

Calcium and Magnesium in Relation to Colorectal Neoplasia, Lipid Profile and Uric Acid

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Dissertation

Submitted to the Faculty of the
Graduate School of Vanderbilt University

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

in

Epidemiology

December, 2016

Nashville, Tennessee

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ACKNOWLEDGEMENTS

As I stand on the finish line, looking back at my journey as a PhD student, I have great admiration and appreciation for several key persons, without their continuous patience, motivation, and immense knowledge, I could not have reached this milestone in my career. I would like to express my sincere gratitude to my mentor and dissertation committee chair, Dr. Qi Dai, for his tremendous support. His creativity and intelligence have greatly influenced this body of work, especially in transforming this multi-faceted dissertation into a well-connected story. Thank you Dr. Dai for allowing me to grow as an epidemiologist under your guidance.

I would also like to express my special appreciation to the other committee members who so graciously afforded me their time and expertise. Dr. Martha J Shrubsole provided me with her insightful comments and suggestions on my works of writing. She is rigorous researcher, who teaches me to be logical, systematical and organized. I learned numerous advanced statistical principles and methodologies from Dr. Chang Yu. His strong intuition in statistics and his ability to explain complicated statistical methodologies with great ease not only made our communications related to statistics inspiring, but also enjoyable. Dr. Todd L. Edwards was always extremely supportive and encouraging especially during challenging times in the dissertation process. I have also benefited from advice of Dr. Thomas J Wang whose extensive experience in cardiovascular disease was an important component of guidance that I need from my committee.

My heartfelt thanks also go to the entire faculty and staff at the division of epidemiology at Vanderbilt University. Interacting with you has greatly enriched my training in epidemiology. I would especially like to thank physicians, nurses, and other staff in Clinical Research Center at Vanderbilt University Medical Center. All of you have been there to support me when I conducted clinic visits for patients and collected data for my Ph.D. thesis. I would like to express my appreciation towards Dr. Xiangzhu Zhu, who is not only my colleague but also a mentor for me in many aspects such as data management and analyses. I owe Dr. Ayush Giri a debt of gratitude for his continuous help with polishing my works of writing and supporting me during the training.

Finally, to my family members, close friends, and fellow students, thank you for your undying support. Words cannot express how grateful I am to my family members for all of the sacrifice that they have made on my behalf during these seven years that I have been studying abroad. Your understanding, tolerance and confidence in me have sustained me thus far. I would also like to thank all of my friends and fellow students who supported me and encouraged me, especially during difficult times when I needed that extra motivation to strive towards my goal.

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LIST OF ABBREVIATIONS

ASR	Age standardized rate
ASU	Annual study update
ATP	Adenosine triphosphate
BMI	Body mass index
BQ	Baseline questionnaire
Ca:Mg	Calcium: magnesium
CaD	Calcium plus vitamin D
CaSR	Calcium-sensing receptor
CI	Confidence interval
CRC	Colorectal cancer
CRP	C-reactive protein
CSFII	Continuing survey of food intakes by individuals
CV	Clinic visit
DHQ	Dietary history questionnaire
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
DQX	Dietary questionnaire
DRI	Dietary reference intake
DRP	Death review process
FSG	Flexible 60-cm sigmoidoscopy
HDL-c	High-density lipoprotein cholesterol
HMG-CoA	2-hydroxy-3-methyl-glutaryl-coenzyme A
HR	Hazards ratios
IGF-1	Insulin-like growth factor 1

IU	International unit
<i>KCNJ1</i>	Potassium channel, inwardly rectifying subfamily J, member 1
LCAT	Lecithin cholesterol acyltransferase
LDL-c	Low-density lipoprotein cholesterol
LPL	Lipoprotein lipase
MRA	Medical record abstraction
NDI	National death index
NDS	Nutrient Data System
NHANES	National Health and Nutrition Examination Survey
NSAIDs	Non-steroidal anti-inflammatory drugs
OR	Odds ratio
PLCO	Prostate, Lung, Colorectal and Ovarian
PPCCT	Personalized Prevention of Colorectal Cancer Trial
SCU	Study of Colonoscopy Utilization
<i>SLC2A9</i>	Solute carrier family 2, member 9
<i>SLC12A1</i>	Solute carrier family 12, member 1
SRSR	Survey Research Shared Resource
T ₀	Entry year
T ₃	The 3-year point
T ₅	The 5-year point
TCPS	Tennessee Colorectal Polyp Study
TIARS	Tennessee-Indiana Metachronous adenoma Study
<i>TRPM7</i>	Transient receptor potential melastatin 7
U.S.	United States
USDA	United States department of agriculture

VLDL Very low-density lipoprotein

WHI Women's Health Initiative

1,25(OH)₂D₃ 1,25-dihydroxyvitamin D₃

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Chapter 1. Introduction

A. Background and significance

Colorectal cancer (CRC) is the third most common incident cancer and the fifth leading cancer killer in 2012 in the world ¹. In the United States (U.S.), the estimated age-standardized rate (ASR) of CRC incidence is more than twice of that in the world (42.4 per 100,000 based on data from 2008-2012 vs 17.2 per 100,000 for both sexes) ^{1,2}, despite the slightly decreasing trend over time ³. Therefore, understanding the etiology of CRC and developing effective preventive strategies are still the most attractive public health goals.

CRC arises from the malignant transformation of adenomas and polyps ⁴. The development of CRC is complex and multi-factorial. Both external factors and/or inherited genetic factors play an essential role in the initiation and progression of the adenoma-carcinoma sequence ⁵. Epidemiological studies have reported inverse ⁶⁻¹⁴ as well as null associations ^{15,16} between calcium intake and the risk of colorectal neoplasia. Although inconsistent, calcium, one of nutrients with chemopreventive effects, was found to reduce 20-30% of risk for colorectal adenoma ¹⁷⁻²⁰ and cancer ^{21,22}. Clinical trials also found that calcium supplementation reduced about 20% risk of metachronous colorectal adenoma ²³⁻²⁵. In addition, recent studies indicate that calcium:magnesium (Ca:Mg) intake ratio modified the associations between intakes of calcium and magnesium and risk of gastrointestinal neoplasia ^{17,26,27}, and total, cardiovascular disease and cancer mortalities ²⁸. These studies also indicated that a ratio between 1.7 and 2.6 is needed for calcium intake to be protective against risk of colorectal neoplasia,

and total, cardiovascular disease and cancer mortalities ^{17,26–28}. It is possible that lack of consideration in Ca:Mg balance may explain some of the inconsistency in previous studies on calcium intake.

The removal of polyps within an endoscopy screening is the most effective prevention strategy to reduce the morbidity and mortality of CRC ²⁹. However, recent evidence suggested that colonoscopy is more related to the reduction of left-side colon deaths, but not right-side ^{30–32}. Furthermore, annual recurrence rates in patients with small and advanced adenomas were as high as 19.3% and 22.9%, respectively ³³. Therefore, additional preventive strategies are necessary.

Studies have shown that individuals with type 2 diabetes ^{34–37} and metabolic syndrome ^{38–40} have an elevated risk of CRC. Magnesium deficiency is a contributor to age-related diseases including cancer and cardiovascular disease. ^{41–44} Calcium and magnesium may share the same homeostatic regulation system ⁴⁵ primarily through absorption in the intestine and reabsorption in the kidneys to maintain the balance of Ca:Mg ^{45,46}. Nevertheless, calcium and magnesium compete with each other in (re)absorption and transport ^{47–51}. Experimental evidence in animals showed that calcium-adequate and magnesium-deficient diets may increase heart lipid peroxidation, plasma levels of triglycerides, and inflammatory markers; whereas both calcium-deficient and magnesium-deficient diets were associated with significant reductions in inflammatory markers, lipid peroxidation and a normal plasma triglyceride level ^{52,53}. However, no previous human study has examined the potential modifying effect of the

Ca:Mg intake ratio in relation to the lipid panel and uric acid, which may have a role in the development of colorectal neoplasia. This is supported by findings from recent studies which indicated that Ca:Mg intake ratio modified the associations between intakes of calcium and magnesium and risk of total, cardiovascular disease and cancer mortalities ²⁸.

B. Study objectives

This thesis is composed of three projects and has three well-connected purposes:

Project A. Calcium: magnesium intake ratio and colorectal carcinogenesis, results from the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial.

1A. To determine the modifying effect of Ca:Mg intake ratio on the association between calcium intake and the risk of colorectal neoplasia.

1B. To measure the association between calcium intake and risks of incident adenoma, metachronous adenoma, and CRC.

1C. To assess whether calcium intake provides additional protection against incident colorectal neoplasia in individuals receiving endoscopic screenings

Project B. Ca:Mg Ratio and Lipid Profile, a Randomized Clinical Trial.

1A. To examine the effect of magnesium supplementation may have in the change of lipid biomarkers in the Personalized Prevention of CRC Trial (PPCCT)

1B. To investigate whether reducing the Ca:Mg intake ratio under 2.6 among participants with high ratios by magnesium supplementation changes the serum lipid level in the PPCCT.

Project C. Ca:Mg Ratio and Uric acid, a Randomized Clinical Trial.

1A. To examine whether the magnesium supplementation may have a role in uric acid metabolism

1B. To investigate whether reducing the Ca:Mg intake ratio under 2.6 among participants with high ratios by magnesium supplementation changes the serum uric acid level in the PPCCT

C. Organization of the thesis

This thesis is divided into five chapters. *Chapter 1* is an overall introduction of the studies including the introduction of background and significance of the studies, the objectives of the thesis and the structure of the thesis. *Chapter 2* reviews the topic of main exposures as well as outcomes in details. *Chapter 3* presents the research methods applied in studies including descriptions of populations, the data collection, power analyses, and the statistical analysis. Chapter 4 is a result section. Chapter 5 discusses and summaries key findings, significance and implications of the study results.

Chapter 2. Literature review

A. Colorectal neoplasia

1. The worldwide and United States burden

CRC is the third most common incident cancer with an estimated worldwide age-standardized rate (ASR) of 17.2 per 100,000 for both sexes in 2012¹. Men usually have slightly higher incidence rates than women (overall sex ratio 1.44:1)¹. Based on cancer statistics from the Surveillance, Epidemiology, and End results program, CRC incidence rates have great geographical variations⁵⁴. The majority (55%) of the cases occur in more developed regions⁵⁴. Australia/New Zealand has the highest estimated incidence rates with ASRs of 44.8 and 32.2 per 100,000 in men and women, respectively. On the other hand, the lowest incidence occurs in Western Africa (ASR: 4.5 and 3.8 per 100,000 for men and women respectively)⁵⁴. However, global trends are changing over time: certain countries such as Japan and Korea with historically low incidence are currently increasing; while historically high-risk countries are variably increasing (Finland, Norway), remaining steady (in most Western and Northern European countries) or decreasing (the U.S.)³.

