# The association between dietary factors and risk of rectal cancer in African Americans and Whites

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## ABSTRACT

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in African Americans and Whites
(Under the direction of Jessie Satia, PhD, MPH)

Colorectal cancer (CRC), a commonly diagnosed malignancy in the U.S., refers to cancers of the colon and rectum. African-Americans have the highest incidence and mortality rates for CRC; many reasons for this disparity remain unknown. Diet is involved in the etiology of CRC. There is an abundance of literature on diet and CRC or colon cancer, while evidence is limited on the role of diet in rectal cancer specifically. This dissertation addresses these issues by examining the relationship between dietary factors and rectal cancer risk, and determining if these associations differ between whites and African-Americans.

We used the North Carolina Colon Cancer Study-Phase II, which included 945 rectal cancer cases (including sigmoid and rectosigmoid) and 959 controls. The Diet History Questionnaire was used to assess dietary intake, and we examined the following dietary factors: macronutrients, micronutrients, food groups, and dietary patterns.

For macronutrients, we observed no association between fat intake in whites or African-Americans; only a possible risk reduction in African-Americans with high intake of polyunsaturated fatty acids. In whites, protein (% energy) was associated with lower rectal

cancer risk. In regards to the micronutrients, statistically significant inverse associations were observed in whites for most micronutrients, but only for selenium in African-Americans. Interestingly, micronutrient intake from dietary supplements did not provide additional risk reduction. Regarding food groups, non-whole grains and white potatoes appeared to elevate rectal cancer risk in whites, while fruits, vegetables, dairy, fish, and poultry were inversely related to risk. In African-Americans, high fruit intake was positively associated with risk for rectal cancer. We identified three dietary patterns in whites and African-Americans. The High fat/Meat/Potatoes pattern was similar in both race groups, and associated with elevated risk in whites.

This work adds to the literature on the relationship between diet and rectal cancer, and suggests that these associations differ by race. It also provides information on the epidemiology of rectal cancer in African-Americans, for which evidence is lacking. Rectal cancer is preventable, partially by dietary modifications; therefore, it is necessary to examine the role of diet in the etiology of rectal cancer, especially in large racially diverse samples.

In loving memory of
my father, Herman M. Payne, Sr.,
my grandfather, Henry G. Stoutermire,
and my grandmother, Mary L. Stoutermire

In honor of
my mother, Bernadine S. Payne

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## LIST OF ABBREVIATIONS

BMI: Body mass index

CI: Confidence interval

CMS: Centers for Medicare & Medicaid Services

CRC: Colorectal cancer

DHQ: Diet History Questionnaire

DMV: Department of Motor Vehicles

NCCCS-Phase II: North Carolina Colon Cancer Study-Phase II

NCI: National Cancer Institute

NSAID: Non-steroidal anti-inflammatory drug

OR: Odds ratio

WCRF/AICR: World Cancer Research Fund / American Institute for Cancer Research

## I. Introduction

### A. Background

Colorectal cancer (cancer of the colon and rectum) is the fourth most common cancer among U.S. men and women. It is widely accepted that diet is involved in the etiology of colon and rectal cancer. Numerous mechanisms have been proposed to explain how components of the diet may play a role in colon and rectal cancer development and progression. In fact, diet is one of the strongest environmental risk factors for colon cancer. However, few studies have investigated the relationship between diet and rectal cancer alone. Therefore, the impact of diet on the risk of rectal cancer specifically is less clear. There are known differences between colon and rectal cancer with respect to tumor development and progression, recurrence, and survival, but little attention has been given to differences in dietary risk factors. Examining rectal cancer as a separate entity, and comparing the associated dietary risk factors to those for colon cancer, is essential to determining the extent of the similarities and differences between these two cancers.

There are more cases of colorectal cancer, as well as the highest mortality rate, among African-Americans than any other U.S. race/ethnic group. Reasons for this disparity are largely unknown. There is evidence that dietary intake differs between whites and African Americans; therefore, it is important to identify dietary factors that may contribute to the racial disparity in colon and rectal cancers. Virtually no studies have included an adequate number of African-Americans and examined rectal cancer risk in this particular race/ethnic group.

This work provides information on possible racial differences in dietary intake and risk of developing rectal cancer. To address the goals of this study, we used data from the North Carolina Colon Cancer Study-Phase II. This is a population-based case-control study of rectal cancer (including sigmoid and rectosigmoid), in which African-Americans were over-sampled. We compared mean dietary intakes between African Americans and whites and used multivariate analyses to assess the relationship between numerous dietary factors and rectal cancer risk.

**B. Research aims**

The overall goal of this project was to assess the relationship between diet and the risk of rectal cancer among African-Americans and whites. This research addresses gaps in the literature by providing valuable information on the etiology of rectal cancer as it relates to diet, rectal cancer risk in African Americans, and how dietary intake may contribute to racial differences in risk.

The **specific aims** of this research were to:

1. Determine the association of nutrients (macronutrients: fat, protein; antioxidant micronutrients: vitamin C, vitamin E, beta-carotene, selenium; DNA methylation-related micronutrients: folate, vitamin B6, vitamin B12) with rectal cancer risk in African Americans and whites.

   **Hypothesis**: The macronutrients fat and protein are associated with elevated risk of rectal cancer, while micronutrients are associated with reduced risk; however, the magnitude of these associations differ by race.

2. Determine the association between food groups and rectal cancer risk in African Americans and whites.

   **Hypothesis**: Food groups such as fruits and vegetables lower rectal cancer risk, while food groups such as red meat elevate risk; there are racial differences in the association between food groups and risk of rectal cancer.

3. Determine the association between dietary patterns and risk of rectal cancer risk in African Americans and whites.

   **Hypothesis**: Dietary patterns, and their relationship with rectal cancer risk, differ between African Americans and whites.

## II. Literature Review

### A. Scope of the problem

Colorectal cancer (CRC) is the fourth most common cancer in the US and worldwide and the second leading cause of cancer-related deaths (1). It is estimated that in 2008 there will be 108,070 colon cancer cases and 40,740 rectal cancer cases, and colon and rectal cancers together would result in approximately 49,960 deaths. Here in North Carolina, the expected number of incident colorectal cancer cases and deaths in 2008 are 4,380 and 1,400, respectively (1). In general, rectal cancers account for approximately 30 percent of all colorectal cancers. Colon and rectal cancers arise from a combination of genetic and environmental factors (2). Established CRC risk factors include age, family history of CRC, history of polyps, and inflammatory bowel diseases such as Crohn's disease and ulcerative colitis. Some environmental risk factors include physical inactivity, smoking, obesity, and diet (2). Given that colorectal cancer is potentially one of the most preventable malignancies, it is necessary to identify factors that contribute to their development, especially modifiable risk factors that could aid in prevention, such as diet.

### B. Diet and colorectal cancer

It is accepted that diet is involved in the etiology of CRC and is considered a strong risk factor for colon cancer (3). Epidemiologic studies suggest that CRC is susceptible to modification by dietary factors. For example, Slattery, et al suggested that about 12% of

colon cancers can be attributed to consumption of a Western-style diet, which is characterized as one high in meat, refined grains, and sugar and low intake of vegetables and fiber (4). It has been estimated that more than 70% of colon cancers could be prevented through diet and lifestyle modifications , and that a third of all cancers could be prevented by diet alone (5). The World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) provides dietary recommendations for cancer prevention, based on epidemiological evidence and recommendations for preventing other chronic diseases (3). Some of these recommendations include limiting consumption of energy-dense foods, eating mostly foods of plant origin, limiting intake of red meat and avoiding processed meat, and limiting alcoholic drinks. Numerous mechanisms have been proposed regarding how these dietary factors may contribute to the development of colorectal cancer.

1. Macronutrients

Fat, carbohydrate, and protein are considered macronutrients because they are relatively large molecules (6). In general, the evidence is not consistent regarding the association between fat and carbohydrate intake and CRC risk, most likely due to the many sub-components of these two macronutrients. Although there are different types of fats (e.g. saturated and unsaturated), overall animal fat intake is associated with increased risk of CRC (3). Animal models have helped elucidate the effect of fat on colon cancer, suggesting that specific types of fat may be important determinants of risk (7). The most compelling explanation is that dietary fat increases production of bile acids which promote tumorigenesis and proliferation, and that free fatty acid damages the intestinal epithelium (8). The effect of carbohydrates is often examined separately as fiber and non-fiber (i.e. effective

carbohydrate) components. Refined carbohydrates can lead to increased intestinal absorption of glucose into the blood, resulting in hyperinsulinemia. This in turn affects insulin-like growth factors that promote proliferation of colorectal cancer (9). Although there has been conflicting evidence regarding the association between fiber intake and risk of CRC, it is likely that fiber could reduce risk by diluting fecal content of dietary components, decreasing transit time of feces through the bowel, increasing stool weight, lowering intestinal pH, and producing short chain fatty acids that can induce apoptosis and cell cycle arrest (10). There is no available epidemiological evidence that overall protein intake increases CRC risk, but it has been proposed that protein degradation results in amino acids that breakdown further into ammonia, which can be carcinogenic (11). Other potentially toxic components of protein degradation include phenolic compounds, amines, and N-nitroso compounds. However, this does not seem to be a well-accepted mechanism. Studies usually focus on meat, which is high in protein, as opposed to overall protein intake.

2. Micronutrients

The micronutrients are vitamins and minerals, and are required in much smaller quantities than macronutrients. There are a number of mechanisms to explain the potential role of micronutrients in CRC development, all of which suggest that micronutrients reduce the risk of CRC. The antioxidant properties of many of these micronutrients (e.g. vitamin C, vitamin E, selenium, and carotenoids such as beta-carotene) are credited for their role in risk reduction (12-14). Antioxidants scavenge free radicals and reactive oxygen molecules, protecting cells against oxidation damage, and vitamins C and E also protect against lipid peroxidation (15). Abberrant DNA methylation patterns are commonly seen in colorectal

tumors (16); therefore, another potentially protective mechanism relates to nutrients involved in modulating DNA synthesis, repair, and methylation (13, 17). These nutrients include folate, vitamin B6, vitamin B12, and methionine. It has been suggested that calcium may reduce CRC risk by inducing apoptosis and binding bile and free fatty acids (12, 14), whereas both calcium and vitamin D may reduce epithelial cell proliferation (18). It is important to note that many of these micronutrients have multiple biological properties that can impact colon and rectal cancer development. For example, carotenoids are also known to effect cell growth regulation, modulate gene expression, and possibly enhance the immune response, thereby preventing CRC development (19). Nutrients such as lycopene and selenium also have anti-inflammatory properties that help reduce risk of inflammatory illnesses such as inflammatory bowel disease (e.g. ulcerative colitis), which is a predisposing risk factor for CRC.

The associations of micronutrients with colon and CRC cancer risk have been extensively studied in large epidemiological studies such as the Nurses Health Study and Health Professionals Follow-Up Study (17, 20), and the Cancer Prevention Study II (21). Most evidence has confirmed the hypotheses of risk reduction due to adequate micronutrient intake. Results from clinical trials, however, question the proposed protective effect of micronutrients. For example, the Aspirin/Folate Polyp Prevention Study did not demonstrate a protective effect of folate on colorectal adenomas, while the Women's Antioxidant Cardiovascular Study found no effect of vitamins C and E and beta-carotene on CRC risk (22).

3. Food groups

The United States Department of Agriculture (USDA) provides dietary recommendations in regards to the types and amounts of food to consume daily. The five primary food groups according to the food guide pyramid are grains, fruit, vegetables, milk (formerly referred to as dairy), and meat (23). The grains food group consists of whole grains (e.g whole wheat bread, brown rice, oatmeal) and refined grains (e.g. white bread, white rice, most cereals). Consumption of whole grain foods are hypothesized to lower cancer risk due to their high content of antioxidants, fiber, and certain phytochemicals (24). Dairy products are a diverse food group that consists of factors that may increase CRC risk (e.g. high-saturated fat) (25), as well as high calcium and vitamin D that may reduce risk (26). It is suggested that low-fat dairy products, in general, have beneficial effects (27).

Of all associations of diet and colorectal cancer, fruit and vegetable intake and meat consumption are the most commonly investigated food groups. Fruits and vegetables may protect against colorectal cancer risk through their anti-carcinogenic components such as antioxidants, folate, flavonoids, organosulfides, isothiocyanates (28). Some of these nutrients that deactivate carcinogens may also act to prevent chromosomal instability, which is considered a precursor to colorectal tumor development (29). Fiber from fruits and vegetables may decrease transit time, lower pH, and produce potentially anti-carcinogenic short-chain fatty acids (28). However, studies on fruit and vegetable consumption and colorectal cancer have been inconsistent, with a recent pooled analysis of 14 cohort studies showing no protective effect (29).

Several large prospective studies (30, 31), as well as meta-analyses of cohort and case-control studies (32, 33) have shown increased consumption of red and processed meat to

correlate with elevated colorectal cancer risk, yet a protective effect of fish and poultry. Several hypotheses have been developed to explain these relationships. One hypothesis is that the fat component of red meat increases bile acid excretion and this product can function in tumor development and cell proliferation in the colonic mucosa (34). Other hypotheses relate to the production of N-nitroso compounds found in processed meat that can induce the formation of DNA adducts in cells in the colon, as well as the potentially carcinogenic heterocyclic amines and polycyclic aromatic hydrocarbons produced by cooking meats at high temperatures. The omega-3 fatty acid content in fish is thought to inhibit tumor growth and reduce the production of eicosanoids that cause inflammation (35).

4. Dietary Patterns

Most research concerning diet and colorectal cancer has focused on individual nutrients and foods, as previously illustrated. Few published studies have examined dietary patterns which take into account the synergistic effect of foods and nutrients, since foods and nutrients are not consumed in isolation. These eating patterns also reflect genetic, cultural, environmental, social, health, and economic influences of eating behavior (36). Dietary patterns may be useful in understanding disease etiology when there is conflicting evidence for diet associations; such is the case for some nutrients and colorectal cancer. A typical "Western" dietary pattern is energy dense and consists of high intakes of meat, refined grains, potatoes, and sugar-containing foods, and less fruits and vegetables. This eating pattern has emerged most frequently from analyses of dietary patterns and colorectal cancer risk. Some studies have shown the "Western" dietary pattern to be positively associated with colorectal cancer (37-39), while others showed inconclusive results regarding eating patterns

and CRC (40, 41). Other commonly observed patterns include a "vegetable", "prudent", and the "healthy" pattern, all of which are inversely related to CRC risk (37, 39, 41). These dietary patterns generally consist of high intakes of fruits and vegetables, fish and poultry, whole-grain products, and low-fat dairy products. In general, dietary patterns may be more easily translated into dietary recommendations for colorectal cancer prevention.

**C. Rationale for examining diet and *rectal cancer***

There has been debate whether colon and rectal cancers should be considered a single entity. These two tumor types are usually combined in diagnoses, as well as epidemiological studies, because they have the same precancerous lesion (i.e. polyp), similar mode of spread, some shared etiology, and of course, anatomical proximity (42). Furthermore, these two carcinomas are often grouped together because the boundary between the colon and rectum is not always clearly delineated (43), making it difficult to determine the exact location of the tumor. However, advances in the diagnosis and staging for rectal cancer (44), and the increasing variety of treatment options have helped to distinguish between tumor development processes for colon and rectal cancers (45). The main drawback of considering colon and rectal cancers as a single entity is that possible differences in epidemiological characteristics are not identifiable. One study pointed out that there is less geographical variation of rectal cancer, as opposed to colon cancer, and that rectal cancers may be less susceptible to environmental influences than colon cancer (46). Furthermore, there are differences between colon and rectal cancer that warrant examining the two carcinomas

separately, and comparing their epidemiological risk factors. There are several reasons why dietary risk factors, in particular, may differentially affect the colon and rectum.

1. Molecular differences

Colon and rectal cancers develop from a combination of genetic and environmental factors. Commonly mutated genes involved in the carcinogenic process include adenomatous polyposis coli (47), Kirsten-ras (k-ras), and p53 (12). The APC and p53 genes are tumor suppressor genes whereas k-ras is an oncogene. Approximately 80% of sporadic colorectal tumors have mutations in the APC gene, 30-50% of colorectal adenomas and carcinomas have k-ras mutations, and the p53 gene is mutated in up to 70% of colorectal cancers (48). Of the few studies that have examined biological differences, it has been suggested that there are different mechanisms of oncogenesis for colon and rectal carcinomas (49). There is evidence that k-ras and APC mutations are more common in colon tumors than rectal tumors (42, 48). On the other hand, mutations in the p53 gene are more frequent in rectal tumors (48, 49). It is suggested that the different bacterial flora in the rectum may alter the contact between potential carcinogens and lead to increased mutations of p53 (49). Regardless of the specific genes involved, the number of mutation in colon tumors is significantly higher than those in rectal tumors (42). Components of the diet may interact with these commonly mutated genes in the process of colon and rectal cancer development and progression.

2. Differences in risk factors for colon and rectal cancer

Statistical analyses with the outcome of colorectal cancer ignore the possibility of heterogeneity between colon and rectal cancer. Due to the different mechanisms by which

colon and rectal carcinomas may develop and other notable distinctions, it may be inappropriate to assume homogeneity, and established risk factors may for colon cancer may not apply (or be relevant to) rectal cancer. Some studies have made the attempt to distinguish between predictors for colon versus rectal cancer. It has been shown that obesity, for example, is a significant predictor of colon cancer, but not rectal cancer (50-52). Likewise studies have consistently shown physical activity to be associated with reduced risk for colon cancer, but no protective effect against rectal cancer has been found to date (53). A possible explanation for this is that insulin sensitivity improves with more physical activity, and the colon is more susceptible to insulin's effects (54). Wei et.al observed that family history of colorectal cancer correlated with a stronger risk for colon cancer than rectal cancer, while smoking was more strongly associated with rectal cancer (55). Based on the different methods by which colon and rectal tumors arise, as well as the observed differences in non-dietary risk factors, it is hypothesized that dietary factors may also differentially affect risk of colon and rectal cancers.

3. Hypotheses for differential effects of diet on colon vs. rectum

It is proposed that dietary factors may differentially affect risk of colon and rectal cancer for several reasons. In addition to the fact that the colon and rectum arise from different embryonic tissue as well as have different molecular aspects of tumor development, they also serve different functions. The colon functions to absorb water and minerals from food and transport them into the bloodstream while the rectum serves as a collection site and stores fecal matter until it is eliminated from the body. The presence of different mutations may result in different diet-gene interactions in colon and rectal cancer development.

Because the rectum is usually empty until wastes are ready to be eliminated from the body, there may be a shorter duration of exposure to potentially carcinogenic dietary components. The different pH levels (55) and bacterial composition (56) of the colon and rectum may also affect their susceptibility to environmental factors such as diet.

**D. Racial differences**

In addition to differences between the colon and rectum, there are racial differences in dietary behaviors and colorectal cancer outcomes.

1. Colorectal cancer outcomes

Colorectal cancer incidence and mortality differ appreciably by race, for many reasons which remain unknown. Specifically, African-Americans have the highest rates of colorectal cancer among all US racial/ethnic groups (1). However, when examining the statistics for colon and rectal cancer separately, it is obvious that the disparity is much greater for colon than rectal cancer. Between 1999 and 2004, colon cancer incidence rates for African-Americans and Whites were 44.3 and 35.1 per 100,000 persons, respectively; corresponding rates for rectal cancer were 13.1 and 13.3. Colon cancer mortality rates for African-Americans and Whites were 21.5 and 14.6, respectively, while corresponding rates for rectal cancer were 3.3 and 2.9 (57). So for rectal carcinomas specifically, incidence is higher among Whites while mortality is greater among African-Americans. African-Americans were also less likely to have localized disease and had increased rates of proximal colon carcinoma (58), and more likely to be diagnosed with advanced stage colorectal cancer

(i.e. Stage III and Stage IV). Some of the overall disparity can be explained by genetic, socioeconomic, and healthcare access differences (59); however, lifestyle factors such as diet may also play a role.

2. Dietary behaviors

Studies have indicated that dietary behaviors differ widely by race and ethnicity (60). Whites have been shown to consume more fruits and dairy products than African-Americans (61), and use lower-fat alternatives in their food preparation (62). African-American women were shown to have significantly lower intakes of vitamin D, vitamin E, folate, and vitamin B-6 compared to White women, mostly due to the increased supplement use among Whites (63). According to data from the National Health and Nutrition Examination Surveys, African-American men and women reported lower intakes of vegetables, potassium, and calcium than their White counterparts (64). Similarly, African-American children were at increased risk of vitamin A, vitamin E, calcium, iron and zinc deficiency based on the UDSA Continuing Survey of Food Intakes by Individuals (65). In their assessment of dietary intake trends among African-Americans and Whites, Kant, et al suggests that previous dietary risk factors for African-Americans have not improved (64).

3. Influence of diet on cancer risk

Furthermore, these dietary behaviors have also been observed to differentially affect risk of disease among these two race subgroups. For example, increased consumption of high animal-fat foods was related to prostate cancer among African-Americans, but not Whites (66). African-American men with indicators of poor vitamin D status had an

appreciably higher risk of cancer incidence and mortality, especially for cancers of the digestive system (67). Also, increased alcohol consumption and tobacco use correlated with a 17-fold risk increase of oral cancer among African-Americans compared to a 9-fold increase among Whites (68). Other findings indicate that dietary intake differences may contribute to higher mortality rates of breast cancer among African-American women (69). Preliminary studies by Satia, et al showed that nutrient intake and associations with colon cancer differed by race (70-72). Given that dietary factors contribute to differences in risk among African-Americans and Whites for other cancer types, such differences may also exist for rectal cancer. It is also important to note that genetic polymorphisms may also vary by race and contribute to differences in disease risk. Therefore, when possible, diet-gene interactions should be assessed. However, the genetic contribution to colon and rectal cancer risk is outside the scope of this research.

