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DIETARY PATTERNS, RED AND PROCESSED MEAT-DERIVED MUTAGEN EXPOSURE, AND COLORECTAL CANCER RISK

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ABSTRACT

Colorectal cancer is the fourth most commonly diagnosed malignancy in developed countries and the second leading cause of cancer death in the U.S. A recent consensus report issued by the World Cancer Research Fund and the American Institute for Cancer Research concluded that the evidence to support a positive association between greater intakes of red and processed meat and colorectal cancer risk was convincing, although the specific components within meat driving these associations remained unclear. The report also stated that there was insufficient evidence to render judgment regarding dietary patterns and colorectal cancer risk. To address these identified gaps in knowledge, we explored associations between both dietary patterns and specific meat components and colorectal cancer risk in a large multi-site population-based case-control study in a high-risk adult population in northeast and central Pennsylvania. Participants completed a 137-item food frequency questionnaire that included a detailed cooked and processed meat module, which allowed for the use of databases of heterocyclic amines (HCAs), polycyclic aromatic hydrocarbons (PAHs), nitrites, and nitrates.

In the first study, our objective was to examine colorectal cancer risk associated with dietary patterns among 431 incident cases (225 men, 206 women) and 726 healthy controls (330 men, 396 women). The majority of previous studies have derived patterns either by measuring compliance with pre-established dietary guidance or by empirical methods such as principal components analysis (PCA). We explored whether dietary patterns identified by both methods were associated with colorectal cancer risk. Three dietary patterns among men (fruits and vegetables, meat and potatoes, alcohol and sweetened beverages) and two among women (fruits and vegetables, meat and potatoes) were identified by PCA. Healthy Eating Index-2005 (HEI-05) scores were generated to assess the degree of adherence to recommendations found in the

Dietary Guidelines for Americans. After adjusting for potential confounders, the PCA-derived fruits and vegetables pattern was inversely associated with colorectal cancer risk among both men (odds ratio (OR) = 0.38, 95% confidence interval (CI) = 0.21-0.69 for the highest compared to the lowest quartile; *P* for trend = 0.006) and women (OR = 0.35, 95% CI = 0.19-0.65; *P* for trend = 0.031). In contrast, the meat and potatoes pattern was positively associated with risk in women (OR = 2.20, 95% CI = 1.08-4.50; *P* for trend = 0.070) and there was a suggestion of a positive association among men (OR = 1.56, 95% CI = 0.84-2.90; *P* for trend = 0.070). Men and women with greater HEI-05 scores had a significant reduction in risk (OR = 0.56, 95% CI = 0.31-0.99; *P* for trend = 0.004; OR = 0.44, 95% CI = 0.24-0.77; *P* for trend <0.001, respectively). In summary, findings from the first study indicate that both the hypothesis-oriented diet index-based approach and the empirically-driven PCA approach are of value in the study of diet and colorectal cancer associations and that following the *Dietary Guidelines* or a primarily plant-based dietary pattern that includes low-fat dairy and fish may be protective.

In the second study, our objective was to explore potential underlying mechanisms for previously observed associations between red and processed meat and colorectal cancer risk. We examined whether increased levels of exposure to mutagens generated through meat cooking and meat processing methods were risk factors for colon and rectal cancer among 726 healthy controls, 287 colon cancer cases, and 128 rectal cancer cases. Associations between meat exposures and colorectal cancer stratified by sub-site of the large intestine were estimated from unconditional logistic regression models. After multivariate adjustment, positive associations with HCAs and PAHs, as measured by total mutagenic activity, were stronger for rectal cancer (OR = 1.75, 95% CI = 1.00, 3.08; *P* for trend = 0.031) than colon cancer, whereas suggestive positive associations with nitrites plus nitrates were stronger for colon cancer (OR = 1.28, 95%

CI = 0.82-2.00; *P* for trend = 0.084). These findings support the hypothesis that greater exposure to HCAs, PAHs, nitrites, and nitrates is a plausible mechanism by which red and processed meat may increase colorectal cancer risk. Our sub-site analyses indicate that associations between meat-derived exposures and colon and rectal cancer may differ, which underscores the need for additional studies that examine dietary risk factors for colon and rectal cancer as separate endpoints.

In conclusion, our research supports the complementary study of both dietary patterns and individual dietary components as cancer risk factors as each can serve a unique purpose in identifying areas to target for colorectal cancer prevention strategies as well as future research studies.

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LIST OF ABBREVIATIONS

A-HEI	Alternate Healthy Eating Index
APC	adenomatous polyposis coli
ATBC	Alpha-Tocopherol Beta-Carotene Study
BCDDP	Breast Cancer Detection Demonstration Project follow-up study
BMI	body mass index (kg/m^2)
CARDIA	Coronary Artery Risk Development in Young Adults
CI	confidence interval
CYP	cytochrome P450 enzymes
DASH	Dietary Approaches to Stop Hypertension Eating Plan
DHQ	National Cancer Institute's Diet History Questionnaire
DFE	dietary folate equivalent
DiMeIQx	2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline
EPIC	European Prospective Investigation into Cancer and Nutrition
F&V	fruits and vegetables
FFQ	food frequency questionnaire
GSTs	glutathione *S*-transferases
GWAS	genome-wide association study
HCA	heterocyclic amines
HEI-05	Healthy Eating Index-2005
HHS	U.S. Department of Health and Human Services
HPFS	Health Professionals Follow-Up Study
HR	hazard ratio
HRT	hormone replacement therapy
IACR	International Agency for Research on Cancer
IQ	2-amino-3-methylimidazo[4,5-*f*]quinoline
JPHC	Japan Public Health Center Cohort
kJ	kilojoule
MED	Mediterranean Diet Score
MeIQ	2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline
MeIQx	2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline
NAS	National Academy of Sciences
NAT	*N*-acetyltransferase
NDMA	*N*-nitrosodimethylamine
NECSS	National Enhanced Cancer Surveillance System
NHANES	National Health and Nutrition Examination Survey
NIH-AARP	National Institutes of Health—AARP Diet and Health Study
NHS	Nurses' Health Study
NLCS	Netherlands Cohort
NOC	*N*-nitroso compound
NRC	National Research Council
NPRO	*N*-nitrosoproline
NPYR	*N*-nitrosopyrrolidine
NS	non-significant
NSAID	non-steroidal anti-inflammatory drug

NTHZ	*N*-nitrosothiazolidine
OR	odds ratio
PA	physical activity
PAH	polycyclic aromatic hydrocarbons
PCA	principal components analysis
PhIP	2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine
PLCO	Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial
ppb	parts per billion
ppm	parts per million
RfD	Reference Dose (established by EPA)
RFS	Recommended Food Score
RR	relative risk
SMC	Swedish Mammography Cohort
SNP	single nucleotide polymorphism
SULT	sulfotransferases
UGT	UDP-glucuronosyltransferases
USDA FG	U.S. Department of Agriculture Food Guide Recommendations
WCRF/AICR	World Cancer Research Fund/American Institute for Cancer Research
WHS	Women's Health Study

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Chapter 1

INTRODUCTION

OVERALL BACKGROUND AND SIGNIFICANCE

Colorectal cancer is the fourth most commonly diagnosed malignancy in developed countries (1) and the second leading cause of cancer death in the U.S. (2). Among non-smokers, colorectal cancer is the leading cause of cancer death (2). Despite advances in colorectal cancer screening and treatment options, the five-year survival rate from the time of diagnosis is only 60%. Incidence rates vary significantly across countries, with the highest rates observed in North America, Europe, Australia, and New Zealand and the lowest rates seen in Asia, Africa, and South America (3). The most recent incidence data provided by the International Agency for Research on Cancer (IACR) indicates that rates have increased rapidly in newly developed countries, which has implicated Westernization as a potential explanation (4).

In combination with international comparisons of incidence rates and observed trends in newly developed countries, findings from migrant studies support an important role of diet in colorectal cancer etiology (5-7). Migrating populations often adopt the dietary patterns as well as the cancer rates of the new host country (8,9), and these changes are often seen within the migrating generation (7,10). This pattern of cancer incidence was observed more than thirty years ago among Japanese populations living in the San Francisco Bay Area, with colon cancer incidence rates among both immigrant and U.S.-born Japanese Americans nearly four-fold greater than rates documented in Japan (9). Populations that experience the greatest incidence rates of colorectal cancer tend to consume more meat and animal fat and less fruits, vegetables, and other fiber- and micronutrient-rich foods than those with lower rates (6).

Compared to the rest of the U.S., Pennsylvania suffers from greater age-adjusted incidence and mortality rates of colorectal cancer (per 100,000 and 23.4 v. 21.0 per 100,000, respectively) (13). The reasons for the higher rates remain unclear but several hypotheses

involving both environmental and genetic factors have been proposed to explain this finding. A large multi-site population-based case-control study including both genders is underway to explore potential environmental and genetic risk factors for colorectal cancer in this high-risk population in Pennsylvania.

BACKGROUND: STUDY 1

Epidemiologic studies have traditionally assessed the effects of single nutrients, foods, and other individual dietary constituents on colorectal cancer risk. Research using this approach is valuable for understanding potential biological mechanisms underlying observed associations, but it is limited by the multicollinearity of dietary intake variables and the inability to detect small effects of single dietary components. The investigation of dietary patterns or overall diet quality is a promising alternative that may prove informative in evaluating diet and colorectal cancer risk (14-17). Dietary patterns encompass the quality, quantity, and proportions of foods and beverages consumed in the diet as well as the frequency in which different items are usually consumed. Examining the totality of diet through dietary pattern analyses reflects the complexity of food and beverage intake and captures synergistic relationships between various dietary constituents that may be related to disease risk (18).

A recent consensus report issued jointly by the World Cancer Research Fund (WCRF) and the American Institute for Cancer Research (AICR) in 2007 stated that the inconsistent definitions used and the insufficient evidence produced in previous studies prevented the expert panel from making a judgment regarding the association between dietary patterns and colorectal cancer risk (19). The majority of previous studies have derived dietary patterns by one of two fundamentally different approaches. One method is defined as hypothesis-oriented (*a priori*) because it relies upon scientific knowledge from previous investigations into health-promoting or

disease-preventing diets (20,21). With this approach, researchers measure compliance with a pre-existing diet quality index, current dietary guidelines, or a specific dietary pattern and assign diet scores that reflect the level of adherence. The Healthy Eating Index-2005 (HEI-05), originally developed in 1995 by the USDA's Center for Nutrition Policy and Promotion (22) and subsequently modified to correspond with recommendations found in the *Dietary Guidelines for Americans* (23,24), is one tool that can be used for this purpose. Greater energy-adjusted dietary intakes of fruit, vegetables, legumes, oil, whole grains, low-fat dairy, and lean meat, and lower energy-adjusted dietary intakes of sodium, saturated fat, and solid fat, alcohol, and added sugar result in higher scores, suggesting greater diet quality. The other commonly used method in dietary pattern analyses is data-driven (*a posteriori*) since statistical methods, such as principal components analysis (PCA), are used to generate patterns empirically. PCA is a variable-oriented technique that aims to reduce the number of explanatory variables into independent factors that capture the primary sources of dietary variation within a study population.

SPECIFIC AIM AND HYPOTHESIS: STUDY 1

Study 1 Specific Aim

In the present multi-site, population-based case-control study in northeast and central Pennsylvania, we explored both PCA-derived dietary patterns and diet-index based scores, as assessed by the HEI-05, and examined whether dietary patterns identified by these two distinct approaches were associated with colorectal cancer risk.

Study 1 Hypothesis

A dietary pattern characterized by higher intakes of fruits, vegetables, whole grains, nuts, seeds, vegetable oils, low-fat dairy, and fish, which is in agreement with current dietary recommendations, will be associated with a reduced risk of colorectal cancer. In contrast, a

pattern representing a Western-style diet high in meat, fried and other white potatoes, high-fat dairy, sweets, and other high-fat and high-sugar food items will be associated with an increased risk.

BACKGROUND: STUDY 2

Preliminary analyses of our pilot data suggested that the population in northeast and central Pennsylvania consumed processed meat more frequently compared to a nationally-representative sample of U.S. adults. These findings generated support for the hypothesis that increased processed meat intake may be a risk factor for colorectal cancer in our study population. Current epidemiological evidence also supports a positive association between processed meat and colorectal cancer (19,25-27), although the recent consensus report issued by the WCRF and the AICR concluded that there was insufficient evidence to implicate specific components within meat. One underlying mechanism whereby processed meat consumption may increase colorectal cancer risk is through increased exposure to *N*-nitroso compounds (NOCs), which have been shown to induce tumors at a variety of sites in over 40 unique animal species, including higher primates (28,29). NOCs can be generated exogenously (in meat) or endogenously (*in vivo*) from the nitrosation of meat-derived amines and amides by sodium nitrites, or nitrates reduced to nitrites by bacteria, that are added to certain meat items as preservatives or curing agents (30). Previous epidemiological studies have been limited in examining the effect of meat-derived NOC exposure due to a lack of detailed data on meat intake and NOC precursors in meat.

SPECIFIC AIMS AND HYPOTHESES: STUDY 2

Study 2 Primary Aim

The primary aim of the second study was to examine whether increased intake of processed meat-derived sodium nitrites and nitrates was a risk factor for colorectal cancer in our study population. We incorporated recently estimated values of nitrites and nitrates (unpublished data from the National Cancer Institute [NCI]) into a database that was tied to the NCI Diet History Questionnaire (DHQ) to address this aim. A validated cooked and processed meat module (31) that was modified to reflect the meat consumption patterns of our population and designed to specifically address our research question was embedded into the DHQ.

Study 2 Primary Hypothesis

Greater intakes of processed meat-derived sodium nitrites and nitrates will be positively associated with an increased risk of colorectal cancer.

Study 2 Secondary Aim

We examined the associations of dietary intakes of meat-derived heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs) with colorectal cancer to explore other potential underlying mechanisms. These highly mutagenic compounds arise from cooking meat well-done at high temperatures or over a direct flame and are known animal carcinogens (32,33). The present study was well-designed to investigate these associations given the detailed meat exposure data collected, the wide variation in reported meat consumption, and the greater average intake of meat compared to a nationally representative sample (34).

Study 2 Secondary Hypothesis

Greater intakes of HCAs and PAHs arising from cooking meat well-done at high temperatures or over a direct flame will be positively associated with an increased risk of colorectal cancer.

SUMMARY

The research presented in this dissertation uses data from an ongoing, multi-site, population-based case-control study in northeast and central Pennsylvania. Two main research questions are addressed: the first in Chapter 3 (Study 1) and the second in Chapter 5 (Study 2). The first question pertains to associations between dietary patterns derived by two fundamentally distinct methods and colorectal cancer risk. A manuscript describing this study and our findings was recently accepted for publication (upon revision) (35). A comprehensive review of the dietary pattern and colorectal cancer risk literature provides a context for the first study (Chapter 2) and is currently in press (36). The second research question focuses on associations between specific red and processed meat compounds and colorectal cancer risk (Chapter 5). The literature that guided the design of the second study is divided into five sections and presented in Chapter 4. The final chapter summarizes the main findings from Study 1 and Study 2, describes the limitations of our research, and provides recommendations for future research efforts (Chapter 6).

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Chapter 2

DIETARY PATTERNS AND COLORECTAL ADENOMA AND CANCER RISK: A REVIEW OF THE EPIDEMIOLOGICAL EVIDENCE[1]

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Miller PE, Lesko SM, Muscat JE, Lazarus P, and Hartman TJ. Dietary patterns and colorectal adenoma and cancer risk: a review of the epidemiological evidence. *Nutrition and Cancer: An International Journal*, 2010; 62(3) (In Press).

ABSTRACT

A number of studies exploring associations between individual dietary components and colorectal adenoma or cancer risk have yielded conflicting results. The study of food-based dietary patterns in relation to chronic disease risk represents an alternative approach to the evaluation of single dietary exposures in epidemiological investigations. Results from prospective cohort and population-based case-control studies examining associations between dietary patterns and colorectal cancer or adenoma risk were evaluated and described in this review. Despite notable differences in population characteristics, study design, and methods used for characterizing dietary patterns across the different studies, two general dietary patterns were found to modestly predict colorectal adenoma and cancer risk. A healthier pattern consisting of greater intakes of fruits and vegetables, and lower intakes of red and processed meat, appeared protective against colorectal adenoma and cancer incidence. Findings also suggest that a less healthy pattern characterized by higher intakes of red and processed meat, as well as potatoes and refined carbohydrates, may increase risk. Continued research efforts are needed to evaluate the cumulative and interactive effects of numerous dietary exposures on colorectal cancer risk.

INTRODUCTION

A number of epidemiological studies have investigated the associations of individual foods, food groups, nutrients, or other dietary components with colorectal cancer risk. The more widely studied individual dietary exposures that may increase the risk of colorectal cancer include high intakes of total energy, saturated and *trans* fat, red and processed meat, and alcohol, whereas greater intakes of fiber, folate, calcium, vitamin D, and fruits and vegetables may be protective (1,2), although results have been largely inconsistent and of relatively modest magnitude. One explanation for this relates to the multicollinearity of the individual dietary exposures, which has challenged methodological efforts to estimate independent effects. A greater intake of fruits and vegetables, for example, has been associated with higher intakes of fiber, folate, calcium, and vitamin D, and lower intakes of alcohol and red meat (3). Furthermore, the influence of different dietary components may be interactive rather than purely additive (4), yet the ability to capture potential interactions of dietary components in statistical analyses may be limited. An additional challenge pertains to nutrient interactions, as various nutrients have been shown to influence the bioavailability and absorption of other nutrients. Estimating the effect on disease risk of any one food is difficult because a greater consumption of one particular food is likely associated with lower consumption of other foods that may also influence cancer risk. Given that dietary intakes of individual nutrients, foods, food groups, and other dietary components are likely interrelated and free-living individuals eat a variety of food items, examining overall diet as a multidimensional environmental exposure in population-based epidemiological studies is valuable (5).

The objective of this review was to systematically explore findings from prospective cohort and population-based case-control studies examining associations between dietary

patterns and colorectal cancer risk. Research that examines the relationship between dietary patterns and risk of colorectal cancer may complement the current evidence base pertaining to the role of single foods, food groups, nutrients, or other dietary components in colorectal carcinogenesis, and improve our understanding of food eating behaviors as risk factors. This in turn may prove useful in the ongoing refinement of food-based dietary guidelines.

MATERIALS AND METHODS

Literature Search and Study Selection

A literature search was conducted in the COCHRANE, PUBMED, and Web of Science databases for articles published through January 2009 with the following search terms: "dietary patterns," "food patterns," "eating patterns," "diet score," "diet index," "factor analysis," "cluster analysis," "colorectal cancer," "colorectal adenoma," "colorectal neoplasm, "colon cancer," "colon adenoma," "colon neoplasm." References from identified articles were examined to ensure inclusion of all pertinent publications. Eligible outcomes for inclusion were colon or colorectal cancer or adenoma incidence. Because adenomas are epithelial polyps that serve as precursors to colorectal cancer, risk factors for the development of colorectal adenoma are likely similar to those for cancer. Publication date was not a criteria for inclusion, but the earliest article that met the criteria for this review was published in 1992 (6). Study inclusion criteria are presented in Table 2.1.

Material

A total of sixteen publications are included in the present review. Studies are divided into three categories according to the methodology used to derive the dietary patterns and are presented chronologically in Tables 2.1, 2.2, and 2.3. The original labels assigned to dietary

patterns identified by researchers are shown in the tables. Publications with findings from gender-stratified analyses appear twice in the tables, with results for males and females shown separately. Table 2.2 presents the characteristics and main findings from one population-based case-control study and two prospective cohort studies evaluating colorectal adenoma or cancer risk using diet scores or indices that assess adherence to different recommended dietary patterns (n = 254,688 females and 313,371 males). Study characteristics and results from four population-based case-control and six prospective cohort studies evaluating dietary patterns by factor analysis and risk of colorectal adenoma or cancer are shown in Table 2.3 (n = 490,638 females and 452,778 males). Wu et al. (7) and Kesse et al. (8) conducted two independent analyses (adenoma risk and cancer risk) in the Health Professionals Follow-up Study (HPFS) and the European Prospective Investigation into Cancer and Nutrition (EPIC), respectively, and therefore the findings appear separately in Table 2.3. Table 2.4 presents the study characteristics and main findings from two population-based case-control studies and one prospective cohort study assessing associations of dietary patterns derived by cluster analysis and risk of colorectal cancer (n = 199,735 females and 294,588 males). Rouillier et al. (9) reported findings from two distinct analyses (adenoma risk and cancer risk), which are presented separately in Table 2.4. In light of the variably defined exposure of dietary patterns across the sixteen publications, as well as the three distinct methods used for determining patterns, study results were not summarized quantitatively in this review.

Methods Used to Determine Dietary Patterns

Two fundamentally different approaches to determine dietary patterns in studies of diet and colorectal cancer relations were identified in the literature reviewed. One method can be defined as largely *a priori*, where the diet is compared to pre-established guidelines, such as a

diet quality index or a specific diet. The *a priori* approach relies upon scientific knowledge from previous investigations into health-promoting or disease-preventing diets. The other approach is considered data-driven or *a posteriori*, where statistical methods such as factor analysis and cluster analysis are used to generate dietary patterns empirically from the study population. Factor analysis is variable-oriented with the objective of reducing the number of explanatory variables into independent factors that capture the primary sources of dietary variation within a study population and are distinct from one another, where foods that load heavily onto one factor do not load appreciably onto others. On the other hand, cluster analysis is subject-oriented with the aim of aggregating similar individuals into mutually exclusive clusters based upon shared dietary characteristics. The objective of cluster analysis is to minimize the within cluster variation while maximizing the between cluster variation.

Six different diet indices or scores were used in the studies reviewed to evaluate dietary patterns: 1) the Recommended Food Score (RFS) (10), 2) the USDA Food Guide Recommendations (USDA FG) (11), 3) the Dietary Approaches to Stop Hypertension (DASH) Eating Plan (11), 4) the Mediterranean Diet Score (MED) (12), 5) the Healthy Eating Index-2005 (HEI-2005) (13), and 6) the Alternate Healthy Eating Index (A-HEI) (14). Briefly, the RFS is a sex- and energy intake-independent measure that is a sum of the number of recommended foods, according to national dietary guidelines, consumed at least weekly. A subset of food items are identified, which varies according to the food frequency questionnaire (FFQ) used in each study, and one point is awarded for the consumption of each food that falls within the recommended food groups: fruits, vegetables, whole grains, low-fat dairy, and lean meats and poultry. The range of diet scores depends upon the number of food items identified by researchers. The 8-point USDA FG measures adherence to food group recommendations for two sex-specific

energy levels in the 2005 *Dietary Guidelines for Americans* (11); higher scores indicate greater intakes of grains, dairy products, fruits, vegetables, and meat or meat equivalents and lower intakes of alcohol, added sugar, and saturated fats. The 2005 *Dietary Guidelines for Americans* also supports an alternative dietary pattern, the DASH Eating Plan. A 9-point DASH diet score is determined by measuring compliance with the daily serving recommendations for whole grains, vegetables, fruits, dairy products, meat or meat equivalents, saturated fat, added sugar, alcohol, and nuts, seeds, and legumes for two sex-specific energy levels.

The 9-point MED scores were calculated according to a previously defined methodology (12) by awarding points for dietary intakes at or greater than sex-specific median values within the study population for whole grains, vegetables, fruit, fish, legumes, nuts, and the ratio of monounsaturated fat to saturated fat in grams. A distinguishing feature between the two studies (15,16) that used MED scores was the treatment of dairy products and meat. Lower intakes of dairy products as well as meat other than fish led to greater scores in the study by Dixon et al. (15), whereas only lower intakes of red and processed meat were rewarded in the study by Reedy et al. (16). The 100-point HEI-2005 (13) includes twelve components that reflect key recommendations found in MyPyramid and the 2005 *Dietary Guidelines for Americans*. Greater energy-adjusted dietary intakes of fruit, vegetables, legumes, oil, low-fat dairy and lean meat, and lower energy-adjusted dietary intakes of sodium, saturated fat, and solid fat, alcohol, and added sugar result in higher scores. Lastly, the A-HEI incorporates several aspects of the original HEI but was modified originally for use in the Nurses' Health Study (NHS) (14) to reflect the scientific evidence concerning diet-related factors associated with reduced chronic disease risk. Individuals receive higher scores on the 87.5-point A-HEI summary measure for unadjusted greater intakes of fruits, vegetables, cereal fiber, nuts, and soy; for higher ratios of

polyunsaturated fat to saturated fat and white meat to red meat; for lower intakes of *trans* fat and alcohol; and for the regular use of a multivitamin.

Evaluation of Dietary Pattern and Colorectal Cancer Risk Associations

Following the assignment of scores to each subject in the diet index studies, subjects were categorized into quartiles or quintiles of diet scores. Subsequently, the score variable was entered into a multivariate logistic regression model or a Cox proportional hazards model to calculate risk estimates and 95% confidence intervals (CIs) for colorectal adenoma or cancer. Statistical models adjusted for body mass index (BMI), energy intake, and smoking, as well as several other potential confounders that varied across studies. A list of other covariates in each of the models is shown in Table 2.2.

Dietary patterns identified by factor analyses were assigned labels that represented the food items or groups with the highest factor loadings or that corresponded to names such as "Western," or "Prudent" that had been used to describe similar patterns in previous investigations. Individual subjects were assigned scores that represented the degree of adherence to each specific pattern by summing the frequency of consumption of food items or groups weighted by the factor loadings. The factor scores were divided into quartiles or quintiles and each factor was entered as an independent categorical variable in separate multivariate logistic regression or Cox proportional hazards regression models to calculate risk estimates and 95% CIs of colorectal adenoma or cancer. A list of covariates in each of the models is shown in Table 2.3. Tests for linear trend were calculated by several different methods, including entering the median factor score of each category as a continuous variable into the multivariate models; entering the quartiles or quintiles of factor scores as ordinal variables into the models; or entering the factor score as a continuous variable into the models.

Dietary patterns determined by cluster analysis were assigned labels according to food items or groups that distinguished the clusters from one another or based on the food items or groups with the greatest energy-adjusted mean intakes for each cluster. One of the clusters was selected to serve as the referent group, either the largest cluster or the cluster considered to be the healthiest, and the other identified clusters were compared to the referent cluster in multivariate logistic regression or Cox proportional hazards regression models used to evaluate associations of clusters with colorectal adenoma and cancer risk. A list of covariates in each of the models is shown in Table 2.4.

RESULTS

Table 2.2: Findings from Diet Index-Derived Dietary Pattern Analyses

A consistently protective effect against colorectal adenoma and cancer incidence of higher scores on all of the diet indices was observed among men (15,16); however, results were less conclusive for women, with statistically significant inverse associations with colorectal cancer observed for higher scores on only two of the indices (15,16). Comparing the highest versus the lowest category of scores among men (2,321 cases, 17,435 controls) aged 55-74y participating in the multisite Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Prevention Trial, odds ratios (ORs) for colorectal adenoma of similar magnitude were reported using the USDA FG (OR = 0.74; 95% CI = 0.64-0.85), the DASH (OR = 0.75; 95% CI = 0.62-0.91), and the MED (OR = 0.79; 95% CI = 0.68-0.92). Comparable findings were observed among 492,382 mostly Caucasian, middle-aged adults (50-71y) from six states and two metropolitan areas enrolled in the National Institutes of Health (NIH)-AARP Diet and Health Study (16). Similar relative risks (RRs) for colorectal cancer comparing the highest versus the lowest quintile of

scores across the four indices were observed among 293,615 male participants (2,151 cases): HEI-2005 (RR = 0.72; 95% CI = 0.62-0.83), A-HEI (RR = 0.71; 95% CI = 0.61-0.82), MED (RR = 0.72; 95% CI = 0.63-0.83), and RFS (RR = 0.75; 95% CI = 0.65-0.87). Statistically significant inverse associations with colorectal adenoma and cancer were observed for higher scores on only two of the indices for women. Comparing the highest to the lowest quartile of scores on the USDA FG, a decreased risk of colorectal adenoma (OR = 0.82; 95% CI = 0.68-0.99) among female participants (1,271 cases, 16,536 controls) in the PLCO trial was observed. Similarly, higher scores on the HEI-2005 among 199,726 women (959 cases) in the NIH-AARP Diet and Health Study cohort also were found to decrease risk (RR = 0.80; 95% CI = 0.64-0.98) for colorectal cancer.

