TOTAL MAGNESIUM INTAKE AND COLORECTAL CANCER INCIDENCE IN MEN

By

Melissa Y. Wei

A THESIS

Presented to the Oregon Health & Science University

Department of Public Health & Preventive Medicine

and School of Medicine

in partial fulfillment of

the requirements for the degree of

Master of Public Health

May 2010

Department of Public Health and Preventive Medicine

School of Medicine

Oregon Health & Science University

CERTIFICATE OF APPROVAL

This is to certify that the Master's thesis of

Melissa Y. Wei

has been approved

[redacted]

[redacted]

Thesis Advisor

[redacted]

Thesis Advisor

[redacted]

Biostatistician

TABLE OF CONTENTS

List of Tables and Figures.....iii

List of Abbreviations.....v

Acknowledgments.....vi

Abstract.....viii

Introduction.....1
Clinical significance.....1
Role of magnesium in colorectal cancer.....2
Calcium and colorectal cancer.....6
Vitamin D and colorectal cancer.....7
Hyperinsulinemia and colorectal cancer.....8
Public health significance.....10

Hypothesis and Specific Aims.....11

Materials and Methods.....13
Study population.....13
Exposure definition and assessment.....13
Assessment of other dietary variables.....16
Assessment of non-dietary variables.....17
Colorectal cancer outcome definition and assessment.....18
Statistical analysis.....19
Sample size and power estimations.....20

Results.....21
Baseline characteristics by magnesium intake.....21
Cumulative updated magnesium intake and colorectal cancer incidence.....22
Baseline magnesium intake and colorectal cancer incidence.....24
Related covariates in the association between magnesium and colorectal cancer.....25
1) Calcium intake.....25
1a) Cox regression stratified by calcium intake.....25
1b) Joint effect of calcium (1000 mg) and magnesium in quintiles at baseline and cumulative update intakes.....26
1c) Joint effect of calcium and magnesium in tertiles at baseline and cumulative updated intakes.....27
1d) Calcium to magnesium ratio.....27

1e) Summary of calcium and magnesium results....28
2) Vitamin D predictor score....28
3) Body mass index....29
Summary of results....30

Discussion....30
Magnesium, calcium and colorectal cancer....32
Magnesium, vitamin D and colorectal cancer....35
Magnesium, BMI and colorectal cancer....37
Strengths and limitations....38
Future studies....41

Summary and Conclusions....42

References....43

Tables and Figures....48

LIST OF TABLES AND FIGURES

Table 1. Age-standardized baseline characteristics according to baseline quintiles of energy-adjusted total magnesium intake in the Health Professionals Follow-up Study of U.S. men, 1986.....48

Table 2a. Age and multivariate adjusted hazard ratios (95% confidence interval) of colorectal cancer according to quintiles of cumulative updated energy-adjusted total magnesium intake in the Health Professionals Follow-up Study of U.S. men, 1986-2004. 50

Table 2b. Age and multivariate adjusted hazard ratios (95% confidence interval) of colorectal cancer according to quintiles of cumulative updated energy-adjusted dietary magnesium intake in the Health Professionals Follow-up Study of U.S. men, 1986-2004. 52

Table 3. Age and multivariate adjusted hazard ratios (95% confidence interval) of colorectal cancer according to quintiles of baseline energy-adjusted total magnesium intake in the Health Professionals Follow-up Study of U.S. men, 1986.....54

Table 4. Multivariate adjusted hazard ratios (95% confidence intervals) of colorectal cancer subsites stratified by varying levels of calcium, vitamin D predictor score and body mass index and quintiles of cumulative updated energy-adjusted total magnesium intake in the Health Professionals Follow-up Study of U.S. men, 1986-2004.....56

Table 5a. Multivariate adjusted hazard ratios (95% confidence intervals) of site-specific colorectal cancer according to the joint effect of different levels of baseline energy-adjusted calcium and magnesium intake in the Health Professionals Follow-up Study of U.S. men, 1986.....59

Table 5b. Multivariate adjusted hazard ratios (95% confidence intervals) of site-specific colorectal cancer according to the joint effect of different levels of energy-adjusted cumulative updated calcium and magnesium intake in the Health Professionals Follow-up Study of U.S. men, 1986-2004.....60

Table 6a. Multivariate adjusted hazard ratios (95% confidence intervals) of site-specific colorectal cancer according to the joint effect of tertiles of baseline energy-adjusted calcium and magnesium intake in the Health Professionals Follow-up Study of U.S. men, 1986.....61

Table 6b. Multivariate adjusted hazard ratios (95% confidence intervals) of site-specific colorectal cancer according to the joint effect of tertiles of energy-adjusted cumulative updated calcium and magnesium intake in the Health Professionals Follow-up Study of U.S. men, 1986-2004.....61

Table 7a. Multivariate adjusted hazard ratios (95% confidence intervals) of site-specific cancer according to the ratio of energy-adjusted calcium to magnesium intake at baseline in the Health Professionals Follow-up Study of U.S. men, 1986.....62

Table 7b. Multivariate adjusted hazard ratios (95% confidence intervals) of site-specific cancer according to the ratio of energy-adjusted calcium to magnesium intake for baseline and cumulative updated intakes in the Health Professionals Follow-up Study of U.S. men, 1986-2004.....62

Figure 1. Fully adjusted hazard ratio of colorectal cancer by joint category of baseline calcium and magnesium intake in tertiles in the Health Professionals Follow-up Study of U.S. men, 1986.....63

LIST OF ABBREVIATIONS

$1,25(OH)_2D$ – 1,25-dihydroxy vitamin D
25(OH)D – 25-hydroxy vitamin D
ATP - adenosine triphosphate
BMI – body mass index
Ca^{2+} – calcium ion
CI – confidence interval
C-peptide - connecting peptide
HPFS – Health Professionals Follow-up Study
HR – hazard ratio
IP_3 – inositol trisphosphate
IGF-1 – insulin-like growth factor-1
IGFBP-3 – insulin-like growth factor binding protein-3
Mg^{2+} – magnesium ion
METs – metabolic equivalents
NHS – Nurses' Health Study
OR – odds ratio
RDA – Recommended Daily Allowance
SFFQ – semi-quantitative food frequency questionnaire
VDR – vitamin D receptor

ACKNOWLEDGEMENTS

Thesis Committee

Edward Giovannucci, MD, ScD, MPH, Professor of Nutrition and Epidemiology at the Harvard the School of Public Health and Associate Professor of Medicine at Harvard Medical School. Thank you for your continued mentorship and support these past since after college and throughout medical school. I admire and am inspired by your boundless knowledge of nutrition, epidemiology and disease and everything in between! Your encouragement and insightful discussion have been paramount to my research experience. I have enjoyed our vitamin D projects and am grateful for your continued support for my MPH thesis. Two World Series titles, one Super Bowl Championship, Isabel and Michael, a new office, Brown Sugar Café outings later…and more to come! I look forward to continued email exchanges, projects and meeting again.

Cynthia Morris, PhD, MPH, Vice Chair of Medical Informatics & Clinical Epidemiology and Professor of Medicine, Public Health and Preventative Medicine. Thank you for your patience, advice and support with the OCTRI OSLER program, this project and preparation for the defense.

Motomi Mori, PhD, Professor and head biostatistician for the OHSU Knight Cancer Institute. Thank you for meeting with me and our conversations on the results and implications.

David Lieberman, MD, Professor of Medicine and Chairman of the Division of Gastroenterology at OHSU/VAMC. I am grateful for connecting with you through your paper on vitamin D and adenoma and in person during MS2 small groups. Thank you for your support and encouragement.

Harvard School of Public Health, Depts. of Nutrition and Epidemiology

Lydia Liu – Thank you for your assistance and dedication with the SAS programming, and for answering all my questions in person and by email.

Kana Wu, MD PhD, my old office mate! Thank you for your friendship and assistance with the colorectal cancer files.

Health Professionals Follow-up Study Group – Thank you to the researchers and staff for their tremendous dedication to this cohort, data collection, follow-up and resulting studies. I am grateful to have worked with the HPFS data and appreciate feedback during the initial proposal meeting and approval for this project.

OHSU Department of Public Health & Preventive Medicine

John Stull, MD MPH, MD/MPH program director. Thank you for your mentorship, support and humor before and throughout my time at OHSU. I have fond memories of the annual kick-off potlucks at your home, stories of you and Carol meeting at the Forest Park track, cherry pies, epi I ("count" but also "do something!"), your expression upon receiving the pirates vs cowboys picture at our MS1 Thai lunch, epi II confounders, epi III (one of the best courses, culmination of MPH courses!) and added meaning to the

Macbeth "Tomorrow" sonnet. You have provided tremendous encouragement, from discussing my career goals in medicine and public health to helping me focus on completing this thesis ("no more reading, no more analysis!"…for now!). We'll keep in touch!

Donald Austin, MD MPH (chronic disease epi and epi III were the best! Thank you for the monkey poem!), *William Lambert, PhD* (Thank you for your feedback on my proposal during the research design course), *Katie Riley, EdD, Rochelle Fu, PhD, Thuan Nguyen, MD PhD, Jodi Lapidus, PhD, Mike Laserav, William Morton, MD DrPH.* Thank you all for your dedication to the MPH program and for your knowledge and inspiration during courses.

Tree Triano and Staff. Thank you for fostering a close community and keeping our program running smoothly!

MD/MPH cohorts, MPH classmates and friends. The past five years would never be the same without your humor, support, knowledge, friendship, and crazy good times (from Pre to pirates and penguins!)! I look forward to keeping in touch and learning of all your adventures personally and professionally!

Funding

NIH National Center for Research Resources
Oregon Clinical & Translational Research Institute (OCTRI), Oregon Students Learn and Experience Research (OSLER) TL1 grant
OHSU School of Medicine Dean's Office
Robert L. Bacon Medical Education Enrichment Fund

Finally, I would like to thank my mother, father and brother Michael for their unwavering love, support, humor, advice, and confidence in me the past 26 years for all my career and life pursuits. You are my role models! I am grateful we have remained close despite the geographic distance. Thank you for listening, advising, and making me laugh when I needed it most! This thesis is but one of many endeavors for which you have provided paramount support…and hopefully more to come! From Urbana to Chicago to Ithaca to Boston to Portland and now onward to Atlanta! Much love, health and happiness.

ABSTRACT

Background: Prior studies report an inverse association between high dietary magnesium and colorectal cancer incidence. This association may be stronger among overweight and obese individuals with probable insulin resistance. Whether magnesium has a primary role or acts in tandem with closely related covariates remains to be elucidated. This study examines magnesium intake for primary and secondary roles through calcium intake, vitamin D status, and body mass index as a proxy for insulin sensitivity.

Methods: Population-based prospective cohort study of men (Health Professionals Follow-up Study, n = 51,529, aged 40-75 in 1986) without previous diagnosis of cancer at baseline was followed through 2004. Baseline and cumulative-updated total energy-adjusted magnesium intake were analyzed; baseline data accounted for the latency period between the protective exposure and development of colorectal cancer while cumulative updated intake enabled multiple dietary measurements and minimized potential for exposure misclassification. Cox proportional hazards modeling was used to evaluate the effect of magnesium intake adjusting for age and potential time-dependent confounders.

Results: Adjusted hazard ratios (95% confidence interval) of colorectal cancer according to quintiles of baseline energy-adjusted total magnesium intake demonstrated no association (adjusted hazard ratio, HR 0.97, 95% CI: 0.78-1.21; p for trend = 0.65). However, there was a significant interaction between baseline calcium and magnesium intake (p = 0.02). There was no significant effect modification by vitamin D status or BMI.

Conclusions: This study is the first to examine potential roles of calcium and vitamin D in the association between magnesium and colorectal cancer. Calcium and magnesium

intake are intricately related, and the balance of these intakes is as important, if not more, than the absolute quantity of magnesium consumed for prevention of colorectal cancer. Further studies would be necessary to support dietary or supplemental magnesium as a simple and cost-effective public health intervention for prevention of colorectal cancer.

INTRODUCTION

Clinical significance

Colorectal cancer is the second leading cause of cancer-related mortality for men and women in the U.S. and Western countries. In 2009 an estimated 9% of cancer deaths were due to colorectal cancer [1]. Incidence has been consistently high among U.S. adults; in 2009 10% of cancers occurred in the colon and rectum. The lifetime risk of developing colorectal cancer is approximately 7% in the U.S.

Globally, colorectal cancer incidence varies 10-fold, with the highest rates in North America, Australia, and northern and western Europe, and lower rates in developing countries [2]. These geographic differences appear attributable to differences in dietary and environmental exposures superimposed on genetically determined susceptibility.

Fortunately this incidence can be reduced with improved screening, monitoring, and lifestyle modification. Compared with other cancers, colorectal cancer ranks second to prostate cancer in cancers modifiable by lifestyle factors. The point estimate and range for colorectal cancers preventable by dietary modification alone were 70% (50-80%) [3, 4]. Factors that increase colorectal cancer risk include age, family history of sporadic cancer or adenomatous polyps, personal history of adenomatous polyps (particularly villous or tubulovillous histology), inflammatory bowel disease, diabetes, obesity, cigarette smoking, alcohol consumption (especially > 45 g/day), and intake of red meat and processed meat [5]. Modifiable factors include screening colonoscopies, regular physical activity, regular use of aspirin or non-steroidal anti-inflammatory drugs, chemoprevention, and dietary factors including folic acid, vitamin B_6, calcium and

vitamin D intake [5].

Role of magnesium in colorectal cancer

Magnesium is the fourth most abundant intracellular cation in the human body (after calcium Ca^{2+}, potassium K^{+}, and sodium Na^{+}). With its strict octahedral symmetry it forms relatively stable complexes with highly charged anions including organic polyphosphates, nucleic acids and some carboxylates. Through enzyme-substrate interactions and stabilization of polymers it facilitates a wide range of physiologic functions including nucleic acid metabolism, DNA repair, gene expression, protein synthesis, and energy production. DNA repair in particular is crucial for genomic stability and prevention of carcinogenic mutations [6, 7].

A review of the literature indicates that while magnesium absorption occurs throughout the intestine, the predominant site is the distal small intestine. Magnesium is absorbed into the intestine through passive diffusion, solvent drag, and active transport, with the majority through passive diffusion through the paracellular pathway. A saturable component has been demonstrated in human and animal studies. Magnesium is then actively transported in the descending colon [8, 9]. Plasma magnesium constitutes 1% of magnesium in the body and is tightly regulated. Plasma levels do not change until extreme deficiency or excess ensues. Bone constitutes two-thirds of total body magnesium and serves as a quickly mobilized protective pool to prevent serious systemic effects from magnesium deficiency.

Magnesium has been shown to inhibit the growth of colon tumors in animal studies. It has been hypothesized to inhibit *c-myc* expression and ornithine decarboxylase

activity in the epithelium of intestinal mucosa. Dietary magnesium hydroxide suppressed the proliferation of carcinogen-induced epithelial cells in the cecum and proximal and distal colon in mice [10]. Magnesium also reduced the exposure of cocarcinogenic bile acids to the distal and proximal colon [11]. Recent reviews of *in vitro* and *in vivo* data support a role for magnesium in multiple steps of carcinogenesis including neoplastic transformation, tumor growth, tumor progression, and pharmacologic treatment [12, 13].

Magnesium has been examined for its independent effects in the prevention of colorectal cancer in human ecologic and prospective cohort studies. Ecological magnesium levels in drinking water were examined in relation to colon cancer deaths and rectal cancer deaths in two matched case-control studies, but adjusted odds ratios did not yield statistically significant relations for either colon [14] or rectal [15] cancer deaths. However, both studies reported statistically significant inverse dose-response relationships between calcium levels and colon (OR = 0.58, 95% CI: 0.47-0.73) and rectal (OR = 0.63, 95% CI: 0.45-0.87) cancer death.

To date, total magnesium intake and colorectal cancer incidence has been examined in five prospective human cohort studies (three of women and two of both sexes) [16-20]. Four found inverse associations for colon cancer including two statistically significant [18, 20], one not statistically significant [16], and one restricted to overweight men and women [19]. Two studies reported no association with colon cancer in women [17, 18]. For rectal cancer, one study reported a significant inverse association with high magnesium intake [16] while four reported no association [17-20].