The worldwide estimated age-standardized mortality rate is 8.5 per 100,000 for both sexes in 2012, which ranked CRC as the fifth leading cancer related death in the world¹. There is less geographical variation in mortality rates; however, more deaths (52%) happen in the less developed regions of the world⁵⁴. The estimated mortality rates are the highest in Central and Eastern Europe (ASR: 20.3 per 100,000 for men, 11.7 per

100,000 for women), and the lowest in Western Africa (ASR: 3.5 and 3.0 for men and women per 100,000, respectively) ⁵⁴.

CRC is the third most common incident cancer in the U.S. ⁵⁵, with an incidence rate of 43.7 per 100,000 men and women per year based on 2007-2011 statistics ⁵⁶. CRC is also the third leading cause of cancer-related death ⁵⁵ with 15.9 deaths per 100,000 for both sexes per year ⁵⁶. It appears that there is an average 3% reduction per year for CRC incidence (**Figure 2.1**) and mortality (**Figure 2.2**) rates from the years 2002 to 2011 ⁵⁷.

CRC incidence and mortality rates vary widely by race and ethnicity within the U.S. ⁵⁵. Generally, both incidence and mortality rates are highest in blacks and lowest in Asians/Pacific Islanders. Based on data from 2006 to 2010, incidence rates in blacks are nearly 50% higher than those in Asians/Pacific Islanders; death rates in blacks (29.4 per 100,000 population) are almost twice as high as those in Asians/Pacific Islanders (13.1) ⁵⁵.

The stage of CRC is determined by “size and extent of the primary tumor, absence or presence of regional lymph node involvement, and absence or presence of distant metastases” ⁵⁸. The stage of cancer at diagnosis is essential in determining the type of therapy and estimating prognosis outcome ³. The earlier CRC is discovered and treated, the better survival rates appear. The five-year survival rates are 90.3%, 70.4% and 12.5% for local, regional and distant stages, respectively ⁵⁵. Forty percent of patients with CRC

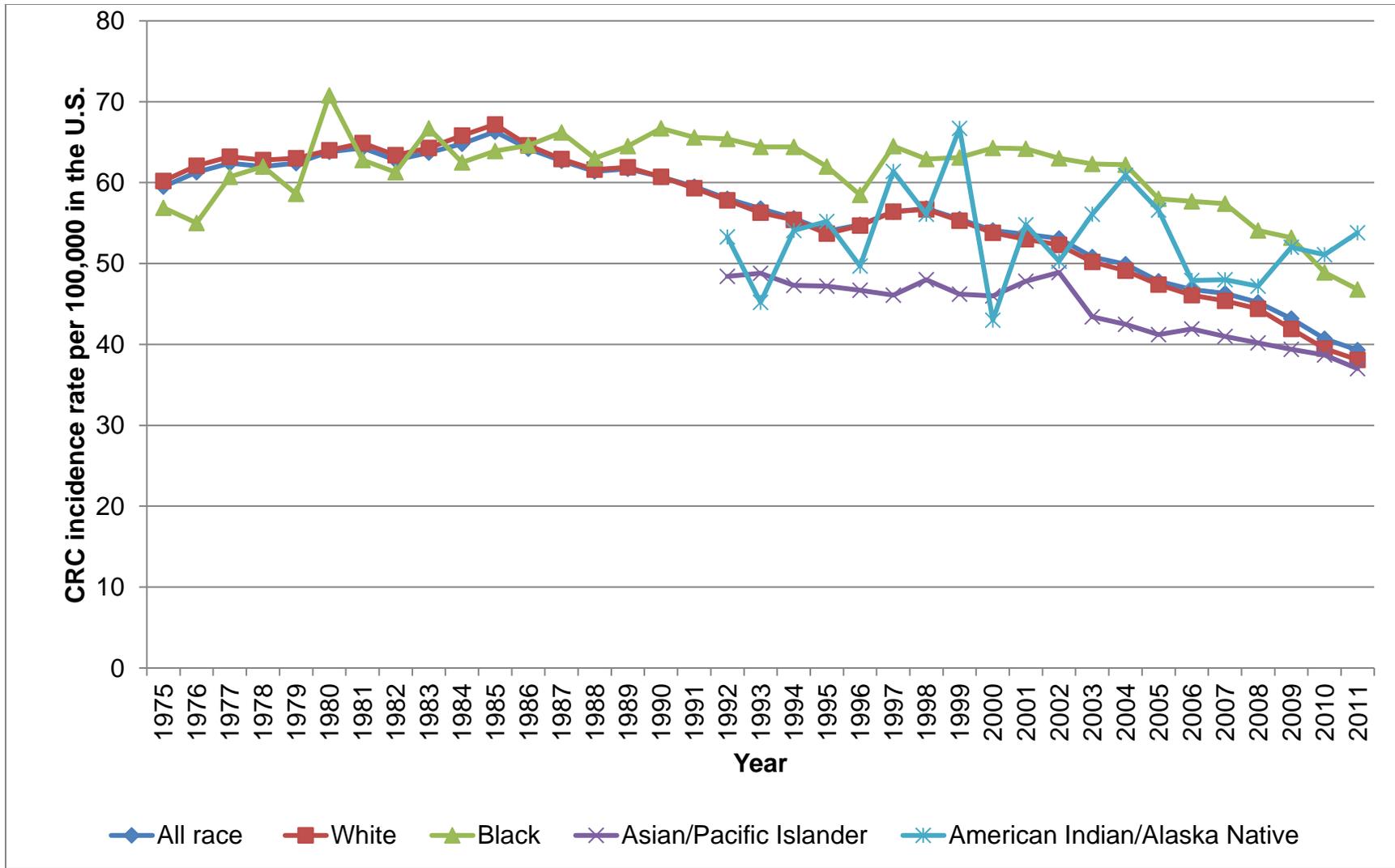


Figure 2.1 The age-standardized CRC incidence rate by ethnicities in the U.S.

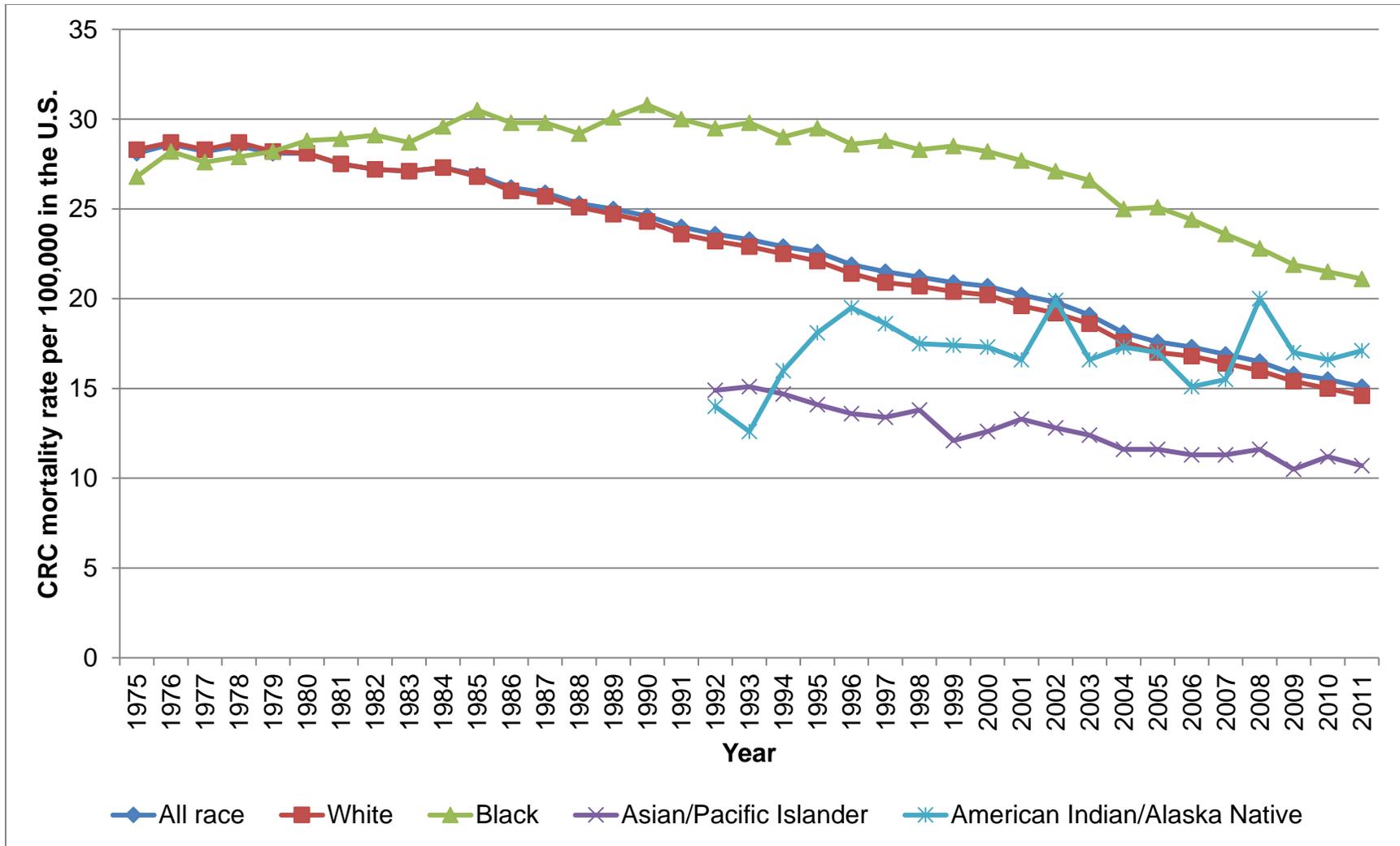


Figure 2.2 The age-standardized CRC mortality rate by ethnicities in the U.S.

are diagnosed with localized disease and 36% and 20% of CRC s are diagnosed with regional and distant disease, respectively ⁵⁵. In an early stage, CRC is usually asymptomatic while symptoms such as a change in bowel habits, rectal bleeding, and blood in stool may appear in a later stage ³. Both the absence of symptoms until later stages and the availability/use of screening affect the stage of diagnosis.