Overall, findings from the diet index-derived pattern studies highlight the importance of fruits and vegetables in a cancer preventive dietary pattern, since greater intakes of these two food groups were awarded points in each of the six indices. A role for animal fats also is suggested as points were deducted for higher intakes of saturated fat directly in two indices, and indirectly through ratios of fat subtypes or the food groups recommended (e.g., lean meats and low-fat dairy) in the other four, which suggests that plant-based dietary patterns lower in animal fats may be protective against colorectal cancer.

Table 2.3: Findings from Factor Analysis-Derived Pattern Analyses

Despite differences in data collection methodology, population characteristics, and decisions made by researchers in relation to food grouping and the number of factors to retain, two distinct dietary patterns emerged across the US (6,7,17-19), European (8,20,21), and Asian investigations (22,23). One pattern was characterized most consistently by fruits and vegetables, and the other by red and processed meats, potatoes, and refined carbohydrates.

Fruit and Vegetable Pattern

Three (17,19,23) of the eight studies in men and three (6,17,18) of the nine studies in women reported statistically significant inverse associations between the highest compared to the lowest category of scores on a high fruit and vegetable dietary pattern and colorectal adenoma or cancer risk (6,17,19). In the earliest published report, Randall et al. (6) observed a statistically significant inverse association between a dietary pattern characterized by many vegetables ("Salad") and colon cancer risk among women (OR = 0.73; 95% CI = 0.60-0.89; 223 controls, 223 cases) but not among men (OR = 0.84; 95% CI = 0.69-1.02; 205 controls, 205 cases) greater than 40 years of age participating in a one-state population-based case-control study (6). Following the work by Randall et al. (6), Slattery and colleagues (17) observed ORs of 0.66 (95% CI = 0.50-0.86) and 0.73 (95% CI = 0.55-0.97) among men (1099 cases, 290 controls) and women (894 cases, 1120 controls), respectively, aged 30-79y with "Prudent" dietary patterns participating in a much larger, multicenter population-based case-control study. Higher intakes of all types of fruits and vegetables, as well as more frequent consumption of fish and poultry compared to red and processed meat, were representative of the "Prudent" dietary pattern. The third report of a statistically significant reduction in colorectal cancer risk associated with a dietary pattern characterized by high intakes of fruits and vegetables was from the NIH-AARP Diet and Health Study cohort (19). Male participants with higher scores on the "Fruit and Vegetables" pattern had a significantly decreased risk of colorectal cancer (RR = 0.81; 95% CI = 0.70-0.93). The results for women, however, were not supportive of an association (RR = 1.06; 95% CI = 0.86-1.30).

Meat and Potatoes Pattern

A second dietary pattern, one characterized by high intakes of meat, potatoes (or French fries), and sweets, desserts, and/or refined grains, was associated with an excess risk of colorectal cancer or adenoma among men in four (6,7,17,19) of the eight studies and among women in five (8,17-20) of the nine studies. The excess risk observed ranged from an OR of 1.18 (95% CI = 1.02-1.35) (19) to 1.80 (95% CI = 1.28-2.15) (17). In the population-based case-control study by Randall et al. (6), researchers identified dietary patterns separately for men and women and observed an elevated colon cancer risk in three dietary patterns among men, whereas there were no consistent associations between patterns and cancer risk among women. The three factors associated with risk in men included a "Traditional" diet distinguished by high intakes of beef, potatoes, cakes, pies, and some vegetables such as green beans (OR = 1.28; 95% CI = 1.04-1.57); a "Snacks" diet characterized by high consumption of cookies, candy, crackers, pastries, hamburgers, ice cream, and baked beans (OR = 1.31; 95% CI = 1.07-1.60); and a "High Fat" diet consisting of high intakes of eggs, bacon, sausage, steak, salami, pepperoni, beer, and other alcohol (OR = 1.28; 95% CI = 1.05-1.58). Notably, red meat was represented consistently in all three dietary patterns, and refined grains and sweets were present in two of the patterns.

Slattery and colleagues (17) observed the greatest elevated risk of colon cancer for both men and women who had higher scores on a "Western" dietary pattern characterized by high intakes of red and processed meat, fast food, and refined grain products. Comparing the highest to the lowest quintile of "Western" pattern scores, men and women had odds of 1.80 (95% CI = 1.28-2.15) and 1.49 (95% CI = 1.05-2.12) for risk of colon cancer, respectively. Following the work by Randall et al. (6) and Slattery et al. (17), Fung and colleagues (18) identified a similar "Western" pattern described by high intakes of red and processed meat, refined grain products,

desserts, high-fat dairy products, and French fries among 76,399 US nurses (546 cases) aged 38-63y enrolled in the NHS cohort, and reported a RR for colorectal cancer of 1.46 (95% CI = 0.97-2.19; *P* for trend 0.02). A "Western" pattern also was identified in the Health Professionals Follow-up Study (HPFS) (7) and was found to increase risk (OR = 1.28; 95% CI = 1.05-1.56) for distal colon adenoma among 20,888 men (2107 cases) aged 40-75y. In a separate analysis by the same authors (7), a similar trend was observed between the "Western" pattern and colon cancer risk (RR = 1.27; 95 CI% 0.96-1.69; *P* for trend = 0.05) among 47,311 men (561 cases) in the HPFS. Dixon and colleagues (20) reported findings from an investigation of 61,463 female participants (586 cases) aged 40-74y in the Swedish Mammography Cohort (SMC) that were fairly analogous to those of Randall et al. (6), Slattery et al. (17), Fung et al. (18), and Wu et al. (7); a "Pork, Processed Meat, Potatoes" pattern was positively associated with colorectal cancer (RR = 1.37; 95% CI = 1.00-1.89; *P* for trend 0.03). Terry et al. (21) also presented findings from an analysis of SMC data (460 cases) three years earlier, but found no clear association between a similar "Western" dietary pattern and colorectal cancer risk (RR = 0.97; 95% CI = 0.66-1.44). The nearly two-fold increase in the number of food groups used in the later investigation for entry into the factor analysis procedures as well as the over 25% increase in colorectal cancer cases, increasing the statistical power to estimate an effect of dietary patterns on colorectal cancer risk, may have contributed to the differential findings.

In a study of women aged 40-65y in the French cohort of the EPIC study (8), researchers investigated associations between dietary patterns and colorectal adenoma and cancer risk separately. Two dietary patterns, with the shared characteristic of high processed meat intake, were each associated with increased colorectal adenoma risk among 5,320 females (516 cases). In addition to processed meat, the "Western" pattern included pizza, pies, sweets, and pasta, and

the “Drinker” pattern included sandwiches, snacks, alcohol, and coffee. The RRs for colorectal adenoma among females with higher scores on the “Western” and “Drinker” patterns were 1.39 (95% CI 1.00-1.94) and 1.42 (95% CI = 1.10-1.83), respectively. Findings from the separate analysis in the EPIC study of associations between dietary patterns and colorectal cancer risk revealed a RR of 1.58 (95% CI = 0.98-2.53; *P* for trend 0.02) among 67,484 women (172 cases) with higher scores on a “Meat Eaters” pattern characterized by high intakes of meat, poultry, potatoes, legumes, coffee, and vegetable oils other than olive. The “Western,” “Drinker,” and “Meat Eaters” patterns all reflected diets high in animal products, snacks and desserts, and low in fruits and vegetables. The most recent investigation of dietary patterns and colorectal cancer risk reported a similar association in the NIH-AARP Diet and Health Study cohort (19). A “Meat and Potatoes” pattern described by high intakes of red and processed meat, potatoes (i.e., French fries and potato salad), and high-fat foods such as gravy and fried fish was associated with RRs of 1.18 (95% CI = 1.02-1.35) and 1.48 (95% CI = 1.20-1.83) for colorectal cancer among men and women, respectively.

Additional factors identified

There were very few clear associations between other factor analysis-derived dietary patterns and colorectal cancer risk in the literature reviewed. Flood et al. (19) reported an inverse association (RR = 0.82; 95% CI = 0.72-0.94) between a “Diet” pattern characterized by greater intakes of fat-reduced/diet foods and lean meats and colorectal cancer risk among male participants in the NIH-AARP Diet and Health Study. Slattery and colleagues (17) identified a similar pattern (“Substituter”) that was not associated with risk among men or women in a large US population-based case-control study. Randall et al. (6) identified a “Light” dietary pattern among women that was characterized by high intakes of lemons, limes, hard cheese, fish, yogurt,

and some fruits and vegetables, and was inversely associated with colon cancer risk (OR = 0.77; 95% CI = 0.63-0.93). Overall, the vast majority of the additional dietary patterns identified were population-specific and not clearly associated with adenoma or cancer risk, limiting efforts to interpret findings and compare patterns across studies.

Table 2.4: Findings from Cluster Analysis-Derived Patterns

Three studies to date have employed cluster analysis to examine dietary patterns and colorectal adenoma or cancer risk: two population-based case-control studies (one in France (9) and one in the US (24)) and a US prospective cohort study (25). Results from the cluster analysis-derived dietary pattern studies are suggestive, although not conclusive, of similar associations to those found in the factor analysis and diet index studies. Overall, findings support a protective effect of a dietary pattern high in micronutrient-rich fruits and vegetables and low in animal fat and high-fat processed meat against colorectal adenoma and cancer incidence.

In a French population-based case-control study (9), Rouillier et al. conducted separate analyses for colorectal adenoma risk (456 cases, 426 polyp-free controls) and colorectal cancer risk (171 cases, 309 population controls) using a unique two-step analytic approach to derive dietary patterns. Factor analysis was performed first to identify important food groups specific to the study population, followed by cluster analysis with the 13 factors that had been retained. Five clusters were identified but not provided with descriptive labels by the authors, thus names that corresponded to descriptions found in the original article were used for the purpose of this review (Table 2.4). A “Low Energy” cluster that most notably was characterized by lower intakes of high-fat processed meats, eggs, bread, starch, wine, pork, beef, and discretionary fats rather than greater intakes of any one food or food group served as the referent group in the multivariate risk analyses of men and women (30-79y) combined. Compared to this “Low

Energy" cluster, a "High Starch and Fat, Low Fruit" pattern, with high intakes of white bread, pork, processed meat, potatoes, rice, and pasta and low intakes of whole grains, fruit, and yogurt, was associated with an OR of 1.5 (95% CI = 1.0-2.2) for colorectal adenoma risk, and a nearly statistically significant OR of 1.5 (95% CI = 0.9-2.5) for colorectal cancer.

Austin and colleagues (24) identified a similar high-risk dietary pattern labeled "High Meat" in a US population-based colorectal adenoma case-control study (179 cases, 466 controls). The "High Meat" pattern was characterized by low intakes of whole grains, fruits, and vegetables (other than potatoes), and comparatively higher intakes of all types of meat. Similar to the "Low Energy" pattern observed by Rouillier et al. (9), a "High Fruit-Low Meat" pattern typified by low intakes of high-fat processed meats, pork, beef, and discretionary fats was identified, but also included higher intakes of fruit and whole grain products. The third cluster, a "High Vegetable-Moderate Meat" cluster, was characterized by less meat consumption than the "High Meat" cluster but more than the "High-Fruit-Low Meat," as well as greater vegetable and starch intakes compared to both of the other clusters. In men and women, the "High Meat" cluster and the "High Vegetable-Moderate Meat" cluster were statistically significantly associated with increased colorectal adenoma risk when compared to the "High Fruit-Low Meat" cluster (OR = 1.70; 95% CI = 1.04-2.80; OR = 2.17; 95% CI = 1.20-3.90, respectively).

Findings from the largest, most recent study using cluster analysis (NIH-AARP Diet and Health Study) (25) corroborate the previously observed protective effect of a dietary pattern characterized by high intakes of fruits and vegetables among men. Four clusters were generated for men and three for women, and the "Many Foods" cluster, distinguished by comparatively high intakes of alcohol and sweets, was selected for both women and men to serve as the referent category. A statistically significant inverse association (HR = 0.85; 95% CI = 0.76-0.94) was

observed among men in a "Vegetables & Fruit" cluster characterized by a greater consumption of fruits, vegetables, poultry, and pasta, and lower intakes of added fat.

DISCUSSION

Two general dietary patterns were observed in the literature reviewed despite a number of differences in study design, methods, and population characteristics. One pattern was in general agreement with current dietary guidance for public health promotion and disease prevention (11,26,27) as it was characterized most consistently by high intakes of fruits and vegetables, and often by higher intakes of one or more of the following food items or groups: whole grains, low-fat dairy products, fish, poultry, olive oil, and legumes. Food items or groups that were not characteristic of this pattern included potatoes, red and processed meat, alcohol, high-sugar and high-fat snacks, and desserts, alcohol, and refined grains such as white bread. Similar to higher scores on recommendation-based indices, a dietary pattern corresponding to general dietary guidance and often labeled accordingly (e.g., "Healthy," "Prudent," or "Fruits and Vegetables") appeared protective against colorectal adenoma and cancer incidence, particularly among men. The reproducibility of these findings in the literature reviewed provides support for the notion that increased adherence to a dietary pattern consistent with current dietary guidance provided by the 2005 *Dietary Guidelines for Americans* (11), or with a Mediterranean-style diet that has previously been associated with reduced morbidity and mortality (28-30), may reduce the risk of colorectal cancer among men, and possibly among women.

The other distinct dietary pattern that emerged from the literature was characterized by greater intakes of animal fat and meat, in particular high-fat processed meat, along with preferential consumption of potatoes over other vegetables, high-sugar and high-fat food items

such as fast food, pizza, and desserts, and refined grain products such as white bread. Accordingly, a dietary pattern with a few to many of these characteristics was often labeled "Western," "Meat and Potatoes," or a close variant, and a modest adverse effect of this pattern was suggested. Many other patterns were identified, some unique to specific populations like the dietary pattern entitled "Japanese" (23), whereas other patterns were common to a few studies, such as a fat-reduced and diet foods pattern.

Although the associations of the two common dietary patterns that emerged with colorectal adenoma and cancer risk were modest and not consistently statistically significant, the directions of the associations were in agreement with much of the previous literature on individual dietary components and colorectal cancer (31). A dietary pattern characterized by high intakes of fruits, vegetables, whole grains, fish, and low-fat dairy and comparatively lower intakes of alcohol, red meat, high-fat processed meats, and high-sugar and high-fat refined grain products would likely be rich in dietary components that may be protective against colorectal carcinogenesis, such as antioxidants, calcium, dietary fiber, folate, and vitamin D. In addition, this pattern would likely be lower in potentially deleterious dietary factors including saturated fat, *trans* fat, alcohol, and meat carcinogens that may arise through food preparation or processing, such as heterocyclic amines and *N*-nitroso compounds (NOCs) (32). Potential mechanisms for the protective or harmful effects of these individual dietary components are numerous. For example, dietary fiber may exert its anticarcinogenic effect through reduced transit time in the gastrointestinal tract, increased binding of carcinogens, increased production of short-chain fatty acids, and decreased concentrations of secondary bile acids (33). Antioxidants can prevent oxidative DNA damage, enhance DNA repair, and inhibit activation of carcinogens such as NOCs (34). The active form of vitamin D, 1,25-dihydroxycholecalciferol,

has been suggested to promote cell differentiation and apoptosis while inhibiting cell proliferation in colonic mucosa (35).

The reason for the differential risk associations for men and women remains uncertain. Perhaps dietary measurement error differs by gender; previous research suggests that women may be more susceptible to self-report bias for reasons of social desirability or approval (36). There may have been underlying differences in the dietary patterns between men and women (37), or differences in other health behaviors not accounted for in the analyses. For example, Wirfält and colleagues (25) identified an additional cluster among men labeled "Fatty Meats" that was not found among women, and a number of other investigations have reported increased meat intakes among men and increased fruit and vegetable intakes among women (38-40). Both Dixon et al. (15) and Reedy et al. (16) found a consistently protective effect against colorectal adenoma and cancer incidence of higher scores on all of the diet indices among men whereas significant inverse associations were observed for higher scores on only two of the indices among women. The authors did not report whether there were statistically significant differences in dietary index scores by gender; however, it is possible that the distribution of scores or the proportion of individuals within each category of scores differed by gender. Diet-related factors could influence the pathogenesis of colorectal cancer differently in men and women. For instance, recent evidence suggests that alcohol consumption is a convincing colorectal cancer risk factor among men, whereas the evidence is less conclusive for women (26). Gender differences in adenoma and tumor location as well as the higher incidence rates experienced by men (41,42) may have contributed to the differential findings.

Strengths of the present review and of dietary pattern analyses include the ability to augment our current understanding of complex diet-cancer relations. Dietary pattern analyses

allow researchers to test whether current dietary recommendations have measurable protective effects on cancer incidence. Factor and cluster analysis can provide insight into food-eating behaviors that may help scientists and policymakers improve current dietary recommendations.

Several limitations of the present review and of dietary pattern analyses are worth noting. Numerous dietary patterns may exist in large or diverse populations, but methodological decisions concerning the grouping of food items and the number of patterns to retain in factor analysis may result in the misclassification of individuals or the oversimplification of actual diets. Dietary indices are more effective in evaluating diet-disease relationships if scores vary considerably in a population (43). Tight clustering around one score may result in a weakened ability to predict disease risk because comparable scores may not actually reflect similar diets. Identified patterns in one population may not be readily transferable to other populations. Dietary patterns may not be reproducible because food patterns may change over time. The study of individual dietary components remains critically important in nutritional epidemiology for the understanding of biological mechanisms underlying observed associations. Findings from dietary pattern and colorectal cancer risk investigations should be interpreted together with results from single nutrient, food, or food group examinations.

In summary, the various analytic tools used in the literature reviewed identified dietary patterns independently of the disease endpoint of interest. Statistical methods such as reduced rank regression (44-46), which group individual foods based on their association with the disease, may provide further insight into diet-cancer relationships. Future studies that validate measures of dietary patterns extracted from FFQ data with multiple 24-hour dietary recalls or food records are needed (47,48). Dietary pattern analyses in diverse populations will be important to evaluate whether various sociodemographic factors modify the dietary pattern-

colorectal cancer relationships. Further analyses are warranted to evaluate whether the observed gender differences in the dietary pattern-colorectal cancer relationship are associated with a gender bias in reporting dietary intake (36), gender differences in food eating patterns (17, 25, 49), or both. Prospective cohort studies with large sample sizes and many years of follow-up will likely be valuable resources in elucidating the role of long-term dietary pattern exposures in colorectal cancer development. Lastly, continued research efforts are needed to evaluate the cumulative and potentially interactive effects of numerous dietary exposures on colorectal cancer risk.

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TABLE 2.1

Criteria for inclusion and exclusion of studies in the selection process

Inclusion criteria
1. Dietary patterns, food patterns, eating patterns, or overall diet scores as the primary exposure of interest
2. Outcome of colon or colorectal cancer or adenoma incidence
3. Prospective cohort or population-based case-control study design
4. All publication dates

TABLE 2.2
Characteristics of population-based epidemiological studies assessing associations between diet index-derived dietary patterns and colorectal adenoma or cancer risk[a]

Author, year (Reference)	Study population	Design	Age (Yr)	Dietary assessment	Outcome	Sex	No. of cases/ controls or cohort size	Results: OR, RR, or HR (95%CI) for highest v. lowest score quartile[b]	Adjustments
Mai et al., 2005 (44)	BCDDP Follow-Up Study, USA	Cohort	35-74	62-item FFQ (abbreviated NCI Block)[c]	Colorectal cancer	F	372/37135	1. RFS: 0.84 (0.62, 1.14)	BMI, energy, NSAID use, previous colonoscopies (yes/no), smoking
Dixon et al., 2007 (15)	PLCO Cancer Prevention Trial, USA	Population-based case-control	55-74	137-item FFQ	Colorectal adenoma	M	2321/17435	1. USDA FG: 0.74 (0.64-0.85); 2. DASH: 0.75 (0.62, 0.91); 3. MED: 0.79 (0.68-0.92)	Age, BMI, calcium supplementation, education, energy, ethnicity, NSAID use, PA, smoking
						F	1271/16536	1. USDA FG: 0.82 (0.68-0.99); 2. DASH: 1.03 (0.81, 1.30); 3. MED: 0.99 (0.81-1.23)	Age, BMI, calcium supplementation, education, energy, ethnicity, HRT, NSAID use, PA, smoking
Reedy et al., 2008 (16)	NIH-AARP Diet and Health Study, USA	Cohort	50-71	124-item FFQ (NCI DHQ)[d]	Colorectal cancer	M	2151/293615	1. HEI-2005: 0.72 (0.62-0.83); 2. A-HEI: 0.71 (0.61-0.82); 3. MED: 0.72 (0.63-0.83); 4. RFS: 0.75 (0.65-0.87)	Age, BMI, education, energy, ethnicity, PA, smoking
						F	959/199726	1. HEI-2005: 0.80 (0.64-0.98); 2. A-HEI: 0.83 (0.66-1.05); 3. MED: 0.89 (0.72-1.11); 4. RFS: 1.01 (0.80-1.28)	Age, BMI, education, energy, ethnicity, HRT, PA, smoking

[a] Abbreviations are as follows: OR, odds ratio; RR, relative risk; HR, hazard ratio; CI, confidence interval; BCDDP, Breast Cancer Detection Demonstration Project; FFQ, food frequency questionnaire; NCI, National Cancer Institute; F, female; RFS, Recommended Food Score; BMI, body mass index, NSAID, non-steroidal anti-inflammatory drug; PLCO, Prostate, Lung, Colorectal, and Ovarian; M, male; USDA FG, US Department of Agriculture Food Guide; DASH, Dietary Approaches to Stop Hypertension Eating Plan; MED, Mediterranean Diet; PA, physical activity; HRT, hormone replacement therapy; NIH-AARP, National Institutes of Health-AARP; NCI DHQ, National Cancer Institute's Diet History Questionnaire; HEI-2005, Healthy Eating Index-2005; A-HEI, Alternate Healthy Eating Index.

[b] Higher scores indicate greater diet quality according to the diet index; multivariate-adjusted risk estimates presented.

[c] FFQ validated in a separate study population.

[d] FFQ validated within the study population.

TABLE 2.3
Characteristics of population-based epidemiological studies assessing dietary patterns by factor analysis and colorectal adenoma or cancer risk[a]

Author, year (Reference)	Study population	Design	Age (Yr)	Dietary assessment	Outcome	Sex	No. of cases/ controls or cohort size	Results: OR, RR, or HR (95% CI) for highest v. lowest factor score quintile or quartile[b]	Adjustments
Randall et al., 1992 (6)	Western New York Diet Study, USA	Population-based case-control	> 40	128-item FFQ[c]	Colon cancer	M	205/205	1. "Salad": 0.84, CI (0.94-1.02);[d] 2. "Fruit": 0.84 (0.69-1.02); 3. "Healthful": 0.96 (0.79-1.17); 4. "Traditional": 1.28 (1.04-1.57); 5. "Snacks": 1.31 (1.07-1.60); 6. "High Fat": 1.28 (1.05-1.58); 7. "Whole Grain": 0.89 (0.73-1.09)	Age, education, energy
						F	223/223	1. "Salad": 0.73 (0.60-0.89); 2. "Healthful": 1.00 (0.83-1.21); 3. "Low Cost: 1.15 (0.94-1.42); 4. "Fruit": 1.08 (0.90-1.31); 5. "High Fat": 0.99 (0.82-1.20); 6. "Light": 0.77 (0.63-0.93); 7. "Whole Grain": 0.88 (0.73-1.07)	Age, education, energy
Slattery et al., 1998 (17)	Multi-site study, USA	Population-based case-control	30-79	CARDIA interviewer-administered diet history questionnaire[e]	Colon cancer	M	1099/1290	1. "Western": 1.80 (1.28-3.15); 2. "Prudent": 0.66 (0.50-0.86); 3. "Drinker": 1.09 (0.84-1.42); 4. "Substituter": 0.84 (0.64-1.10); (3 other factors identified but not analyzed)	Age, BMI, energy, PA
						F	894/1120	1. "Western": 1.49 (1.05-2.12); 2. "Prudent": 0.73 (0.55-0.97); 3. "Drinker": 0.91 (0.68-1.22); 4. "Substituter": 0.86 (0.63-1.17); (2 other factors identified but not analyzed)	Age, BMI, energy, PA
Terry et al., 2001 (21)	SMC, Sweden	Cohort	40-74	67-item FFQ[c]	Colorectal cancer	F	460/61143	1. "Healthy": 0.79 (0.56-1.10); 2. "Western": 0.97 (0.66-1.44); 3. "Drinker": 1.13 (0.84-1.53)	Age, BMI, education, energy
Fung et al., 2003 (18)	NHS, USA	Cohort	38-63	131-item FFQ (Willet)[c]	Colorectal cancer	F	546/76399	1. "Prudent": 0.71 (0.50-1.00); 2. "Western": 1.46 (0.97, 2.19)	Age, alcohol, aspirin use, BMI, energy, family history, missing FFQ, multivitamin use, PA, smoking, time
Dixon et	ATBC,	Cohort	50-	276-item FFQ[c]	Colorectal	M	322/29133	1. "Vegetables": 1.22 (0.87-1.73);	Age, BMI, education,

al., 2004 (20)	Finland		69		cancer			2. “Pork, Processed Meats, Potatoes”: 1.49 (0.93-2.39)	energy, occupational activity, smoking, treatment group
	SMC, Sweden	Cohort	40-74	67-item FFQ[c]	Colorectal cancer	F	586/61463	1. “Vegetables”: 0.99 (0.77-1.27); 2. “Pork, Processed Meats, Potatoes”: 1.37 (1.00-1.89)	Age, BMI, education, energy
	NLCS, Netherlands	Cohort	55-69	150-item FFQ[c]	Colorectal cancer	M	660/58279	1. “Vegetables”: 1.04 (0.78-1.39); 2. “Pork, Processed Meats, Potatoes”: 0.90 (0.65-1.26)	Age, BMI, education, energy, family history, PA, smoking
						F	512/62573	1. “Vegetables”: 0.91 (0.65-1.27); 2. “Pork, Processed Meats, Potatoes”: 0.89 (0.64-1.23)	Age, BMI, education, energy, family history, PA, smoking
Wu et al., 2004 (7)	HPFS, USA	Cohort	40-75	131-item FFQ (Willet)[c]	Colon cancer	M	561/47311	1. “Prudent”: 0.84 (0.64-1.10); 2. “Western”: 1.21 (0.91, 1.60)	Age, aspirin use, BMI, energy, family history, history of endoscopy, PA, race, smoking
					Distal colon adenoma	M	1207/20888	1. “Prudent”: 0.89 (0.73-1.08); 2. “Western”: 1.26 (1.03, 1.53)	Age, aspirin use, BMI, energy, family history, indication of last endoscopy, PA, race, smoking
Kim et al., 2005 (22)	JHPC, Japan	Cohort	40-59	44-item FFQ[c]	Colorectal cancer	M	231/20300	1. “Healthy”: 0.81 (0.52-1.24); 2. “Traditional”: 0.88 (0.55-1.42); 3. “Western”: 0.93 (0.62-1.41)	Age, alcohol intake, BMI, education, energy, family history, PA, smoking, study area
						F	139/21812	1. “Healthy”: 0.98 (0.58-1.65); 2. “Traditional”: 1.53 (0.93-2.52); 3. “Western”: 1.45 (0.85-2.48)	Age, BMI, education, energy, family history, PA, smoking, study area
Mizoue et al., 2005 (23)	Self-Defense Forces Health Study, Japan	Population-based case-control	47-59	74-item FFQ	Colorectal adenoma	M	346/995	1. “High-Dairy, High-Fruit and -Vegetable, High-Starch, Low-Alcohol”: 0.62 (0.43-0.90); 2. “Animal Food”: 0.86 (0.60-1.23); 3. “Japanese”: 1.18 (0.83-1.69)	Age, BMI, occupational rank, PA, site of colonoscopy examination, smoking
Kesse et al., 2006 (8)	EPIC, France	Cohort	40-65	208-item FFQ[c] + qualitative questions	Colorectal adenoma	F	516/5320	1. “Healthy”: 0.85 (0.65-1.10); 2. “Western”: 1.39 (1.00-1.94); 3. “Drinker”: 1.42 (1.10-1.83); 4. “Meat Eater”: 1.13 (0.87-1.46)	BMI, education, energy, family history, PA, smoking

					Colorectal cancer	F	172/67484	1. “Healthy”: 0.77 (0.49-1.20); 2. “Western”: 1.09 (0.60-2.00); 3. “Drinker”: 1.36 (0.85-2.17); 4. “Meat Eater”: 1.58 (0.98-2.53)	BMI, education, energy, family history, PA, smoking
Flood et al., 2008 (19)	NIH-AARP Diet and Health Study, USA	Cohort	50-71	124-item FFQ[c] (NCI DHQ)[c]	Colorectal cancer	M	2151/293615	1. “Fruits and Vegetables”: 0.81 (0.70-0.93); 2. “Fat-Reduced/Diet Foods”: 0.82 (0.72, 0.94); 3. “Meat and Potatoes”: 1.18 (1.02-1.35)	BMI, education, ethnicity, family history, NSAID use, PA, smoking
						F	959/198767	1. “Fruits and Vegetables”: 1.06 (0.86-1.30); 2. “Meat and Potatoes”: 1.48 (1.20-1.83) 3. “Fat-Reduced/Diet Foods”: 0.87 (0.71, 1.07)	BMI, education, ethnicity, family history, HRT, NSAID use, PA, smoking

[a] Abbreviations are as follows: OR, odds ratio; RR, relative risk; HR, hazard ratio; CI, confidence interval; FFQ, food frequency questionnaire; M, male; F, female; CARDIA, Coronary Artery Risk Development in Young Adults; BMI, body mass index; PA, physical activity; SMC, Swedish Mammography Cohort; NHS, Nurses’ Health Study; ATBC, Alpha-Tocopherol Beta-Carotene Cancer Prevention Study; NLCS, Netherlands Cohort Study on Diet and Cancer; HPFC, Health Professionals Follow-up Study; JPHC, Japan Public Health Center cohort; EPIC, European Prospective Investigation into Cancer and Nutrition; NIH-AARP, National Institutes of Health-AARP Diet and Health Study; NCI DHQ, National Cancer Institute’s Diet History Questionnaire; NSAID, non-steroidal anti-inflammatory drug; HRT, hormone replacement therapy.

