| | Paleolithic Diet Score | | Mediterranean Diet Score | | Paleolithic Diet Score | | Mediterranean Diet Score | |
| | Adjusted | | Adjusted | | Adjusted | | Adjusted | |
| **Quintile, by Number of Adenomas** | OR[b] | 95% CI | OR[b] | 95% CI | OR[b] | 95% CI | OR[b] | 95% CI |
| One adenoma | | | | | | | | |
| 1 | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| 2 | 0.93 | 0.65, 1.33 | 0.76 | 0.53, 1.10 | 1.03 | 0.69, 1.55 | 0.69 | 0.46, 1.04 |
| 3 | 0.74 | 0.51, 1.08 | 0.91 | 0.63, 1.30 | 0.76 | 0.50, 1.15 | 0.80 | 0.53, 1.20 |
| 4 | 0.66 | 0.44, 0.98 | 0.53 | 0.35, 0.80 | 0.67 | 0.44, 1.03 | 0.53 | 0.34, 0.82 |
| 5 | 0.80 | 0.54, 1.18 | 0.81 | 0.56, 1.16 | 0.97 | 0.63, 1.49 | 0.85 | 0.57, 1.26 |
| *P for trend* | *0.10* | | *0.09* | | *0.40* | | *0.21* | |
| Two or more adenomas | | | | | | | | |
| 1 | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| 2 | 1.00 | 0.60, 1.68 | 0.71 | 0.42, 1.20 | 1.07 | 0.62, 1.84 | 0.73 | 0.42, 1.25 |
| 3 | 0.97 | 0.58, 1.61 | 0.70 | 0.41, 1.19 | 0.92 | 0.54, 1.57 | 0.66 | 0.38, 1.16 |
| 4 | 0.72 | 0.41, 1.25 | 0.88 | 0.53, 1.46 | 0.77 | 0.43, 1.36 | 0.99 | 0.58, 1.69 |
| 5 | 0.43 | 0.23, 0.82 | 0.53 | 0.31, 0.92 | 0.54 | 0.28, 1.05 | 0.55 | 0.31, 0.97 |
| *P for trend* | *0.01* | | *0.07* | | *0.04* | | *0.13* | |
| **Quintile, by Subtype** | | | | | | | | |
| Tubular | | | | | | | | |
| 1 | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| 2 | 0.93 | 0.64, 1.36 | 0.86 | 0.59, 1.24 | 1.03 | 0.68, 1.56 | 0.78 | 0.52, 1.17 |
| 3 | 0.95 | 0.65, 1.37 | 0.96 | 0.67, 1.40 | 0.92 | 0.61, 1.39 | 0.85 | 0.56, 1.28 |
| 4 | 0.77 | 0.52, 1.15 | 0.73 | 0.49, 1.08 | 0.78 | 0.51, 1.20 | 0.73 | 0.47, 1.13 |
| 5 | 0.80 | 0.53, 1.19 | 0.86 | 0.59, 1.24 | 0.95 | 0.61, 1.47 | 0.87 | 0.58, 1.31 |
| *P for trend* | *0.17* | | *0.29* | | *0.48* | | *0.44* | |
| Tubulovillous or villous | | | | | | | | |
| 1 | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| 2 | 0.97 | 0.60, 1.57 | 0.57 | 0.34, 0.94 | 1.09 | 0.65, 1.81 | 0.58 | 0.34, 0.97 |
| 3 | 0.59 | 0.35, 0.98 | 0.63 | 0.38, 1.04 | 0.60 | 0.35, 1.03 | 0.61 | 0.36, 1.04 |
| 4 | 0.51 | 0.29, 0.89 | 0.49 | 0.29, 0.84 | 0.55 | 0.31, 0.98 | 0.54 | 0.31, 0.95 |
| 5 | 0.52 | 0.29, 0.91 | 0.52 | 0.31, 0.86 | 0.66 | 0.36, 1.19 | 0.56 | 0.33, 0.96 |
| *P for trend* | *<0.01* | | *0.01* | | *0.02* | | *0.03* | |

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval.

[a] Endoscopy controls included those who had a colonoscopy and those who had only a flexible sigmoidoscopy

[b] From unconditional regression model; covariates included age (continuous), sex, family history of colon cancer in a first-degree relative (yes/no), regular (≥ once/week) non-steroidal anti-inflammatory use, body mass index (kg/m$^2$), physical activity (MET-hrs./wk.), total energy intake (kcal/day), and hormone replacement therapy use (in women)

# CHAPTER 4: ASSOCIATIONS OF PALEOLITHIC AND MEDITERRANEAN DIET PATTERNS WITH ALL-CAUSE AND CAUSE-SPECIFIC MORTALITY

Kristine A. Whalen[1], Marjorie L. McCullough[2], W. Dana Flanders[1,3],

Terryl J. Hartman[1,4], Suzanne Judd[5], Roberd M. Bostick[1,4]

[1] Emory University Rollins School of Public Health, Department of Epidemiology

Atlanta, Georgia, United States

[1] American Cancer Society, Epidemiology Research Program, Atlanta, Georgia, United States

[1] Emory University Rollins School of Public Health, Department of Biostatistics and Bioinformatics

Atlanta, United States

[1] Emory University, Winship Cancer Institute Atlanta, Georgia, United States

[1] University of Alabama at Birmingham, Biostatistics Birmingham, Alabama, United States

## Abstract

Poor diet quality is associated with higher risk of many chronic diseases that are among the leading causes of death in the United States. Evolutionary discordance may account for some of the higher incidence and mortality from these diseases. We investigated associations of two diet pattern scores, the "Paleolithic" and the Mediterranean, with all-cause and cause-specific mortality in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study, a longitudinal cohort of black and white adults ≥45 years of age. Participants completed questionnaires, including a Block food-frequency questionnaire (FFQ), at baseline and were contacted every 6 months to determine their health status. Of the analytic cohort (n = 21,423), a total of 2,513 participants died during a median follow-up of 6.25 years. We created diet scores from FFQ responses and assessed their associations with mortality using multivariable Cox proportional hazards regression models adjusting for major risk factors. For those in the highest relative to the lowest quintiles of the Paleolithic and Mediterranean diet scores the multivariable adjusted hazard ratios for all-cause mortality were, respectively, 0.77 (95% confidence interval [CI] 0.67, 0.89; $P_{trend}<0.01$) and 0.63 (95% CI 0.54, 0.73; $P_{trend}<0.01$). Results for all-cancer and all-cardiovascular disease mortality were similar to those for all-cause mortality. Findings from this biracial prospective study suggest that diets closer to Paleolithic or Mediterranean diet patterns may be inversely associated with all-cause and cause-specific mortality.

## Introduction

Cardiovascular disease and cancer are the two leading causes of death in the United States (3). Diet has been strongly associated with risk for these and other chronic diseases (10, 91, 238), and investigations into which foods or nutrients may affect risk led to several large clinical trials of the effects of various nutritional supplements on risk for cardiovascular disease (79, 80, 82, 83, 167-169, 172-188), cancer (77-80, 82, 83, 167-173, 175-178, 180, 182-187, 189), and mortality (77-80, 82, 83, 167-173, 175-182, 185-188). Most of these trials yielded null, or sometimes harmful, results (77-80). Given these findings, nutritional supplements are not generally recommended for preventing cardiovascular disease or cancer. There are several potential explanations for the null results from supplementation trials, including a possible inability of supplements to mimic the complex composition and interacting components of whole foods and diets (14, 91, 212, 213).

To assess the totality of diet rather than the influence of single nutrients, foods, and other individual dietary constituents, nutrition researchers have utilized dietary pattern analysis. There are several methods for constructing or representing dietary patterns for analysis, from agnostic data-driven methods, to *a priori* methods in which food groups and consumption patterns are characterized based on, for example, published dietary quality indices or current dietary recommendations (91).

A dietary pattern of growing interest is the Paleolithic diet pattern. The substantial differences between modern diet and lifestyle and those of our hunter-gatherer ancestors, termed evolutionary discordance, has been hypothesized to account for some of the dramatic increases in chronic disease over the past century (149). Modeled after the diet of pre-agricultural hunter-gatherer humans, the "Paleolithic diet" was estimated from anthropological evidence from fossils and extant hunter-gather groups (6). The diet pattern is characterized as a predominantly plant-food based diet (with a wide diversity of fruits, nuts, and vegetables, including wild greens that contained high amounts of calcium and other minerals (6)) and includes lean meat, while being low in dairy, grains, sugar, and salt. While several clinical studies reported beneficial effects of the Paleolithic diet

on cardiovascular and other metabolic risk biomarkers (157-162, 164, 166), few studies explored its association with chronic disease endpoints (214).

A more commonly studied dietary pattern with many similarities to the Paleolithic diet is the Mediterranean diet (98, 99, 104-106, 111, 121-126, 128-137, 162, 214). It has been strongly and consistently linked with health benefits (209), especially in relation to cardiovascular disease (104-106, 129, 130, 145, 148). These apparent health benefits make the Mediterranean diet a model with which to compare the Paleolithic pattern. The many similarities between the Mediterranean and Paleolithic diet patterns include a high consumption of fruits, vegetables, and nuts and little added sugar. However, the idealized Mediterranean diet pattern includes grains and moderate amounts of alcohol and dairy foods, whereas the Paleolithic diet includes none (105, 123, 148).

We previously reported finding similar inverse associations of the Paleolithic and Mediterranean diet scores with incident, colorectal adenomas (214). Also, in a yet unpublished study, we found similar inverse associations of both scores with biomarkers of inflammation and oxidative balance. Motivated partly by these results, and by the growing popularity of the "Paleolithic diet", herein we report an investigation of associations of both the Paleolithic diet score and the Mediterranean diet score (as comparison), in relation to all-cause and cause-specific mortality endpoints. This study was conducted using data from the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study, a prospective cohort of 30,183 white and black adults from the Southeastern U.S.

## Methods

### Study population and data collection

The study design and recruitment for REGARDS was described previously (196-200). Briefly, REGARDS is a prospective cohort study designed to investigate the causes of racial and geographic disparities in stroke. The national cohort was constructed using oversampling from the US "stroke belt" and "stroke buckle" regions such that there was equal representation by sex and by white or African American ancestry. From January 2003 to October 2007, 30,183 individuals ≥45 years of age were enrolled. Of these participants, 8,547 did not complete the food frequency

questionnaire at baseline, and an additional 213 were lost to follow-up and excluded from the present analyses, leaving an analytic cohort of 21,423 individuals. The institutional review boards of all participating institutions reviewed and approved the study methods. All participants involved in the present study gave written informed consent at the time of recruitment.

Computer-assisted telephone interviews were conducted to collect demographic information and medical history, followed 3-4 weeks later by an in-home visit during which study staff collected blood and urine samples, measured blood pressure, and conducted an ECG, a medication audit, and anthropometry. At the in-home visit, self-administered questionnaires, including a Block 98 Food Frequency Questionnaire (FFQ) that included 109 food and beverage items to assess habitual consumption of over the past year (201, 239, 240), were left with the participants to fill out and send back.

Assessment of exposures

The Paleolithic and Mediterranean diet pattern scores were constructed as described previously (214). Briefly, using the habitual food consumption information obtained at baseline, each study participant was assigned a quintile rank (a score from 1-5) based on the sex-specific distribution of intake for each food group included in the score. As shown in Table 4.1, more points were awarded for higher intakes of foods considered characteristic of a diet pattern, and fewer points were awarded for higher intakes of foods considered uncharacteristic that pattern. The points for each of the food groups comprising a diet score were then summed, so that the final Paleolithic diet score (14 components) could range from 14 to 70, while the Mediterranean diet score (11 components) could range from 11 to 55.

Cohort follow-up

Study staff contacted cohort participants by phone every six months to ascertain any stroke events or deaths. If a death occurred, the death certificate and any associated medical records for the 28-day period prior to death were collected. Possible deaths of participants who could not be

contacted, and thus considered lost to follow-up, were ascertained using the social security death index and/or the National Death Index.

Causes of death

We defined cardiovascular disease mortality as death from a myocardial infarction, stroke, sudden death, heart failure, pulmonary embolism, other cardiac causes of death, and non-cardiac but other cardiovascular disease deaths. Cause of death was adjudicated by a committee of physicians using information from death certificates, hospital records, and proxy interviews. We defined cancer mortality as death attributed to any type of cancer. We also created a category for all injuries or accidents, and a category that included all other deaths, most of which were due to respiratory illness, infection, liver disease, or kidney failure.

Statistical analysis

The characteristics of the study population at baseline, by quintile of each diet pattern score, were summarized and compared using chi-square tests for categorical variables and analysis of variance for continuous variables following a normal distribution, or the Kruskal-Wallis nonparametric test for continuous variables that did not follow a normal distribution. Hazard ratios (HR) and 95% confidence intervals (CI) for mortality were estimated with Cox proportional-hazards regression models, with participants' age as the underlying time scale (the results calculated using time on study after adjusting for age were similar to those using age as the underlying time scale). We tested each exposure and potential covariate for the proportional hazards assumption using Log-Log Kaplan-Meier curves, goodness-of-fit tests, and extended Cox models.