**E. Limitations of current studies**

While there is an abundance of evidence for the role of diet on risk of colon cancer and colorectal cancer, much fewer studies have examined the relationship between diet and rectal cancer. Even the comprehensive review done by the World Cancer Research Fund/American Institute for Cancer Research stated that they had less evidence on risk factors for rectal cancer (3). Of the studies that have attempted to examine epidemiologic risk factors by sub-sites of the colorectum, they often had very few rectal cancer cases, thereby limiting the statistical power to detect significant associations. This study will help fill this gap in the literature by providing evidence for dietary risk factors for rectal cancer specifically in a large sample of rectal cancer cases and controls. Furthermore, it will

provide information on rectal cancer in African Americans, for which the available literature is lacking, and how diet may contribute to racial differences in risk of rectal cancer.

**F. Summary and significance**

Colon and rectal cancers are preventable, partially by dietary modifications. To aid in the prevention of these cancers and elimination of the racial disparity, it is necessary to determine environmental and genetic factors that contribute to elevated risk for both tumor types in racially/ethnically diverse study samples. Because there are few published epidemiological studies that solely focus on rectal cancer and associations with diet, and none that include adequate representation of African-Americans, this study aims to determine dietary factors associated with risk of rectal cancer and assess racial differences. Currently, these relationships have not been examined in a racially heterogeneous population. This project contributes appreciably to the knowledge of the etiology of rectal cancer, especially in African-Americans, and provides possible explanations for racial disparities.

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## III. Study Design and Methods

### A. Overview

This proposed study seeks to 1) determine the association between nutrients and risk of rectal cancer in whites and African Americans, 2) determine the association between food groups and risk of rectal cancer in whites and African Americans, and 3) determine the association between dietary patterns and risk of rectal cancer in whites and African Americans. These aims were accomplished using data from the North Carolina Colon Cancer Study-Phase II (NCCCS- Phase II). This population-based case-control study of rectal cancer (including sigmoid and rectosigmoid) was conducted between May 2001 and September 2006. One of the overall goals of the NCCCS-Phase II was to identify environmental and lifestyle risk factors for rectal cancer in African-Americans and whites; therefore the specific aims of this proposed research fits well into this broad objective.

### B. Study area and population

*Study area*

The study area consists of 33 contiguous counties in the central and eastern portion of North Carolina. These counties represent the major urban areas of the state, as well as large

segments of rural areas. African-Americans make up about one-third of the population of these counties, and this area provides a good socioeconomic mix of African-Americans and Whites in order to make comparisons. All 59 hospitals in the 33-county study area participated. Border counties were intentionally excluded to limit the referral of patients to non-participating hospitals.

*Cases*

Cases were identified using the rapid ascertainment system of the North Carolina Central Cancer Registry (CCR). This system required hospitals in the 33 counties to forward pathology reports and identifying data for newly diagnosed cases to the North Carolina CCR staff in Raleigh, NC within one month of a case's diagnosis. The CCR staff performed initial screening checks to determine if the case met the study eligibility requirements. Eligible cases were forwarded to the NCCCS-Phase II study staff weekly. Case eligibility criteria included: 40-79 years of age (inclusive) at time of diagnosis, resided in one of the 33 target counties, African-American or White, and had a North Carolina driver's license or identification card if under 65 (because controls under 65 were selected from Department of Motor Vehicle rosters). They also had to be proficient in English and able to complete an interview. Cases had a diagnosis of rectal adenocarcinoma (including cancers of the sigmoid and rectosigmoid junction to increase the number of available subjects) between May 1, 2001 and September 30, 2006. All diagnoses were confirmed by the study pathologist using pathology slides and medical records. Cases were excluded if they had diagnoses of non-invasive carcinoma, a previous diagnosis of colorectal cancer, or were deceased at time of

identification. Permission was obtained from each case's primary physician before contacting them about study participation.

*Controls*

Controls were selected from two sources: 1) North Carolina Division of Motor Vehicles (DMV) records for those under the age of 65; 2) Center for Medicaid and Medicare Services (CMS, formerly known as the Health Care Financing Administration) for those 65 and older. The DMV and CMS have the most comprehensive databases of NC residents between the ages of 40 and 79. The DMV and CMS can provide a list of NC residents with a license or ID card and a list of Medicare recipients, respectively, to health researchers for studies that are likely to benefit citizens of NC. All controls were 40-79 years of age, resided within the 33 target counties, had no previous diagnosis of colorectal cancer, African-American or White, alive at time of selection and interview, and proficient in English and able to complete the interview. The study team received lists of recruitable controls from the DMV and CMS, containing age, sex, ethnicity, as well as their contact information. Only a random sub-sample of eligible controls was randomized to recruitment. Control sampling and randomization to recruitment was done at the beginning of the study and at the midpoint of data collection, using updated lists.

**C. Sampling and recruitment**

A randomized process was used to determine which cases and controls to contact regarding participation. This randomized recruitment strategy was used to control for potential confounding by race, age, and sex, and to achieve a race ratio sufficient for statistical efficiency to assess interaction by race (1). Cases were sampled to yield a 3:1 ratio

of Whites to African-Americans. One particular benefit of randomized recruitment is that the main effect of the design variables (race, sex, age) can be estimated using maximum likelihood estimation in logistic models. A main goal of the NCCCS-Phase II is to assess whether colon/rectal cancer risk indicators differ by race; therefore African-American cases and controls were over-sampled since the African-American population in North Carolina is only about 20%.

To implement randomized recruitment, estimates of the relative risks for the matched design variables were used to derive recruitment probabilities, which in turn affect the case and control distributions. All eligible cases and control subjects were assigned a random number between 0 and 1, and this number was compared to the recruitment probability. If the random number was less than or equal to the recruitment probability, then the potential participant was recruited to participate in the study. As previously mentioned, a unique feature of this study was that African-American cases and controls were over-sampled. The recruitment probability for African-American cases was 1.0 (i.e. all were recruited) and African-American controls had higher recruitment probabilities than White controls. The overall population distribution of cases and controls is shown in Table 1.

Table 1: Population distribution of the NCCCS-Phase II

| | Study sample | |
|---|---|---|
| | Cases | Controls |
| Sampled | 1831 | 2345 |
| Eligible | 1417 | 1827 |
| Interviewed | 1057 | 1019 |
| Analyzed | 945 | 959 |
| Response rate* | 74% | 56% |

*response rate=number interviewed/number eligible

**D. Data collection**

Data were collected by trained nurse-interviewers during in-person interviews using two main questionnaires: the participant questionnaire and the diet questionnaire. Interviews were conducted in participants' homes or another convenient location, such as the local hospital or health department.

1. Exposure assessment

Diet is the exposure of interest in this proposed research study. The NCCCS-Phase II used a food frequency questionnaire (FFQ) that assessed portion size and frequency of consumption. Food frequency questionnaires are thought be a practical tool for collecting dietary data in large epidemiological studies (2). Compared to other methods such as the 24 hour recall, the FFQ collects less detail regarding the foods consumed, cooking methods, and portion size. However, the FFQ is designed to assess usual dietary intake and the nutrient values obtained can be used to rank individuals based on their nutrient intakes (3). Therefore, it was a suitable dietary assessment tool for this study.

The FFQ used in the NCCCS-Phase II was the Diet History Questionnaire (DHQ). The DHQ was developed by the National Cancer Institute and its validity was assessed in racially diverse samples. The race/ethnicity distribution in the validation study by Thompson, et al was 79% White, 10% African American, 5% Latino, and 5% Other (4); the validation study done by Subar, et al. was 76% White, 14% African American, 4% Latino, and 6% Other (2). The DHQ is a cognitively-based FFQ and an extension of the NCI Block FFQ. It has been shown that the cognitive improvements in the DHQ provide better measures of frequency than the Block FFQ (4), and have similar or higher correlations compared to the

Block FFQ (2). The DHQ consists of 124 separate food items and assesses the frequency of consumption and portion size consumed for each food item. In addition, it contains ten questions about the frequency and dose of dietary supplements used.

The questionnaire was administered by a nurse-interviewer and referred to participants' food and beverage intake in the past 12 months to take into account seasonal variation in food consumption. Cases were asked to estimate their usual intake 1 year prior to diagnosis, and controls were asked to estimate consumption 1 year prior to interview. Food and nutrient estimates were determined using the NCI's Diet*calc analysis program which estimates intake based on reported frequency and serving size of each food item.

2. Covariate assessment

The participant questionnaire collected data on many characteristics, and the following covariates were used in this study:

*Demographic characteristics*

Demographic information that was collected included age, sex, race, and education (less than high School, high School or GED equivalent, some college, college or advanced degree), and annual household income.

*Lifestyle factors*

Detailed information was collected regarding cigarette, cigar, and pipe smoking because smoking has been shown to increase risk for colorectal cancer (5). Smoking status was categorized as never smoker, former smoker, or current smoker. Information on subjects' physical activity level (very hard, hard, moderate, light, sleeping/relaxing) for occupational and non-occupational activities was collected, as well as the amount of time

spent during typical work and non-work days in different activities during the one-year referent period. Metabolic equivalent task minutes per day for each level of physical activity was categorized into quartiles and used to assess confounding by physical activity.

*Non-steroidal anti-inflammatory drug (NSAID) use*

NSAID use was assessed because the literature supports a protective effect of regular use (3-4 times a week) of NSAIDs such as aspirin (6). The questionnaire asked about prescription and non-prescription NSAIDs, including the frequency, duration, and use during the past five years. NSAID use was categorized as regular (more than 15 times a month) and non-regular users (15 or less times a month).

*Family history of CRC*

Participants indicated if there was a family history of colorectal cancer (yes/no). The questionnaire asked for information on the vital status, current age or age at death, and history of cancer for all first-degree relatives. For those with a history of cancer, information was collected regarding the site and age at diagnosis.

*Anthropometric measurements*

Study participants' height and weight were measured by the interviewer to calculate their BMI (weight (kg)/height ($m^2$)). Their waist and hip circumference were also measured. Participants were asked to recall their weight 1 year and 5 years ago. Data from the NCCCS show that many cases lost weight, possibly as a consequence of their illness; therefore, BMI 1 year ago was assessed as a potential confounder. BMI was categorized as normal weight (18-24.9kg/$m^2$), overweight (25-29.9 kg/$m^2$) or obese ($\geq$30 kg/$m^2$) (7).

**E. Statistical analyses**

All analyses were performed using SAS software (8). Descriptive statistics (means, standard deviations, and frequencies) were computed for all study variables by case-control status and race. A 5% significance level was used for all statistical tests, and 95% confidence intervals were constructed. Table 2 shows all variables considered in this study.

Table 2: Study variables

| **Dietary exposures** | **Outcome** | **Covariates** |
|---|---|---|
| Macronutrients<br>Fat (saturated, monounsaturated, polyunsaturated)<br>Protein<br><br>Micronutrients<br>Antioxidant nutrients<br>Vitamin C, Vitamin E, beta-carotene, selenium<br>DNA methylation-related nutrients<br>Folate, Vitamin B6, Vitamin B12<br><br>Food groups<br>Grains, Dairy, Fruits, Vegetables, Meat, Other<br><br>Dietary patterns | Rectal cancer<br><br>(including sigmoid and rectosigmoid cancers) | Age<br>Sex<br>Race<br>Education<br>Income<br>Smoking status<br>Physical activity<br>BMI<br>NSAID use<br>Family history of CRC |

1. **Aim 1:** Determine the association of nutrients (*macronutrients* (fat, protein), antioxidant *micronutrients* (vitamin C, vitamin E, beta-carotene, selenium), and DNA methylation-related *micronutrients* (folate, vitamin B6, vitamin B12)) with rectal cancer risk in African Americans and whites.

a) Macronutrients

The goal of this sub-aim was to estimate the risk of rectal cancer associated with fat (total, saturated, monounsaturated, and polyunsaturated) and protein, as well as the percent of energy from fat and protein. Descriptive statistics included means and standard deviations of

intake for each macronutrient among cases and controls. The Wilcoxon rank sum procedure was used to determine if mean intake differed between cases and controls.

Logistic regression analyses were used to examine the relationship between macronutrients and rectal cancer risk. We categorized macronutrient intake into quartiles to reduce the impact of extreme values. Cut-points for quartiles were based on the distribution among race-specific controls. Odds ratios were estimated by comparing each quartile to the lowest. A test for linear trend was conducted by incorporating a variable containing the median levels observed for each quartile of macronutrient intake into a logistic regression model, and using the resulting p value to determine the presence/absence of a significant trend. The tests for linear trend models were weighted by the inverse of the variance to account for the variance within each quartile. Each macronutrient was analyzed in separate models, and all analyses were adjusted for potential confounders (see III.E.4b).

b) Micronutrients

The goal of this sub-aim was to estimate the risk of rectal cancer associated with two categories of micronutrients based on their proposed mechanism of effect: antioxidant micronutrients (vitamin C, vitamin E, beta-carotene, selenium) and DNA methylation-related micronutrients (folate, vitamin B6, and vitamin B12). Descriptive statistics included means and standard deviations of intake for each micronutrient among cases and controls. The Wilcoxon rank sum test was used to determine if mean intake differed between cases and controls. To assess racial differences in micronutrient intake, this test was also used to compare mean micronutrient intakes between White and African American controls.

Logistic regression analyses were used to determine the association of these micronutrients with rectal cancer risk. The micronutrients were categorized into quartiles to

reduce the impact of extreme values. Cut-points for quartiles of micronutrient intakes were based on the distribution among race-specific controls. Odds ratios were estimated by comparing each quartile to the lowest. A test for trend was conducted by incorporating a continuous variable representing the median levels observed for each quartile of micronutrient intake into a logistic regression model, and using the resulting p value to determine the presence/absence of a significant trend. These trend test models were weighted by the inverse of the quartile variances. The micronutrients were first examined in separate logistic regression models. We then examined the effect of each micronutrient while adjusting for the other micronutrients in the same category to simulate the combined (and highly correlated) effect of these nutrients in food. All analyses will be adjusted for potential confounders (see III.E.4b). We performed these analyses with and without adjustment for fruits and vegetables, which are the primary food sources of these nutrients.

Since a large proportion of micronutrient intake comes from dietary supplements (9), these analyses were done for micronutrient intake from foods only, as well as total micronutrient intake from the combination of foods and supplements. The Diet History Questionnaire included an additional ten questions on the frequency and dose of single vitamin/mineral supplement use as well as the use of multivitamins/minerals.

2. **Aim 2:** Determine the association between food groups and rectal cancer risk in African Americans and whites

The goal of this aim was to estimate the risk of rectal cancer associated with food groups defined by the United States Department of Agriculture (USDA). The five main

USDA pyramid food groups are grains, fruits, vegetables, dairy, and meat. We examined total intake of these food groups and numerous sub-groups; therefore, our analysis was based on 29 food groups. Descriptive statistics included means and standard deviations of intake for each food group among cases and controls. The t-test procedure was used to determine if mean intake differs between cases and controls.

Logistic regression analyses were used to obtain odds ratios and 95% confidence intervals for the relationship between each food group and rectal cancer risk. These food groups were categorized into quartiles and the cut-points for these quartiles were based on the distribution among race-specific controls. Odds ratios were estimated by comparing each quartile to the lowest. A test for linear trend was conducted by incorporating a variable containing the median levels observed for each quartile of intake into a logistic regression model, and using the resulting p value to determine the presence/absence of a significant trend. These logistic regression models for the trend test were weighted by the inverse of the variance for the quartiles. Each food group was analyzed in separate models, and all analyses were adjusted for potential confounders (see III.E.4b).

3. **Aim 3:** Determine the association between dietary patterns and risk of rectal cancer risk in African Americans and whites

The goal of this aim was to create dietary patterns using control subjects who, we assume, represent the general population, and estimate the risk of rectal cancer associated with these dietary patterns. We created the dietary patterns separately for whites and African Americans to account for possible racial differences in consumption and correlations between food groups. The dietary patterns were based on the same food groups as in Aim 2,

with some exclusions. The food group totals (i.e. total grains, total fruit, etc.) were excluded so the dietary patterns would be based on mutually exclusive food groups. The organ meat and soy products were excluded and yogurt was combined with the milk group because there was a large percentage of non-consumers of organ meat, soy, and yogurt. Alcohol was also excluded because it may be a part of an overall behavior pattern and not just a dietary pattern.

There are several methods used to determine dietary patterns. One of the data-driven methods is factor analysis (10). Factor analysis reduces data into 'factors' based on correlations between foods and assigns factor scores for each factor. Most studies using factor analysis have used the principal components analysis (PCA) method, which is used when variables are highly correlated, as is the case for most dietary data. PCA reduces the number of observed variables to a smaller number of principal components that account for most of the variance of the observed variables. For this aim, we used the PCA method to identify dietary patterns among race-specific controls. We retained factors with eigenvalues >1.0, which indicate that the factor describes more of the variability in the data than a single variable (11). We also assessed the scree plot and interpretability of the factors. Based on these criteria, we extracted the factors to be used in subsequent analyses. Factors were rotated using a varimax (orthogonal) rotation to obtain a more easily interpretable solution and uncorrelated factors. The factor loading matrix illustrates how each food group correlated with each factor. These factors represent the dietary patterns in the study sample. Once the dietary patterns were determined by PCA and labeled based on foods with high factor loadings, factor scores were obtained and applied to each observation, then used as the exposure variables in logistic regression models.

The relationship between factor scores and other dietary and lifestyle variables were analyzed using Pearson and Spearman correlations for continuous and categorical variables, respectively. Partial Pearson correlations adjusted for total energy were obtained for all dietary variables. Odds ratios and 95% confidence intervals for risk of rectal cancer for each dietary pattern was estimated using logistic regression analyses. The linear trend test was done by incorporating the median factor scores for each quartile as a continuous variable in models, which were weighted by the inverse of the variance. All analyses were adjusted for potential confounders (see III.E.4b).

4. Common statistical methods for all aims

There are several common statistical methods that were applied to all previous analyses.

a) Effect modification

Effect measure modification by race was assessed in all analyses. This was done using two methods. One method involved using the Breslow-Day test for homogeneity to test the homogeneity of binary covariates across the two race categories. The p value associated with the Breslow-Day statistic tests the null hypothesis that the covariates are constant across race strata. The other method was the likelihood ratio test to compare logistic regression models with and without interaction terms. The likelihood ratio test helped determine whether the full model including the interaction terms, which allowed the model to depart from constancy, maximized the likelihood of the observed data better than the reduced model, which contained no interaction terms. If these tests supported the constancy assumption, we still presented the results stratified by race. Because data are lacking

regarding the epidemiology of rectal cancer in African-Americans, it is valuable to present the data separately by race and fill this gap in the literature.

There are different incidence and mortality rates for colon and rectal cancer for males and females. In addition, the literature suggests that sex modifies the effect of risk factors for colorectal cancer (12); therefore we assessed effect measure modification by sex.

b) Confounding

To assess confounding, we conducted bivariate analyses of each potential confounder in a logistic regression model and covariate inclusion was based on a 10 percent or greater alteration in the parameter coefficient of continuous dietary variables. All covariates that met this criterion were simultaneously included in a model, and a backwards stepwise procedure was done to obtain the final model. This method is most useful when there are a large number of predictors, as was the case in these analyses. The potential confounding factors included age, sex, education, income, prior BMI, smoking status, physical activity, family history of CRC, and NSAID use.

All analyses were adjusted for total energy and other dietary variables where appropriate. Absolute nutrient intake is a function of total energy intake and the composition of the diet (3). Therefore, most nutrients, especially macronutrients, are highly correlated with total energy intake. It is necessary to adjust for total energy in diet-disease associations in order to distinguish the effects of a particular nutrient from that of total energy intake, which is mainly determined by body size and physical activity(13).

c) Offset term

All statistical analyses included an offset term to account for the sampling probabilities. This resulted in unbiased odds ratios and allowed estimation of the main effects for matching variables (14). The offset term used in the analyses was based on the original age-sex-race strata from the rosters used to ascertain cases and controls. It was necessary to include this term in all analyses because recruitment was conditioned on age, sex, and race, in addition to disease status; thus the odds ratios without the offset term would have been biased compared with a traditional design in which recruitment was conditioned on disease status alone.

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**IV.** Antioxidant and DNA methylation-related micronutrients and risk of rectal cancer

## A. Abstract

Objective: To investigate the relationship between antioxidant nutrients (vitamins C and E, β-carotene, selenium) and DNA methylation-related nutrients (folate, vitamins B6 and B12) and rectal cancer risk in whites and African Americans, and to examine intakes from food only versus total (food plus dietary supplements) intakes. Methods: Data are from the North Carolina Colon Cancer Study-Phase II, a case-control study of 945 rectal cancer (including sigmoid and rectosigmoid junction) cases and 959 controls. In-person interviews captured usual dietary intake (using the Diet History Questionnaire) and various covariates. Multivariate logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (95% CI). Results: High intakes of all antioxidant nutrients were associated with reduced risk in whites, except total vitamin E intake. Only selenium had a statistically significant inverse association in African Americans (OR: 0.25, 95% CI 0.08-0.84). All DNA methylation-related nutrients had independent inverse associations with rectal cancer risk in whites; there were no statistically significant associations in African Americans. Supplements did not provide additional risk reduction beyond intakes from food. Conclusions: Our findings provide evidence that micronutrients may lower the risk of rectal cancer, and that optimal micronutrient intakes from food alone may be more beneficial than supplementation.