CRC is most commonly diagnosed in the proximal colon (42%) followed by the rectum (28%) and distal colon (23%) ⁵⁵. The etiologies and carcinogenesis of CRC varies substantially by site of diagnosis ^{55,59-61}. The frequency of CRC by subsites varies with age and sex. In the population with age over 65 years, women have higher rates of CRC incidence and mortality, compared with men ⁶². Women, especially aged 80 years and older, have a higher proportion of proximal colon cancer, while men are more likely to be at risk of rectal cancer ⁶³⁻⁶⁵. It was hypothesized that oral contraceptive and hormone related-factors may explain potential differences in CRC risk by subsite and sex ^{63,66}. However, the mechanism of sex differences in subsite-specific CRC has not been fully understood.

2. The development of colorectal neoplasia

2.1 Anatomic features and physical function of the colon and rectum

An integral part of the digestive system, the colon is the first part of the large bowel, is connected to the small bowel at the lower right abdomen and is about 5 feet long ⁶⁷. The four sections of the colon include ascending, transverse, descending, and sigmoid regions. The colon absorbs mineral nutrients and water from the food passed through

this tube, and retains the waste matter which is eventually expelled it through the rectum and anus, which is the end of the large intestine that is about 6-8 inches long ⁶⁷.

The wall of colon and rectum is made up of several layers. From the inner to the outer, its layers include mucosa, submucosa, thick muscle layers, subserosa and serosa (only for colon but not for rectum) ⁶⁸. The mucosa is the inner lining of colon and rectum and contains three layers (epithelium, connective tissue, and thin muscle layer). The submucosa is a layer of fibrous tissue under the mucosa ⁶⁸. A thick muscle layer beneath submucosa is vital in expelling fecal matter ⁶⁸.

2.2 The natural history of adenoma-carcinoma sequence

A polyp is a small cluster of nonmalignant cells and can form in the lining of colon or rectum ⁶⁹. Adenomas and hyperplastic polyps are two broad types of polyps that may develop. The colorectal adenoma is a benign glandular tumor of the colon and rectum ^{70,71}. Previous studies have identified the potential precursor role of adenomas in CRC development, thus a hypothesis of adenoma to carcinoma sequence was first reported in 1951 ⁷². Although it is still debatable, geographical, pathological as well as epidemiological evidence indicate the possibility of the adenoma-carcinoma sequence. The adenoma prevalence in geographical areas was correlated with CRC incidence in those same regions ⁷³. The evidence of reduction in CRC incidence after endoscopic removal of adenomas supports the theory of the adenoma-cancer sequence because removal of adenomas by polypectomy has been shown to reduce the incidence and mortality of CRC ^{74,75}. Moreover, colorectal carcinomas were observed to arise at the

site of polyps left *in situ* and ⁷⁰. In addition, there is evidence of a temporal relationship between adenoma and CRC development: the average age of diagnosis with an adenoma is, on average, about 4 or 5 years younger than the average age of cancer diagnosis ⁷⁰. The time length of the adenoma-carcinoma sequence varies among individuals ⁷⁰. The whole period may range from 10 to 15 years ⁶⁷.

The removal of polyps within an endoscopy screening is the most effective prevention strategy to reduce the morbidity and mortality of CRC ²⁹. However, polypectomy has been shown to be ineffective in inhibiting the progression of CRC carcinogenesis in around 24% of adenoma cases ⁷⁶. Recent evidence also suggests that colonoscopy is more effective in reduction of colorectal cancer deaths associated with the distal colon, but not proximal colon ³⁰⁻³².

Adenomas are very common and the prevalence varies from 7.4 to 52.5% ⁷⁷. A meta-analysis of 18 studies reported that the pooled prevalence of adenomas was 30.2% in an average at-risk adult population ⁷⁸. Approximately one third to one half of people will develop one or more adenomas in their lifetime ⁷⁹. However, the risk of transformation from adenoma to carcinoma in an individual is low. Colonoscopy screening is used to locate and remove adenomas. Follow-up studies among participants with negative colonoscopy findings at baseline suggested the incidence rate of subsequent CRC within 10 years was 0.006% ⁸⁰ while the CRC incidence rate after four years of follow up increased to around 0.6% for participants with advanced adenoma at the baseline ⁴.

Even though the malignancy rate for an adenoma is low, adenomas, compared with hyperplastic polyps, are most likely to develop into CRC ⁶⁷.

The characteristics of adenomas are important predictors for subsequent carcinogenesis. Adenoma size was one of main predictors in the progression to malignancy ^{70,71}. Recent reviews indicated that the proportion of malignancy for adenomas under 1 cm is around 1.3%, the malignant potential for adenomas with a diameter of 1-2 cm increases to 9.7%, and nearly one third to half of large-size adenomas with a diameter over 2 cm develop to invasive cancers ⁷⁰. In addition, villous adenoma was more likely to progress to carcinoma compared to tubular adenoma ^{70,71}. Other known factors that predisposed to CRC were adenomas with advanced features, familial polyposis and ulcerative colitis ⁷⁰. Advanced features include one of the following: >25% villous histology, size larger than 1 cm, and/or presence of high-grade dysplasia ^{81,82}. The coexistence of two or more adenomas diagnosed at the same time or within 6 months is defined as synchronous adenomas ⁸³. The incidence of advanced adenomas is higher in individuals who have synchronous adenomas ⁸⁴. The U.S. population undergoing endoscopy screening has a prevalence of 3.4 to 7.6% for advanced adenomas ⁴. Sessile serrated polyps are saw-toothed and differ from traditional adenomas ⁴. The accurate prevalence for sessile serrated polyps is unknown but current best estimate was approximately 6 to 12% ⁴. Metachronous adenomas are adenomas detected half to one year after the previous polypectomy ⁸³. Thus, it is possible that individuals with synchronous, large, advanced adenomas and sessile serrated polyps are at a higher risk of subsequent metachronous and advanced

adenomas as well as CRC ⁴. A cohort study based on private practice settings reported that the number of polyps at baseline was the only significant predictor for metachronous adenoma ⁸⁵. Additionally, another cohort study in the Netherlands has reported that three or more adenomas identified at initial colonoscopy was a significant risk factor for further lesions at subsequent surveillance ⁸¹.

CRC includes malignancies originating from the epithelial cells of the gastrointestinal tract, which includes the colon, rectum and appendix ⁸². The carcinogenesis progress is usually accumulated defects in tumor suppressor genes, oncogenes, and deoxyribonucleic acid (DNA) repair genes, which control cell growth, division, and apoptosis ⁸⁶. However, the majority of genetic abnormalities that is involved in the formation of carcinoma is believed to start with somatic genetic mutations rather than heritable mutations ³. The genetic abnormalities can be caused by endogenous factors such as hormones and cellular nutrient metabolism or exogenous factors such as radiations, toxins and chemicals ³. Genetic components such as aforementioned accumulated defects may influence an individual's propensity to develop polyps or CRC. Lifestyle or environmental factors could also initiate and promote the formation of polyps and subsequent CRC ⁸⁷.

3. Risk factors for colorectal neoplasia

There are several unmodifiable risk factors for colorectal neoplasia such as age over 50 years ⁵⁵, male gender, a family history of CRC including multiple first-degree relatives with CRC or relatives with CRC diagnosed at a young age, and personal history of

chronic inflammatory bowel disease ⁸⁴. A large number of studies have investigated modifiable risk factors associated with the development of and/or death due to CRC. Although it is inconsistent, behavioral factors which seem to decrease risk include being physically active, eating healthy, keeping an optimal body mass index (18.5 to 25), abstinence from smoking, no to light alcohol consumption, hormonal replacement therapy for postmenopausal women, regular endoscopy screening to remove polyps, and use of non-steroidal anti-inflammatory drugs (NSAIDs) ⁶⁷. Oral intake of NSAIDs is considered to be one of the most promising prevention strategies for colorectal carcinogenesis chemoprevention ⁸⁸. However, the long-term use of NSAIDs also carries risks for side effects.

Over the past few decades, a large number of studies have investigated the role diet has on the risk of CRC. Nutrients such as fiber ⁸⁹, vegetables ⁹⁰, vitamin C and E ⁹¹, carotenoids and other antioxidants ^{90,92-94} have been extensively studied and the results are inconsistent. Recent large epidemiologic studies and/or clinical trials regarding the chemopreventive effects of these nutrients did not provide further evidence to support the potential benefits of these nutrients. Contrary to the original hypothesis, folic acid supplementation appeared to increase rather than reduce the risk of colorectal neoplasia ⁹⁵. Similarly, although debatable, calcium alone or along with vitamin D probably protects against cancer ⁹⁶⁻⁹⁹.

4. Calcium and carcinogenesis

4.1 Calcium intake and ionized calcium

Calcium is a vital mineral in human metabolism and can be obtained from the diet ¹⁰⁰. Ionized calcium balance is a comprehensive function of oral intake, intestinal absorption, renal reabsorption and skeletal modeling ¹⁰¹. Ionized calcium is vital for cellular functions and initiation of numerous physiological metabolic pathways ¹⁰². Intracellular calcium functions as a secondary messenger in pathways that involve secretion of many hormones and neurotransmitters ¹⁰².

4.2 Mechanisms of calcium in relation to cancer

The homeostasis of cancer is regulated by molecular-calcium pathways including: active energy-dependent calcium transporters, calcium-permeable ion channels, calcium-binding and storage proteins, and calcium-dependent effectors ¹⁰³. Despite inconsistencies, calcium may also function by reducing cell proliferation, stimulating differentiation, inducing apoptosis and regulating the cell-cycle of normal and tumor colorectal cells ^{104–107}. Therefore, aberrant calcium signaling may be associated with uncontrolled proliferation, unregulated apoptosis, and metastasis of cancer cells.

Although it may be assertive to state that aberrant calcium signaling is a cause of molecular and tissue homeostasis change and subsequent carcinogenesis; it would be better to consider aberrant calcium signaling as a dynamic consequence of genome, epigenetics, environmental factors, adaptation and compensation that triggers cancer initiation or progression¹⁰³. In other words, mutations, over/under expression,

regulations of calcium handling toolkits of proteins are potential causes of aberrant calcium signaling in cancer ¹⁰³.