After 17 years of follow-up, the Iowa Women's Health Study of 35,196 women reported hazard ratios of 1.00, 0.96, 0.83, 0.80 across quintiles of total magnesium intake

(mean intake 302 mg/day, including 289 mg/day dietary and 13 mg/day supplemental) after adjusting for age, energy, other nutrients and risk factors for colorectal cancer. There was an inverse association for colon (hazard ratios across quintiles 1.00, 1.00, 0.88, 0.85 and 0.77; p for trend = 0.04) but not rectal cancer. The adjusted hazard ratio was even stronger in diabetic (0.70, 95% CI not provided) versus non-diabetic (HR 0.92, 95% CI not provided) women although the interaction was not statistically significant ($p > 0.05$) [20].

The Swedish Mammography Cohort of 61,433 women aged 40-75 years reported a 41% reduction in colorectal cancer incidence for the highest (≥ 255 mg/day) versus lowest (< 209 mg/day) quintiles of total magnesium intake after 14.8 years of follow-up and 805 incident colorectal cancer cases (adjusted HR = 0.59, 95% CI: 0.40-0.87, p trend = 0.006) [16]. As a continuous variable, an incremental 50 mg increase in magnesium daily correlated with an adjusted hazard ratio of 0.78 (95% CI: 0.62-0.99) for colorectal cancer. Results were significant for rectal (HR 0.59, 95% CI: 0.40-0.87, p trend = 0.02) but not colon (HR 0.66, 95% CI: 0.41-1.07, p trend = 0.08) cancer, with a similar magnitude of effect at proximal and distal colon sites.

A third study on women, the Women's Health Initiative, reported no relationship between total magnesium intake (mean intake 338 mg/day, including 323 mg/day dietary and 14 mg/day supplemental) in quartiles and incidence of colorectal cancer after 11 years of follow-up and 259 cases (HR 0.97, 95% CI: 0.63-1.49) [17]. Neither hazard ratios for colon (HR 1.04, 95% CI: 0.64-1.69) nor rectal (HR 0.65, 95% CI: 0.24-1.78) cancer was significant. The cohort was comprised of 38,345 women at least 45 years of age after exclusion for women with cancer at the time of enrollment or without data

available for magnesium intake and colorectal risk factors.

The Netherlands Cohort Study on Diet and Cancer is one of two studies that included men (n = 58,297 men, n = 62,573 women). The mean energy-adjusted magnesium intake was 332 mg/day for men and 292 mg/day for women. The authors reported similar and non-statistically significant inverse relations for colorectal cancer risk and magnesium intake among men (HR 0.91, 95% CI: 0.62-1.35; p for trend = 0.50) and women (HR 0.89, 95% CI: 0.59-1.35; p for trend = 0.77) after 13 years of follow-up and 1380 male and 948 female colorectal cancer cases [19]. However, a statistically significant inverse dose-response trend was found for increasing quintiles of total magnesium intake and colon but not rectal cancer risk among overweight subjects (HRs across quintiles 1.0, 0.72, 0.69, 0.60 and 0.67; p for trend = 0.05). Among overweight subjects, findings was significant for proximal (HRs across quintiles 1.0, 0.69, 0.65, 0.48 and 0.54; p for trend = 0.02) but not distal colon cancer (p for trend = 0.98).

Most recently, Ma et al. examined magnesium intake in the Japan Public Health Center-based Prospective Study of 40,830 men and 46,287 women followed for 7.9 and 8.3 years, respectively [18]. Mean magnesium intake was 284 mg/day for men and 279 mg/day for women. This was the only cohort to adjust for diabetes status, as diabetes was a risk factor for colon cancer and more prevalent among higher magnesium consumers in this cohort. Significant inverse associations were reported for colon (HR 0.48, 95% CI: 0.26-0.89; p for trend = 0.01), distal colon (HR 0.43, 95% CI: 0.19-0.95; p for trend = 0.02), invasive colon (HR 0.44, 95% CI: 0.21-0.92; p for trend = 0.02), and colorectal cancer (HR 0.65, 95% CI: 0.40-1.03; p for trend = 0.04) for the highest vs. lowest quintile of magnesium intake among men but not women. Individuals in this cohort were

leaner than individuals in Western cohorts. Stratification by BMI < 25kg/m^2 was non-significantly inversely associated with colorectal cancer for men with the highest compared with lowest tertile of magnesium intake (HR 0.68, 95% CI: 0.46-1.04; p for trend = 0.06). There was no evidence of effect modification by regular alcohol consumption (≥ 300 g/week) or smoking status in men or women.

Examining the magnesium and colorectal cancer association in a large population-based cohort of U.S. men (Health Professionals Follow-up Study, HFPS) will enable corroboration with prior cohort studies. The HPFS cohort has an extensive and longer follow-up period for dietary intake, non-dietary exposures, and colorectal cancer outcomes compared to prior studies. Further, with mixed findings in the two prior studies of men, examination using the HPFS cohort of 51,529 men will help contribute to accumulating evidence of an association and any differences by sex. This study further examines possible heterogeneity across other population characteristics.

Calcium and colorectal cancer

There are close physiologic relationships between calcium and magnesium; among them, Ca^{2+} and Mg^{2+} share ion channels [21]. Studies also suggest calcium may directly or indirectly compete with magnesium for intestinal absorption and transport [9], and that ionized magnesium counters [22] or has a negative feedback effect on ionized calcium in many physiologic activities [21, 23]. Higher Mg^{2+} concentrations may also decrease Ca^{2+} by two mechanisms: 1) Noncompetitive inhibition of IP_3 binding to its receptor, and 2) inhibited release of Ca^{2+} through IP_3-gated channels [24].

Several studies have implicated a protective effect of calcium in colorectal cancer.

In the HPFS and Nurses' Health Study (NHS) there was an inverse association between higher total calcium intake (> 1250 mg/day versus ≤ 500 mg/day) and distal colon cancer: pooled HR = 0.65 (95% CI: 0.43-0.98). This association was not reported for proximal colon cancer [25]. Cohort studies on the association between magnesium intake and colorectal cancer incidence either did not examine or specifically report results for effect modification by calcium. We will thus explore whether magnesium works in tandem or is inhibited by high calcium intake by examining both the ratio of calcium to magnesium (Ca:Mg) intake and the interaction between magnesium and calcium in relation to colorectal cancer incidence.

Vitamin D and colorectal cancer

Magnesium and vitamin D are also intricately associated, and several epidemiological studies provide evidence that vitamin D reduces the risk of colorectal cancer. A role for vitamin D in the prevention of colorectal cancer was first hypothesized in 1980 based on ecological patterns of colorectal cancer mortality by latitude as an indication of regional variation in solar radiation, which is required for vitamin D synthesis [26]. The vitamin D receptor (VDR) and the enzyme 1α-hydroxylase, which converts 25(OH)D to the active form $1,25(OH)_2D$ are expressed in the colon, rectum, and nearly all cell tissues [27, 28]. When activated by $1,25(OH)_2D$, the VDR is a transcription factor that has been demonstrated to decrease epithelial cell proliferation and to induce differentiation in colorectal cell cultures [29] and apoptosis in colorectal tumor and adenoma cell lines [30, 31].

We recently reported a significant inverse linear dose-response relationship for

both dietary vitamin D intake and circulating 25-hydroxyvitamin D with colorectal cancer risk. Individuals with vitamin D intake ≥ 1,000 IU/day or circulating 25(OH)D ≥ 33 ng/ml had 50% lower incidence of colorectal cancer compared with reference values ($p < 0.0001$ and $p < 0.01$, respectively) [32]. In a pooled quantitative meta-analysis there was a 50% decreased risk of colorectal cancer with the highest quantile of serum 25(OH)D (≥ 33 ng/ml) compared with the lowest quantile (≤ 12 mg/ml) (OR = 0.49, 95% CI: 0.35-0.68) [33].

Most recently we confirmed these associations for colorectal adenomas, although with smaller magnitude: circulating 25(OH)D was inversely associated with colorectal adenomas with an OR = 0.70 (95% CI: 0.56-0.87) for high versus low circulating 25(OH)D, and the highest quintile of vitamin D intake conferred an 11% marginally decreased risk of colorectal adenomas compared with low vitamin D intake (OR = 0.89; 95% CI: 0.78-1.02) [34].

We investigated for potential effect modification by vitamin D status through the vitamin D predictor score, which is based on multiple determinants of vitamin D exposure including dietary and supplementary vitamin D, skin pigmentation, adiposity, geographic residence, and leisure-time physical activity to estimate sunlight exposure [35].

Hyperinsulinemia and colorectal cancer

Hyperinsulinemia has been hypothesized as the biological mechanism for the well-established associations between obesity, physical inactivity, highly processed and refined diet, and increased risk for colorectal cancer [36] and precursor adenomas [37].

Colorectal cancer risk is elevated in individuals with higher levels of insulin, circulating C-peptide (a marker of insulin secretion), insulin-like growth factor (IGF)-1, and IGF-1/IGF binding protein (IGFBP)-3. Women with high levels of circulating C-peptide, a marker for hyperinsulinemia, had a non-statistically significant increased risk of colorectal cancer that was stronger for colon than rectal cancer and at the proximal site (adjusted HR = 2.62, 95% CI: 0.91-7.53, p for trend = 0.17). There was also an inverse association between increased (second through fourth quartiles) fasting levels of IGFBP-1 and colon cancer incidence (adjusted HR = 0.28, 95% CI: 0.11-0.75, p for trend = 0.05) [36].

Magnesium is implicated in central pathways of carbohydrate, lipid, and protein metabolism and in mitochondrial ATP synthesis. Magnesium is a necessary cofactor for several enzymes in glucose metabolism [38], and hypomagnesemia has been observed to negatively impact post-receptor insulin signaling in animals [39]. Magnesium deficiency has been shown to cause insulin hypersecretion in humans. For example, hypomagnesemia is a common occurrence among patients with type 2 diabetes, and it has been proposed as a risk factor for type 2 diabetes. A significant inverse association between magnesium intake and risk of type 2 diabetes was reported in the HPFS and NHS cohorts [40]. Magnesium supplementation has been shown to improve insulin sensitivity and glucose metabolism in short-term human metabolic studies [41-43] and randomized controlled trials [44]. Most recent support for a role of magnesium in hyperinsulinemia include findings that increased dietary magnesium intake decreased the incidence of gallstones, which may be facilitated by insulin hypersecretion [45].

Two studies linked magnesium, hyperinsulinemia, and colorectal cancer. The

Netherlands Cohort Study on Diet and Cancer reported a statistically significant inverse dose-response relationship with increasing energy-adjusted dietary magnesium intake and colon and proximal colon cancer among overweight subjects (p for trend = 0.05 and 0.02, respectively) [19]. The Iowa Women's Study also reported a stronger although not statistically significant hazard ratio of colon cancer among diabetic compared to non-diabetic women [20]. These results suggest a role for magnesium in decreasing insulin hypersecretion.

We hypothesize that magnesium may be acting through insulin resistance. This study examines whether the effect of higher magnesium intake is stronger among individuals with probable insulin resistance (overweight, obese).

Public health significance

Magnesium is ubiquitous in a variety of natural food sources; however, the content of magnesium in foods varies substantially. Sources with the highest magnesium content include green leafy vegetables, unpolished whole grains, nuts, legumes, and tofu. Magnesium is greatly diminished through processing [46]. Refined foods have the lowest magnesium content, and with greater consumption of highly processed foods, magnesium consumption has decreased in the U.S. and industrialized countries by half since 1900. Concomitantly, intake of micronutrients that increase magnesium requirements, including calcium, vitamin D and phosphorous, have been increasing [47].

Dietary intake levels are the best indicator of magnesium status; serum levels are tightly regulated and thus do not reflect adequate magnesium status. The mean estimated magnesium intakes for men and women in the U.S. are 323 mg/day and 228 mg/day,

respectively [48]. An estimated 80% of the U.S. population does not meet the Recommended Daily Allowance (RDA) for magnesium [49], established at 420 mg/day for adult men and 320 mg/day for adult women by the Institute of Medicine in 1997 [50].

Magnesium deficiency is associated with numerous human pathologies including gallstones [45], coronary heart disease [51], and various nervous and musculoskeletal system diseases. These associations may be primary or secondary to other factors intricately related to magnesium such as calcium, vitamin D, insulin action and glucose metabolism. Subsequent supplementation with magnesium has proven helpful in prevention or treatment of hypertension, atherosclerosis, heart failure, arrhythmias [52], and numerous diseases of the nervous and musculoskeletal systems [53-57].

The ultimate significance of this study is to help prevent colorectal cancer through modifiable risk factors. Findings from this study could potentially have direct implication for simple and inexpensive dietary intervention in the prevention of colorectal cancer. A dietary increase of 50 mg of magnesium can be obtained through one small serving of spinach, one banana, half a serving of beans, one serving of oatmeal, or two slices of whole grain bread. Magnesium supplementation could additionally be considered as a safe and efficient public health intervention for prevention of colorectal cancer.

HYPOTHESIS AND SPECIFIC AIMS

The goal of this study is to elucidate the role of magnesium in the prevention of colorectal cancer. Several hypotheses will be addressed to ascertain primary and secondary roles of magnesium in colorectal cancer prevention. We will determine the association between magnesium intake and colorectal cancer incidence using the

prospective Health Professionals Follow-up Study cohort of U.S. men. We will further examine whether there is an independent association or joint effects of magnesium with other dietary covariates, particularly calcium intake and vitamin D status. Finally, we will examine for effect modification by BMI as a marker for hyperinsulinemia. Hyperinsulinemia markers have been associated with increased colorectal cancer incidence, and we will examine whether magnesium attenuates this association. The culmination of these specific aims will help elucidate the role of magnesium independently and jointly with intimately linked covariates in colorectal cancer prevention.

Specific Aim #1: To examine the association between total magnesium intake and colorectal cancer incidence.

Specific Aim #2: To examine for effect modification by related covariates of magnesium in the association with colorectal cancer incidence.

2.A Calcium intake and ratio of calcium to magnesium (Ca:Mg) intake as effect modifiers of the magnesium and colorectal cancer association.

2.B Vitamin D status (vitamin D predictor score) is an effect modifier of the magnesium and colorectal cancer association.

Specific Aim #3: To examine for effect modification by markers of hyperinsulinemia (overweight, obese) in colorectal cancer incidence.

MATERIALS AND METHODS

Study population

The Health Professionals Follow-up Study (HPFS) is a population-based prospective cohort study comprised of 51,529 U.S. male health professionals aged 40-75 years when data collection began in 1986. All surviving cohort members receive questionnaires by mail regarding diet, medications, and medical history. Dietary assessment takes place biannually and includes a 131-item semi-quantitative food frequency questionnaire with intakes for magnesium, calcium, zinc, vitamin D, folic acid, vitamin B_6 and multivitamins. All other variables are obtained biennially to update information on exposures (anthropometric measurements, smoking status, colorectal cancer screening, aspirin and non-steroidal anti-inflammatory drug use, physical activity) and newly diagnosed conditions including colorectal cancer and adenoma.

The follow-up rate for biennial questionnaires has historically been greater than 94% for each 2-year follow-up cycle. Men with a diagnosis of cancer (except non-melanoma skin cancer) or familial adenomatous polyposis at baseline, incomplete data for diet, anthropometry or covariates, and implausible energy intakes (< 800 or > 4200 kcal/day) were excluded, leaving a subset of the study population followed from 1986 to 2004.

Exposure definition and assessment

The comprehensive exposure definition was baseline or cumulative-updated semi-quantitative food frequency questionnaire (SFFQ) measured total energy-adjusted magnesium intake in quintiles. Each feature will be described below.

Baseline or cumulative-updated refers to the time period magnesium intake was assessed. Given the latency period of potentially decades for colorectal cancer, total magnesium intake at baseline in 1986 was utilized as an exposure.