## B. Introduction

Colorectal cancer (CRC) is the third most common cancer in the U.S. (1) and results from a combination of genetic, epigenetic, and environmental factors (2, 3). Diet is widely believed to play an important role in the development of CRC, and a number of dietary micronutrients have been associated with CRC risk (4-7). However, results in epidemiologic studies are often conflicting. Some observational studies have reported CRC risk reductions associated with micronutrient intake (4, 7), while clinical trials found null or positive associations (8, 9). Therefore, an exploration of these associations driven by knowledge of mechanisms of action can be very informative. Several biological mechanisms provide a theoretical link between micronutrients and reduced risk of CRC (10, 11) .

Oxidative stress plays a major role in CRC development and progression (12, 13), and results from an excess production of free radicals or insufficient antioxidant defenses (14, 15). Free radicals are unstable, highly reactive, oxygen-containing molecules that can cause tissue damage. Therefore, the balance between free radicals and antioxidants is critical. Numerous dietary nutrients, such as vitamin C, vitamin E, carotenoids, and selenium, have antioxidant properties (11, 16). Antioxidant nutrients protect against the damaging effects of free radicals, thereby reducing oxidative stress and ultimately preventing CRC.

Another well-known process involved in colorectal carcinogenesis is DNA hypomethylation, which is consistently observed in colon neoplasms (3, 17). Hypomethylation is a result of low levels of S-adenosyl methionine (SAM), and the production of SAM depends on dietary factors such as folate, vitamin B6, and vitamin B12. The main role of folate is to provide one-carbon units in several reactions necessary for DNA methylation and synthesis, while vitamins B12 and B6 serve as cofactors in some of these

reactions (18). Therefore, sustained low levels of these nutrients can lead to disturbances in DNA methylation, synthesis, and repair, thus influencing colorectal carcinogenesis.

Despite the biological and mechanistic rationale for the hypothesis that these nutrients could reduce the risk of CRC development, epidemiological studies have yielded inconsistent results. This may in part be due to different methods of diet assessment, or that few studies had complete data on both dietary and supplemental sources of these nutrients. It is particularly important to include intakes from vitamin and mineral supplements, as they contribute appreciably to micronutrient intakes (19). Compared to colon cancer, evidence is limited regarding the association between these micronutrients and rectal cancer (4-7, 20-22), although it has been suggested that dietary risk factors may differ for these two carcinomas. Most published studies have had few rectal cases (5-7, 20, 22); none have reported on these associations in African Americans.

In this report, we examined associations of total intake of selected micronutrients (from food only and food plus dietary supplements) with risk of rectal cancer among White and African American participants. Specifically, we evaluated the relationships between antioxidant nutrients (vitamin C, vitamin E, β-carotene, selenium) and DNA methylation-related nutrients (folate, vitamin B6, vitamin B12) and the risk of rectal cancer in a population-based case-control study. We chose these micronutrients based on a plausible biological rationale, and we further examine the combined effects of nutrients hypothesized to function in similar ways to affect rectal cancer.

## C. Methods

1. Study design and population

Data were obtained from the North Carolina Colon Cancer Study-Phase II, which was conducted between May 2001 and September 2006. Subjects were eligible for the study if they resided in one of 33 counties in central and eastern North Carolina, were African American or White, were 40-79 years of age, had a North Carolina driver's license, had no previous diagnosis of colon or rectal cancer, and were able to give informed consent and complete the interview. African Americans were over-sampled to increase their representation in the study. This study was approved by the institutional review board at the University of North Carolina-Chapel Hill.

Cases had a primary diagnosis of rectal (including sigmoid and rectosigmoid junction) cancer during the study period. Cases were obtained from the rapid ascertainment system of the North Carolina Central Cancer Registry and diagnoses were confirmed our study pathologist. Permission was received from the primary physician before contacting cases. There were a total of 1,831 potentially eligible cases identified. Fifty-seven (3%) of these were excluded for physician refusal and 357 (19%) were found to be ineligible. Of the remaining 1,417 eligible cases, 118 (8%) could not be contacted and 242 (17%) refused; therefore, 1,057 (75%) had an in-person interview. The overall response rate (number of persons interviewed divided by the total number of eligible persons) for cases was 74% (76% and 70% for White and African American cases, respectively).

Controls under the age of 65 were identified using lists provided by the North Carolina Division of Motor Vehicles and the Center for Medicaid and Medicare Services for those 65 and older. Controls were selected using a randomized recruitment procedure based on sampling probabilities within blocks defined by 5-year age group, sex, and race (23). There were a total of 2,345 potentially eligible controls, but 518 (22%) were found to be

ineligible. Of the 1,827 eligible controls identified, 325 (18%) could not be contacted, 483 (26%) refused to be contacted; therefore, 1,019 (56%) were interviewed. The overall response rate for controls was 56% (58% and 46% for White and African American controls, respectively).

The analyses were restricted to those who completed all components of the study (n=1987). We further excluded 83 participants with implausible values for total energy intake (<800 kcal/day and >5000 kcal/day for men and <600 kcal/day and >4000 kcal/day for women) (24). Therefore, the analytic sample for this report included 1520 Whites (720 cases, 800 controls) and 384 African Americans (225 cases, 159 controls).

2. Data Collection

Trained nurse-interviewers collected all data in participants' home or another convenient location using standard questionnaires. We collected information on age at diagnosis, socioeconomic indicators, household information, physical activity, medical history, non-steroidal anti-inflammatory drug use, smoking history, and first degree family history of colorectal cancer. Dietary information was obtained using the 124-item Diet History Questionnaire (DHQ), developed by the National Cancer Institute (NCI) (25, 26). Participants were asked to recall their intake in the 12 months prior to diagnosis (cases) or interview (controls). There were 10 frequency options for each food, as well as 3 choices to estimate portion size. Nutrient and total energy intakes were based on the nutrient content of each food item, frequency of consumption, and portion size, and were determined using software provided by the NCI. The DHQ also collected detailed information on the type, dose, and frequency of dietary supplement use. The nutrients of interest for this study were

antioxidant nutrients (vitamin C, vitamin E, β-carotene, selenium) and DNA methylation-related nutrients (folate, vitamin B12, vitamin B6) from food and supplements.

3. Statistical Analyses

All analyses were done using SAS 9.1 software (SAS Institute, Inc., Cary, NC). We stratified the analyses by race and compared characteristics of cases and controls. We used chi-square and Wilcoxon rank-sum tests to make these comparisons with regard to categorical and continuous variables, respectively. Each nutrient was categorized into quartiles based on intake among race-specific controls. Unconditional logistic regression models were used to determine odds ratios (OR) and 95% confidence intervals (95% CI) for the association between nutrient intake and risk of rectal cancer. We examined these associations for nutrient intake from foods only, as well as total intake (food plus dietary supplements). Nutrients were examined in separate models. We also simultaneously controlled for other micronutrients to examine the combined effect of nutrients in both categories (i.e. all antioxidant nutrients and all DNA methylation-related nutrients). All logistic models included an offset term to adjust for the sampling probability. To assess confounding, the following covariates were tested in a bivariate model with each nutrient: age (continuous), sex, education (less than or equal to high school, some college, college graduate/advanced degree), smoking status (never, current, former), prior BMI (i.e., in the year prior to interview for controls and diagnosis for cases) (normal, overweight, obese), physical activity (quartiles of metabolic equivalent (MET)-minutes/day), first-degree family history of colorectal cancer (yes, no), non-steroidal anti-inflammatory drug use (yes, no), and total energy intake (continuous). All models were adjusted for total energy intake to account

for differences in total energy between cases and controls and whites and African Americans. Covariates that produced at least a 10% change in any of the nutrient coefficients were considered potential confounders, and a backwards-stepwise procedure was done to obtain the final model. Any variable that was a confounder in any model was retained in all models. A linear trend test was conducted using median quartile values among race-specific controls, which were incorporated into the logistic regression model as a continuous predictor and weighted by the inverse of the variance. Interactions were tested by including a cross-product term for the variables of interest in the model**.**

**D. Results**

Table 3 presents demographic and lifestyle characteristics of rectal cancer cases and respective controls stratified by race. In both Whites and African Americans, cases were slightly younger, had a higher mean BMI one year ago, and greater total daily energy intakes than their respective controls. Fewer White cases reported using non-steroidal anti-inflammatory drugs compared to controls (35.1% vs. 45.7%, $p<0.0001$). In African Americans, significantly more cases had a first-degree family history of CRC ($p=0.03$). In controls, a larger proportion of African Americans were obese and more whites were college graduates.

Mean nutrient intakes for White and African American rectal cancer cases and controls are given in Table 4. Nutrient intake was evaluated by the contribution from food sources only and from food and dietary supplements combined. There were significant differences in intake between cases and controls in both racial groups. In general, White cases had *lower* mean nutrient intakes than controls, while African American cases had

*higher* nutrient intakes than their respective controls. In both whites and African Americans, total (food plus supplements) mean intakes for most nutrients were significantly different between cases and controls (all p-values < 0.05). More specifically, White controls had higher intakes of all nutrients, except selenium, compared to White cases; African American cases reported higher consumption of vitamin C, vitamin E, folate, vitamin B6, and vitamin B12 than their respective controls. The contribution of supplements to total selenium intake was negligible in both racial groups. In controls, African Americans reported significantly lower total mean intakes for most nutrients compared to whites, and the higher intakes in whites was mainly due to contributions from dietary supplements. For example, daily vitamin E levels from food sources only in White and African American controls were similar (12.0 mg αTE and 11.1 mg αTE, respectively); however, total vitamin E intake was 103 mg αTE among Whites and 47 mg αTE among African Americans.

Tables 5 and 6 give the associations (OR and 95% CI) between rectal cancer and nutrients in our study population, stratified by race. The ORs presented are based on race-specific quartile cut-points, although ORs estimated using identical cut-points for both races were similar. Table 3 presents results for antioxidant nutrients (vitamins C and E, β-carotene, and selenium). In whites, the highest quartiles of all nutrients were associated with a statistically significant lower risk of rectal cancer compared to the lowest quartile, except for total vitamin E intake. The greatest risk reduction was observed for total β-carotene intake (Q4 vs. Q1 OR: 0.47, 95%CI 0.33-0.66). For vitamins C and E, the association with risk was stronger for food sources only than from total intake. For example, the OR for high vitamin C intake from food only was 0.49 (95% CI 0.35-0.69) and 0.62 (95% CI 0.45-0.86) from food and supplements combined. In African Americans, high selenium intake had a

strong inverse association with rectal cancer risk: total selenium intake was associated with a 75% lower risk (OR: 0.25, 95% CI 0.08-0.84). The combined effect of all antioxidant nutrients on risk reduction in whites was less than the significant associations observed for single nutrients.

Table 6 gives results for DNA methylation-related nutrients (folate, vitamin B6, vitamin B12). There were significantly lower risks associated with all nutrient intakes in whites when contrasting the highest and lowest quartiles of intake, but only marginally significant for total folate intake (OR: 0.71, 95% CI 0.50-1.01). High intake of vitamin B12 from food in Whites had the strongest (58%) reduction in risk (OR: 0.42, 95% CI 0.28-0.63, $p < 0.0001$). The combined effect of all DNA methylation-related nutrients (Q4 vs. Q1 OR: 0.62, 95%CI 0.44-0.88) was stronger than the independent associations for total folate and total vitamin B6. In African Americans, total folate, vitamin B6, and vitamin B12, as well as the combined effect of all DNA methylation-related nutrients were suggestive of elevated risk, although odds ratios were not statistically significant.

Since whites had higher nutrient intakes and less total energy intakes compared to African Americans, the nutrient densities were also greater in whites. When we examined the effect of energy adjustment, there were slightly stronger associations for energy-adjusted estimates compared to ORs not adjusted for total energy. For example, the energy-adjusted odds ratio for the highest category of vitamin C intake in whites was 0.62 (95% CI 0.45-0.86); however, the non-energy-adjusted estimate was 0.69 (95% CI 0.50-0.94). In African Americans, the energy-adjusted and non-energy-adjusted estimates for total vitamin C were 1.45 and 1.91, respectively, although neither was statistically significant.

**E. Discussion**

In this large population-based case-control study, all antioxidant nutrients were associated with reduced rectal cancer risk in whites, and selenium reduced risk in African Americans. Inverse associations with DNA methylation-related nutrients were only observed in whites yet appeared to elevate risk in African Americans. To our knowledge, this is the first study to report associations between micronutrients and rectal cancer risk in African Americans.

There were notable differences in mean nutrient intakes between whites and African Americans. In general, African American controls reported lower mean intakes than White controls, primarily due to the greater contribution to intake from dietary supplements in whites. The prevalence of any dietary supplement use in the last 12 months among our control population was 72% in Whites and 53% in African Americans. It has been estimated that approximately 50-70% of non-institutionalized U.S. adults take dietary supplements in the form of multivitamin/mineral or single nutrient supplements (19, 27, 28), and Radimer, et al also noted that supplement use patterns differ by race (19). Therefore, it is necessary to collect detailed information on supplement use when assessing the effect of micronutrients on disease risk, especially in diverse populations.

Findings in this present study for whites are consistent with the hypotheses that dietary antioxidants may reduce the risk of rectal cancer. In addition to their antioxidant properties, these nutrients may also inhibit tumor development by stimulating the immune system and (2) and regulating cell growth (29, 30). Our results are in agreement with other observational studies reporting significant inverse associations for dietary antioxidant intake and colon cancer (4, 7, 31, 32) and colorectal cancer (4, 7). Kune and colleagues reported rectal cancer risk reductions for high intakes of vitamin C, vitamin E, and selenium (7), and

elevated risk of rectal cancer has been observed for low vitamin E intakes in women (21). On the contrary, there was no effect of vitamin E on colon cancer in the Women's Health Study clinical trial (33). Most of the current evidence has been limited to non-African American populations; however, Satia et. al. noted significant inverse associations with colon cancer for high intakes of β-carotene, vitamin C, and vitamin E in African Americans (31).

We also found intakes of DNA methylation-related nutrients to be associated with reduced risk of rectal cancer in whites. Results are conflicting regarding the effect of folate on colorectal cancer development. In a recent report of the Netherlands Cohort Study, the authors did not find folate to be significantly associated with colorectal cancer risk in men or women (34). Null findings have also been reported for folate and colon cancer (5, 7, 31, 35, 36). The most recent report from the World Cancer Research Fund/American Institute for Cancer Research indicated that there is only limited suggestive evidence that folate reduces the risk of colorectal cancer (37). Epidemiologic studies of vitamin B6 and B12 are limited in comparison to studies on folate intake. The present study is in agreement with findings from an Australian case-control study in which there was a significant rectal cancer risk reduction for the highest category of vitamin B6 and B12 intake (7). On the other hand, two large prospective studies observed an elevated risk of rectal cancer in women for high intake of vitamin B6 (5, 34). These discrepant findings may be due to inherent biases in case-control studies, the method of dietary assessment, or variation in intakes of these micronutrients. We did not observe effect modification by alcohol for any of these DNA methylation-related nutrients, although alcohol is a known to interact with these nutrients (38). This may be because the average alcohol intake in our study population was low (<10g/day), thereby limiting our ability to detect any modifying effects by alcohol intake.

The reasons why the associations between micronutrients and rectal cancer differ for whites and African Americans are not totally clear. Surprisingly, high total intakes of all nutrients except selenium appeared to elevate the risk of rectal cancer in African Americans, although odds ratios were not significant. This direct association may be due to the source of these nutrients; however, after controlling for fruit and vegetable consumption there was still a non-significant positive association with risk. To our knowledge, this is the first study to report such strong rectal cancer risk reduction in African Americans for high intake of selenium (75% risk reduction); however, this could be a chance finding. Also, due to our small sample of African Americans, we may have missed other statistically significant associations, and these small sample size may also have led to unstable estimates Results from other epidemiologic studies with adequate African American representation are needed to confirm (or dispute) these findings.

It is interesting to note that for all DNA methylation-related nutrients in whites, the risk reduction was greater for intake from food sources only compared to total intake (food plus supplements). This phenomenon was also seen for vitamins C and E intakes. In African Americans, the suggested risk elevation was actually greater for total intake than from food alone. Other studies have reported null effects of supplement use on colorectal cancer (8, 39) and adenomas (9, 40). For example, compared to the placebo, 1mg/day of folic acid did not reduce the risk of colorectal adenomas, the precursor to colon and rectal cancer, and actually increased the risk of advanced adenomas in the Aspirin/Folate Polyp Prevention Study (9). There are several possible explanations for these findings. This may be due to the dual effect of folate, depending on dosage and time of exposure. While adequate folate intake may suppress tumor development, excessive intake may not offer additional benefit or even

enhance carcinogenesis, especially when there are pre-existing lesions (41). These disparate findings may also reflect the different chemical structures and biological pathways of natural folate and synthetic folic acid. Folic acid is more bioavailable and therefore more readily absorbed than natural folate found in food (42). However, high circulating levels of unmetabolized folic acid may reduce the immune response against carcinogenic cells by reducing the amount of natural killer cells (42). Clinical trials have also found no evidence for associations of vitamin C, vitamin E, or β-carotene with reduced risk of CRC (8, 39). One trial reported a significant inverse association of vitamin E supplementation and colon cancer risk, but there was no statistically significant association with rectal cancer (8). Therefore, these supplements may have different effects on colon and rectal cancer. Also, vitamins C and E may exert pro-oxidant effects, promoting oxidative DNA damage, at high concentrations. Our study results suggest that nutrient intake from dietary supplements may not help reduce rectal cancer risk, and that intake from food sources alone may be more relevant for risk reduction. This could be because supplement use only benefits those with suboptimal nutrient intakes, while providing no benefit for those with adequate intakes. In our study, the mean intake of these micronutrients from food alone was above the daily recommended intakes for both whites and African Americans (43). In addition, other compounds of natural foods such as phytochemicals and fiber may be chemopreventive and act in synergy with these nutrients to reduce rectal cancer risk, and it is likely that past and long-term supplement use may be associated with rectal cancer risk as opposed to recent use. Currently, the overall evidence for recommending supplements for rectal cancer is weak (44, 45).

A major strength of this study was our large sample size, especially the number of rectal cancer cases. This allowed us to observe associations that would be undetectable in studies with fewer participants. Our study is among the first reports of micronutrient intake and rectal cancer risk in African Americans. We collected detailed information on dietary supplement use to include in our assessment of total nutrient intake.

There are some limitations worth noting. Our study was subject to potential biases in case-control studies such as recall bias. It is possible that there was differential recall between cases and controls. Differential response rates between cases and controls, as well as between whites and African Americans, could have biased our results. There was also the potential of measurement error; however the diet history questionnaire has been validated, although not in African American populations (25). Due to our small sample size of African Americans, some significant associations may have been missed because of low statistical power.

In summary, the present findings add to the evidence that dietary antioxidants (vitamin C, vitamin E, β-carotene, selenium) and DNA methylation-related nutrients (folate, vitamin B6, vitamin B12) are associated with lower risk of rectal cancer in whites. Our results also support the hypotheses of mechanisms by which these nutrients may play a role in preventing colorectal cancer. This study provides evidence that selenium may reduce risk in African Americans. We observed striking differences in the relationship between the micronutrients and rectal cancer in whites and African Americans. This stresses the importance of examining these associations by race in large racially diverse samples. Furthermore, intakes from dietary supplements appeared to reduce the risk reduction for

some nutrients, suggesting that optimal intakes of these nutrients from food sources alone may be sufficient to lower risk of rectal cancer.