Newmark *et al.* first reported that calcium may have a chemopreventive effect on CRC in 1984 ¹⁰⁸. Calcium is known to bind secondary bile acids and ionized fatty acids in the colon lumen to form insoluble calcium soaps, preventing bile acids and fatty acids from damaging the mucosa of the intestinal lumen by inhibiting their proliferative effects ¹⁰⁸. Several studies ¹⁰⁸⁻¹¹⁰ have supported that dietary calcium may decrease cytotoxicity, nitroso compounds, and lipoperoxidation in feces of dimethylhydrazine-initiated rats and human volunteers, thereby subsequently reducing CRC risk attributed to processed meat intake.

4.3 Epidemiological studies on calcium and colorectal neoplasia

A few studies have reported a significant protective effect of calcium on colorectal neoplasia ^{18,110,11,8} while a few studies have not detected such beneficial effect ^{125,126}. A pooled-analysis of 10 cohort studies suggested that the highest quintile of total calcium consumption from both dietary and supplemental sources may confer a 14% reduction of risk in CRC versus the lowest quintile, with the association only being significant among people with high intake of vitamin D ²². Epidemiologic studies found high calcium consumption was associated with approximate 20-30% reduction in risk of colorectal adenoma ¹⁷⁻²⁰, metachronous colorectal adenoma ²³⁻²⁵, and overall CRC risk ^{21,22}. The protective effect of calcium supplementation may extend up to 5 years after cessation of

active treatment ¹²⁷. **Figure 2.3** is a hypothesized diagram of calcium intake in relation to adenoma-carcinoma sequence.

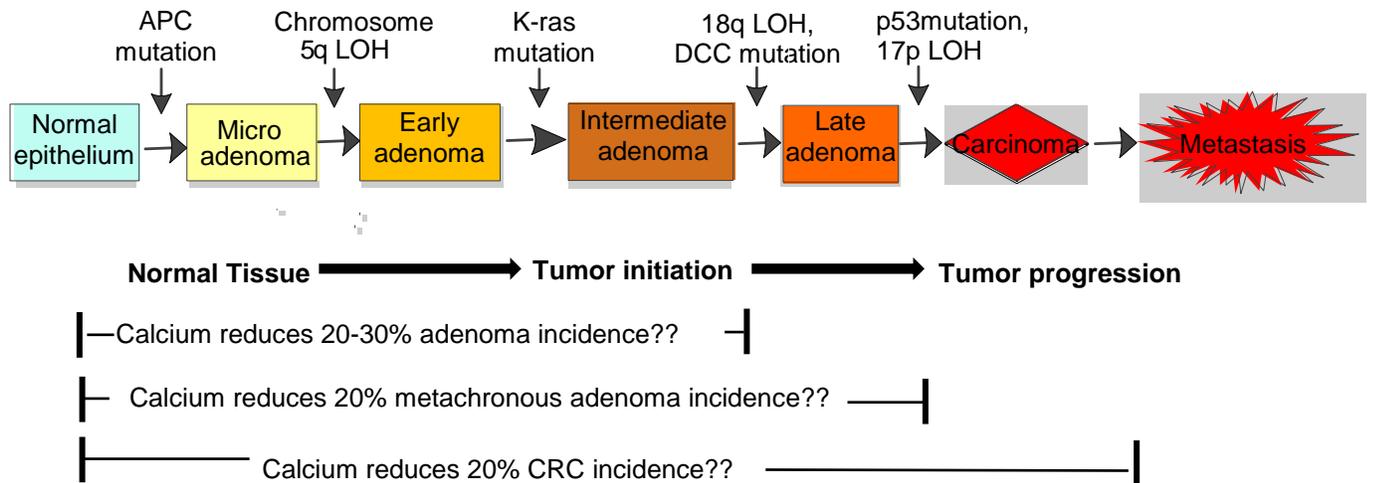


Figure 2.3 Calcium intake on adenoma-carcinoma sequence derived from Morán A et al. 2010

Consistent with the results from these epidemiologic studies, two large intervention trials observed that calcium supplementation moderately reduced the risk of metachronous colorectal adenoma ^{23,24}, while one trial found that this protective effect may only be present in those with a high blood level of vitamin D ²⁵. Baron *et al.* ¹²⁷ found in the observational phase of a clinical trial that the protective effect of calcium supplementation on metachronous colorectal adenoma rate extends up to 5 years after cessation of active treatment. However, results from a very recent large-scale randomized clinical trial (Women's Health Initiative, WHI) do not support an effect of calcium plus vitamin D (CaD) supplementation for 7 years on the incidence of CRC ¹⁵. An updated analysis of WHI study showed that among participants with no personal calcium or vitamin D supplements, CaD was significantly associated with decrease the risk of total cancers and non-significantly reduces the risk of CRC ; however, CaD did

not alter cancer risk in women who took personal calcium or vitamin D supplements ¹²⁸. Clinical trials regarding calcium supplementation for at least 6 months on risk of colorectal neoplasia is summarized in **Table 2.1**.

Calcium sensing receptor (*CaSR*), mainly expressed in the parathyroid gland and the renal tubules of the kidney, controls calcium homeostasis by regulating the release of parathyroid hormone and by inhibiting the reabsorption of calcium in the kidney ^{111,112}. Polymorphisms of the *CaSR* gene were reported to affect serum calcium concentrations ^{111,113,114}. However, genetic epidemiological studies have reported both significant ^{115–119} and null association ^{120–123} between variant alleles of the *CaSR* gene and risk of colorectal tumors. A meta-analysis ¹²⁴ of the *CaSR* gene found that polymorphisms of rs1042636 were associated with reduced risk of proximal and distal CRC; variant alleles of rs1801726 were associated with increased risk of distal but not proximal CRC; and rs1801725 polymorphisms were not associated with risk of CRC.

5. Magnesium and carcinogenesis

Magnesium, involved in more than 300 metabolic functions ¹²⁹, is an essential element for the body. Similar to the distribution of calcium in humans, approximately 60% of the magnesium is reserved in the bones and teeth, 20% in the muscle tissue, 19% in soft tissues such as heart, brain, liver and kidney, and less than 1% in the extracellular fluid including erythrocytes and serum ¹³⁰. An analysis of data from the National Health and Nutrition Examination Survey (NHANES) of 2005-2006 found that a majority of

Table 2.1 Randomized clinical trials of calcium supplementation and the risk of colorectal neoplasia

Authors	Study characteristics	Interventions	Outcomes	Baseline calcium (mg/day)	Main findings Highest vs lowest category of calcium OR (95%CI)
Baron J, et al. 2015	N=2259, Multicenter, Patients aged 45-75 and with recently diagnosed adenomas and no known colorectal polyps remaining after complete colonoscopy	Daily vitamin D3 (1000 IU) N=420, calcium as carbonate (1200 mg) N=419, both N=421, or neither N=415, for a mean duration of 4 years	Colorectal metachronous adenoma	--	Ca vs no Ca: 0.95 (0.85-1.06) Ca by baseline Ca≤597 mg/day: 0.90 (0.78-1.05) Ca by baseline Ca>597 mg/day: 0.97 (0.82-1.15) Ca by Ca:Mg ≤ median ^a : 0.68 (0.52, 0.90) Ca by Ca:Mg > median ^a : 0.98 (0.75, 1.28)
Bonithon Kopp, 2000	665 patients with a history colorectal adenomas	2 g elemental calcium, or 3.5 g fibre, or placebo daily for 3 years	Colorectal metachronous adenoma	944±364	Calcium /placebo: 0.66 (0.38,1.17)

^a: results among 930 subjects were previously published by Dai Q¹¹, 2012.

Americans at all ages have less magnesium intake than recommended daily intakes ¹³¹. Adult men aged 71 years and older and adolescent females are most likely to have low magnesium intakes ¹³¹. A low magnesium diet in the long term is associated with reduced serum magnesium and intracellular free magnesium in red blood cells ¹³².

Magnesium is a necessary nutrient needed to provide energy supply to cells. It plays a vital role in the active transport of calcium and potassium ions across cell membranes ¹²⁹. Magnesium is also inversely associated with anti-inflammatory biomarkers ¹³³.

Larsson *et al.* ¹³⁴ proposed evidence that magnesium may prevent colon cancer by reducing oxidative stress, improving insulin sensitivity, or by other means of decreasing colonic epithelial cell proliferation.

Fewer studies have investigated possible associations between magnesium and colorectal neoplasia than have been conducted for association with calcium. A case-control study found that magnesium level in drinking water was not associated with death from cancers of the colon ¹³⁵ and rectum ¹³⁶. Two prospective studies observed an inverse association between total magnesium intake and incident CRC ^{134,137}, although one study reported that the inverse association was only limited to colon cancer ¹³⁷. A cohort study in Japanese has indicated the inverse association was only significant for men but not for women ¹³⁸. However, two prospective studies have reported null results ^{139,140}.

6. Calcium to magnesium ratio

Micronutrients of calcium, magnesium, phosphorus, and vitamin D share common food sources and are considered to be “metabolically interactive”¹⁴¹. Magnesium has an active role in adenosine triphosphatase, which is the energy source for ion exchange pump¹⁴². Intracellular magnesium deficiency may result in an increase in intracellular calcium¹⁴³. Examining the effect of a single micronutrient without taking into account the influence of complementary or opposing nutrients may lead to residual confounding and incomplete risk assessment¹⁴⁴.

Calcium and magnesium share the same homeostatic regulatory system⁴⁵ primarily through absorption in intestine and reabsorption in kidney to keep the balance of Ca:Mg^{45,46}. Nevertheless, magnesium and calcium compete with each other in (re)absorption and transport⁴⁷⁻⁵¹. High calcium intake reduces absorption of both magnesium and calcium¹⁴⁵ whereas moderate magnesium deprivation results in negative magnesium balance while increased calcium retention¹⁴⁶. Extracellular concentration of magnesium has been shown to competitively inhibit the transportation of calcium¹⁴⁷. In addition, magnesium supplementation has been associated with increased urinary calcium excretion when calcium intake was less than 800 mg/day¹⁴⁸, suggesting that magnesium may suppress calcium reabsorption when Ca:Mg intake ratio is very low²⁸. Therefore, the Ca:Mg intake ratio may take an important role in regulating calcium and magnesium (re)absorption. Several recent studies have postulated and preliminarily confirmed that the dietary ratio of calcium to magnesium modifies the effects of calcium and magnesium intakes on carcinogenesis^{17,28,149}. In addition, one animal study has

indicated that the severity of inflammation caused by magnesium deficiency could be reduced by calcium deficiency⁵². Growing evidence strongly suggests that the balance of calcium and magnesium plays an essential role in many diseases⁵². Considering that more than 45% of the U.S. population aged 40 or older are users of calcium supplements, an investigation into the balance of calcium and magnesium (Ca:Mg ratio) in colorectal carcinogenesis is particularly important.