Based on previous literature and biological plausibility, potential confounding variables considered included sex, race, body mass index (BMI), waist circumference, regular aspirin use, regular other nonsteroidal anti-inflammatory drug (NSAID) use, income, health insurance coverage, region of residence in the US, rural/urban classification, personal history of comorbidities such as cancer or diabetes, education, self-reported health, vitamin or mineral supplement use, and physical activity, all measured at baseline. The criteria for inclusion in the final models included: biological plausibility, statistical significance, and/or whether inclusion or exclusion of the variable from the

model changed the adjusted hazard ratios for the primary exposure variable by ≥10%. The final multivariate-adjusted model controlled for sex, race (black/white), total energy intake (kcal, continuous), BMI (kg/m$^2$, categorized by WHO criteria into underweight, normal, overweight, and obese), physical activity (0, 1-3, or 4+ exercise sessions on average per week), smoking (current, former, and never), annual income (refused, <$20,000, $20,000-$34,000, $35,000-$74,000, and >$75,000), and hormone replacement therapy use (in women).

To assess potential effect modification, separate analyses for all-cause mortality were conducted within categories of race (white/black), sex (male/female), age (≤65 yrs./>65 yrs.), self-reported health at baseline (poor, fair/good, very good, excellent), BMI (underweight & normal/overweight & obese), length of follow-up (≤6.25 yrs./>6.25 yrs.), smoking status (never/former/current), and region of US (Southern US/Western, Midwest, and Eastern US), and alcohol drinking status (current drinkers/former and never drinkers).

We conducted several sensitivity analyses to assess the sensitivity of the observed associations to how we defined the scores. We removed each food component from each *a priori* score one at a time to determine whether any one component overly influenced the observed associations. Diet scores were also constructed using medians rather than quintiles for the food groups, using an alternative construction of the fat ratio variable (monounsaturated + polyunsaturated:saturated fats) in the Mediterranean diet score, and using sex- and race-specific cut-points of food group consumption to create the final scores. BMI was also excluded as a covariate in the models since it could be considered both a confounder and a mediator of the association of diet with mortality. Last, we excluded deaths that occurred during the first 1-3 years of follow-up to assess the potential of diet at baseline being different from long-term usual dietary intakes because of pre-morbid health conditions. We conducted all analyses using SAS statistical software (SAS version 9.3, SAS Statistical Institute, Cary, NC). Two-sided tests were considered statistically significant if $p \leq 0.05$.

## Results

Selected characteristics of the participants by diet score quintiles are presented in Table 4.2. Compared to those in the lowest quintile of the Paleolithic diet score, those in the highest quintile were, on average, three years older; more likely to be female, white, obese, or a non-smoker; more likely to exercise more frequently, regularly take aspirin, have a higher annual income, have health insurance, to have been previously diagnosed with cancer or diabetes, and, if a woman, more likely to currently take hormone replacement therapy; and, on average, to consume less total energy. The distribution of baseline characteristics across Mediterranean diet score quintiles was similar to those for the Paleolithic diet, except that age and total energy intakes were generally similar across quintiles of the Mediterranean diet score, the proportion of men and women in each quintile were similar, and those in the upper quintile were less likely to reside in the Southern US or to have previously been diagnosed with cancer or diabetes. The Paleolithic diet scores ranged from 21 to 65, while the Mediterranean diet scores ranged from 14 to 50. These ranges did not differ appreciably by sex and were correlated (Pearson correlation coefficient = 0.66; despite the similar constructions of the scores, there were also differences, such as in scoring of grains, dairy, and alcohol, as described further above). The percentages of the participants who were in the same or different quintiles for each quintile of the Paleolithic and Mediterranean diet scores are shown in Table 4.3. Among those in the lowest and highest quintiles of the Paleolithic diet score, 55.5% and 50.0%, respectively, were in the corresponding quintile of the Mediterranean diet score. Also, among those in each quintile of the Paleolithic diet score, there were persons who were in one of each of the quintiles of the Mediterranean diet score.

During the 11 years of follow-up (median, 6.25 years) 2,513 participants died. Adjusting only for age, sex, race, and total energy intake, all-cause mortality among those in the upper relative to lower quintiles of the Paleolithic and Mediterranean diet scores, was 41% (95% CI 49% to 33%) and 52% (95% CI 59% to 44%) lower, respectively (Table 4.4). After additional adjustment for potential confounders, particularly smoking status, the association of each score with all-cause mortality was somewhat attenuated (to 23% and 37% for those in the upper relative to the lowest

quintiles of the Paleolithic and Mediterranean diet scores, respectively) but the point estimates for the extreme quintile comparisons and the tests for trend remained statistically significant. For each diet score, the findings for cardiovascular-specific, cancer-specific, and other non-injury/accident mortality, were similar to those for all-cause mortality. The associations of the scores with injury/accident-specific mortality were essentially null.

The associations of the Paleolithic diet score with all-cause mortality stratified by various participant characteristics at baseline are summarized in Figure 4.1. The associations comparing those in the upper with those in the lowest quintile differed minimally across the various strata. One exception was that, among those who were never or former smokers the scores were inversely associated with mortality, but among current smokers they were directly associated. Although the test for multiplicative interaction for this finding was statistically significant (p=0.04), the 95% CIs for the point estimates for the never and current smoker strata included 1.0.

The associations of the Mediterranean diet score with all-cause mortality according to various participant characteristics are summarized in Figure 4.2. The findings for those in the highest relative to those in the lowest quintile differed minimally across the various strata except that the inverse association tended to be stronger among women than among men ($p_{interaction}$ = 0.06), and stronger among former drinkers than among current or never drinkers ($p_{interaction}$ = 0.06). Unlike the findings for the Paleolithic diet score, all of the estimated associations according to smoking status were inverse.

In sensitivity analyses, removal of most individual score components one at a time did not materially alter the estimated inverse associations. However, for the Paleolithic diet score, removal of the nuts and the red and processed meats components attenuated the observed associations for all-cause mortality for those in the fifth relative to those in the first quintile by 15.6% and 14.3%, respectively, to HR 0.89 (95% CI 0.77, 1.02; $P_{trend}$=0.04) and HR 0.88 (95% CI 0.76, 1.02; $P_{trend}$<0.01), respectively. Removal of nuts and red and processed meats from the Mediterranean diet score also attenuated the observed associations, but more modestly, by 9.5% and 6.3%, respectively.

Modifying the construction of the scores by using the medians rather than the quintiles of intake for each food group, using sex-and race-specific quintile cut points, or for the Mediterranean diet using an alternative fat ratio variable did not materially alter the observed associations. Finally, excluding persons with chronic diseases at baseline, including baseline chronic diseases in the models, removing BMI from the models, or excluding those who died during the first 1-3 years of follow-up did not materially affect the estimated associations.

## Discussion

Our results suggest that diets that are more "Paleolithic-" or Mediterranean-like may be associated with lower risk of all-cause, cardiovascular-specific, cancer-specific, and other non-injury/accident-specific mortality, although the observed inverse associations were slightly stronger for the Mediterranean diet pattern. As expected, neither diet pattern was associated with injury/accident-specific mortality, although the number of deaths in this category was small. To our knowledge, the current study is the first to examine the association of a Paleolithic diet pattern with all-cause or cause-specific mortality.

The Paleolithic and Mediterranean diets may reduce the risk of chronic disease mortality by several potential mechanisms. The foods that characterize both diets, including high intakes of fruits, vegetables, fish, and nuts, are associated with lower inflammation and less oxidative stress, biochemical processes that are associated with cardiovascular disease and cancer (42, 209). Also, the Paleolithic and Mediterranean diets, relative to the Western diet (241), feature more low energy-dense foods, thereby facilitating energy balance and weight management. Indeed, in previous pilot trials it was found that participants on a Paleolithic-like diet relative to a standard diet reported greater satiety (66, 48) and had a greater release of anorectic gut hormone (164). Adiposity is associated with higher systemic inflammation and oxidative stress, and may influence chronic disease risk by other, independent mechanisms (12, 20). Therefore, one mechanism by which the Paleolithic and Mediterranean diets may reduce mortality is through energy balance. To address this, in the present study, we included BMI in the models for our primary analyses as a potential confounder, but assessed excluding it in our sensitivity analyses since it could also be a mediator of the associations.

Inclusion/exclusion of BMI from the models had no material impact on the estimated associations, suggesting that the possible effects of the Paleolithic and Mediterranean diets on chronic disease mortality may indeed include mechanisms beyond improving energy balance, such as reducing inflammation and improving oxidative balance.

There have been few reported studies on the health effects of following a Paleolithic diet pattern. Reported studies include seven small pilot trials and one slightly larger long-term weight loss trial (157-162, 164). Most of the pilot trials examined the effects of the diet on cardiovascular and glycemic control biomarkers, including HbA1c, plasma insulin, and serum lipids as well as on satiety; in general, all reported improved values/effects (160-162, 164), although only four of the trials had a control group. A larger study (n = 70) of the effects of a Paleolithic diet on long-term weight loss in post-menopausal women found a statistically significant weight loss at 6 months relative to those in the control group, but this difference became attenuated and was no longer statistically significant after 24 months (166).

The Mediterranean diet has been associated with lower mortality (242) and greater longevity, mainly through lower risk for cardiovascular disease (130, 144, 145, 148, 243-246) and possibly cancer (134). There have been several trials of a Mediterranean diet to improve health and prevent cardiovascular disease. The Lyon Diet Heart study (145) randomized 605 free-living individuals who previously had had a recent myocardial infarction to receive either dietary advice to follow a Mediterranean-type diet or standard care, which included the general recommendation to follow a "prudent diet" for the secondary prevention of myocardial infarction. After a mean follow-up of 46 months, 275 recurrent nonfatal and fatal cardiac events occurred—14 in the Mediterranean diet group and 44 in the control group (RR 0.28 95% CI 0.15, 0.53) (145). Also, a second, larger trial (n=7,447) of the Mediterranean diet for cardiovascular disease primary prevention, PREDIMED, found reduced cardiovascular disease risk in the two study intervention arms, which included following a Mediterranean diet and supplying participants with either nuts or olive oil, relative to a

low fat diet arm (288 events; HR 0.70; 95% CI 0.54, 0.92) (148). The results of the current study support these previously reported findings.

Contrary to expectations, we found that, although the Paleolithic diet was inversely associated with all-cause mortality among those who were not current smokers at baseline, it was directly associated among those who were current smokers. Although the test for multiplicative interaction was statistically significant, there were multiple comparisons, the point estimates for current smokers and former smokers were not statistically significant, and the biological plausibility is unclear.

While our findings support our hypothesis that multiple aspects of diet, via multiple mechanisms, collectively affect risk for chronic diseases more strongly than does any single aspect of diet, we do note that we found that consumption of nuts and red and processed meats more strongly contributed to the associations of the scores, especially the Paleolithic diet score, with mortality than did other individual components of the scores. Further investigation of the components of the score in relation to the overall score in other cohorts is needed.

This study has several strengths and limitations. Strengths include that is the first investigation of associations of a Paleolithic-like diet pattern with all-cause or cause-specific mortality, its prospective design, and the diverse study population. For most cases, cause of death reported on the death certificate was confirmed using medical records. The Paleolithic and Mediterranean diet patterns were constructed using similar methods so that differences in the observed diet-mortality associations between the two diets would be attributable to differences in the underlying diet patterns rather than the score construction methods. Limitations include the potential for residual confounding from insufficiently characterized or unmeasured aspects of a healthy lifestyle. However, although the number deaths due to injuries/accidents was small, as was hypothesized, there were no indications that the diets were associated with injury/accident-specific mortality, suggesting that our findings for all-cause, cardiovascular-specific, cancer-specific, and other non-injury/accident-specific mortality were plausibly related to the aggregate of the biological

mechanisms of the diets. In addition, it is unlikely that few, if any, of the participants' diets were strongly concordant with either pattern, likely resulting in underestimating the potential of strong adherence to the diets for reducing risk for chronic disease mortality. Although the use of self-reported food frequency questionnaires in prospective cohort studies is well established, there are known limitations in using them, such as the limited number of specific food items included, limited differentiation of important variants of certain major foods (e.g., high- vs. low-fat red meat), limited information on food cooking methods, and recall error. Finally, while cause-specific mortality is subject to competing risks, all-cause mortality is not, and the consistency of our findings across both types of outcomes lends support to the validity of the cause-specific associations.

In conclusion, our findings, taken together with those from previous studies, suggest that diets that are more Paleolithic- or Mediterranean-like may be associated with lower risk of all-cause, cardiovascular-specific, cancer-specific, and other non-injury/accident-specific mortality.