## F. Tables

**Table 3** Characteristics (means and standard deviations, percents) of cases and controls in the North Carolina Colon Cancer Study-Phase II (2001-2006), by race

| | Whites (N=1520) | | African Americans (N=384) | |
|---|---|---|---|---|
| | Cases (n=720) | Controls (n=800) | Cases (n=225) | Controls (n=159) |
| Sex (%) | | | | |
| Male | 58.3 | 60.5 | 52.4 | 52.2 |
| Age (years) (%) | | | | |
| 40-49 | 19.2 | 12.1 | 21.3 | 17.5 |
| 50-59 | 27.5 | 26.6 | 29.2 | 22.7 |
| 60-69 | 31.5 | 34.4 | 33.8 | 41.6 |
| 70-79 | 21.8 | 27.0 | 15.7 | 18.2 |
| Mean(SD) | 59.6(10.3) | 61.7(9.8) | 58.0(10.0) | 60.3(9.8) |
| Education (%) | | | | |
| <=High School | 50.3 | 39.0 | 61.8 | 58.5 |
| Some College | 25.1 | 25.9 | 22.2 | 25.8 |
| College graduate/Advanced degree | 24.6 | 35.1 | 16.0 | 15.7 |
| Body Mass Index (1yr ago) (%) | | | | |
| Normal (18.5-24.9 kg/m$^2$) | 22.7 | 30.3 | 17.4 | 18.1 |
| Overweight (25.0-29.9 kg/m$^2$) | 38.8 | 40.7 | 31.6 | 36.2 |
| Obese (≥30.0 kg/m$^2$) | 38.5 | 29.0 | 50.9 | 45.6 |
| Mean(SD) | 29.2(6.3) | 28.0(5.5) | 31.6(7.7) | 29.9(6.5) |
| Physical activity (MET-min/day [a]) (%) | | | | |
| Quartile 1 | 25.4 | 24.5 | 30.7 | 28.9 |
| Quartile 2 | 24.4 | 23.5 | 25.5 | 28.9 |
| Quartile 3 | 21.1 | 26.5 | 16.0 | 19.5 |
| Quartile 4 | 29.1 | 25.3 | 27.8 | 22.8 |
| Mean(SD) | 2250.0(661.8) | 2152.7(473.4) | 2178.4(545.5) | 2152.8(494.2) |
| Total energy intake (kcal/day) | | | | |
| Mean(SD) | 2245.9(826.2) | 2143.0(790.9) | 2423.6(953.3) | 2207.7(891.6) |
| Smoking Status (%) | | | | |
| Current Smoker | 15.6 | 13.5 | 22.7 | 17.0 |
| Former Smoker | 47.3 | 48.7 | 38.2 | 42.1 |
| Never Smoker | 37.1 | 37.8 | 39.1 | 40.9 |
| NSAID use [b](%) | | | | |
| Yes | 35.1 | 45.7 | 24.4 | 22.8 |
| First degree family history of CRC (%) | | | | |
| Yes | 13.2 | 11.3 | 11.8 | 5.2 |

[a] metabolic equivalent minutes per day
[b] greater than or equal to 15 non-steroidal anti-inflammatory drugs (NSAID) per month in the past 5 years

**Table 4** Mean (standard deviation) nutrient intakes of participants in the North Carolina Colon Cancer Study-Phase II (2001-2006), by case/control status and race

| Nutrient | Whites (N=1520) | | | African Americans (N=384) | | | |
|---|---|---|---|---|---|---|---|
| | Cases (N=720) | Controls (N=800) | p[a] | Cases (N=225) | Controls (N=159) | p[a] | p[b] |
| Vitamin C (mg/day) | | | | | | | |
| From foods only | 132(82) | 146(77) | <0.0001 | 183(107) | 152(86) | 0.003 | 0.76 |
| From foods + supplements | 266(314) | 313(346) | <0.0001 | 259(228) | 215(212) | 0.001 | 0.0004 |
| Vitamin E (mg αTE [c]/day) | | | | | | | |
| From foods only | 11.5(5.5) | 12.0(6.2) | 0.38 | 11.9(6.0) | 11.1(5.0) | 0.35 | 0.14 |
| From foods + supplements | 79(133) | 103(153) | 0.0003 | 56(122) | 47(91) | 0.05 | <0.0001 |
| β-carotene (µg/day) | | | | | | | |
| From foods only | 3881(2610) | 4595(3012) | <0.0001 | 5794(4637) | 5247(4233) | 0.31 | 0.30 |
| From foods + supplements | 4185(2712) | 4945(3077) | <0.0001 | 6042(4678) | 5426(4277) | 0.21 | 0.75 |
| Selenium (µg/day) | | | | | | | |
| From foods only | 104(41) | 105(43) | 0.81 | 112(51) | 107(50) | 0.42 | 0.91 |
| From foods + supplements | 105(41) | 106(44) | 0.75 | 112(50) | 108(49) | 0.45 | 0.94 |
| Folate (µg/day) | | | | | | | |
| From foods only | 433(157) | 450(176) | 0.14 | 483(206) | 432(177) | 0.02 | 0.20 |
| From foods + supplements | 619(262) | 665(270) | 0.002 | 649(280) | 549(252) | 0.0004 | <0.0001 |
| Vitamin B6 (mg/day) | | | | | | | |
| From foods only | 2.1(0.8) | 2.2(0.9) | 0.08 | 2.3(1.0) | 2.1(0.9) | 0.01 | 0.14 |
| From foods + supplements | 4.8(8.1) | 5.6(9.0) | 0.0004 | 4.3(5.9) | 3.7(5.9) | 0.001 | <0.0001 |
| Vitamin B12 (µg/day) | | | | | | | |
| From foods only | 5.4(3.2) | 5.7(3.3) | 0.06 | 6.5(5.2) | 6.2(4.7) | 0.20 | 0.62 |
| From foods + supplements | 8.2(4.3) | 8.8(4.3) | 0.004 | 9.0(5.9) | 7.9(5.5) | 0.01 | 0.0004 |

[a] based on Wilcoxon rank sum test for comparisons between race-specific cases and controls

[b] based on Wilcoxon rank sum test for comparisons between White and African American controls

[c] alpha-tocopherol equivalents

**Table 5** Multivariate odds ratios (OR)[a] and 95% confidence intervals for the association of rectal cancer with antioxidant nutrients, by race (North Carolina Colon Cancer Study-Phase II, 2001-2006)

| | Whites (n=1520) | | | | African Americans (n=384) | | | |
|---|---|---|---|---|---|---|---|---|
| Nutrient quartiles | No. of Cases | Median intake/day in controls | OR | 95%CI | No. of Cases | Median intake/day in controls | OR | 95%CI |
| Vitamin C (mg) (food only) | | | | | | | | |
| Q1 | 260 | 72 | 1.00 | | 36 | 62 | 1.00 | |
| Q2 | 171 | 112 | 0.71 | 0.53-0.96 | 43 | 111 | 1.08 | 0.57-2.70 |
| Q3 | 145 | 152 | 0.59 | 0.43-0.81 | 65 | 163 | 1.51 | 0.86-3.01 |
| Q4 | 144 | 233 | 0.49 | 0.35-0.69 | 81 | 260 | 1.58 | 0.76-3.29 |
| P for linear trend | | | | 0.85 | | | | 0.92 |
| Vitamin C (mg) (food + supplements) | | | | | | | | |
| Q1 | 253 | 94 | 1.00 | | 34 | 64 | 1.00 | |
| Q2 | 171 | 159 | 0.72 | 0.53-0.97 | 55 | 130 | 1.22 | 0.61-2.44 |
| Q3 | 153 | 225 | 0.64 | 0.46-0.87 | 53 | 193 | 0.98 | 0.48-2.02 |
| Q4 | 143 | 664 | 0.62 | 0.45-0.86 | 83 | 336 | 1.45 | 0.71-2.96 |
| P for linear trend | | | | 0.92 | | | | 1.0 |
| Vitamin E (mg αTE) (food only) | | | | | | | | |
| Q1 | 198 | 6.4 | 1.00 | | 53 | 5.9 | 1.00 | |
| Q2 | 161 | 9.3 | 0.80 | 0.58-1.11 | 51 | 8.6 | 0.69 | 0.35-1.38 |
| Q3 | 194 | 12.3 | 0.81 | 0.57-1.15 | 59 | 12.4 | 0.65 | 0.30-1.44 |
| Q4 | 167 | 18.1 | 0.60 | 0.39-0.91 | 62 | 17.2 | 0.42 | 0.15-1.15 |
| P for linear trend | | | | 0.09 | | | | 0.50 |
| Vitamin E (mg α TE) (food +supplements) | | | | | | | | |
| Q1 | 208 | 9 | 1.00 | | 53 | 7 | 1.00 | |
| Q2 | 207 | 21 | 0.92 | 0.67-1.25 | 34 | 11 | 0.53 | 0.26-1.06 |
| Q3 | 172 | 33 | 0.94 | 0.68-1.29 | 59 | 20 | 0.87 | 0.43-1.74 |
| Q4 | 133 | 298 | 0.76 | 0.55-1.05 | 79 | 41 | 1.09 | 0.56-2.11 |
| P for linear trend | | | | 0.96 | | | | 0.72 |

**Table 5** continued

| | | Whites (n=1520) | | | African Americans (n=384) | | | |
|---|---|---|---|---|---|---|---|---|
| Nutrient quartiles | No. of Cases | Median intake/day in controls | OR | 95%CI | No. of Cases | Median intake/day in controls | OR | 95%CI |
| β-carotene ( µg) (food only) | | | | | | | | |
| Q1 | 253 | 1876 | 1.00 | | 49 | 1701 | 1.00 | |
| Q2 | 201 | 3201 | 0.83 | 0.62-1.12 | 62 | 3386 | 0.99 | 0.52-1.90 |
| Q3 | 135 | 4716 | 0.60 | 0.44-0.83 | 43 | 5133 | 0.58 | 0.29-1.17 |
| Q4 | 131 | 7453 | 0.49 | 0.35-0.67 | 71 | 8959 | 1.11 | 0.55-2.23 |
| P for linear trend | | | | 0.99 | | | | 1.0 |
| β-carotene ( µg) (food + supplements) | | | | | | | | |
| Q1 | 262 | 2113 | 1.00 | | 48 | 1808 | 1.00 | |
| Q2 | 188 | 3554 | 0.76 | 0.56-1.03 | 62 | 3561 | 0.98 | 0.50-1.90 |
| Q3 | 141 | 5110 | 0.66 | 0.48-0.91 | 40 | 5481 | 0.52 | 0.26-1.07 |
| Q4 | 129 | 7920 | 0.47 | 0.33-0.66 | 75 | 9212 | 1.18 | 0.58-2.41 |
| P for linear trend | | | | 1.0 | | | | 1.0 |
| Selenium (µg) (food only) | | | | | | | | |
| Q1 | 181 | 60 | 1.00 | | 50 | 51 | 1.00 | |
| Q2 | 183 | 86 | 0.92 | 0.66-1.27 | 53 | 84 | 0.65 | 0.32-1.31 |
| Q3 | 179 | 112 | 0.72 | 0.49-1.07 | 67 | 119 | 0.58 | 0.24-1.37 |
| Q4 | 177 | 154 | 0.54 | 0.32-0.90 | 55 | 165 | 0.28 | 0.08-0.95 |
| P for linear trend | | | | 0.77 | | | | 0.89 |
| Selenium (µg) (food + supplements) | | | | | | | | |
| Q1 | 178 | 60 | 1.00 | | 53 | 52 | 1.00 | |
| Q2 | 192 | 86 | 0.98 | 0.70-1.35 | 49 | 85 | 0.53 | 0.26-1.08 |
| Q3 | 175 | 114 | 0.67 | 0.45-1.00 | 68 | 120 | 0.54 | 0.23-1.26 |
| Q4 | 175 | 155 | 0.51 | 0.30-0.85 | 55 | 165 | 0.25 | 0.08-0.84 |
| P for linear trend | | | | 0.75 | | | | 0.87 |

**Table 5** continued

| | Whites (n=1520) | | | | African Americans (n=384) | | | |
|---|---|---|---|---|---|---|---|---|
| Nutrient quartiles | No. of Cases | Median intake/day in controls | OR | 95%CI | No. of Cases | Median intake/day in controls | OR | 95%CI |
| All antioxidant nutrients (mg) (food + supplements) | | | | | | | | |
| Q1 | 237 | 111 | 1.00 | | 36 | 80 | 1.00 | |
| Q2 | 184 | 199 | 0.84 | 0.62-1.14 | 50 | 153 | 1.01 | 0.50-2.03 |
| Q3 | 166 | 318 | 0.72 | 0.53-0.98 | 60 | 232 | 1.07 | 0.53-2.17 |
| Q4 | 133 | 942 | 0.66 | 0.47-0.91 | 79 | 446 | 1.29 | 0.64-2.61 |
| P for linear trend | | | | 0.97 | | | | 0.99 |

[a] adjusted for age, sex, education, BMI, family history of CRC, non-steroidal anti-inflammatory drug use, and total energy

**Table 6** Multivariate odds ratios (OR)[a] and 95% confidence intervals for the association of rectal cancer with DNA methylation-related nutrients, by race (North Carolina Colon Cancer Study-Phase II, 2001-2006)

| | Whites (n=1520) | | | | African Americans (n=384) | | | |
|---|---|---|---|---|---|---|---|---|
| Nutrient quartiles | No. of Cases | Median intake/day in controls | OR | 95%CI | No. of Cases | Median intake/day in controls | OR | 95%CI |
| Folate (µg) (food only) | | | | | | | | |
| Q1 | 203 | 276 | 1.00 | | 45 | 241 | 1.00 | |
| Q2 | 156 | 373 | 0.77 | 0.56-1.07 | 46 | 354 | 0.84 | 0.42-1.70 |
| Q3 | 211 | 476 | 0.91 | 0.64-1.30 | 65 | 467 | 0.87 | 0.39-1.93 |
| Q4 | 150 | 640 | 0.50 | 0.32-0.78 | 69 | 641 | 0.81 | 0.31-2.10 |
| P for linear trend | | | | 0.97 | | | | 1.0 |
| Folate (µg) (food + supplements) | | | | | | | | |
| Q1 | 213 | 332 | 1.00 | | 43 | 282 | 1.00 | |
| Q2 | 195 | 566 | 0.82 | 0.60-1.13 | 32 | 434 | 0.68 | 0.32-1.45 |
| Q3 | 157 | 769 | 0.84 | 0.62-1.16 | 46 | 587 | 0.82 | 0.41-1.67 |
| Q4 | 155 | 966 | 0.71 | 0.50-1.01 | 104 | 916 | 2.06 | 0.98-4.33 |
| P for linear trend | | | | 0.98 | | | | 0.98 |
| Vitamin B6 (mg) (food only) | | | | | | | | |
| Q1 | 204 | 1.3 | 1.00 | | 43 | 1.11 | 1.00 | |
| Q2 | 179 | 1.8 | 0.91 | 0.67-1.26 | 41 | 1.72 | 0.92 | 0.45-1.89 |
| Q3 | 179 | 2.3 | 0.85 | 0.60-1.22 | 67 | 2.20 | 1.32 | 0.63-2.76 |
| Q4 | 158 | 3.1 | 0.58 | 0.38-0.88 | 74 | 3.10 | 1.34 | 0.53-3.39 |
| P for linear trend | | | | <0.0001 | | | | <0.0001 |
| Vitamin B6 (mg) (food + supplements) | | | | | | | | |
| Q1 | 214 | 1.6 | 1.00 | | 48 | 1.4 | 1.00 | |
| Q2 | 204 | 2.8 | 0.90 | 0.66-1.23 | 32 | 2.0 | 0.55 | 0.27-1.13 |
| Q3 | 157 | 3.9 | 0.83 | 0.60-1.14 | 47 | 3.0 | 0.74 | 0.37-1.48 |
| Q4 | 145 | 5.3 | 0.68 | 0.48-0.96 | 98 | 4.7 | 1.57 | 0.78-3.18 |
| P for linear trend | | | | <0.0001 | | | | 0.87 |

**Table 6** continued

| Nutrient quartiles | Whites (n=1520) No. of Cases | Median intake/day in controls | OR | 95%CI | African Americans (n=384) No. of Cases | Median intake/day in controls | OR | 95%CI |
|---|---|---|---|---|---|---|---|---|
| Vitamin B12 (µg) (food only) | | | | | | | | |
| Q1 | 218 | 2.7 | 1.00 | | 35 | 2.0 | 1.00 | |
| Q2 | 170 | 4.2 | 0.68 | 0.50-0.94 | 61 | 3.6 | 1.44 | 0.73-2.84 |
| Q3 | 165 | 6.0 | 0.58 | 0.41-0.81 | 58 | 6.5 | 1.15 | 0.54-2.47 |
| Q4 | 167 | 9.1 | 0.42 | 0.28-0.63 | 71 | 12.5 | 1.42 | 0.63-3.24 |
| P for linear trend | | | | <0.0001 | | | | 0.62 |
| Vitamin B12 (µg) (food + supplements) | | | | | | | | |
| Q1 | 235 | 3.7 | 1.00 | | 39 | 2.3 | 1.00 | |
| Q2 | 170 | 7.4 | 0.67 | 0.49-0.91 | 41 | 5.4 | 0.74 | 0.36-1.51 |
| Q3 | 157 | 10.0 | 0.73 | 0.53-1.00 | 64 | 8.5 | 1.12 | 0.57-2.21 |
| Q4 | 158 | 13.3 | 0.60 | 0.43-0.84 | 81 | 15.5 | 1.56 | 0.75-3.27 |
| P for linear trend | | | | 0.01 | | | | 0.80 |
| All DNA methylation-related nutrients (mg) (food + supplements) | | | | | | | | |
| Q1 | 214 | 2.0 | 1.00 | | 49 | 1.7 | 1.00 | |
| Q2 | 208 | 3.4 | 0.89 | 0.66-1.21 | 30 | 2.4 | 0.40 | 0.19-0.84 |
| Q3 | 160 | 4.7 | 0.77 | 0.56-1.06 | 46 | 3.7 | 0.66 | 0.33-1.33 |
| Q4 | 138 | 6.3 | 0.62 | 0.44-0.88 | 100 | 5.5 | 1.32 | 0.65-2.66 |
| P for linear trend | | | | 0.12 | | | | 0.95 |

[a] adjusted for age, sex, education, BMI, family history of CRC, non-steroidal anti-inflammatory drug use, and total energy

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**V.** Associations of red meat, fat, and protein intake with rectal cancer risk

## A. Abstract

Studies suggest that red and processed meat consumption elevate the risk of colorectal cancer; however, the relationship between red meat, as well as fat and protein, and rectal cancer specifically is not clear. We determined the risk of rectal cancer associated with red and processed meat, fat, and protein intakes in whites and African Americans. There were 945 cases of rectal cancer (including sigmoid and rectosigmoid) cases and 959 controls. We assessed dietary intake in the 12 months prior to diagnosis for cases and interview for controls. Multivariate logistic regression analyses were used to obtain odds ratios (OR) and 95% confidence intervals (95% CI). There was no association between total fat, saturated fat, or monounsaturated fat and rectal cancer risk. In African Americans, the OR of rectal cancer for polyunsaturated fat was 0.28 (95% CI 0.08-0.96). The percent of energy from protein was associated with a 47% risk reduction in whites (OR: 0.53, 95% CI 0.37-0.77). Total red meat intake was not related to rectal cancer in either race group, but beef/pork/lamb consumption in whites was associated with a marginally significant risk reduction (OR: 0.66, 95% CI 0.43-1.00). Our results do not support the hypotheses that fat, protein, and red meat increase the risk of rectal cancer. These findings demonstrate the potential value of examining these associations by race.

**B. Introduction**

Colorectal cancer (CRC) is the fourth most common cancer in the U.S. and accounts for approximately 9% of all cancer deaths (1). Diet is widely believed to be associated with CRC development, and is a modifiable risk factor. Therefore, there is great interest in better understanding which dietary factors may be associated with higher or lower CRC risk. In particular, increased consumption of dietary fat, protein (mainly animal fat and protein), and have shown strong correlations with CRC cancer incidence in ecological studies (2-5). Observational studies in the U.S. have generally reported that high intakes of red meat and processed meat may increase risk for CRC (6, 7). A comprehensive review by the World Cancer Research Fund/American Institute for Cancer Research concluded that there was convincing evidence that red meat and processed meat increases CRC risk, but that the evidence regarding the role of foods containing animal fat is limited (8). Several hypotheses have been proposed to explain a possible relationship between red meat and CRC risk. These hypotheses relate to two primary nutrients in red meat, i.e. fat (9-11) and protein (3, 12), as well as components of processed red meat such as N-nitroso compounds (9, 13), and factors produced while cooking red meat at high temperatures, namely heterocyclic amines and polycyclic aromatic hydrocarbons (5, (3, 9, 13).

Colorectal cancer consists of carcinomas of the colon and rectum, and rectal cancer comprises approximately one-third of all colorectal cancers. It has been suggested that there are different etiologies for colon and rectal cancer (14, 15); therefore, it is important to examine risk factors separately for both sites. Some investigators have studied associations between meat intake and sub-sites of the colorectum (16-18); however, the currently

available evidence regarding the associations of red meat, fat, and protein with rectal cancer risk is inconclusive.

In this study, we examined associations of red meat, fat, and protein intake with risk for rectal cancer in African Americans and whites in a large case-control study in North Carolina (NC). This study adds to the literature in two ways: it contributes to the body of knowledge regarding diet and rectal cancer risk and is, to our knowledge, the first study to examine these associations in African Americans.

## C. Methods

1. Study design and population

The North Carolina Colon Cancer Study-Phase II is a population-based study conducted between May 2001 and September 2006. Cases and controls were selected through a randomized recruitment approach that used age-, sex-, and race-specific incidence rates to calculate selection probabilities (19, 20). African Americans were over-sampled to increase their representation in the study. The eligibility criteria for all subjects were: age 40-79, resident in one of 33 target counties in central and eastern NC, a NC driver's license, no history of colon or rectal cancer, able to give informed consent, and able to complete the interview.

Rectal (including sigmoid and rectosigmoid) cancer cases were selected through the rapid ascertainment system (21) of the NC Central Cancer Registry. Cases were diagnosed with a primary adenocarcinoma between May 2001 and September 2006. Our study pathologist confirmed these diagnoses using pathology slides and medical records. Controls

were randomly selected from the NC Department of Motor Vehicles if under age 65 and the Centers for Medicare and Medicaid Services if 65 and older.

A total of 1057 out of 1417 eligible cases and 1019 out of 1827 eligible controls had an interview. Among those eligible to participate, the overall response rate (number interviewed/number eligible) for cases was 74% (76% for Whites, 70% African Americans) and 56% in controls (58% for Whites, 46% for African Americans). For this analysis, we further excluded 89 participants who did not complete all components of the study, and an additional 86 participants who had implausible energy intake values (<800 kcal/d and >5000 kcal/d for men and <600 kcal/d and > 4000 kcal/d for women (22). The final analytic sample included 945 cases (720 White, 225 African American) and 959 controls (800 White, 159 African American). This study was approved by the Institutional Review Board at the University of North Carolina-Chapel Hill.

2. Data collection

The National Cancer Institute's (NCI) Diet History Questionnaire (DHQ) was used to assess dietary intake. The DHQ is a 124-item food frequency questionnaire that includes questions on dietary supplement use and fat added to foods (23, 24). The questionnaire was administered by trained nurse-interviewers, who asked subjects to recall their usual dietary intake over the 1 year prior to diagnosis for cases or interview for controls. Nutrient intakes were determined using software provided by the NCI, and were based on the nutrient content of each food item, the frequency of consumption, and portion size. The nutrients of interest for this study were total fat, saturated fat, monounsaturated fat (MUFA), polyunsaturated fat (PUFA), protein, and red meat. The two categories of red meat were beef/pork/lamb

(including veal, lamb, beef steaks, beef roast, beef mixtures, burgers, ham (not luncheon meat), pork, bacon, ribs) and processed meat (including sausage, hot dogs, and all cold-cuts). Interviewers administered a separate questionnaire to collect data on covariates including demographic and household information, medical history, medication use, physical activity, smoking status, and family history of colorectal cancer.