6.1 Calcium to magnesium ratio and chronic diseases

Magnesium and calcium also potentially antagonize each other in many physiologic activities^{47,48}, including insulin resistance¹⁵⁰, oxidative stress¹⁵¹, systematic inflammation¹³⁴, cell proliferation, differentiation, angiogenesis, and apoptosis^{47,49,52,108,152,153}. These activities may subsequently result in carcinogenesis. Dai *et al.* first reported that dietary intake ratio of Ca:Mg modified the association between intakes of calcium and magnesium in relation to colorectal adenoma¹⁷. Ca:Mg ratio also interacted with a functional common polymorphism in the gene encoding *TRPM7*, a channel regulating Ca²⁺ and Mg²⁺¹⁵⁴, in relation to both colorectal adenoma and hyperplastic polyps¹⁷. A subsequent randomized clinical trial reported that calcium supplementation only reduced risk of colorectal metachronous adenoma when the baseline Ca:Mg was less than or equal to 2.63²⁶. In addition, a high serum Ca:Mg was significantly associated with an increased risk of high-grade prostate cancer even after adjusting for serum levels of calcium and magnesium¹⁴⁹. In support of these findings, one case-control study in Belgians has reported a high calcium but low magnesium was associated with increased risk of bladder cancer¹⁴⁴. East Asian populations have a

similar intake level of magnesium as the U.S. population ^{155,156}. A high magnesium intake at U.S. recommended dietary allowance level was associated with increased risk of total mortality, cardiovascular diseases and cancer mortality in East-Asian populations with a very low Ca:Mg ratio (1.7(median)) ^{28,156}; whereas in the U.S. population with a high Ca:Mg ratio (median=3.0) ¹⁵⁵, high magnesium intake was significantly linked to reduced risk of these diseases ^{134,139}. In the same cohort study conducted in east Asians, the association between intakes of calcium and magnesium with the risk of total, cardiovascular disease and/or cancer mortalities were modified by Ca:Mg ratio, but not by calcium or magnesium intake alone ²⁸. These findings were consistent with that from the Nurses' Health Study which reported that the interaction between total calcium and magnesium intakes was not statistically significant in relation to the risk of CRC incidence ¹³⁹. The direct associations between Ca:Mg ratio and chronic diseases are summarized in **Table 2.2** and the modification effect of Ca:Mg ratio on associations between calcium intake or serum calcium levels and the risk of common chronic diseases are presented in **Table 2.3**.

7. Hypothesized explanations for inconsistent associations between calcium and colorectal neoplasia

Factors such as the dose and duration of calcium and vitamin D treatment have been proposed to explain the inconsistency in findings across studies ^{99,157}. Prospective studies of CRC and calcium intake suggested that 600-1000mg of calcium per day may be ideal for CRC risk reduction, with no further protection beyond this range ¹⁵⁸. The Nurses' Health Study and the Health Professionals Follow-up Study also indicated a

Table 2.2 The direct effect of Ca:Mg ratio on the risk of common chronic diseases

Authors	Population	Exposures		Outcomes	Study Design	Main findings Highest vs lowest category of calcium OR (95%CI)
		Dietary Ca/Mg	Serum Ca/Mg			
Dai Q, et al ¹ . 2010	494 NMHS participants including 331 cases and 163 controls	--	Yes	Prostate cancer cases	Case-control	Higher VS lower Ca:Mg ratio: 2.81 (1.24, 6.36)
Takata Y, et al. 2013	71, 267 females From SWHS in China	Yes	--	Lung cancer	Cohort	Higher VS lower Ca:Mg ratio: 0.62 (0.47, 0.82)
Sun, Y, et al ¹⁸ . 2013	--	--	Yes	Proliferation of prostate cancer cells	In-vitro study	An increase in the serum ratio of Ca ²⁺ /Mg ²⁺ promoted proliferation for the initiation/progression of prostate cancer cells by activating TRPM7 channel
Ziniewicz HK, 2015	48 neonates	--	Yes	Cardiovascular disease markers, insulin sensitivity/resistance markers (e.g. glucose, insulin, HOMA)	Cross-sectional study	The Ca:Mg ratio is negatively related to arylesterase (AE) (p<0.01), AE/HDL-c (p<0.05), and low levels of insulin Serum calcium negative correlated with HDL-c (p<0.05), arylesterase (AE) (p<0.01), the Apo A1/Apo B (p<0.05) and AE/HDL-c (p<0.05) ratios Neonates within the highest quartile for Mg displayed significantly higher levels of LDL-c and homocysteine (p<0.05)

Table 2.3 Studies of the modification effect of Ca:Mg ratio on associations between calcium and the risk of diseases

Authors	Population	Exposures		Outcomes	Study Design	Main findings Highest vs lowest category of calcium OR (95%CI)
		Components				
		Diet	Supplement			
Dai Q, et al ² . 2007	898 cases and 1306 controls from the TCPS	Yes	Yes	Colorectal adenoma cases , hyperplastic polyp cases	Case-control	Diet Ca: 1.12 (0.76, 1.65) Total Ca: 0.98 (0.68, 1.40) Total Ca by Ca:Mg ≤ 2.78: 0.72 (0.30, 1.69) Total Ca by Ca:Mg > 2.78: 1.32 (0.69, 2.53)
Dai Q ¹¹ . 2012	930 subjects	--	Yes (Treatment)	Colorectal metachronous adenoma	Randomized clinical trials	Ca by Ca:Mg ≤ median: 0.68 (0.52, 0.90) Ca by Ca:Mg > median: 0.98 (0.75, 1.28)
Dai et al, 2013	Over 130,000 participants. From SWHS and SMHS in China	Yes	Yes	All-cause mortality and disease-specific mortality	Cohort	Men Total mortality by Ca:Mg ≤1.7: 0.95 (0.66, 1.36) Total mortality by Ca:Mg >1.7: 0.59 (0.44, 0.80) CHD mortality by Ca:Mg ≤1.7: 1.64 (0.73, 3.70) CHD mortality by Ca/Mg>1.7: 0.48 (0.21, 1.09) Women Total mortality by Ca:Mg ≤1.7: 1.03 (0.78, 1.36) Total mortality by Ca:Mg >1.7: 1.06 (0.81, 1.39) CHD mortality by Ca:Mg ≤1.7: 1.92 (1.00, 3.70) CHD mortality by Ca/Mg>1.7: 0.85 (0.41, 1.76)

daily intake of 700-800mg calcium is beneficial for CRC prevention⁹⁰. These findings may explain the null effects found in the WHI, in which women had a total calcium intake of approximately 2150 mg/day (1000 mg of Ca carbonate plus a baseline mean intake of 1151 mg)¹⁵⁸, which is far from the beneficial range summarized from prospective studies. However, this does not explain why another clinical trial using similar or even higher levels of calcium supplementation (1200 mg/day) and total calcium intake (>2100 mg/day) found calcium intake was associated with reduced risk of metachronous adenoma²³. It is plausible that there is a population difference. Participants recruited in these metachronous adenoma trials have a high risk of colorectal polyps, whereas participants included in the WHI are generally post-menopausal female subjects.

Furthermore, evidence from earlier studies suggested that there is a substantial variation of the ability to (re)absorb calcium in healthy people, which is mostly attributed to genetic variation¹⁵⁹. Although not consistent, several previous studies found the association between calcium intake and CRC risk may be modified by genetic polymorphisms in *vitamin D receptor*^{160,161} and *calcium-sensing receptor*^{162,163}. Dai *et al.* also reported the association of calcium intake and risk of colorectal adenoma may differ by polymorphism in the *transient receptor potential melastatin 7 (TRPM7)* gene¹⁷. Moreover, high calcium intake was significantly associated with reduced adenoma risk among carriers with variant alleles in genes of potassium channel, inwardly rectifying subfamily J, member 1 (*KCNJ1*) and solute carrier family 12, member 1 (*SLC12A1*)¹⁶⁴. Future research on adequate nutrients intake and targeting for subpopulations with high

risk such as certain genetic profiles (gene-nutrient interactions) is going to advance the field of cancer chemoprevention and prevention of other common chronic diseases.

It is also reasonable to investigate whether the balance of calcium and other nutrients could help to explain the discrepancy. The beneficial effect of nutrient supplementation was reported in a large clinical trial conducted in a population in China, at high risk of nutritional deficiency ¹⁶⁵. This indicated that supplementation of nutrients may only be effective among high-risk populations with nutritional deficiency. Furthermore, from these trials, we learned that excessive supplementation of nutrients also could lead to adverse effects ¹⁶⁶. High levels of calcium consumption or serum levels were associated with a reduced risk of CRC or adenoma²² but an increased risk of advanced/fatal prostate cancer ^{167–174}. Growing evidence showed that some nutrients or metals, including calcium, showed protective effects on cancer at a normal range while harmful effects at a high dose ¹⁶⁵. These evidences further support the hypothesis of nutrients balance. The Ca:Mg intake ratio, a hypothesized marker for calcium and magnesium balance, has not been considered in most of previous studies, except for some recent publications ^{17,26}, in which the inverse association between calcium intake and adenoma prevalence and recurrence only appeared when the Ca:Mg intake ratio is low. Thus, studies on Ca:Mg ratio ^{17,26,149} provide further evidence for the theory of nutrients balance.