Cumulative updated intake was also utilized and provides the best assessment of long-term intake because it minimizes variation due to true dietary changes and measurement error. To help illustrate this point, nutrient data from the 1986 questionnaire was used for the 1986-1990 follow-up, and the average nutrient data from the 1986 and 1990 questionnaires was used for the 1990-1994 follow-up. Magnesium status is best indicated through measurements of intake rather than serum, which is tightly regulated and less reflective of long term status. This study analyzed total magnesium intake, which included dietary magnesium from food sources and supplemental magnesium from multivitamins and minerals. For the majority of participants, the predominant source of magnesium was dietary sources.

Magnesium intake information from HPFS was assessed through a validated SFFQ [58]. A full description of the SFFQ and procedures utilized to compute nutrient intake have been published previously [58, 59]. The current SFFQ includes 131 food items, vitamin and mineral supplements, and open-ended sections for other foods and supplements not specifically listed. Food items are highly specific, for example the exact breakfast cereal, multivitamin, margarine, and vegetable oil used for frying or baking. Participants were asked to indicate the average frequency of consumption of selected foods during the previous year. Respondents selected from nine options ranging from never or less than once per month to six or more times per day. Nutrient scores were computed by multiplying the frequency of intake of each food unit from the SFFQ by its

nutrient content according to food-composition tables from the Harvard Food Composition Database and the U.S. Department of Agriculture [60] that contain more than 130 nutrients. The SFFQ and databases are continually modified to reflect dietary trends over the 30-year period.

Reproducibility and validity of the SFFQ data were assessed in studies that compared multiple-week dietary records corrected for within-person variation in diet. The correlation coefficient for magnesium intake between two SFFQs was 0.69, between two diet records was 0.75, and between the SFFQ and dietary records on two measurements was 0.67 and 0.71 [58, 59].

Supplemental intake of magnesium was ascertained at baseline in 1986 and updated biennially. The frequency of intake and specific brand and type of multivitamins were recorded to compute the amount of supplemental magnesium. Total magnesium represents the sum of magnesium intake computed from the SFFQ and food-composition tables and supplemental sources.

Magnesium was energy-adjusted to account for the *a priori* biologic consideration that larger, more physically active individuals require higher caloric intake, which is associated with higher absolute intake of all nutrients. This same absolute intake may have a different effect on a smaller, less active person. Thus, adjusting for energy intake enables us to examine the composition of the diet independent of their energy requirements and utilization.

Participants were divided into five categories (quintiles) based on baseline or cumulative updated total magnesium intake adjusted for total energy intake using the residual method and multivariate nutrient densities for macronutrients [61]. Magnesium

was treated as a categorical variable given there is no known linear association between magnesium intake and reduced colorectal cancer risk. Quintiles were used since there is further no known cutoff or threshold effect whereby a certain level of magnesium intake is effective. The use of medians of quintiles is also less subject to influence by outliers. Finally, an individual's exact quantity of magnesium intake may be subject to measurement error but a range or quintile of magnesium intake can be approximated with high certainty.

Assessment of other dietary variables

The assessment of other dietary covariates was similar to magnesium: nutrient intake was computed based on the SFFQ, energy-adjusted and examined in quintiles of total intake. Nutrients measured by the SFFQ and detailed dietary records in a sub-sample of the cohort were correlated with an average of $r = 0.65$. Nutrients from the SFFQ have also been correlated with corresponding biochemical markers [58, 59].

Calcium was assessed at baseline and for cumulative updated intakes when examined as an effect modifier and as a cumulative updated intake when assessed as a confounder. Given greater knowledge of the effects of calcium and colorectal cancer and adenoma, calcium intake was divided into clinically significant categories listed in Table 2.

Vitamin D status is based on dietary intake and solar ultra-violet B radiation exposure. The vitamin D predictor score was computed based on six quantifiable determinants of vitamin D exposure including simple updated dietary and supplementary vitamin D intake, geographic residence, season, race as an indicator of skin pigmentation,

BMI, and leisure-time physical activity as a proxy for sunlight exposure. Details of the methods have been published previously [35]. In a reproducibility study the correlation between two predicted 25(OH)D levels (correlation coefficient 0.83) exceeded the correlation between two actual plasma 25(OH)D measurements (correlation coefficient 0.70) [58, 59]. Vitamin D intake was not available from dietary records but the predominant sources of dietary vitamin D were highly correlated with the FFQ (skim or lowfat milk, r = 0.88, whole milk, r = 0.67, cold breakfast cereal, r = 0.86, dark meat fish, r = 0.58) [62].

Assessment of non-dietary variables

This study hypothesized *a priori* that anthropometric measures, physical activity, aspirin intake, and race/ethnicity would be related to colorectal cancer. Data on several non-dietary exposures were collected biennially including current body weight, waist circumference, tobacco use, aspirin and medication use, screening practices including endoscopy, and physical activity (leisure time and vigorous). Variables were updated throughout follow-up and these time-varying covariates were utilized in the hazards models.

Aspirin use was assessed at baseline (1986) and followed biennially. Participants reported the average frequency and dose of tablets taken on each day, week or month. Low-dose aspirin (81 mg) was added to the questionnaire in 1996.

Race was assessed at baseline, and categorical variables have been created in HPFS. These data were additionally utilized to ascertain skin pigmentation for the vitamin D predictor score.

This study utilized proxies for serum data including BMI for insulin resistance and the vitamin D predictor score estimate of serum 25(OH)D. BMI and other anthropometric indices have been examined as markers for metabolic syndrome and hyperinsulinemia. BMI has been reported to have moderate correlation with the insulin resistance by homeostasis model assessment (HOMA-IR): age- and sex-adjusted partial correlation coefficient for men and women combined = 0.49 [63].

Physical activity was assessed through the average time per week spent engaging in each of eight groups of activity ranging from moderate to vigorous activity. In addition participants reported the number of flights of stairs climbed daily and usual walking pace. The reported time spent on each activity was multiplied by the typical energy expenditure required in metabolic equivalents (METs) [64]. For leisure-time and vigorous physical activity, participants reported the average hours per week spent doing specific activities. Each activity was weighted by intensity level, and this was used to estimate the cumulative average amount of physical activity of each participant.

The reproducibility and validity of self-reported physical activity and body weight in the HPFS cohort were reported previously [65-67]. The correlation coefficient between self-reported and measured body weight was r = 0.96 [66].

Colorectal cancer outcome definition and assessment

Colorectal cancer was the major outcome for this study. The eligible population at risk was composed of those without diagnosis of cancer (except non-melanoma skin cancer) or familial adenomatous polyposis at the beginning of each two-year follow-up interval, assessed through questionnaire biennially and confirmed with medical records

and pathology reports.

Participants (or next of kin for decedents) were sent questionnaires biennially that asked whether they had a diagnosis of colorectal cancer during the prior two years. Incident diagnoses were confirmed with medical records and pathology reports for participants who provided consent. A HPFS study physician (e.g. Edward Giovannucci, MD (pathology), ScD, MPH; Charles Fuchs, MD (oncology), MPH) reviewed all medical records related to colorectal cancer and extracted detailed information on tumor location.

For non-respondents, the National Death Index was searched for decedents and whether colorectal cancer was a primary contributor to death or secondary diagnosis. For a small number of cases where records were unavailable, state tumor registries were searched for specific histology and other data. These searches were conducted routinely following each biennial follow-up cycle.

Person-years of follow-up began on the date the baseline questionnaire was returned and ended on whichever date came first: date of diagnosis of colorectal cancer, date of death, or termination of the HPFS. Participants lost to follow-up were censored on the last confirmed date of their presence in the study.

Statistical analysis

All analyses will be performed using Statistical Analysis System software, release 9.2 (SAS Institute, Cary, NC, 2008). Univariate analyses were conducted using SAS procedures (proc means, proc univariate, proc corr, proc freq, proc glm). Baseline characteristics were age-standardized, and dietary covariates were adjusted for total energy intake with the residual model.

A multiplicative model was utilized. Using a conservative approach, all risk factors hypothesized *a priori* to have an association with colorectal cancer but not in the causal pathway were included as model covariates in the analyses. Risk factors discovered during the study were also added to the analyses. All covariates were treated as time-dependent variables using cumulative updating throughout cohort follow-up.

Age-adjusted models were examined initially and based on cumulative updated person-time. Non-dietary, followed by dietary covariates except calcium and vitamin D were added through Cox proportional hazards modeling [68, 69]. Calcium intake and the vitamin D predictor score were then added individually and simultaneously. The salient models examined are included in Table 2a.

The hazard ratio was analyzed according to strata of calcium intake, vitamin D predictor score and BMI to assess for potential effect modification of these covariates with magnesium intake. The strata are shown in Table 4.

Sample size and power estimations

This study used cases confirmed through 2004. Cases by sub-sites:

Subsite	N
Colon	667
Proximal colon	329
Distal colon	308
Rectum	215
Advanced	426
Colorectal	1013

For magnesium intake in quintiles, the power to detect a significant linear trend ($p < 0.05$) in risk across quintiles for the lowest versus highest quintile of intake was computed using the formula of Chapman and Nam [70]. For dichotomous exposure

classifications, statistical power was computed for a range of possible hazard ratios with 95% confidence intervals [71]. Based on the projected colorectal cancer cases, this study has a power of 80% to detect a hazard ratio of 1.25 and power of 90% to detect a hazard ratio of 1.30 between low versus high quintiles of magnesium intake.

RESULTS

After 18 years and 401,498 person-years of follow-up until December 2004, there were 1,013 colorectal, 667 colon, 329 proximal colon, 308 distal colon, 215 rectal and 426 advanced incident cancer cases documented. 7.2% of the cohort was excluded for reasons including missing magnesium or birth date information at baseline, implausible data for diet (e.g. energy intakes < 800 or > 4200 kcal/day) or other covariates, repeated data on a single individual, and diagnosis of cancer (except non-melanoma skin cancer) or death at baseline.

Baseline characteristics by magnesium intake

At baseline and for cumulative-updated averages, magnesium intake predominantly originated from dietary sources. At baseline the energy-adjusted dietary magnesium mean was 343 mg/day (standard deviation, SD 58.6 mg/day) and accounted for more than 96% of total magnesium intake (mean 354, SD 83.6 mg/day). Supplemental intake (mean 11.0, SD 31.6 mg/day) was uncommon in the population. 18% of individuals received some source of magnesium supplementation from either magnesium supplements or multivitamins, and among these individuals, the mean supplemental magnesium intake was 62.8 mg/day.

Cumulative updated magnesium intake was similar to baseline values: total (mean 351, SD 66.6 mg/day), dietary (mean 340, SD 58.6 mg/day), and supplemental mean intake 12.2, SD 27.2 mg/day).

Dietary sources of magnesium in the HPFS included a variety of food sources, with each contributing less than 7% of an individual's total magnesium intake. The top sources of magnesium were skim milk (6.8%), cold breakfast cereal (5.9%), coffee (4.5%), multivitamins (3%) and the following that each contributed less than 3% of total magnesium intake: beer, dark bread, orange juice, nuts and beef.

Given dietary sources predominated, the results presented will focus on total magnesium. The age-standardized baseline characteristics of the HPFS according to quintiles of energy-adjusted total magnesium intake are shown in Table 1. Men with a high intake of total magnesium engaged in longer and more intense physical activity (total physical activity and vigorous physical activity), were less likely to be current smokers and more likely to have never smoked or quit smoking, and were more likely to have received endoscopy screening and take multivitamins and aspirin. They were also slighter taller, had modestly smaller BMIs, and consumed less red meat, processed meat and saturated fat, but had higher consumption of fiber, folate, zinc, calcium, vitamin D, vitamin B_6 and vitamin B_{12}. There was no difference in waist to hip ratio, race/ethnicity, family history of colorectal cancer, alcohol consumption and total energy intake as magnesium consumption increased.

Cumulative updated magnesium intake and colorectal cancer incidence

There was a decreased risk of colorectal cancer in the age-adjusted model for

individuals in the highest compared with lowest quintile of total energy-adjusted magnesium intake (HR 0.76, 95% CI: 0.63-0.93; p for trend = 0.003) (Table 2a). Non-dietary covariates including BMI, height, race, vigorous physical activity, smoking status, alcohol consumption, family history of colorectal cancer, history of endoscopic screening and current aspirin use attenuated the association; the hazard ratio for men in the highest compared with lowest quintiles of intake was 0.90 (95% CI: 0.74-1.10; p for trend = 0.26). Following additional adjustment for dietary covariates (multivitamin use, total caloric intake, energy-adjusted intakes of red meat, folate and vitamin B_6) there was no association between colorectal cancer and total magnesium intake: HR 1.00 (95% CI: 0.78-1.26; p for trend = 0.92) for men in the highest versus lowest quintile of magnesium intake. Cumulative-updated energy-adjusted total calcium intake and vitamin D predictor score individually and simultaneously did not change the association (fully adjusted HR 1.02, 95% CI: 0.80-1.30; p for trend = 0.92). There was no association between colorectal cancer and the combined quintiles (2 through 5) of magnesium intake compared with the lowest quintile at baseline (HR 0.88, 95% CI: 0.74-1.04) and for cumulative updated intakes of magnesium (HR 0.98, 95% CI: 0.82-1.18).

Cumulative-updated total magnesium intake was neither associated with colon (HR 1.06, 95% CI: 0.78-1.44; p for trend = 0.64) nor rectal cancer (HR 1.08, 95% CI: 0.64-1.84; p for trend = 0.78) cancer for individuals with the highest versus lowest quintile of magnesium intake in the fully adjusted multivariate model (Table 2a). There was similarly no association for proximal colon (HR 1.17, 95% CI: 0.74-1.82; p for trend = 0.54) or distal colon cancer (HR 1.02, 95% CI: 0.65-1.59; p for trend = 0.93). Advanced cancers were not associated with total magnesium intake (HR 1.16, 95% CI:

0.79-1.70; p for trend = 0.70).

In addition to total magnesium intake, this study analyzed the dietary and supplemental magnesium intake and colorectal cancer subsites. Results for cumulative updated dietary and total magnesium intake were comparable for colorectal subsites except a nonsignificant inverse association for rectal cancer (HR 0.74, 95% CI: 0.47-1.18; p for trend = 0.12) and weak nonsignificant inverse association for colorectal cancer (HR 0.95, 95% CI: 0.76-1.18; p for trend = 0.56) for the highest compared with lowest quintiles of magnesium intake (Table 2b). There was no association between supplemental magnesium intake and colorectal subsites. Results hereafter will focus on total magnesium.

Baseline magnesium intake and colorectal cancer incidence

Given the latency period of possibly decades for colorectal cancer, total magnesium intake was also assessed at baseline. As with cumulative updated total magnesium intake, there was attenuation of the association with colorectal association with inclusion of additional covariates. Adjustment by age yielded a non-significant inverse association: HR 0.84 (95% CI: 0.66-1.06; p for trend = 0.07. In the full multivariate model there was no association between colorectal cancer incidence and magnesium for individuals with the highest compared with lowest quintiles of total intake (HR 0.97, 95% CI: 0.78-1.21; p for trend = 0.65) (Table 3).

Baseline magnesium intake was not associated with colon cancer, proximal and distal colon subsites, or advanced cancer (Table 3). However, in contrast with cumulative updated magnesium intake, baseline magnesium was nonsignficantly inversely associated

with rectal cancer: hazard ratio 0.85 (95% CI: 0.54-1.33; p for trend = 0.58).

Related covariates in the association between magnesium and colorectal cancer

To examine whether the association between total magnesium intake and colorectal cancer was modified by related covariates, stratified Cox regression analysis was used to examine potential effect modifiers including 1) calcium intake, 2) vitamin D status and 3) BMI.