[b] Multivariate-adjusted risk estimates presented.

[c] FFQ validated within the study population.

[d] Risk estimates in the study by Randall et al. are for an increase of 1 standard deviation in factor score.

[e] FFQ validated in a separate study population.

TABLE 2.4
Characteristics of population-based epidemiological studies assessing dietary patterns by cluster analysis and colorectal adenoma or cancer risk[a]

Author, year (Reference)	Study population	Design	Age (Yr)	Dietary assessment	Outcome	Sex	No. of cases/ controls or cohort size	Results: OR, RR, or HR (95% CI) by cluster[b]	Adjustments
Rouillier et al., 2005 (9)	Burgundy, France	Population-based case-control	30-79	Interviewer-administered food history questionnaire[c]	Colorectal cancer	M + F	171/309	1. "Low Energy" (low intake of unhealthy foods): referent cluster;[c] 2. "High Starch and Fat, Low Fruit": 1.5 (0.9-2.5); 3. "High Processed Meat, Energy, Alcohol, and Starch ": 1.0 (0.5-2.2); 4. "High Fish, Cereals, Honey, Olive Oil, Fruits and Vegetables": 1.4 (0.6-3.1); 5. "High Flour, Sugar, Chocolate, Animal Fats, and Eggs": 1.5 (0.6-3.8)	Age, alcohol, BMI, energy, PA, smoking
					Colorectal adenoma	M + F	465/426	1. "Low Energy": referent cluster; 2. "High Starch and Fat, Low Fruit": 1.5 (1.0-2.2); 3. "High Processed Meat, Energy, Alcohol, and Starch": 0.9 (0.5-1.5); 4. "High Fish, Cereals, Honey, Olive Oil, Fruits and Vegetables": 0.7 (0.4-1.2); 5. "High Flour, Sugar, Chocolate, Animal Fats, and Eggs": 0.7 (0.4-1.2)	Age, alcohol, BMI, energy, PA, smoking
Austin et al., 2007 (24)	The Diet and Health Study IV, USA	Population-based case-control	30-80	124-item FFQ (NCI DHQ)[e]	Colorectal adenoma	M + F	179/466	1. "High Fruit-Low Meat": referent cluster; 2. "High Vegetable-Moderate Meat": 2.17 (1.20-3.90); 3. "High Meat": 1.70 (1.04-2.80)	Age, alcohol, BMI, ethnicity, gender, NSAID use, smoking
Wirfält et al., 2008 (25)	NIH-AARP Diet and Health Study, USA	Cohort	50-71	124-item FFQ (NCI DHQ)[c]	Colorectal cancer	M	2151/293576	1. "Many Foods": referent cluster; 2. "Vegetables & Fruit": 0.85 (0.76-0.94); 3. "Fatty Meats": 0.94 (0.80-1.10); 4. "Fat-Reduced Foods": 0.88 (0.70-1.11)	Age, BMI, education, energy, ethnicity, PA, smoking
						F	959/198730	1. "Many Foods": referent cluster; 2. "Vegetables & Fruit": 0.90 (0.77-1.06); 3. "Diet Foods, Lean Meats": 1.04 (0.87-1.24)	Age, BMI, education, energy, ethnicity, HRT, PA, smoking

[a] Abbreviations are as follows: OR, odds ratio; RR, relative risk; HR, hazard ratio; CI, confidence interval; M, male; F, female; BMI, body mass index; PA, physical activity; FFQ, food frequency questionnaire; NCI DHQ, National Cancer Institute's Diet History Questionnaire; NIH-AARP, National Institutes of Health-AARP Diet and Health Study; NSAID, non-steroidal anti-inflammatory drug; HRT, hormone replacement therapy.
[b] Multivariate-adjusted risk estimates presented.
[c] FFQ validated within the study population.
[d] Clusters were not named in the article and are labeled here according to descriptions provided by the authors.
[e] FFQ validated in a separate study population.

Chapter 3

DIET INDEX-BASED AND EMPIRICALLY-DERIVED DIETARY PATTERNS ARE ASSOCIATED WITH COLORECTAL CANCER RISK[1]

[1] This manuscript was accepted upon revision:

Miller PE, Lazarus P, Lesko SM, Muscat JE, Harper G, Cross AJ, Sinha R, Ryczak K, Escobar G, Mauger D, and Hartman TJ. Diet index-based and empirically-derived dietary patterns are associated with colorectal cancer risk. *Journal of Nutrition* (Accepted upon Revision).

ABSTRACT

Previous studies have derived patterns by measuring compliance with pre-established dietary guidance or empirical methods such as principal components analysis (PCA). Our objective was to examine colorectal cancer risk associated with patterns identified by both methods. The study included 431 incident colorectal cancer cases (225 men, 206 women) and 726 healthy controls (330 men, 396 women) participating in a population-based case-control study. PCA identified sex-specific dietary patterns and the Healthy Eating Index-2005 (HEI-05) assessed adherence to the *Dietary Guidelines for Americans*. Three dietary patterns among men (fruits and vegetables, meat and potatoes, alcohol and sweetened beverages) and two among women (fruits and vegetables, meat and potatoes) were identified. After adjusting for potential confounders, the fruits and vegetables pattern was inversely associated with colorectal cancer risk among both men (OR = 0.38, 95% CI = 0.21-0.69 for the highest compared to the lowest quartile; *P* for trend = 0.006) and women (OR = 0.35, 95% CI = 0.19-0.65; *P* for trend = 0.031). The meat and potatoes pattern was positively associated with risk in women (OR = 2.20, 95% CI = 1.08-4.50; *P* for trend = 0.070) and there was a suggestion of a positive association among men (OR = 1.56, 95% CI = 0.84-2.90; *P* for trend = 0.070). Men and women with greater HEI-05 scores had a significantly reduced risk of colorectal cancer (OR = 0.56, 95% CI = 0.31-0.99; *P* for trend = 0.004; OR = 0.44, 95% CI = 0.24-0.77; *P* for trend <0.001, respectively). Following the *Dietary Guidelines* or a dietary pattern lower in meat and potatoes and higher in fruits and vegetables may reduce colorectal cancer risk.

INTRODUCTION

Epidemiologic studies have traditionally assessed the effects of single nutrients, foods, and other individual dietary constituents on colorectal cancer risk. Research using this approach is valuable for understanding potential biological mechanisms underlying observed associations, but it is limited by the multicollinearity of dietary intake variables and the inability to detect small effects of single dietary components. The investigation of dietary patterns or overall diet quality is a promising alternative that may prove informative in evaluating diet and colorectal cancer risk (1-4). Examining the totality of diet reflects the complexity of food intake and captures synergistic relationships between various dietary constituents (5).

The majority of studies have derived dietary patterns by one of two fundamentally different approaches. One method is defined as hypothesis-oriented (*a priori*) because it relies upon scientific knowledge from previous investigations into health-promoting or disease-preventing diets (6, 7). With this approach, researchers measure compliance with a pre-existing diet quality index, current dietary guidelines, or a specific dietary pattern and assign diet scores that reflect the level of adherence. The other commonly used method is data-driven (*a posteriori*) since statistical methods, such as principal components analysis (PCA), are used to generate dietary patterns empirically. PCA is a variable-oriented technique that aims to reduce the number of explanatory variables into independent factors that capture the primary sources of dietary variation within a study population. In the present population-based case-control study, we explored both PCA-derived dietary patterns and diet index-based scores, as assessed by the Healthy Eating Index-2005 (HEI-05) (8, 9), and examined whether dietary patterns identified by these two distinct approaches were associated with colorectal cancer risk.

METHODS

Study Design and Population. This study included incident colorectal cancer cases and healthy controls participating in a population-based case-control study in a contiguous 19-county area in central and northeast Pennsylvania. The study was designed to investigate risk factors for colorectal cancer among adult residents of this area. Newly diagnosed cases (identified within 12 months of diagnosis) with histologically-confirmed colon or rectal cancer were identified between June 2007 and November 2009 from records of the Pennsylvania State Cancer Registry. Individuals were eligible for inclusion if they were fluent in English and had no history of previous colorectal cancer. A letter introducing the study was sent to potential participants, followed by a telephone call from a study coordinator to further explain the study, answer questions, and obtain informed consent. Controls residing in the same region who were fluent in English and had no history of colorectal cancer were identified by random digit dialing. After written consent was obtained from a potential case or control, a personal interview was scheduled and a self-administered food frequency questionnaire (FFQ) was mailed with instructions to complete the FFQ prior to the interview. Data regarding sociodemographic factors, medical history, alcohol use, lifetime tobacco exposure, physical activity behavior, height, weight, medication use, and other lifestyle-related factors were collected by trained interviewers during in-person interviews. Information prior to diagnosis was obtained for cases. The previously mailed FFQ was reviewed during the in-person interview. For the present analysis, we excluded participants who were less than 35 y (n = 6), who had missing body mass index (BMI; kg/m^2) data (n = 5), or who reported implausible energy intakes (< 2093 kJ or >

20,930 kJ (10, 11)) (n = 29). After these exclusions, we included 431 cases (225 men, 206 women) and 726 controls (330 men, 396 women) in this analysis. The institutional review boards at the Northeast Regional Cancer Institute, the Penn State College of Medicine, and the Lehigh Valley Health Systems (Allentown, PA) approved this study.

Dietary Assessment Method. Participants completed a modified version of the Diet History Questionnaire (DHQ), a validated FFQ developed by the National Cancer Institute (NCI) (12, 13). The reference period was the past year for controls and the year prior to diagnosis for cases. We modified the DHQ for our study population using previously collected 24-h dietary recall data from a similar Pennsylvania study population (14). These modifications included the addition of processed meat items commonly consumed in this population. The DHQ included a meat module (15) that was modified to capture the meat consumption patterns of Pennsylvania residents. The DHQ and visual materials, which were designed to facilitate the recall of portion sizes, were mailed with instructions to complete the DHQ prior to the scheduled interview. Respondents were queried about their usual intake and portion size of 137 separate food items, 49 of which contained additional embedded questions. Respondents selected from 10 frequency categories that ranged from never to two or more times per day for each food and from nine frequency categories that ranged from never to six or more times per day for each beverage. Three food- and beverage-specific portion size ranges were available for each question. The DHQ included questions that addressed variations in food type (e.g., regular vs. low-fat), seasonal intake, and added fats. Data pertaining to dietary supplement use were collected with the DHQ. Trained interviewers reviewed the DHQ during in-person interviews. Energy and nutrient intake values were calculated with Diet*Calc (version 1.4.3), nutrient analysis software developed by the NCI for use with this instrument and configured to accommodate our

questionnaire modifications. Portion size and frequency of food intake information were used to calculate the average daily servings, according to standard USDA serving sizes (16), for each food item consumed.

Dietary Patterns Derived by Principal Components Analysis. Single food items were aggregated into predefined food groups based on similarity of nutrient content, culinary use, or potential relevance to colorectal cancer etiology. Individual food or beverage items were preserved if they were thought to represent distinct dietary behaviors or they had unique nutrient profiles (**Supplemental Table 1**). PCA was performed on the 35 mutually-exclusive food group and food item variables to identify sex-specific dietary patterns using the Proc Factor command in SAS (version 9.2, SAS Institute, Inc., Cary, North Carolina). We used an orthogonal (varimax) rotation procedure to generate uncorrelated factors and to obtain a simpler structure with greater interpretability (17). Oblique rotation also was explored, but similar patterns emerged. Three dietary patterns were identified for men and two for women after consideration of eigenvalues that were greater than 1.5, the Scree test, and the interpretability of the factors (17). Food groups were considered as contributing substantially to a pattern if the absolute factor loadings were $\geq$ 0.30 (18-20). A positive factor loading indicates that the food group is positively associated with the pattern, whereas a negative loading indicates an inverse association.

The PCA-derived dietary patterns were labeled according to the food groups or food items that loaded most strongly on the respective factors. The first factor for men was labeled fruits and vegetables, which accounted for 13.8% of the variance in dietary intake, followed by a meat and potatoes pattern (9.6%), and an alcohol and sweetened beverages pattern (6.1%). A similar meat and potatoes pattern was the first factor derived for women, which accounted for the largest amount of variance in dietary intake (17.1%), followed by a fruits and vegetables pattern

(10.9%). A factor score was created for participants by summing intakes of food groups weighted by their factor loadings for each of the identified components. Factor scores were categorized into quartiles based on the distribution among the control population for men and women separately.

Diet Index Based-Patterns. We calculated HEI-05 component and summary scores based on a previously published method (9) using food intake data and nutrient intake estimates generated with Diet*Calc. A recent evaluation of the HEI-05 with dietary data collected in NHANES 2001-2002 found the index to be a reliable and valid measure of diet quality (21). The HEI-05, originally developed in 1995 by the USDA's Center for Nutrition Policy and Promotion (8), was recently modified to measure compliance with the key recommendations found in MyPyramid and the *Dietary Guidelines for Americans* (9, 22). Twelve individual components that correspond to these recommendations are included in the revised HEI: total fruit; whole fruit; total vegetables; dark green and orange vegetables and legumes; total grains; whole grains; milk; meat and beans; oils; saturated fat; sodium; and calories from solid fat, alcohol, and added sugars. Solid fat refers to both saturated and *trans* fat and alcohol refers to alcoholic beverages, including beer, wine, and liquor. All components are adjusted for energy on a density basis (amount consumed per 4,186 kJ or % of total kJ) (**Supplemental Table 2**). Greater energy-adjusted dietary intakes of fruit, vegetables, legumes, oil, whole grains, low-fat dairy, and lean meat, and lower energy-adjusted dietary intakes of sodium, saturated fat, and solid fat, alcohol, and added sugar result in higher HEI-05 scores, indicating higher diet quality. HEI-05 scores, which range from a minimum of 0 to a maximum of 100 points, were assigned to all participants and were categorized into quartiles based on the distribution among the control population for men and women separately.

Statistical Methods. Characteristics of cases and controls were compared with *t* tests for continuous variables and χ^2 tests for categorical variables. Food intakes were energy-adjusted by the residual method for comparisons of mean intakes between cases and controls (23). Spearman rank correlation coefficients were calculated to assess the relationship between the dietary pattern factor scores and HEI-05 scores. Odds ratios (ORs) and 95% confidence intervals (CI) for each quartile of factor and HEI-05 scores were estimated by logistic regression to determine whether the PCA-derived patterns and the diet index-derived patterns were associated with colorectal cancer risk. Age-adjusted and multivariate-adjusted logistic regression analyses were conducted separately for men and women. We calculated *P* for trend by modeling the factor and HEI-05 scores as continuous variables. The following potential confounders were included in the final multivariate logistic regression models: age (y); educational attainment (< high school, high school/some college, and college graduate/advanced degree); BMI (kg/m^2); smoking status (never, current, or past); total energy intake (kJ/d); family history of colorectal cancer (yes, no); nonsteroidal anti-inflammatory drug (NSAID) use (yes, no); and physical activity (< 1 h vs. $\geq$ 1 h/wk of vigorous activity). Postmenopausal hormone use (yes, no) was included in the final multivariate regression model for women. NSAID and postmenopausal hormone use were defined as ever having been a regular user ($\geq$ 1/wk for at least 1 y). Alcohol intake was not considered in the multivariate regression model because it was one of the HEI-05 sub-components and an input variable in the PCA. We explored the possibility of effect modification by creating cross-product terms of dietary pattern scores and potential modifiers, including age, BMI, physical activity, and smoking. No statistically significant interactions were identified. Reported *P* values are 2-sided and $P < 0.05$ was considered significant for all tests. All statistical analyses were performed with SAS (version 9.2, SAS Institute, Inc., Cary, North Carolina).

RESULTS

Demographics. Racial distribution, smoking status, regular NSAID use, dietary supplement use, family history of colorectal cancer, total energy intake, and energy-adjusted intakes of fruits and vegetables, red and processed meat, and low-fat dairy products did not differ significantly between cases and controls in both men and women (**Table 3.1**). Energy-adjusted intakes of white and fried potatoes and whole grains were significantly greater in male controls compared to cases, but were similar for female cases and controls. Female and male cases were older, less physically active, and less likely to have a college or advanced degree compared to controls. Female cases had greater BMIs and alcohol intake compared to controls, whereas BMI and alcohol intake did not differ significantly between male cases and controls. Regular menopausal hormone use was similar between female cases and controls.

***Dietary patterns*.** PCA identified three major dietary patterns among men (fruits and vegetables [12 items], meat and potatoes [13 items], and alcohol and sweetened beverages [9 items]); two of these patterns were also identified among women (fruits and vegetables [12 items] and meat and potatoes [16 items]) (**Table 3.2**). The fruits and vegetables pattern was characterized by greater intakes of all subgroups of fruits and vegetables other than potatoes, as well as nuts, seeds, legumes, whole grains, vegetable oils, and low-fat dairy in women and fish in men. The meat and potatoes pattern was characterized by greater consumption of red and processed meat, poultry, fried and white potatoes, high-fat dairy, sweets, salty snacks, butter, mayonnaise, gravy, pizza, and refined grains for both men and women. Starchy vegetables, sugar-sweetened beverages, and margarine were also represented in the meat and potatoes pattern among women, whereas sugar-

free beverages contributed to the meat and potatoes pattern among men. In addition to sweetened beverages, liquor, and beer, the third pattern derived in men (alcohol and sweetened beverages) was characterized by greater consumption of refined grains, sweets, high-fat dairy products, mayonnaise, and fruit. Higher scores on the fruits and vegetables pattern were positively associated with HEI-05 scores for men ($r = 0.59$; $P < 0.001$) and women ($r = 0.58$; $P < 0.001$). Higher scores on the meat and potatoes and alcohol and sweetened beverage patterns in men were inversely associated with HEI-05 scores ($r = -0.36$ and -0.16, respectively; $P < 0.001$ for both). An inverse association was observed between the meat and potatoes pattern and HEI-05 scores for women ($r = -0.48$; $P < 0.001$).

***Dietary patterns and colorectal cancer risk in men*.** We observed an inverse association between the fruits and vegetables pattern and risk of colorectal cancer (OR = 0.38 for the highest compared with the lowest quartile; 95% CI = 0.21-0.69; *P* for trend = 0.006) after adjusting for potential confounders (**Table 3.3**). In contrast, higher scores on the meat and potatoes pattern were positively associated with colorectal cancer risk in the age-adjusted model (OR = 1.92; 95% CI = 1.15-3.20; *P* for trend = 0.005), but this association was attenuated in the fully-adjusted model (OR = 1.56; 95% CI = 0.84-2.90; *P* for trend = 0.070). There was no evidence of an association between scores on the alcohol and sweetened beverages pattern and risk. A protective effect (OR = 0.56; 95% CI = 0.31-0.99; *P* for trend = 0.004) was seen with higher scores on the HEI-05, suggesting that greater adherence to the *Dietary Guidelines* was associated with a decreased risk of colorectal cancer.

***Dietary patterns and colorectal cancer risk in women*.** Diets that were more consistent with the fruits and vegetables pattern and the *Dietary Guidelines* appeared protective against colorectal cancer among women (**Table 3.4**). The multivariate ORs (95% CI) of colorectal cancer across

increasing quartiles of fruits and vegetables pattern scores were 0.46 (0.27-0.78), 0.45 (0.26-0.76), and 0.35 (0.19-0.65) (*P* for trend = 0.031). By contrast, we observed a significant positive association between the meat and potatoes pattern and colorectal cancer risk when comparing the highest to the lowest quartile of factor scores (OR = 2.20; 95% CI = 1.08-4.50; *P* for trend = 0.170). Greater HEI-05 scores were associated with a significantly reduced risk of colorectal cancer (OR = 0.44; 95% CI = 0.24-0.77; *P* for trend < 0.001).

DISCUSSION

Our results support the use of both hypothesis-oriented and empirical methods for deriving dietary patterns to evaluate diet and colorectal cancer risk. Dietary patterns that captured the primary sources of variation in dietary intake as well as those derived by measuring adherence to the *Dietary Guidelines* provided insight into the association between diet and colorectal cancer risk. We observed significant inverse associations between higher scores on the fruits and vegetables factor and the HEI-05 and colorectal cancer risk in both men and women. The diet index and PCA approaches have been used in previous studies of diet and colorectal cancer. Findings have been consistent in men, but have been mixed in women. Higher scores on all diet indices investigated have been associated with a reduced risk of colorectal cancer in men (24, 25). On the other hand, significant inverse associations with colorectal cancer have been observed with higher scores on some (24, 25) but not all diet indices (24-26) among women. Greater levels of adherence with the *Dietary Guidelines*, measured by the HEI-05 (25) or a USDA Food Guide Index (24), have been shown to reduce colorectal cancer risk in women, whereas higher scores on the Recommended Food Score (RFS) (27), the Alternate-Healthy

Eating Index (A-HEI) (28), the Mediterranean Diet Score (MDS) (24, 25), and the DASH Score (24) have not been found to be protective.

Findings have been suggestive but not conclusive for both men and women in studies examining the risk of colorectal cancer associated with PCA-derived dietary patterns. A healthy or prudent pattern characterized by high intakes of fruits and vegetables, and often higher intakes of whole grains, low-fat dairy products, fish, poultry, vegetable oils, and/or legumes, has been identified in several studies (19, 29-37) and is comparable to the fruits and vegetables pattern described in our population. Greater adherence to this pattern has been associated with a reduced risk of colorectal cancer among men in two U.S. case-control studies (29, 31), two U.S. cohort studies (30, 33), one Japanese case-control (19), and one Japanese cohort study (37). Not all associations have been statistically significant (29, 30, 37) and results from a study of two European cohorts in the Dietary Patterns and Cancer (DIETSCAN) Project found no association between a vegetables pattern and colorectal cancer risk (35). Differences in the dietary patterns identified and the study populations may partially explain the inconsistent findings. A significant inverse association between a fruits and vegetables pattern and colorectal cancer risk has been reported in two U.S. case-control studies (29, 31), one U.S. cohort study (32), and two European cohort studies (34, 36) among women. Similar to findings among men, not all associations were statistically significant (32, 34, 36) and results from DIETSCAN were not suggestive of an inverse association (35).

Our findings support a protective effect for both men and women of a largely plant-based dietary pattern characterized by higher intakes of fruits, vegetables, whole grains, nuts, seeds, vegetable oils, and low-fat dairy in women and fish in men. It is not unexpected that higher fruits and vegetables pattern scores were positively associated with higher HEI-05 scores since our

fruits and vegetables pattern is in general agreement with current dietary guidance for public health promotion and disease prevention (22, 38, 39). Greater scores on the fruits and vegetables pattern and the HEI-05 would likely reflect diets rich in components that may be protective against colorectal carcinogenesis, such as antioxidants, calcium, vitamin D, folate, and dietary fiber. Exposure to potentially harmful dietary factors including *trans* fat, saturated fat, and meat-related compounds (e.g., heterocyclic amines, polycyclic aromatic hydrocarbons, *N*-nitroso compounds, and heme iron) would likely be lower.

A second PCA-derived dietary pattern analogous to the meat and potatoes pattern found in our study has been identified in a number of investigations (30-37). This pattern has been characterized most often by greater intakes of red and processed meat, fried and white potatoes, fast food, pizza, high-fat dairy products, desserts, refined grain products, and other high-fat and high-sugar food items. The meat and potatoes pattern derived in the present study was associated with a statistically significant increased risk of colorectal cancer in women, which is in agreement with some (31, 33-35) but not all previous studies (29, 32, 36, 37). Men with diets more closely aligned to the meat and potatoes pattern in our study had a non-significant increased risk. Similar to results from previous investigations in women, findings among men have been mixed (19, 29-31, 33, 35, 37). Differences in study design, population characteristics, and researcher-dependent decisions in relation to food grouping and the number of factors to retain may partially explain the differences in findings.

Both the hypothesis-oriented index-based approach and the empirically-driven PCA approach proved informative in our examination of diet and colorectal cancer risk in a population-based case-control study. The more useful tool in future studies may depend upon the objective of the investigation. Diet index-based approaches are valuable in assessing compliance

with recommended dietary patterns, while data-driven analyses are useful in examining actual dietary behavior within a population. The strengths and limitations of each approach should be considered when selecting a method to examine dietary patterns. Dietary patterns generated by PCA in one population may not be replicable in other populations, whereas a diet index that measures adherence to established dietary guidance is more easily transferrable across study populations. An additional reservation often cited with the PCA relates to researcher-dependent decisions concerning the grouping of food items and the number of factors to retain. A strength of PCA is its ability to overcome issues pertaining to the multicollinearity of food, nutrients, and other dietary constituents. Dietary indices may have limited use in evaluating diet-disease relationships when scores do not vary considerably in a population (40). Tight clustering around one score may result in a weakened ability to predict disease risk because comparable scores may not reflect similar diets. The effectiveness of a particular diet index in the study of diet-disease associations depends largely upon the underlying evidence used in its development.

The strengths of our study include the population-based design, the use of a validated FFQ (12, 13), and the detailed exposure information captured. Trained interviewers reviewed the FFQs to ensure completeness and were blind to the research hypothesis to minimize interviewer bias. Several limitations of the present study should also be considered. Measurement error associated with FFQs may lead to non-differential misclassification of respondents into dietary exposure categories, thereby attenuating risk estimates. The case-control design used in this study is susceptible to recall bias; cases may report past diet differently than controls if certain dietary exposures are preconceived to be risk factors for cancer. Although the major dietary patterns identified in our study are consistent with those generated in previous investigations, the generalizability of our findings may be limited due to a fairly homogenous sample consisting of

a predominately white and rural population. Potential confounders were considered and included in our multivariate models, but residual or unknown confounding remains possible.

Previous investigations into dietary patterns and colorectal cancer risk have relied upon either the diet index-based approach or an empirical method such as PCA. Our study examined dietary patterns derived from these two fundamentally different approaches to predict colorectal cancer risk within the same population after controlling for the same potential confounders. Our findings indicate that both the hypothesis-oriented diet index-based approach and the empirically-driven PCA approach are of value in the assessment of diet and colorectal cancer risk. In addition, following the *Dietary Guidelines for Americans* or a primarily plant-based dietary pattern that includes low-fat dairy and fish may reduce colorectal cancer risk.

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Author Contributions

P. L., T. J. H., S. M. L., J. E. M., G. H., A. J. C., R. S., and P. E. M designed research; K. R. and G. E. conducted research; A. J. C. and R. S. provided essential materials; P. E. M., T. J. H., and D.M. analyzed data; P. E. M. wrote the paper; P. E. M. had primary responsibility for final content. All authors read and approved the final manuscript.