8. Chronic diseases related to colorectal neoplasia

Epidemiological studies have shown that diabetes ^{34–37} and metabolic syndrome ^{38–40} are strongly associated with risk for CRC development. The metabolic syndrome is a

cluster of metabolic abnormalities including dyslipidemia (hypertriglyceridemia and decreased fasting serum high-density lipoprotein cholesterol (HDL-c)), glucose intolerance, hypertension, insulin resistance, and central obesity. Higher blood pressure, total cholesterol, triglyceride, and lower HDL-c levels have been associated with increased risk of colorectal adenoma ¹⁷⁵. Hypertension, one of main components in metabolic syndrome, is a risk factor for recurrent colorectal adenoma ¹⁷⁶. A recent meta-analysis has reported that metabolic syndrome was associated with 34% increase in the risk of colorectal neoplasia ¹⁷⁷. Diabetes was associated with an elevated risk for both proximal and distal colon adenomas, but was more strongly associated with proximal colon adenoma and with large adenomas (≥ 5 mm in diameter) ¹⁷⁸. The mechanism underlying these relations is not completely understood.

B. The lipid profile

1. The composition and physical function of lipid profile

The lipid profile or lipid panel is a pattern of lipids in the blood, which is also a group of blood tests used to assess the risk of developing cardiovascular disease or to monitor treatment effect. Typically, the lipid profile includes low-density lipoprotein cholesterol (LDL-c), HDL-c, triglycerides, and total cholesterol ¹⁷⁹. Some lipid measurements also include very low-density lipoprotein (VLDL) if the LDL-c is very high.

LDL-c mainly facilitates the transportation of fat and a small amount protein from liver to other peripheral tissues ¹⁷⁹. VLDL is produced in the liver primarily from dietary triglycerides ¹⁷⁹. It is usually estimated by a percentage of the triglyceride. The main

function of VLDL is to distribute the triglyceride produced in the liver. HDL-c is called "good" cholesterol ¹⁷⁹. It was postulated that HDL-c helps transport cholesterol from peripheral tissues back to the liver, where the majority of cholesterol were catabolized and excreted ¹⁸⁰⁻¹⁸². Higher levels of HDL-c is associated with lower risk for cardiovascular disease ¹⁷⁹. Triglycerides are the fats absorbed in the blood through diet or made by the liver in response to diets rich in sugars, refined carbohydrates, or fats ¹⁷⁹. The body utilizes triglycerides to store energy and provide a source energy to muscles. Only small amounts are found in the blood. Cholesterol is necessary for constructing the lipid layers in cells and for hormone production ¹⁷⁹. Excessive blood cholesterol accelerates blockages of inside arteries, which can increase risk for cardiovascular disease.

2. The development of cardiovascular disease

Initially, cholesterol deposits and accumulates in the arteries that supply to the heart ¹⁸³. With gradual accumulation over time, the lumen of the artery begin to narrow. This process is called atherosclerosis. Atherosclerosis in the coronary artery is known as coronary artery disease, which is the most common type of cardiovascular disease ¹⁸⁴.

3. Contributing factors of cardiovascular heart disease

Heart disease is the leading cause of death in the U.S. ¹⁹⁷. Multiple factors (i.e. diabetes, smoking, high blood pressure, obesity, physical inactivity, and genetics) could affect lipid panel and are deemed as contributing factors of cardiovascular disease ¹⁹⁸. Greater control of risk factors and improved treatment are considered main contributors

to the reduction of mortality ^{199,200}. The administration of statin treatment is associated with a 20% reduction in total cholesterol, 28% reduction in LDL-c, 13% reduction in triglycerides, and 5% increase in HDL-c ¹⁸⁸. Combinations of fibrate, niacin (North and South America), omega-3 fatty acids or ezetimibe with a statin further reduced cardiovascular risk ²⁰¹. However, a large residual risk of cardiovascular disease remains unexplained ^{187,201}.

4. Magnesium and lipids

Kobayashi first reported in an ecological study that the hardness of drinking water was inversely associated with mortality rate due to stroke ³⁰⁵. Since then, numerous studies have investigated the associations of calcium and magnesium content in drinking water ³⁰⁶ or dietary intake ^{213,220,236,307–309} on lipid metabolism and the risk of subsequent cardiovascular disease. Early observational studies reported positive ²²⁷, inverse ²²⁸, and null ²²⁹ associations between blood magnesium and cholesterol.

Early experimental data from animal studies have shown that magnesium plays a role in the pathogenesis and treatment of vascular disease ^{202–204}. In several species, magnesium deficiency was associated with marked hypertriglyceridemia, hypercholesterolemia, increased LDL-c, and a decrease in the percentage of cholesterol transported by HDL-c ^{202–204}. Increased magnesium intake has been shown to improve insulin sensitivity, hyperglycemia, hyperlipidemia, and oxidative stress and has been shown to reduce lipid peroxidation in fructose-fed rats ²⁰⁵. These findings indicate that magnesium deficiency worsens several parameters of lipids.

A number of small-scale clinical trials have also generated inconsistent results on the effects of magnesium supplementation on lipid profile. Magnesium supplementation significantly improved lipid profile, including a reduction in total cholesterol, LDL-c and triglycerides in a few trials ^{206,207}, but not in others ^{208,209 210,211}. A meta-analysis of nine clinical trials found magnesium treatment significantly, but weakly, increased HDL-c, and had no effect on LDL-c and triglycerides ²¹². A clinical trial among patients with type 2 diabetes mellitus has observed a significant fall in serum total cholesterol, LDL-c and triglycerides as well as a rise in HDL-c levels in 4 to 8 weeks after the initiation of magnesium supplementation. This effect continued to be observed till the end of the study 12 weeks ²¹³. However, none of these previous studies have considered the Ca:Mg balance and this may explain a part of the inconsistency in previous studies.

5. Calcium and magnesium with lipid

High calcium supplementation was linked to increased risk of myocardial infarction ^{232,233}. Conversely, some studies found that high calcium from dietary sources were associated with a reduced risk of cardiovascular disease ^{234,235}. Two grams of calcium carbonate supplementation was associated with decreased triglycerides and possibly total cholesterol in a small clinical trial with 92 colorectal adenoma patient ²³⁶. Previous intervention trials also generated inconsistent results on the effect of calcium supplementation on lipid profile ^{307,313,314}. It was hypothesized that dietary sources of calcium usually goes together with reasonably high magnesium dietary intake, which leads to a reasonable Ca:Mg ratio. In contrast, calcium supplements alone substantially increase the Ca:Mg ratio. This may explain the differential effects of calcium from

dietary ^{234,235} and supplemental sources ^{232,233} on cardiovascular disease risk and mortality.

Experimental studies have suggested that the balance between calcium and magnesium may modify markers of lipid profile. Evidence from animal studies reported that calcium-adequate and magnesium-deficient diets lead to increases in heart lipid peroxidation, plasma levels of triglycerides and inflammatory markers; whereas a combination of calcium-deficient and magnesium-deficient diets cause significant reductions in inflammatory markers, lipid peroxidation and a normalization of plasma triglyceride concentration ^{52,53}. These findings highlight the critical role of the dietary Ca:Mg balance in the development of dyslipidemia and subsequent cardiovascular disease.

Patients with cardiovascular disease such as variant angina ²³⁷ and myocardial infarction ²³⁸ usually have magnesium deficiency ²³⁹. Calcium channel-blocker therapy was proved to increase serum or fluid magnesium ^{237,238}. Dai *et al.* proposed that calcium and/or magnesium intake may be associated with an increased risk of cardiovascular disease mortality if the Ca:Mg ratio is either ≥ 2.6 or ≤ 1.7 , but associated with a reduced risk or no risk when the Ca:Mg ratio is between 1.7 and 2.6 ²⁸. The modifying effect of the Ca:Mg ratio may explain the inconsistent results of previous studies pertaining to calcium and magnesium intakes and cardiovascular disease risk ^{216,220} or between magnesium supplementation and lipid profile change ^{206–209,211,212}.

Previous trials of magnesium supplementation enrolled subjects with both high and low Ca:Mg ratios; the high doses of magnesium generally used in these trials may have resulted in a very low Ca:Mg ratio (<1.7), particularly for those with a low ratio at baseline ²¹². These findings indicate the critical role of the dietary Ca:Mg balance in cardiovascular disease development. In other words, the effects of dietary magnesium and calcium on cardiovascular disease may differ by Ca:Mg intake ratio levels in the diet.

6. Potential mechanisms for magnesium efficacy on cardiovascular disease

The enzyme activity of lecithin cholesterol acyltransferase (LCAT) ²⁴⁰ and lipoprotein lipase (LPL) ²⁰², which lowers triglycerides levels and raises HDL-c levels, requires the involvement of magnesium ²⁴¹. The lipid lowering effect of magnesium could be attributed to improved activity of LPL or insulin, or decreased actions of lipogenic liver enzymes ^{242,243}. Moreover, magnesium²⁺- adenosine triphosphate (ATP) determines the rate-limiting enzyme in the cholesterol biosynthesis, which is associated with cholesterol levels ²⁴¹. In the pathway of cholesterol biosynthesis, the conversion of 2-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) to mevalonate requires the enzyme, HMG-CoA reductase, which is the rate-limiting enzyme ²⁴⁴. The statins and magnesium inhibit that enzyme ²⁴⁴.

The role of Ca:Mg intake ratio in lipid metabolism is less frequently studied. Thus, there are no reports of whether the change of Ca:Mg intake ratio by magnesium supplementation may have a role in lipid metabolism. No previous studies investigated

the hypothesis that lipid profile is an potential intermediate biomarker between Ca:Mg ratio and risk of colorectal neoplasia and cardiovascular disease.

C. Uric acid

1. Known factors that affect uric acid

Uric acid is a metabolic product of purine nucleotides. The serum uric acid reflects the balance between production and excretion ²⁴⁵. Both exogenous (dietary) and endogenous purines are the major sources of uric acid for humans. Approximately 70% of daily excretion of uric acid is through the kidney ²⁴⁶.

1.1 Increase of uric acid

Diets rich in purines, high-fructose corn syrup, and table sugar are a risk factor of elevated uric acid ^{247,248}. Liver and kidney ²⁴⁹ have a high amount of purines. A moderate amount of purines is found in meats such as beef, lamb, pork, poultry and seafood ²⁴⁹. Certain vegetables such as asparagus, beans, cauliflower, green peas, mushrooms, oatmeal, spinach, etc., contain a moderate level of purines ²⁴⁹. In addition, hereditary factors, rapid weight loss, and diuretic medications could cause elevation of uric acid ²⁵⁰. Certain drugs including thiazide diuretics and inosine can increase uric acid levels in the blood by interfering with renal clearance ²⁵¹. Behavioral factors such as fasting and rapid weight loss can temporarily elevate uric acid levels. Correcting low or deficient zinc levels can help elevate serum uric acid in Wilson's disease by ameliorating liver function and uric acid synthesis ²⁵². A higher serum uric acid level was associated with diabetes mellitus, hypertension, and increased triglycerides level ⁴³.