1) Calcium intake

Examination of the effect of calcium was examined using stratified Cox regression analysis, joint analysis for the combined effect of calcium (1000 mg) and magnesium in quintiles, joint analysis for the combined effect of calcium and magnesium both in tertiles, and the ratio of calcium to magnesium intake as a continuous variable and in quintiles. For all analyses, baseline and cumulative updated intakes of calcium and magnesium were considered as well as all colorectal cancer subsites. A summary of the salient findings follows:

1a) Cox regression stratified by calcium intake

The association between magnesium intake and colorectal cancer varied by total calcium intake. Men with cumulative updated total calcium intake ≥1000 mg/day had a decreased risk of colorectal cancer, colon cancer, proximal colon cancer and rectal cancer with cumulative updated magnesium intake beyond the first quintile. There was a U-shaped association for increased cumulative updated magnesium intake and colorectal

cancer subsites, with the strongest inverse associations observed for the third quintile of magnesium intake (Table 4). Among the strongest associations observed was a significant 59% reduction in rectal cancer risk.

For men with calcium intake < 1000 mg/day at baseline or with cumulative updating, there was no association between total magnesium intake and colorectal cancer subsites (Table 4).

1b) Joint effect of calcium (1000 mg) and magnesium in quintiles at baseline and cumulative updated intakes

The joint effect of baseline total calcium intake ≥ 1000 mg/day and all levels of magnesium intake was inversely related to colorectal, colon and rectal cancer (Table 5a). There was no significant association between the joint effect of cumulative updated total calcium and magnesium intake and colorectal or colon cancer (Table 5b). There was a U-shaped association between colon cancer and the joint variable of cumulative updated calcium intake ≥ 1000mg/day total magnesium intake. For the lowest magnesium intake there was a non-significant increased risk of rectal cancer.

For the joint effect of baseline calcium < 1000 mg/day and magnesium intake, non-signficant U-shaped associations were observed with colorectal and colon cancer. For rectal cancer, a significant inverse association was observed with the joint effect of baseline calcium < 1000 mg/day and magnesium intake for the second through fourth quintiles compared with the first quintile: HR 0.58 (95% CI: 0.36-0.93), 0.68 (95% CI: 0.42-1.08), 0.48 (95% CI: 0.27-0.84), respectively (Table 5a).

1c) Joint effect of calcium and magnesium in tertiles at baseline and cumulative updated intakes

There was a significant interaction between baseline calcium and magnesium intake in tertiles in the association with colorectal cancer (p for interaction = 0.02) (Table 6a, Figure 1). The highest tertile of baseline calcium intake was inversely related with colorectal cancer for all ranges of magnesium intake (p for trend = 0.01), particularly at low to middle range intakes. There was a significant 36% decreased risk of colorectal cancer for men in the highest tertile of calcium and lowest tertile of magnesium (HR = 0.63, 95% CI: 0.45-0.88). Low and middle range baseline calcium intakes were inversely related with colorectal cancer as magnesium intake increased. Thus, combinations of both high magnesium with low calcium and high calcium with low magnesium intake at baseline significantly reduced risk of colorectal cancer. For colon and rectal cancer the calcium-magnesium interaction terms were marginally and non-statistically significant, respectively.

The associations between cumulative updated tertiles of calcium and magnesium intake and colorectal cancer subsites were attenuated and not statistically significant (Table 6b).

1d) Calcium to magnesium ratio

The ratio of calcium intake to magnesium intake was examined as a continuous variable and in quintiles.

As a continuous variable, there was no association with colorectal or colon cancer (Table 7a). For rectal cancer, there was an inverse association with the continuous ratio of

calcium to magnesium intake (HR 0.92, 95% CI: 0.81-1.05). The association was unchanged when calcium intake was also low (< 1000 mg/day) but stronger for individuals with calcium intake ≥ 1000 mg/day (HR 0.78, 95% CI: 0.76-1.22; p = 0.77).

Quintiles of the ratio of calcium to magnesium intake were not associated with colorectal cancer incidence for baseline or cumulative updated intakes (Table 7b).

1e) Summary of calcium and magnesium results

This study reports a significant interaction between calcium intake and magnesium intake in the association with colorectal cancer. Individuals with a combination of low calcium and high magnesium intake, or high calcium and low magnesium intake, or mid-range intakes of both conferred the greatest reduction in colorectal cancer risk.

2) Vitamin D predictor score

Cox regression hazard ratios stratified by low and high vitamin D predictor score did not suggest effect modification by vitamin D in the association between magnesium and colorectal cancer risk.

For individuals with a high vitamin D predictor score there was no statistically significant association between total magnesium intake and colorectal cancer (Table 4). There was a non-significant positive association between colon cancer and high magnesium intake: HR 1.22 (95% CI: 0.82-1.81) and 1.23 (95% CI: 0.80-1.87) for quintiles four and five of magnesium intake, respectively, compared with the first quintile. For rectal cancer there was an inverse association with magnesium intake for the

second through fifth quintiles compared with the first quintile: HR 0.75 (95% CI: 0.39-1.45), 0.65 (95% CI: 0.33-1.27), 0.89 (95% CI: 0.47-1.69) and 0.83 (95% CI: 0.41-1.65), respectively.

Among individuals with a low vitamin D predictor score, the association between magnesium intake and colorectal cancer was U-shaped: HR 1.11 (95% CI: 0.85-1.44), 0.85 (95% CI: 0.62-1.16), 0.86 (95% CI: 0.61-1.21), 0.91 (95% CI: 0.61-1.35) for the second through fourth quintiles, respectively, compared with the first quintile (Table 4). There was an inverse association between colon cancer and magnesium intake for the second through fourth quintiles of magnesium intake: HR 0.79 (95% CI: 0.54-1.17), 0.95 (95% CI: 0.63-1.43) and 0.81 (95% CI: 0.49-1.34), respectively. There was no association between rectal cancer and magnesium intake for individuals with a low vitamin D predictor score.

3) Body mass index

Stratified Cox regression was performed to examine for potential effect modification by BMI in the association between magnesium and colorectal cancer risk. Overall, there was no statistically significant evidence of effect modification for colorectal cancer subsites.

Among individuals with BMI $\geq$ 25 kg/m^2 there were non-significant inverse associations for the middle quintile but not higher doses of magnesium intake for colorectal (HR 0.87, 95% CI: 0.66-1.15), colon (HR 0.83, 95% CI: 0.59-1.17) and advanced (HR 0.79, 95% CI: 0.51-1.23) cancer (Table 4). For rectal cancer there were inverse associations for the third (HR 0.81, 95% CI: 0.43-1.53) and fourth (HR 0.85, 95%

CI: 0.44-1.66) quintiles of magnesium intake compared with the first quintile.

For individuals with BMI < 25 kg/m^2 there was a modest non-significant inverse association between colorectal cancer and increasing magnesium intake for the second through fourth quintiles compared with the first quintile. There was no association between magnesium and colon or rectal cancers for any level of magnesium intake. There was a non-significant positive association between rectal cancer and magnesium for all but the third quintile of intake.

Summary of results

This study demonstrates no significant association between total magnesium intake (baseline and cumulative updated intakes) and colorectal cancer. There was a significant interaction between baseline calcium and magnesium intake with colorectal cancer. There was no evidence of effect modification by vitamin D predictor score or BMI.

DISCUSSION

This large cohort study of U.S. men reports a non-significant inverse association between higher magnesium intake at baseline in 1986 and colorectal cancer after full adjustment for potential confounders. There was no association with cumulative updating of total magnesium intake after adjusting for age and other confounders of colorectal cancer. This study further examined the association according to subgroups of calcium intake, vitamin D status, and BMI to assess for potential interactions of these covariates with magnesium. To our knowledge this is the first study in humans to examine for

potential effect modification by calcium and vitamin D in the magnesium and colorectal cancer association.

Magnesium intake in the HPFS closely resembled magnesium intake in U.S. adult men (mean 323 mg/day), the majority of whom do not meet the RDA for magnesium intake (420 mg/day). While men in the highest quintile of total magnesium intake who met the RDA did not confer colorectal cancer risk reduction, our study found an inverse dose response relationship with rectal cancer and total magnesium intake through the fourth quintile. That medium-high but not the highest intake of magnesium was inversely related with rectal cancer suggests individuals with the highest intakes of magnesium differ from individuals with medium levels of magnesium intake in ways beyond dietary and non-dietary covariates adjusted for in the analyses. However, this finding is unlikely due to uncontrolled confounding, as failure to adjust for positive confounders would bias away from null.

These results may suggest the source of magnesium matters. While magnesium intake was predominantly from dietary sources, the quantity from supplemental sources increased as total magnesium intake increased. In particular, individuals in the fifth quintile of total magnesium received a greater quantity (mean 36.6 mg/day) but also proportion (7.6%) of their total magnesium from supplemental sources compared with individuals with lower total magnesium intake (supplements mean 0.32 to 10.7 mg/day and supplement proportion 0.32 to 2.8% for the first through the fourth quintiles) (Table 1). In the hazards models, magnesium supplements were adjusted for when examining dietary but not total magnesium, which would result in over-adjustment.

Prior studies on the relationship between magnesium and colorectal cancer have

been mixed. The Japan Prospective Study reported significant inverse associations with colon, distal colon and invasive colon cancer for men but not women. Total magnesium intake in the HPFS was greater than in the Japanese cohort (mean 284, SD 105, median quintiles 216-308). However, diets differ and the RDAs for magnesium in the U.S. are higher than Japan: 370, 350, and 310 mg/day for Japanese men aged 30-49, 50-69, and > 70 years, respectively. The Japanese cohort was the only study that adjusted for diabetes, which was a risk factor for colon cancer and more prevalent among higher magnesium consumers in the Japanese cohort. Controlling for diabetes status, a positive confounder, further strengthens the inverse association between magnesium intake and colon cancer reported by Ma et al.

The Netherlands Cohort reported a non-significant inverse association with colon cancer for men. Total magnesium intake was similar in the HPFS and Netherlands Cohort (mean 332, SD 58, median quintiles 264-401 mg/day), although a greater proportion of U.S. men received magnesium from supplements. The Iowa Women's Study, Women's Health Study, and Japan Public Health Center-based Prospective Study but neither the Swedish Mammography nor Netherlands Cohort studies adjusted for smoking status, a known risk factor for colorectal cancer and potential positive confounder that would over-estimate the true association if left unaccounted. All prior studies used single measurements of magnesium intake self-reported at baseline, which has potential for exposure misclassification that would bias the estimate toward null.

Magnesium, calcium and colorectal cancer

This study found a significant interaction between calcium and magnesium intake

in the association with colorectal cancer. Individuals with a combination of low calcium and high magnesium intake, or high calcium and low magnesium intake, or mid-range intakes of both conferred the greatest reduction in colorectal cancer risk. These findings allude to a few potential explanations for magnesium and calcium in relation to colorectal cancer prevention.

There is strong biologic plausibility for the calcium and magnesium interaction given the inverse relationship of the concentration of these cations in the plasma. Magnesium was once referred to as "nature's physiologic Ca^{2+} channel blocker" [22]. The release of ionized calcium (Ca^{2+}) from intracellular stores is inversely related to ionized magnesium (Mg^{2+}) concentrations. Mg^{2+} has been shown to decrease the inward Ca^{2+} flux through slow calcium channels and decrease transport of Ca^{2+} from the sarcoplasmic reticulum into the cytosol [72, 73]. In contrast, decreases in Mg^{2+} concentration result in increased intracellular Ca^{2+}. This may be due to uptake from extracellular calcium and release from intracellular calcium stores. Thus, there may be an inflection point at which high intakes of magnesium begin to interfere with the protective effects of calcium on colorectal cancer.

Alternatively, the absolute quantity of magnesium intake may not be as important as the balance with other protective factors, namely calcium. In this study, the ratio of calcium to magnesium intakes both as a continuous variable and in quintiles did not show an association with colorectal cancer risk. This study may not have been adequately powered to demonstrate a significant interaction. A prior study that examined the calcium to magnesium ratio in colorectal adenoma showed calcium intake was protective in individuals with a low calcium to magnesium intake ratio [74].

In understanding the balance between calcium and magnesium homeostasis it may be more informative to examine the relationship of intakes with corresponding serum levels, although this is unlikely to be achieved through human metabolic studies given calcium and magnesium are both tightly regulated. Further, at present there is no lab test to quantify Mg^{2+}.

Human cohort studies have suggested baseline calcium intake may be more important than magnesium intake in colorectal cancer risk reduction. The relationship between higher calcium intake and reduction of colorectal cancer and adenoma incidence has been well documented in the HPFS and NHS cohorts [25]. Randomized clinical trials have also demonstrated calcium (1200 mg) is protective against recurrent and large colorectal adenoma [75], suggesting calcium is protective in early stages of carcinogenesis. The Women's Health Initiative showed no effect for 1000 mg of calcium intake and 400 IU of vitamin D_3 on colorectal cancer after 7 years of follow-up [76] although this study was limited by inadequate dosing of vitamin D to affect a change in 25(OH)D, insufficient difference in intakes between cases and controls to detect a protective effect, and inadequate study duration for colorectal cancer latency.

Dietary magnesium and calcium intake were recently examined in relation to all-cause mortality, cancer mortality and cardiovascular disease mortality in the population-based Swedish cohort of men [77]. Dietary calcium intake was associated with a statistically lower rate of all-cause mortality and nonsignificant decreased rates of cancer and cardiovascular disease mortality while dietary magnesium intake was not inversely associated with all-cause, cancer or cardiovascular disease mortality. Dietary intakes of calcium and magnesium were high in the Swedish cohort.

Calcium intake has increased dramatically with increased supplementation while magnesium intake has decreased from 500 mg/day in the early 1900s to 175-225 mg/day at present [78]. Concomitant increased calcium intake and decreased magnesium intake have resulted in a shift toward a higher ratio of calcium to magnesium intake of 5:1 to 15:1 in the current U.S. diet compared with 1:1 in the Paleolithic diet [47]. Calcium has been demonstrated to be protective against colorectal cancer but further studies are necessary to determine the role of magnesium with calcium and perhaps ratio of calcium to magnesium intake to maximize colorectal cancer prevention.

Magnesium, vitamin D and colorectal cancer

As with calcium, vitamin D is also intricately linked with magnesium intake. Individuals with a high vitamin D predictor score had a non-statistically significant reduction in rectal cancer with increasing quintiles of magnesium intake although not in a linear dose relationship. For colon cancer, however, the findings were opposite; low vitamin D status was inversely associated with colon cancer for increasing quintiles of magnesium intake although the reduction was smaller and not statistically significant.

This is the first study to examine for effect modification by vitamin D in the magnesium and colorectal cancer association. However, Dai et al reported a decreased risk of colorectal adenoma in subjects who consumed high intakes of magnesium and dietary vitamin D and low ratio of calcium to magnesium: OR 0.32 (95% CI: 0.13-0.80) [74].

The relationship between vitamin D and magnesium is less clearly understood, although studies suggest magnesium absorption depends on vitamin D status. There are

vitamin D dependent and independent mechanisms for intestinal magnesium transport [8]. In rats with sufficient vitamin D, high [79, 80] but not low [81] doses of $1,25(OH)_2D_3$ increased magnesium absorption. In vitamin D deficient rats fed dietary magnesium, one study demonstrated increased intestinal absorption while another showed a net depression in magnesium absorption [82].

In humans, vitamin D has been shown to enhance magnesium absorption in normal subjects [80] and those with chronic renal failure [83]. Short-term metabolic studies of orally administered ergocalciferol, 25(OH)D or $1,25(OH)_2D_3$ for 1 to 6 months enhanced intestinal magnesium absorption in patients with various calcium or bone metabolism disorders; however, urinary magnesium excretion also increased such that there was no net change in mean magnesium balance [84]. In a similar study of high doses of oral cholecalciferol, urinary magnesium increased so overall magnesium balance was unaffected [85].

Other studies have shown high doses of vitamin D resulted in a net decrease in magnesium balance due to increased magnesium excretion [79, 86]. A limitation of short term human studies is that they may not have accounted for adaption to vitamin D, calcium and magnesium status, which likely occurs over months. Long-term human studies would help elucidate the effect of vitamin D on magnesium absorption, excretion and net balance and how the balance of these intricately linked micronutrients may help protect against colorectal cancer.

Cohort studies and meta-analyses have demonstrated vitamin D reduces colorectal cancer [32, 33] and adenoma risk [34]. This study did not suggest a significant interaction between magnesium and vitamin D with colorectal cancer.