3. Statistical analysis

Analyses were conducted using SAS (version 9.1; SAS Institute, Inc., Cary, NC) and based on 2-sided p-values. Participants were stratified by race and case-control status. The Wilcoxon non-parametric rank sum test was used to assess differences in mean nutrient intakes between White and African American controls. We calculated adjusted odds ratios (OR) and 95% confidence intervals (95% CI) using unconditional logistic regression models. PROC LOGISTIC in SAS was used with an option in the MODEL statement to include offsets. The offset term takes into account the selection probabilities based on age, race, and sex, which were used to identify eligible participants (20). Quartiles were constructed for nutrient and red meat intakes based on the distribution among race-specific controls for stratified analyses. This was done to account for possible differences in the variation in range of intake for whites and African Americans. The following covariates were considered for inclusion in the multivariate models: age (continuous), sex, education (≤ high school, some college, college graduate/advanced degree), smoking status (never, current, former), prior body mass index (i.e. BMI in the 1 year prior to interview for controls and diagnosis for cases)(normal, overweight, obese), physical activity (continuous), first degree family history of CRC (yes, no), non-steroidal anti-inflammatory drug (NSAID) use (yes, no), calcium

(continuous), folate (continuous), fiber (continuous), and total energy intake (continuous). Multivariate models were created using backward elimination and included covariates that changed the odds ratios of interest by ≥ 10%. All covariates met the criteria for inclusion, except smoking status and folate. P-values for trend were obtained using the median intake values in controls of each quartile as a continuous variable in the model, which was weighted by the inverse of the variance. For each red meat category, we constructed 3 multivariate models: model 1 included age, sex, education, prior BMI, family history, NSAID use, physical activity, calcium, fiber, and total energy; model 2 consisted of the covariates in model 1 and energy-adjusted saturated fat; model 3 consisted of the covariates in model 1 and energy-adjusted protein. We examined these 3 different models to determine the extent to which the association between red meat and rectal cancer can be attributed to overall saturated fat or protein intake since it has been suggested that these nutrients in red meat contribute to the elevated risk of rectal cancer. Therefore, these 3 different model specifications test the hypothesis that fat and protein intake mediate the association between red meat and rectal cancer.

**D. Results**

Table 7 summarizes characteristics of cases and controls by race with respect to potential confounders and dietary intake. Cases in both race groups were younger, had less education, and a higher mean BMI than their respective controls. Among whites, regular use of non-steroidal anti-inflammatory drugs was much more frequent in controls compared to cases, and a greater proportion of African American cases had a family history of CRC than African American controls. Some nutrient intakes and meat consumption patterns varied by

race; among controls, on average, whites had significantly greater calcium, fiber, and alcohol intakes than African Americans. There were no appreciable differences by race in total energy, dietary folate, and fat. The percent of energy from protein was greater for whites, although absolute intakes did not differ significantly by race. Whites reported a slightly higher mean intake of beef/pork/lamb than African Americans (52.1 versus 50.1 g/d, respectively (p=0.05)), while African Americans had greater processed meat consumption than whites (24.5 versus 18.7 g/d, respectively (p=0.006)).

As shown in Table 8, absolute intakes of total fat and the percent of energy from total fat had null associations with risk in whites and non-statistically significant inverse associations in African Americans. The ORs for saturated fat and the percent of energy from saturated fat were not statistically significant in either race group; however, we observed a significant inverse trend in African Americans for the percent of energy from saturated fat (p-value for trend = 0.004). With regards to the unsaturated fats (MUFA and PUFA), there were no associations with risk in whites. In African Americans, the highest category of PUFA intake was associated with a considerable reduced risk (OR: 0.28, 95% CI 0.08-0.96). The ORs for protein intake were less than one in both race groups, although there was no association in African Americans. In whites, high absolute protein intake was suggestive of lower rectal cancer risk (OR: 0.57, 95% CI 0.32-1.01). Also in whites, high percent of energy from protein yielded a significant risk reduction (OR: 0.53, 95% CI 0.37-0.77), and the odds ratios decreased progressively with increasing percent of energy from protein (p-value for trend=0.003).

Table 9 shows the relationship between rectal cancer risk and total red meat, beef/pork/lamb, and processed meat. For total red meat, we did not find any statistically

significant associations with risk in whites or African Americans in any of the models. The Model 1 ORs for the highest category of beef/pork/lamb consumption were similar in both race groups, yet only approached statistical significance in whites (OR: 0.66, 95% CI 0.43-1.00). There was a slightly stronger risk reduction in whites when we controlled for energy from saturated fat (OR: 0.60, 95% CI 0.39-0.93). Moderately high processed meat consumption had a significant positive association with risk in whites (Q3 vs. Q1 OR: 1.43, 95% CI 1.02-2.02), which was even stronger when we adjusted for energy from protein (Q3 vs. Q1 OR: 1.56, 95% CI 1.10-2.20). There were no statistically significant associations in African Americans, although odds ratios suggest lower risk for all red meat.

**E. Discussion**

In this large case-control study of 945 rectal (including sigmoid and rectosigmoid) cancer cases and 959 controls, we did not find any evidence of associations between total and saturated fat and rectal cancer risk, although monounsaturated fatty acids appeared to reduce risk in African Americans. Our study does not support the hypothesis that high red meat or processed meat consumption increases the risk of rectal cancer. Rather, we found that that protein intake and beef/pork/lamb consumption reduces the risk of rectal cancer in whites.

The results for total fat intake are in agreement with several previous case-control (25, 26) and cohort (27, 28) studies, which generally found no statistically significant association with rectal cancer risk. Several investigations found the relationship between overall fat intake and rectal cancer to vary by gender. For example, an early study by Freudenheim, et al. observed an approximately 2-fold higher rectal cancer risk in males (OR: 1.96, 95% CI 1.19-3.24), but no clear association in females (29). Similarly, a more recent

and larger case-control study in Canada found a positive association among male participants (OR: 1.7, 95% CI 1.1-2.6), but no association in females (30). We did not find evidence of effect modification by gender in our analyses of total fat and rectal cancer.

When examining the association between dietary fat and rectal cancer, it is important to consider different types of fatty acids because they can have different and opposite effects on risk. Experimental studies have shown that high saturated fat and omega-6 PUFAs increase the incidence of chemically induced colon cancer in animal models (31), while omega-3 PUFAs inhibit colorectal carcinogenesis in rodents (32, 33). It has been suggested that the fat content, particularly saturated fat, in red meat may influence CRC risk by increasing the production of secondary bile acids that can promote colon carcinogenesis (11, 34). Postulated mechanisms regarding the protective role for omega-3 PUFAs include their ability to inhibit tumor growth and modulate the expression of pro-inflammatory genes (35, 36). There is limited evidence that foods containing animal fat increase the risk of CRC (8). For example, a combined analysis of 13 case-control studies found no evidence of an association between CRC and saturated fat, PUFAs, or MUFAs (37). This is also the case for rectal cancer specifically, as studies have observed no association between saturated fat and rectal cancer (25-28, 38) as we did in the present study. High intake of PUFAs in our study was inversely related to risk, but only reached statistical significance in African Americans, suggesting a strong risk reduction (72%) in this race group. However, it is possible that this was a chance finding. Unfortunately, we were not able to distinguish between omega-3 and omega-6 PUFAs.

Total consumption of protein was significantly associated with reduced risk in whites and had a non-statistically significant association with lower risk in African Americans in the

present study. There is limited epidemiologic evidence for a relationship between overall protein intake and rectal cancer risk. A few studies reported no association between protein intake and rectal cancer risk (26, 28) while a case-control study in Italy reported a marginally significant risk reduction of rectal cancer for 100 calories/day from protein (39). These results are contrary to the hypothesis that increased protein intake may elevate rectal cancer risk due to components of protein degradation such as ammonia, phenolic compounds, amines, N-nitroso compounds, and possibly sulfides that are known to exert toxic effects in animal models and in vitro (12).

Meta-analyses of meat consumption and CRC risk have concluded that red meat and processed meat increase the risk of CRC, colon cancer, and rectal cancer (3, 9, 40), and that processed meat may be a stronger risk factor than fresh red meat (40, 41). In contrast, findings from individual studies have not been consistent (16, 18, 25, 42-45); therefore, the biological mechanisms relating red meat intake to CRC risk remain speculative. Individual studies investigating the relationship between red meat and rectal cancer specifically are also conflicting. Some studies have reported a significantly higher risk of rectal cancer with increased red meat consumption (16, 43, 45) while others did not find any statistically significant associations (17, 18, 25, 28, 44, 46, 47). Our study did not suggest that high total red meat intake elevates the risk of rectal cancer, although moderately high intakes of processed meat appear to be associated with significantly higher risk in Whites. Surprisingly, we found high beef/pork/lamb consumption to be associated with lower risk in whites.

It was initially hypothesized that the saturated fat and protein content of red meat increases rectal cancer risk for reasons previously mentioned. This hypothesis has been explored in animal studies, and it was found that lean beef did not promote colon

carcinogenesis in rats (48). When we controlled for energy from saturated fat in our analyses the risk estimates for the associations between red meat and rectal cancer were not appreciably altered; however, in whites, there was a trend toward a slightly stronger risk reduction for high beef/pork/lamb consumption. Therefore, there was no evidence to indicate that the association of red meat and rectal cancer was mediated by saturated fat intake. Protein metabolism is another mechanism to explain the relationship between rectal cancer risk and red meat intake. Meat is a major source of protein and products of protein metabolism such as ammonia and N-nitroso compounds are known to have toxic effects (12). High protein intakes in the present study appeared to reduce the risk of rectal cancer in whites, mainly as the percent of total energy. Controlling for protein intake in the analyses of red meat and rectal cancer risk in whites resulted in elevated risk estimates for total red meat, removed the significant risk reduction for high beef/pork/lamb intake, and strengthened the positive association with processed meat consumption. Therefore, the protein content in red meat appears to contribute to rectal cancer risk reduction in whites. No statistically significant changes for were observed in African Americans, although there were generally less favorable risk estimates when adjusting for protein.

Recently, more attention has been given to the potentially carcinogenic effects of heterocyclic amines (HCA) and polycyclic aromatic hydrocarbons (PAH) in an effort to explain the association between red meat and increased rectal cancer risk. HCAs are mutagenic compounds formed when cooking meat at high temperatures such as grilling, frying, or oven-broiling, while PAHs are produced when grilling or broiling over an open flame (46). In general, studies have shown well-done red meat and high mutagen indices to have strong positive associations with CRC risk (49, 50). However, the risk posed by these

compounds may depend on the extent to which they are activated by metabolic enzymes (13).

Our findings do not support the hypotheses that fat, protein, and red meat increase the risk of rectal cancer, although these dietary components have generally been associated with elevated colon cancer risk. There are several possible explanations for these findings. Our results may simply reflect differences in colon and rectal cancer development. There is evidence that colon and rectal cancer may have distinct etiologies (15, 51) as well as differences with regards to the metabolism of bile acids (14), expression of metabolizing enzymes (14), bacterial composition and pH (52), and genetic profile (15, 16). Another explanation may be that red meat intake in our study population was relatively low, and therefore perhaps below the level necessary to elevate risk. For example, Larsson et al. reported a 63% increase in rectal cancer risk associated with 120 g/d of red meat; the mean total red meat intake in our study was 76 g/d. These results may also reflect our inability to determine the amount of red meat consumed according to doneness and cooking methods, and thereby estimate the amount of HCAs and PAHs in the red meat. These mutagenic compounds may be the culpable substances moreso than overall red meat consumption.

The reasons why some of our results differed by race are not totally clear. No other available literature has reported the associations between diet and rectal cancer in African Americans. An early study of diet and colon cancer in African Americans did not observe any statistically significant associations between colon cancer and beef and pork consumption (53). A population-based case-control study of colon cancer did not report any associations between colon cancer risk in African Americans and energy-adjusted saturated fat, protein, or red meat intakes (54, 55). This study did find a significant colon cancer risk

reduction for high total fat intake in African Americans. We realize that the relatively small sample of African Americans in our study may have resulted in reduced power to detect real associations and resulted in unstable estimates. The risk differences remained after we estimated odds ratios using the same quartile cut-points in whites and African Americans; therefore, variation in the range of nutrient intake is also not a likely explanation for the racial differences in risk.

The population-based design and large sample size are among the strengths of this study. It is noteworthy that this is among the first published reports of associations between fat, protein, red meat and rectal cancer risk in African Americans. All data were collected in-person using standard questionnaires administered by our nurse-interviewers, thereby minimizing the potential for misclassification. Recall and response bias could have been introduced in our study and affected our results. We also cannot exclude the possibility of measurement error due to the use of the food frequency questionnaire.

In summary, this study did not provide evidence that total or saturated fat is related to rectal cancer risk in whites and African Americans. High intake of polyunsaturated fatty acids may reduce rectal cancer risk in African Americans, while protein intake may lower risk in whites. There was no association between total red meat intake and rectal cancer, although beef/pork/lamb appeared reduce risk and processed meat may elevate risk in whites. These findings highlight the importance of examining these associations in large racially diverse populations and add to the knowledge base for dietary risk factors for rectal cancer.

**Table 7: Characteristics[1] of cases and controls, by race (North Carolina Colon Cancer Study-Phase II, N=1904)**

| | Whites (N=1520) | | African Americans (N=384) | | P-value[4] |
|---|---|---|---|---|---|
| | Cases | Controls | Cases | Controls | |
| N | 720 | 800 | 225 | 159 | |
| Mean age (years) | 59.6 (10.3) | 61.7(9.8) | 58.0(10.0) | 60.3(9.8) | |
| Male (%) | 58.3 | 60.5 | 52.4 | 52.2 | |
| > High school education (%) | 49.7 | 61.0 | 38.2 | 41.5 | |
| Mean body mass index (kg/m$^2$) | 29.2(6.3) | 28.0(5.5) | 31.6(7.7) | 29.9(6.5) | |
| Mean physical activity (MET-min/d)[2] | 2250(661) | 2152(473) | 2178(545) | 2152(494) | |
| Current smokers (%) | 15.6 | 13.5 | 22.7 | 17.0 | |
| Regular NSAID use[3] (%) | 35.1 | 45.7 | 24.4 | 22.8 | |
| Family history of CRC (%) | 13.2 | 11.3 | 11.8 | 5.2 | |
| Mean daily intake | | | | | |
| Total energy (kcal) | 2245(826) | 2143(790) | 2423(953) | 2207(891) | 0.56 |
| Calcium (mg) | 814(351) | 860(393) | 788(354) | 725(354) | <0.0001 |
| Dietary folate (mcg) | 432(157) | 450(176) | 483(205) | 432(177) | 0.20 |
| Fiber (g) | 21.0(8.2) | 22.1(8.5) | 22.1(10.4) | 20.7(9.0) | 0.02 |
| Alcohol (g) | 8.8(22.2) | 8.3(17.1) | 7.9(24.1) | 4.8(13.3) | <0.0001 |
| Total fat (g) | 91.1(39.2) | 86.0(39.3) | 93.1(43.7) | 89.8(43.5) | 0.43 |
| Total fat (% energy) | 36.2(6.7) | 35.5(7.0) | 34.1(7.0) | 36.0(6.8) | 0.40 |
| Saturated fat (g) | 28.2(13.2) | 26.3(13.4) | 28.0(13.6) | 26.9(14.2) | 0.76 |
| Saturated fat (% energy) | 11.2(2.7) | 10.8(2.8) | 10.3(2.5) | 10.6(2.4) | 0.94 |
| Monounsaturated fat (g) | 35.5(15.9) | 33.3(15.7) | 36.1(17.7) | 35.0(17.5) | 0.38 |
| Polyunsaturated fat (g) | 20.8(9.3) | 20.0(9.2) | 22.0(10.7) | 21.2(10.4) | 0.27 |
| Protein (g) | 78.0(29.5) | 79.9(31.9) | 82.3(44.8) | 78.1(36.8) | 0.24 |
| Protein (% energy) | 14.1(2.7) | 15.0(2.8) | 13.6(2.8) | 14.2(3.1) | 0.001 |
| Beef/pork/lamb (g) | 55.3(40.7) | 52.1(40.5) | 52.7(44.8) | 50.1(46.2) | 0.05 |
| Processed meat (g) | 20.8(19.3) | 18.7(18.2) | 25.2(25.9) | 24.5(25.3) | 0.006 |

[1] Means (standard deviations) and percents
[2] Metabolic equivalent minutes per day
[3] Greater than or equal to 15 non-steroidal anti-inflammatory drugs per month in the last 5 years
[4] Based on Wilcoxon rank sum test for comparisons between White and African American controls

**Table 8: Odds ratios[1] and 95% confidence intervals for fat and protein intake and risk of rectal cancer (North Carolina Colon Cancer Study-Phase II, N=1904)**

| | Whites (n=1520) | | | | African Americans (n=384) | | | |
|---|---|---|---|---|---|---|---|---|
| Nutrient quartiles | Cases/ controls | Median intake/day in controls | OR | 95%CI | Cases/ controls | Median intake/day in controls | OR | 95%CI |
| Total fat (g) | | | | | | | | |
| Q1 | 146/200 | 45.2 | 1.00 | | 55/40 | 42.7 | 1.00 | |
| Q2 | 156/200 | 67.7 | 0.98 | 0.68-1.39 | 46/40 | 69.8 | 0.55 | 0.26-1.14 |
| Q3 | 206/200 | 91.5 | 1.21 | 0.80-1.82 | 67/40 | 99.6 | 0.55 | 0.23-1.36 |
| Q4 | 212/200 | 132.3 | 1.01 | 0.56-1.81 | 57/39 | 145.2 | 0.32 | 0.10-1.15 |
| P for linear trend | | | | 0.97 | | | | 0.89 |
| Total fat (% energy) | | | | | | | | |
| Q1 | 144/200 | 27.3 | 1.00 | | 70/40 | 28.1 | 1.00 | |
| Q2 | 207/201 | 33.6 | 1.36 | 0.98-1.90 | 70/40 | 34.5 | 1.07 | 0.56-2.01 |
| Q3 | 169/199 | 38.1 | 0.92 | 0.65-1.30 | 50/40 | 38.4 | 0.55 | 0.29-1.07 |
| Q4 | 200/200 | 43.3 | 1.00 | 0.71-1.42 | 35/39 | 44.3 | 0.59 | 0.29-1.21 |
| P for linear trend | | | | 0.25 | | | | 0.27 |
| Saturated fat(g) | | | | | | | | |
| Q1 | 144/200 | 12.9 | 1.00 | | 46/40 | 11.8 | 1.00 | |
| Q2 | 156/200 | 20.0 | 0.96 | 0.67-1.37 | 58/40 | 19.4 | 0.81 | 0.39-1.66 |
| Q3 | 202/200 | 27.9 | 1.14 | 0.76-1.70 | 55/40 | 29.6 | 0.54 | 0.22-1.34 |
| Q4 | 218/200 | 42.1 | 1.05 | 0.61-1.81 | 66/39 | 44.0 | 0.60 | 0.18-1.97 |
| P for linear trend | | | | 0.66 | | | | 0.56 |
| Saturated fat (%energy) | | | | | | | | |
| Q1 | 133/200 | 7.7 | 1.00 | | 64/40 | 7.8 | 1.00 | |
| Q2 | 184/200 | 9.7 | 1.12 | 0.80-1.58 | 71/40 | 9.8 | 1.13 | 0.60-2.15 |
| Q3 | 173/202 | 11.5 | 0.95 | 0.67-1.35 | 43/40 | 11.4 | 0.52 | 0.26-1.04 |
| Q4 | 230/198 | 13.8 | 1.27 | 0.88-1.84 | 47/39 | 13.6 | 0.69 | 0.33-1.42 |
| P for linear trend | | | | 0.27 | | | | 0.004 |

**Table 8 continued**

| | Whites (n=1520) | | | | African Americans (n=384) | | | |
|---|---|---|---|---|---|---|---|---|
| Nutrient quartiles | Cases/ controls | Median intake/day in controls | OR | 95%CI | Cases/ controls | Median intake/day in controls | OR | 95%CI |
| Monounsaturated fat(g) | | | | | | | | |
| Q1 | 151/200 | 17.2 | 1.00 | | 57/40 | 15.5 | 1.00 | |
| Q2 | 161/200 | 26.4 | 0.98 | 0.69-1.39 | 48/40 | 27.1 | 0.56 | 0.27-1.13 |
| Q3 | 192/200 | 35.1 | 1.03 | 0.69-1.53 | 60/40 | 39.0 | 0.57 | 0.24-1.37 |
| Q4 | 216/200 | 51.8 | 0.89 | 0.51-1.55 | 60/39 | 56.7 | 0.41 | 0.12-1.40 |
| P for linear trend | | | | 0.62 | | | | 0.68 |
| Polyunsaturated fat (g) | | | | | | | | |
| Q1 | 150/200 | 10.3 | 1.00 | | 45/40 | 10.3 | 1.00 | |
| Q2 | 180/200 | 15.8 | 1.12 | 0.79-1.58 | 57/40 | 16.5 | 0.77 | 0.38-1.58 |
| Q3 | 185/201 | 21.4 | 1.03 | 0.70-1.51 | 76/40 | 23.0 | 0.78 | 0.33-1.86 |
| Q4 | 205/199 | 30.6 | 0.94 | 0.58-1.55 | 47/39 | 31.8 | 0.28 | 0.08-0.96 |
| P for linear trend | | | | 0.68 | | | | 0.78 |
| Protein (g) | | | | | | | | |
| Q1 | 187/200 | 47.0 | 1.00 | | 45/40 | 38.4 | 1.00 | |
| Q2 | 176/200 | 66.1 | 0.90 | 0.64-1.27 | 55/40 | 60.7 | 1.05 | 0.51-2.16 |
| Q3 | 183/200 | 84.4 | 0.86 | 0.57-1.30 | 66/40 | 85.0 | 0.81 | 0.32-2.05 |
| Q4 | 174/200 | 115.4 | 0.57 | 0.32-1.01 | 59/39 | 122.0 | 0.58 | 0.16-2.10 |
| P for linear trend | | | | 0.74 | | | | 0.93 |
| Protein (% energy) | | | | | | | | |
| Q1 | 272/200 | 11.9 | 1.00 | | 67/40 | 10.7 | 1.00 | |
| Q2 | 191/201 | 14.2 | 0.82 | 0.60-1.12 | 61/40 | 13.0 | 0.99 | 0.51-1.92 |
| Q3 | 140/199 | 15.6 | 0.65 | 0.47-0.91 | 59/40 | 15.2 | 0.95 | 0.47-1.88 |
| Q4 | 117/200 | 18.1 | 0.53 | 0.37-0.77 | 38/39 | 17.2 | 0.60 | 0.28-1.30 |
| P for linear trend | | | | 0.003 | | | | 0.59 |