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TABLE 3.1 Selected characteristics of cases and controls, by gender[1]

	Men			Women		
	Controls	Cases	*P*-value	Controls	Cases	*P*-value[2]
n	330	225		396	206	
Age, *y*	61.4 ± 10.4	66.2 ± 10.7	<0.001	57.5 ± 10.7	65.8 ± 11.9	<0.001
Race, *% white*	97.6	96.9	0.624	98.0	98.1	0.948
Education, *%*			<0.001			<0.001
< High school	3.6	12.4		3.5	11.2	
High school/some college	60.3	59.1		65.7	69.9	
College graduate/advanced degree	36.1	28.4		30.8	18.9	
BMI, kg/m^2	29.0 ± 5.4	29.7 ± 5.4	0.128	28.2± 6.3	29.9 ± 7.6	0.005
Smoking status, *%*			0.121			0.270
Never	40.0	39.6		51.8	52.4	
Former	45.2	51.1		33.6	37.4	
Current	14.9	9.3		14.7	10.2	
Alcohol, *g/d*	17.3 ± 42.8	14.7 ± 36.9	0.080	6.8 ± 15.3	3.7 ± 11.8	<0.001
NSAID use, *% yes*	59.7	56.4	0.445	51.0	46.6	0.305

Physical activity, *% ≥ 1 h/wk*	37.3	28.9	0.041	39.1	19.4	<0.001
Family history of colorectal cancer, *% yes*	10.9	15.6	0.108	12.4	16.0	0.216
Menopausal hormone use, *% yes*	–	–	–	18.9	17.0	0.557
Multivitamin use, *% yes*	51.5	50.2	0.765	56.3	48.5	0.070
Supplemental calcium use, *% yes*	13.6	11.6	0.471	47.2	42.2	0.244
Healthy Eating Index-2005 (HEI-05), *score*	63.3 ± 12.7	60.7 ± 11.6	0.014	67.7 ± 11.0	63.9 ± 11.7	<0.001
Dietary intakes[3]						
Total energy, *kJ/d*	8472 ± 3415	8535 ± 3749	0.729	6714 ± 2833	6836 ± 3060	0.969
Fruits and vegetables,[4] *servings/d*	5.3 ± 3.4	5.0 ± 3.7	0.318	5.3 ± 3.0	5.1 ± 3.6	0.263
Red and processed meat, *servings/d*	3.2 ± 2.3	3.3 ± 2.3	0.376	1.8 ± 1.3	1.8 ± 1.7	0.903
White and fried potatoes, *servings/d*	0.79 ± 0.60	0.91 ± 0.73	0.043	0.64 ± 0.60	0.69 ± 0.58	0.304
Low-fat dairy products, *servings/d*	0.54 ± 0.84	0.46 ± 0.84	0.261	0.59 ± 0.70	0.51 ± 0.62	0.167
Whole grains, *servings/d*	1.3 ± 1.2	1.1 ± 1.0	0.025	1.0 ± 0.9	1.0 ± 1.0	0.419

[1] Values are means ± SD for continuous variables and percents for categorical variables.

[2] *P*-values for differences in means were calculated with *t* tests and differences in proportions were calculated with χ^2 tests.

[3] Dietary intakes were adjusted for total energy intake by the residual method.

[4] Fruits and vegetables category excludes white and fried potatoes.

TABLE 3.2 Factor loadings of food groups in the dietary patterns derived from principal components analysis[1]

	Men			Women	
Food group	Factor 1: fruits and vegetables	Factor 2: meat and potatoes	Factor 3: alcohol and sweetened beverages	Factor 1: meat and potatoes	Factor 2: fruits and vegetables
Other vegetables	0.77	–	–	–	0.80
Yellow and orange vegetables	0.68	–	–	–	0.70
Dark green and cruciferous vegetables	0.60	–	–	–	0.73
Other fruit	0.59	–	0.35	–	0.62
Tomatoes	0.54	–	–	–	0.46
Citrus, berries, melons	0.48	–	0.45	–	0.54
Starchy vegetables	0.45	–	–	0.41	0.49
Legumes	0.45	–	–	–	0.50
Vegetable oils	0.41	–	–	–	0.39
Fish	0.36	–	–	–	–
Nuts and seeds	0.32	–	–	–	0.34
Whole grains	0.31	–	–	–	0.37
Fresh red meat	–	0.70	–	0.57	–
Fried potatoes	–	0.67	–	0.50	–
White potatoes	–	0.63	–	0.70	–
Refined grains	–	0.55	0.40	0.68	–
Processed meat	–	0.53	–	0.48	–
Gravy	–	0.40	–	0.43	–
Poultry	–	0.39	–	0.35	–
Sweets	–	0.38	0.32	0.51	–
Butter	–	0.38	–	0.48	–
Pizza	–	0.38	–	0.34	–
Salty snacks	–	0.37	–	0.43	–
Sugar-free beverages	–	0.33	–	–	–
Sugar-sweetened beverages	–	–	0.64	0.35	–
Liquor	–	–	0.59	–	–

Mayonnaise	–	–	0.42	0.49	–
High-fat dairy	–	0.30	0.34	0.49	–
Beer	–	–	0.31	–	–
Margarine	–	–	–	0.43	–
Low-fat dairy	–	–	–	–	0.31

[1] Factor loadings $\leq$ |0.30| are omitted for simplicity.

TABLE 3.3 Odds ratios (95% CI) of colorectal cancer risk according to quartiles of dietary pattern and diet index scores in men

		Age-adjusted	Multivariate-adjusted[1]
Dietary pattern scores	Cases/controls	OR (95% CI)	OR (95% CI)
Fruits and vegetables pattern			
Quartile 1	65/82	1.00 (ref)	1.00 (ref)
Quartile 2	70/83	0.96 (0.60-1.54)	0.89 (0.53-1.49)
Quartile 3	50/82	0.76 (0.46-1.24)	0.64 (0.37-1.10)
Quartile 4	40/83	0.54 (0.32-0.90)	0.38 (0.21-0.69)
P for trend		0.046	0.006
Meat and potatoes pattern			
Quartile 1	48/83	1.00 (ref)	1.00 (ref)
Quartile 2	64/82	1.37 (0.83-2.25)	1.39 (0.83-2.32)
Quartile 3	47/82	1.08 (0.65-1.83)	1.03 (0.58-1.81)
Quartile 4	66/83	1.92 (1.15-3.20)	1.56 (0.84-2.90)
P for trend		0.005	0.070
Alcohol and sweetened beverages pattern			
Quartile 1	51/83	1.00 (ref)	1.00 (ref)

Quartile 2	53/82	1.07 (0.65-1.77)	1.13 (0.67-1.91)
Quartile 3	69/83	1.39 (0.86-2.52)	1.37 (0.83-2.27)
Quartile 4	52/82	1.14 (0.69-1.89)	1.02 (0.58-1.80)
P for trend		0.161	0.441
HEI-05			
Quartile 1	60/83	1.00 (ref)	1.00 (ref)
Quartile 2	80/82	1.23 (0.77-1.97)	1.24 (0.76-2.03)
Quartile 3	49/82	0.72 (0.44-1.19)	0.74 (0.43-1.26)
Quartile 4	36/83	0.52 (0.31-0.88)	0.56 (0.31-0.99)
P for trend		0.002	0.004

[1] Adjusted for age, BMI, education, energy intake, family history of colorectal cancer, NSAID use, physical activity, and smoking.

TABLE 3.4 Odds ratios (95% CI) of colorectal cancer risk according to quartiles of dietary pattern and diet index scores in women

		Age-adjusted	Multivariate-adjusted[1]
Dietary pattern scores	Cases/controls	OR (95% CI)	OR (95% CI)
Fruits and vegetables pattern			
Quartile 1	76/99	1.00 (ref)	1.00 (ref)
Quartile 2	45/99	0.52 (0.31-0.85)	0.46 (0.27-0.78)
Quartile 3	47/99	0.53 (0.33-0.87)	0.45 (0.26-0.76)
Quartile 4	38/99	0.47 (0.28-0.79)	0.35 (0.19-0.65)
P for trend		0.108	0.031
Meat and potatoes pattern			
Quartile 1	30/99	1.00 (ref)	1.00 (ref)
Quartile 2	64/99	2.51 (1.45-4.33)	2.34 (1.32-4.17)
Quartile 3	51/99	2.34 (1.33-4.14)	2.02 (1.09-3.75)
Quartile 4	61/99	2.85 (1.63-4.97)	2.20 (1.08-4.50)
P for trend		0.007	0.170
HEI-05			
Quartile 1	69/99	1.00 (ref)	1.00 (ref)

Quartile 2	55/99	0.78 (0.48-1.26)	0.80 (0.48-1.33)
Quartile 3	50/99	0.61 (0.37-0.99)	0.59 (0.35-0.99)
Quartile 4	32/99	0.37 (0.21-0.62)	0.44 (0.24-0.77)
P for trend		<0.001	<0.001

[1] Adjusted for age, BMI, education, energy intake, family history of colorectal cancer, postmenopausal hormone use, NSAID use, physical activity, and smoking.

Online Supporting Material

TABLE 3.5 (Supplemental Table 1) Description of food groups explored to derive dietary patterns in the principal components analysis[1]

Food group	Food items
Yellow and orange vegetables	Carrots, sweet potatoes, and yams
Dark green and cruciferous vegetables	Broccoli, Brussels sprouts, cabbage, cauliflower, coleslaw, greens, sauerkraut, and spinach
Starchy vegetables	Corn and green peas
Other vegetables	Green beans, lettuce, onions, olives, other mixed vegetables, peppers, and string beans
Tomatoes	Tomatoes (includes tomatoes in mixed dishes, sauces, soups, and 100% juice)
Fried potatoes	French fries, hash browned potatoes, home fries, and tater tots
White potatoes	Baked, boiled, or mashed potatoes and potato salad
Citrus, berries, melons	Berries, cantaloupes, grapefruits, oranges, tangelos, tangerines, other melons, and other citrus fruits (includes fresh, frozen, or dried fruit and 100% juice)
Other fruit	Apples, apricots, avocados, bananas, grapes, nectarines, peaches, pears, pineapple, plantains, plums, and fruit other than citrus, berries, or melons (includes fresh, frozen, or dried fruit, apple sauce, and 100% juice)
Legumes	Beans, soybeans, and tofu/meat substitutes
Nuts and seeds	Peanut butter, other nut butters, nuts, and seeds
Fresh red meat	Beef, pork, organ meats, and lamb (not processed)
Processed meat	Bacon, corned beef, hot-dogs, jerky products, pepperoni, salami, sausage, smoked meats and fish, and deli-style chicken, ham, roast beef, and turkey
Fish	Canned tuna, canned salmon, fish, and other seafood
Poultry	Chicken and turkey (not processed)
Eggs	Eggs and egg substitutes
High-fat dairy	Cream cheese, cheese/white sauce, custard/pudding, full-fat cheese, frozen dairy desserts, whole and 2% dairy-fat milk, sour cream
Low-fat dairy	Skim and 1% dairy-fat milk, yogurt, cottage cheese, ricotta cheese, non-fat and reduced-fat cheese
Whole grains	Whole grain breads, hot cereals, whole grain/high-fiber cold cereal
Refined grains	Biscuits, cornbread, dumplings, muffins, pancakes, pasta, refined grain breads,

	refined grain cold cereal, rolls, stuffing, waffles, and white rice
Salty snacks	Crackers, chips, popcorn, and pretzels
Sweets	Brownies, cakes, candy, candied dessert toppings and syrups, cheesecake, cakes, cobblers, dessert breads, Danishes, doughnuts, pop tarts, sweet rolls
Pizza	Pizza
Gravy	Gravy
Butter	Full-fat and reduced-fat butter
Margarine	Full-fat, reduced-fat, and fat-free margarine
Mayonnaise	Full-fat and reduced-fat mayonnaise
Vegetable oil	Vegetables oils, including oil-based salad dressings
Sugar-free beverages	Sugar-free soft drinks and fruit drinks
Sugar-sweetened beverages	Sugar-sweetened soft drinks and fruit drinks
Liquor	Liquor
Beer	Beer
Wine	Wine
Coffee	Caffeinated and decaffeinated coffee
Tea	Caffeinated and decaffeinated hot tea and iced tea

Online Supporting Material

TABLE 3.6 (Supplemental Table 2) Assignment of scores and calculations for the HEI-05

	Range of score	Calculation
Total HEI-05 score	0-100	Sum of individual components
1. Total fruit (includes 100% juice)	0-5	Cup equivalent per 4,186 kJ[1]
2. Whole fruit (not juice)	0-5	Cup equivalent per 4,186 kJ
3. Total vegetables	0-5	Cup equivalent per 4,186 kJ
4. Dark green and orange vegetables and legumes	0-5	Cup equivalent per 4,186 kJ
5. Total grains	0-5	Ounce equivalent per 4,186 kJ
6. Whole grains	0-5	Ounce equivalent per 4,186 kJ
7. Milk	0-10	Cup equivalent per 4,186 kJ
8. Meat and beans	0-10	Ounce equivalent per 4,186 kJ
9. Oils	0-10	g per 4,186 kJ
10. Saturated fat[1]	0-10	% of total energy
11. Sodium	0-10	g per 4,186 kJ
12. Calories from solid fat, alcohol, and added sugar	0-20	% of total energy

[1] 4,186 kJ = 1,000 kilocalories

[2] Greater intakes of saturated fat, sodium, solid fat, alcohol, and added sugar result in lower total scores.

Chapter 4

RED AND PROCESSED MEAT CONSUMPTION AND COLORECTAL CANCER RISK: A REVIEW OF THE EPIDEMIOLOGICAL EVIDENCE AND MECHANISMS UNDERLYING ASSOCIATIONS

INTRODUCTION

Current evidence suggests that a dietary pattern characterized by comparatively higher intakes of sugar, salt, refined grain products, alcohol, and high-fat foods of animal origin, often termed a Western-style dietary pattern, increases the risk of chronic diseases such as colorectal cancer (1). By contrast, a dietary pattern characterized by relatively higher intakes of fiber and micronutrient-rich whole grains, fruits, vegetables, legumes, nuts, seeds, and other foods of plant origin has been found to decrease risk for chronic diseases. Findings from comparative epidemiological and migrant studies (2,3) indicate that the adoption of a Western-style dietary pattern correlates with increasing incidence rates of breast, prostate, and colorectal cancer (4).

One component of a Western-style diet that may be an independent risk factor for colorectal cancer is processed meat (5), broadly defined as meat preserved by methods other than freezing. Included within this definition are meats that are cured, salted, smoked, or treated with preserving agents. These preserving agents are most commonly sodium nitrites and nitrates. Specific food items classified as processed meat include ham, bacon, sausages, bratwursts, frankfurters, hot dogs, liverwurst (or liver pâté), dried meat products, canned meat, corned beef, salami, pastrami, deli-style turkey, and other cold cuts. Processed meat items come primarily, but not exclusively, from beef and pork in the U.S. and have often been included within the category of red meat in epidemiological investigations (6-13). A review of the evidence implicating red meat as a risk factor for colorectal cancer (Section 4.2) will follow a comprehensive review of the processed meat and colorectal cancer risk literature (Section 4.1).

Both red and processed meat are rich sources of several essential nutrients, such as iron, zinc, vitamin B12, and high-quality protein, but they also contain several components that may have harmful effects in the colon and rectum. Higher levels of dietary fat lead to an increased

production and excretion of membrane-damaging secondary bile acids and fatty acids, which in turn may increase cellular proliferation (14). Results from epidemiological studies that control for key confounders, such as energy intake, obesity, and physical activity, do not support a consistent positive association between total fat intake and colorectal cancer risk (7,15-21). Saturated fat is the primary fat subtype found in red and processed meat, and fat composition rather than total fat may be important (22). Red and processed meat contain modest amounts of naturally-occurring *trans* fatty acids that may adversely influence levels of systemic inflammation, insulin resistance, and cellular proliferation (23). Dietary protein that escapes digestion and absorption in the small intestine can be degraded by bacteria, resulting in increased nitrosatable amines and amides, as well as ammonia, which can damage epithelial cells (24). Iron-induced oxidative damage to colonic epithelial cells and the promotion of endogenous *N*-nitroso compound formation by heme iron may also be involved (25-29).

An additional biologic mechanism whereby red and processed meat consumption may increase colorectal cancer risk is through increased exposure to HCAs and PAHs. These mutagens have been shown to induce DNA adducts and tumors in a number of animal models (30,31). HCAs are generated from the reaction between amino acids, sugars, and creatine or creatinine from muscle tissue. Longer cooking times, higher internal temperatures, and greater external charring result in greater HCA formation (32,33). Cooking methods such as grilling, barbequing, and pan-frying provide optimal conditions for the generation of HCAs (34,35). Grilling or barbequing meat over a direct flame and smoking meat also provide a conducive environment for the production of PAHs (24). The fat or meat juices that fall on the fire produce flames containing PAHs that can coat the surface of meat. Studies examining associations between these meat mutagens and colorectal cancer risk will be reviewed (Section 4.3). Methods

that reduce the formation of these mutagens, such as marinating and pre-cooking meat in a microwave, will also be covered.

Sodium nitrites, sodium nitrates, and nitrogen oxides are used in the curing, smoking, and salting of meats (36). The reaction of these compounds with protein-derived amines, amides, or amine-like compounds such as creatine or creatinine (present in high concentrations in meat) (37) can lead to the formation of *N*-nitroso compounds (NOCs) (38). These compounds have been shown to induce tumors at a variety of sites in over 40 unique animal species, including higher primates (39,40). A review of the evidence implicating NOC exposure as a risk factor for colorectal cancer will be included in this literature review (Section 4.4).

The degree to which greater consumption of processed meat elevates colorectal cancer risk is likely influenced by genetic variability in the expression and activity of enzymes involved in the metabolism of specific meat compounds. Section 4.5 covers the current scientific knowledge pertaining to genetic variability in NOC metabolism and susceptibility to the potentially carcinogenic components in processed meat.

A host of dietary and health-related factors are correlated with red and processed meat consumption and may confound observed associations with colorectal cancer risk. Greater intakes of red and processed meat have been associated with increased consumption of animal fat, white and fried potatoes, refined grains, and a variety of high-sugar and high-fat food items, and with lower consumption of fruits, vegetables, whole grains, legumes, low-fat dairy, and fish (Chapter 3) (41,42). Diets characterized by high intakes of red and processed meat have been associated with increased body mass index, alcohol consumption, and rates of smoking, and with lower levels of physical activity and educational attainment (6,10,43).

SECTION 4.1

Processed Meat Consumption and Colorectal Cancer Risk

A number of epidemiologic studies have investigated the association of dietary intakes of processed meat, as well as individual processed meat subtypes, with colorectal cancer risk. The magnitude of the observed associations and the types of meat items included in the exposure category have varied across studies. The present section of this review aims to explore the current epidemiological evidence supporting an association of processed meat intake and colorectal cancer risk and to identify gaps in knowledge warranting further investigation.

Literature Review Selection Procedure

An initial literature search conducted in the COCHRANE, PUBMED, and Web of Science databases for prospective cohort and population-based case-control studies revealed that findings from three meta-analyses were published in the years 2001 (44), 2002 (45), and 2006 (46). The WCRF and the AICR report published in 2007 also presented results from a meta-analysis of studies investigating processed meat intake and colorectal cancer risk (5). The four meta-analyses met the following *a priori* criteria: the main exposure of processed meat was investigated independently of red meat intake; the outcome was colon, rectal, or colorectal cancer incidence; sufficient quantitative information for statistical analyses including risk estimates and CIs was available; and the study design was either a prospective cohort or a population-based case-control. The meta-analyses evaluated a combined 21 unique prospective cohort studies and 16 unique case-control studies from 1973 to 2005. The main findings from the meta-analyses are summarized in Table 4.1. Original articles that reported results inconsistent with the direction of the summary estimate in each meta-analysis were critically evaluated. An additional literature search for articles published after the last meta-analysis was performed to

ensure comprehensiveness of this review. The following search terms were used: processed meat, cured meat, preserved meat, smoked meat, colorectal cancer, colorectal neoplasm, colon cancer, colon neoplasm, rectal cancer, and rectal neoplasm. A total of five publications were identified that met the inclusion criteria (Table 4.2) (9,10,47-49).

Characteristics of the Meta-Analyses

The meta-analyses were not mutually exclusive; several cohort studies appeared in two or more of the meta-analyses and one study among participants in the New York University Women's Health Study (50) was included in all four meta-analyses. Epidemiologic studies from five continents (Asia, Australia, Europe, North America, and South America) were included and eight prospective cohort studies (6,7,15,16,19,50-52) were common to the two most recent meta-analyses (5,46). Only one meta-analysis (45) provided a summary risk estimate from case-control studies. The WCRF/AICR-review summarized 44 case-control studies qualitatively and reported an increased risk of colorectal cancer with increasing processed meat consumption.

Exposure Assessment and Categorization

The category of processed meat was defined similarly in the meta-analyses, but the definition varied in the individual studies included within the meta-analyses. These differences may be attributable to variations in dietary assessment methodology or to differences in food supply and dietary intake patterns across study populations. Processed meat intake was most often estimated by food frequency questionnaires (FFQs) that ranged in length from 20 to 276 items. Less detailed FFQs may have been unable to sufficiently differentiate between processed meat and fresh meat. For example, the abbreviated NCI Block FFQ (7) combines one processed meat item and two fresh meat items into one question.

Results from the Meta-Analyses

The four meta-analyses of epidemiological studies conducted in the past decade support a statistically significant positive association between processed meat intake and colorectal cancer risk (5,44-46). Risk estimates from all four meta-analyses were greater than 1.0 and statistically significant, ranging from 1.20 (95% CI = 1.11-1.31) (46) to 1.31 (95% CI = 1.13-1.51) (45) when comparing the highest to the lowest category of intake. The largely consistent results across the diverse populations studied provide evidence of a positive association between processed meat intake and colorectal cancer risk.

TABLE 4.1
Selected Characteristics and Findings from Four Meta-Analyses: Processed Meat Intake and Colorectal Cancer Risk

Lead author, year	Years	No. of studies	Countries/regions (*n*)	RR/OR (95%CI), highest v. lowest intake category[1]	RR (95%CI), per-unit analysis[1,2]
Sandhu, 2001	1980-1999	8 cohorts	Western Europe (4), U.S. (4)	Not available	1.49 (1.22-1.81) for a 25 g/d increase in consumption
Norat, 2002	1990-1999	7 cohorts	Western Europe (3), U.S. (4),	1.39 (1.09-1.76)	1.36 (1.15-1.61) for a 30 g/d increase in consumption (cohort + case-control studies)
	1973-1999	16 case-control studies	Western Europe (6), U.S. (4), Japan (2), Argentina (1), Australia (1), Serbia (1), Singapore (1)	1.29 (1.09-1.52)	
Larsson, 2006	1994-2005	14 cohorts	Western Europe (6), U.S. (6), Japan (3), Australia (1)	1.20 (1.11-1.31)	1.09 (1.05-1.13) for a 30 g/d increase in consumption
WCRF/ AICR, 2007	1975-2005	13 cohorts	Western Europe (5), U.S. (7), Australia (1)	1.30 (1.14-1.47)	1.21 (1.04-1.42) for a 50 g/d increase in consumption

Abbreviations: CI, confidence interval, g/d, grams per day, OR, odds ratio, M, men, RR, relative risk, W, women, WCRF/AICR, World Cancer Research Fund/American Institute for Cancer Research
[1] Risk estimates shown were generated with random effect models

[2] Sufficient data were provided for a per-unit analysis in all 7 cohort studies and 9 of the 16 case-control studies in Norat *et al*; 11 of the 14 studies in Larsson *et al*.; and 5 of the 12 studies in the WCRF/AICR meta-analysis

Meta-Analysis by Sandhu *et al*. (44)

Eight prospective cohort studies published between 1980 and 1999 were reviewed by Sandhu *et al.* (44). Two studies (16,18) provided risk estimates for men and women separately. A formal assessment of potential heterogeneity was not statistically significant ($P = 0.81$), and both a random effects model and a fixed effects model produced identical risk estimates and confidence intervals. A random effects model provides identical weights to each study whereas the weights provided in a fixed effects model are dependent upon the sample size of each study (53). Comparable summary estimates produced by these two models indicate low heterogeneity among the individual studies included in the meta-analysis. Nine of the ten estimated RRs were positive and the pooled RR for both case-control and cohort studies was 1.49 (95% CI = 1.22-1.81) for a daily increase of 25 grams.

One study included in this analysis presented results that were stratified by family history (54). Women participating in the Women's Health Study who had no reported family history of colon cancer ($n = 180$) had an RR of 1.0 (95% CI = 0.7-1.4 for the highest v. the lowest tertile), whereas women with a family history of colon cancer ($n = 61$) had an RR of 0.88 (95% CI = 0.49-1.57). The increased risk of colon cancer experienced by women with a positive family history may be largely influenced by genetic rather than environmental factors (55). Familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal cancer (HNPCC) are two well-established genetic syndromes that result in an excess risk of colorectal cancer (56).

Meta-Analysis by Norat *et al*. (45)

Norat and colleagues reviewed seven prospective cohort (1990-1999) and 16 case-control studies (1973-1999) (45). A statistically significant RR of 1.31 (95% CI = 1.13-1.51) comparing

the highest to the lowest category of intake, and 1.36 (95% CI = 1.15-1.61) for a 30 gram per day increase in intake were calculated. An evaluation of each individual prospective cohort study revealed that all RRs were positive, but only one was statistically significant (18). Thirteen of the case-control studies found positive associations, seven of which were statistically significant, whereas two reported null associations (57,58) and one reported a statistically significant inverse association (59).

The statistically significant inverse association between processed meat intake and colorectal cancer was observed in an Argentinean case-control study (OR = 0.43; 95% CI = 0.21-0.89 for Q4 [>198 times/year] v. Q1 [<16 times/year]) (59). This OR was estimated in a univariate conditional logistic regression model. A relatively small number of cases (n = 110) is an additional consideration when interpreting the results of this study. The study differs from the others included in the meta-analysis because it was the only one conducted in South America. The Argentinean population has been estimated to consume substantially more red meat compared to other regions (45,59) and different dietary patterns in Argentina may partially explain the inverse association.

Meta-Analysis by Larsson and Wolk (46)

Findings from an additional nine prospective cohort studies published after the investigations of Norat *et al.* (45) and Sandhu *et al.* (44) were included in a meta-analysis conducted by Larsson and Wolk in 2006 (46). In addition to the nine new studies, five cohort studies were also present in one or both of the previous reviews. In contrast to findings from the previous two meta-analyses, Larsson and Wolk observed positive associations in all fourteen studies, although only three of the observed associations were statistically significant (16,51,60). The summary risk estimates from the meta-analysis were statistically significant (RR = 1.20

(95% CI = 1.18-1.39) for the highest compared to the lowest quantile of intake; RR = 1.09 (95% CI = 1.05-1.13) for a daily increase of 25 grams).

The use of more recent studies that adjusted for a larger number of confounders may have contributed to the smaller effect size and the more precise summary risk estimates calculated by Larsson and Wolk (46) compared to those calculated by the earlier meta-analyses (44,45). Results from a stratified analysis to test for the possible influence of publication year on the magnitude of observed associations found a considerably higher RR of 1.44 (95% CI = 1.10-1.90) for studies published between 1994 and 2000 compared to an RR of 1.18 (95% CI = 1.08-1.29) for studies published between 2001 and 2006.

Meta-Analysis by the WCRF/AICR (5)

A total of 13 cohort studies were included in the WCRF/AICR meta-analysis. An RR of 1.30 (95% CI = 1.14-1.47) in a random effect model and an RR of 1.28 (95% CI = 1.16-1.42) in a fixed effect model when comparing the highest versus the lowest category of processed meat consumption were estimated. A summary RR of 1.21 (95% CI = 1.04-1.42) for a daily increase of 50 grams in both a fixed effect model and a random effect model was calculated. Two studies reported null associations (7,61), which were inconsistent with the direction of the 14 other risk estimates. Limitations in exposure assessment and sample size may underlie these observed differences. Only sausage intake was investigated in the Dutch nested case-control study (61) and an abbreviated FFQ was used in the U.S. cohort study (7). The exposure of interest may have been inadequately captured or imprecisely measured, and individuals may have been misclassified into quintiles of consumption. In addition, only 48 female colorectal cancer cases were included in the Dutch study, limiting the statistical power to detect associations.