Table 4.1. Paleolithic and Mediterranean diet score constituents and point assignments[a]

| Intake category | Scoring | Paleolithic diet score[b] | Mediterranean diet score[c] |
|---|---|---|---|
| **Higher intake 'best'** | Points assigned = quintile rank | Vegetables | Vegetables |
| | | Fruits | Fruits |
| | (e.g., highest and lowest quintiles scored +5 and +1 points, respectively) | Lean meats[e] | Lean meats[e] |
| | | Fish | Fish |
| | | Nuts | Nuts |
| | | Fruit & vegetable diversity[d] | Monounsaturated:saturated fat ratio |
| | | Calcium[f] | |
| **Lower intake 'best'** | Points assigned = reverse quintile rank | Red and processed meats[g] | Red and processed meats[g] |
| | | Sodium (mg) | Sodium (mg) |
| | (e.g., highest and lowest quintiles scored +1 and +5 points, respectively) | | |
| | | Dairy | |
| | | Grain and starches | |
| | | Baked goods[h] | |
| | | Sugar sweetened beverages | |
| | | Alcohol | |
| **Moderate intake 'best'** | 3rd quintile scored +5, | | Dairy |
| | 2nd and 4th quintiles scored +3, and | | Grains and starches |
| | 1st and 5th quintiles scored +1 points | | |
| **Other** | | | Alcohol: |
| | | | Women: 5 – 15 g/day (+5 points) |
| | | | Men: 10—25 g/day (+5 points) |
| | | | Otherwise (+1 point) |

[a] All constituents measured in grams/week unless otherwise indicated.
[b] Paleolithic diet score: 14 components, range of possible scores 14 – 70.
[c] Mediterranean diet score: 11 components, range of possible scores 11 – 55.
[d] Fruit & vegetable diversity calculated by summing the total number of types of fruits and vegetables that the participant reported eating more than 1-3 servings of per month.
[e] Lean meats include skinless chicken or turkey, lean beef.
[f] Calcium intake from sources other than dairy; calculated as residuals from linear regression of total calcium intake (mg/day) on dairy foods intake.
[g] Nitrate processed meats and non-lean red meat consumption together.
[h] Baked goods include items such as cake, pie, and other pastry-type food.

Table 4.2. Selected characteristics of participants at baseline (N=21,423); REGARDS.

| | Paleolithic diet score[a] | | | | | Mediterranean diet score[a] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Quintile 1 | | Quintile 5 | | | Quintile 1 | | Quintile 5 | | |
| | N=5,073 n or Mean | Range (21-37) % or (SD) | N=3,819 n or Mean | Range (48-65) % or (SD) | *P-value*[e] | N=4,837 n or Mean | Range (14-26) % or (SD) | N=3,629 n or Mean | Range (36-50) % or (SD) | *P-value*[e] |
| Age (yrs.)[c] | 63.1 | 9.4 | 66.1 | 8.8 | <0.01 | 64.3 | 9.5 | 65.3 | 8.9 | 0.05 |
| Male | 2,344 | 46.2 | 1,581 | 41.4 | <0.01 | 2,127 | 44.0 | 1,611 | 44.4 | 0.99 |
| White | 3,180 | 62.7 | 2,695 | 70.6 | <0.01 | 3,014 | 62.3 | 2,647 | 72.9 | <0.01 |
| Current smoker[d] | 1,215 | 24.1 | 193 | 5.1 | <0.01 | 1,062 | 22.1 | 213 | 5.9 | <0.01 |
| Body Mass Index (kg/m$^2$)[b] | | | | | | | | | | |
| Underweight (<18.5) | 75 | 1.5 | 31 | 0.8 | | 73 | 1.5 | 19 | 0.5 | |
| Normal (18.5 - 24.9) | 1,200 | 23.8 | 1,060 | 27.9 | | 1,143 | 23.8 | 1,032 | 28.6 | |
| Overweight (25 - 29.9) | 1,802 | 35.8 | 1,483 | 39.1 | | 1,676 | 34.9 | 1,475 | 40.8 | |
| Obese (30+) | 1,959 | 38.9 | 1,223 | 32.2 | <0.01 | 1,906 | 39.7 | 1,089 | 30.1 | <0.01 |
| Exercise sessions[d] | | | | | | | | | | |
| None | 2,039 | 40.9 | 884 | 23.4 | | 1,953 | 41.1 | 811 | 22.5 | |
| 1-3/week | 1,666 | 33.4 | 1,473 | 39.0 | | 1,573 | 33.1 | 1,442 | 40.1 | |
| 4+ /week | 1,279 | 25.7 | 1,423 | 37.7 | <0.01 | 1,231 | 25.9 | 1,345 | 37.4 | <0.01 |
| Regular aspirin user[d] | 2,009 | 39.6 | 1,822 | 47.7 | <0.01 | 2,045 | 42.3 | 1,677 | 46.3 | <0.01 |
| Regular NSAID user[d] | 784 | 15.5 | 548 | 14.4 | 0.58 | 725 | 15.0 | 531 | 14.7 | 0.94 |
| Income < $20k/year | 1,110 | 21.9 | 388 | 10.2 | <0.01 | 1,109 | 22.9 | 310 | 8.5 | <0.01 |
| Relationship status | | | | | | | | | | |
| Married | 2,946 | 58.1 | 2,464 | 64.5 | | 2,685 | 55.5 | 2,436 | 67.1 | |
| Widowed | 861 | 17.0 | 633 | 16.6 | <0.01 | 967 | 20.0 | 519 | 14.3 | <0.01 |
| Has health insurance[d] | 4,608 | 90.9 | 3,665 | 96.1 | <0.01 | 4,446 | 92.0 | 3,493 | 96.3 | <0.01 |
| Region | | | | | | | | | | |
| Southern US | 2,872 | 56.6 | 2,090 | 54.7 | | 2,866 | 59.3 | 1,875 | 51.7 | |
| Western, Midwest, Eastern US | 2,201 | 43.4 | 1,729 | 45.3 | 0.19 | 1,971 | 40.8 | 1,754 | 48.3 | <0.01 |
| Ever diagnosed with cancer | 381 | 12.4 | 363 | 16.3 | <0.01 | 414 | 14.5 | 335 | 15.6 | 0.44 |
| Diabetes at baseline | 854 | 17.4 | 758 | 20.5 | <0.01 | 997 | 21.4 | 509 | 14.5 | <0.01 |
| HRT use (in women) | 1,485 | 54.6 | 1,384 | 62.0 | <0.01 | 1,475 | 54.7 | 1,290 | 64.1 | <0.01 |
| Total energy intake (kcal/day)[c] | 1,904.6 | 722.6 | 1,539.9 | 591.4 | <0.01 | 1,628.2 | 736.5 | 1,776.2 | 624.9 | <0.01 |

Abbreviations: SD, standard deviation; NSAID, non-steroidal anti-inflammatory drug.

[a] Unequal sample sizes in quintiles due to ranking ties.

[b] P values calculated using chi-square tests for categorical variables and ANOVA for continuous variables unless otherwise noted.

[c] P values calculated using Kruskal-Wallis non-parametric test.

[d] Missing data: Smoking status (n=82), BMI (n=140), Exercise sessions (n=292), Regular aspirin use (n=10), Regular NSAID use (n=69), Insurance status (n=14).

Table 4.3. For each quintile of the Paleolithic diet score, the number and percentages of study participants who were in the same and different quintiles of the Mediterranean diet score; REGARDS.

| | | Paleolithic diet score quintile | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| Mediterranean diet score quintile | 1 | 2,816 (55.5%) | 963 (25.8%) | 664 (16.1%) | 321 (6.9%) | 73 (1.9%) |
| | 2 | 1,321 (26.0%) | 1,194 (32.0%) | 1,062 (25.7%) | 749 (16.1%) | 239 (6.3%) |
| | 3 | 664 (13.1%) | 923 (24.8%) | 1,181 (28.6%) | 1,237 (26.5%) | 636 (16.7%) |
| | 4 | 230 (4.5%) | 493 (13.2%) | 797 (19.3%) | 1,268 (27.2%) | 963 (25.2%) |
| | 5 | 42 (0.8%) | 155 (4.2%) | 433 (10.5%) | 1,091 (23.4%) | 1,908 (50.0%) |
| | | 5,073 (100%) | 3,728 (100%) | 4,137 (100%) | 4,666 (100%) | 3,819 (100%) |

Table 4.4. Associations of Paleolithic and Mediterranean diet scores with total and cause-specific mortality (N=21,423); REGARDS.

| Cause of death | Paleolithic diet score quintile | | | | | | | | Mediterranean diet score quintile | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | 1 (N=5,073) | 2 (N=3,728) | 3 (N=4,137) | 4 (N=4,666) | 5 (N=3,819) | *P trend*[c] | | N | 1 (N=4,837) | 2 (N=4,565) | 3 (N=4,641) | 4 (N=3,751) | 5 (N=3,629) | *P trend*[c] | |
| All causes | | | | | | | | | | | | | | | | |
| No. of deaths (%) | 2,513 | 626 (24.9%) | 482 (19.2%) | 500 (19.9%) | 536 (21.3%) | 369 (14.7%) | | | 2,513 | 708 (28.2%) | 602 (24.0%) | 520 (20.7%) | 395 (15.7%) | 288 (11.5%) | | |
| Minimally adjusted HR (95% CI)[a] | | 1.00 | 0.89 (0.78, 1.01) | 0.80 (0.71, 0.91) | 0.71 (0.63, 0.80) | 0.59 (0.51, 0.67) | <0.01 | | | 1.00 | 0.85 (0.75, 0.95) | 0.71 (0.63, 0.81) | 0.67 (0.59, 0.76) | 0.48 (0.41, 0.56) | <0.01 | |
| Fully adjusted HR (95% CI)[b] | | 1.00 | 0.95 (0.84, 1.08) | 0.94 (0.83, 1.07) | 0.87 (0.77, 0.99) | 0.77 (0.67, 0.89) | <0.01 | | | 1.00 | 0.92 (0.82, 1.03) | 0.81 (0.71, 0.91) | 0.80 (0.70, 0.92) | 0.63 (0.54, 0.73) | <0.01 | |
| Cardiovascular[d] | | | | | | | | | | | | | | | | |
| No. of deaths (%) | 863 | 199 (23.1%) | 154 (17.8%) | 198 (22.9%) | 186 (21.6%) | 126 (14.6%) | | | 863 | 230 (26.7%) | 212 (24.6%) | 183 (21.2%) | 140 (16.2%) | 98 (11.4%) | | |
| Minimally adjusted HR (95% CI)[a] | | 1.00 | 0.95 (0.76, 1.19) | 1.04 (0.84, 1.29) | 0.79 (0.64, 0.98) | 0.63 (0.49, 0.80) | <0.01 | | | 1.00 | 0.91 (0.74, 1.12) | 0.81 (0.66, 1.00) | 0.76 (0.60, 0.95) | 0.53 (0.41, 0.68) | <0.01 | |
| Fully adjusted HR (95% CI)[b] | | 1.00 | 1.01 (0.80, 1.27) | 1.18 (0.95, 1.46) | 0.94 (0.75, 1.18) | 0.78 (0.61, 1.00) | 0.06 | | | 1.00 | 0.98 (0.80, 1.20) | 0.89 (0.72, 1.10) | 0.90 (0.71, 1.13) | 0.67 (0.51, 0.86) | <0.01 | |
| Cancer | | | | | | | | | | | | | | | | |
| No. of deaths (%) | 728 | 184 (25.3%) | 151 (20.7%) | 134 (18.4%) | 166 (22.8%) | 93 (12.8%) | | | 728 | 204 (28.0%) | 161 (22.1%) | 146 (20.1%) | 130 (17.9%) | 87 (12.0%) | | |
| Minimally adjusted HR (95% CI)[a] | | 1.00 | 0.91 (0.72, 1.14) | 0.73 (0.57, 0.92) | 0.75 (0.60, 0.94) | 0.52 (0.40, 0.67) | <0.01 | | | 1.00 | 0.79 (0.64, 0.99) | 0.69 (0.55, 0.86) | 0.76 (0.60, 0.96) | 0.49 (0.37, 0.64) | <0.01 | |
| Fully adjusted HR (95% CI)[b] | | 1.00 | 1.00 (0.80, 1.27) | 0.87 (0.69, 1.11) | 0.96 (0.76, 1.21) | 0.72 (0.55, 0.95) | 0.03 | | | 1.00 | 0.87 (0.70, 1.09) | 0.79 (0.63, 0.99) | 0.93 (0.73, 1.17) | 0.65 (0.49, 0.85) | 0.01 | |
| Injury/Accident | | | | | | | | | | | | | | | | |
| No. of deaths (%) | 100 | 22 (22.0%) | 17 (17.0%) | 20 (20.0%) | 24 (24.0%) | 17 (17.0%) | | | 100 | 29 (29.0%) | 24 (24.0%) | 21 (21.0%) | 10 (10.0%) | 16 (16.0%) | | |
| Minimally adjusted HR (95% CI)[a] | | 1.00 | 1.11 (0.56, 2.21) | 1.08 (0.56, 2.10) | 1.00 (0.52, 1.90) | 0.93 (0.47, 1.85) | 0.74 | | | 1.00 | 0.95 (0.53, 1.71) | 0.79 (0.43, 1.46) | 0.50 (0.24, 1.05) | 0.76 (0.40, 1.47) | 0.15 | |
| Fully adjusted HR (95% CI)[b] | | 1.00 | 1.24 (0.62, 2.50) | 1.30 (0.66, 2.56) | 1.17 (0.60, 2.30) | 1.21 (0.59, 2.47) | 0.69 | | | 1.00 | 0.99 (0.54, 1.81) | 0.89 (0.48, 1.66) | 0.60 (0.28, 1.27) | 0.95 (0.49, 1.87) | 0.50 | |
| Other Cause of Death | | | | | | | | | | | | | | | | |
| No. of deaths (%) | 822 | 221 (26.9%) | 160 (19.5%) | 148 (18.0%) | 160 (19.5%) | 133 (16.2%) | | | 822 | 245 (29.8%) | 205 (24.9%) | 170 (20.7%) | 115 (14.0%) | 87 (10.6%) | | |
| Minimally adjusted HR (95% CI)[a] | | 1.00 | 0.80 (0.64, 1.00) | 0.64 (0.51, 0.80) | 0.58 (0.47, 0.72) | 0.58 (0.46, 0.73) | <.001 | | | 1.00 | 0.82 (0.68, 1.00) | 0.64 (0.52, 0.79) | 0.53 (0.42, 0.67) | 0.41 (0.31, 0.53) | <.001 | |
| Fully adjusted HR (95% CI)[b] | | 1.00 | 0.84 (0.67, 1.05) | 0.76 (0.61, 0.96) | 0.71 (0.57, 0.89) | 0.77 (0.60, 0.98) | 0.01 | | | 1.00 | 0.89 (0.73, 1.09) | 0.74 (0.60, 0.91) | 0.64 (0.50, 0.81) | 0.54 (0.41, 0.71) | <.001 | |

Abbreviations: HR, hazards ratio; 95% CI, 95% confidence interval.