1.2 Decrease of uric acid

A purine-free diet was associated with approximately 35% decline in plasma urate for healthy males ²⁵³. A cohort study with hemodialysis patients has indicated that factors such as cardiovascular diseases, increased age, creatinine, fasting blood glucose, corrected calcium, phosphate, corrected calcium plus phosphate product, and LDL-c levels were associated with lower serum uric acid levels ⁴³.

2. Uric acid and disease

Abnormal concentrations of uric acid may be an indicator of certain diseases ²⁴⁵.

Hyperuricemia is associated with a variety of diseases such as all-site cancer incidence and mortality ³²¹, hypertension ^{254–256}, cardiovascular disease ^{257–260}, diabetes ²⁶¹, gout, kidney disease ^{262–264}, and obesity ²⁶⁵. Hypouricemia was linked with multiple sclerosis ^{266,267}. Although epidemiologic studies have indicated rich evidence that elevated serum uric acid is independently associated with metabolic abnormalities, the mechanisms behind these associations have not been fully understood and are not well-established ²⁶⁸.

2.1 Magnesium, uric acid and type 2 diabetes

Whether elevated serum uric acid is a risk factor for diabetes has long been a matter of debate. Even since the early 1980s, uric acid has been associated with the development of diabetes although these associations were not adjusted for potential confounders ²⁶⁹. Moreover, this report showed that serum uric acid levels were higher in prediabetics than in nondiabetics ^{269,270}. A prospective follow-up study showed high

serum uric acid measured at the baseline is associated with higher risk of type 2 diabetes, independent of obesity, dyslipidemia, and hypertension ²⁷¹. For each 1 mg/dl increment in uric acid levels, approximately 60% increased risk of incident type 2 diabetes was observed ²⁷². Although statistically significant associations have been extensively reported, one study in Japanese men reported a null association ²⁷³. Another study has found a negative association between serum uric acid and diabetes ²⁷⁴. Changes in serum urate (salt of uric acid) were associated with changes in serum magnesium in women with type 2 diabetes and non-diabetic men ²⁷⁵.

2.2 Magnesium, uric acid and metabolic syndrome

Hyperuricemia, a relatively new risk factor of metabolic syndrome, has been associated with a higher prevalence of metabolic syndrome ²⁶¹. A study has suggested fructose-induced hyperuricemia may play a pathogenic role in metabolic syndrome ²⁷⁶. This is consistent with the increased consumption of fructose-containing beverages (such as fruit juices and soft drinks sweetened with sugar and high-fructose corn syrup) and the epidemic of diabetes and obesity in recent decades ²⁷⁷. Serum uric acid is closely associated with metabolic syndrome in females than males ²⁷⁸. One study has demonstrated elevated uric acid and lower serum magnesium were significantly associated with insulin resistance ²⁷⁹.

2.3 Calcium, magnesium, uric acid and cardiovascular disease

Viazzi *et al.* found that serum uric acid was associated with a positive history of cardiovascular disease and high Framingham risk score, which is independent of

metabolic syndrome ²⁸⁰. A meta-analysis reported that 1 mg/dl increase in uric acid level was associated with 13% increased risk of incident hypertension ²⁸¹. Elevated serum uric acid level is positively associated with cardiovascular mortality ²⁵⁷. In addition, elevated levels of serum uric acid was associated with the presence of coronary artery calcium ⁴², a marker of atherosclerosis. A number of studies adjusted for multiple risk factors suggested that uric acid may be an independent risk factor for cardiovascular disease ^{257,282–284}. Another set of studies have indicated that uric acid is not independent of other established risk factors, especially hypertension, for the development of cardiovascular disease ^{285–288}. The role of uric acid in cardiovascular disease is still debated ²⁶⁵.

However, the majority of studies evaluating uric acid are observational studies. Moreover, our previous findings suggest a modifying effect of the Ca:Mg ratio on associations between calcium or magnesium intake and risk of colorectal ^{17,26} and esophageal neoplasia ²⁷, total, cardiovascular disease and cancer mortalities ²⁸. To our knowledge, no previous randomized trial has evaluated the effect of magnesium on uric acid status. Further, no previous study has examined whether the Ca:Mg intake ratio plays a role in uric acid metabolism and whether uric acid is an intermediate biomarker between Ca:Mg ratio and risk of colorectal neoplasia and cardiovascular disease.

Chapter 3. Research strategies

A. The Prostate, Lung, Colorectal, and Ovarian (PLCO) screening trial (project A)

1. Study design: overall description

The PLCO is a ten-study-center, randomized, controlled trial comparing cancer screening tests and usual care to determine the effects of screening on cancer-related mortality. The details of the study design were described elsewhere ^{289,290}. Nine of ten study centers (Denver, CO; Detroit, MI; Honolulu, HI; Marshfield, WI; Minneapolis, MN; Pittsburgh, PA; Salt Lake City, UT; St. Louis, MO; and Washington, DC) began enrollment of eligible participants in November 1993. The University of Alabama began enrollment in January 1998. About 155,000 subjects were recruited between November 1993 and July 2001. After stratification by age and gender within each study center, participants were individually randomized to the intervention arm or control arm in equal proportions (block randomization). The date of enrollment is the date of randomization. Participants assigned to the intervention arm were invited to receive screening exams for prostate, lung, colorectal and ovarian cancers, whereas participants assigned to the control arm received routine medical care. The screening methods were digital rectal examination plus serum prostate-specific antigen (for prostate), chest X-ray (for lung), flexible 60-cm sigmoidoscopy (FSG) (for colorectum), cancer antigen 125 and transvaginal ultrasound (for ovary). The data analyses for this proposed project were primarily within the screening arm of the PLCO trial (n=77,444). The control arm of the PLCO initially had 77,453 participants.

2. Study population

According to the original PLCO study design^{289,290}, the exclusion criteria used to select eligible subjects were listed as follows:

- 1) Aged < 60 or >74 at the time of randomization; Beginning in January 1996, participants aged < 55 or >74 at the time of randomization were excluded;
- 2) Under cancer treatment (excluding basal and squamous cell skin cancer) at the entry of the trial;
- 3) With known diagnosis of colorectal, lung, ovarian or prostate cancer;
- 4) With previous surgical removal of the entire prostate, one lung or the entire colon;
- 5) With prior surgical removal of both ovaries. Starting in October 1995, this criteria was deleted;
- 6) Participating in another cancer screening or cancer primary prevention trial;
- 7) Males who have taken Proscar in the past 6 months;
- 8) Starting in April 1995, males who had more than one prostate specific antigen (PSA) test in the three years prior to enrollment;
- 9) Starting in April 1995, participants who had a colonoscopy, sigmoidoscopy, or barium enema in the three years prior to enrollment;
- 10) Unable or unwilling to provide informed consent.

3. Data collection

3.1 Baseline Questionnaire (BQ)

At the entry of the trial, participants were asked to complete a BQ. The BQ collected information on demography (e.g., age, sex, race, education, and marital status), family cancer history, personal medical history (including the history of colorectal polyp, nonsteroidal anti-inflammatory drugs use, cancer screening history within 3 years), anthropometry (height, weight), lifestyle factors including smoking history and physical activities. In the intervention arm, 75,611 (97.6%) of participants finished the BQ. In the control arm, 74,366 (96.0%) of subjects has a valid BQ.

3.2 Dietary Information

3.2.1 Dietary Questionnaire (DQX)

The DQX which included 137 food items at baseline was administered among intervention arm participants to obtain dietary intake over the 12 months before enrollment, and 14 questions about supplement intake. In the intervention arm, 60,358 (77.9%) of participants completed the DQX and 1,721 of subjects were further excluded because of invalid DQX. A questionnaire was invalid for any of the following four criteria²⁹¹: 1) Missing date of completion; 2) Death before the completion of the DQX; 3) Eight or more missing items in food frequency questionnaire; and 4) Extreme (highest or lowest 1% for each gender) caloric intake.

3.2.2 Dietary History Questionnaire (DHQ)

In December 1998, a second dietary questionnaire, the DHQ with 114 food items, was implemented. The DHQ was administered to participants of the control arm at T₀ (time

of year 0) and intervention arm at T₃ (time of year 3). Participants who had already passed these windows received the DHQ at their randomization anniversary in either 1999 or 2000. Among enrolled participants, 111,477 (72.0%) individuals have completed the DHQ. Four criteria were used to confirm the validation of questionnaires: 1) A date of completion must exist; 2) The date of completion must not be later than the date of death; 3) Fewer than 8 missing frequencies; 4) Caloric intake is within normal range (excluding top and bottom 1% for each gender). Only valid food frequency questionnaires (N=106,522) were used for dietary analyses, of which 58,637 were in the intervention arm and 47,885 were in the control arm.

4. Nutrients

4.1 Dietary nutrients

Usual frequency of intake (less than once a month, once a month, twice to thrice a month, once a week, twice a week, three to four times a week, five to six times a week, once a day, two or more times a day) and portion size (small, medium, large) were queried. The usual dietary intakes of nutrients, including calcium, magnesium, and vitamin D, were derived from frequency and portion-size responses from DHQ and DQX, in which nutrient values per portion were multiplied by the daily frequency of intake and summed across all relevant food items. Nutrient composition databases which were constructed based on U.S. Department of Agriculture (USDA) food composition tables (USDA's 1994-96 Continuing Survey of Food Intakes by Individuals [CSFII])²⁹². Cut points between small and medium portions and between medium and large portions corresponded to the 25th and 75th percentiles, respectively, for portion sizes reported by

participants in the CSFII, 51 years or older ²⁹². Dietary calcium or dietary vitamin D intakes were calculated from all dietary sources, without supplements, respectively.