Summary of Meta-Analyses

A review of the findings from four large meta-analyses conducted in the past decade provides evidence of a positive association between processed meat intake and colorectal cancer risk. The relatively narrow range of the summary risk estimates across the meta-analyses, as well as the narrow 95% CIs surrounding each summary estimate, further supports this relationship. An exploration of the several studies reporting risk estimates inconsistent with the overall summary estimates revealed considerable limitations. Nevertheless, the calculated risk estimates were fairly modest, with the largest estimate around a 50% increased risk (44) and the smallest just below 10% (46) in the per-unit analyses.

Several strengths and limitations of meta-analyses should be considered given their increasing popularity in epidemiological research (62). Meta-analyses are able to quantitatively summarize large bodies of research on key public health issues. The use of meta-analytic methods can highlight inconsistent findings that may be present in the literature, which may help in the design of new research studies. One limitation of meta-analyses is the potential for bias in the summary estimates, resulting from selection bias, publication bias, or bias present in the original studies. Another concern relates to the potential for heterogeneity across the individual studies included in the meta-analyses, attributable to factors such as differences in study design, exposure assessment, exposure categorization, study population characteristics, and study results. Varying degrees of adjustment for confounding factors across studies is an additional limitation.

Studies Published after the Meta-Analyses (2006-2009)

Findings from two prospective cohort studies (10,47), one case-cohort study (49), and two population-based case-control studies (9,48) that investigated processed meat and colorectal cancer have been published since the most recent study evaluated in the meta-analyses (63).

Selected characteristics and gender-stratified results (when available) are presented in Table 4.2. The cohort studies were conducted in the U.S. (NIH-AARP) (10) and Japan (47); the case-cohort study was carried out in the Netherlands (NLCS) (49); and the case-control studies were performed in Canada (48) and Japan (9). Common to the five studies were the inclusion of both sexes and fairly large sample sizes, ranging from 1,575 (782 cases) (9) to 494,036 (5,107 cases) (10). All but one of the studies used definitions for the exposure of processed meat similar to those found in the meta-analyses. Kimura *et al.* (9) did not provide a clear description of the exposure, although processed meat was investigated independently of fresh meat.

Summary of Recent Evidence

Findings from the recently published studies strengthen the evidence of an increased risk of colorectal cancer associated with higher intakes of processed meat to a greater degree among men than women (9,10,47,48,63). The largest study reviewed (*n* = 494,036; 5,107 cases) found a statistically significant increased risk of colorectal cancer among both men and women participating in the NIH-AARP Diet and Health Study (HR = 1.20; 95% CI = 1.09-1.32 for Q5 [median intake of 22.6 grams/day] v. Q1 [1.6 grams/day]) (10). In the Japanese cohort study, greater consumption of processed meat elevated the risk of colon cancer significantly (RR = 1.98; 95% CI = 1.24-3.16 for Q4 [20.3 g/d] v. Q1 [3.9 g/d]) among male participants (*n* = 13,894; 111 cases) but not among female participants (RR = 0.85; 95% CI = 0.50-1.43 for Q4 [16.3 g/d] v. Q1 [7.3 g/d]; *n* = 16,327; 102 cases) (47). The reason for the observed gender differences is unclear but perhaps dietary measurement error, underlying food intake patterns, or other health-related behaviors that were not unaccounted for in the models may partially explain these findings. Hu *et al*. (48) performed analyses stratified by site of the large intestine and reported equal ORs for colon (1.5; 95% CI = 1.2-1.8) and rectal cancer (1.5; 95% CI 1.2-1.8) in a

population-based case-control study among men and women in Canada (5,039 controls, 1,727 colon cancer cases, and 1,447 rectal cancer cases) (48).

Conclusion

The review of four meta-analyses supports the hypothesis that higher consumption of processed meat may increase the risk of colorectal cancer, which corresponds with current dietary advice for cancer prevention from leading independent cancer organizations including the American Cancer Society (ACS) and the WCRF/AICR to limit consumption of processed meat (5,64). The findings from the individual studies were not as conclusive despite the consistently positive associations determined by the meta-analyses. Differences in cancer risk associated with processed meat consumption across studies may be attributable not only to differences in the level of consumption of processed meat but also to variations in the level of consumption of other dietary variables. Greater intakes of potentially protective factors, such as food groups rich in fiber and antioxidants, or lower intakes of other possible dietary risk factors, such as red meat, may modify associations between processed meat and colorectal cancer risk. Differences may also be related to variations in exposure to specific components in processed meat between populations, including NOCs, NOC precursors, HCAs, and PAHs.

TABLE 4.2

Selected Characteristics and Findings from Recent Prospective Cohort and Population-Based Case-Control Studies: Processed Meat Intake and Colorectal Cancer Risk[1]

Lead author, year	Study design, country	Sample size	Age (y)	Years	Exposure definition[2]	Highest v. lowest intake category (median g/d)	RR/OR (95%CI), highest v. lowest intake category	Adjustments
Oba, 2006	Prospective cohort, Japan	M: *n* = 13,894 (111 cases[3]) W: *n* = 16,327 (102 cases[3])	≥ 35	1992-2000	Ham, sausage, bacon, and Chinese roasted pork	M: 20.3 v. 3.9 W: 16.3 v. 7.3	M: 1.98 (1.24-3.16) W: 0.85 (0.50-1.43)	Age, alcohol, BMI, height, physical activity, smoking
Balder, 2006	Case-control, The Netherlands[4]	M: 2,156 controls, 896 cases W: 2,215 controls, 666 cases	55-69	1986-1995	Smoked, fermented, and cured meat	M/W: 32.0 v. 0	M: 1.18 (0.84-1.64) W: 1.05 (0.74-1.48)	Age, alcohol, BMI, energy intake, family history, physical activity, smoking, vegetable intake
Kimura, 2007	Case-control, Japan[5]	M: 495 controls, 473 cases W: 298 controls, 309 cases	20-74	2000-2003	Processed meat (undefined)	M/W: 14.9 v. 0.4	M/W: 1.15 (0.83, 1.60)	Age, alcohol, BMI, calcium intake, employment, energy intake, family history, fiber intake, physical activity, residential area, sex, smoking

Cross, 2007	Prospective cohort, U.S.[6]	M: *n* = 294,724 (472 cases) W: *n* =199,312 (418 cases)	50-71	1995-2003	Bacon, sausage, luncheon meats, cold cuts, ham, and hot dogs	M/W: 22.6 v. 1.6	M/W: 1.20 (1.09-1.32)	Age, alcohol, BMI, education, energy intake, family history, fruit and vegetable intake, marital status, physical activity, race, sex, smoking
Hu, 2008	Case-control, Canada[7]	M: 2,547 controls, 959/858 colon/rectal cases W: 2,492 controls, 768/589 colon/rectal cases	$\geq$ 20	1994-1997	Hot dogs, luncheon meats, smoked meat, corned beef, bacon, and sausage	M/W: controls 4.1 $\pm$ 6.2; colon/rectal cases 4.5 $\pm$ 5.5/4.5 $\pm$ 5.4 servings/wk (mean $\pm$ SD)	M/W: colon/rectal cancer 1.5 (1.2-1.8)/ 1.5 (1.2-2.0)	Age, alcohol, BMI, education, energy intake, fruit and vegetable intake, physical activity, sex, smoking

Abbreviations: BMI, body mass index (kg/m^2), CI, confidence interval, g/d, grams per day, OR, odds ratio, M, men, RR, relative risk, W, women

[1] Study details and findings are stratified by gender when sufficient data were available

[2] Meat exposures were captured by FFQ

[3] Colon cancer only

[4] The Netherlands Cohort Study

[5] Fukuoka Colorectal Cancer Case-Control Study

[6] National Institutes of Health (NIH)-AARP Diet and Health Study

[7] National Enhanced Cancer Surveillance System Case-Control Study

SECTION 4.2

Red Meat Consumption and Colorectal Cancer Risk

A recent consensus report issued jointly by the WCRF and the AICR concluded that the evidence to support a positive association between greater intakes of red meat and colorectal cancer was convincing, but the evidence for specific meat components explaining these associations remained inconclusive (5). Several components of red meat, including saturated fat, naturally-occurring *trans* fat, and heme iron, have been investigated as independent risk factors for colorectal cancer (5,7,23), although results have been mixed. Increased exposure to HCAs and PAHs remains a plausible mechanism by which red meat may increase colorectal cancer risk. The exposures of red meat and processed meat have often been collapsed into one category in epidemiological studies (6-13), and it may be that increased intake of processed meat is responsible for the elevated risk associated with red meat intake.

Literature Review Selection Procedure

The four meta-analyses described in the preceding section (5,44-46) quantitatively assessed the association between red meat and colorectal cancer risk. Selected characteristics and the main findings are shown in Table 4.3. An additional search for articles published after the meta-analyses (2006 through 2009) was performed to evaluate the recent evidence using the following search terms: red meat, beef, pork, colorectal cancer, colorectal neoplasm, colon cancer, colon neoplasm, rectal cancer, and rectal neoplasm. A total of seven publications (9-12,47,48,65) were identified that met the following *a priori* inclusion criteria: the exposure included more than one type of red meat; the outcome was colon, rectal, or colorectal cancer incidence; risk estimates and CIs were available; the study design was either a prospective cohort or a population-based case-control; and the study population was not included in one of the

meta-analyses. The more recent publication was included in the present review (11) when more than one publication presented findings from examinations of red meat intake in the same population (11,66). Selected characteristics and main findings from the seven studies are presented in Table 4.4; study details and findings are shown by gender or tumor site when sufficient data were provided by the authors.

Characteristics of the Meta-Analysis

The meta-analyses were not mutually exclusive since sixteen of the individual cohort studies were represented in two or more of the meta-analyses. Overall, 20 prospective cohort studies and 14 population-based case-control studies were analyzed and five continents were represented. The majority of studies were from the U.S. (n = 18) and Western Europe (n = 11); the remaining studies were from Australia (n = 2), China (n = 1), Argentina (n = 1), and Singapore (n = 1). Red meat was broadly defined by the authors of the meta-analyses to include red meat consumed fresh as well as processed. The exposure of red meat in some of the individual studies included processed meat from all sources, including poultry. For example, both red and white meat cold cuts fall into the category of processed meat. Information pertaining to the specific meat sub-types included in the red meat category was not consistently provided in the individual studies. Of the four meta-analyses, only Norat *et al*. (45) performed analyses stratified by the type of red meat (i.e., all red meat, which included processed meat, and fresh red meat).

Results from the Meta-Analyses

Results from the four meta-analyses conducted in the past decade support the hypothesis that greater red meat intake is associated with a modest increased risk of colorectal cancer. Risk estimates from all four meta-analyses were greater than 1.0 and statistically significant, ranging

from 1.27 (95% CI = 1.11-1.45) (45) to 1.35 (95% CI = 1.19-1.53) (5) when comparing the highest to the lowest category of intake. These summary findings are complicated by the variable definition of the exposure and should be interpreted with some degree of caution. A review of the individual studies revealed that most of the estimated associations were not statistically significant. Only five (17,21,51,63,67) of the 20 prospective cohort studies reported RRs that reached statistical significance, although all of the risk estimates were greater than 1.0. The extent to which including processed meat in the red meat category influenced the positive associations observed remains unclear. The meta-analysis by Norat *et al*. (45) was the only one of the four to stratify by the type of red meat. An RR of 1.28 (95% CI = 1.11-1.47) was estimated when comparing the highest to the lowest category of fresh red meat intake in this study.

TABLE 4.3

Selected Characteristics and Findings from Four Meta-Analyses: Red Meat Intake and Colorectal Cancer Risk

Lead author, year	**Years**	**No. of studies**	**Countries/regions (*n*)**	**RR/OR (95%CI), highest v. lowest intake category (all red meat)[1]**	**RR (95%CI), highest v. lowest intake category (fresh red meat) [1]**	**RR/OR (95%CI), per-unit analysis (all red meat) [1,2]**	**RR (95%CI), per-unit analysis of (fresh red meat)[1]**
Sandhu, 2001	1980-1999	7 cohorts	U.S. (7)	Not available	Not available	1.17 (1.05-1.21) for a 100 g/d increase in consumption	Not available
Norat, 2002	1990-1999	9 cohorts	U.S. (8), Western Europe (1)	1.27 (1.11-1.45)	1.28 (1.11-1.47)[3]	1.22 (1.05-1.41) for a 120 g/day increase in consumption	1.19 (0.91-1.55) for a 120 g/d increase in consumption[4]
	1984-1999	14 case-control studies	Western Europe (6), U.S. (5), Argentina (1), Australia (1), Singapore (1)	1.36 (1.17-1.59)		1.26 (1.02-1.55) for a 120 g/d increase in consumption	
Larsson, 2006	1994-2005	15 cohorts	Western Europe (6), U.S. (8), Australia (1)	1.28 (1.15-1.42)	Not available	1.28 (1.18-1.39) for a 120 g/d increase in consumption	Not available
WCRF/ AICR, 2007	1975-2005	12 cohorts	U.S. (6), Western Europe (4), Australia (1), China (1)	1.35 (1.19-1.53)	Not available	1.29 (1.05-1.59) for a 100 g/d increase in consumption	Not available

Abbreviations: CI, confidence interval, g/d, grams/day, OR, odds ratio, M, men, RR, relative risk, WCRF/AICR, World Cancer Research Fund/American Institute for Cancer Research

[1] Risk estimates shown were generated with random effect models

[2] Sufficient data were provided for a per-unit analysis in all 9 cohort studies and 8 of the 14 case-control studies in Norat *et al*; 14 of the 15 studies in Larsson *et al*.; and 3 of the 12 studies in the WCRF/AICR meta-analysis

[3] Thirteen of the 23 individual studies in Norat *et al*. investigated also investigated the fresh red meat; case-control and cohort studies were combined by the authors for this analysis

[4] Sufficient data were provided for a per-unit analysis of fresh red meat in 8 of the 23 studies in Norat *et al*; case-control and cohort studies were combined by the authors for this analysis

Studies Published after the Meta-Analyses (2006-2009)

Results from two prospective cohorts (10,47) and five population-based case-control studies (9,11-13,48) that examined red meat and colorectal cancer risk (Table 4.4) have been published since the most recent meta-analysis (5). One prospective cohort study (10) and one case-control study (11) were conducted in the U.S.; two case-control studies (12,48) were performed in Canada; one case-control study was carried out in Denmark (13); and one prospective cohort (47) and one case-control study (9) were from Japan. Three studies reported statistically significant positive associations between red meat and colorectal cancer (10-12). The red meat exposure included processed meat in these studies. Oba *et al*. (47) performed gender-stratified analyses in a Japanese prospective cohort study and found no association between greater intakes of fresh red meat and colon cancer among men and a non-significant inverse association among women (RR = 0.79; 95% CI = 0.49-1.28). This finding should be interpreted with caution the study included only 102 female colon cancer cases. Hu *et al*. (48) also examined fresh red meat intake and colorectal cancer and reported statistically significant positive associations (OR for colon cancer = 1.4; 95% CI = 1.1-1.8; OR for rectal cancer = 1.2; 95% CI = 1.0-1.5) (48). The mixed results across the individual studies may be partially attributable to the differential adjustment for potential confounders and the variably defined exposure of red meat, as well as population differences. Sample size and the number of cases ranged substantially, from 102 female cases in a gender-stratified analysis to 1,095 cases in an analysis with both men and women (12). Overall, findings from the recent studies do not oppose those from the meta-analyses, yet they do not provide convincing evidence of a significant positive association between red meat and colorectal cancer.

Conclusion

Whether the excess risk estimated in the four meta-analyses and in several of the recent studies is driven by processed meat is unclear. Red meat is a broad category that includes both lean and fattier cuts of fresh beef, pork, lamb, veal, and other meat from mammals, as well as processed red meat (and in several studies, white processed meat). A variety of cooking methods are used to prepare red meat, including those resulting in the generation of HCAs and PAHs. The evidence implicating HCAs and PAHs as risk factors for colorectal cancer is reviewed in the next section.

TABLE 4.4

Selected Characteristics and Findings from Recent Prospective Cohort and Population-Based Case-Control Studies: Red Meat Intake and Colorectal Cancer Risk[1]

Lead author, year	Study design, country	Sample size	Age (y)	Years	Exposure definition[2]	Highest v. lowest intake category (median g/d)	RR/OR (95%CI), highest v. lowest intake category	Adjustments
Oba, 2006	Prospective cohort, Japan	M: *n* = 13,894 (111 cases)[3] W: *n* = 16,327 (102 cases)[3]	≥ 35	1992-2000	Beef and pork (no processed meat)	M: 56.6 v. 18.7 W: 42.3 v. 10.7	M: 1.03 (0.64-1.66) W: 0.79 (0.49-1.28)	Age, alcohol, BMI, height, physical activity, smoking
Kimura, 2007	Case-control, Japan[4]	M: 495 controls, 473 cases W: 298 controls, 309 cases	20-74	2000-2003	Beef and pork (included processed meat)	M/W: 78.9 v. 18.0	M/W: 1.13 (0.80-1.61)	Age, alcohol, BMI, calcium intake, employment, energy intake, family history, fiber intake, physical activity, residential area, sex, smoking
Cross, 2007	Prospective cohort, U.S.[5]	M: *n* = 294,724 (472 cases) W: *n* =199,312 (418 cases)	50-71	1995-2003	Bacon, pork, and lamb (included processed meat)	M/W: 62.7 v. 9.8	M/W: 1.24 (1.12-1.36)	Age, alcohol, BMI, education, energy intake, family history, fruit and vegetable intake, marital status, physical activity, race, sex, smoking
Hu, 2008	Case-control, Canada[6]	M: 2,547 controls, 959/858 colon/rectal cases W: 2,492 controls, 768/589 colon/rectal cases	≥ 20	1994-1997	Beef, pork, and lamb (no processed meat)	M/W: controls 4.1 ± 6.2; colon/rectal cases 4.5 ± 5.5/ 4.5 ± 5.4 servings/wk (mean ± SD)	M/W: colon cancer 1.4 (1.1-1.8); rectal cancer 1.2 (1.0-1.5)	Age, alcohol, BMI, education, energy intake, fruit and vegetable intake, physical activity, sex, smoking

Girard, 2008	Case-control, U.S.[7]	M/W: 866 controls, 537 cases[3]	40-84	1996-2000	Hamburger, steak, pork chop, sausage, and bacon	M/W: < 28.5 (median) 213/433 cases/controls; > 28.5 324/433 cases/controls	M/W: 1.3 (1.0-1.8)	Age, fat intake, fiber intake, offsets,[8] race, sex, energy intake, total meat intake
Cotterchio, 2008	Case-control, Canada[9]	M/W: 1,890 controls, 1,095 cases	20-74	1997-2001	Beef, pork, veal, lamb, and venison (included processed meat)	M/W: > 5 v. 0-2 servings/wk	M/W: 1.67 (1.36-2.05)	Age and sex
Sørensen, 2008	Case-cohort, Denmark[10]	M: 425 sub-cohort members, 212 cases W: 344 sub-cohort members, 167 cases	51-70	1993-2003	Beef, veal, pork, and organ meat (included processed meat)	M/W: sub-cohort members 105 (40-228); cases 105 (45-229) [median (5-95 percentiles]	M/W: 1.09 (0.96-1.23) per 25 g/day	BMI, hormone replacement therapy, intakes of poultry, fish, alcohol, and fiber, and smoking status

Abbreviations: BMI, body mass index (kg/m^2), CI, confidence interval, g/d, grams per day, OR, odds ratio, M, men, RR, relative risk, W, women

[1] Study details and findings are stratified by gender or sub-site when sufficient data were available

[2] Meat exposures were captured by FFQ

[3] Colon cancer only

[4] Fukuoka Colorectal Cancer Case-Control Study

[5] National Institutes of Health (NIH)-AARP Diet and Health Study

[6] National Enhanced Cancer Surveillance System Case-Control Study

[7] North Carolina Colon Cancer Study

[8] Offsets account for the selection probability by age, race, and sex

[9] Participants were from the Ontario Family Colorectal Cancer Registry

[10] Diet, Cancer and Health prospective follow-up study

SECTION 4.3

Meat-Derived Heterocyclic Amine and Polycyclic Aromatic Hydrocarbon Exposure and Colorectal Cancer Risk

Several plausible hypotheses have been proposed to explain the observed associations between red and processed meat and colorectal cancer risk. Chief among them is increased exposure to compounds derived through cooking meat well-done at high temperatures or over a direct flame (HCAs and PAHs) (24). These compounds have been shown to induce DNA adducts and tumors in numerous animal models (30,31). HCAs are generated from the reaction between amino acids, sugars, and creatine or creatinine (found in meat muscle). Longer cooking times, higher internal temperatures, and greater external charring result in greater HCA formation (32,33). Cooking methods such as grilling, barbequing, and pan-frying provide optimal conditions for the generation of HCAs (34,35). Modulating factors in the production of HCAs include marinating and pre-cooking meat in the oven or microwave prior to grilling or barbequing (32,68). Grilling meat over a direct flame and smoking meat are conducive to the production of PAHs (24). The fat or meat juices that fall on the fire produce flames that contain PAHs, which can coat the surface of meat. It is likely that lower fat meat items lessen the amount of PAH-containing flames.

Literature Review Selection Procedure

A search for articles reporting on associations between HCA and PAH exposure and colorectal cancer risk was performed using the following search terms: heterocyclic amine, polycyclic aromatic hydrocarbon, colorectal cancer, colorectal neoplasm, colon cancer, colon neoplasm, rectal cancer, and rectal neoplasm. Five publications (69-73) were identified that met the following *a priori* inclusion criteria: the exposure included at least one HCA or PAH (based

on previously measured values rather than surrogate measures); the outcome was colon, rectal, or colorectal cancer incidence; risk estimates and CIs were available; and the study design was either a prospective cohort or a population-based case-control study. The study which provided data most relevant to the present review was included (71) when more than one publication presented findings from examinations in the same population (11,66,71). Selected characteristics and main findings from the five studies (four population-based case-control studies (69-71,73) and one nested case-control study (72)) are presented in Table 4.5; study details were stratified by tumor site when sufficient data were provided by the authors.

Results

Of the five studies that examined exposure to HCAs and/or PAHs in relation to colorectal cancer risk (69-73), one reported a strong statistically significant elevated risk of colorectal cancer with increased exposure to 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) (OR = 4.09; 95% CI = 1.94-9.08) (69). Caution is warranted in interpreting this finding since adjustment for energy intake was not performed, and it is likely that total energy intake is a confounding factor in the association between meat-derived mutagen exposure and cancer risk. Two studies reported statistically significant positive associations between HCAs and rectal cancer (70,73). One of these studies (73) reported findings for men only because null associations were observed between HCAs and both colon and rectal cancer among women. The strength of the associations between HCAs and rectal cancer in these two studies varied widely, from an OR of 1.5 (95% CI = 1.0-2.1) in the Swedish population-based case-control study (70) to an OR of 3.1 (95% CI = 1.3-7.7) in the population-based case-control study conducted in a multiethnic population in Hawaii (73). Both of these studies (70,73) reported no association between HCAs and colon cancer.

TABLE 4.5
Selected Characteristics and Findings from Prospective Cohort and Population-Based Case-Control Studies of Heterocyclic Amines and Polycyclic Aromatic Hydrocarbons and Colorectal Cancer Risk

Lead author, year	Study design, country	Sample size	Age (y)	Years	Exposure definition[1]	HCA intake (ng/d)	OR (95%CI), highest v. lowest intake category	Adjustments
Augustsson, 1999	Case-control, Sweden	M/W: 553 controls, 352 colon cases	51-77	1992-1994	IQ MeIQ MeIQx DiMeIQx PhIP Total HCA	Not available	1.1 (0.7-1.6) 1.3 (0.9-1.8) 0.6 (0.4-1.0) 0.6 (0.4-0.9) 0.6 (0.4-0.9) 0.6 (0.4-1.0)	Age, energy intake, sex
		M/W: 249 rectal cases			IQ MeIQ MeIQx DiMeIQx PhIP Total HCA		0.8 (0.5-1.3) 1.5 (1.0-2.1) 0.7 (0.4-1.2) 0.6 (0.4-1.1) 0.6 (0.4-1.1) 0.7 (0.4-1.1)	
Le Marchand, 2002	Case-control, U.S.[2]	M: 426 controls, 289 colon cases	< 85	1994-1998	MeIQx DiMeIQx PhIP Total HCA	Not available	1.0 (0.6-1.1) 1.1 (0.7-1.7) 1.0 (0.6-1.6) 1.1 (0.7-1.7)	Age, aspirin use, BMI, calcium, education, ethnicity, non-starch polysaccarhides from vegetables, physical activity, sex, smoking
		M: 137 rectal cases			MeIQx DiMeIQx PhIP Total HCA		3.1 (1.3-7.7) 2.7 (1.1-6.3) 1.7 (0.3-3.8) 2.2 (1.0-4.7)	
Nowell, 2002	Case-control, U.S.[3]	M/W: 380 controls, 155 cases	20-88	1993-1999	MeIQx	Median (range): 54 (0-994) controls; 88 (0-717) cases	4.09 (1.94-9.08)	Age, ethnicity, sex

Butler, 2003	Case-control, U.S.[4]	M/W: 1,038 controls, 620 colon cases	40-84	1996-2000	MeIQx DiMeIQx PhIP benzo[*a*]pyrene Mutagenicity[5]	124 v. 4 10 v. 0 219 v. 0 78 v. 0.5 18 v. 0.8 (highest v. lowest quintile)	1.1 (0.6-2.0) 1.8 (1.3, 3.1) 0.9 (0.6-1.5) 1.2 (0.8-1.7) 1.4 (1.0-2.0)	Age, energy intake, ethnicity, fat intake, fiber intake, offsets,[6] sex
Nöthlings, 2009	Nested case-control, U.S.[7]	M/W: 1,552 controls, 1,009 cases	45-75	1993-2000	DiMeIQx MeIQx PhIP Total HCA	$\geq$ 6.2 v. < 1.8[8] $\geq$ 94 v. < 30 $\geq$ 461 v. < 171 $\geq$ 567 v. < 217 (highest v. lowest tertile)	1.18 (0.88-1.59) 1.09 (0.81-1.47) 1.03 (0.77-1.39) 1.03 (0.77-1.39)	Age, BMI, energy-adjusted calcium, fiber, folic acid, and vitamin D, ethanol, ethnicity, family history, physical activity, smoking

Abbreviations: CI, confidence interval, DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline, HCA, heterocyclic amine, IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline, M, men, MeIQ, 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline, MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline, ng/d, nanograms per day, OR, odds ratio, PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, PAH, polycyclic aromatic hydrocarbon, W, women

[1] Mutagen exposures were estimated using the NCI reference database (74,75) in the four U.S. studies

[2] Study population was from Hawaii (60% Japanese, 26% Caucasian, 14% Hawaiian)

[3] Study population was from Arkansas and Tennessee

[4] North Carolina Colon Cancer Study

[5] Measured in revertant colonies/d

[6] Offsets account for the selection probability by age, race, and sex

[7] The Multiethnic Cohort Study (19% African-American, 29% Japanese-American, 6% Native Hawaiian, 22% Latino, 20% Caucasian)

[8] Values are ng/1,000 kcal/d

Results (Continued)

Only one study (71) examined exposure to both HCAs and PAHs, as well as total mutagenicity, a measure of overall mutagenic potential that accounts for differences in mutagenic activity between the various compounds. Butler and colleagues (71) observed statistically significant positive associations for colon cancer (rectal cancer was not examined) with increasing exposure to DiMeIQx and total mutagenicity. In contrast, no relationship between benzo[*a*]pyrene and colon cancer was observed. The null findings with this PAH should be interpreted with some degree of caution since over 100 unique PAHs are known to exist yet estimates of meat-derived exposure are currently available for only one (benzo[*a*]pyrene) (24).

Conclusion

In summary, greater HCA exposure was positively associated with colorectal cancer in one of two studies, with colon cancer in one of three studies, and with rectal cancer in the two studies that investigated this endpoint separately. The inconsistent results across studies may be attributable to the variable adjustment for potential confounders (covariates included in the final multivariate models ranged from three to 12), the different endpoints, and differences in meat-derived mutagen exposure. Meat consumption patterns, meat cooking methods, meat doneness preferences, and thus meat-mutagen exposure likely vary between populations. For example, both studies conducted in the southeastern region of the U.S. reported elevated risks of colon (71) and colorectal cancer (69) with increasing intakes of HCAs, whereas both studies carried out in multiethnic populations in Hawaii and southern California found null associations between HCAs and colon (73) and colorectal cancer (72). Overall, the findings are suggestive of an association between HCAs and rectal cancer but results are mixed for associations between these mutagens and colon cancer.