Magnesium, BMI and colorectal cancer

Cox regression stratified analysis by the BMI cutoff of 25 kg/m^2 conferred no difference in colorectal risk. Overall there was no evidence of effect modification by BMI in the association between colorectal cancer subsites and magnesium for baseline and cumulative updated intakes.

Prior studies report a significant inverse association for colon cancer [19] and colorectal cancer [18] with increased magnesium intake among overweight and obese individuals. In the Netherlands Cohort Study there was a dose-response relationship through the fourth but not fifth quintile of magnesium intake although the highest magnesium intake was also significantly inversely related to colon and proximal colon cancer risk [19]. In the Japan Public Health Center-based Prospective Study, there was a non-significant inverse association between magnesium and colorectal cancer for men with BMI < 25 kg/m^2 but not women [18]. The Iowa Women's Study demonstrated a stronger inverse relationship between magnesium intake and colon cancer among diabetic women [20]. Magnesium intake was also examined in relation to pancreatic cancer in the HPFS, and a statistically significant inverse association was observed only among overweight men (HR 0.67, 95% CI: 0.46, 0.99) [87]. Given individuals with higher BMI have greater insulin resistance, these studies suggest magnesium may help improve insulin hypersensitivity.

Animal studies [39, 88], short-term human metabolic studies [41, 42], cohorts [40] and a meta-analysis [89] have implicated roles for magnesium in improving insulin secretion and glucose metabolism and reducing the incidence of type II diabetes.

Strengths and limitations

Strengths of this study include the large prospective cohort design, detailed assessment and consistently high follow-up of dietary and non-dietary characteristics, confirmed colorectal cases, reduced potential for reverse causation, and rigorous control for potential confounders in the analysis. In this prospective study, magnesium and other nutrient intake were reported prior to the diagnosis of colorectal cancer, thus minimizing the potential for recall bias. There was no pre-existing established association between magnesium intake and colorectal cancer risk that would motivate individuals with a predisposition to colorectal cancer (e.g. those with a family history of colorectal cancer) to alter their intake and reporting. Participation rates on questionnaires were consistently high, thus also minimizing the potential for selection bias from attrition. To minimize the potential for reverse causation, individuals who were diagnosed with cancer (except non melanoma skin cancer) or who died prior to 1986 were excluded from the analysis. All colorectal cancer cases were confirmed by pathology report.

Magnesium intake was measured at baseline and every four years throughout follow-up. Cumulative updated measurements are less subject to exposure misclassification compared with a single measurement. Repeated measurements also reflect dietary trends and long term dietary habits, and enabled us to capture these changes since baseline. In the HPFS there was high validity and reproducibility of the SFFQ for measuring magnesium intake and other dietary variables. All prior studies used baseline total magnesium intake, which has potential for exposure misclassification that would bias the estimate toward null. Given the latency period of possibly decades for colorectal cancer, baseline magnesium intake may serve as a better exposure than

cumulative updated intake and was thus also assessed as a primary exposure.

Potential confounders were carefully assessed and rigorously controlled for in the analysis. Dietary and non-dietary covariates were measured and averaged throughout follow-up, along with magnesium intake, and treated as time-dependent covariates in the Cox proportional hazards models. This HPFS, Iowa Women's Study and Women's Health Study but neither the Swedish Mammography nor Netherlands Cohort studies adjusted for smoking status, a known risk factor for colorectal cancer and potential positive confounder that would over-estimate the true association if left unaccounted.

This study was adequately powered to detect a difference in colorectal cancer risk based on magnesium intake. However, it is possible that the overall cohort was not at an adequate range for potential benefits of magnesium intake or at an ideal ratio of calcium to magnesium intake.

This study focused mainly on dietary magnesium intake. There was limited ability to analyze supplemental magnesium, as few individuals took supplements and the quantity of magnesium consumed and fraction of the total intake were generally low. The proportion of magnesium from dietary sources was similar to prior studies. In the Iowa Women's Health Study the mean magnesium intake was 302 mg/day, comprised of 289 mg/day from food sources and 13 mg/day from supplements and multivitamins [20], and studies by Lin [17] and van den Brandt [19] reported similar magnitude patterns of magnesium intake. Prior studies on magnesium supplementation have demonstrated improved insulin sensitivity [44] and glucose metabolism [43] but did not examine colorectal cancer as an endpoint.

Limitations of this study include the potential for exposure misclassification,

residual confounding, and use of proxies for serum measurements. Exposure misclassification cannot be excluded given individual nutrient intakes were computed based on self-administered questionnaire data. This would be nondifferential with respect to the outcome and bias toward the null association. Thus it is plausible that a true inverse association was attenuated. Dietary records with actual weighing of food consumed may be a superior measure of nutrient intake, and magnesium intake from the SFFQ was highly but not perfectly correlated with dietary records.

Self-administered questionnaire was used to capture other dietary and lifestyle factors, and the potential for residual confounding cannot be excluded. For categorical variables, strata were carefully determined using clinical reasoning. Unknown potential confounders warranting further consideration include phosphorous, zinc, free fatty acids and oxalate. High dietary levels of these dietary components may bind magnesium and decrease its biologic availability; however, to date there has been no established association of these dietary factors with colorectal cancer risk.

This study utilized proxies for serum data including BMI for insulin resistance and the vitamin D predictor score estimate of serum 25(OH)D. BMI has been moderately correlated with insulin resistance by homeostasis model assessment (HOMA-IR) [63]. Additional anthropometric measures that may predict glucose and insulin resistance disorders include the supine sagittal abdominal diameter and waist circumference. The vitamin D predictor score, computed from six quantifiable determinants of vitamin D exposure, was highly correlated with although not equivalent to actual 25(OH)D measures.

Finally, the exact latency period of colorectal cancer is unknown. This study

utilized magnesium at baseline and cumulative updated intakes. However, a lagged analysis at two-year intervals would enable greater precision in determining when the greatest protective effects from magnesium intake occur along the progression of colorectal carcinogenesis.

Future studies

Further studies are necessary to elucidate the role of dietary and supplemental magnesium and related covariates in colorectal cancer prevention. Findings from this study should be corroborated with other prospective cohorts such as the Nurses' Health Study of 126,699 women. Serum data are also available in the HPFS and NHS cohorts to analyze hyperinsulinemia markers and 25(OH)D in a nested case-control study.

Colorectal adenoma, with its shorter latency period and larger number of cases in the HPFS and NHS cohorts, should also be assessed. One prior study examined total magnesium intake and colorectal adenomas and reported a significant 46% reduced risk [74]. In particular, a large number of colorectal adenoma outcomes would increase power to examine for interactions between magnesium and related covariates.

Prior studies demonstrated significant and marginally significant inverse associations between total magnesium intake and colorectal cancer. In the HPFS and prior studies, supplemental magnesium was a small component of total magnesium intake. If findings of reduced colorectal cancer risk are corroborated by observational studies, a clinical trial would be necessary to examine whether magnesium supplements confer the same benefit as dietary sources.

SUMMARY AND CONCLUSIONS

This study does not provide support for increasing total magnesium intake for prevention of colorectal cancer. This association was not modified by vitamin D status or BMI. However, there was a significant interaction between baseline calcium and magnesium intake. Individuals with a combination of high calcium and low magnesium intake, low calcium and high magnesium intake, or mid-range intakes of both conferred the greatest colorectal cancer risk reduction.

The ideal quantity of magnesium intake for reduced risk of colorectal cancer remains to be elucidated. Further, the absolute quantity of magnesium intake may not be as important as the ratio of magnesium intake with related covariates, particularly calcium.

Magnesium intake in the HPFS cohort was representative of U.S. men, with an estimated 80% who do not meet the RDA for magnesium. In the past century, magnesium intake has decreased concomitantly with increased intakes of calcium, vitamin D, and phosphorus, which all potentially further decrease the bioavaibility of magnesium.

Regardless of the potential role for magnesium in reducing colorectal cancer risk, magnesium remains an essential macromineral involved in over 325 enzymatic functions with health benefits that span beyond cell cycle regulation and anticarcinogenesis. Numerous studies implicate a role for magnesium in glucose metabolism, cardiac and neuromuscular function, and bone and mineral metabolism. Magnesium deficiency has been associated with increased risk of human pathologies while magnesium supplementation has been helpful in the treatment or prevention of certain health outcomes, including growing evidence on specific cancers.

REFERENCES

1. American Cancer Society. *Cancer Statistics 2009.* 2009 [cited 2010 April]; Available from: http://www.cancer.org/docroot/PRO/content/PRO_1_1_ Cancer_Statistics_2009_Presentation.asp.
2. Bingham, S. and E. Riboli, *Diet and cancer--the European Prospective Investigation into Cancer and Nutrition.* Nat Rev Cancer, 2004. **4**(3): p. 206-15.
3. Nasca, P.C. and H. Pastides, *Fundamentals of cancer epidemiology.* 2nd ed. 2008, Sudbury, Mass.: Jones and Bartlett Publishers. xiv, 541 p.
4. Willett, W.C., *Diet, nutrition, and avoidable cancer.* Environ Health Perspect, 1995. **103 Suppl 8**: p. 165-70.
5. Wei, E.K., et al., *Comparison of risk factors for colon and rectal cancer.* Int J Cancer, 2004. **108**(3): p. 433-42.
6. Klungland, A., et al., *Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage.* Proc Natl Acad Sci U S A, 1999. **96**(23): p. 13300-5.
7. Stipanuk, M.H., *Biochemical and physiological aspects of human nutrition.* 2000, Philadelphia: W.B. Saunders. xxx, 1007 p.
8. Hardwick, L.L., et al., *Site and mechanism of intestinal magnesium absorption.* Miner Electrolyte Metab, 1990. **16**(2-3): p. 174-80.
9. Hardwick, L.L., et al., *Magnesium absorption: mechanisms and the influence of vitamin D, calcium and phosphate.* J Nutr, 1991. **121**(1): p. 13-23.
10. Mori, H., et al., *Preventive effect of magnesium hydroxide on carcinogen-induced large bowel carcinogenesis in rats.* Basic Life Sci, 1993. **61**: p. 111-8.
11. Wang, A., et al., *The inhibitory effect of magnesium hydroxide on the bile acid-induced cell proliferation of colon epithelium in rats with comparison to the action of calcium lactate.* Carcinogenesis, 1994. **15**(11): p. 2661-3.
12. Maier, J.A., et al., *Insights into the mechanisms involved in magnesium-dependent inhibition of primary tumor growth.* Nutr Cancer, 2007. **59**(2): p. 192-8.
13. Wolf, F.I., et al., *Magnesium and neoplasia: from carcinogenesis to tumor growth and progression or treatment.* Arch Biochem Biophys, 2007. **458**(1): p. 24-32.
14. Yang, C.Y., et al., *Calcium and magnesium in drinking water and risk of death from colon cancer.* Jpn J Cancer Res, 1997. **88**(10): p. 928-33.
15. Yang, C.Y. and H.F. Chiu, *Calcium and magnesium in drinking water and risk of death from rectal cancer.* Int J Cancer, 1998. **77**(4): p. 528-32.
16. Larsson, S.C., L. Bergkvist, and A. Wolk, *Magnesium intake in relation to risk of colorectal cancer in women.* Jama, 2005. **293**(1): p. 86-9.
17. Lin, J., et al., *Total magnesium intake and colorectal cancer incidence in women.* Cancer Epidemiol Biomarkers Prev, 2006. **15**(10): p. 2006-9.
18. Ma, E., et al., *High dietary intake of magnesium may decrease risk of colorectal cancer in Japanese men.* J Nutr, 2010. **140**(4): p. 779-85.
19. van den Brandt, P.A., et al., *Magnesium intake and colorectal cancer risk in the Netherlands Cohort Study.* Br J Cancer, 2007. **96**(3): p. 510-3.
20. Folsom, A.R. and C.P. Hong, *Magnesium intake and reduced risk of colon cancer in a prospective study of women.* Am J Epidemiol, 2006. **163**(3): p. 232-5.
21. Hoenderop, J.G. and R.J. Bindels, *Epithelial Ca2+ and Mg2+ channels in health and disease.* J Am Soc Nephrol, 2005. **16**(1): p. 15-26.

22. Iseri, L.T. and J.H. French, *Magnesium: nature's physiologic calcium blocker.* Am Heart J, 1984. **108**(1): p. 188-93.
23. Hoenderop, J.G., B. Nilius, and R.J. Bindels, *Molecular mechanism of active Ca2+ reabsorption in the distal nephron.* Annu Rev Physiol, 2002. **64**: p. 529-49.
24. Volpe, P., B.H. Alderson-Lang, and G.A. Nickols, *Regulation of inositol 1,4,5-trisphosphate-induced Ca2+ release. I. Effect of Mg2+.* Am J Physiol, 1990. **258**(6 Pt 1): p. C1077-85.
25. Wu, K., et al., *Calcium intake and risk of colon cancer in women and men.* J Natl Cancer Inst, 2002. **94**(6): p. 437-46.
26. Garland, C.F. and F.C. Garland, *Do sunlight and vitamin D reduce the likelihood of colon cancer?* Int J Epidemiol, 1980. **9**(3): p. 227-31.
27. Cross, H.S., et al., *Vitamin D metabolism in human colon adenocarcinoma-derived Caco-2 cells: expression of 25-hydroxyvitamin D3-1alpha-hydroxylase activity and regulation of side-chain metabolism.* J Steroid Biochem Mol Biol, 1997. **62**(1): p. 21-8.
28. Holt, P.R., et al., *Colonic epithelial cell proliferation decreases with increasing levels of serum 25-hydroxy vitamin D.* Cancer Epidemiol Biomarkers Prev, 2002. **11**(1): p. 113-9.
29. Xue, L., et al., *Influence of dietary calcium and vitamin D on diet-induced epithelial cell hyperproliferation in mice.* J Natl Cancer Inst, 1999. **91**(2): p. 176-81.
30. Diaz, G.D., et al., *Apoptosis is induced by the active metabolite of vitamin D3 and its analogue EB1089 in colorectal adenoma and carcinoma cells: possible implications for prevention and therapy.* Cancer Res, 2000. **60**(8): p. 2304-12.
31. Miller, E.A., et al., *Calcium, vitamin D, and apoptosis in the rectal epithelium.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(2): p. 525-8.
32. Gorham, E.D., et al., *Vitamin D and prevention of colorectal cancer.* J Steroid Biochem Mol Biol, 2005. **97**(1-2): p. 179-94.
33. Gorham, E.D., et al., *Optimal vitamin D status for colorectal cancer prevention: a quantitative meta analysis.* Am J Prev Med, 2007. **32**(3): p. 210-6.
34. Wei, M.Y., et al., *Vitamin D and prevention of colorectal adenoma: a meta-analysis.* Cancer Epidemiol Biomarkers Prev, 2008. **17**(11): p. 2958-69.
35. Giovannucci, E., et al., *Prospective study of predictors of vitamin D status and cancer incidence and mortality in men.* J Natl Cancer Inst, 2006. **98**(7): p. 451-9.
36. Wei, E.K., et al., *A prospective study of C-peptide, insulin-like growth factor-I, insulin-like growth factor binding protein-1, and the risk of colorectal cancer in women.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(4): p. 850-5.
37. Wei, E.K., et al., *C-peptide, insulin-like growth factor binding protein-1, glycosylated hemoglobin, and the risk of distal colorectal adenoma in women.* Cancer Epidemiol Biomarkers Prev, 2006. **15**(4): p. 750-5.
38. Paolisso, G., et al., *Magnesium and glucose homeostasis.* Diabetologia, 1990. **33**(9): p. 511-4.
39. Suarez, A., et al., *Impaired tyrosine-kinase activity of muscle insulin receptors from hypomagnesaemic rats.* Diabetologia, 1995. **38**(11): p. 1262-70.
40. Lopez-Ridaura, R., et al., *Magnesium intake and risk of type 2 diabetes in men and women.* Diabetes Care, 2004. **27**(1): p. 134-40.