[1] adjusted forage, sex, education, BMI, family history, NSAID use, physical activity, calcium, fiber, and total energy

**Table 9. Odds ratios[1] and 95% confidence intervals for red meat consumption and risk of rectal cancer (North Carolina Colon Cancer Study-Phase II, N=1904)**

| | Whites (n=1520) | | | | | | African Americans (n=384) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cases/ controls | Median intake/day in controls | OR[1] | 95%CI | OR[2] | OR[3] | Cases/ controls | Median intake/day in controls | OR[1] | 95%CI | OR[2] | OR[3] |
| Total red meat (g) | | | | | | | | | | | | |
| Q1 | 127/200 | 24.4 | 1.00 | | 1.00 | 1.00 | 55/41 | 21.3 | 1.00 | | 1.00 | 1.00 |
| Q2 | 190/202 | 46.9 | 1.30 | 0.93-1.83 | 1.20 | 1.39 | 40/39 | 43.9 | 0.50 | 0.24-1.01 | 0.55 | 0.53 |
| Q3 | 212/198 | 73.7 | 1.24 | 0.86-1.78 | 1.05 | 1.47[4] | 76/40 | 73.1 | 1.01 | 0.51-2.03 | 1.25 | 1.18 |
| Q4 | 191/200 | 123.7 | 0.88 | 0.57-1.37 | 0.68 | 1.33 | 54/39 | 148.3 | 0.55 | 0.22-1.38 | 0.72 | 0.75 |
| P for linear trend | | | | 0.99 | | | | | | 0.99 | | |
| Beef/pork/lamb (g) | | | | | | | | | | | | |
| Q1 | 149/207 | 16.2 | 1.00 | | 1.00 | 1.00 | 58/41 | 12.7 | 1.00 | | 1.00 | 1.00 |
| Q2 | 186/195 | 32.9 | 1.09 | 0.78-1.52 | 1.06 | 1.17 | 39/39 | 27.8 | 0.54 | 0.27-1.09 | 0.61 | 0.58 |
| Q3 | 199/198 | 53.6 | 1.05 | 0.74-1.49 | 0.99 | 1.23 | 65/40 | 45.5 | 0.83 | 0.42-1.63 | 0.95 | 0.95 |
| Q4 | 186/200 | 94.8 | 0.66 | 0.43-1.00 | 0.60[4] | 0.92 | 63/39 | 108.6 | 0.64 | 0.27-1.50 | 0.80 | 0.84 |
| P for linear trend | | | | 0.90 | | | | | | 0.94 | | |
| Processed meat (g) | | | | | | | | | | | | |
| Q1 | 131/204 | 3.4 | 1.00 | | 1.00 | 1.00 | 44/41 | 3.7 | 1.00 | | 1.00 | 1.00 |
| Q2 | 178/202 | 9.6 | 1.15 | 0.82-1.62 | 1.14 | 1.21 | 85/40 | 12.2 | 1.47 | 0.76-2.85 | 1.65 | 1.54 |
| Q3 | 208/198 | 19.1 | 1.43 | 1.02-2.02 | 1.42[4] | 1.56[4] | 42/40 | 24.9 | 0.54 | 0.24-1.18 | 0.62 | 0.59 |
| Q4 | 203/196 | 37.7 | 1.16 | 0.80-1.68 | 1.14 | 1.36 | 54/38 | 42.7 | 0.86 | 0.38-1.96 | 0.59 | 1.02 |
| P for linear trend | | | | 0.57 | | | | | | 0.54 | | |

[1] adjusted for age, sex, education, BMI, family history, NSAID use, physical activity, calcium, fiber, and total energy
[2] adjusted for age, sex, education, BMI, family history, NSAID use, physical activity, calcium, fiber, total energy, and energy-adjusted saturated fat
[3] adjusted for age, sex, education, BMI, family history, NSAID use, physical activity, calcium, fiber, total energy, and energy-adjusted saturated protein
[4] $p<0.05$

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**VI.** Dietary patterns, food groups, and rectal cancer risk in Whites and African Americans

## A. Abstract

Background: Associations between individual foods and nutrients and colorectal cancer have been inconsistent, and few studies have examined associations between food, nutrients, dietary patterns, and rectal cancer. We examined the relationship between food groups and dietary patterns and risk of rectal cancer in non-Hispanic Whites and African Americans. Methods: Data were from the North Carolina Colon Cancer Study-Phase II and included 1520 Whites (720 cases, 800 controls) and 384 African Americans (225 cases, 159 controls). Diet was assessed using the Diet History Questionnaire. Multivariate logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (95% CI). Results: Among Whites, non-whole grains and white potatoes were associated with elevated risk of rectal cancer, while fruit, vegetables, dairy, fish, and poultry were associated with reduced risk. In African Americans, high consumption of citrus fruit and added sugar suggested elevated risk. We identified three major dietary patterns in Whites and African Americans. The High Fat/Meat/Potatoes pattern was observed in both race groups, but was only positively associated with risk in Whites (OR: 1.84, 95% CI 1.03-3.15). The Vegetable/Fish/Poultry and Fruit/Whole-Grain/Dairy patterns in Whites had significant inverse associations with risk. In African Americans, there was a positive dose-response for the Fruit/Vegetables pattern ($P_{trend}$ <0.0001), and an inverse linear trend for the Legumes/Dairy pattern ($P_{trend}$ <0.0001). Conclusion: Our findings indicate that associations

of certain food groups and overall dietary patterns with rectal cancer risk differ between Whites and African Americans, highlighting the importance of examining diet and cancer relationships in racially diverse populations.

**B. Introduction**

Colorectal cancer (CRC) is the fourth most common cancer in the United States (U.S.) among men and women (1). Incidence and mortality rates are highest among African Americans compared to other U.S. race/ethnic groups. While some of this disparity can be attributed to access to care and socioeconomic differences (2), other reasons remain largely unknown. It is generally accepted that diet plays an etiologic role in colorectal cancer development; however, studies examining associations of specific foods and nutrients with CRC risk have been inconsistent. Moreover, most studies have focused on colon cancer only or the combination of colon and rectal cancer, while less attention has been given to the risk of rectal cancer specifically.

The majority of diet and cancer studies examine associations of individual nutrients with disease risk. Examining individual nutrients in relationship to cancer risk is beneficial for gaining insight into possible mechanisms of dietary components. This individual nutrient approach, however, is not adequate for considering the synergistic effect of highly correlated nutrients and other compounds found in foods (3). Other studies have focused on food groups, which take into account the way the foods are typically consumed. Nonetheless, it has been suggested that dietary patterns represent a more logical approach, as the analysis of dietary patterns takes into consideration the synergistic effect of both foods and nutrients, neither of which is consumed in isolation. Dietary patterns include numerous dietary exposures and are often associated with other health behaviors, such as physical activity, smoking, and cancer screening (4). A common approach to identifying dietary patterns is factor analysis, which reduces a large number of variables into a small number of factors based on their degree of correlation (5). These factors then represent dietary patterns in the

study population and are used as predictors in subsequent analyses of risk. Comparisons between the food/nutrient and dietary pattern approaches among previous studies have been difficult due to differences in study design, study populations, and statistical methods.

As noted above, few studies have examined associations of diet with rectal cancer risk separately, as most have combined rectal and colon cancers. However, true mechanisms underlying the etiology of colon and rectal tumors may be different (6, 7). The objective of this work is to examine associations of food groups and dietary patterns (based on factor analysis) with risk of rectal cancer in a population-based case-control study of non-Hispanic Whites (Whites) and African Americans in North Carolina. To our knowledge, this is the first population-based study to examine these relationships in a racially diverse U.S. population.

## C. Methods

1. Study design and population

The North Carolina Colon Cancer Study-Phase II is a population-based case control study in a 33-county area in central North Carolina. These counties include rural, suburban, and urban areas and are socioeconomically diverse. Participants were selected using a randomized recruitment strategy that over-sampled African Americans and involved matching on 5-year age, sex, and race. This study was approved by the University of North Carolina's Institutional Review Board.

*Cases*

Rectal cancer cases were identified by the North Carolina Central Cancer Registry rapid ascertainment system and included those with cancers of the rectum, sigmoid, and rectosigmoid junction (ICD 154). Eligibility criteria for cases included: age 40-79 at time of

diagnosis, diagnosed with a primary adenocarcinoma between May 2001 and September 2006, have a North Carolina driver's license or identification (because controls under 65 were selected from Department of Motor Vehicle rosters), and able to give informed consent and complete the interview. All diagnoses were confirmed by the study pathologist through review of pathology slides and reports. Cases with a non-invasive carcinoma or a previous diagnosis of colorectal cancer were excluded. After notification of the primary physician, eligible cases were sent a letter describing the study and a race-matched enrollment specialist contacted them to explain the study and obtain their consent to participate. Interviews were scheduled for consenting cases. There were a total of 1831 cases sampled, 1417 of whom were eligible to participate. Of the eligible cases, 118 (8%) were unable to be contacted, 242 (17%) refused, and 1057 (75%) were interviewed. The response rate, (number of persons interviewed divided by the total number of eligible persons), was 76% and 70% for Whites and African Americans, respectively.

*Controls*

Using lists provided by the agencies, controls were randomly identified from the North Carolina Department of Motor Vehicles (NC DMV) (for those less than age 65) and from the Center for Medicaid and Medicare Services (CMS, formerly known as the Health Care Financing Administration), for those age 65 and older. Eligible controls were 40-79 years old at the time of selection, resided in the 33-county study area, and had no previous diagnosis of colorectal cancer. Similar to cases, potential controls were sent an introductory letter and contacted by a race-matched enrollment specialist and in-person interviews were scheduled for controls who agreed to participate. Among eligible controls (1,827 out of 2,345 sampled), 325 (18%) could not be contacted, 483 (26%) refused, and 1019 (56%) were

interviewed. The response rates were 58% and 46% for White and African American controls, respectively.

2. Data collection

All data were collected by trained nurse-interviewers in participants' home or other convenient location.

Usual dietary intake was assessed using the Diet History Questionnaire (DHQ) developed and tested for validity by the National Cancer Institute (8-10). This instrument was validated in study samples that were racially diverse, with African Americans representing 10-14% of these study samples (9, 10). The DHQ consists of 124 separate food items and assesses the frequency of consumption and portion size consumed for each food item. Participants were asked to estimate their food and beverage intake in the past 12 months. The 12-month period was chosen to take into account seasonal variation in food consumption. Cases were asked to estimate their usual frequency and portion size over the 12 month period prior to diagnosis, and controls were asked to estimate consumption during the 1 year prior to interview. Daily intakes of nutrients and total energy were calculated with software provided by the NCI and developed for the survey instrument. Nutrient intakes were determined using the frequency of consumption, reported portion size, and nutrient content. For the food group analysis, we examined the following U.S. Department of Agriculture (USDA) pyramid food groups (11) : total grains, whole grains, non-whole grains (e.g. white bread, pasta, cereal), total vegetables, dark green vegetables, deep yellow vegetables, dry beans and peas, white potatoes, starchy vegetables, tomatoes, other vegetables(e.g. cabbage, cauliflower, Brussels sprouts, onions), total fruits, citrus fruits(including melons and berries), other fruits, total

dairy, milk, yogurt, cheese, total meat, beef/pork/lamb (i.e. red meat), processed meats, organ meats, fish and other seafood, poultry, eggs(i.e. eggs, egg whites, egg substitutes), soy products, nuts (e.g. peanuts, walnuts, seeds), added sugar (sugars added during processing, cooking, or at the table), and discretionary fat (i.e. excess fat in foods and fat added to foods). Average weekly intakes were calculated for each food group. There was a large proportion of non-consumers for the yogurt, organ meat, and soy food groups (58%, 49%, 76%, respectively). For this reason, we dichotomized (consumers vs. non-consumers) these foods in the food group analysis, and combined the yogurt group with the milk food group and excluded the organ meat and soy food groups in the factor analysis.

The participant questionnaire queried age at diagnosis, sex, race, education, annual income, use of non-steroidal anti-inflammatory drugs, smoking history, and first degree family history of colorectal cancer.

The analyses were restricted to participants who completed all components of the study (n=1987). Participants with unreliable reported energy intakes (<800 kcal/day and >5000 kcal/day for men and <600 kcal/day and >4000 kcal/day for women) were also excluded (n=83 (50 men, 33 women)) because they were considered implausible based on daily energy requirements (12). Thus, the analytic sample for this report included 1520 Whites (720 cases, 800 controls) and 384 African Americans (225 cases, 159 controls).

3. Statistical analyses

Descriptive statistics (means, standard deviations, and frequencies) were computed for all study variables by case-control status and race to describe the demographic and dietary characteristics of the study population. Results were stratified by race because tests for

interaction indicated the presence of effect modification by race for some of the demographic and dietary variables. Each food group was categorized into quartiles based on the distribution among race-specific controls. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated using unconditional logistic regression models to determine the association between the food groups and rectal cancer risk. These food group models included an offset term to account for the randomized recruitment and to allow us to obtain unbiased odds ratios, as well as the following covariates: age (continuous), sex, socioeconomic status (represented by education (less than or equal to high school, some college, college graduate/advanced degree) and income (categorized)), BMI 1 year ago (i.e., in the year prior to interview for controls and diagnosis for cases) (normal, overweight, obese), physical activity (continuous), family history (yes, no), non-steroidal anti-inflammatory drug use (yes, no), and total energy intake (continuous).

Dietary patterns were identified separately among White and African American controls using 21 predefined food groups in a principal components factor analysis. This analysis was conducted using the PROC FACTOR procedure in SAS. To determine the number of factors to retain, we considered eigenvalues >1, the scree plot, and the interpretability of the factors. Extraction of these factors was followed by orthogonal rotation (the varimax rotation option in SAS) to obtain uncorrelated factors and enhance interpretability. For each dietary pattern (factor), a factor score was calculated for cases and controls by summing intakes of the food items weighted by their factor loadings. Pearson and Spearman correlation coefficients were used to examine the correlation of factor scores for each dietary pattern with other participant characteristics and dietary variables. Partial Pearson correlation coefficients adjusted for energy were obtained for the dietary variables.

Factor scores were categorized into quartiles based on the distribution in the control population for African Americans and Whites separately. To determine the relationship between these dietary patterns and rectal cancer, we used unconditional logistic regression models to obtain odds ratios and 95% confidence intervals. Test for trend was conducted by incorporating a variable for the median values of factor scores among race-specific controls observed for each food group quartile into a logistic regression model. The trend test was weighted by the inverse of the variance for the quartiles. All logistic regression models were adjusted for the same covariates as in the food group models.

All analyses were performed using SAS 9.1 software (SAS Institute, Inc., Cary, NC). Statistical tests were two-sided and $p<0.05$ was considered statistically significant.

**D. Results**

The distribution of cases and controls by race is shown in Table 10. Among Whites, controls were older and more educated, had a slightly lower mean BMI 1 year ago, and used more non-steroidal anti-inflammatory drugs than cases. Among African Americans, the mean age was less for cases than controls, while cases had a higher mean BMI. In addition, a larger proportion of African American cases had a family history of CRC compared to controls. All of these participant characteristics were significantly associated with the risk of rectal cancer in multivariate models, except annual income, smoking status, and family history (data not shown).

Tables 11 and 12 give the covariate-adjusted race-specific odds ratios (OR) and 95% confidence intervals for each food group among Whites and African Americans, respectively. The odds ratios presented are not mutually adjusted for the other food groups, although

estimates were similar when we controlled for the other primary food groups. For Whites, high intakes of non-whole grains and white potatoes were significantly positively associated with rectal cancer risk (Table 11). Conversely, fruit, dark green vegetables, deep yellow vegetables, other starchy vegetables, other vegetables, dairy foods, fish, and poultry were significantly associated with reduced risk for rectal cancer. High consumption of dark green vegetables had the strongest inverse association (OR: 0.41, 95% CI 0.29-0.58). The highest quartile of red meat intake had an OR less than 1, but was not statistically significant. High intake of other fruit and added sugar were associated with elevated risk in African Americans (OR: 3.25 95% CI 1.52-6.96 for other fruit; OR: 2.65 95% CI 1.11-6.34 for added sugar) (Table 12). There was a significant lower risk associated with the second quartile of intake of total vegetables, other vegetables, total meat, and discretionary fat in African Americans.

Three dietary patterns were identified separately among White and African American controls using principal components analysis. These 3 patterns explained 39% of the variance in Whites and 43% of the variance in African Americans. Table 13 presents the factor loadings for the food groups on each dietary pattern for each race group. The first dietary pattern, High Fat/Meat/Potatoes, was similar for both Whites and African Americans and had strong positive loadings for discretionary fat, non-whole grains, white potatoes, red and processed meat, cheese, and added sugar. The second and third factors were only slightly different for Whites and African Americans. For Whites, the second dietary pattern was characterized by high loadings of most vegetables, as well as fish and poultry, and was therefore labeled the "Vegetable/Fish/Poultry" (abbreviated as Veg/Fish/Poultry) pattern. The third dietary factor in Whites was labeled "Fruit/Whole Grain/Dairy" because of its high positive loadings of fruit, whole grains, and milk/yogurt. In African Americans, fruits also

loaded heavily on the second factor in addition to vegetables. This factor was labeled "Fruit/Vegetables". The third factor in African Americans had strong loadings of nuts, beans and peas, and milk/yogurt, and was labeled "Legumes/Dairy".

Table 14 shows correlations of the three separate dietary patterns in Whites and African Americans with selected participant characteristics and dietary variables. Age was inversely correlated with the High Fat/Meat/Potatoes pattern for both race groups, whereas education and income were positively correlated with the Veg/Fish/Poultry pattern in Whites. The dietary variables presented are those related to energy intake (i.e. total energy, fat, carbohydrate, protein, alcohol). Folate and fiber were included because of their high content in fruits and vegetables. The High Fat/Meat/Potatoes pattern had the highest correlation with total energy in Whites (r=0.86) and African Americans (r=0.82), while inversely related to carbohydrates, alcohol, folate, and fiber. The Veg/Fish/Poultry pattern in Whites had a strong positive correlation with protein, and the Fruit/Vegetable pattern in African Americans was highly correlated with folate and fiber.

Associations (odds ratios and their 95% CI) of the dietary patterns (according to quartiles of factor scores) with rectal cancer risk, stratified by race, are given in Table 15. Estimates based on race-specific quartile cut-points are shown, although similar associations were observed when quartile cut-points were matched across ethnic groups. Among Whites, high factor scores for the High Fat/Meat/Potatoes pattern had odds ratios suggestive of elevated rectal cancer risk (OR: 1.84, 95% CI 1.08-3.15). The second and third patterns in Whites were significantly associated with reduced risk of rectal cancer. The ORs for the highest quartiles for the Veg/Fish/Poultry and Fruit/Whole-grain/Dairy patterns were 0.47 (95% CI 0.33-0.67) and 0.65 (95% CI 0.45-0.93), respectively. In African Americans, the

High Fat/Meat/Potatoes and Legumes/Dairy patterns were suggestive of reduced risk, while the Fruit/Vegetables pattern suggested elevated risk. None of the quartile estimates reached statistical significance. There was, however, evidence of a positive linear trend for the Fruit/Vegetables pattern and an inverse dose-response for the Legumes/Dairy patterns ($p<0.0001$ for both). We did not observe any effect modification by gender for any of the food group totals or dietary patterns.

**E. Discussion**

This population-based case-control study examined the relationship of food groups and dietary patterns with the risk of rectal cancer in Whites and African Americans. High intakes of fruit, vegetables, and dairy were associated with reduced rectal cancer risk in Whites, while African Americans had an elevated risk associated with other fruit and added sugar. We identified three major dietary patterns and investigated the relationship between these patterns and rectal cancer. The first dietary pattern, High Fat/Meat/Potatoes, was similar for Whites and African Americans, while the other two patterns differed slightly. To our knowledge, this is the first study to examine these associations in African Americans.