SECTION 4.4

Meat-Derived *N*-Nitroso Compounds: Mechanisms and Risk of Colorectal Cancer

Increasing concern in the late 1960s and early 1970s over the presence of preformed NOCs in preserved meats and possible risks to human health (76), including cancer of the gastrointestinal tract (77), prompted the USDA to convene a scientific panel in 1973 to address current meat curing practices. The panel recommended that the maximum allowable amount of nitrites should be reduced; the use of sodium and potassium nitrates should be prohibited; and the maximum allowable amount of the reducing agents sodium ascorbate or erythorbate should be mandatory additions. The maximum allowable amount of nitrites decreased from 200 to 120 parts per million (ppm) (i.e., 200 to 120 micrograms (mcg) of sodium nitrites per gram (g) of meat) and the mandatory addition of 550 ppm of a nitrosation inhibitor (sodium ascorbate or erythorbate) was established (78). The USDA also commenced a Nitrosamine Monitoring Program to ensure that nitrosamine levels were below 10 parts per billion (ppb) in bacon, which translates to 10 micrograms (mcg) per kilogram (kg) of bacon.

Following the new regulations, the National Research Council (NRC) of the National Academy of Sciences (NAS) conducted an expert review of available data that resulted in a two-part report summarizing the current evidence (79) and providing recommendations for minimizing health risks (80). The reports indicated that although meat treated with nitrite below current allowable levels was safe and the level of detectable nitrosamines was low, continued research into alternative approaches for the preservation of meat was warranted. Additional recommendations included the periodic review by the USDA of nitrite use procedures to ensure current use practices were consistent with the most recent scientific evidence. These steps

resulted in an appreciable decrease in observed NOC concentrations in fried bacon (40), the most commonly tested dietary contributor to NOC intake (81-85). Nevertheless, preformed NOCs have still been detected in meat processed by direct fire-drying and smoking and meat preserved by the addition of sodium nitrites since the NAS expert reports were released in 1981 and 1982 (40).

Literature Review Selection Procedure

The primary objectives of the present section were to review the experimental animal and human volunteer studies that examine NOC exposure in the colon and rectum and to evaluate diet-induced NOC exposure in the U.S. via processed meat intake. Findings from experimental animal and human volunteer studies evaluating mechanisms of action were reviewed to address the first aim. Original investigations that measured NOC concentrations in specific food items, as well as review studies that compiled data on NOC concentrations, were evaluated to accomplish the second objective. In light of the multiple diet-related factors that may influence NOC exposure (fiber, dietary antioxidants, etc.), studies exploring promoters and inhibitors of exogenous NOC generation and endogenous formation in human populations were reviewed. Studies measuring NOC concentrations in food supplies outside of the U.S. were excluded for this section of the review since regulation of meat preserving procedures occurs at the national level. Studies conducted prior to 1978 were excluded since the present USDA regulations pertaining to the use of additives in the meat and poultry processing industries became effective in that year. A literature search was performed in the COCHRANE, PUBMED, and Web of Science databases for articles published from 1978 through September 2008 with the following search terms: colorectal cancer, colorectal neoplasm, colon cancer, colon neoplasm, rectal cancer, rectal neoplasm, processed meat, cured meat, preserved meat, nitrosation, N-nitroso

compound, nitrosamines, nitrosamides, food content, and dietary sources. References from identified articles were examined to ensure inclusion of all pertinent publications.

NOC Carcinogenesis

The potent and organ-specific carcinogenicity of NOCs is well-supported by an abundance of scientific evidence (86-88). The first report of the carcinogenic potential of NOCs dates back to 1956 (89). A previous review of the published literature found that NOCs were carcinogenic in over 40 unique animal species, including higher primates (39). More than 300 NOCs have been examined in animal models, and 85% of nitrosamines and 92% of nitrosamides have been found to induce tumors at a variety of sites (40). The most recent *Report on Carcinogens* (90) released by the National Toxicology Program (NTP) of the U.S. Department of Health and Human Services (HHS) describes NOCs as probable human carcinogens.

Findings from experimental animal studies provide insight into the mechanisms underlying NOC carcinogenesis in the colon and rectum (86,88,91). Nitrosamines undergo α-hydroxylation by cytochrome P450 (CYP) enzymes (mainly CYP2E1 and CYP2A6) to biologically active metabolites that can bind with DNA to form adducts that may produce mutations and tumors if not detoxified (92). Mutagenic adducts (e.g., O^6-methylguanine) that may cause G→A transitions in codons 12 or 13 of the K-*ras* gene have previously been detected in human colon cells (93). On the other hand, nitrosamides spontaneously form alkylating agents and can form adducts with DNA without metabolic activation.

Overview of Diet-Induced NOC Exposure

Data on the potential carcinogenicity of diet-induced NOC exposure in humans, including both endogenously and exogenously generated NOCs, are lacking. Possible nitrosating agents include nitrites, nitrogen oxides, and nitrates, which are readily reduced to nitrite by bacteria in

saliva or along the gastrointestinal tract (38). The nitrosatable substrate may be a secondary amine or amide derived from protein, or an amide-like compound such as guanidine (37). Examples of guanidines include creatine and creatinine, which are present in high concentrations in meat (94).

Epidemiological investigations have not evaluated total diet-related exposure to NOC in relation to colorectal cancer risk, likely because accurate measurement and identification of total NOC exposure is difficult to achieve. One epidemiologic investigation (95) assessed quantitative intake of a specific preformed nitrosamine (*N*-nitrosodimethylamine [NDMA]) and risk of colorectal cancer in a cohort of 9,985 Finnish adults (RR = 2.12; 95% CI = 1.04-4.33 for the highest compared with the lowest quartile). Measurement of exposure to endogenous NOC formation was not performed.

Exogenous NOC Exposure

Studies have indicated that diet provides the main exposure to NOCs among the general population (96,97). Non-dietary exposure may arise in occupational settings such as the leather, metal machining, rubber, and tire industries and from the inhalation of tobacco smoke (98). Diet-related exogenous formation of NOCs may occur in meats that are preserved with sodium nitrites or heat-treated by smoking or direct fire-drying, the latter two processes prompting nitrogen oxides from the drying air or smoke to directly nitrosate available amines. These NOCs are considered preformed since they develop in meat prior to human consumption. Sodium nitrites are added to meat during the curing process for a number of reasons. Nitrites inhibit the growth of *clostridium botulinum*, an anaerobic bacterium that preferentially develops in low oxygen environments (vacuum-packaging, canning, etc.) and can produce the potentially fatal toxin botulin (99). Nitrites are responsible for the characteristic flavor and pink color associated with

cured meats through the prevention of oxidation. Smoking and direct fire-drying are performed to prevent rancidity through inhibiting fatty acid oxidation.

Methodology for Measuring Preformed NOC in Meat

One of three methods was used to estimate nitrosamine concentrations in the studies included in this review: a gas chromatographic (GC) system combined with mass spectrometry (MS); a GC system combined with a nitrosamine-specific thermal energy analysis (TEA) method; or chemical denitrosation with hydrogen bromide (HBr) combined with TEA. Estimated nitrosamine concentrations in meat items are presented in parts per billion (ppb), which translates to micrograms (mcg) of NOC per kilogram (kg) of meat (Table 4.6).

Data on nitrosamide concentration in food is limited. These compounds are highly unstable and are thought to exist in smaller quantities in meat items than nitrosamines. Findings from several laboratory experiments (100-103) suggest that nitrosamide precursors such as methylurea are generated from creatinine in meat and are readily converted to nitrosamides in a high-nitrite and acidic environment. One study (104) measured nitrosamides along with nitrosamines in meat, but total NOC concentrations were reported rather than concentrations of each NOC subtype separately.

Table 4.6
Estimated Concentrations of Preformed *N*-Nitroso Compounds in Sampled Meat Items

Lead author, year	Meat item	NOC	Detection method	ppb (mcg of NOC per kg of meat)	
				mean	range
Kimoto, 1982	Cooked bacon	NDMA, NPYR	GC-MS	__	ND-32
Fiddler, 1995	Bacon	NPRO	GC-TEA	50	__
Canas, 1986	Fried bacon	NDMA, NPYR	GC-TEA	21	1.0-65
Vecchio, 1986	Fried bacon	NDMA, NPYR, NTHZ	GC-MS	__	ND-4.2
Pensabene, 1990	Fried bacon	NTHZ	GC-TEA	__	ND-2.0
Fiddler, 1995	Canned corned beef	NPRO	GC-TEA	7,880	__
Fiddler, 1995	Canned ham	NPRO	GC-TEA	79	__
Fiddler, 1995	Dried beef	NPRO	GC-TEA	1,170	__
Fiddler, 1995	Frankfurter	NPRO	GC-TEA	27	__
Haorah, 2001	Frankfurter	Total NOCs	HBr-TEA	27.5	0.5-101
Fiddler, 1995	Salami	NPRO	GC-TEA	1,310	__
Haorah, 2001	Smoked, dried herring	Total NOCs	HBr-TEA	104	__
Haorah, 2001	Summer sausage	Total NOCs	HBr-TEA	18.5	17.5-21
Haorah, 2001	Ground beef, chicken, and pork	Total NOCs	HBr-TEA	2.5	1.5-3.5

Abbreviations: GC, gas chromatographic system, HBr, hydrogen bromide treatment, MS, mass spectrometry, mcg/kg, micrograms of NOC per 1 kilogram of meat, ND, not detectable, NDMA, *N*-nitrosodimethylamine, NOC, N-nitroso compounds, NPYR, *N*-nitrosopyrrolidine, NPRO, *N*-nitrosoproline, NTHZ, *N*-nitrosothiazolidine, ppb, parts per billion, TEA, thermal energy analysis method

Summary of Laboratory Analyses of Exogenous NOC Exposure

A review of the findings from the laboratory analyses revealed that not all samples contained detectable levels of nitrosamines, although three (81-83) of the five studies (81-85) measuring NOCs in bacon reported concentrations well above the 10 ppb mark established by the USDA Nitrosamine Monitoring Program for bacon. Furthermore, the measurement by Fiddler *et al*. (82) was for uncooked bacon, and it has been shown that frying and other high-temperature cooking methods induce nitrosation reactions (105). A wide range of values were reported, both within studies and between studies. Between study variation is not unexpected due

to the preservation, cooking, measurement, and food compositional factors that can influence NOC formation (106). The within study variation may have been true differences in the food samples analyzed. For example, Haorah *et al.* (104) found that NOC concentrations in the samples of a single frankfurter brand varied by the time of purchase, from 1.0 to 5.6 ppb in a five-month span of time (104). The comparatively high levels of NOCs among several specific processed meat items, notably salami, dried beef, and canned corned beef, are cause for concern.

Promoters of Exogenous NOC Formation

Several factors may influence the generation of NOCs in meat (106). The use of high temperature methods of cooking, such as frying, charbroiling, and barbequing, as well as longer duration of cooking, are conducive to the production of NOCs (107). Acidic aqueous solutions may promote nitrosation reactions through increased formation of the nitrosating agent nitrous anhydride from nitrite (106). Greater concentration of amines, longer duration of storage, and higher storage temperatures are additional factors that prompt nitrosation reactions (24).

Inhibitors of Exogenous NOC Formation

A variety of factors may reduce or inhibit NOC formation in meat. Nitrosation inhibitors may act as nitrite scavengers, destroying the nitrosating agent, or they may compete for amine substrates of nitrosation. Mirvish *et al.* (108) discovered in the early 1970s that ascorbate can reduce nitrosation reactions by scavenging nitrosating agents, which led to the requirement for the addition of either ascorbate or its isomer erythorbate to nitrite-treated meat products in the U.S. (78). This mandatory change has contributed to an estimated five-fold decrease in the levels of detectable NOCs in meat from the mid-1970s to the mid-1990s (99). The use of lower temperature cooking methods, such as baking and microwaving (107), as well as cooking meat for shorter durations of time (109), may limit nitrosation reactions.

Diet-Induced Endogenous NOC Formation

It has been suggested that endogenous synthesis may account for 45-75% of total human NOC exposoure (110). NOCs can be formed within the gastrointestinal tract or can reach the large intestine via the blood supply (24). Nitrogenous residues from protein metabolism and nitrosating agents from bacterial nitrate reduction or nitrite intake within the colon may promote nitrosation reactions. Evidence of endogenous nitrosation comes from early human studies (111), where a nitrosamine (*N*-nitrosoproline [NPRO]) was measured in urine after a nitrosatable substrate (proline) and a nitrosating agent (nitrite) were provided. Total urinary NPRO was found to increase proportionally with the proline dose and exponentially with the nitrite dose.

Measurement of Diet-Induced Endogenous NOC Formation

Several small studies have examined potential exposure to NOCs via diet-induced endogenous pathways among human subjects (n = 4-18) (27,112-115). Pignatelli *et al.* (116) describes a method for identifying NOC concentrations in gastric juices that has been applied to recent studies of endogenous nitrosation in fecal samples. Practical and analytical difficulties have limited evaluations of colonic endogenous nitrosation in large study samples.

Factors Involved in Endogenous NOC Generation

1. *Acidic Environment*

Gastric juices provide an acidic environment that is conducive to the production of NOCs. Pignatelli *et al.* (117) measured NOC levels in the gastric juice of 211 subjects and found that NOC levels increased proportionally with increasing nitrite concentration.

2. *Bacteria*

Bacteria can decarboxylate amino acids, generating amines and amides (36), and can reduce nitrate to nitrite (118). Research has shown that the activity of bacterial nitrate reductase

is positively related to its nitrosating ability and its activity varies widely between individuals (119). Differences in dietary fiber intake and other dietary variables may influence the expression and activity of these colonic bacteria. Bacteria can also synthesize nitrosating agents via the nitric oxide synthase pathway.

3. *Inflammation*

Inflammatory bowel diseases including ulcerative colitis and Crohn's disease have been shown to increase colorectal cancer risk (120). Underlying the observed association may in part be the elevated generation of nitric oxide during inflammation (40). Nitric oxide is produced from the amino acid arginine by activated macrophages and bacteria under conditions of inflammation.

4. *Dietary Intake of Protein, Total Meat, Red Meat, Iron, and Heme*

It is well-established that the supply of nitrogenous residues in the colon increases with higher levels of protein intake (29,112,121), but whether this increased pool of nitrogenous residues translates directly to increased NOC formation remains unclear (29,112,113). Hughes *et al.* (113) observed a dose-dependent effect of total meat intake on fecal NOC concentrations, a proxy for endogenous NOC formation, among eight healthy participants in a randomized controlled trial (RCT). Two later RCTs reported no effect of total protein on measured fecal NOC concentrations (29,112). Bingham *et al.* (112) measured a dose-response increase of fecal NOCs with increasing red meat intake among twelve healthy males; an equivalent amount of white meat had no effect on fecal NOC levels. The following year, Cross and colleagues (29) further examined the effect of a high red meat diet on endogenous NOC formation among 21 healthy male subjects and found that the heme in red meat rather than the iron or protein was responsible for the increased NOC generation.

5. ***Additional Dietary Factors***

Additional dietary factors likely influence rates of endogenous nitrosation. Ascorbic acid, alpha-tocopherol, glutathione, polyphenols, carotenoids, and other dietary antioxidants may reduce or inihibt endogenous nitrosation by scavenging nitrites (122,123). Cruciferous vegetables such as broccoli, cauliflower, and cabbage contain isothiocyanates that may decrease cytochrome P450-mediated metabolic activation of nitrosamines (124). Sulfur-containing allium vegetables such as garlic and onions may reduce bacterial nitrate reduction (125). Diets characterized by higher fiber intakes may reduce transit time (fewer opportunities for nitrosation reactions) and decrease bacterial protein degradation (less nitrosatable substrates) (36,113).

Dietary Intake of NOC Precursors

Appreciable quantities of both nitrates and nitrites were recently measured in a sample of meat items examined by Sinha and colleagues at the NCI (personal communication, October 1, 2008). These meat items represented 90% of the processed meats reported according to nationally representative survey data. Vegetables are a significant source of nitrate, accounting for an estimated 85% of current U.S. dietary intake (126,127), but they also contain naturally-occurring nitrosation inhibitors. The extent to which nitrate reduction to nitrite translates into NOC generation is unclear, although findings from a clinical trial of eight healthy subjects suggests that endogenous formation of NOCs from nitrate intake can contribute significantly to total NOC exposure (measured by fecal NOC concentrations) (114). Preserved meats are the predominate source of dietary nitrite among the general U.S. population (128). Current estimates of nitrite and nitrate intakes in the U.S. are limited since these compounds are not present in standard food composition databases.

SECTION 4.5

Genetic Variability in *N*-Nitroso Compound-Metabolizing Enzymes and Interactions with Processed Meat in Relation to Colorectal Cancer

The ability of various dietary components to modify the process of colorectal carcinogenesis is well-supported by the scientific literature (129). The extent of this contribution is likely dependent upon underlying genetic factors. Several inheritable single-gene mutations with high-penetrance, including *APC* and *MLH1* gene mutations, significantly increase the RR ($\geq$ 5.0) of developing colorectal cancer (130). These altered cancer susceptibility single-gene mutations are rare and account for less than 5% of all colorectal cancer cases (130). Polymorphisms in genes coding for carcinogen metabolizing enzymes have lower penetrance but are considerably more prevalent (131,132). We may augment our current understanding of the role of dietary factors in carcinogenesis by accounting for these inter-individual metabolic differences.

Cytochrome P450 (CYP) enzymes (Phase I enzymes) metabolize NOCs into mutagenic compounds that can damage DNA and form adducts if not conjugated and detoxified by Phase II enzymes, such as UDP-glucuronidases (UGTs) (92). Variability in genes coding for either Phase I or Phase II enzymes may be an important factor underlying colorectal cancer risk. Genetic polymorphisms conferring enhanced enzymatic activation or decreased detoxification of nitrosamines (increased carcinogen exposure) may promote DNA adduct formation in colon and rectal tissue (133). Variant genotypes of CYPs, UGTs, or other enzyme families expressed in the liver may modify relations between processed meat and colorectal cancer risk because nitrosamines may reach the large intestine via the blood supply post-hepatic activation. The extent of nitrosamine distribution from the liver to the colon is currently unknown (134).

Methods to Evaluate Polymorphic Metabolic Enzymes

Several approaches can be used to evaluate the role of polymorphic metabolic enzymes on processed meat and colorectal cancer associations: metabolic genotyping (135) and metabolic phenotyping (136,137). When a previously identified allelic variant is uncommon in a population, but inter-individual metabolic activity differs considerably, phenotyping analyses may capture variation not explained by the known genetic polymorphisms (136). Genotyping analyses are often performed on a previously identified important region of the gene with polymorphisms such as single nucleotide polymorphisms (SNPs) that occur with reasonable frequency for a given sample size (133). The analysis of haplotypes is an additional approach in epidemiologic association studies, which allows for combinations of polymorphisms (e.g., a set of SNPs) within a particular chromosomal location to be assessed for their association with disease risk (138). With the advent of the International HapMap Project, which has genotyped more than 3.1 million human SNPs (139,140), future epidemiologic studies may be able to investigate combinations of genetic metabolic variants involved in metabolizing meat carcinogens. Lastly, genome-wide association studies (GWAS) aim to investigate variation across the entire human genome in relation to disease risk (141).

Each approach has its strengths and limitations. The assumption inherent with phenotype analyses is that the metabolic phenotype was present prior to the disease, although it is possible that the observed phenotype (e.g., increased enzymatic activity) may be a consequence of the disease state. Genotype analyses can provide insight into the biological function of a specific allele, but do not capture the interplay of multiple polymorphisms and may not be useful if the variant has both a low frequency and penetrance in the study population. Haplotype analyses are able to identify combinations of genetic variants that tend to be inherited together, thereby

capturing potential interactions between polymorphisms and their combined and synergistic influence on risk. Information pertaining to which alleles are responsible for the increased or decreased risk may be unclear. GWAS tend to be non-hypothesis driven and largely exploratory in nature, but may provide insight into genomic function and disease risk, generating hypotheses for future studies.

Literature Selection Procedure

Prospective cohort and population-based case-control studies examining associations of genetic polymorphisms in hepatic and colonic enzymes involved in nitrosamine metabolism and interactions with processed meat intake and colorectal cancer or adenoma risk were reviewed. Studies investigating metabolic phenotypes, genotypes, haplotypes, and genome-wide associations and risk of colorectal cancer were considered. A literature search was conducted in the COCHRANE, PUBMED, and Web of Science databases for articles published through January 2010 with the following search terms: colorectal cancer, colorectal neoplasm, colorectal adenoma, colon cancer, colon neoplasm, colon adenoma, rectal cancer, rectal neoplasm, rectal adenoma, genetic polymorphism, nitrosamine metabolism, genotype, phenotype, haplotype, genome-wide association, processed meat, cured meat, preserved meat, and smoked meat. References from identified articles were examined to ensure inclusion of all pertinent publications.

Results: Genetic Polymorphisms, Processed Meat Intake, and Colorectal Cancer

Enzyme Families Investigated

Genetic polymorphisms in the following enzyme systems involved in meat-derived carcinogen activation and detoxification have been evaluated for their influence on associations of meat intake and colorectal cancer risk: CYPs, *N*-acetyltransferases (NATs), glutathione *S*-

transfereases (GSTs), and UGTs. Most studies focused on the potential modifying effects of these enzymes on associations between meat-derived HCA and PAH exposure and colorectal cancer risk. Only three studies were identified that evaluated the effect of metabolic polymorphisms on observed associations between processed meat intake and colorectal cancer (Table 4.6) (135-137). No studies were published on the interaction of processed meat intake (or meat-derived nitrosamine exposure) and NAT, GST, UGT, or SULT polymorphisms and colorectal cancer risk. An overview of UGT or SULT polymorphisms and colorectal cancer risk is provided in this review to explore future research directions in the area of processed meat-derived carcinogens, metabolic gene polymorphisms, and colorectal cancer risk since other environmentally-derived nitrosamines are substrates for the UGT enzymes in hepatic and extra-hepatic tissue (142) and may be substrates for the SULT enzymes (143).

Comparison of Findings

Three case-control studies evaluated the effect of metabolic genotype (135) or metabolic phenotype (136,137) on associations between processed meat intake and colorectal cancer or adenoma risk. In these studies, the investigators stratified by metabolic genotype or phenotype and examined processed meat intake and colorectal cancer risk associations for each genetic variant or for each tertile of enzymatic activity. Le Marchand *et al*. (135) evaluated the potential modifying effect of two genetic polymorphisms in *CYP2E1* (a 96-bp [base pairs] insertion and a *Rsa*I substitution polymorphism [G125C]) in an ethnically diverse sample from Hawaii (60% Japanese, 26% Caucasian, and 14% Native Hawaiian; 639 controls and 356 colon cancer cases). By contrast, both Nowell *et al*. (136) and Ward *et al*. (137) examined the influence of CYP2A6 activity (assessed by urinary caffeine metabolites) among primarily Caucasian populations (333 controls and127 colorectal cancer cases; 228 controls and 146 colorectal adenoma cases,

respectively). An additional difference is that CYP2A6 is expressed predominately in the liver, whereas CYP2E1 is expressed in both the colon and the liver.

The *Rsa*I substitution polymorphism was found to confer lower CYP2E1 activity (decreased nitrosamine activation), whereas the 96-bp insertion induced higher activity (increased nitrosamine activation). The authors (135) hypothesized that the low-activity allele would weaken the positive association of processed meat intake and colon cancer risk and the high-activity allele would strengthen the positive association. Only a slight modulating effect was observed between the *CYP2E1* genetic polymorphisms and the association of processed meat intake and colon cancer risk (interaction tests were not significant). Ward *et al*. (137) also found little evidence of a modifying effect of CYP2A6 activity on associations of processed meat intake and adenoma risk (interaction tests were not significant). In contrast, findings from the work by Nowell *et al*. (136) indicated that greater CYP2A6 enzyme activity (second and third tertiles of activity) modestly but statistically significantly increased the associations of processed meat intake and colorectal cancer risk ($P = 0.01$ for interaction).

Discussion

Few previous epidemiologic investigations into processed meat intake and colorectal cancer risk have evaluated underlying genetic factors that may influence the observed diet-disease associations. The suggestive findings from this review warrant further investigation. Ward *et al*. (137) observed a trend of increasing colorectal adenoma risk from processed meat intake by tertile of CYP2A6 activity. A similar pattern of increasing risk from processed meat intake among individuals with a variant allele conferring greater CYP2E1 activity was reported by Le Marchand *et al*. (135). The three studies that investigated these interactions may have lacked statistical power to detect interactions due to the relatively small sample sizes. For

example, only 22 cases and 19 controls were in the top tertile of processed meat intake (> 24 grams per day) and CYP2A6 activity (representing greater levels of nitrosamine activation) in the case-control study conducted by Ward *et al*. (137). The subgroup of individuals with higher intakes of processed meat (> median of 14.8 g/d) and the 96-bp insertion variant high-activity allele was comprised of 57 cases and 82 controls in the case-control study by Le Marchand and colleagues (135).

TABLE 4.7

Selected Characteristics and Findings from Population-Based Case-Control Studies: Polymorphic Metabolic Genes, Processed Meat Intake, and Colorectal Cancer Risk

Lead author, year	Study design, state	Sample size	Age (y)	Years	Exposure definition	Polymorphic gene or enzyme	Gene-processed meat interaction and colorectal cancer risk[1]
Le Marchand, 2002	Case-control study, Hawaii[1]	M/W: 639 controls, 356 cases	56-74	1994-98	Ham, bacon, sausage, luncheon meats	*CYP2E1*: low-activity allele (G125C substitution) / high-activity allele (96-bp insertion)	Limited evidence of a modifying effect of low-activity allele or high-activity allele on associations (interaction tests NS)
Nowell, 2002	Case-control study, Arkansas	M/W: 333 controls, 127 CRC cases	28-83	1993-99	Bacon, sausage, hot dogs, ham, bologna, salami	CYP2A6 activity assessed by caffeine phenotyping assay (tertiles of activity)	Statistically significant evidence of a slight modifying effect of CYP2A6 activity on associations ($P = 0.01$ for interaction)
Ward, 2007	Case-control study, Maryland[2]	M/W: 228 controls, 146 cases	18-74[3]	1994-96	Bacon, sausage, hot dogs, ham steaks/pork chops[4], luncheon meats, liverwurst	CYP2A6 enzyme activity assessed by caffeine phenotyping assay (tertiles of activity)	Limited evidence of a modifying effect of CYP2A6 activity on associations (interaction tests NS)

Abbreviations: CYP, cytochrome P-450 enzymes, M/W, Men/Women, NS, not significant

[1] colon cancer

[2] colorectal adenoma

[3] 10^{th} to 90^{th} percentiles of age: 46-70y (controls) and 46-71y (cases)

[4] Pork chops were included by author because they were grouped with ham steaks on the FFQ

A Potential Modifying Effect of UGT Polymorphisms

Whether the glucuronidation pathway is a mechanism of detoxification for meat-derived nitrosamines in the colon and rectum remains to be determined. A number of UGT enzyme isoforms are understood to be expressed in both human colon, rectal, and hepatic tissues, whereas others are thought to be expressed exclusively in one organ (144). Research indicates that several variant low-activity alleles of UGT enzymes expressed in the colon may be associated independently with an increased risk of colorectal cancer (145). A recent U.S. population-based case-control study (400 colon cancer cases and 412 controls) reported that a low activity *UGT1A7* genotype increased the susceptibility for colon cancer among individuals with higher intakes of HCAs (66). Additional work examining gastrointestinal glucuronidation rates of HCAs and PAHs has revealed great inter-individual variability that was attributed in part to SNPs identified in the UGT coding region, thereby altering rates of detoxification (11). Other UGT polymorphisms that decrease hepatic enzymatic expression have been associated with reduced detoxification of carcinogenic intermediates of meat-derived HCA metabolism (146).