41. Paolisso, G., et al., *Daily magnesium supplements improve glucose handling in elderly subjects.* Am J Clin Nutr, 1992. **55**(6): p. 1161-7.
42. de Valk, H.W., *Magnesium in diabetes mellitus.* Neth J Med, 1999. **54**(4): p. 139-46.
43. Paolisso, G., et al., *Changes in glucose turnover parameters and improvement of glucose oxidation after 4-week magnesium administration in elderly noninsulin-dependent (type II) diabetic patients.* J Clin Endocrinol Metab, 1994. **78**(6): p. 1510-4.
44. Rodriguez-Moran, M. and F. Guerrero-Romero, *Oral magnesium supplementation improves insulin sensitivity and metabolic control in type 2 diabetic subjects: a randomized double-blind controlled trial.* Diabetes Care, 2003. **26**(4): p. 1147-52.
45. Tsai, C.J., et al., *Long-term effect of magnesium consumption on the risk of symptomatic gallstone disease among men.* Am J Gastroenterol, 2008. **103**(2): p. 375-82.
46. Saris, N.E., et al., *Magnesium. An update on physiological, clinical and analytical aspects.* Clin Chim Acta, 2000. **294**(1-2): p. 1-26.
47. Dean, C., *The magnesium miracle : discover the essential nutrient that will lower therisk of heart disease, prevent stroke and obesity, treat diabetes, and improve mood and memory*. Ballantine books trade pbk. ed. 2007, New York: Ballantine Books. xxii, 309 p.
48. Cleveland, L.E., Goldman, J.D., and Borrdu, L.G., *Data Tables: Results from USDA's 1994 Continuing Survey of Food Intakes by Individuals and 1994 Diet and Health Knowledge Survey*. 1996, Agricultural Research Service, U.S. Department of Agriculture: Riverdale, MD.
49. Ervin, R.B., et al., *Dietary intake of selected minerals for the United States population: 1999-2000.* Adv Data, 2004(341): p. 1-5.
50. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, F.a.N.B., Institute of Medicine, *Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride*. 1997, Washington, D.C.: National Academy Press.
51. Al-Delaimy, W.K., et al., *Magnesium intake and risk of coronary heart disease among men.* J Am Coll Nutr, 2004. **23**(1): p. 63-70.
52. Zehender, M., et al., *Antiarrhythmic effects of increasing the daily intake of magnesium and potassium in patients with frequent ventricular arrhythmias. Magnesium in Cardiac Arrhythmias (MAGICA) Investigators.* J Am Coll Cardiol, 1997. **29**(5): p. 1028-34.
53. Ravn, H.B., T.L. Korsholm, and E. Falk, *Oral magnesium supplementation induces favorable antiatherogenic changes in ApoE-deficient mice.* Arterioscler Thromb Vasc Biol, 2001. **21**(5): p. 858-62.
54. Shechter, M., *Does magnesium have a role in the treatment of patients with coronary artery disease?* Am J Cardiovasc Drugs, 2003. **3**(4): p. 231-9.
55. Aagaard, N.K., et al., *Magnesium supplementation and muscle function in patients with alcoholic liver disease: a randomized, placebo-controlled trial.* Scand J Gastroenterol, 2005. **40**(8): p. 972-9.
56. Henyan, N.N., et al., *Impact of intravenous magnesium on post-cardiothoracic*

surgery atrial fibrillation and length of hospital stay: a meta-analysis. Ann Thorac Surg, 2005. **80**(6): p. 2402-6.
57. Tsuji, A., et al., *Effectiveness of oral magnesium in a patient with ventricular tachycardia due to hypomagnesemia.* J Cardiovasc Pharmacol Ther, 2005. **10**(3): p. 205-8.
58. Rimm, E.B., et al., *Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals.* Am J Epidemiol, 1992. **135**(10): p. 1114-26; discussion 1127-36.
59. Willett, W.C., et al., *Reproducibility and validity of a semiquantitative food frequency questionnaire.* Am J Epidemiol, 1985. **122**(1): p. 51-65.
60. U.S.D.o. Agriculture, *Composition of foods-raw, processed, and prepared, 1963-1992.* Agricultural handbook series. 1993, Washington, D.C.: Department of Agriculture, U.S. Government Printing Office.
61. Willett, W., *Nutritional epidemiology.* 2nd ed. Monographs in epidemiology and biostatistics ;. 1998, New York: Oxford University Press. xiv, 514 p.
62. Feskanich, D., et al., *Reproducibility and validity of food intake measurements from a semiquantitative food frequency questionnaire.* J Am Diet Assoc, 1993. **93**(7): p. 790-6.
63. Bosy-Westphal, A., et al., *Value of body fat mass vs anthropometric obesity indices in the assessment of metabolic risk factors.* Int J Obes (Lond), 2006. **30**(3): p. 475-83.
64. Ainsworth, B.E., et al., *Compendium of physical activities: classification of energy costs of human physical activities.* Med Sci Sports Exerc, 1993. **25**(1): p. 71-80.
65. Chasan-Taber, S., et al., *Reproducibility and validity of a self-administered physical activity questionnaire for male health professionals.* Epidemiology, 1996. **7**(1): p. 81-6.
66. Rimm, E.B., et al., *Validity of self-reported waist and hip circumferences in men and women.* Epidemiology, 1990. **1**(6): p. 466-73.
67. Wolf, A.M., et al., *Reproducibility and validity of a self-administered physical activity questionnaire.* Int J Epidemiol, 1994. **23**(5): p. 991-9.
68. Cox, D.R., *Regression Models and Life-Tables.* J Roy Statist Soc Ser B (Methodological), 1972. **34**(2): p. 187-220.
69. D'Agostino, R.B., et al., *Relation of pooled logistic regression to time dependent Cox regression analysis: the Framingham Heart Study.* Stat Med, 1990. **9**(12): p. 1501-15.
70. Chapman, D.G. and J.M. Nam, *Asymptotic power of chi square tests for linear trends in proportions.* Biometrics, 1968. **24**(2): p. 315-27.
71. Rosner, B., *Fundamentals of biostatistics.* 6th ed. 2006, Belmont, CA: Thomson-Brooks/Cole. xix, 868 p.
72. O'Rourke, B., *Ion channels as sensors of cellular energy. Mechanisms for modulation by magnesium and nucleotides.* Biochem Pharmacol, 1993. **46**(7): p. 1103-12.
73. White, R.E. and H.C. Hartzell, *Magnesium ions in cardiac function. Regulator of ion channels and second messengers.* Biochem Pharmacol, 1989. **38**(6): p. 859-67.

74. Dai, Q., et al., *The relation of magnesium and calcium intakes and a genetic polymorphism in the magnesium transporter to colorectal neoplasia risk.* Am J Clin Nutr, 2007. **86**(3): p. 743-51.
75. Baron, J.A., et al., *Calcium supplements for the prevention of colorectal adenomas. Calcium Polyp Prevention Study Group.* N Engl J Med, 1999. **340**(2): p. 101-7.
76. Wactawski-Wende, J., et al., *Calcium plus vitamin D supplementation and the risk of colorectal cancer.* N Engl J Med, 2006. **354**(7): p. 684-96.
77. Kaluza, J., et al., *Dietary calcium and magnesium intake and mortality: a prospective study of men.* Am J Epidemiol, 2010. **171**(7): p. 801-7.
78. Altura, B.M., *Introduction: importance of Mg in physiology and medicine and the need for ion selective electrodes.* Scand J Clin Lab Invest Suppl, 1994. **217**: p. 5-9.
79. Hanna, S., *Influence of large doses of vitamin D on magnesium metabolism in rats.* Metabolism, 1961. **10**: p. 735-43.
80. Krejs, G.J., et al., *Effect of 1,25-dihydroxyvitamin D3 on calcium and magnesium absorption in the healthy human jejunum and ileum.* Am J Med, 1983. **75**(6): p. 973-6.
81. Karbach, U., *Cellular-mediated and diffusive magnesium transport across the descending colon of the rat.* Gastroenterology, 1989. **96**(5 Pt 1): p. 1282-9.
82. Levine, B.S., et al., *Effects of vitamin D and diet magnesium on magnesium metabolism.* Am J Physiol, 1980. **239**(6): p. E515-23.
83. Schmulen, A.C., et al., *Effect of 1,25-(OH)2D3 on jejunal absorption of magnesium in patients with chronic renal disease.* Am J Physiol, 1980. **238**(4): p. G349-52.
84. Hodgkinson, A., D.H. Marshall, and B.E. Nordin, *Vitamin D and magnesium absorption in man.* Clin Sci (Lond), 1979. **57**(1): p. 121-3.
85. Brickman, A.S., et al., *Clinical effects of 1,25-dihydroxyvitamin D3 in uremic patients with overt osteodystrophy.* Contrib Nephrol, 1980. **18**: p. 29-41.
86. Heaton, F.W. and F.M. Parsons, *The metabolic effect of high magnesium intake.* Clin Sci, 1961. **21**: p. 273-84.
87. Kesavan, Y., et al., *A prospective study of magnesium and iron intake and pancreatic cancer in men.* Am J Epidemiol, 2010. **171**(2): p. 233-41.
88. Balon, T.W., et al., *Magnesium supplementation reduces development of diabetes in a rat model of spontaneous NIDDM.* Am J Physiol, 1995. **269**(4 Pt 1): p. E745-52.
89. Larsson, S.C. and A. Wolk, *Magnesium intake and risk of type 2 diabetes: a meta-analysis.* J Intern Med, 2007. **262**(2): p. 208-14.

Table 1. Age-standardized baseline characteristics according to baseline quintiles of energy-adjusted total magnesium intake in the Health Professionals Follow-up Study of U.S. men, 1986.

	Quintiles of energy-adjusted total magnesium intake (median, mg/d)				
Characteristic	Q1	Q2	Q3	Q4	Q5
Median intake, mg/day	262	307	343	384	457
Mean intake, mg/day	256	306	342	384	478
No. of participants	9491	9526	9755	9492	9525
Person-years	79554	80557	81938	79739	79710
Age at baseline, mean, y	53.6	53.8	54.0	54.1	54.2
BMI at baseline, mean, kg/m^2	25.1	25.2	25.0	24.8	24.5
Height at baseline, mean, in	70.0	70.1	70.2	70.2	70.2
Waist circumference in 1987, mean, cm	36.4	36.4	36.3	36.0	35.8
Waist to hip ratio in 1987, mean	0.92	0.92	0.92	0.92	0.92
Race/ethnicity					
White, %	90.6	91.0	91.3	91.0	90.4
Black, %	1.4	0.89	0.83	0.86	0.10
Asian or Pacific Islander, %	1.6	1.7	1.5	1.6	2.0
Other race, %	2.4	2.6	2.2	2.4	2.6
Physical activity, mean, MET-h/wk	15.8	18.6	21.0	22.8	26.7
Vigorous physical activity, mean, MET-h/wk	7.7	9.6	11.2	12.4	14.7
Smoking status					
Current smoker in 1986, %	12.8	11.0	9.2	8.6	7.0
Past smoker in 1986, %	37.4	40.9	42.5	43.8	44.9
Never smoker in 1986, %	45.8	44.5	44.2	43.6	44.0
Family history of colorectal cancer in parent prior to age 60 years, %	1.9	2.2	2.0	1.8	2.2
History of endoscopic screening, %	15.6	16.6	17.3	18.4	20.3
Aspirin use in 1986, %	25.6	28.6	29.1	30.9	32.7
Alcohol consumption, mean, g/day	11.8	11.4	11.5	11.8	10.5
Multivitamin use in 1986, %	28.0	32.5	38.3	46.1	64.0
Vitamin D predictor score* mean	21.0	21.6	22.2	22.8	23.8

Total energy intake, mean, kcal/day	1949	1992	2020	1999	1963
Energy-adjusted dietary intake, mean					
Red meat intake, mg/day	0.76	0.70	0.63	0.54	0.39
Processed meat, mg/day	0.49	0.42	0.37	0.29	0.20
Saturated fat, g/day	27.4	26.2	24.8	23.0	20.6
Fiber, g/day	15.8	18.4	20.5	22.8	27.6
Folate, mcg/day	351.6	409.6	456.0	514.8	674.6
Vitamin D, IU/day	274	332	382	445	605
Calcium, mg/day	685.7	803.5	889.0	972.6	1136
Zinc, mg/day	15.2	17.0	18.6	22.0	32.3
Vitamin B_6, mg/day	4.6	5.5	6.9	9.8	16.4
Vitamin B_{12}, mcg/day	10.8	11.4	12.0	12.8	16.4
Magnesium from cereals, mg/day	46.4	56.0	65.4	77.2	108.0
Magnesium from fruit, mg/day	25.7	32.0	37.0	41.6	47.8
Magnesium from vegetables, mg/day	38.4	48.0	54.8	62.8	80.7
Magnesium from food sources, mg/day	256	304	338	373	438
Magnesium from supplements, mg/day	0.84	2.2	4.7	10.7	36.6

Abbreviations: Q, quintile

* based on determinants of vitamin D exposure including simple updated dietary and supplementary vitamin D intake, skin pigmentation, adiposity, geographic residence, and leisure-time physical activity to estimate sunlight exposure

Table 2a. Age and multivariate adjusted Cox proportional hazard ratios (95% confidence interval) of colorectal cancer according to quintiles of cumulative updated energy-adjusted total magnesium intake in the Health Professionals Follow-up Study of U.S. men, 1986-2004.

Covariate	Quintiles of cumulative updated energy-adjusted total magnesium intake (median, mg/d)					
	Q1 (262)	Q2 (307)	Q3 (343)	Q4 (384)	Q5 (457)	P for trend
Colorectal cancer cases, No.	205	214	181	204	209	
HR (95% CI)						
Age-adjusted	1.0	0.94 (0.78, 1.15)	0.74 (0.61, 0.92)	0.79 (0.65, 0.96)	0.76 (0.63, 0.93)	0.003
MV1[a]	1.0	1.00 (0.82, 1.21)	0.82 (0.66, 1.00)	0.90 (0.74, 1.09)	0.90 (0.74, 1.10)	0.26
MV2[b]	1.0	1.02 (0.84, 1.24)	0.86 (0.69, 1.06)	0.96 (0.76, 1.18)	1.00 (0.78, 1.26)	0.92
MV3[c]	1.0	1.05 (0.86, 1.28)	0.88 (0.71, 1.10)	0.98 (0.78, 1.24)	1.02 (0.80, 1.30)	0.92
Colon cancer cases, No.	134	139	116	142	136	
HR (95% CI)						
Age-adjusted	1.0	0.94 (0.74, 1.19)	0.73 (0.57, 0.94)	0.84 (0.66, 1.06)	0.76 (0.60, 0.96)	0.02
MV1[a]	1.0	0.98 (0.77, 1.24)	0.79 (0.62, 1.02)	0.94 (0.74, 1.20)	0.89 (0.70, 1.14)	0.38
MV2[b]	1.0	1.02 (0.79, 1.30)	0.84 (0.64, 1.10)	1.02 (0.78, 1.33)	1.00 (0.74, 1.32)	0.94
MV3[c]	1.0	1.06 (0.82, 1.36)	0.88 (0.68, 1.16)	1.08 (0.82, 1.43)	1.06 (0.78, 1.44)	0.64
Proximal colon cancer cases, No.	54	64	70	73	68	
HR (95% CI)						
Age-adjusted	1.0	1.05 (0.73, 1.50)	1.08 (0.76, 1.54)	1.04 (0.73, 1.48)	0.92 (0.64, 1.32)	0.55
MV1[a]	1.0	1.09 (0.76, 1.56)	1.14 (0.80, 1.64)	1.12 (0.78, 1.60)	1.00 (0.70, 1.46)	0.94
MV2[b]	1.0	1.08 (0.74, 1.56)	1.14 (0.78, 1.67)	1.14 (0.77, 1.69)	1.09 (0.71, 1.67)	0.72
MV3[c]	1.0	1.12 (0.76, 1.62)	1.19 (0.80, 1.76)	1.20 (0.80, 1.80)	1.17 (0.74, 1.82)	0.54
Distal colon cancer cases, No.	69	70	43	64	62	
HR (95% CI)						
Age-adjusted	1.0	0.94 (0.67, 1.31)	0.54 (0.36, 0.78)	0.76 (0.54, 1.07)	0.69 (0.49, 0.98)	0.02
MV1[a]	1.0	0.99 (0.70, 1.38)	0.58 (0.40, 0.86)	0.88 (0.62, 1.24)	0.85 (0.60, 1.22)	0.36
MV2[b]	1.0	1.08 (0.76, 1.52)	0.66 (0.44, 1.00)	1.00 (0.68, 1.48)	0.96 (0.63, 1.46)	0.85
MV3[c]	1.0	1.13 (0.80, 1.60)	0.71 (0.47, 1.08)	1.08 (0.72, 1.62)	1.02 (0.65, 1.59)	0.93
Rectal cancer cases, No.	43	45	38	42	47	
HR (95% CI)						