Increased consumption of whole grain foods, as well as fruit, vegetables, and dairy products, has generally been associated with reduced colon and rectal cancer risk in epidemiologic studies (13-15), although results have not been entirely consistent. The potentially protective role of these food groups has been attributed to their fiber content and micronutrients such as vitamins, carotenoids, calcium, and folate (16-18). Our study showed that fruit, some vegetables, and dairy foods were associated with reduced risk in Whites. Our findings support evidence from a case-control study by Slattery, et al. that reported

significant rectal cancer risk reductions for high consumption of fruit and vegetables in a predominantly White population (15).

The relationship between fruit and vegetables and rectal cancer risk in our study varied by race and food subgroups. Contrary to our results which showed risk reductions associated with specific fruits and vegetables among Whites, Michels et al. did not find a protective effect for total fruit and vegetable intake, or any subgroups of fruit and vegetables, on colon and rectal cancer incidence (19). High fruit consumption in African Americans correlated with significantly higher risk of rectal cancer. This strong positive association remained after adjustment for other dietary variables such as citrus fruit, added sugar, and total carbohydrate intake. The elevated risk may be due to high intakes of high-calorie fruit juice or low intakes of fresh fruit.

Interestingly, high intake of the red meat in our study population was not significantly associated with rectal cancer risk. It has been hypothesized that the high heme iron content in red meat enhances free radical production and tumor cell proliferation (20, 21), and that the fat content of red meat may increase the production of bile acids, also causing cellular proliferation (22). Some studies have shown elevated rectal cancer risk to be associated with high consumption of red meat (23, 24) and processed meat (24, 25). Our results are in agreement with findings by Wei, et al. which also showed no association between increased consumption of red meat and rectal cancer risk (7), although our findings do suggest elevated risk for high intake of processed meat in Whites.

Fish and other seafood may play an important role in rectal cancer risk reduction perhaps due to their rich omega-3 polyunsaturated fatty acid (PUFA) content , which may reduce the production of pro-inflammatory eicosanoids (26, 27). Although the effects of fish

and poultry on colon and rectal cancer risk have been examined less often compared to red meat, at least five studies have shown fish and poultry to be associated with reduced risk of colorectal cancer (23, 24, 28-30). Three of these studies reported an inverse relationship between these food groups and risk of rectal cancer specifically (23, 24, 28), as we did in this present study among Whites. Fish and poultry had a non-significant positive association with risk in African Americans, which may reflect how these foods were prepared. However, the results did not change when we adjusted for total fat intake.

Three dietary patterns were identified separately among White and African American controls using principal components factor analysis. The High Fat/Meat/Potatoes dietary pattern was similar in both race groups. Comparable dietary patterns in some cohort studies have found no association of this pattern with colon or rectal cancer risk (31, 32). However, other studies in which this pattern was labeled "Western" and "red meat" have reported significant elevated risk of colon cancer and CRC, respectively (4,33). Our results for Whites are consistent with these findings because high factor scores among Whites for the High Fat/Meat/Potatoes pattern were associated with elevated risk.

In addition to a type of "Western" dietary pattern, researchers have often identified a presumably healthy pattern that has been labeled "healthy", "prudent", and "vegetable" patterns in some studies (34-38). Among Whites in our study, potentially healthy patterns emerged as two distinct dietary patterns, i.e. the Veg/Fish/Poultry and the Fruit/Whole Grain patterns; both were associated with reduced risk of rectal cancer. Interestingly, the Veg/Fish/Poultry pattern had weak factor loadings for fruits and dairy products, and the Fruit/Whole Grain pattern had only weak to modest loadings for most vegetables. This suggests that it may not be appropriate to combine fruit and vegetables as an individual food

group. In African Americans, the two presumably healthy patterns were the Fruit/Vegetables pattern and the Legumes/Dairy pattern. There was a positive linear relationship between the Fruit/Vegetable pattern and rectal cancer. This could be due to the heavy loadings of fruit, especially citrus fruit, which also showed a significant positive trend in risk in the food group analysis. The Legumes/Dairy pattern in African Americans suggested a protective effect on risk, as was expected.

These dietary patterns only accounted for 39% and 43% of the total variance in Whites and African Americans, respectively, which suggests that other patterns exist. There were a total of 5 factors in Whites and 7 factors in African Americans that had eigenvalues greater than 1.0, and together these factors explained 50% and 65% of the variance, respectively. However, these factors not presented were difficult to interpret. The low proportion of variance explained by the 3 factors in race group could also be due, in part, to the limited number of foods entered in the factor analysis, or a reflection of the overall complexity of the diet.

Our findings provide evidence that rectal cancer risk differs between African Americans and Whites for certain foods and dietary patterns. Unfortunately, there are virtually no studies of diet and colon and rectal cancer associations in African Americans. Similar racial differences were reported by Satia-Abouta, et al. in a population-based study of food groups and colon cancer (14). Few studies have conducted comparisons of dietary patterns for Whites and African Americans (39-41). The dietary patterns in our study were similar to those identified in the Multiethnic Cohort Study, which also used the USDA food groups for the factor analysis (41). Bell and colleagues reported that food patterns among Whites and African Americans did not differ. Although the patterns were generally similar

in both race groups in our study, there were some different associations with rectal cancer risk. The observed heterogeneity in risk may in part be due to racial variation in dietary intake of certain foods and nutrients as reported in some studies (42-44). We used race-specific cut-points for food groups and dietary patterns to account for possible differences in consumption, although this could have affected our assessment of racial differences in risk. We cannot exclude the possibility that socioeconomic status contributes to this racial disparity; however, we controlled for both education and income in our analyses.

Our study has many strengths, including its population-based design and the inclusion of a large number of rectal cancer cases. Also, the randomized recruitment strategy used to select participants minimized the possibility of selection bias in our results. Over-sampling allowed us to increase the number of African Americans in our study sample in an effort to assess racial differences. Both food group analysis and factor analysis were examined in the same population and included the same covariates.

There are also some limitations to our study. The use of predefined food groups in the factor analysis may have introduced error in our risk estimates. Grouping foods prevents the food items within the group from having different loadings on the dietary patterns identified and may obscure differences in consumption. However, the consistent use of food groupings may enable us to better compare studies of dietary patterns. Food frequency questionnaires, like that used in this study, are subject to measurement error and may not have included some typically consumed Southern foods (45) or foods common to certain races/ethnicities. Due to our case-control study design, recall bias is a possibility. Response bias may also have been introduced in our study, especially because the response rate was lower among African Americans than Whites, and lower among controls compared to cases.

Although we over-sampled African Americans, the sample size for this subpopulation was relatively small (N=384). This resulted in less power to detect significant associations in African Americans and unstable risk estimates. Therefore, these findings should be interpreted with caution and need to be confirmed in a larger sample of African Americans.

In summary, this study used two different approaches to investigate the relationship between diet and rectal cancer risk: food group analysis and factor analysis. Our results showed that several food groups and dietary patterns are associated with rectal cancer risk. Some of the food groups yielded different associations with risk than the overall pattern with which it was highly correlated. Complex correlations between foods may be better captured by dietary patterns, which may also prove to be more amenable to translation into dietary recommendations, and easier to apply to improve the efficacy of nutrition intervention and prevention programs. Notably, our results suggest that dietary risk factors may differ by race, which highlights the importance of examining diet and cancer associations in racially diverse study populations.

## F. Tables

**Table 10: Characteristics of participants by case/control status and race (North Carolina Colon Cancer Study-Phase II)**

| | Whites (N=1520) | | African Americans (N=384) | |
|---|---|---|---|---|
| | Cases (n=720) | Controls (n=800) | Cases (n=225) | Controls (n=159) |
| Sex (%) | | | | |
| Male | 58 | 61 | 52 | 52 |
| Age (years) (%) | | | | |
| 40-49 | 19 | 12 | 21 | 18 |
| 50-59 | 28 | 27 | 29 | 23 |
| 60-69 | 32 | 34 | 34 | 42 |
| 70-79 | 22 | 27 | 16 | 18 |
| Mean(SD) | 59.6(10.3) | 61.7(9.8) | 58.0(10.0) | 60.3(9.8) |
| Education (%) | | | | |
| <=High School | 50 | 39 | 62 | 59 |
| Some College | 25 | 26 | 22 | 26 |
| College grad/Adv degree | 25 | 35 | 16 | 16 |
| Annual Income (%) | | | | |
| <$20,000 | 21 | 18 | 47 | 52 |
| $20,000-$34,999 | 21 | 18 | 19 | 16 |
| $35,000-$49,999 | 15 | 15 | 11 | 8 |
| $50,000-$74,999 | 20 | 23 | 13 | 15 |
| >$75,000 | 24 | 27 | 11 | 10 |
| Body Mass Index (1yr ago) (%) | | | | |
| Normal (18.5-24.9 $kg/m^2$) | 23 | 30 | 18 | 18 |
| Overweight (25.0-29.9 $kg/m^2$) | 39 | 41 | 32 | 36 |
| Obese ($\geq$30.0 $kg/m^2$) | 39 | 29 | 51 | 46 |
| Mean(SD) | 29.2(6.3) | 28.0(5.5) | 31.6(7.7) | 29.9(6.5) |
| Smoking Status (%) | | | | |
| Current Smoker | 16 | 14 | 23 | 17 |
| Former Smoker | 47 | 49 | 38 | 42 |
| Never Smoker | 37 | 38 | 39 | 41 |
| Mean(SD) years of smoking | 26.9(15.6) | 25.5(16.7) | 24.3(16.3) | 25.2(17.9) |
| Physical activity (MET-min/day*) (%) | | | | |
| Quartile 1 | 25.4 | 24.5 | 30.7 | 28.9 |
| Quartile 2 | 24.4 | 23.5 | 25.5 | 28.9 |
| Quartile 3 | 21.1 | 26.5 | 16.0 | 19.5 |
| Quartile 4 | 29.1 | 25.3 | 27.8 | 22.8 |
| Mean(SD) | 2250.0(661.8) | 2152.7(473.4) | 2178.4(545.5) | 2152.8(494.2) |
| NSAID use† (%) | | | | |
| Yes | 35 | 46 | 24 | 23 |
| First-degree family history of colorectal cancer (%) | | | | |
| Yes | 13 | 11 | 12 | 5 |

*Metabolic equivalent minutes per day
†greater than or equal to 15 non-steroidal anti-inflammatory drugs (NSAID) per month in the past 5 years

**Table 11: Odds ratios and 95% confidence intervals for rectal cancer among Whites according to food groups (North Carolina Colon Cancer Study-Phase II)***

| Food Group (servings/week) | Q1 | Q2 | Q3 | Q4 | P for trend |
|---|---|---|---|---|---|
| Total grains | 154/202 (20.7)‡ | 161/199 (32.3) | 181/199 (41.5) | 224/200 (60.7) | |
| OR (95% CI) | 1.00 | 1.09 (0.77-1.55) | 1.21 (0.84-1.75) | 1.44 (0.92-2.25) | 0.09 |
| Whole grains | 204/200 (2.8) | 182/203 (6.3) | 174/198 (10.2) | 160/199 (16.4) | |
| OR (95% CI) | 1.00 | 1.03 (0.74-1.42) | 0.92 (0.66-1.27) | 0.93 (0.66-1.31) | 0.55 |
| Non-whole grains | 140/200 (14.7) | 149/201 (23.6) | 200/200 (32.3) | 231/199 (48.0) | |
| OR (95% CI) | 1.00 | 1.19 (0.83-1.71) | 1.46 (1.01-2.12) | 1.60 (1.01-2.53) | 0.04 |
| Total fruit | 243/204 (7.35) | 190/201 (14.3) | 136/199 (21.0) | 151/199 (32.2) | |
| OR (95% CI) | 1.00 | 0.83 (0.60-1.13) | 0.63 (0.45-0.87) | 0.62 (0.44-0.86) | 0.0021 |
| Citrus fruit | 223/200 (1.89) | 218/199 (5.6) | 145/201 (9.7) | 134/200 (16.4) | |
| OR (95% CI) | 1.00 | 0.97 (0.71-1.33) | 0.71 (0.51-0.99) | 0.61 (0.43-0.86) | 0.0012 |
| Other fruit | 232/202 (3.01) | 161/198 (7.1) | 177/200 (11.5) | 150/200 (18.5) | |
| OR (95% CI) | 1.00 | 0.74 (0.54-1.03) | 0.83 (0.60-1.14) | 0.67 (0.48-0.94) | 0.04 |
| Total vegetables | 207/202 (14.7) | 186/202 (23.7) | 149/165 (31.4) | 178/201 (44.6) | |
| OR (95% CI) | 1.00 | 0.97 (0.70-1.34) | 0.76 (0.53-1.09) | 0.73 (0.50-1.06) | 0.07 |
| Tomato | 197/201 (1.3) | 190/205 (2.4) | 168/197 (3.6) | 165/197 (6.5) | |
| OR (95% CI) | 1.00 | 1.00 (0.73-1.38) | 0.89 (0.63-1.25) | 0.86 (0.60-1.23) | 0.35 |
| Dark green vegetables | 277/206 (0.6) | 173/196 (1.7) | 152/198 (3.1) | 118/200 (6.4) | |
| OR (95% CI) | 1.00 | 0.68 (0.50-0.93) | 0.59 (0.43-0.81) | 0.41 (0.29-0.58) | <0.0001 |
| Deep yellow vegetables | 286/229 (0.5) | 149/181 (1.0) | 148/196 (1.8) | 137/194 (3.6) | |
| OR (95% CI) | 1.00 | 0.72 (0.52-0.99) | 0.60 (0.43-0.83) | 0.65 (0.46-0.90) | 0.02 |
| Beans and peas | 169/179 (0.1) | 211/233 (0.6) | 176/188 (1.2) | 164/200 (2.7) | |
| OR (95% CI) | 1.00 | 1.02 (0.74-1.41) | 0.97 (0.69-1.37) | 0.91 (0.64-1.30) | 0.52 |
| White potatoes | 112/209 (1.3) | 168/198 (3.3) | 178/189 (5.6) | 262/204 (9.3) | |
| OR (95% CI) | 1.00 | 1.57 (1.10-2.23) | 1.83 (1.27-2.63) | 2.55 (1.74-3.73) | <0.0001 |
| Other starchy vegetables | 204/204 (0.8) | 167/186 (1.8) | 185/210 (3.0) | 164/200 (5.2) | |
| OR (95% CI) | 1.00 | 0.77 (0.56-1.07) | 0.84 (0.61-1.17) | 0.64 (0.45-0.91) | 0.026 |
| Other vegetables | 232/204 (5.0) | 159/197 (8.3) | 173/200 (11.8) | 156/199 (18.5) | |
| OR (95% CI) | 1.00 | 0.76 (0.54-1.05) | 0.79 (0.56-1.09) | 0.66 (0.47-0.94) | 0.04 |
| Total dairy | 203/202 (3.6) | 208/201 (6.7) | 170/198 (10.9) | 139/199 (17.4) | |
| OR (95% CI) | 1.00 | 0.82 (0.59-1.12) | 0.66 (0.47-0.93) | 0.47 (0.32-0.69) | <0.0001 |
| Cheese | 189/191 (0.6) | 208/214 (1.5) | 155/194 (2.6) | 168/201 (5.9) | |
| OR (95% CI) | 1.00 | 1.02 (0.74-1.41) | 0.69 (0.48-0.99) | 0.73 (0.50-1.06) | 0.06 |
| Milk | 183/205 (1.4) | 190/198 (3.7) | 204/197 (6.6) | 143/200 (12.7) | |
| OR (95% CI) | 1.00 | 0.97 (0.70-1.35) | 1.02 (0.73-1.42) | 0.66 (0.46-0.95) | 0.017 |
| Yogurt | 435/430 (0.0) | 285/370 (0.42) | | | |
| OR (95% CI)† | 1.00 | 0.69 (0.53-0.89) | | | --- |
| Total meat | 154/200 (4.2) | 208/202 (7.0) | 184/198 (10.2) | 174/200 (15.7) | |
| OR (95% CI) | 1.00 | 1.29 (0.92-1.82) | 0.97 (0.67-1.40) | 0.78 (0.50-1.21) | 0.07 |
| Red meat | 148/199 (1.30) | 187/203 (2.7) | 198/198 (4.4) | 187/200 (7.8) | |
| OR (95% CI) | 1.00 | 1.14 (0.81-1.60) | 1.22 (0.85-1.74) | 0.85 (0.56-1.28) | 0.26 |
| Organ meat† | 380/425 (0.0) | 340/375 (0.23) | | | |
| OR (95% CI)† | 1.00 | 0.89 (0.70-1.13) | | | --- |
| Processed meat | 131/204 (0.3) | 178/202 (0.8) | 208/198 (1.6) | 203/196 (3.1) | |
| OR (95% CI) | 1.00 | 1.16 (0.82-1.64) | 1.45 (1.03-2.05) | 1.27 (0.87-1.85) | 0.26 |
| Fish | 233/194 (0.3) | 194/209 (0.9) | 157/197 (1.5) | 136/200 (2.7) | |
| OR (95% CI) | 1.00 | 0.72 (0.53-0.99) | 0.68 (0.48-0.94) | 0.52 (0.36-0.73) | 0.0004 |
| Poultry | 185/202 (0.6) | 210/199 (1.3) | 175/194 (2.2) | 150/205 (4.0) | |
| OR (95% CI) | 1.00 | 1.15 (0.83-1.59) | 0.96 (0.68-1.34) | 0.68 (0.47-0.98) | 0.01 |
| Eggs | 175/192 (0.6) | 175/209 (1.4) | 149/202 (2.5) | 221/197 (4.2) | |
| OR (95% CI) | 1.00 | 1.05 (0.75-1.47) | 0.81 (0.57-1.14) | 1.07 (0.76-1.50) | 0.86 |

**Table 11 continued**

| Food Group (servings/week) | Q1 | Q2 | Q3 | Q4 | P for trend |
|---|---|---|---|---|---|
| Nuts | 192/216 (0.2) | 188/189 (0.7) | 199/198 (1.5) | 141/197 (4.2) | |
| OR (95% CI) | 1.00 | 1.24 (0.90-1.71) | 1.26 (0.90-1.76) | 0.92 (0.64-1.32) | 0.24 |
| Soy | 558/578 (0.0) | 162/222 (0.07) | | | |
| OR (95% CI)† | 1.00 | 0.91 (0.70-1.20) | | | --- |
| Added sugar (g) | 163/200 (177.5) | 144/200 (314.0) | 171/200 (489.0) | 242/200 (832.7) | |
| OR (95% CI) | 1.00 | 0.84 (0.60-1.19) | 0.90 (0.63-1.28) | 1.19 (0.80-1.77) | 0.19 |
| Discretionary fat (g) | 146/200 (237.6) | 153/200 (373.7) | 205/200 (514.2) | 216/200 (745.9) | |
| OR (95% CI) | 1.00 | 0.99 (0.69-1.42) | 1.37 (0.92-2.05) | 1.32 (0.76-2.28) | 0.21 |

*****adjusted for age, sex, education, income. BMI 1 year ago, physical activity, family history, non-steroidal anti-inflammatory drug use, and total energy intake

† OR represents consumers vs. non-consumers (referent)

‡ number of cases/number of controls (median intake in controls)