A Potential Modifying Effect of SULT Polymorphisms

Members of the SULT family of enzymes have been implicated for their involvement in activating nitrosamines to alkylating agents in the hepatic tissue of animal models (143,147). Meat-derived nitrosamines have not been directly evaluated in relation to SULT genotype and colorectal cancer risk, but it remains possible that SULT polymorphisms might modify nitrosamine-cancer associations. A recent clinic-based study examined interactions between polymorphic SULT genes, meat intake, and risk of colorectal polyps (651 cases, 556 controls) (148). Increased risk was observed among those with a high-activity *SULT1A1* genotype (translating to greater activation) and greater intakes of fried, broiled, and baked meat (148).

A Potential Modifying Effect of Multiple Phase I and Phase II Metabolic Gene Polymorphisms

The interplay of a number of genetic polymorphisms with one another as well as with diet and other environmental exposures may be involved in the etiology of colorectal cancer. The extent to which various combinations of genetic polymorphisms modify associations of processed meat intake and colorectal cancer risk has yet to be examined. Findings from investigations of SNPs on diet-cancer relations highlight the need for large epidemiological studies to explore interactions of multiple polymorphisms and environmental exposures on colorectal cancer risk. One approach that may prove effective in capturing these relationships involves haplotype association analyses since complex diseases are likely caused by more than one common, low-penetrance genetic polymorphism.

Summary

Findings from this review lend support for the hypothesis that associations between processed meat intake and colorectal cancer may be modified by genetic variability in carcinogen-metabolizing enzymes. Previous sections of this review suggest that these diet-gene interactions are likely complicated by additional dietary factors that may influence enzyme expression or activity. For example, metabolites of glucosinolates found in cruciferous vegetables such as broccoli and cabbage have been shown to alter CYP-mediated metabolic activation of nitrosamines (124).

Very few studies have explored interactions of processed meat-derived nitrosamine exposure with metabolic polymorphisms and colorectal cancer risk. A host of factors may explain the paucity of data, including methodological challenges inherent with capturing total nitrosamine exposure and collecting biological samples in large epidemiologic studies. The large

sample size required to consider multiple interactions and subgroups of exposure may deter such investigations. This review indicates a need for large epidemiologic studies examining interactions of multiple metabolic polymorphisms with sufficiently-captured diet-related exposures of interest in relation to colorectal cancer risk.

SECTION 4.6

CONCLUSION

Several recent meta-analyses have consistently shown greater red and processed meat intakes to be risk factors for colorectal cancer, although results from individual studies have been less conclusive. Observed inconsistencies may be related to variations in exposure to specific components in meat between populations, including NOCs, NOC precursors, HCAs, and PAHs. Exposure to these meat components is difficult to estimate in large epidemiological studies and the surrogate measures used have generated mixed results. The development of a database that includes measured values of several HCAs and one PAH by NCI researchers has led to recent investigations into meat cooking mutagens and colorectal cancer. Results from these studies have been suggestive, but several null findings have generated new hypotheses regarding the involvement of diet-gene interactions. The investigation of interactions between multiple genetic polymorphisms and meat exposures in relation to colorectal cancer risk is underway in a large multi-site population-based case-control study including both genders

Very few studies have examined exposure to NOCs and NOC precursors and colorectal cancer risk. The recent measurement of nitrites and nitrates in processed meat items by NCI researchers (personal communication, October 1, 2008) allowed for the incorporation of these estimated values into a database that we tied to our modified Diet History Questionnaire (DHQ).

The following chapter investigates whether increased exposure to NOC precursors is associated with colorectal cancer, and whether associations differ by tumor site.

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Chapter 5

RED AND PROCESSED MEAT-DERIVED MUTAGEN EXPOSURE AND COLORECTAL CANCER RISK IN A POPULATION-BASED CASE-CONTROL STUDY[1]

[1] This manuscript is in preparation for submission to the *American Journal of Epidemiology*:
Miller PE, Lazarus P, Lesko SM, Muscat JE, Harper G, Cross AJ, Sinha R, Ryczak K, Escobar G, Liao J, and Hartman TJ. Red and processed meat-derived mutagen exposure and colorectal cancer risk in a population-based case-control study. *American Journal of Epidemiology* (In Preparation).

ABSTRACT

To explore potential underlying mechanisms for previously observed associations between red and processed meat and colorectal cancer, we examined associations of mutagens that are generated through certain meat cooking and meat processing methods with colorectal cancer in a large multi-site population-based case-control study including both genders. Participants (726 healthy controls, 287 colon cancer cases, and 128 rectal cancer cases) completed a 137-item food frequency questionnaire, which included a detailed cooked and processed meat module that allowed for the use of databases of heterocyclic amines (HCAs), polycyclic aromatic hydrocarbons (PAHs), nitrites, and nitrates. Odds ratios (ORs) and 95% confidence intervals (CIs) for associations between meat exposures and colorectal cancer stratified by sub-site of the large intestine were estimated from unconditional logistic regression models. After multivariate adjustment, positive associations with HCAs and PAHs, as measured by total mutagenic activity, were stronger for rectal cancer (OR = 1.75, 95% CI: 1.00, 3.08; *P* for trend = 0.031) than colon cancer, whereas suggestive positive associations with nitrites plus nitrates were stronger for colon cancer (OR = 1.28, 95% CI: 0.82-2.00; *P* for trend = 0.084). Our findings support the hypothesis that greater exposure to HCAs, PAHs, nitrites, and nitrates is a plausible mechanism by which red and processed meat may increase colorectal cancer risk. In addition, our sub-site analyses indicate that associations between meat-derived exposures and colon and rectal cancer may differ, which underscores the need for additional studies that examine dietary risk factors for colon and rectal cancer as separate endpoints.

INTRODUCTION

Colorectal cancer is the fourth most commonly diagnosed malignancy in developed countries (1) and the second leading cause of cancer death among Americans (2). Among non-smokers, colorectal cancer is the leading cause of cancer death and the five-year survival rate from the time of diagnosis is only 60% (2). Epidemiologic evidence indicates that a substantial proportion of all colorectal cancer cases are attributable to diet (3-5). Red and processed meats, larger contributors to the diet in populations with comparatively greater colorectal cancer incidence rates, have been implicated as risk factors for colorectal cancer. A recent consensus report issued jointly by the World Cancer Research Fund (WCRF) and the American Institute for Cancer Research (AICR) concluded that the evidence to support a positive association between greater intakes of red and processed meat and colorectal cancer was convincing, but the evidence for specific meat components explaining these associations remained inconclusive (6). Several individual components have been suggested to explain the underlying mechanisms by which red and processed meat may elevate the risk of colorectal cancer, including saturated fat, animal protein, and heme iron (7,8), although findings have been largely inconsistent. Enduring among the proposed hypotheses is increased exposure to heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs) arising from cooking meat well-done at high temperatures or over a direct flame and the addition of sodium nitrites and nitrates as preservatives (9). HCAs and PAHs are highly mutagenic compounds and known animal carcinogens (10,11). The reaction of nitrosating agents such as nitrites (or nitrates that are readily reduced to nitrites by bacteria) with amines or amides derived from protein leads to the formation of *N*-nitroso compounds (NOCs), which are potent and organ-specific animal carcinogens (12).

A number of studies have examined associations between red and processed meat intake and colorectal cancer, leading to four large meta-analyses of the epidemiologic evidence (6,13-15). Fewer studies have examined the effects of increased exposure to meat-derived HCAs, PAHs, and sodium nitrites and nitrates on colorectal cancer risk. No studies to date have captured exposure to all three classes of carcinogens and examined associations with colon and rectal cancer as separate endpoints, although there is biologic (16) and epidemiologic evidence (17) to suggest that risk factors for colorectal cancer differ by sub-site.

To explore potential underlying mechanisms for previously observed associations between red and processed meat and colorectal cancer, we examined the associations of dietary intakes of meat-derived HCAs, PAHs, and sodium nitrites and nitrates with colon and rectal cancer in a large multi-site population-based case-control study including both genders.

MATERIALS AND METHODS

Study population

This study included incident colorectal cancer cases and healthy controls participating in a population-based case-control study in a contiguous 19-county area in central and northeast Pennsylvania. The study was designed to investigate risk factors for colorectal cancer among adult residents of this area. Newly diagnosed cases (identified within 12 months of diagnosis) with histologically-confirmed colon or rectal cancer were identified between June 2007 and November 2009 from records of the Pennsylvania State Cancer Registry. Individuals were eligible for inclusion if they were fluent in English and had no history of previous colorectal cancer. Controls residing in the same 19-county region were identified by random digit dialing. Eligible controls were screened for fluency in English and no previous history of colorectal

cancer. Written consent was obtained from a potential case or control, a personal interview was scheduled at the home of the participant, and a self-administered food frequency questionnaire (FFQ) was mailed with instructions to complete the FFQ prior to the interview. Data regarding sociodemographic factors, medical history, alcohol use, lifetime tobacco exposure, physical activity behavior, height, weight, medication use, and other lifestyle-related factors were collected by trained interviewers during in-person interviews. For health and lifestyle-related factors, such as weight and physical activity, data prior to diagnosis were collected for cases. The completed FFQ was reviewed during the in-person interview. For the present analysis, we excluded participants who were less than 35 y (n = 6), who had missing body mass index ((BMI) weight (kg)/height (m^2)) data (n = 5), who reported implausible energy intakes (< 500 kcal or > 5,000 kcal) (18,19)) (n = 29), or who had missing tumor site data (n = 16). After these exclusions, 1,141 participants (415 cases, 726 controls) were included in this analysis. Thirty-one percent of the 415 cases were rectal cancer cases (n = 128) and the remaining 69% were colon cancer cases (n = 287). The institutional review boards at the Northeast Regional Cancer Institute, Penn State College of Medicine, and Lehigh Valley Health Systems (Allentown, PA) approved this study.

Dietary assessment method

Participants completed a modified version of the Diet History Questionnaire (DHQ), a validated FFQ developed by the National Cancer Institute (NCI) (20). The reference period was the prior year for controls and the year prior to diagnosis for cases. We modified the DHQ for our study population using previously collected 24-h dietary recall data from a similar Pennsylvania study population (21). The DHQ also included a meat module (22) that was modified to capture the meat eating patterns of Pennsylvania residents. These modifications

included the addition of processed meat items commonly consumed in this population. The meat module contained questions on preferred meat cooking methods and doneness levels for individual meat subtypes.

The DHQ and visual materials, which were designed to facilitate the recall of portion sizes and preferences for meat doneness levels, were mailed with instructions to complete the DHQ prior to the scheduled interview. Respondents were queried about their usual intake and portion size of 137 separate food and beverage items, 49 of which contained additional embedded questions. Thirty-two of the 137 items were related to meat consumption. Respondents selected from 10 frequency categories that ranged from never to two or more times per day for each food and from nine frequency categories that ranged from never to six or more times per day for each beverage. Three food- and beverage-specific portion size ranges were available for each question. The DHQ included questions that addressed variations in food type (e.g., regular vs. low-fat), seasonal intake, and added fats. Data pertaining to dietary supplement use were also collected with the DHQ. Energy and nutrient intake values were calculated with Diet*Calc (version 1.4.3), nutrient analysis software developed by the NCI for use with this instrument and configured to accommodate our questionnaire modifications. Portion size and frequency of food intake data were used to calculate the average daily servings according to standard USDA serving sizes (23) for each food item consumed. Similarly, portion size and frequency of intake information was used to estimate total intake of red meat and processed meat in grams per day. The total red meat variable included the following beef and pork items: bacon, sausage, cold cuts (ham, bologna, salami, pepperoni, beef luncheon meat, dried or chipped beef), beef jerky, corned beef, hot dogs, hamburgers, roast beef, pot roast, roast pork, steak, pork chops, pork or beef spare ribs, liver, ham, and meat added to mixed dishes such as chili and spaghetti.

The total processed meat variable included the following processed beef, pork, poultry, and fish items: bacon, sausage, all cold cuts, beef jerky, corned beef, hot dogs, ham, smoked fish, smoked turkey, and processed meat added to mixed dishes such as pizza.

Data on frequency of intake, portion sizes, and cooking and doneness preferences were used to generate estimated exposure to meat-related mutagens with the Computerized Heterocyclic Amines Resource for Research in Epidemiology of Disease (CHARRED) software application (22). In addition to meat variables, meat-based gravy intakes were also used. The CHARRED program estimated exposure (ng/day) to three HCAs (2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MelQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)) and one PAH (benzo[a]pyrene). In addition, the CHARRED application generated total mutagenic activity (revertant colonies/day), a measure of overall mutagenic potential that accounts for differences in mutagenic activity between the various compounds. Frequency and portion size data were used to generate estimated processed meat-derived nitrate and nitrite intakes based on an NCI database (unpublished data) (22). The database contained measured values from 10 commonly consumed meat items in the U.S. that accounted for 90% of the processed meats consumed according to nationally representative survey data.

Statistical analysis

Characteristics of cases and controls were compared using *t* tests for continuous variables and χ^2 tests for categorical variables. Spearman rank correlation coefficients were calculated to assess the relationship between the individual dietary variables. Odds ratios (ORs) and 95% confidence intervals (CIs) for associations between meat exposures and colorectal cancer were estimated from unconditional logistic regression models. Linear trend tests were calculated using

the median intake values for each quartile. We also investigated associations stratified by tumor site (colon or rectum). For the site-specific associations, both colon cases and rectal cases were compared to the entire sample of controls. Dietary variables were adjusted for total energy intake by the nutrient density method (24). For micronutrients with both dietary and supplemental intakes, dietary intakes were energy-adjusted using the residual method (24) and then combined with supplemental intakes. Energy-adjusted intakes of red and processed meat and meat-related compounds were categorized into quartiles according to the distribution among the controls. Non-normally distributed intake variables were logarithmically transformed to normalize their distributions.

All logistic regression models included age (years) and sex. In addition, we evaluated the following variables as potential confounders: alcohol intake (g/day); educational attainment (< high school, high school/some college, and college graduate/advanced degree); BMI (<25, 25-29.9, and ≥30); smoking status (never, current, or past smoker); total energy intake (kcal/day); fruit and vegetable intake (servings/1,000 kcal); low-fat dairy intake (servings/1,000 kcal); whole grain intake (servings/1,000 kcal); saturated fat intake (g/1,000 kcal); fiber intake (g/1,000 kcal); total folate intake (dietary folate equivalents (DFE)/ 1,000 kcal); total calcium intake (mg/day); total vitamin D intake (mcg/day); family history of colorectal cancer (yes, no); past regular nonsteroidal anti-inflammatory drug (NSAID) use (yes, no); and physical activity (< 1 hour and ≥ 1 hour/week of vigorous activity). NSAID use was defined as ever having been a regular user (≥ 3/week for at least 1 year prior to the interview for controls or diagnosis for cases). According to a 10% change-in-estimate criterion (25), BMI, fruit and vegetable intake, and past regular NSAID use were important covariates in our analyses; thus, these variables along with age, total energy intake, and sex were included in the final multivariate logistic regression models. We

explored the possibility of effect modification by creating cross-product terms of meat variables and potential modifiers, including age, sex, BMI, physical activity, NSAID use, and smoking. Likelihood ratio tests were used to evaluate the fit of a multiplicative interaction term in each model. Reported *P* values are 2-sided and $P < 0.05$ was considered significant for all tests. All statistical analyses were performed with SAS (version 9.2, SAS Institute, Inc., Cary, North Carolina).

RESULTS

Characteristics of the study population are presented in Table 1. Cases were older, more likely to be obese (BMI > 30 kg/m^2), more likely to have a family history of colorectal cancer, were less physically active, less likely to have a college or advanced degree, and less likely to be regular users of NSAIDs orscalcium or vitamin D supplements compared to controls. Racial distribution, smoking status, and multivitamin use did not differ significantly between cases and controls.

Table 2 summarizes dietary intakes of cases and controls. Controls reported greater consumption of low-fat dairy products, dietary fiber, and total calcium intake, whereas cases reported greater intake of red meat. Intakes of meat-derived nitrates and the individual HCA compounds DiMeIQx and MeIQx were higher among cases compared to controls. Controls tended to consume more fruits and vegetables and less processed meat compared to cases. Other dietary intakes examined did not differ appreciably between cases and controls.

Associations between red and processed meat intake and colorectal cancer are shown in Table 3. We observed a positive association between greater consumption of red meat and colorectal cancer when comparing the highest to the lowest quartile in the age- and sex-adjusted model (OR = 1.47, 95% CI: 1.02, 2.12; *P*-trend = 0.017). This association was attenuated after

further adjustment for BMI, fruit and vegetable intake, total energy intake, and NSAID use. Sub-site analyses showed that red meat consumption was more strongly associated with colon compared to rectal cancer (age- and sex-adjusted OR = 1.50, 95% CI: 0.99-2.28; *P*-trend = 0.041; OR = 1.34, 95% CI: 0.78, 2.30; *P*-trend 0.110, respectively). Multivariate adjustment weakened the association between red meat and colon cancer and no evidence remained for an association between red meat intake and rectal cancer. Processed meat intake increased the risk of colorectal cancer in the age- and sex-adjusted models (OR = 1.31, 95% CI: 0.90, 1.91; *P*-trend = 0.088), although the association did not achieve statistical significance in the fully adjusted model. Analyses stratified by tumor site revealed a nonsignificant positive association between processed meat and colon cancer (multivariate OR = 1.22, 95% CI: 0.78, 1.93; *P*-trend = 0.148). There was little evidence of an association between processed meat and rectal cancer.

The ORs for colorectal cancer across quartiles of meat-derived nitrite and nitrate intake are shown in Table 4. Increasing intakes of nitrites combined with nitrates elevated the risk of colorectal cancer after controlling for age and sex (OR = 1.35, 95% CI: 0.94-1.94; *P*-trend = 0.014); this positive association was attenuated in the multivariate models (OR = 1.17, 95% CI: 0.80-1.72; *P*-trend = 0.098). Results from stratified analyses revealed stronger positive associations between nitrite and nitrate intake and colon compared to rectal cancer. The OR of colon cancer for the highest versus the lowest quartile of nitrite plus nitrate intake was 1.43 (95% CI: 0.94, 2.19; *P*-trend = 0.024) in the age- and sex-adjusted model and 1.28 (95% CI: 0.82, 2.00; *P*-trend = 0.084) in the multivariate model. There was little evidence that nitrites or nitrates were associated with rectal cancer.

Table 5 presents associations between meat cooking-related mutagens and colorectal cancer. In contrast to our findings with nitrites and nitrates, the individual HCA compounds, as

well as total mutagenic activity, were more strongly associated with rectal compared to colon cancer. The multivariate ORs (95% CIs) for rectal cancer comparing the highest to the lowest quartile of DiMeIQx intake, PhIP intake, and total mutagenic activity were 1.74 (1.00, 3.03; *P*-trend = 0.034), 1.76 (1.03, 3.01; *P*-trend = 0.004), and 1.75 (1.00, 3.08; *P*-trend = 0.031). Positive associations were observed between greater intakes of DiMeIQx and MeIQx and colon cancer, but these associations did not achieve statistical significance. No statistically significant interactions were identified in any of the logistic regression models examining associations between meat exposures and colorectal cancer as one endpoint.

DISCUSSION

Accumulating evidence supports the association between red and processed meat and colorectal cancer risk (6,13-15). Several plausible hypotheses have been proposed to explain these observed associations; chief among them is increased exposure to three main classes of carcinogens derived through cooking meat well-done at high temperatures or over a direct flame (HCAs and PAHs) and NOCs formed from the addition of sodium nitrites and nitrates as preservatives (9). Few studies have captured exposure to all three families of carcinogens and no studies to date have examined these three classes of carcinogens in relation to colon and rectal cancer as separate endpoints despite biologic (16) and epidemiologic evidence (17) that risk factors may differ between these sites. Our study is the first to report on associations between meat-derived HCA, PAH, and sodium nitrite and nitrate intakes and colorectal cancer stratified by tumor site. Given the detailed meat exposure data collected, the wide variation in reported meat consumption, and the greater average intake of meat compared to a nationally representative sample (for example, 67 g of red meat/day in our sample v. 40 g/day in NHANES 1999-2004 (26)), our study was well-designed to investigate these associations.

Results from our examination of red and processed meat and colorectal cancer (considered as a single endpoint) are consistent with the direction and magnitude of the associations reported by four recent meta-analyses (6,13-15). Of the three meta-analyses that summarized results stratified by tumor site (6,14,15), one reported that red meat was more strongly associated with rectal compared to colon cancer (14), but no differential associations were found with processed meat in this meta-analysis. Results from a recent U.S. prospective cohort study among men and women residing in six states and two metropolitan areas suggested that both red and processed meat consumption were slightly greater risk factors for rectal cancer (27), although the differences between cancer sites were not statistically significant. On the other hand, similar to findings from our study, Wei *et al*. (17) reported positive associations between red and processed meat and colon cancer, but found no significant associations with rectal cancer in the Nurses' Health Study and the Health Professionals Follow Up Study. These inconsistent findings may be due to limited statistical power in stratified analyses since less than one-third of all colorectal cancer tumors in developed countries are found in the rectum (2).

A recent U.S. clinic-based case-control study reported a two-fold increased risk of colorectal adenoma with greater meat-derived nitrite and nitrate intakes (28). We also found a positive association between nitrite and nitrate intake and colorectal cancer, although the association did not reach statistical significance after multivariate adjustment. Since evidence from our study and others suggests that risk factors may be different for colon and rectal cancer, examining associations between meat-related compounds and sub-sites of the large intestine will further our understanding of underlying mechanisms. Bacterial content, rates of xenobiotic metabolism, transit time, morphology, enzymatic expression, and the level of different pro-carcinogenic DNA-adducts differ between the colon and rectum (16,29). The degree of

susceptibility to the effects of different potential carcinogens may vary between these sites (16) and it is possible that the colon may be more susceptible to the harmful effects of nitrites. Greater bacterial decarboxylation of amino acids into nitrosatable amines and amides, as well as reduction of nitrates to nitrites, occurs in the colon due to its greater bacterial population (30). The reaction of nitrites with amines and amides results in the formation of NOCs, which have been shown to induce tumors at a variety of sites in over 40 unique animal species, including higher primates (12).

An additional biologic mechanism whereby red and processed meat consumption may increase colorectal cancer risk is through increased exposure to HCAs and PAHs, which have been shown to induce DNA adducts and tumors in animal studies (10,11). In our study, examining association separately for colon and rectal cancer revealed that risk estimates were of greater magnitude for rectal compared to colon cancer. Our findings provide evidence that greater HCA and PAH exposure elevate the risk of rectal cancer after accounting for other risk factors. The differential associations between exposure to HCAs and PAHs and cancer risk by sub-site warrant further investigation.

Findings from our investigation are consistent with an earlier report of positive associations between different individual HCAs (as well as total HCAs) and rectal cancer (31). Le Marchand *et al*. (31) reported that total HCA intake was significantly associated with rectal cancer, but not colon cancer, among men and women in a multi-ethnic U.S. population-based case-control study. Augustsson *et al*. (32) examined associations between HCAs and colorectal cancer stratified by tumor site In a Swedish population-based case-control study, but found no evidence of an association between meat-mutagens and colon or rectal cancer. The inconsistent results across studies may be attributable to differences in meat-derived mutagen exposure since

consumption patterns, meat cooking methods, meat doneness preferences, and thus meat-mutagen exposure likely vary between populations.

An important strength of the present study was our dietary assessment method, which was specifically designed to address our hypothesis. We modified a validated cooked and processed meat module (33) to reflect the meat consumption patterns of our population. This meat module, which is linked to a database of HCAs and PAHs, was embedded within a validated comprehensive FFQ (20). We incorporated recently estimated values of processed meat-derived nitrites and nitrates (22) into a database that we tied to our FFQ. The use of these databases allowed us to examine specific mechanisms underlying previously observed associations between meat and colorectal cancer (6,13-15). In addition, participants were provided with colored photographs of six meat items often cooked by high-temperature methods to reduce misclassification of reported cooking and doneness preferences (34). Additional strengths of our study include the population-based design and the detailed exposure information collected during the in-person interviews. Trained interviewers reviewed the FFQs to ensure completeness and were blind to the research hypothesis to minimize interviewer bias.

Several limitations of the present study should also be considered. Measurement error associated with FFQs may lead to non-differential misclassification of respondents into dietary exposure categories, thereby attenuating risk estimates. The case-control design used in this study is susceptible to recall bias; cases may have reported past meat consumption differently than controls if meat intake was preconceived to be a risk factor for colorectal cancer. Another limitation is that respondents reported diet for the past year (prior to diagnosis for cases), but long-term dietary exposure is likely important in the development of cancer. Dietary habits have been shown to be relatively stable over time, suggesting that a diet history questionnaire

administered at one time point may reliably rank individuals according to long-term dietary intakes (35). Potential confounders were considered and included in our multivariate models, but residual or unknown confounding remains possible. The comparatively small number of rectal cancer cases likely reduced our power to reach statistical significance in analyses stratified by tumor site, and it is possible that a larger sample would have provided more definitive evidence for the observed associations. Our ability to determine the independent effects of specific meat variables was limited due to the multicollinearity among various meat constituents and total energy; for example, red meat and total energy were significantly correlated in our study (r = 0.52).

The degree to which greater consumption of specific meat compounds elevates the risk of colorectal cancer is likely affected by genetic variability in the expression and activity of the enzymes responsible for their activation and detoxification. Findings from several studies that have investigated interactions between meat compounds, genetic polymorphisms, and colorectal polyps (36), adenomas (28,37), or cancer (38,39) have been inconsistent, which may be due to insufficient statistical power to examine these diet-gene interactions. The investigation of interactions between multiple genetic polymorphisms and meat exposures in relation to colorectal cancer risk is underway in our study population.

In summary, our findings underscore the importance of collecting detailed meat cooking and consumption data to allow for the study of specific mechanisms involved in colon and rectal carcinogenesis. Results from the present study support the hypothesis that increased exposure to HCAs, PAHs, nitrites, and nitrates is a plausible mechanism by which red and processed meat may increase colorectal cancer risk. Our sub-site analyses indicate that associations between meat-derived exposures and colon and rectal cancer may differ, which highlights the need for

additional studies that examine dietary risk factors for colon and rectal cancer as separate endpoints.

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Table 1. Descriptive characteristics of cases and controls

Characteristic	Cases (n = 415)		Controls (n = 726)		P-Value[a]
	No.	%	No.	%	
Age, years					
<50	39	9.4	168	23.1	<0.001
50-59	86	20.7	193	26.6	
60-69	123	29.6	225	31.0	
≥70	167	40.2	140	19.3	
Sex					
Men	219	52.8	330	45.5	0.017
Women	196	47.2	396	54.6	
Race					
White	404	97.4	710	97.8	0.633
Non-white	11	2.6	16	2.2	
Education					
< High school	48	11.6	26	3.6	<0.001
High school/some college	266	64.1	459	63.2	
College graduate/advanced degree	101	24.3	241	33.2	
Body mass index[b]					
<25	91	21.9	213	29.3	<0.001
25-29.9	139	33.5	264	36.4	
≥30	185	44.6	249	34.3	
Smoking status					
Never	192	46.3	337	46.4	0.223
Former	184	44.3	282	38.8	
Current	39	9.4	107	14.7	
Past regular NSAID use[c]					
Yes	221	53.3	436	60.1	0.025
No	194	46.8	290	39.9	
Physical activity[d]					
<1 hour/week	313	75.4	448	61.7	<0.001
≥1 hour/week	102	24.6	278	38.3	
Family history of colorectal cancer					
Yes	67	16.1	85	11.7	0.034
No	348	83.9	641	88.3	

Multivitamin use					
Yes	206	49.6	393	54.1	0.144
No	209	50.4	333	45.9	
Calcium supplement use[e]					
Yes	109	26.3	232	32.0	0.043
No	306	73.7	494	68.0	
Vitamin D supplement use[f]					
Yes	116	28.0	247	34.0	0.034
No	299	72.1	479	66.0	

Abbreviations: NSAID, nonsteroidal anti-inflammatory drug

[a] *P*-values for differences in means were calculated with *t* tests and differences in proportions were calculated with χ^2 tests.

[b] Weight (kg)/height $(m)^2$.

[c] NSAID use is defined as ever having been a regular user ($\geq$ 3 time/week for at least 1 year prior to the interview for controls and diagnosis for cases).

[d] Physical activity defined as vigorous.

[e] Includes individual calcium and calcium/vitamin D combination supplements.

[f] Includes individual vitamin D and calcium/vitamin D combination supplements.