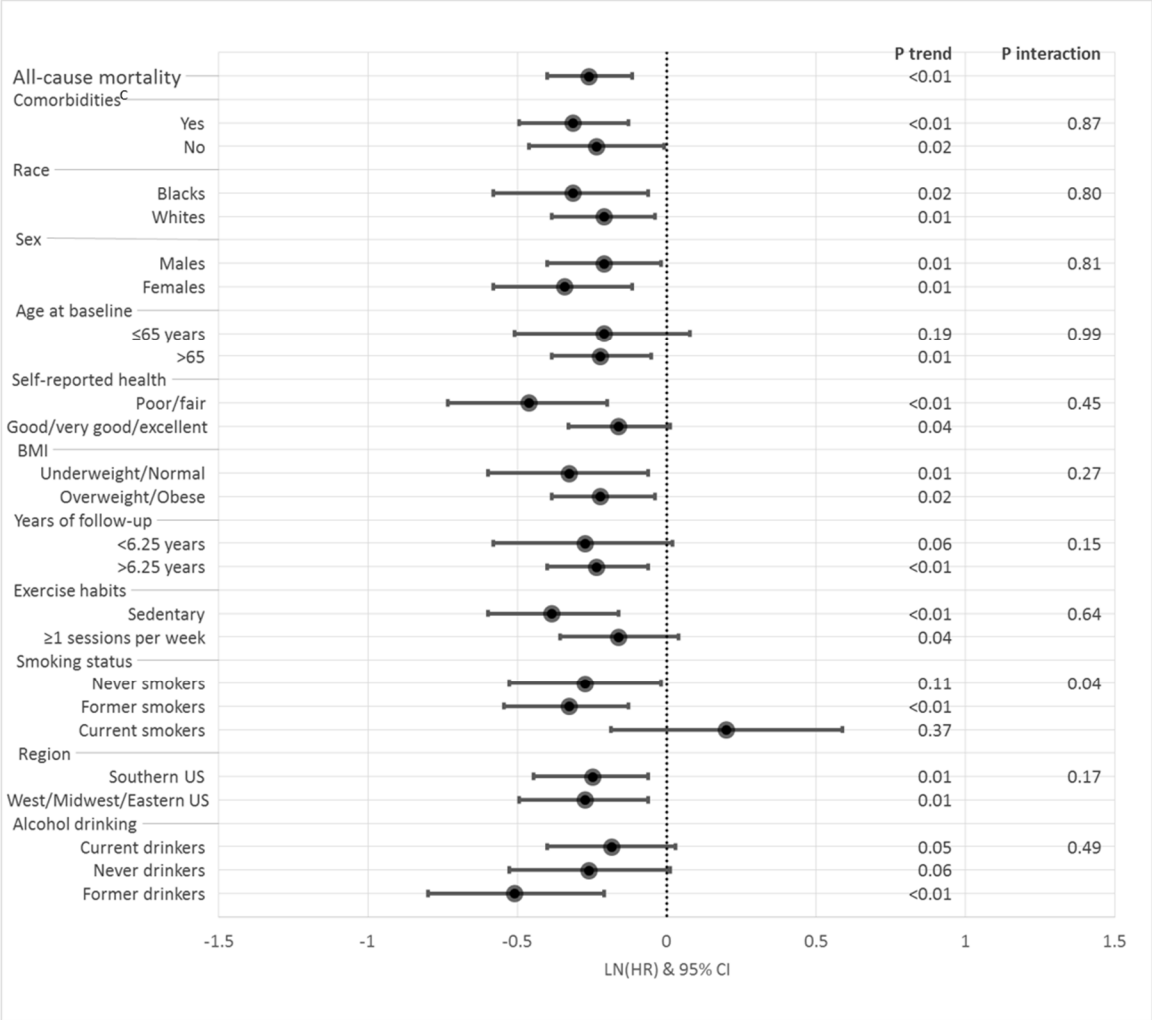
[a] Adjusted for sex, race (black/white), total energy intake (kcal) at baseline in an age-as-time-scale Cox model.

[b] Adjusted for sex, race (black/white), total energy intake (kcal), body mass index (kg/m$^2$, categorized by WHO criteria into underweight, normal, overweight, and obese), physical activity (0, 1-3, or 4+ exercise sessions per week), smoking (current, former, never), annual income (refused, <$20,000, $20,000-$34,000, $35,000-$74,000, >$75,000 per year), and hormone replacement therapy use (in women) at baseline in an age (in months)-as-time-scale model.

[c] P for trend calculated by assigning median of each diet score quintile to each quintile, and treating this quintile exposure as continuous.

[d] Cardiovascular disease deaths include deaths attributed to myocardial infarction, stroke, sudden death, heart failure, pulmonary embolism, other cardiac causes of death, and other non-cardiac cardiovascular disease deaths.

*Figure 4.1. Associations of the Paleolithic diet score with all-cause mortality[a], according to selected participant characteristics at baseline (N=21,423); REGARDS.*



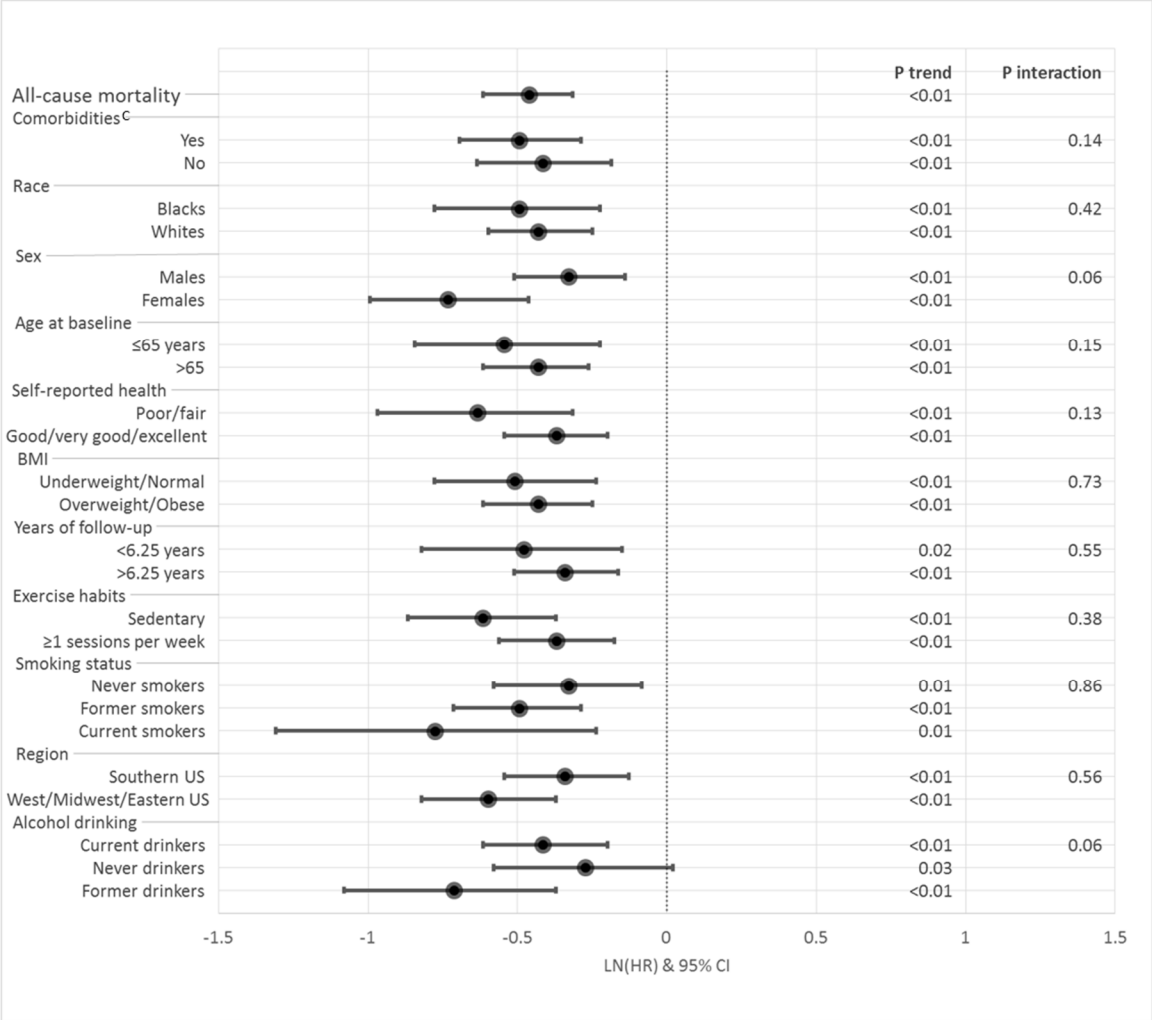

Abbreviations: BMI, body mass index; HR, hazard ratio; LN, natural logarithm; 95% CI, 95% confidence interval.

[a] From Cox model; only the comparison of the 5th relative to the 1st quintile of each diet score with all-cause mortality is shown; model covariates included sex, race (black/white), total energy intake (kcal), body mass index (kg/m$^2$, categorized by WHO criteria into underweight, normal, overweight, and obese), physical activity (0, 1-3, or 4+ exercise sessions per week), smoking (current, former, never), annual income (refused, <$20,000, $20,000-$34,000, $35,000-$74,000, >$75,000 per year), and hormone replacement therapy use (in women) at baseline in an age-as-time-scale.

[b] P for trend calculated by assigning median of each diet score quintile to each quintile, and treating this quintile exposure as continuous.

[c] Comorbidities include history of any cancer, kidney failure, type II diabetes, stent, heart surgery, aneurysm, or myocardial infarction.

*Figure 4.2. Associations of the Mediterranean diet score with all-cause mortality[a], according to selected participant characteristics at baseline (N=21,423); REGARDS.*




Abbreviations: BMI, body mass index; HR, hazard ratio; LN, natural logarithm; 95% CI, 95% confidence interval.

[a] From Cox model; only the comparison of the 5th relative to the 1st quintile of each diet score with all-cause mortality is shown; model covariates included sex, race (black/white), total energy intake (kcal), body mass index (kg/m$^2$, categorized by WHO criteria into underweight, normal, overweight, and obese), physical activity (0, 1-3, or 4+ exercise sessions per week), smoking (current, former, never), annual income (refused, <$20,000, $20,000-$34,000, $35,000-$74,000, >$75,000 per year), and hormone replacement therapy use (in women) at baseline in an age-as-time-scale.

[b] P for trend calculated by assigning median of each diet score quintile to each quintile, and treating this quintile exposure as continuous.

[c] Comorbidities include history of any cancer, kidney failure, type II diabetes, stent, heart surgery, aneurysm, or myocardial infarction.

## Conclusions and Public Health Implications

The goal for my dissertation was to 1) develop a Paleolithic diet pattern score; 2) investigate its associations with biomarkers of inflammation and oxidative balance, colorectal adenomas, and all-cause and cause-specific mortality; and 3) compare the observed associations to those from parallel analyses for a Mediterranean diet pattern score.

In the first dissertation project, a pooled cross-sectional study of an elective outpatient population, we found that higher Paleolithic or Mediterranean diet pattern scores were similarly inversely associated with serum CRP and $F_2$-isoprostane levels (Study #1). In the second dissertation project, based on data from a case-control study of colorectal polyps, we found that higher Paleolithic or Mediterranean diet pattern scores were inversely associated with colorectal adenoma. Our findings also suggested that these inverse associations may be stronger among persons with higher inflammation or oxidative stress levels (Study #2). For my third dissertation project I analyzed data from a large national prospective cohort study, Reasons for Geographic and Racial Differences in Stroke (REGARDS) to investigate associations of the Paleolithic or Mediterranean diet pattern scores with all-cause and cause-specific mortality. We found that overall, higher Paleolithic or Mediterranean diet pattern scores were similarly associated with lower all-cause mortality. The findings for all-cancer and all-cardiovascular disease mortality were similar to those for all-cause mortality. As we hypothesized, neither diet score was associated with mortality due to injuries or accidents, suggesting that our findings for all-cause, cardiovascular, cancer, and other non-injury/accident mortality were plausibly related to the underlying biological mechanisms of the diets (Study #3).

Overall, the results of this dissertation support my hypothesis that a Paleolithic diet pattern is associated with a lower risk of cardiovascular disease or cancer. The diet score was inversely associated with biomarkers of two of the hypothesized biological mechanisms (inflammation, oxidative stress) by which diet modulates chronic disease risk. It was also inversely associated with a known pre-malignant neoplasm, colorectal adenomas, and all-cause and cause-specific mortality.