Age-adjusted	1.0	0.96 (0.63, 1.46)	0.75 (0.48, 1.16)	0.79 (0.52, 1.21)	0.84 (0.55, 1.27)	0.33
MV1[a]	1.0	1.03 (0.68, 1.57)	0.85 (0.54, 1.32)	0.92 (0.60, 1.42)	1.01 (0.66, 1.54)	0.96
MV2[b]	1.0	1.06 (0.69, 1.64)	0.90 (0.56, 1.44)	1.01 (0.62, 1.62)	1.20 (0.72, 1.98)	0.50
MV3[c]	1.0	1.04 (0.66, 1.60)	0.86 (0.53, 1.38)	0.94 (0.58, 1.54)	1.08 (0.64, 1.84)	0.78
Advanced cancer cases, No.	89	100	76	76	85	
HR (95% CI)						
Age-adjusted	1.0	1.00 (0.76, 1.34)	0.71 (0.52, 0.96)	0.67 (0.50, 0.92)	0.71 (0.52, 0.96)	0.004
MV1[a]	1.0	1.08 (0.81, 1.44)	0.80 (0.58, 1.09)	0.80 (0.58, 1.08)	0.87 (0.64, 1.18)	0.14
MV2[b]	1.0	1.18 (0.88, 1.60)	0.94 (0.67, 1.30)	0.97 (0.68, 1.36)	1.12 (0.78, 1.60)	0.84
MV3[c]	1.0	1.22 (0.90, 1.64)	0.96 (0.68, 1.35)	1.00 (0.70, 1.44)	1.16 (0.79, 1.70)	0.70

Abbreviations: CI, confidence interval; MV, multivariate; HR, hazard ratio; BMI, body mass index

[a] MV1: age (months), BMI (< 21.0, 21-22.9, 25-27.4, 27.5-29.9, ≥30 kg/m^2), height (quintiles <66, 66 to <68, 68 to <70, 70 to <72, ≥72 in), race (white, black, Asian or Pacific Islander, other race), vigorous physical activity (quintiles), smoking status (never smoker, past smoker who quit > 10 years ago, current or recent smoker who quit ≤ 10 years ago), cumulative updated alcohol consumption (none, 0.1-9.9, 10-19.9, 20-29, ≥30 g/day), family history of colorectal cancer (mother or father diagnosed before age 60), history of endoscopic screening, current aspirin use

[b] MV2: all the variables in MV1 plus multivitamin use, total caloric intake (cumulative updated quintiles), and cumulative-updated, energy-adjusted intakes of red meat, folate and vitamin B_6 (quintiles)

[c] MV3: all the variables in MV2 plus cumulative-updated, energy-adjusted calcium intake (<500, 500-749, 750-999, 1000-1499, 1500-1999, ≥2000 mg/day) and vitamin D predictor score (based on multiple determinants of vitamin D exposure including simple updated dietary and supplementary vitamin D intake, skin pigmentation, adiposity, geographic residence, and leisure-time physical activity to estimate sunlight exposure)

Table 2b. Age and multivariate adjusted Cox proportional hazard ratios (95% confidence interval) of colorectal cancer according to quintiles of cumulative updated energy-adjusted dietary magnesium intake in the Health Professionals Follow-up Study of U.S. men, 1986-2004.

Covariate	Quintiles of cumulative updated energy-adjusted dietary magnesium intake (median, mg/d)					
	Q1 (260)	Q2 (302)	Q3 (335)	Q4 (371)	Q5 (432)	P for trend
Colorectal cancer cases, No.	219	188	221	175	210	
HR (95% CI)						
Age-adjusted	1.0	0.79 (0.66, 0.96)	0.88 (0.73, 1.06)	0.66 (0.54, 0.81)	0.76 (0.64, 0.92)	0.003
MV1[a]	1.0	0.82 (0.68, 1.00)	0.94 (0.78, 1.13)	0.72 (0.59, 0.88)	0.86 (0.71, 1.05)	0.10
MV2[b]	1.0	0.84 (0.69, 1.02)	0.97 (0.80, 1.18)	0.76 (0.62, 0.94)	0.94 (0.76, 1.16)	0.45
MV3[c]	1.0	0.86 (0.70, 1.04)	0.99 (0.81, 1.20)	0.78 (0.62, 0.96)	0.95 (0.76, 1.18)	0.56
Colon cancer cases, No.	137	114	152	127	137	
HR (95% CI)						
Age-adjusted	1.0	0.77 (0.60, 0.99)	0.97 (0.77, 1.22)	0.77 (0.60, 0.98)	0.80 (0.63, 1.02)	0.10
MV1[a]	1.0	0.80 (0.62, 1.02)	1.03 (0.82, 1.30)	0.84 (0.66, 1.08)	0.90 (0.71, 1.16)	0.58
MV2[b]	1.0	0.82 (0.64, 1.06)	1.08 (0.85, 1.38)	0.90 (0.70, 1.16)	1.00 (0.76, 1.30)	0.78
MV3[c]	1.0	0.84 (0.66, 1.09)	1.12 (0.88, 1.44)	0.94 (0.72, 1.22)	1.06 (0.80, 1.39)	0.52
Proximal colon cancer cases, No.	58	51	88	67	65	
HR (95% CI)						
Age-adjusted	1.0	0.80 (0.54, 1.16)	1.31 (0.94, 1.82)	0.94 (0.66, 1.34)	0.88 (0.62, 1.25)	0.60
MV1[a]	1.0	0.82 (0.56, 1.20)	1.37 (0.98, 1.92)	1.00 (0.70, 1.42)	0.94 (0.65, 1.34)	0.86
MV2[b]	1.0	0.82 (0.56, 1.20)	1.38 (0.97, 1.94)	1.01 (0.70, 1.46)	1.00 (0.67, 1.48)	0.80
MV3[c]	1.0	0.84 (0.57, 1.23)	1.42 (1.00, 2.01)	1.05 (0.72, 1.54)	1.05 (0.70, 1.58)	0.62
Distal colon cancer cases, No.	69	61	58	54	66	
HR (95% CI)						
Age-adjusted	1.0	0.84 (0.59, 1.18)	0.74 (0.52, 1.05)	0.66 (0.46, 0.95)	0.78 (0.56, 1.10)	0.12
MV1[a]	1.0	0.86 (0.60, 1.22)	0.79 (0.56, 1.12)	0.74 (0.51, 1.06)	0.93 (0.66, 1.32)	0.59
MV2[b]	1.0	1.08 (0.76, 1.52)	0.66 (0.44, 1.00)	1.00 (0.68, 1.48)	0.96 (0.63, 1.46)	0.85
MV3[c]	1.0	0.95 (0.66, 1.36)	0.91 (0.63, 1.32)	0.88 (0.59, 1.29)	1.11 (0.74, 1.66)	0.68
Rectal cancer cases, No.	56	42	44	26	47	
HR (95% CI)						

Age-adjusted	1.0	0.70 (0.47, 1.04)	0.69 (0.46, 1.03)	0.39 (0.24, 0.62)	0.68 (0.46, 1.00)	0.02
MV1[a]	1.0	0.72 (0.48, 1.08)	0.74 (0.49, 1.10)	0.42 (0.26, 0.68)	0.74 (0.49, 1.10)	0.05
MV2[b]	1.0	0.74 (0.49, 1.11)	0.76 (0.50, 1.14)	0.44 (0.27, 0.72)	0.83 (0.54, 1.29)	0.22
MV3[c]	1.0	0.72 (0.48, 1.08)	0.72 (0.47, 1.09)	0.41 (0.25, 0.68)	0.74 (0.47, 1.18)	0.12
Advanced cancer cases, No.	97	81	87	74	87	
HR (95% CI)						
Age-adjusted	1.0	0.78 (0.58, 1.04)	0.78 (0.58, 1.04)	0.64 (0.46, 0.86)	0.72 (0.54, 0.96)	0.02
MV1[a]	1.0	0.80 (0.60, 1.08)	0.83 (0.62, 1.11)	0.69 (0.50, 0.94)	0.80 (0.60, 1.08)	0.10
MV2[b]	1.0	0.87 (0.64, 1.18)	0.94 (0.70, 1.28)	0.82 (0.60, 1.14)	1.00 (0.72, 1.14)	0.98
MV3[c]	1.0	0.88 (0.65, 1.19)	0.96 (0.71, 1.32)	0.85 (0.61, 1.18)	1.04 (0.74, 1.45)	0.86

Abbreviations: CI, confidence interval; MV, multivariate; HR, hazard ratio; BMI, body mass index

[a] MV1: age, BMI, height, race, vigorous physical activity, smoking status, cumulative updated alcohol consumption, family history of colorectal cancer, history of endoscopic screening, current aspirin use

[b] MV2: all the variables in MV1 plus multivitamin use, total caloric intake, and cumulative-updated, energy-adjusted intakes of red meat, folate and vitamin B_6

[c] MV3: all the variables in MV2 plus cumulative-updated, energy-adjusted calcium intake and vitamin D predictor score

Table 3. Age and multivariate adjusted Cox proportional hazard ratios (95% confidence interval) of colorectal cancer according to quintiles of baseline energy-adjusted total magnesium intake in the Health Professionals Follow-up Study of U.S. men, 1986.

Covariate	Quintiles of baseline energy-adjusted total magnesium intake (median, mg/d)					
	Q1	Q2	Q3	Q4	Q5	P for trend
Mean (SD), mg/day	262	307	343	384	457	
Person-years in cohort	152247	154292	156884	152508	152450	
Colorectal cancer cases, No.	219	194	211	171	218	
HR (95% CI)						
Age-adjusted	1.0	0.87 (0.68, 1.11)	0.94 (0.75. 1.20)	0.72 (0.56, 0.92)	0.84 (0.66, 1.06)	0.07
MV1[a]	1.0	0.85 (0.70, 1.04)	0.88 (0.73, 1.07)	0.70 (0.58, 0.86)	0.88 (0.73, 1.08)	0.14
MV2[b]	1.0	0.87 (0.72, 1.06)	0.92 (0.76, 1.12)	0.74 (0.60, 0.92)	0.96 (0.77, 1.18)	0.56
MV3[c]	1.0	0.88 (0.72, 1.08)	0.94 (0.76, 1.14)	0.76 (0.60, 0.94)	0.97 (0.78, 1.21)	0.65
Colon cancer cases, No.	136	127	147	116	141	
HR (95% CI)						
Age-adjusted	1.0	0.83 (0.68, 1.00)	0.84 (0.70, 1.02)	0.66 (0.54, 0.80)	0.80 (0.66, 0.96)	0.01
MV1[a]	1.0	0.90 (0.70, 1.14)	0.99 (0.78, 1.26)	0.76 (0.60, 0.98)	0.92 (0.72, 1.17)	0.36
MV2[b]	1.0	0.92 (0.72, 1.18)	1.04 (0.82, 1.34)	0.82 (0.63, 1.07)	1.02 (0.78, 1.33)	0.93
MV3[c]	1.0	0.95 (0.74, 1.22)	1.08 (0.84, 1.39)	0.86 (0.65, 1.12)	1.06 (0.80, 1.41)	0.82
Proximal colon cancer cases, No.	59	60	80	60	70	
HR (95% CI)						
Age-adjusted	1.0	0.93 (0.65, 1.34)	1.18 (0.84, 1.64)	0.84 (0.58, 1.20)	0.94 (0.66, 1.32)	0.54
MV1[a]	1.0	0.96 (0.66, 1.37)	1.22 (0.86, 1.70)	0.88 (0.61, 1.26)	0.98 (0.69, 1.40)	0.73
MV2[b]	1.0	0.96 (0.66, 1.38)	1.22 (0.86, 1.73)	0.90 (0.61, 1.32)	1.06 (0.72, 1.57)	0.90
MV3[c]	1.0	0.98 (0.68, 1.41)	1.25 (0.88, 1.78)	0.93 (0.62, 1.37)	1.11 (0.74, 1.67)	0.72
Distal colon cancer cases, No.	67	62	64	50	65	
HR (95% CI)						
Age-adjusted	1.0	0.88 (0.62, 1.24)	0.84 (0.60, 1.19)	0.64 (0.44, 0.92)	0.80 (0.56, 1.13)	0.10
MV1[a]	1.0	0.90 (0.64, 1.28)	0.90 (0.64, 1.26)	0.70 (0.48, 1.00)	0.92 (0.65, 1.30)	0.44
MV2[b]	1.0	0.96 (0.68, 1.38)	0.99 (0.69, 1.42)	0.78 (0.52, 1.15)	1.02 (0.68, 1.51)	0.86
MV3[c]	1.0	1.00 (0.70, 1.44)	1.04 (0.72, 1.50)	0.82 (0.54, 1.22)	1.06 (0.70, 1.61)	0.97
Rectal cancer cases, No.	57	38	36	31	53	
HR (95% CI)						

Age-adjusted	1.0	0.62 (0.42, 0.94)	0.56 (0.36, 0.84)	0.46 (0.30, 0.72)	0.76 (0.52, 1.10)	0.20
MV1[a]	1.0	0.64 (0.42, 0.98)	0.58 (0.38, 0.90)	0.50 (0.32, 0.78)	0.84 (0.56, 1.22)	0.42
MV2[b]	1.0	0.66 (0.43, 1.00)	0.60 (0.39, 0.94)	0.52 (0.33, 0.84)	0.94 (0.60, 1.46)	0.85
MV3[c]	1.0	0.64 (0.42, 0.97)	0.57 (0.36, 0.89)	0.49 (0.30, 0.79)	0.85 (0.54, 1.33)	0.58
Advanced cancer cases, No.	100	81	87	69	89	
HR (95% CI)						
Age-adjusted	1.0	0.76 (0.56, 1.01)	0.76 (0.56, 1.01)	0.58 (0.42, 0.78)	0.71 (0.54, 0.95)	0.01
MV1[a]	1.0	0.78 (0.58, 1.04)	0.80 (0.60, 1.08)	0.63 (0.46, 0.86)	0.80 (0.60, 1.08)	0.11
MV2[b]	1.0	0.83 (0.62, 1.12)	0.90 (0.66, 1.21)	0.72 (0.52, 1.00)	0.96 (0.68, 1.34)	0.74
MV3[c]	1.0	0.84 (0.62, 1.14)	0.91 (0.67, 1.24)	0.74 (0.52, 1.03)	0.98 (0.69, 1.38)	0.84

Abbreviations: CI, confidence interval; MV, multivariate; HR, hazard ratio; BMI, body mass index

[a] MV1: age, BMI, height, race, vigorous physical activity, smoking status, cumulative updated alcohol consumption, family history of colorectal cancer, history of endoscopic screening, current aspirin use

[b] MV2: all the variables in MV1 plus multivitamin use, total caloric intake, and cumulative-updated, energy-adjusted intakes of red meat, folate and vitamin B_6

[c] MV3: all the variables in MV2 plus cumulative-updated, energy-adjusted calcium intake and vitamin D predictor score

Table 4. Multivariate* adjusted Cox proportional hazard ratios (95% confidence intervals) of colorectal cancer subsites stratified by varying levels of calcium intake, vitamin D predictor score and body mass index and quintiles of cumulative updated energy-adjusted total magnesium intake in the Health Professionals Follow-up Study of U.S. men, 1986-2004.