Table 2. Dietary intakes of cases and controls

Dietary variable	Cases (*n* = 415) Mean (SD)	Controls (*n* = 726) Mean (SD)	*P*-Value
Total energy intake, kcal/day	1,853 (851)	1,795 (772)	0.632
Alcohol, g/day	8.4 (24.5)	10.2 (26.4)	<0.001
Red meat, g/1,000 kcal	37.6 (26.4)	34.9 (25.0)	0.032
Processed meat, g/1,000 kcal	15.5 (15.4)	14.3 (14.9)	0.059
Fruit and vegetables, servings/1,000 kcal	3.2 (1.8)	3.4 (1.8)	0.066
Low-fat dairy products, servings/1,000 kcal	0.29 (0.43)	0.34 (0.44)	0.025
Whole grains, servings/1,000 kcal	0.61 (0.59)	0.66 (0.63)	0.292
Saturated fat, g/1,000 kcal	12.9 (3.3)	12.6 (3.6)	0.095
Fiber, g/1,000 kcal	9.3 (3.4)	9.8 (3.5)	0.047
Total folate, DFE[a]	798 (392)	827 (382)	0.142
Total vitamin D µg[a]	10.2 (7.2)	11.3 (8.2)	0.137
Total calcium, mg[a]	905 (435)	1,006 (512)	<0.001
Meat-derived nitrites, µg/1,000 kcal	121 (116)	112 (129)	0.156
Meat-derived nitrates, µg/1,000 kcal	229 (249)	201 (187)	0.046
Meat-derived nitrites and nitrates, µg/1,000 kcal	350 (333)	314 (293)	0.020
DiMeIQx, ng/1,000 kcal	1.6 (2.0)	1.4 (1.9)	0.021
MeIQx, ng/1,000 kcal	19.1 (23.1)	15.9 (20.1)	0.020
PhIP, ng/1,000 kcal	47.1 (78.7)	46.8 (65.9)	0.334
Benzo[a]pyrene, ng/1,000 kcal	10.3 (17.0)	10.4 (15.1)	0.085
Total mutagenic activity, revertant colonies/1,000 kcal	2,842 (3,899)	2,655 (3,318)	0.562

Abbreviations: DFE, dietary folate equivalents; DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; SD, standard deviation.

[a] Dietary intakes were energy-adjusted by the residual method and then combined with supplemental intake.

Table 3. Odds ratios of colon and rectal cancer by quartile of red and processed meat intake

	Quartile of intake[a]								*P*-Trend
	Q1[b]	Q2		Q3		Q4			
		OR	95% CI	OR	95% CI	OR	95% CI		
Red meat, g/1,000 kcal									
Colorectal cancer									
No. of cases	91	96		104		124			
Age- and sex-adjusted	1	1.01	0.70,1.46	1.26	0.87, 1.82	1.47	1.02, 2.12		0.017
Multivariate[c]	1	0.94	0.64, 1.38	1.18	0.80, 1.73	1.24	0.84, 1.81		0.159
Colon cancer									
No. of cases	63	74		65		85			
Age- and sex-adjusted	1	1.08	0.71, 1.65	1.18	0.76, 1.81	1.50	0.99, 2.28		0.041
Multivariate	1	1.01	0.65, 1.55	1.12	0.72, 1.75	1.30	0.84, 2.00		0.178
Rectal cancer									
No. of cases	28	22		39		39			
Age- and sex-adjusted	1	0.77	0.42, 1.39	1.38	0.81, 2.36	1.34	0.78, 2.30		0.110
Multivariate	1	0.70	0.38, 1.31	1.25	0.71, 2.18	1.07	0.61, 1.90		0.434
Processed meat, g/1,000 kcal									
Colorectal cancer									
No. of cases	88	90		123		114			
Age- and sex-adjusted	1	0.95	0.66, 1.39	1.36	0.94, 1.95	1.31	0.90, 1.91		0.088
Multivariate	1	0.92	0.62, 1.37	1.28	0.88, 1.88	1.17	0.79, 1.74		0.290
Colon cancer									
No. of cases	58	67		86		76			
Age- and sex-adjusted	1	1.02	0.65, 1.61	1.42	0.91, 2.20	1.31	0.83, 2.08		0.216
Multivariate	1	0.96	0.61, 1.49	1.31	0.85, 2.03	1.22	0.78, 1.93		0.148
Rectal cancer									
No. of cases	30	23		37		38			
Age- and sex-adjusted	1	0.72	0.40, 1.30	1.10	0.64, 1.89	1.13	0.65, 1.94		0.342
Multivariate	1	0.68	0.37, 1.25	0.96	0.54, 1.68	0.94	0.53, 1.67		0.724

[a] Quartiles of intake (g/1,000 kcal) were as follows: red meat, quartile 1 (< 17.0), quartile 2 (17.0-29.8), quartile 3 (29.9-47.3), quartile 4 (> 47.3); for processed meat, quartile 1 (< 5.1), quartile 2 (5.2-9.5), quartile 3 (9.6-19.0), quartile 4 (> 19.0).

[b] Referent quartile.

[c] ORs were adjusted for age, sex, total energy intake, body mass index, past regular NSAID use, and fruit and vegetable consumption.

Table 4. Odds ratios of colon and rectal cancer by quartile of meat-derived nitrite and nitrate intake in the study population

	Quartile of intake[a]								*P*-Trend
	Q1[b]	Q2		Q3		Q4			
		OR	95% CI	OR	95% CI	OR	95% CI		
Nitrites, µg/1,000 kcal									
Colorectal cancer									
No. of cases	91		92		106		126		
Age- and sex-adjusted	1	1.07	0.74, 1.56	1.11	0.77, 1.61	1.43	0.99, 2.06		0.038
Multivariate[c]	1	1.02	0.69, 1.51	1.04	0.71, 1.54	1.27	0.87, 1.86		0.153
Colon cancer									
No. of cases	63		65		75		84		
Age- and sex-adjusted	1	1.14	0.74, 1.76	1.10	0.72, 1.68	1.49	0.98, 2.27		0.058
Multivariate	1	1.09	0.70, 1.71	1.07	0.69, 1.67	1.35	0.87, 2.09		0.157
Rectal cancer									
No. of cases	28		27		31		42		
Age- and sex-adjusted	1	0.94	0.48, 1.67	0.99	0.56, 1.74	1.34	0.78, 2.30		0.165
Multivariate	1	0.85	0.47, 1.54	0.88	0.49, 1.57	1.12	0.64, 1.97		0.412
Nitrates, µg/1,000 kcal									
Colorectal cancer									
No. of cases	94		90		113		118		
Age- and sex-adjusted	1	0.91	0.71, 1.20	1.21	0.84, 1.74	1.30	0.90, 1.89		0.048
Multivariate	1	0.88	0.60, 1.29	1.16	0.79, 1.70	1.15	0.77, 1.69		0.218
Colon cancer									
No. of cases	63		68		76		80		
Age- and sex-adjusted	1	1.01	0.66, 1.55	1.20	0.79, 1.83	1.39	0.90, 2.13		0.081
Multivariate	1	0.99	0.54, 1.53	1.15	1.74, 1.80	1.25	0.80, 1.96		0.226
Rectal cancer									
No. of cases	31		22		37		38		
Age- and sex-adjusted	1	0.69	0.38, 1.25	1.14	0.67, 1.94	1.11	0.65, 1.91		0.256
Multivariate	1	0.64	0.35, 1.17	1.02	0.58, 1.78	0.91	0.52, 1.61		0.642
Nitrites plus nitrates, µg/1,000 kcal									
Colorectal cancer									
No. of cases	99		76		112		128		
Age- and sex-adjusted	1	0.74	0.51, 1.09	1.08	0.75, 1.56	1.35	0.94, 1.94		0.014
Multivariate	1	0.71	0.48, 1.06	1.03	0.70, 1.51	1.17	0.80, 1.72		0.098

Colon cancer								
No. of cases	67		59		73		88	
Age- and sex-adjusted	1	0.83	0.54, 1.28	1.05	0.69, 1.60	1.43	0.94, 2.17	0.024
Multivariate	1	0.81	0.52, 1.27	1.03	0.66, 1.60	1.28	0.82, 2.00	0.084
Rectal cancer								
No. of cases	32		17		39		40	
Age- and sex-adjusted	1	0.51	0.27, 0.96	1.10	0.65, 1.86	1.14	0.67, 1.94	0.177
Multivariate	1	0.47	0.25, 0.90	0.96	0.55, 1.68	0.91	0.52, 1.60	0.567

[a] Quartiles of intake (µg/1,000 kcal) were as follows: nitrites, quartile 1 (< 35), quartile 2 (35-72), quartile 3 (73-146), quartile 4 (> 146); nitrates, quartile 1 (< 82), quartile 2 (83-149), quartile 3 (150-260), quartile 4 (> 261); nitrites plus nitrates, quartile 1 (< 130), quartile 2 (130-224), quartile 3 (225-412), quartile 4 (> 412).

[b] Referent quartile.

[c] ORs were adjusted for age, sex, total energy intake, body mass index, past regular NSAID use, and fruit and vegetable consumption.

Table 5. Odds ratios of colon and rectal cancer by quartile of meat-derived mutagen intake in the study population

	Quartile of intake[a]								*P*-Trend
	Q1[b]	Q2		Q3		Q4			
		OR	95% CI	OR	95% CI	OR	95% CI		
DiMeIQx, ng/1,000 kcal									
Colorectal cancer									
No. of cases	89	101		100		125			
Age- and sex-adjusted	1	1.25	0.87, 1.81	1.13	0.78, 1.63	1.60	1.12, 2.29		0.015
Multivariate[c]	1	1.23	0.84, 1.79	1.14	0.78, 1.67	1.48	1.02, 2.14		0.057
Colon cancer									
No. of cases	64	72		70		81			
Age- and sex-adjusted	1	1.29	0.85, 1.95	1.12	0.73, 1.70	1.50	0.99, 2.27		0.091
Multivariate	1	1.30	0.84, 1.99	1.13	0.73, 1.73	1.40	0.92, 2.15		0.205
Rectal cancer									
No. of cases	25	29		30		44			
Age- and sex-adjusted	1	1.20	0.67, 2.14	1.17	0.66, 2.08	1.83	1.07, 3.14		0.019
Multivariate	1	1.16	0.64, 2.09	1.18	0.65, 2.12	1.74	1.00, 3.03		0.034
MeIQx, ng/1,000 kcal									
Colorectal cancer									
No. of cases	101	78		107		129			
Age- and sex-adjusted	1	0.83	0.57, 1.21	1.12	0.78, 1.60	1.35	0.95, 1.91		0.021
Multivariate	1	0.32	0.55, 1.21	1.15	0.80, 1.67	1.19	0.82, 1.71		0.135
Colon cancer									
No. of cases	74	57		57		86			
Age- and sex-adjusted	1	0.84	0.55, 1.28	1.00	0.66, 1.50	1.26	0.85, 1.88		0.100
Multivariate	1	0.85	0.55, 1.31	1.03	0.68, 1.57	1.12	0.74, 1.70		0.355
Rectal cancer									
No. of cases	27	31		37		43			
Age- and sex-adjusted	1	0.81	0.44, 1.50	1.38	0.80, 2.37	1.57	0.92, 2.66		0.027
Multivariate	1	0.76	0.41, 1.41	1.40	0.80, 2.44	1.37	0.79, 2.38		0.092
PhIP, ng/1,000 kcal									
Colorectal cancer									
No. of cases	116	84		107		108			
Age- and sex-adjusted	1	0.74	0.51, 1.06	1.08	0.76, 1.54	1.33	0.93, 1.90		0.016
Multivariate	1	0.72	0.49, 1.05	1.04	0.72, 1.49	1.27	0.87, 1.83		0.035

Colon cancer								
No. of cases	86		60		79		62	
Age- and sex-adjusted	1	0.72	0.48, 1.08	1.17	0.79, 1.74	1.12	0.74, 1.71	0.246
Multivariate	1	0.70	0.46, 1.07	1.14	0.76, 1.71	1.08	0.70, 1.66	0.327
Rectal cancer								
No. of cases	30		24		28		46	
Age- and sex-adjusted	1	0.78	0.44, 1.40	0.98	0.56, 1.71	1.84	1.09, 3.09	0.002
Multivariate	1	0.79	0.44, 1.43	0.92	0.52, 1.64	1.76	1.03, 3.01	0.004
benzo[a]pyrene, ng/1,000 kcal								
Colorectal cancer								
No. of cases	135		94		85		101	
Age- and sex-adjusted	1	0.87	0.61, 1.23	0.78	0.55, 1.12	1.12	0.79, 1.61	0.224
Multivariate	1	0.85	0.59, 1.21	0.82	0.57, 1.19	1.12	0.77, 1.61	0.236
Colon cancer								
No. of cases	101		65		60		61	
Age- and sex-adjusted	1	0.81	0.55, 1.20	0.77	0.52, 1.56	0.99	0.65, 1.49	0.674
Multivariate	1	0.80	0.53, 1.19	0.84	0.55, 1.27	1.02	0.67, 1.55	0.552
Rectal cancer								
No. of cases	34		29		25		40	
Age- and sex-adjusted	1	0.95	0.55, 1.64	0.79	0.45, 1.40	1.43	0.85, 2.42	0.070
Multivariate	1	0.90	0.51, 1.57	0.83	0.46, 1.47	1.40	0.82, 2.39	0.078
Total mutagenic activity, revertant colonies/1,000 kcal								
Colorectal cancer								
No. of cases	104		96		106		109	
Age- and sex-adjusted	1	0.94	0.66, 1.35	1.14	0.79, 1.62	1.33	0.93, 1.90	0.059
Multivariate	1	0.93	0.64, 1.34	1.11	0.77, 1.61	1.21	0.84, 1.76	0.183
Colon cancer								
No. of cases	79		68		73		67	
Age- and sex-adjusted	1	0.88	0.59, 1.32	1.07	0.71, 1.59	1.16	0.77, 1.75	0.319
Multivariate	1	0.89	0.57, 1.32	1.07	0.70, 1.61	1.05	0.69, 0.61	0.605
Rectal cancer								
No. of cases	25		28		33		42	
Age- and sex-adjusted	1	1.16	0.65, 2.07	1.35	0.77, 2.38	1.87	1.09, 3.23	0.015
Multivariate	1	1.11	0.61, 2.01	1.32	0.74, 2.36	1.75	1.00, 3.08	0.031

Abbreviations: DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine.

[a] Quartiles of intake (ng/1,000 kcal) were as follows: DiMeIQx, quartile 1 (< 0.27), quartile 2 (0.27-0.84), quartile 3 (0.85-1.83), quartile 4 (> 1.83); MeIQx, quartile 1 (< 4.8), quartile 2 (4.8-10.1), quartile 3 (10.2-20.0), quartile 4 (> 20.0); PhIP, quartile 1 (< 9.8), quartile 2 (9.8-21.7), quartile 3 (21.8-56.3), quartile 4 (> 56.3); benzo[a]pyrene, quartile 1 (< 0.5), quartile 2 (0.5-3.9), quartile 3 (4.0-13.3), quartile 4 (> 13.3); total mutagenic activity (revertant colonies/1,000 kcal), quartile 1 (< 758), quartile 2 (758-1701), quartile 3 (1702-3504), quartile 4 (> 3504).

[b] Referent quartile.

[c] ORs were adjusted for age, sex, total energy intake, body mass index, past regular NSAID use, and fruit and vegetable consumption.

Chapter 6

CONCLUSION

SUMMARY

A recent consensus report issued jointly by the World Cancer Research Fund (WCRF) and the American Institute for Cancer Research (AICR) concluded that the evidence to support a positive association between greater intakes of red and processed meat and colorectal cancer was convincing, but the evidence for specific meat components explaining these associations remained inconclusive (1). The report also stated that the inconsistent definitions used and the insufficient evidence produced in previous studies investigating dietary patterns prevented the expert panel from making a judgment regarding dietary patterns and colorectal cancer risk (1). We explored associations between both dietary patterns and specific meat components and colorectal cancer risk to identify modifiable risk factors in a high-risk population and to determine directions for future research efforts.

Colorectal cancer is a complex and multifactorial disease that likely develops from the combined and interactive influence of environmental factors such as diet and lifestyle and inherited and acquired genetic mutations. It is unlikely that the dietary components involved operate in isolation as colorectal carcinogenesis involves a number of biological mechanisms (2). The combined and potentially synergistic relationships among a number of individual dietary components can be examined collectively for associations with colorectal cancer by studying the totality of diet through dietary pattern analyses (3). Dietary pattern analyses may be able to overcome one of the most significant limitations in epidemiological investigations of individual dietary components by accounting for the multicollinearity of dietary intake variables.

The majority of studies to date have derived dietary patterns by one of two fundamentally different approaches: principle components analysis and diet-index methods. In the first study, we explored both approaches within the same population and examined whether dietary patterns

identified by these two distinct methods were associated with colorectal cancer risk. Our findings indicated that following the *Dietary Guidelines for Americans* (4) or a primarily plant-based dietary pattern that included low-fat dairy and fish was associated with a reduced risk of colorectal cancer. In contrast, a pattern representing a Western-style diet high in meat, fried and other white potatoes, high-fat dairy, sweets, and other high-fat and high-sugar food items was associated with an increased risk of colorectal cancer. These results confirmed our hypothesis and highlighted the importance of further examinations into individual dietary components to elucidate biological mechanisms underlying observed associations. Two components that were present in the higher-risk dietary pattern and absent in the protective diets in both men and women were red and processed meat. Findings from our pilot study suggested that the population in northeast and central Pennsylvania consumed red and processed meat more frequently compared to a nationally-representative sample of U.S. adults. These results (5) in combination with those from our investigation of dietary patterns (Chapter 3) supported previously observed associations between red and processed meat intake and colorectal cancer risk. Two hypotheses were generated from these findings and were subsequently tested in the second study presented in this dissertation.

Our main hypothesis in the second study was that greater intakes of processed meat-derived sodium nitrites and nitrates would be positively associated with an increased risk of colon and rectal cancer. *N*-nitroso compounds (NOCs), which are known animal carcinogens (6,7), can be generated exogenously (in meat) or endogenously (*in vivo*) from the nitrosation of meat-derived amines and amides by sodium nitrites, or nitrates reduced to nitrites by bacteria, that are added to meat items as preservatives or curing agents (8). A secondary hypothesis was that greater exposure to highly mutagenic heterocyclic amines (HCAs) and polycyclic aromatic

hydrocarbons (PAHs) arising from cooking meat well-done at high temperatures or over a direct flame would also be positively associated with an increased risk of colon and rectal cancer. Earlier epidemiological studies have examined exposure to HCAs and PAHs in relation to colorectal cancer risk (9-12), but previous studies have been limited in examining the effect of meat-derived NOC exposure due to a lack of detailed data on processed meat intake and NOC precursors in meat.

To overcome these methodological limitations, we modified a detailed and previously validated cooked and processed meat module (13) to reflect the meat consumption patterns of our population. This meat module, which is linked to a database of HCA and PAH compounds in variably cooked meat, was embedded within a validated comprehensive FFQ (14). We incorporated recently estimated values of processed meat-derived nitrites and nitrates (15) into a database that we tied to our FFQ. This study is the first to use the cooked and processed meat module in combination with the database of nitrite and nitrate values to examine associations with colon and rectal cancer risk.

Results from the second study suggested that greater intakes of meat-derived nitrites and nitrates may be a risk factor for colorectal cancer and that the increased risk of cancer associated with nitrite and nitrate intake may be more pronounced in the colon compared to the rectum. Our second study also provided evidence that the effect of increased exposure to meat-derived HCAs may differ between sub-sites of the large intestine, with risk estimates for rectal cancer of greater magnitude than those estimated for colon cancer. These observations of sub-site differences in risk factors, supported by evidence from previous studies (16,17), underscores the value in examining associations between meat-related compounds and sub-sites of the large intestine to improve upon our current understanding of underlying mechanisms. It is possible that the degree

of susceptibility to the effects of different potential carcinogens varies between these sites because bacterial content, rates of xenobiotic metabolism, transit time, morphology, enzymatic expression, and the level of different pro-carcinogenic DNA-adducts differ between the colon and rectum (17,18). Our findings suggest that the colon may be more susceptible to the harmful effects of nitrites compared to the rectum, which could be explained in part by the greater bacterial decarboxylation of amino acids into nitrosatable amines and amides, as well as the increased reduction of nitrates to nitrites that occurs in the colon due to its greater bacterial population (19). On the other hand, the carcinogenic effect of HCAs appeared stronger in the rectum compared to the colon. These findings have generated hypotheses to be tested in future diet-gene investigations. For example, previous research has indicated that *GSTM1* null genotypes, conferring reduced detoxification of compounds such as HCAs by the glutathione *S*-transferase (GST)M1 enzyme, are found with greater frequency in the rectum compared to the colon (20). Additional research is needed to corroborate our findings related to sub-site differences in associations with meat-derived compounds since our study was limited by the number of rectal cancer cases (n = 128).

LIMITATIONS

An essential consideration with epidemiological studies is that observations of diet-disease associations do not provide sufficient evidence to determine causality but rather should be interpreted in concert with findings from randomized controlled trials and mechanistic studies to understand complex diet-disease relationships. The multicollinearity of diet is a significant limitation in nutritional epidemiology and is not easily reconciled with traditional statistical adjustments. We examined dietary patterns derived by principle components analysis to overcome this limitation in our first study, but our ability to determine the independent effects of

specific meat variables in our second study was limited by the multicollinearity among various meat constituents and total energy intake. Measurement error associated with food frequency questionnaires (FFQs) may lead to non-differential misclassification of respondents into dietary exposure categories, thereby attenuating risk estimates. Measurement error is difficult to address, particularly in large, multi-site epidemiological studies where biomarkers of dietary exposure that could be used to improve estimations of the contribution of diet to cancer risk are challenging to obtain due to practical and economic constraints. We attempted to minimize the measurement error associated with dietary assessment in our study by using portion size visuals and colored photographs of six meat items often cooked by high-temperature methods (21), by carefully reviewing completed FFQs, and by adjusting for total energy intake in our multivariate regression models (22).

The case-control design used in this study is susceptible to recall bias; cases may report past diet differently than controls if certain dietary exposures, such as meat intake, are preconceived to be risk factors for cancer. Another limitation is that respondents reported diet for the past year (prior to diagnosis for cases), but long-term dietary exposure is likely important in the development of cancer. Dietary habits have been shown to be relatively stable over time, suggesting that a diet history questionnaire administered at one time point may reliably rank individuals according to long-term dietary intakes (23). Although the major dietary patterns identified in our first study are consistent with those generated in a number of other studies (24), the generalizability of our findings may be limited due to a fairly homogenous sample consisting of a predominately white and rural population. The comparatively small number of rectal cancer cases likely reduced our power to reach statistical significance in analyses stratified by tumor site in our second study, and it is possible that a larger sample would have provided more definitive

evidence for the observed associations. The inability to stratify by both tumor-site and gender was also a limitation of our study. Potential confounders were considered and included in our multivariate models, but residual or unknown confounding remains possible.

FUTURE DIRECTIONS

We explored both food-based dietary patterns and specific food constituents to better understand complex relationships between diet and colorectal cancer. Our findings support the complementary study of both overall dietary patterns and individual dietary components as cancer risk factors because each can serve a unique purpose in identifying areas to target for cancer prevention strategies as well as future research studies. Continued research efforts employing improved methodological approaches are warranted to evaluate the cumulative and likely interactive effects of numerous dietary exposures on colorectal cancer risk. One future direction is to explore the multidimensionality of diet through the use of new methods and statistical approaches, such as reduced rank regression (25) and classification tree analysis (26). These methods may provide additional insight into diet-cancer associations as well as new strategies for cancer prevention efforts. One possibility is to use reduced rank regression to determine combinations of food intake variables that explain the greatest amount of variation in a set of potential intermediate markers of colorectal cancer, such as aberrant crypt foci or diet-induced DNA adducts. This approach could identify high-risk individuals who may greatly benefit from dietary modification. Another opportunity in the area of dietary pattern analyses is to use classification tree analysis to evaluate multilevel interactions among dietary variables and other risk factors, which may help to identify important cancer pathways and multifaceted prevention strategies.

A very promising future research direction includes the examination of potential interactions between exposure to meat-derived mutagens and genetic polymorphisms. The degree to which greater consumption of specific meat compounds elevates the risk of colorectal cancer is likely affected by genetic variability in the expression and activity of the enzymes responsible for their activation and detoxification. The limited number of studies that have investigated interactions between processed meat and colorectal cancer thus far (27,28) have focused on single-gene polymorphisms involved in nitrosamine activation, but recent advances including the International HapMap Project and genome-wide association studies may allow for comprehensive examinations of the influence of interactive and synergistic relationships between multiple genetic polymorphisms and environmental exposures on colorectal cancer risk. Indeed, recent reports from genome-wide association studies provide evidence that combinations of genetic polymorphisms are likely involved in colorectal carcinogenesis (29-31). These studies remain exploratory in nature, but as the science progresses, evaluations of the interplay between genome-wide variation and dietary exposures may improve the precision of cancer risk estimations as well as generate new hypotheses to be tested in future studies.

Continued research efforts that explore approaches to reduce public exposure to potentially carcinogenic meat-derived compounds are warranted. Developing strategies that target broader environmental change as well as those that focus on individual behavior change may prove more effective than relying on one approach alone. A possible environmental-level change to investigate would be modification of current meat curing and processing methods. Research is needed to identify safe alternatives that mimic the desired effects of sodium nitrite, including its taste, color, flavor, and antimicrobial characteristics (32). The current replacements for synthetic nitrites and nitrates that are used on a voluntary basis by certain processed meat

manufacturers include naturally occurring nitrates found in vegetables (e.g., celery juice or beet extracts). The extent to which these substitutions reduce exposure to NOCs is unclear since these nitrates can be readily reduced to nitrite. If alternatives are discovered and determined safe by the FDA, a role for governmental regulation may be warranted. This environmental-level approach has the potential to positively impact public health without requiring individual behavioral change.

Interventions that focus on increasing public awareness and promoting behavior change to prevent colorectal cancer should also be explored. It is likely that some individuals will still opt to consume more red and processed meat than is recommended (1,4) and to fry, grill, and barbeque meats. Increasing awareness of simple practices such as pre-cooking meat in a microwave, marinating meat prior to grilling, increasing the distance between the meat and flame, and pairing processed meat items with antioxidant-rich foods may help reduce individual exposure to these compounds. The most effective strategies for achieving sustainable diet-related behavior change and creating a healthier food environment remain to be determined, and continued research in these two areas will be critically important in moving the field of cancer prevention research forward.

In conclusion, the research presented in this dissertation has provided valuable insight into specific meat compounds and dietary patterns related to colorectal cancer in a high-risk population of adults. Our findings have contributed to the knowledge base of diet-related risk factors for colorectal cancer and have elucidated distinct areas for future research efforts.

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EDUCATION

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HONORS AND AWARDS

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2009	American Dietetic Association Foundation Scholarship in Memory of Dora E. Colver
2008	Nutrition and Dietetics Alumni Society Travel Grant, The Pennsylvania State University
2007-2009	Student Competitive Research Travel Grant, The Pennsylvania State University
2006-2007	President, Nutrition Graduate Student Association, The Pennsylvania State University

SELECTED PEER-REVIEWED PUBLICATIONS

1. **Miller PE**, Lesko SM, Muscat JE, Lazarus P, and Hartman TJ. Dietary patterns and colorectal adenoma and cancer risk: a review of the epidemiological evidence. *Nutr Cancer* (In Press).
2. **Miller PE**, Lazarus P, Lesko SM, Muscat JE, Harper G, Cross AJ, Sinha R, Ryczak K, Escobar G, Mauger D, Hartman TJ. Diet index-based and empirically-derived dietary patterns are associated with colorectal cancer risk. *J Nutr* (Accepted).
3. **Miller PE**, Mitchell DC, Harala PL, Pettit JM, Smiciklas-Wright H, and Hartman TJ. Evaluation of the Healthy Eating Index-2005 calculated with the Nutrition Data System for Research (NDSR). *Public Health Nutr* (Accepted).
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12. Snyder DC, Sloane R, Haines PS, **Miller PE**, Clipp EC, Morey MC, Pieper C, Cohen H, and Demark-Wahnefried, W. The Diet Quality Index-Revised (DQI-R): a tool to promote and evaluate dietary change among older cancer survivors enrolled in a home-based intervention trial. *J Am Diet Assoc*, 2007: 107(9):1519-29.