Furthermore, we repeated the analysis with another similarly constructed diet score, the Mediterranean diet pattern score, to assess the relative strengths of the observed associations for the two diet patterns. The Mediterranean diet pattern has been extensively in other studies, which generally found inverse associations of the diet with biomarkers of inflammation and oxidative balance, risk of various cancers, and risk of mortality. The results of randomized diet trials of the diet pattern support these observational findings. The associations of the Paleolithic and the Mediterranean diet scores with the biomarkers of inflammation and oxidative stress and with colorectal adenoma were virtually indistinguishable. The associations of the two diet scores with mortality were also similarly inverse, although those for the Mediterranean diet tended to be a little stronger.

Taken together, the results from this dissertation suggest that Paleolithic- or Mediterranean-like diet patterns may be inversely associated with risk for chronic diseases such as cardiovascular disease or cancer.

## FUTURE DIRECTIONS

In the three studies included in this dissertation, the Paleolithic and Mediterranean diet pattern scores were similarly inversely associated with biomarkers of inflammation and oxidative balance, risk of colorectal adenomas, and all-cause and cause-specific mortality. The Mediterranean diet pattern has been extensively studied for associations with a variety of chronic diseases in many populations, and provided support for several successful randomized diet trials. In order to follow the Mediterranean diet pattern example, the Paleolithic diet pattern's associations with other chronic diseases in other populations still needs to be assessed.

Researchers can also examine the Paleolithic diet in randomized trials to assess the risk of chronic diseases. Currently, there have been few, mostly small pilot trials of a Paleolithic diet and their effect on blood pressure, dyslipidemia, glucose control, and weight loss (157-162, 164, 166). While large-scale diet trials are expensive, particularly when the outcome of interest is the

development of a chronic disease, there several short-term endpoints that can be used to provide evidence for possible beneficial effects of the Paleolithic diet.

Based on the results of my dissertation, I propose to test, in a pilot, randomized controlled trial, the effects of a Paleolithic diet and a Mediterranean diet relative to a healthy control diet and to each other on tissue and systemic biomarkers of risk for colorectal neoplasms and other chronic diseases in patients who have had a prior colorectal adenoma. The biomarkers will be measured in biopsies of normal-appearing colorectal mucosa and in blood and urine samples. Biomarkers of risk for colorectal neoplasms will include 1) tissue markers of inflammation and oxidative stress, markers of the major colon carcinogenesis pathways, cell cycle markers, autocrine-paracrine growth factors, and others; and 2) systemic markers of inflammation and oxidative balance (there will be a more extensive set of markers than I investigated in my dissertation in order to provide a more comprehensive profile of inflammation and oxidative stress). Biomarkers of risk for other chronic diseases will include lipid panels and other cardiometabolic markers. In this pilot trial, we would be able to assess directly the relative strengths of the Paleolithic diet and Mediterranean diets, and more definitely assess potential lifestyle, and genetic modifiers of the diet effects.

# REFERENCES


1. Arias E. United States life tables, 2009. *National vital statistics reports : from the Centers for Disease Control and Prevention, National Center for Health Statistics, National Vital Statistics System* 2014;62(7):1-63.

2. Omran AR. The epidemiologic transition. A theory of the epidemiology of population change. *The Milbank Memorial Fund quarterly* 1971;49(4):509-38.

3. Heron M. Deaths: leading causes for 2010. *National vital statistics reports : from the Centers for Disease Control and Prevention, National Center for Health Statistics, National Vital Statistics System* 2013;62(6):1-96.

4. Monteiro CA, Moubarac JC, Cannon G, et al. Ultra-processed products are becoming dominant in the global food system. *Obesity reviews : an official journal of the International Association for the Study of Obesity* 2013;14 Suppl 2:21-8.

5. Lieberman D. *The story of the human body : evolution, health, and disease*. First edition. ed. New York: Pantheon Books; 2013.

6. Eaton SB, Konner M. Paleolithic nutrition. A consideration of its nature and current implications. *The New England journal of medicine* 1985;312(5):283-9.

7. Keys A, Menotti A, Karvonen MJ, et al. The diet and 15-year death rate in the seven countries study. *American journal of epidemiology* 1986;124(6):903-15.

8. Critchley J, Liu J, Zhao D, et al. Explaining the increase in coronary heart disease mortality in Beijing between 1984 and 1999. *Circulation* 2004;110(10):1236-44.

9. Zhou BF, Stamler J, Dennis B, et al. Nutrient intakes of middle-aged men and women in China, Japan, United Kingdom, and United States in the late 1990s: the INTERMAP study. *Journal of human hypertension* 2003;17(9):623-30.

10. Mente A, de Koning L, Shannon HS, et al. A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. *Archives of internal medicine* 2009;169(7):659-69.

11. Tomasetti C, Vogelstein B. Cancer etiology. Variation in cancer risk among tissues can be explained by the number of stem cell divisions. *Science (New York, NY)* 2015;347(6217):78-81.

12. World Cancer Research Fund / American Institute for Cancer Research. Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective. Washington DC: AICR, 2007.

13. Bostick RM. Diet and nutrition in the etiology and primary prevention of colon cancer. In: Bendich A, Deckelbaum RJ, eds. *Preventative Nutrition: The Comprehensive Guide for Health Professionals, 2nd Edition*. Totowa, NJ: Humana Press Inc., 2001:47-96.

14. Miller PE, Lesko SM, Muscat JE, et al. Dietary patterns and colorectal adenoma and cancer risk: a review of the epidemiological evidence. *Nutrition and cancer* 2010;62(4):413-24.

15. Randi G, Edefonti V, Ferraroni M, et al. Dietary patterns and the risk of colorectal cancer and adenomas. *Nutrition reviews* 2010;68(7):389-408.

16. Ogden CL, Carroll MD, Kit BK, et al. Prevalence of childhood and adult obesity in the United States, 2011-2012. *JAMA : the journal of the American Medical Association* 2014;311(8):806-14.

17. Malik VS, Willett WC, Hu FB. Global obesity: trends, risk factors and policy implications. *Nature reviews Endocrinology* 2013;9(1):13-27.

18. Hubert HB, Feinleib M, McNamara PM, et al. Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. *Circulation* 1983;67(5):968-77.

19. Rabkin SW, Mathewson FA, Hsu PH. Relation of body weight to development of ischemic heart disease in a cohort of young North American men after a 26 year observation period: the Manitoba Study. *The American journal of cardiology* 1977;39(3):452-8.

20. Bastien M, Poirier P, Lemieux I, et al. Overview of epidemiology and contribution of obesity to cardiovascular disease. *Progress in cardiovascular diseases* 2014;56(4):369-81.

21. Kwok CS, Pradhan A, Khan MA, et al. Bariatric surgery and its impact on cardiovascular disease and mortality: a systematic review and meta-analysis. *International journal of cardiology* 2014;173(1):20-8.

22. Maestro A, Rigla M, Caixas A. Does bariatric surgery reduce cancer risk? A review of the literature. *Endocrinologia y nutricion : organo de la Sociedad Espanola de Endocrinologia y Nutricion* 2015;62(3):138-43.

23. Rosin O. Weight-loss dieting behavior: an economic analysis. *Health economics* 2012;21(7):825-38.

24. Johansson K, Neovius M, Hemmingsson E. Effects of anti-obesity drugs, diet, and exercise on weight-loss maintenance after a very-low-calorie diet or low-calorie diet: a systematic review and meta-analysis of randomized controlled trials. *The American journal of clinical nutrition* 2014;99(1):14-23.

25. Swift DL, Johannsen NM, Lavie CJ, et al. The role of exercise and physical activity in weight loss and maintenance. *Progress in cardiovascular diseases* 2014;56(4):441-7.

26. Andersen RE, Wadden TA, Bartlett SJ, et al. Effects of lifestyle activity vs structured aerobic exercise in obese women: a randomized trial. *JAMA : the journal of the American Medical Association* 1999;281(4):335-40.

27. Church TS, Martin CK, Thompson AM, et al. Changes in weight, waist circumference and compensatory responses with different doses of exercise among sedentary, overweight postmenopausal women. *PloS one* 2009;4(2):e4515.

28. Fogelholm M, Kukkonen-Harjula K. Does physical activity prevent weight gain--a systematic review. *Obesity reviews : an official journal of the International Association for the Study of Obesity* 2000;1(2):95-111.

29. Irving BA, Davis CK, Brock DW, et al. Effect of exercise training intensity on abdominal visceral fat and body composition. *Medicine and science in sports and exercise* 2008;40(11):1863-72.

30. Jakicic JM, Winters C, Lang W, et al. Effects of intermittent exercise and use of home exercise equipment on adherence, weight loss, and fitness in overweight women: a randomized trial. *JAMA : the journal of the American Medical Association* 1999;282(16):1554-60.

31. Martin CK, Church TS, Thompson AM, et al. Exercise dose and quality of life: a randomized controlled trial. *Archives of internal medicine* 2009;169(3):269-78.

32. Ross R, Dagnone D, Jones PJ, et al. Reduction in obesity and related comorbid conditions after diet-induced weight loss or exercise-induced weight loss in men. A randomized, controlled trial. *Annals of internal medicine* 2000;133(2):92-103.

33. Ross R, Janssen I, Dawson J, et al. Exercise-induced reduction in obesity and insulin resistance in women: a randomized controlled trial. *Obesity research* 2004;12(5):789-98.

34. Thomas DM, Bouchard C, Church T, et al. Why do individuals not lose more weight from an exercise intervention at a defined dose? An energy balance analysis. *Obesity reviews : an official journal of the International Association for the Study of Obesity* 2012;13(10):835-47.

35. Deighton K, Stensel DJ. Creating an acute energy deficit without stimulating compensatory increases in appetite: is there an optimal exercise protocol? *The Proceedings of the Nutrition Society* 2014;73(2):352-8.

36. Mekary RA, Feskanich D, Hu FB, et al. Physical activity in relation to long-term weight maintenance after intentional weight loss in premenopausal women. *Obesity (Silver Spring, Md)* 2010;18(1):167-74.

37. Anderson AS, Craigie AM, Caswell S, et al. The impact of a bodyweight and physical activity intervention (BeWEL) initiated through a national colorectal cancer screening programme: randomised controlled trial. *BMJ (Clinical research ed)* 2014;348:g1823.

38. Atallah R, Filion KB, Wakil SM, et al. Long-term effects of 4 popular diets on weight loss and cardiovascular risk factors: a systematic review of randomized controlled trials. *Circulation Cardiovascular quality and outcomes* 2014;7(6):815-27.

39. Trigueros L, Pena S, Ugidos AV, et al. Food ingredients as anti-obesity agents: a review. *Critical reviews in food science and nutrition* 2013;53(9):929-42.

40. Foster GD, Wyatt HR, Hill JO, et al. Weight and metabolic outcomes after 2 years on a low-carbohydrate versus low-fat diet: a randomized trial. *Annals of internal medicine* 2010;153(3):147-57.

41. Foster GD, Wyatt HR, Hill JO, et al. A randomized trial of a low-carbohydrate diet for obesity. *The New England journal of medicine* 2003;348(21):2082-90.

42. Bosma-den Boer MM, van Wetten ML, Pruimboom L. Chronic inflammatory diseases are stimulated by current lifestyle: how diet, stress levels and medication prevent our body from recovering. *Nutrition & metabolism* 2012;9(1):32.

43. Barbaresko J, Koch M, Schulze MB, et al. Dietary pattern analysis and biomarkers of low-grade inflammation: a systematic literature review. *Nutrition reviews* 2013;71(8):511-27.

44. Alcock J, Franklin ML, Kuzawa CW. Nutrient signaling: evolutionary origins of the immune-modulating effects of dietary fat. *The Quarterly review of biology* 2012;87(3):187-223.

45. Labonte ME, Couture P, Richard C, et al. Impact of dairy products on biomarkers of inflammation: a systematic review of randomized controlled nutritional intervention studies in overweight and obese adults. *The American journal of clinical nutrition* 2013;97(4):706-17.

46. de Punder K, Pruimboom L. The dietary intake of wheat and other cereal grains and their role in inflammation. *Nutrients* 2013;5(3):771-87.

47. Alemany M. Relationship between energy dense diets and white adipose tissue inflammation in metabolic syndrome. *Nutrition research (New York, NY)* 2013;33(1):1-11.

48. Ruiz-Nunez B, Pruimboom L, Dijck-Brouwer DA, et al. Lifestyle and nutritional imbalances associated with Western diseases: causes and consequences of chronic systemic low-grade inflammation in an evolutionary context. *The Journal of nutritional biochemistry* 2013;24(7):1183-201.

49. Medzhitov R. Origin and physiological roles of inflammation. *Nature* 2008;454(7203):428-35.

50. Jones DP. Extracellular redox state: refining the definition of oxidative stress in aging. *Rejuvenation Res* 2006;9(2):169-81.

51. Reuter S, Gupta SC, Chaturvedi MM, et al. Oxidative stress, inflammation, and cancer: how are they linked? *Free radical biology & medicine* 2010;49(11):1603-16.

52. Hussain SP, Harris CC. Inflammation and cancer: an ancient link with novel potentials. *International journal of cancer Journal international du cancer* 2007;121(11):2373-80.