	Quintiles of cumulative updated total magnesium intake (median, mg/day)										
	Q1 (262)	Q2 (307)		Q3 (343)		Q4 (384)		Q5 (457)		P for trend	P for interaction
	HR	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI		
Colorectal cancer											
Calcium intake											0.72
<1000 mg/day	1.0	1.05	0.85, 1.31	0.97	0.77, 1.24	1.02	0.78, 1.32	1.00	0.74, 1.36	0.94	
≥1000 mg/day	1.0	0.88	0.54, 1.42	0.57	0.35, 0.94	0.76	0.47, 1.22	0.80	0.30, 1.28	0.89	
Vitamin D predictor score											0.06
Low	1.0	1.11	0.85, 1.44	0.85	0.62, 1.16	0.86	0.61, 1.21	0.91	0.61, 1.35	0.32	
High	1.0	0.98	0.72, 1.34	0.91	0.66, 1.26	1.06	0.77, 1.45	1.07	0.76, 1.49	0.48	
Body mass index											0.54
<25 kg/m^2	1.0	1.11	0.79, 1.56	0.94	0.65, 1.35	0.92	0.63, 1.33	0.94	0.63, 1.39	0.52	
≥25 kg/m^2	1.0	1.03	0.80, 1.32	0.87	0.66, 1.15	1.05	0.79, 1.39	1.08	0.79, 1.48	0.57	
Colon cancer											
Calcium intake											0.94
<1000 mg/day	1.0	1.02	0.77, 1.33	0.96	0.71, 1.30	1.15	0.84, 1.58	0.97	0.66, 1.42	0.84	
≥1000 mg/day	1.0	0.96	0.52, 1.76	0.56	0.30, 1.06	0.73	0.40, 1.32	0.84	0.46, 1.53	0.98	
Vitamin D predictor score											0.02
Low	1.0	1.05	0.76, 1.45	0.79	0.54, 1.17	0.95	0.63, 1.43	0.81	0.49, 1.34	0.34	
High	1.0	1.08	0.72, 1.60	1.00	0.67, 1.50	1.22	0.82, 1.81	1.23	0.80, 1.87	0.24	
Body mass index											0.55
<25 kg/m^2	1.0	1.28	0.83, 1.98	1.05	0.66, 1.69	1.00	0.62, 1.62	1.06	0.64, 1.75	0.81	
≥25 kg/m^2	1.0	0.97	0.71, 1.32	0.83	0.59, 1.17	1.16	0.83, 1.63	1.05	0.71, 1.55	0.53	
Proximal colon cancer											
Calcium intake											0.60
<1000 mg/day	1.0	1.08	0.71, 1.65	1.42	0.92, 2.17	1.30	0.84, 2.10	1.03	0.59, 1.80	0.64	
≥1000 mg/day	1.0	0.85	0.37, 1.93	0.49	0.21, 1.17	0.61	0.27, 1.38	0.80	0.36, 1.79	0.86	

Vitamin D predictor score											0.14
Low	1.0	1.02	0.60, 1.70	0.99	0.56, 1.75	0.96	0.51, 1.80	0.66	0.30, 1.47	0.36	
High	1.0	1.22	0.69, 2.14	1.41	0.81, 2.47	1.44	0.82, 2.53	1.51	0.83, 2.74	0.20	
Body mass index											0.28
<25 kg/m^2	1.0	1.16	0.60, 2.24	1.61	0.85, 3.07	1.15	0.58, 2.27	1.25	0.61, 2.55	0.80	
≥25 kg/m^2	1.0	1.12	0.71, 1.79	1.01	0.61, 1.67	1.30	0.78, 2.18	1.12	0.62, 2.02	0.61	
Distal colon cancer											
Calcium intake											0.57
<1000 mg/day	1.0	1.02	0.70, 1.50	0.67	0.42, 1.07	1.08	0.68, 1.70	0.91	0.52, 1.58	0.76	
≥1000 mg/day	1.0	1.60	0.58, 4.37	0.83	0.28, 2.40	1.16	0.42, 3.16	1.81	0.42, 3.24	0.99	
Vitamin D predictor score											0.20
Low	1.0	1.22	0.78, 1.91	0.74	0.42, 1.30	1.04	0.58, 1.87	0.98	0.48, 1.97	0.79	
High	1.0	1.04	0.58, 1.84	0.69	0.37, 1.30	1.08	0.60, 1.95	1.00	0.54, 1.85	0.82	
Body mass index											0.45
<25 kg/m^2	1.0	1.28	0.70, 2.32	0.49	0.22, 1.07	0.86	0.42, 1.76	0.74	0.35, 1.58	0.32	
≥25 kg/m^2	1.0	1.06	0.68, 1.63	0.84	0.51, 1.38	1.20	0.73, 1.97	1.16	0.67, 2.02	0.50	
Rectal cancer											
Calcium intake											0.90
<1000 mg/day	1.0	1.16	0.72, 1.86	0.98	0.58, 1.68	0.99	0.55, 1.77	1.43	0.76, 2.71	0.45	
≥1000 mg/day	1.0	0.62	0.22, 1.74	0.56	0.20, 1.55	0.82	0.32, 2.14	0.74	0.28, 1.97	0.98	
Vitamin D predictor score											0.88
Low	1.0	1.37	0.75, 2.50	1.16	0.59, 2.28	0.80	0.35, 1.85	1.58	0.69, 3.63	0.60	
High	1.0	0.75	0.39, 1.45	0.65	0.33, 1.27	0.89	0.47, 1.69	0.83	0.41, 1.65	0.96	
Body mass index											0.50
<25 kg/m^2	1.0	1.24	0.63, 2.47	0.97	0.46, 2.03	1.16	0.55, 2.44	1.37	0.61, 3.08	0.51	
≥25 kg/m^2	1.0	0.98	0.55, 1.74	0.81	0.43, 1.53	0.85	0.44, 1.66	0.99	0.49, 1.99	0.97	
Advanced cancer											
Calcium intake											0.37
<1000 mg/day	1.0	1.17	0.84, 1.63	1.09	0.75, 1.56	0.90	0.59, 1.37	1.27	0.81, 2.00	0.62	
≥1000 mg/day	1.0	1.09	0.54, 2.20	0.52	0.23, 1.13	0.93	0.45, 1.89	0.86	0.41, 1.78	0.83	
Vitamin D											

predictor score											0.86
Low	1.0	1.31	0.88, 1.94	1.00	0.63, 1.61	0.92	0.53, 1.60	1.54	0.86, 2.76	0.40	
High	1.0	1.11	0.69, 1.76	0.92	0.56, 1.50	1.00	0.61, 1.63	0.96	0.57, 1.62	0.77	
Body mass index											0.20
<25 kg/m^2	1.0	1.51	0.91, 2.51	1.39	0.81, 2.38	0.80	0.43, 1.49	1.05	0.56, 1.97	0.48	
≥25 kg/m^2	1.0	1.10	0.76, 1.60	0.79	0.51, 1.23	1.17	0.76, 1.82	1.29	0.80, 2.09	0.26	

Abbreviations: Q, quintile; HR, Cox-proportional hazard ratio; CI, confidence interval; BMI, body mass index

* Multivariate model adjusted for age, BMI, height, vigorous physical activity, smoking status, alcohol consumption, race, first-degree relative with colorectal cancer, %, history of endoscopic screening, current aspirin use, red meat intake, multivitamin use, total caloric intake, vitamin D predictor score, and energy-adjusted intakes of calcium, folate and vitamin B_6. The variable used for stratification was excluded from the model.

Table 5a. Multivariate* adjusted Cox proportional hazard ratios (95% confidence intervals) of site-specific colorectal cancer according to the joint effect of different levels of baseline energy-adjusted calcium and magnesium intake in the Health Professionals Follow-up Study of U.S. men, 1986.

Baseline total calcium intake	Quintiles of baseline total magnesium intake (median, mg/day)									
	Q1 (262)		Q2 (307)		Q3 (343)		Q4 (384)		Q5 (457)	
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Colorectal cancer										
Calcium <1000 mg/day	1.0	Reference	0.86	0.69, 1.06	0.92	0.74, 1.14	0.68	0.52, 0.86	0.89	0.68, 1.16
Calcium ≥1000 mg/day	0.60	0.35, 1.01	0.76	0.52, 1.08	0.79	0.58, 1.08	0.76	0.57, 1.02	0.93	0.72, 1.20
Colon cancer										
Calcium <1000 mg/day	1.0	Reference	0.94	0.72, 1.22	1.04	0.79, 1.35	0.77	0.57, 1.04	0.92	0.66, 1.26
Calcium ≥1000 mg/day	0.58	0.29, 1.14	0.66	0.40, 1.06	0.90	0.62, 1.30	0.79	0.55, 1.14	1.01	0.73, 1.40
Proximal colon cancer										
Calcium <1000 mg/day	1.0	Reference	1.01	0.68, 1.49	1.26	0.86, 1.84	0.78	0.50, 1.22	0.90	0.56, 1.46
Calcium ≥1000 mg/day	0.71	0.28, 1.78	0.58	0.27, 1.22	0.92	0.54, 1.58	0.96	0.59, 1.58	1.10	0.70, 1.75
Distal colon cancer										
Calcium <1000 mg/day	1.0	Reference	0.94	0.64, 1.36	0.92	0.62, 1.37	0.80	0.51, 1.24	0.94	0.58, 1.50
Calcium ≥1000 mg/day	0.54	0.19, 1.48	0.84	0.44, 1.62	0.98	0.58, 1.67	0.64	0.36, 1.14	0.98	0.60, 1.58
Rectal cancer										
Calcium <1000 mg/day	1.0	Reference	0.58	0.36, 0.93	0.68	0.42, 1.08	0.48	0.27, 0.84	0.96	0.58, 1.62
Calcium ≥1000 mg/day	0.94	0.40, 2.20	0.96	0.50, 1.86	0.41	0.18, 0.92	0.60	0.32, 1.14	0.90	0.52, 1.54
Advanced cancer										
Calcium <1000 mg/day	1.0	Reference	0.87	0.63, 1.20	0.96	0.68, 1.32	0.70	0.48, 1.02	0.94	0.64, 1.41
Calcium ≥1000 mg/day	0.74	0.36, 1.52	0.61	0.33, 1.12	0.71	0.43, 1.16	0.75	0.48, 1.18	0.96	0.64, 1.42

Abbreviations: Q, quintile; HR, hazard ratio; CI, confidence interval; BMI, body mass index

* Multivariate model adjusted for age, BMI, height, vigorous physical activity, smoking status, alcohol consumption, race/ethnicity, first-degree relative with colorectal cancer, %, history of endoscopic screening, current aspirin use, red meat intake, multivitamin use, total caloric intake, vitamin D predictor score, and energy-adjusted intakes of calcium, folate and vitamin B_6.

Table 5b. Multivariate* adjusted Cox proportional hazard ratios 95% confidence intervals) of site-specific colorectal cancer according to the joint effect of different levels of energy-adjusted cumulative updated calcium and magnesium intake in the Health Professionals Follow-up Study of U.S. men, 1986-2004.

Cumulative updated total calcium intake	**Quintiles of cumulative updated total magnesium intake (median mg/day)**									
	Q1 (262)		Q2 (307)		Q3 (343)		Q4 (384)		Q5 (457)	
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Colorectal cancer										
Calcium <1000 mg/day	1.0	Reference	1.04	0.83, 1.29	0.95	0.74, 1.20	0.98	0.76, 1.26	0.94	0.70, 1.27
Calcium ≥1000 mg/day	1.26	0.83, 1.90	1.14	0.82, 1.58	0.74	0.52, 1.04	1.02	0.76, 1.35	1.11	0.84, 1.45
Colon cancer										
Calcium <1000 mg/day	1.0	Reference	1.00	0.76, 1.31	0.93	0.69, 1.25	1.12	0.82, 1.51	0.92	0.64, 1.33
Calcium ≥1000 mg/day	1.17	0.69, 1.96	1.22	0.83, 1.80	0.72	0.47, 1.10	0.97	0.68, 1.38	1.12	0.80, 1.56
Proximal colon cancer										
Calcium <1000 mg/day	1.0	Reference	1.09	0.71, 1.65	1.41	0.93, 2.14	1.37	0.88, 2.14	1.06	0.61, 1.81
Calcium ≥1000 mg/day	1.64	0.80, 3.36	1.41	0.80, 2.50	0.81	0.43, 1.50	1.03	0.61, 1.73	1.26	0.77, 2.06
Distal colon cancer										
Calcium <1000 mg/day	1.0	Reference	0.99	0.68, 1.45	0.64	0.40, 1.01	0.98	0.64, 1.54	0.80	0.47, 1.38
Calcium ≥1000 mg/day	0.68	0.28, 1.72	1.24	0.71, 2.14	0.68	0.36, 1.26	0.98	0.59, 1.62	1.04	0.64, 1.69
Rectal cancer										
Calcium <1000 mg/day	1.0	Reference	1.19	0.74, 1.92	1.02	0.60, 1.73	1.00	0.56, 1.76	1.32	0.71, 2.45
Calcium ≥1000 mg/day	1.71	0.76, 3.88	1.01	0.46, 2.20	0.88	0.42, 1.82	1.28	0.70, 2.34	1.31	0.73, 2.37
Advanced cancer										
Calcium <1000 mg/day	1.0	Reference	1.20	0.87, 1.67	1.13	0.79, 1.62	0.95	0.63, 1.43	1.29	0.83, 2.00
Calcium ≥1000 mg/day	1.38	0.75, 2.54	1.44	0.90, 2.31	0.66	0.38, 1.17	1.16	0.75, 1.80	1.11	0.72, 1.72

Abbreviations: Q, quintile; HR, hazard ratio; CI, confidence interval; BMI, body mass index

* Multivariate model adjusted for same covariates as Table 5a.

Table 7a. Multivariate* adjusted Cox proportional hazard ratios (95% confidence intervals) of site-specific cancer according to the ratio of energy-adjusted calcium to magnesium intake at baseline in the Health Professionals Follow-up Study of U.S. men, 1986.

Total calcium intake	Ratio of calcium to magnesium intake		
	HR	95% CI	P value
Colorectal cancer			
Calcium	0.96	0.91, 1.02	
Calcium <1000 mg/day	0.96	0.89, 1.03	0.30
Calcium ≥1000 mg/day	0.98	0.88, 1.09	0.74
Colon cancer			
Calcium	0.99	0.92, 1.07	
Calcium <1000 mg/day	1.00	0.91, 1.10	0.98
Calcium ≥1000 mg/day	0.97	0.84, 1.10	0.66
Rectal cancer			
Calcium	0.92	0.81, 1.05	
Calcium <1000 mg/day	0.91	0.77, 1.06	0.24
Calcium ≥1000 mg/day	0.78	0.76, 1.22	0.77

Abbreviations: HR, hazard ratio; CI, confidence interval
* Multivariate model adjusted for same covariates as Table 5a

Table 7b. Multivariate* adjusted Cox proportional hazard ratios (95% confidence intervals) of site-specific cancer according to the ratio of energy-adjusted calcium intake to magnesium intake for baseline and cumulative updated intakes in the Health Professionals Follow-up Study of U.S. men, 1986-2004.

	Quintiles of the ratio of baseline calcium to magnesium intake								
	Q1	Q2		Q3		Q4		Q5	
	HR	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Colorectal cancer	1.0	0.95	0.77, 1.16	1.06	0.86, 1.28	1.02	0.84, 1.24	1.02	0.83, 1.24
	Quintiles of the ratio of cumulative updated calcium to magnesium intake								
Colorectal cancer	1.0	0.98	0.80, 1.20	0.97	0.79, 1.18	1.10	0.90, 1.34	1.06	0.86, 1.30

Abbreviations: Q, quintile; HR, hazard ratio; CI, confidence interval
* Multivariate model adjusted for same covariates as Table 5a

Figure 1. Fully adjusted hazard ratio of colorectal cancer by joint category of baseline calcium and magnesium intake in tertiles in the Health Professionals Follow-up Study of U.S. men, 1986.