**Table 12: Odds ratios and 95% confidence intervals for rectal cancer among African Americans according to food groups (North Carolina Colon Cancer Study-Phase II)***

| Food Group (servings/week) | Q1 | Q2 | Q3 | Q4 | P for trend |
|---|---|---|---|---|---|
| Total grains | 64/40 (20.1)‡ | 60/40 (35.5) | 44/40 (45.5) | 57/39 (65.4) | |
| OR (95% CI) | 1.00 | 0.70 (0.35-1.41) | 0.55 (0.24-1.28) | 0.52 (0.19-1.40) | 0.19 |
| Whole grains | 72/41 (2.9) | 59/40 (6.3) | 52/39 (10.6) | 42/39 (18.9) | |
| OR (95% CI) | 1.00 | 1.19 (0.59-2.39) | 0.91 (0.45-1.83) | 0.67 (0.21-1.42) | 0.20 |
| Non-whole grains | 44/40 (14.4) | 71/40 (25.7) | 49/40 (37.5) | 61/39 (53.5) | |
| OR (95% CI) | 1.00 | 1.18 (0.58-2.43) | 0.83 (0.35-2.00) | 1.08 (0.37-3.12) | 0.99 |
| Total fruit | 42/40 (7.9) | 33/40 (13.7) | 73/41 (22.8) | 77/38 (38.5) | |
| OR (95% CI) | 1.00 | 0.91 (0.42-1.97) | 2.22 (1.05-4.72) | 1.90 (0.88-4.10) | 0.05 |
| Citrus fruit | 37/40 (2.3) | 60/40 (5.7) | 57/41 (10.6) | 71/38 (21.7) | |
| OR (95% CI) | 1.00 | 1.97 (0.94-4.17) | 1.67 (0.79-6.54) | 1.54 (0.71-3.35) | 0.68 |
| Other fruit | 41/40 (3.1) | 41/39 (7.4) | 43/41 (11.7) | 100/39 (20.4) | |
| OR (95% CI) | 1.00 | 1.18 (0.53-2.62) | 1.33 (0.61-2.90) | 3.25 (1.52-6.96) | 0.0004 |
| Total vegetables | 64/40 (11.7) | 26/40 (19.3) | 60/40 (27.4) | 75/39 (45.9) | |
| OR (95% CI) | 1.00 | 0.36 (0.17-0.79) | 0.79 (0.38-1.64) | 0.90 (0.40-2.04) | 0.58 |
| Tomato | 63/46 (0.6) | 55/31 (1.4) | 47/43 (2.4) | 60/39 (4.2) | |
| OR (95% CI) | 1.00 | 0.83 (0.40-1.72) | 0.58 (0.29-1.19) | 0.85 (0.40-1.81) | 0.64 |
| Dark green vegetables | 61/40 (0.7) | 39/40 (1.8) | 50/40 (3.6) | 75/39 (8.7) | |
| OR (95% CI) | 1.00 | 0.54 (0.25-1.15) | 0.58 (0.28-1.20) | 1.00 (0.48-2.08) | 0.42 |
| Deep yellow vegetables | 63/47 (0.3) | 45/31 (0.8) | 59/42 (1.5) | 58/39 (3.4) | |
| OR (95% CI) | 1.00 | 1.08 (0.52-2.26) | 0.72 (0.35-1.48) | 0.78 (0.36-1.66) | 0.45 |
| Beans and peas | 70/46 (0.1) | 71/35 (0.6) | 37/39 (1.3) | 47/39 (2.6) | |
| OR (95% CI) | 1.00 | 1.18 (0.60-2.31) | 0.57 (0.27-1.17) | 0.49 (0.23-1.07) | 0.02 |
| White potatoes | 50/41 (1.0) | 63/39 (2.8) | 45/40 (4.5) | 67/39 (8.9) | |
| OR (95% CI) | 1.00 | 0.96 (0.46-1.99) | 0.51 (0.23-1.14) | 0.97 (0.42-2.26) | 0.89 |
| Other starchy vegetables | 62/40 (0.8) | 52/42 (1.5) | 43/37 (2.7) | 68/40 (5.3) | |
| OR (95% CI) | 1.00 | 0.94 (0.46-1.94) | 0.61 (0.29-1.29) | 0.87 (0.40-1.87) | 0.75 |
| Other vegetables | 54/42 (3.6) | 38/38 (6.5) | 60/39 (8.8) | 73/40 (17.7) | |
| OR (95% CI) | 1.00 | 0.39 (0.18-0.82) | 0.75 (0.36-1.57) | 0.87 (0.39-1.90) | 0.66 |
| Total dairy | 37/40 (1.5) | 49/40 (3.4) | 66/40 (6.8) | 73/39 (13.3) | |
| OR (95% CI) | 1.00 | 0.93 (0.44-1.97) | 1.04 (0.47-2.32) | 1.18 (0.53-2.62) | 0.55 |
| Cheese | 49/36 (0.2) | 46/46 (0.8) | 68/39 (1.7) | 62/38 (4.6) | |
| OR (95% CI) | 1.00 | 0.633 (0.30-1.31) | 0.84 (0.39-1.81) | 1.04 (0.44-2.46) | 0.50 |
| Milk | 37/36 (0.6) | 62/44 (2.1) | 60/39 (4.1) | 66/40 (8.6) | |
| OR (95% CI) | 1.00 | 0.94 (0.45-1.96) | 0.78 (0.35-1.75) | 0.90 (0.41-1.95) | 0.85 |
| Yogurt | 142/104 (0.0) | 83/55 (0.21) | | | |
| OR (95% CI)† | 1.00 | 1.08 (0.62-1.87) | | | --- |
| Total meat | 56/40 (4.2) | 34/39 (7.0) | 78/41 (11.6) | 57/39 (18.9) | |
| OR (95% CI) | 1.00 | 0.42 (0.19-0.92) | 1.03 (0.50-2.14) | 0.59 (0.22-1.56) | 0.65 |
| Red meat | 58/41 (1.0) | 39/39 (2.3) | 65/39 (3.7) | 63/40 (8.8) | |
| OR (95% CI) | 1.00 | 0.52 (0.25-1.08) | 0.97 (0.48-1.97) | 0.72 (0.30-1.71) | 0.70 |
| Organ meat | 65/56 (0.0) | 160/103 (0.09) | | | |
| OR (95% CI)† | 1.00 | 1.09 (0.63-1.87) | | | --- |
| Processed meat | 44/41 (0.3) | 84/38 (1.0) | 43/42 (2.0) | 54/38 (3.5) | |
| OR (95% CI) | 1.00 | 1.73 (0.86-3.49) | 0.48 (0.21-1.08) | 0.89 (0.37-2.11) | 0.23 |
| Fish | 43/39 (0.3) | 61/41 (0.9) | 69/41 (2.0) | 52/38 (3.2) | |
| OR (95% CI) | 1.00 | 1.68 (0.80-3.54) | 1.29 (0.62-2.58) | 1.14 (0.51-2.54) | 0.88 |
| Poultry | 49/40 (0.7) | 69/43 (1.7) | 52/36 (2.9) | 55/40 (5.0) | |
| OR (95% CI) | 1.00 | 1.27 (0.63-2.55) | 1.18 (0.57-2.44) | 1.17 (0.53-2.59) | 0.82 |
| Eggs | 57/42 (0.7) | 45/38 (1.8) | 57/40 (3.1) | 66/39 (6.6) | |
| OR (95% CI) | 1.00 | 0.78 (0.38-1.60) | 1.18 (0.59-2.35) | 1.53 (0.73-3.20) | 0.16 |

**Table 12 continued**

| Food Group (servings/week) | Q1 | Q2 | Q3 | Q4 | P for trend |
|---|---|---|---|---|---|
| Nuts | 60/41 (0.1) | 62/37 (0.4) | 36/40 (0.9) | 67/41 (2.4) | |
| OR (95% CI) | 1.00 | 0.90 (0.44-1.81) | 0.40 (0.18-0.86) | 0.73 (0.34-1.58) | 0.57 |
| Soy | 176/128 (0.0) | 49/31 (0.04) | | | |
| OR (95% CI)† | 1.00 | 0.97 (0.52-1.81) | | | --- |
| Added sugar (g) | 38/40 (188.7) | 41/39 (351.7) | 55/41 (645.1) | 91/39 (1036.3) | |
| OR (95% CI) | 1.00 | 1.20 (0.57-2.50) | 1.64 (0.74-3.66) | 2.65 (1.11-6.34) | 0.02 |
| Discretionary fat (g) | 57/40 (222.5) | 42/40 (387.7) | 67/40 (551.2) | 59/39 (823.2) | |
| OR (95% CI) | 1.00 | 0.45 (0.21-0.97) | 0.51 (0.21-1.25) | 0.31 (0.09-1.11) | 0.10 |

*****adjusted for age, sex, education , income, BMI 1 year ago, physical activity, family history, non-steroidal anti-inflammatory drug use, and total energy intake

† OR represents consumers vs. non-consumers (referent)

‡ number of cases/number of controls (median intake in controls)

**Table 13: Factor loading matrix for the 3 major dietary patterns identified among race-specific controls in the North Carolina Colon Cancer Study-Phase II***

| | Whites | | | African Americans | | |
|---|---|---|---|---|---|---|
| | Factor 1: "High fat/ Meat/Potatoes" | Factor 2: "Veg/Fish/ Poultry" | Factor 3: "Fruit/Whole-grain/Dairy" | Factor 1: "High fat/ Meat/Potatoes" | Factor 2: "Fruit/ Vegetables" | Factor 3: "Legumes/ Dairy" |
| Discretionary fat | 0.86 | - | - | 0.80 | - | 0.45 |
| Non-Whole grains | 0.77 | - | 0.22 | 0.73 | - | 0.39 |
| Beef/Pork/Lamb | 0.72 | 0.21 | - | 0.76 | - | - |
| White potatoes | 0.65 | - | - | 0.60 | - | - |
| Added sugar | 0.57 | -0.31 | - | 0.47 | - | - |
| Processed meat | 0.49 | - | - | 0.68 | - | - |
| Cheese | 0.49 | 0.24 | - | 0.55 | 0.20 | - |
| Eggs | 0.40 | - | -0.20 | 0.50 | - | - |
| Nuts | 0.31 | - | 0.28 | - | - | 0.72 |
| Beans and peas | 0.27 | 0.22 | 0.26 | 0.26 | - | 0.69 |
| Other vegetables | 0.21 | 0.73 | 0.26 | 0.20 | 0.69 | 0.35 |
| Dark green vegetables | - | 0.71 | - | -0.30 | 0.61 | 0.30 |
| Poultry | 0.22 | 0.54 | - | 0.37 | 0.30 | - |
| Fish | - | 0.51 | - | 0.25 | - | - |
| Deep yellow vegetables | - | 0.47 | 0.37 | - | 0.70 | 0.24 |
| Tomato | 0.34 | 0.37 | 0.27 | 0.38 | 0.50 | - |
| Other fruit | - | - | 0.70 | - | 0.68 | - |
| Citrus fruit | - | - | 0.56 | - | 0.48 | -0.21 |
| Whole grains | - | - | 0.56 | - | 0.20 | 0.31 |
| Milk/Yogurt | - | - | 0.51 | - | - | 0.48 |
| Other starchy vegetables | 0.28 | 0.27 | 0.37 | 0.37 | 0.59 | - |
| *variance explained* | *3.75%* | *2.39%* | *2.14%* | *4.12%* | *2.89%* | *2.10%* |

*factor loadings < $|0.20|$ are indicated by "-"

**Table 14: Correlation coefficients for relations between dietary patterns and other selected variables**

| | Whites | | | Africans Americans | | |
|---|---|---|---|---|---|---|
| | Factor 1: "High fat/ Meat/Potatoes" | Factor 2: "Veg/Fish/ Poultry" | Factor 3: "Fruit/Whole-grain/Dairy" | Factor 1: "High fat/ Meat/Potatoes" | Factor 2: "Fruit/ Vegetables" | Factor 3: "Legumes/ Dairy" |
| Age† | -0.18 (-0.23, -0.13) | -0.08 (-0.13, -0.03) | 0.17 (0.12, 0.22) | -0.15 (-0.24, -0.05) | 0.11 (0.01, 0.21) | 0.09 (-0.01, 0.19)‡ |
| Education* | -0.14 (-0.19, -0.09) | 0.30 (0.25, 0.35) | 0.05 (-0.00, 0.10) | -0.07 (-0.17, 0.03)‡ | 0.12 (0.02, 0.21) | -0.04 (-0.14, 0.06)‡ |
| BMI 1 yr ago† | 0.11 (0.06, 0.16) | 0.02 (-0.03, 0.07)‡ | -0.07 (-0.12, -0.02) | -0.09 (-0.19, 0.01)‡ | 0.08 (-0.02, 0.18)‡ | -0.05 (-0.15, 0.05)‡ |
| Annual income* | 0.01 (-0.04, 0.07)‡ | 0.26 (0.22, 0.31) | -0.07 (-0.12, -0.02) | 0.03 (-0.08, 0.13)‡ | -0.01 (-0.11, 0.10)‡ | 0.07 (-0.03, 0.18)‡ |
| Daily intakes | | | | | | |
| Energy(kcal)† | 0.86 (0.85, 0.87) | 0.10 (0.05, 0.15) | 0.35 (0.31, 0.40) | 0.82 (0.79, 0.85) | 0.28 (0.18, 0.37) | 0.43 (0.35, 0.51) |
| Total fat(g)† | 0.51 (0.47, 0.54) | 0.09 (0.04, 0.14) | -0.43 (-0.47, -0.39) | 0.28 (0.19, 0.37) | -0.16 (-0.25, -0.06) | 0.27 (0.17, 0.36) |
| Carbohydrates(g)† | -0.28 (-0.33, -0.24) | -0.35 (-0.40, -0.31) | 0.60 (0.56, 0.63) | -0.25 (-0.34, -0.14) | 0.18 (0.09, 0.28) | -0.17 (-0.27, -0.07) |
| Protein(g)† | 0.10 (0.05, 0.15) | 0.58 (0.55, 0.61) | -0.10 (-0.15, -0.05) | 0.18 (0.08, 0.27) | 0.29 (0.19, 0.38) | 0.15 (0.05, 0.24) |
| Alcohol(g)† | -0.31 (-0.35, -0.26) | 0.15 (0.10, 0.20) | -0.20 (-0.25, -0.15) | -0.18 (-0.27, -0.08) | -0.13 (-0.23, -0.03) | -0.10 (-0.20, 0.00) |
| Folate(μg)† | -0.43 (-0.47, -0.39) | 0.46 (0.42, 0.50) | 0.49 (0.45, 0.53) | -0.45 (-0.53, -0.37) | 0.66 (0.60, 0.71) | 0.29 (0.19, 0.38) |
| Fiber(g)† | -0.52 (-0.55, -0.48) | 0.50 (0.46, 0.54) | 0.66 (0.63, 0.68) | -0.54 (-0.61, -0.46) | 0.77 (0.72, 0.80) | 0.41 (0.32, 0.49) |

*Spearman correlation coefficients presented for these variables.

†Pearson correlation coefficients presented for these variables. Correlations for all nutrients are partial correlations adjusted for energy.

‡ P-value ≥0.05

**Table 15: Odds ratios and 95% confidence intervals for rectal cancer according to dietary pattern quartiles, by race (North Carolina Colon Cancer Study-Phase II)***

| Dietary Pattern | Q1 | Q2 | Q3 | Q4 | P for trend |
|---|---|---|---|---|---|
| Whites | | | | | |
| High fat/Meat/Potatoes | | | | | |
| Cases/controls | 126/200 | 148/200 | 221/200 | 225/200 | |
| OR (95% CI) | 1.00 | 1.25 (0.86-1.80) | 1.80 (1.21-2.68) | 1.84 (1.08-3.15) | <0.0001 |
| Veg/Fish/Poultry | | | | | |
| Cases/controls | 266/200 | 214/200 | 118/200 | 122/200 | |
| OR (95% CI) | 1.00 | 1.00 (0.74-1.35) | 0.57 (0.40-0.80) | 0.47 (0.33-0.67) | <0.0001 |
| Fruit/Whole-grain/Dairy | | | | | |
| Cases/controls | 221/200 | 196/200 | 155/200 | 148/200 | |
| OR (95% CI) | 1.00 | 1.04 (0.76-1.43) | 0.78 (0.56-1.09) | 0.65 (0.45-0.93) | <0.0001 |
| African Americans | | | | | |
| High fat/Meat/Potatoes | | | | | |
| Cases/controls | 45/39 | 59/41 | 59/39 | 62/40 | |
| OR (95% CI) | 1.00 | 0.81 (0.39-1.70) | 0.79 (0.33-1.91) | 0.89 (0.27-3.00) | 0.80 |
| Fruit/Vegetables | | | | | |
| Cases/controls | 52/40 | 37/40 | 59/39 | 77/40 | |
| OR (95% CI) | 1.00 | 0.77 (0.35-1.70) | 1.01 (0.49-2.07) | 1.50 (0.71-3.18) | <0.0001 |
| Legumes/Dairy | | | | | |
| Cases/controls | 57/39 | 46/40 | 57/41 | 65/39 | |
| OR (95% CI) | 1.00 | 0.83 (0.40-1.73) | 0.79 (0.39-1.59) | 0.74 (0.35-1.59) | <0.0001 |

*****adjusted for age, sex, education , income, BMI 1 year ago, physical activity, family history, non-steroidal anti-inflammatory drug use, and total energy intake

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## VII. Conclusions

### A. Review of aims

The overall objective of this dissertation was to investigate the relationship between dietary factors and rectal cancer in whites and African Americans in North Carolina. The North Carolina Colon Cancer Study-Phase II was a very appropriate dataset that provided extensive information on dietary intake in a racially heterogeneous population in a specific geographic area, with a large and fairly equal number of rectal cancer cases and controls. The specific aims of this research were to: 1) determine the association between nutrients and risk of rectal cancer, 2) determine the association between food groups and risk of rectal cancer, and 3) determine the association between dietary patterns and risk of rectal cancer.

### B. Summary of findings

1. Nutrient intake and rectal cancer risk

a) Micronutrients

Antioxidants micronutrients (vitamin C, vitamin E, beta-carotene, selenium) had significant independent inverse associations with rectal cancer risk in whites, suggesting a 24-53% reduction in risk. The combined effect of these antioxidant nutrients in whites was associated with a 34% risk reduction (OR: 0.66, 95% CI 0.47-0.91). With the exception of selenium, there were no statistically significant associations in African Americans. The odds ratio for total selenium intake in African Americans was 0.25 (95% CI 0.06-0.68).

High intakes of DNA methylation-related nutrients (folate, vitamin B6, vitamin B12) appeared to have a protective effect on rectal cancer in whites, with independent risk reductions ranging from 29% to 58%. The combined effect of these nutrients was also associated with lower risk (OR: 0.62, 95% CI 0.44-0.88). In African Americans, there were no statistically significant odds ratios, but we did observe a positive linear trend for vitamin B6 intake from food ($p<0.0001$).

For most of the micronutrients, the risk estimates associated with intake from food only was more favorable than the risk associated with total intake (i.e. from food and supplements). This finding challenges the notion that supplement use aids in rectal cancer risk reduction and supports the idea that adequate intakes from food may be sufficient for risk reduction.

b) Macronutrients

In regards to macronutrient intake, neither total fat nor any subtypes of fat were associated with risk of rectal cancer in whites. In African Americans, there was a possible risk reduction associated with high polyunsaturated fatty acid intake (OR: 0.28, 95% CI 0.08-0.96). In whites, absolute intake of protein suggested lower rectal cancer risk, while the risk reduction was stronger for the percent of energy from protein (OR: 0.53, 95% CI 0.37-0.77).

2. Food groups and rectal cancer risk

In whites, non-whole (refined) grains and white potatoes were positively associated with the risk of rectal cancer. The following food groups had statistically significant inverse associations with risk: fruit, vegetables (specifically, dark green vegetables, deep yellow vegetables, other starchy vegetables (i.e. excluding white potatoes), other vegetables), dairy,

fish, and poultry. In African Americans, other (non-citrus) fruit and added sugar were associated with elevated risk, yet no statistically significant risk reductions were observed. These findings suggest that there are racial differences in rectal cancer risk associated with many of the USDA predefined food groups.

3. Dietary patterns and rectal cancer risk

We identified three major dietary patterns among race-specific controls. In whites, the following three patterns emerged: High fat/Meat/Potatoes, Vegetables/Fish/Poultry, and Fruit/Whole grain/Dairy. The High fat/Meat/Potatoes dietary pattern was associated with elevated risk of rectal cancer (OR: 1.84, 95% CI 1.08-3.15), while the other two patterns were associated with reduced risk. In African Americans, the following three patterns resulted: High fat/Meat/Potatoes, Fruit/Vegetables, and Legumes/Dairy. There were no statistically significant risk estimates observed in African Americans; however, there was a positive trend related to the Fruit/Vegetables patterns and there was an inverse trend associated with the Legumes/Dairy dietary pattern. The High fat/Meat/Potatoes pattern in both whites and African Americans was highly correlated with total energy (Pearsons' $r$=0.86 and 0.82, respectively), and inversely correlated with alcohol, folate, and fiber.

**C. Limitations**

One limitation is the possibility for response bias resulting from those who refuse to participate. The response rate in controls was less than that in cases, and the response rate in African Americans was much less than the rate in whites. Non-responders may be different from those who chose to participate, and this could have jeopardized the validity of the study.

Recall bias of past exposures is also possible. Recall bias could also affect these analyses if cases recalled their dietary intake differently than controls as a consequence of their illness. Dietary information was limited to the one year prior to interview or diagnosis. This short interval helped to limit recall errors. Cases were interviewed well after they recovered from surgery in an effort to minimize differential recall. Although diet in the more distant past may be more relevant to cancer development, there is greater potential for inaccurate recall when assessing diet in the remote past.

Measurement error was also possible due to the use of a food frequency questionnaire, such as the Diet History Questionnaire (DHQ), and the nutrient databases. Possibly, this error was reduced by using trained nurse-interviewers, in an attempt to standardize the way the questions were asked and interpreted. It is important to note that measurement error from food frequency questionnaires usually attenuates estimates of disease risk. Also, it is possible that the DHQ possibly did not include certain food items common to certain race/ethnic groups or southern diets.

Another limitation is that we had a small sample of African Americans, despite our efforts to over-sample and increase their representation in the study. This could have prevented us from observing more statistically significant results, thereby limiting our ability to accurately assess racial differences in risk.

**D. Strengths**

The major strength of this study is that it is one of the first studies to examine the association of dietary factors and rectal cancer risk in a large sample of African-Americans and Whites recruited from the same geographic area. This will enable us to observe associations that may not have been detectable in smaller studies. It will further allow us to assess possible

racial differences in risk for rectal cancer, for which there is currently no evidence. The case-control study design is useful for exploring exposure-disease relationships when the disease is rare and/or has a long latency period, such as cancer. The population-based feature of our study improves the generalizability of our findings and the randomized recruitment feature minimized potential selection bias.

**E. Public health significance**

Colorectal cancer (CRC) is the fourth most common cancer in the U.S. Rectal cancer accounts for approximately one-third of all colorectal cancers. Diet plays a major role in CRC development because everything we consume comes in contact with the lining of the colon and rectum to some extent. While there is abundant evidence regarding the effect of diet on colorectal and colon cancer, much less is known about the relationship between dietary factors and risk of rectal cancer. In addition, African Americans have the highest incidence and mortality of colon and rectal cancer. While some of this disparity is due to socioeconomic status and access to care, these factors do not fully explain why African Americans are disproportionately affected by CRC. Nationally representative data has shown that dietary intake differs between whites and African Americans; therefore, it is logical to assume that differences in consumption may correlate with differences in risk. It was important to investigate this possibility since colon and rectal cancers are partially preventable by dietary modifications.

This dissertation supports the hypothesis that there are some differences in dietary intake and risk associated with rectal cancer between whites and African Americans, which may contribute to racial disparities. It further emphasizes the need to examine diet-cancer associations in large racially diverse samples to confirm (or dispute) these findings. This

study adds to the literature on the epidemiology and etiology of rectal cancer, especially in African Americans. Because some of the associations we observed for rectal cancer in this study contradict what has been suggested for colon cancer, this stresses the importance of examining these carcinomas separately when trying to identify risk factors.