53. Federico A, Morgillo F, Tuccillo C, et al. Chronic inflammation and oxidative stress in human carcinogenesis. *International journal of cancer Journal international du cancer* 2007;121(11):2381-6.

54. Goodman M, Bostick RM, Kucuk O, et al. Clinical trials of antioxidants as cancer prevention agents: past, present, and future. *Free radical biology & medicine* 2011;51(5):1068-84.

55. Kritharides L, Stocker R. The use of antioxidant supplements in coronary heart disease. *Atherosclerosis* 2002;164(2):211-9.

56. Ridker PM, Morrow DA. C-reactive protein, inflammation, and coronary risk. *Cardiology clinics* 2003;21(3):315-25.

57. Kim YW, West XZ, Byzova TV. Inflammation and oxidative stress in angiogenesis and vascular disease. *Journal of molecular medicine (Berlin, Germany)* 2013;91(3):323-8.

58. Mantovani A, Allavena P, Sica A, et al. Cancer-related inflammation. *Nature* 2008;454(7203):436-44.

59. Valinluck V, Sowers LC. Inflammation-mediated cytosine damage: a mechanistic link between inflammation and the epigenetic alterations in human cancers. *Cancer research* 2007;67(12):5583-6.

60. Allin KH, Nordestgaard BG. Elevated C-reactive protein in the diagnosis, prognosis, and cause of cancer. *Critical reviews in clinical laboratory sciences* 2011;48(4):155-70.

61. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell* 2010;140(6):883-99.

62. Hawk ET, Umar A, Viner JL. Colorectal cancer chemoprevention--an overview of the science. *Gastroenterology* 2004;126(5):1423-47.

63. Sandler RS, Halabi S, Baron JA, et al. A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. *The New England journal of medicine* 2003;348(10):883-90.

64. Sturmer T, Glynn RJ, Lee IM, et al. Aspirin use and colorectal cancer: post-trial follow-up data from the Physicians' Health Study. *Annals of internal medicine* 1998;128(9):713-20.

65. Ridker PM, Cushman M, Stampfer MJ, et al. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *The New England journal of medicine* 1997;336(14):973-9.

66. Benamouzig R, Deyra J, Martin A, et al. Daily soluble aspirin and prevention of colorectal adenoma recurrence: one-year results of the APACC trial. *Gastroenterology* 2003;125(2):328-36.

67. Benamouzig R, Uzzan B, Deyra J, et al. Prevention by daily soluble aspirin of colorectal adenoma recurrence: 4-year results of the APACC randomised trial. *Gut* 2012;61(2):255-61.

68. Burn J, Gerdes AM, Macrae F, et al. Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: an analysis from the CAPP2 randomised controlled trial. *Lancet* 2011;378(9809):2081-7.

69. Arber N, Eagle CJ, Spicak J, et al. Celecoxib for the prevention of colorectal adenomatous polyps. *The New England journal of medicine* 2006;355(9):885-95.

70. Arber N, Spicak J, Racz I, et al. Five-year analysis of the prevention of colorectal sporadic adenomatous polyps trial. *The American journal of gastroenterology* 2011;106(6):1135-46.

71. Baron JA, Cole BF, Sandler RS, et al. A randomized trial of aspirin to prevent colorectal adenomas. *The New England journal of medicine* 2003;348(10):891-9.

72. Baron JA, Sandler RS, Bresalier RS, et al. A randomized trial of rofecoxib for the chemoprevention of colorectal adenomas. *Gastroenterology* 2006;131(6):1674-82.

73. Morrow JD. Quantification of isoprostanes as indices of oxidant stress and the risk of atherosclerosis in humans. *Arteriosclerosis, thrombosis, and vascular biology* 2005;25(2):279-86.

74. Fortmann SP, Burda BU, Senger CA, et al. U.S. Preventive Services Task Force Evidence Syntheses, formerly Systematic Evidence Reviews. *Vitamin, Mineral, and Multivitamin Supplements for the Primary Prevention of Cardiovascular Disease and Cancer: A Systematic Evidence Review for the US Preventive Services Task Force*. Rockville (MD): Agency for Healthcare Research and Quality (US), 2013.

75. Xie M, Shan Z, Zhang Y, et al. Aspirin for primary prevention of cardiovascular events: meta-analysis of randomized controlled trials and subgroup analysis by sex and diabetes status. *PloS one* 2014;9(10):e90286.

76. Funk CD, FitzGerald GA. COX-2 inhibitors and cardiovascular risk. *Journal of cardiovascular pharmacology* 2007;50(5):470-9.

77. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. *The New England journal of medicine* 1994;330(15):1029-35.

78. Virtamo J, Pietinen P, Huttunen JK, et al. Incidence of cancer and mortality following alpha-tocopherol and beta-carotene supplementation: a postintervention follow-up. *JAMA : the journal of the American Medical Association* 2003;290(4):476-85.

79. Omenn GS, Goodman GE, Thornquist MD, et al. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *The New England journal of medicine* 1996;334(18):1150-5.

80. Goodman GE, Thornquist MD, Balmes J, et al. The Beta-Carotene and Retinol Efficacy Trial: incidence of lung cancer and cardiovascular disease mortality during 6-year follow-up after stopping beta-carotene and retinol supplements. *Journal of the National Cancer Institute* 2004;96(23):1743-50.

81. Bischoff-Ferrari HA. Optimal serum 25-hydroxyvitamin D levels for multiple health outcomes. *Advances in experimental medicine and biology* 2014;810:500-25.

82. Combs GF, Jr., Clark LC, Turnbull BW. Reduction of cancer mortality and incidence by selenium supplementation. *Medizinische Klinik (Munich, Germany : 1983)* 1997;92 Suppl 3:42-5.

83. Clark LC, Combs GF, Jr., Turnbull BW, et al. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group. *JAMA : the journal of the American Medical Association* 1996;276(24):1957-63.

84. Clark LC, Dalkin B, Krongrad A, et al. Decreased incidence of prostate cancer with selenium supplementation: results of a double-blind cancer prevention trial. *British journal of urology* 1998;81(5):730-4.

85. Bolland MJ, Grey A, Gamble GD, et al. Calcium and vitamin D supplements and health outcomes: a reanalysis of the Women's Health Initiative (WHI) limited-access data set. *The American journal of clinical nutrition* 2011;94(4):1144-9.

86. Jacobs DR, Jr., Steffen LM. Nutrients, foods, and dietary patterns as exposures in research: a framework for food synergy. *The American journal of clinical nutrition* 2003;78(3 Suppl):508s-13s.

87. Jacques PF, Tucker KL. Are dietary patterns useful for understanding the role of diet in chronic disease? *The American journal of clinical nutrition* 2001;73(1):1-2.

88. Reedy J, Wirfalt E, Flood A, et al. Comparing 3 dietary pattern methods--cluster analysis, factor analysis, and index analysis--With colorectal cancer risk: The NIH-AARP Diet and Health Study. *American journal of epidemiology* 2010;171(4):479-87.

89. Schulze MB, Hoffmann K. Methodological approaches to study dietary patterns in relation to risk of coronary heart disease and stroke. *The British journal of nutrition* 2006;95(5):860-9.

90. Gibson GR. *Principles of Nutritional Assessment*. 2nd ed.: Oxford University Press; 2005.

91. Shim JS, Oh K, Kim HC. Dietary assessment methods in epidemiologic studies. *Epidemiology and health* 2014;36:e2014009.

92. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. *American journal of epidemiology* 1985;122(1):51-65.

93. Hu FB, Rimm E, Smith-Warner SA, et al. Reproducibility and validity of dietary patterns assessed with a food-frequency questionnaire. *The American journal of clinical nutrition* 1999;69(2):243-9.

94. Nanri A, Yoshida D, Yamaji T, et al. Dietary patterns and C-reactive protein in Japanese men and women. *The American journal of clinical nutrition* 2008;87(5):1488-96.

95. Nanri H, Nakamura K, Hara M, et al. Association between dietary pattern and serum C-reactive protein in Japanese men and women. *Journal of epidemiology / Japan Epidemiological Association* 2011;21(2):122-31.

96. Liu S, Manson JE, Buring JE, et al. Relation between a diet with a high glycemic load and plasma concentrations of high-sensitivity C-reactive protein in middle-aged women. *The American journal of clinical nutrition* 2002;75(3):492-8.

97. Jonasson L, Guldbrand H, Lundberg AK, et al. Advice to follow a low-carbohydrate diet has a favourable impact on low-grade inflammation in type 2 diabetes compared with advice to follow a low-fat diet. *Annals of medicine* 2014;46(3):182-7.

98. Gaskins AJ, Rovner AJ, Mumford SL, et al. Adherence to a Mediterranean diet and plasma concentrations of lipid peroxidation in premenopausal women. *The American journal of clinical nutrition* 2010;92(6):1461-7.

99. Dai J, Jones DP, Goldberg J, et al. Association between adherence to the Mediterranean diet and oxidative stress. *The American journal of clinical nutrition* 2008;88(5):1364-70.

100. Chen Y, McClintock TR, Segers S, et al. Prospective investigation of major dietary patterns and risk of cardiovascular mortality in Bangladesh. *International journal of cardiology* 2013;167(4):1495-501.

101. Anderson AL, Harris TB, Tylavsky FA, et al. Dietary patterns and survival of older adults. *Journal of the American Dietetic Association* 2011;111(1):84-91.

102. Huffman FG, Zarini GG, McNamara E, et al. The Healthy Eating Index and the Alternate Healthy Eating Index as predictors of 10-year CHD risk in Cuban Americans with and without type 2 diabetes. *Public health nutrition* 2011;14(11):2006-14.

103. McCullough ML, Feskanich D, Stampfer MJ, et al. Diet quality and major chronic disease risk in men and women: moving toward improved dietary guidance. *The American journal of clinical nutrition* 2002;76(6):1261-71.

104. Alkerwi A, Vernier C, Crichton GE, et al. Cross-comparison of diet quality indices for predicting chronic disease risk: findings from the Observation of Cardiovascular Risk Factors in Luxembourg (ORISCAV-LUX) study. *The British journal of nutrition* 2014:1-11.

105. Garcia-Fernandez E, Rico-Cabanas L, Rosgaard N, et al. Mediterranean Diet and Cardiodiabesity: A Review. *Nutrients* 2014;6(9):3474-500.

106. Sofi F, Macchi C, Abbate R, et al. Mediterranean diet and health status: an updated meta-analysis and a proposal for a literature-based adherence score. *Public health nutrition* 2014;17(12):2769-82.

107. Santos FL, Esteves SS, da Costa Pereira A, et al. Systematic review and meta-analysis of clinical trials of the effects of low carbohydrate diets on cardiovascular risk factors. *Obesity reviews : an official journal of the International Association for the Study of Obesity* 2012;13(11):1048-66.

108. Nimptsch K, Malik VS, Fung TT, et al. Dietary patterns during high school and risk of colorectal adenoma in a cohort of middle-aged women. *International journal of cancer Journal international du cancer* 2014;134(10):2458-67.

109. Makambi KH, Agurs-Collins T, Bright-Gbebry M, et al. Dietary patterns and the risk of colorectal adenomas: the Black Women's Health Study. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2011;20(5):818-25.

110. Agnoli C, Grioni S, Sieri S, et al. Italian Mediterranean Index and risk of colorectal cancer in the Italian section of the EPIC cohort. *International journal of cancer Journal international du cancer* 2013;132(6):1404-11.

111. Bamia C, Lagiou P, Buckland G, et al. Mediterranean diet and colorectal cancer risk: results from a European cohort. *European journal of epidemiology* 2013;28(4):317-28.

112. Miller PE, Cross AJ, Subar AF, et al. Comparison of 4 established DASH diet indexes: examining associations of index scores and colorectal cancer. *The American journal of clinical nutrition* 2013;98(3):794-803.

113. Fung TT, Hu FB, Schulze M, et al. A dietary pattern that is associated with C-peptide and risk of colorectal cancer in women. *Cancer causes & control : CCC* 2012;23(6):959-65.

114. Bravi F, Edefonti V, Bosetti C, et al. Nutrient dietary patterns and the risk of colorectal cancer: a case-control study from Italy. *Cancer causes & control : CCC* 2010;21(11):1911-8.

115. Kurotani K, Budhathoki S, Joshi AM, et al. Dietary patterns and colorectal cancer in a Japanese population: the Fukuoka Colorectal Cancer Study. *The British journal of nutrition* 2010;104(11):1703-11.

116. Meyerhardt JA, Niedzwiecki D, Hollis D, et al. Association of dietary patterns with cancer recurrence and survival in patients with stage III colon cancer. *JAMA : the journal of the American Medical Association* 2007;298(7):754-64.

117. Berentzen NE, Beulens JW, Hoevenaar-Blom MP, et al. Adherence to the WHO's healthy diet indicator and overall cancer risk in the EPIC-NL cohort. *PloS one* 2013;8(8):e70535.

118. Chuang SC, Jenab M, Heck JE, et al. Diet and the risk of head and neck cancer: a pooled analysis in the INHANCE consortium. *Cancer causes & control : CCC* 2012;23(1):69-88.

119. Helen-Ng LC, Razak IA, Ghani WM, et al. Dietary pattern and oral cancer risk--a factor analysis study. *Community dentistry and oral epidemiology* 2012;40(6):560-6.

120. Edefonti V, Randi G, La Vecchia C, et al. Dietary patterns and breast cancer: a review with focus on methodological issues. *Nutrition reviews* 2009;67(6):297-314.

121. Cade JE, Taylor EF, Burley VJ, et al. Does the Mediterranean dietary pattern or the Healthy Diet Index influence the risk of breast cancer in a large British cohort of women? *European journal of clinical nutrition* 2011;65(8):920-8.

122. Couto E, Sandin S, Lof M, et al. Mediterranean dietary pattern and risk of breast cancer. *PloS one* 2013;8(2):e55374.

123. Verberne L, Bach-Faig A, Buckland G, et al. Association between the Mediterranean diet and cancer risk: a review of observational studies. *Nutrition and cancer* 2010;62(7):860-70.

124. Couto E, Boffetta P, Lagiou P, et al. Mediterranean dietary pattern and cancer risk in the EPIC cohort. *British journal of cancer* 2011;104(9):1493-9.

125. Bosetti C, Turati F, Dal Pont A, et al. The role of Mediterranean diet on the risk of pancreatic cancer. *British journal of cancer* 2013;109(5):1360-6.

126. Serra-Majem L, Roman B, Estruch R. Scientific evidence of interventions using the Mediterranean diet: a systematic review. *Nutrition reviews* 2006;64(2 Pt 2):S27-47.

127. Azzini E, Polito A, Fumagalli A, et al. Mediterranean Diet Effect: an Italian picture. *Nutrition journal* 2011;10:125.

128. Abiemo EE, Alonso A, Nettleton JA, et al. Relationships of the Mediterranean dietary pattern with insulin resistance and diabetes incidence in the Multi-Ethnic Study of Atherosclerosis (MESA). *The British journal of nutrition* 2013;109(8):1490-7.

129. Agnoli C, Krogh V, Grioni S, et al. A priori-defined dietary patterns are associated with reduced risk of stroke in a large Italian cohort. *The Journal of nutrition* 2011;141(8):1552-8.

130. de Lorgeril M. Mediterranean diet and cardiovascular disease: historical perspective and latest evidence. *Current atherosclerosis reports* 2013;15(12):370.

131. Buckland G, Agudo A, Lujan L, et al. Adherence to a Mediterranean diet and risk of gastric adenocarcinoma within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort study. *The American journal of clinical nutrition* 2010;91(2):381-90.

132. Fung TT, Hu FB, Wu K, et al. The Mediterranean and Dietary Approaches to Stop Hypertension (DASH) diets and colorectal cancer. *The American journal of clinical nutrition* 2010;92(6):1429-35.

133. Demetriou CA, Hadjisavvas A, Loizidou MA, et al. The mediterranean dietary pattern and breast cancer risk in Greek-Cypriot women: a case-control study. *BMC cancer* 2012;12:113.

134. Giacosa A, Barale R, Bavaresco L, et al. Cancer prevention in Europe: the Mediterranean diet as a protective choice. *European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation (ECP)* 2013;22(1):90-5.

135. Zazpe I, Sanchez-Tainta A, Toledo E, et al. Dietary patterns and total mortality in a Mediterranean cohort: the SUN project. *Journal of the Academy of Nutrition and Dietetics* 2014;114(1):37-47.

136. Sjogren P, Becker W, Warensjo E, et al. Mediterranean and carbohydrate-restricted diets and mortality among elderly men: a cohort study in Sweden. *The American journal of clinical nutrition* 2010;92(4):967-74.

137. Bonaccio M, Di Castelnuovo A, Costanzo S, et al. Adherence to the traditional Mediterranean diet and mortality in subjects with diabetes. Prospective results from the MOLI-SANI study. *European journal of preventive cardiology* 2015.

138. Ambring A, Friberg P, Axelsen M, et al. Effects of a Mediterranean-inspired diet on blood lipids, vascular function and oxidative stress in healthy subjects. *Clinical science (London, England : 1979)* 2004;106(5):519-25.

139. Mitjavila MT, Fandos M, Salas-Salvado J, et al. The Mediterranean diet improves the systemic lipid and DNA oxidative damage in metabolic syndrome individuals. A randomized, controlled, trial. *Clinical nutrition (Edinburgh, Scotland)* 2013;32(2):172-8.

140. Carter P, Achana F, Troughton J, et al. A Mediterranean diet improves HbA1c but not fasting blood glucose compared to alternative dietary strategies: a network meta-analysis. *Journal of human nutrition and dietetics : the official journal of the British Dietetic Association* 2014;27(3):280-97.

141. Damasceno NR, Perez-Heras A, Serra M, et al. Crossover study of diets enriched with virgin olive oil, walnuts or almonds. Effects on lipids and other cardiovascular risk markers. *Nutrition, metabolism, and cardiovascular diseases : NMCD* 2011;21 Suppl 1:S14-20.

142. Chiva-Blanch G, Badimon L, Estruch R. Latest evidence of the effects of the mediterranean diet in prevention of cardiovascular disease. *Current atherosclerosis reports* 2014;16(10):446.

143. Rees K, Hartley L, Flowers N, et al. 'Mediterranean' dietary pattern for the primary prevention of cardiovascular disease. *The Cochrane database of systematic reviews* 2013;8:Cd009825.

144. de Lorgeril M, Renaud S, Mamelle N, et al. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. *Lancet* 1994;343(8911):1454-9.

145. de Lorgeril M, Salen P, Martin JL, et al. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. *Circulation* 1999;99(6):779-85.

146. de Lorgeril M, Salen P, Martin JL, et al. Mediterranean dietary pattern in a randomized trial: prolonged survival and possible reduced cancer rate. *Archives of internal medicine* 1998;158(11):1181-7.

147. Urpi-Sarda M, Casas R, Chiva-Blanch G, et al. Virgin olive oil and nuts as key foods of the Mediterranean diet effects on inflammatory biomakers related to atherosclerosis. *Pharmacological research : the official journal of the Italian Pharmacological Society* 2012;65(6):577-83.

148. Estruch R, Ros E, Salas-Salvado J, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. *The New England journal of medicine* 2013;368(14):1279-90.

149. Konner M, Eaton SB. Paleolithic nutrition: twenty-five years later. *Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition* 2010;25(6):594-602.

150. M G, H K. Longevity Among Hunter-Gatherers: A Cross-Cultural Examination. *Population and Development Review* 2007;33(2):321-65.

151. Price WA. *Nutrition and Physical Degeneration*. 22nd Printing, 2014; 1939.

152. Fodor GJ, Helis E, Yazdekhasti N, et al. "Fishing" for the origins of the "Eskimos and heart disease" story. Facts or wishful thinking? A review. *Canadian Journal of Cardiology* 2014.

153. O'Dea K. Marked improvement in carbohydrate and lipid metabolism in diabetic Australian aborigines after temporary reversion to traditional lifestyle. *Diabetes* 1984;33(6):596-603.

154. Eilat-Adar S, Mete M, Nobmann ED, et al. Dietary patterns are linked to cardiovascular risk factors but not to inflammatory markers in Alaska Eskimos. *The Journal of nutrition* 2009;139(12):2322-8.

155. Labonte ME, Dewailly E, Lucas M, et al. Traditional dietary pattern is associated with elevated cholesterol among the Inuit of Nunavik. *Journal of the Academy of Nutrition and Dietetics* 2014;114(8):1208-15.e3.

156. Schnorr SL, Candela M, Rampelli S, et al. Gut microbiome of the Hadza hunter-gatherers. *Nature communications* 2014;5:3654.

157. Frassetto LA, Schloetter M, Mietus-Synder M, et al. Metabolic and physiologic improvements from consuming a paleolithic, hunter-gatherer type diet. *European journal of clinical nutrition* 2009;63(8):947-55.

158. Osterdahl M, Kocturk T, Koochek A, et al. Effects of a short-term intervention with a paleolithic diet in healthy volunteers. *European journal of clinical nutrition* 2008;62(5):682-5.

159. Smith M, Trexler E, Sommer A, et al. Unrestricted Paleolithic Diet is Associated with Unfavorable Changes to Blood Lipids in Healthy Subjects. *International Journal of Exercise Science* 2014;7(2).

160. Jonsson T, Granfeldt Y, Ahren B, et al. Beneficial effects of a Paleolithic diet on cardiovascular risk factors in type 2 diabetes: a randomized cross-over pilot study. *Cardiovascular diabetology* 2009;8:35-49.

161. Boers I, Muskiet FA, Berkelaar E, et al. Favourable effects of consuming a Palaeolithic-type diet on characteristics of the metabolic syndrome: a randomized controlled pilot-study. *Lipids in health and disease* 2014;13(1):160.

162. Lindeberg S, Jonsson T, Granfeldt Y, et al. A Palaeolithic diet improves glucose tolerance more than a Mediterranean-like diet in individuals with ischaemic heart disease. *Diabetologia* 2007;50(9):1795-807.

163. Jonsson T, Granfeldt Y, Lindeberg S, et al. Subjective satiety and other experiences of a Paleolithic diet compared to a diabetes diet in patients with type 2 diabetes. *Nutrition journal* 2013;12:105.

164. Bligh HF, Godsland IF, Frost G, et al. Plant-rich mixed meals based on Palaeolithic diet principles have a dramatic impact on incretin, peptide YY and satiety response, but show little effect on glucose and insulin homeostasis: an acute-effects randomised study. *The British journal of nutrition* 2015:1-11.

165. Becker W. [New Nordic nutrition recommendations 2004. Physical activity as important as good nourishing food]. *Lakartidningen* 2005;102(39):2757-8, 60-2.

166. Mellberg C, Sandberg S, Ryberg M, et al. Long-term effects of a Palaeolithic-type diet in obese postmenopausal women: a 2-year randomized trial. *European journal of clinical nutrition* 2014;68(3):350-7.

167. Hercberg S, Galan P, Preziosi P, et al. The SU.VI.MAX Study: a randomized, placebo-controlled trial of the health effects of antioxidant vitamins and minerals. *Archives of internal medicine* 2004;164(21):2335-42.

168. Hercberg S, Kesse-Guyot E, Druesne-Pecollo N, et al. Incidence of cancers, ischemic cardiovascular diseases and mortality during 5-year follow-up after stopping antioxidant vitamins and minerals supplements: a postintervention follow-up in the SU.VI.MAX Study. *International journal of cancer Journal international du cancer* 2010;127(8):1875-81.

169. Gaziano JM, Sesso HD, Christen WG, et al. Multivitamins in the prevention of cancer in men: the Physicians' Health Study II randomized controlled trial. *JAMA : the journal of the American Medical Association* 2012;308(18):1871-80.

170. Hennekens CH, Buring JE, Manson JE, et al. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *The New England journal of medicine* 1996;334(18):1145-9.

171. Cook NR, Le IM, Manson JE, et al. Effects of beta-carotene supplementation on cancer incidence by baseline characteristics in the Physicians' Health Study (United States). *Cancer causes & control : CCC* 2000;11(7):617-26.

172. Lee IM, Cook NR, Manson JE, et al. Beta-carotene supplementation and incidence of cancer and cardiovascular disease: the Women's Health Study. *Journal of the National Cancer Institute* 1999;91(24):2102-6.

173. Greenberg ER, Baron JA, Karagas MR, et al. Mortality associated with low plasma concentration of beta carotene and the effect of oral supplementation. *JAMA : the journal of the American Medical Association* 1996;275(9):699-703.

174. Green A, Williams G, Neale R, et al. Daily sunscreen application and betacarotene supplementation in prevention of basal-cell and squamous-cell carcinomas of the skin: a randomised controlled trial. *Lancet* 1999;354(9180):723-9.

175. Sesso HD, Buring JE, Christen WG, et al. Vitamins E and C in the prevention of cardiovascular disease in men: the Physicians' Health Study II randomized controlled trial. *JAMA : the journal of the American Medical Association* 2008;300(18):2123-33.

176. Lippman SM, Klein EA, Goodman PJ, et al. Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *JAMA : the journal of the American Medical Association* 2009;301(1):39-51.

177. Lee IM, Cook NR, Gaziano JM, et al. Vitamin E in the primary prevention of cardiovascular disease and cancer: the Women's Health Study: a randomized controlled trial. *JAMA : the journal of the American Medical Association* 2005;294(1):56-65.

178. Stranges S, Marshall JR, Trevisan M, et al. Effects of selenium supplementation on cardiovascular disease incidence and mortality: secondary analyses in a randomized clinical trial. *American journal of epidemiology* 2006;163(8):694-9.

179. Omenn GS, Goodman GE, Thornquist MD, et al. Risk factors for lung cancer and for intervention effects in CARET, the Beta-Carotene and Retinol Efficacy Trial. *Journal of the National Cancer Institute* 1996;88(21):1550-9.

180. Goodman GE, Omenn GS, Thornquist MD, et al. The Carotene and Retinol Efficacy Trial (CARET) to prevent lung cancer in high-risk populations: pilot study with cigarette smokers. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 1993;2(4):389-96.

181. Omenn GS, Goodman G, Thornquist M, et al. The beta-carotene and retinol efficacy trial (CARET) for chemoprevention of lung cancer in high risk populations: smokers and asbestos-exposed workers. *Cancer research* 1994;54(7 Suppl):2038s-43s.

182. Gaziano JM, Glynn RJ, Christen WG, et al. Vitamins E and C in the prevention of prostate and total cancer in men: the Physicians' Health Study II randomized controlled trial. *JAMA : the journal of the American Medical Association* 2009;301(1):52-62.

183. Cole BF, Baron JA, Sandler RS, et al. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. *JAMA : the journal of the American Medical Association* 2007;297(21):2351-9.

184. Figueiredo JC, Grau MV, Haile RW, et al. Folic acid and risk of prostate cancer: results from a randomized clinical trial. *Journal of the National Cancer Institute* 2009;101(6):432-5.

185. Avenell A, MacLennan GS, Jenkinson DJ, et al. Long-term follow-up for mortality and cancer in a randomized placebo-controlled trial of vitamin D(3) and/or calcium (RECORD trial). *The Journal of clinical endocrinology and metabolism* 2012;97(2):614-22.

186. Trivedi DP, Doll R, Khaw KT. Effect of four monthly oral vitamin D3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial. *BMJ (Clinical research ed)* 2003;326(7387):469.

187. Wactawski-Wende J, Kotchen JM, Anderson GL, et al. Calcium plus vitamin D supplementation and the risk of colorectal cancer. *The New England journal of medicine* 2006;354(7):684-96.

188. Bolland MJ, Barber PA, Doughty RN, et al. Vascular events in healthy older women receiving calcium supplementation: randomised controlled trial. *BMJ (Clinical research ed)* 2008;336(7638):262-6.

189. Lappe JM, Travers-Gustafson D, Davies KM, et al. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. *The American journal of clinical nutrition* 2007;85(6):1586-91.

190. Fung TT, Hu FB, McCullough ML, et al. Diet quality is associated with the risk of estrogen receptor-negative breast cancer in postmenopausal women. *The Journal of nutrition* 2006;136(2):466-72.

191. MacIntosh DL, Williams PL, Hunter DJ, et al. Evaluation of a food frequency questionnaire-food composition approach for estimating dietary intake of inorganic arsenic and methylmercury. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 1997;6(12):1043-50.

192. Morrow JD, Roberts LJ, 2nd. Mass spectrometry of prostanoids: F2-isoprostanes produced by non-cyclooxygenase free radical-catalyzed mechanism. *Methods in enzymology* 1994;233:163-74.

193. Smith-Warner SA, Elmer PJ, Fosdick L, et al. Fruits, vegetables, and adenomatous polyps: the Minnesota Cancer Prevention Research Unit case-control study. *American journal of epidemiology* 2002;155(12):1104-13.

194. Goodman M, Bostick RM, Dash C, et al. A summary measure of pro- and anti-oxidant exposures and risk of incident, sporadic, colorectal adenomas. *Cancer causes & control : CCC* 2008;19(10):1051-64.

195. O'Brien MJ, Winawer SJ, Zauber AG, et al. The National Polyp Study. Patient and polyp characteristics associated with high-grade dysplasia in colorectal adenomas. *Gastroenterology* 1990;98(2):371-9.

196. Tsivgoulis G, Psaltopoulou T, Wadley VG, et al. Adherence to a Mediterranean Diet and Prediction of Incident Stroke. *Stroke; a journal of cerebral circulation* 2015.

197. Howard VJ, Cushman M, Pulley L, et al. The reasons for geographic and racial differences in stroke study: objectives and design. *Neuroepidemiology* 2005;25(3):135-43.

198. Wadley VG, McClure LA, Howard VJ, et al. Cognitive status, stroke symptom reports, and modifiable risk factors among individuals with no diagnosis of stroke or transient ischemic attack in the REasons for Geographic and Racial Differences in Stroke (REGARDS) Study. *Stroke; a journal of cerebral circulation* 2007;38(4):1143-7.

199. Tsivgoulis G, Alexandrov AV, Wadley VG, et al. Association of higher diastolic blood pressure levels with cognitive impairment. *Neurology* 2009;73(8):589-95.

200. Wadley VG, Unverzagt FW, McGuire LC, et al. Incident cognitive impairment is elevated in the stroke belt: the REGARDS study. *Annals of neurology* 2011;70(2):229-36.

201. Block G, Hartman AM, Dresser CM, et al. A data-based approach to diet questionnaire design and testing. *American journal of epidemiology* 1986;124(3):453-69.

202. Otamiri T, Sjodahl R. Increased lipid peroxidation in malignant tissues of patients with colorectal cancer. *Cancer* 1989;64(2):422-5.

203. Morrow JD, Roberts LJ, 2nd. The isoprostanes. Current knowledge and directions for future research. *Biochemical pharmacology* 1996;51(1):1-9.

204. Rodrigo R, Libuy M, Feliu F, et al. Oxidative stress-related biomarkers in essential hypertension and ischemia-reperfusion myocardial damage. *Disease markers* 2013;35(6):773-90.

205. Barocas DA, Motley S, Cookson MS, et al. Oxidative stress measured by urine F2-isoprostane level is associated with prostate cancer. *The Journal of urology* 2011;185(6):2102-7.

206. Epplein M, Franke AA, Cooney RV, et al. Association of plasma micronutrient levels and urinary isoprostane with risk of lung cancer: the multiethnic cohort study. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2009;18(7):1962-70.

207. Nowsheen S, Aziz K, Kryston TB, et al. The interplay between inflammation and oxidative stress in carcinogenesis. *Current molecular medicine* 2012;12(6):672-80.

208. Milne GL, Musiek ES, Morrow JD. F2-isoprostanes as markers of oxidative stress in vivo: an overview. *Biomarkers : biochemical indicators of exposure, response, and susceptibility to chemicals* 2005;10 Suppl 1:S10-23.

209. Katz DL, Meller S. Can we say what diet is best for health? *Annual review of public health* 2014;35:83-103.

210. Bjelakovic G, Nikolova D, Simonetti RG, et al. Systematic review: primary and secondary prevention of gastrointestinal cancers with antioxidant supplements. *Alimentary pharmacology & therapeutics* 2008;28(6):689-703.

211. Albanes D, Malila N, Taylor PR, et al. Effects of supplemental alpha-tocopherol and beta-carotene on colorectal cancer: results from a controlled trial (Finland). *Cancer causes & control : CCC* 2000;11(3):197-205.

212. Yusof AS, Isa ZM, Shah SA. Dietary patterns and risk of colorectal cancer: a systematic review of cohort studies (2000-2011). *Asian Pacific journal of cancer prevention : APJCP* 2012;13(9):4713-7.

213. Millen BE, Quatromoni PA, Copenhafer DL, et al. Validation of a dietary pattern approach for evaluating nutritional risk: the Framingham Nutrition Studies. *Journal of the American Dietetic Association* 2001;101(2):187-94.

214. Whalen KA, McCullough M, Flanders WD, et al. Paleolithic and Mediterranean Diet Pattern Scores and Risk of Incident, Sporadic Colorectal Adenomas. *American journal of epidemiology* 2014.

215. Gong YL, Xie DW, Deng ZL, et al. Vitamin D receptor gene Tru9I polymorphism and risk for incidental sporadic colorectal adenomas. *World journal of gastroenterology : WJG* 2005;11(31):4794-9.

216. Daniel CR, Bostick RM, Flanders WD, et al. TGF-alpha expression as a potential biomarker of risk within the normal-appearing colorectal mucosa of patients with and without incident sporadic adenoma. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2009;18(1):65-73.

217. Kong SY, Bostick RM, Flanders WD, et al. Oxidative balance score, colorectal adenoma, and markers of oxidative stress and inflammation. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2014;23(3):545-54.

218. Goodman M, Bostick RM, Gross M, et al. Combined measure of pro- and anti-oxidant exposures in relation to prostate cancer and colorectal adenoma risk: an update. *Annals of epidemiology* 2010;20(12):955-7.

219. Pendyala S, Neff LM, Suarez-Farinas M, et al. Diet-induced weight loss reduces colorectal inflammation: implications for colorectal carcinogenesis. *The American journal of clinical nutrition* 2011;93(2):234-42.

220. Hopkins MH, Fedirko V, Jones DP, et al. Antioxidant micronutrients and biomarkers of oxidative stress and inflammation in colorectal adenoma patients: results from a randomized, controlled clinical trial. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2010;19(3):850-8.

221. Itzkowitz SH, Yio X. Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. *American journal of physiology Gastrointestinal and liver physiology* 2004;287(1):G7-17.

222. American Cancer Society. Colorectal Cancer Facts & Figures 2011-2013. Atlanta: American Cancer Society, 2011.

223. Potter JD, Hunter D. Colorectal Cancer. In: Adami H, Hunter D, D. T, eds. *Textbook of Cancer Epidemiology*. New York: Oxford University Press, 2008:275-307.

224. Roberston I, Bound R, Segal L. Colorectal cancer, diet and lifestyle factors: opportunities for prevention. *Health Promotion International* 1998;13(2):141-50.

225. Schatzkin A, Lanza E, Corle D, et al. Lack of effect of a low-fat, high-fiber diet on the recurrence of colorectal adenomas. Polyp Prevention Trial Study Group. *The New England journal of medicine* 2000;342(16):1149-55.

226. Sillars-Hardebol AH, Carvalho B, van Engeland M, et al. The adenoma hunt in colorectal cancer screening: defining the target. *The Journal of pathology* 2012;226(1):1-6.

227. Vargas AJ, Thompson PA. Diet and nutrient factors in colorectal cancer risk. *Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition* 2012;27(5):613-23.

228. Dixon LB, Subar AF, Peters U, et al. Adherence to the USDA Food Guide, DASH Eating Plan, and Mediterranean dietary pattern reduces risk of colorectal adenoma. *The Journal of nutrition* 2007;137(11):2443-50.

229. Reedy J, Mitrou PN, Krebs-Smith SM, et al. Index-based dietary patterns and risk of colorectal cancer: the NIH-AARP Diet and Health Study. *American journal of epidemiology* 2008;168(1):38-48.

230. Cottet V, Bonithon-Kopp C, Kronborg O, et al. Dietary patterns and the risk of colorectal adenoma recurrence in a European intervention trial. *European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation (ECP)* 2005;14(1):21-9.

231. Dahm CC, Keogh RH, Spencer EA, et al. Dietary fiber and colorectal cancer risk: a nested case-control study using food diaries. *Journal of the National Cancer Institute* 2010;102(9):614-26.

232. Hauret KG, Bostick RM, Matthews CE, et al. Physical activity and reduced risk of incident sporadic colorectal adenomas: observational support for mechanisms involving energy balance and inflammation modulation. *American journal of epidemiology* 2004;159(10):983-92.

233. Aleksandrova K, Nimptsch K, Pischon T. Obesity and colorectal cancer. *Frontiers in bioscience (Elite edition)* 2013;5:61-77.

234. Ho GY, Wang T, Gunter MJ, et al. Adipokines linking obesity with colorectal cancer risk in postmenopausal women. *Cancer research* 2012;72(12):3029-37.

235. Bloomer RJ, Fisher-Wellman KH. Lower postprandial oxidative stress in women compared with men. *Gender medicine* 2010;7(4):340-9.

236. Kim S, Keku TO, Martin C, et al. Circulating levels of inflammatory cytokines and risk of colorectal adenomas. *Cancer research* 2008;68(1):323-8.

237. Mellberg C, Sandberg S, Ryberg M, et al. Long-term effects of a Palaeolithic-type diet in obese postmenopausal women: a 2-year randomized trial. *Eur J Clin Nutr* 2014.

238. Kushi LH, Doyle C, McCullough M, et al. American Cancer Society Guidelines on nutrition and physical activity for cancer prevention: reducing the risk of cancer with healthy food choices and physical activity. *CA: a cancer journal for clinicians* 2012;62(1):30-67.

239. Boucher B, Cotterchio M, Kreiger N, et al. Validity and reliability of the Block98 food-frequency questionnaire in a sample of Canadian women. *Public health nutrition* 2006;9(1):84-93.

240. Johnson BA, Herring AH, Ibrahim JG, et al. Structured measurement error in nutritional epidemiology: applications in the Pregnancy, Infection, and Nutrition (PIN) Study. *Journal of the American Statistical Association* 2007;102(479):856-66.

241. Kaiser KA, Brown AW, Bohan Brown MM, et al. Increased fruit and vegetable intake has no discernible effect on weight loss: a systematic review and meta-analysis. *The American journal of clinical nutrition* 2014.

242. Trichopoulou A, Bamia C, Trichopoulos D. Anatomy of health effects of Mediterranean diet: Greek EPIC prospective cohort study. *BMJ (Clinical research ed)* 2009;338:b2337.

243. de Lorgeril M, Salen P. Dietary prevention of coronary heart disease: the Lyon diet heart study and after. *World review of nutrition and dietetics* 2005;95:103-14.

244. de Lorgeril M, Salen P. The Mediterranean diet in secondary prevention of coronary heart disease. *Clinical and investigative medicine Medecine clinique et experimentale* 2006;29(3):154-8.

245. Ibarrola-Jurado N, Bullo M, Guasch-Ferre M, et al. Cross-sectional assessment of nut consumption and obesity, metabolic syndrome and other cardiometabolic risk factors: the PREDIMED study. *PloS one* 2013;8(2):e57367.

246. Nordmann AJ, Suter-Zimmermann K, Bucher HC, et al. Meta-analysis comparing Mediterranean to low-fat diets for modification of cardiovascular risk factors. *The American journal of medicine* 2011;124(9):841-51.e2.