4.2 Nutrients from supplement use

Supplement use was calculated from single and/or multi-vitamins based on the responses to the supplement questions on the DQX or DHQ. The food frequency questionnaire also collected information on multivitamin, single vitamin, and mineral supplement use with questions about current use, past use (2 and 5 years ago), dosage, and years of intake ²⁹³. Values for multivitamins were derived from the third National Health and Nutrition Examination Survey (NHANES III) database. Supplemental calcium, magnesium, or vitamin D intake were calculated by summing the intake from the single vitamin supplement calcium (the specific doses for calcium, dolomite, Tums, and so forth.) or vitamin D and multivitamins (162 magnesium calcium and 400 international unit (IU) of vitamin D per one-a-day multivitamin pill; 400 IU of vitamin D per therapeutic or high-dose types), as defined by the generic multivitamins most frequently reported by participants ages 55 to 74 years in the NHANES III cohort ²⁹⁴. In this dissertation project, analysis of calcium intake from the supplements is mainly based on current intake (0-2 years before enrollment). Total calcium and vitamin D intake is defined as dietary plus supplemental intake.

5. Screening and diagnostics

FSG was performed at entry (T_0) and then at the 3 (T_3) or 5 (T_5)-year point. Participants randomized before Oct 1994 underwent a second screen at T_3 . For participants who

were randomized between Oct 1994 and Nov 1995, a T₃ FSG were scheduled; however, T₅ FSG were offered if T₃ were missed ²⁹⁵. Intervention arm participants randomized on/after Dec 1995 were scheduled for FSG at T₅. In the intervention arm, 65,000 subjects underwent baseline FSG and 42,000 received T₃ or T₅ FSG. There were 39,000 participants had FSGs both at baseline and at the T₃ or T₅ FSG. The screening results were divided into three mutually exclusive result categories. An FSG was defined as “positive FSG” if one or more of the following were found: rectal nodule, rectal and/or colon mass, and/or colon polyp. An FSG was defined as “inadequate” if a <50-cm depth of insertion was achieved or visual inspection was limited to <90% of the mucosal surface due to inadequate bowel preparation. Otherwise, an FSG was defined “negative” group if there was no detection of a polyp or mass. Subjects with inadequate screening could return for a second examination. Among 7,522 inadequate cases, 632 returned and 7,099 continued to be defined as inadequate after the second screening.

Participants with a positive screening were referred to their health care providers for further diagnostic evaluation and follow-up. Within 12 months after a positive screening, the PLCO abstracted medical records pertaining to diagnostics. Information on date of diagnosis, the anatomic location, the size and histology of polyps and masses were collected. In addition, classifications of malignant tumors (TNM) clinical and pathologic stages were abstracted for participants with CRC.

6. Follow-up

Study centers actively tracked subjects to collect, assemble, organize, and abstract medical record information related to diagnosis during the follow-up. Participants would

be followed until death or termination of the study (13 years from randomization) to ascertain all cancers of the prostate, lung, colorectal, and ovary, as well as deaths from all causes. The main sources by which study personnel learned of participants' diagnosis/death were (1) abnormal FSG screening, (2) the administration of the Annual Study Update (ASU) questionnaires, (3) reports from relatives, friends, or physicians, and (4) National Death Index (NDI) plus searches. Study centers also attempted to obtain a death certificate for each death that occurred on or before December 31, 2009. Information from the death certificate was abstracted and coded in the trial database. The underlying cause of death from death certificate was derived according to rules established by the National Center for Health Statistics. In order to ensure a more accurate assessment of the trial endpoints, a Death Review Process (DRP) was conducted and medical records were reviewed for all deaths that might have been due to prostate, lung, colorectal and ovarian cancers. The DRP cause of death was considered authoritative and will be used in statistical analyses of the primary endpoints.

In addition, cancer incidence, stages, and case survival data were also collected using the ASU questionnaire. The ASU was followed by linkage to medical records to confirm the diagnosis. The diagnosis of colorectal neoplasia was through medical record abstraction (MRA).

7. Outcomes in the PLCO trial

7.1 Incidence of colorectal adenoma

Participants (n=22,521) with a negative T₀ FSG in the intervention arm of the PLCO trial were eligible to be included in the sub-cohort for evaluation of incident colorectal

adenoma risk. In addition, an adequate T_{3/5} screen was required to further classify an incident adenoma case or control. Cases must have a positive T_{3/5} screen which led to the discovery of a left-sided adenoma²⁹⁶. Controls must have a negative T_{3/5} FSG. Filters including a valid BQ (n=22,500), no cancer history before BQ (n=21,693), complete DQX (n=19,503), a valid DQX (n=19,088), no cancer history before DQX (n=19,076) were further applied. Finally, 1,147 incident colorectal adenoma cases and 17,929 control participants were identified. An adenoma with ≥1 cm in size, high-grade dysplasia, or villous component was defined as an advanced adenoma (**Figure 3.1**).

7.2 Metachronous colorectal adenoma (recurrent adenoma)

The Study of Colonoscopy Utilization (SCU) was designed as an ancillary study nested within the PLCO trial, which aimed to investigate the utilization of subsequent colonoscopy in the PLCO screening arm. Participants with a positive baseline FSG screening, diagnostic endoscopy within 6 months from baseline, and no cancer findings were invited to complete interviewer-administered telephone-based SCU questionnaire²⁹⁶. A baseline adenoma was defined as an adenoma found within the first 18 months following a positive T₀ FSG screen, on the first endoscopy that followed the screen, or on an endoscopy within six months of the first endoscopy following the screen. A questionnaire collected information on all known colonoscopy after randomization.

Medical record abstraction was performed to verify the collected questionnaire information. Individuals who were diagnosed with an adenoma in the second endoscopy were defined as recurrent colorectal adenoma cases. Participants not in SCU but with a

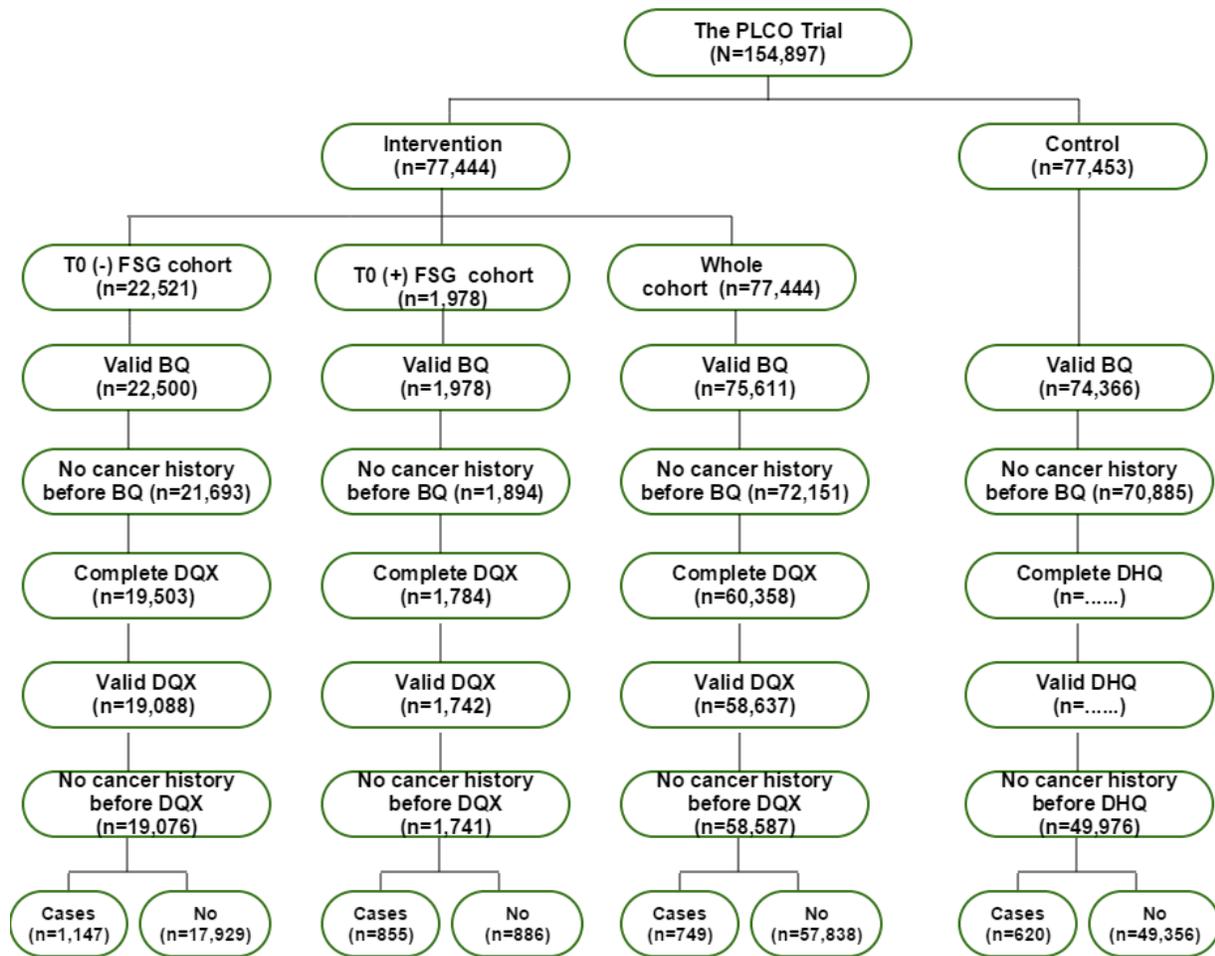


Figure 3.1 The flow chart of the population sample size in the PLCO

positive T_{3/5} screen which resulted in an endoscopy that discovered recurrence were also included.

We further restricted the population to those who were diagnosed with adenoma at baseline endoscopy, had a second endoscopy, completed a valid BQ (n=1,978), no cancer history before BQ (n=1,894), complete DQX (n=1,784), a valid DQX (n=1,742), and who had no cancer history before DQX (n=1,741). The final analysis included 855 colorectal metachronous adenoma cases and 886 controls (**Figure 3.1**).

7.3 Incident CRC

Medical records related to diagnostic follow-up, a diagnosis of CRC, and cancer complications were collected. The intervention arm of the PLCO trial was further restricted to a valid BQ (n=75,611), no history of any cancer prior to BQ (n=72,151), completed a DQX (n=60,358), has valid DQX (n=58,637), and no history of any cancer prior to DQX (n=58,587). The control arm of the PLCO trial was further restricted to a valid BQ (n=74,366), no history of any cancer prior to BQ (n=70,885), and no history of any cancer prior to DQX (n=49,976). The final analysis included 58,587 subjects in the intervention arm, of which 749 were incident CRC cases and 57,838 CRC free controls. The control arm was reduced to 49,976 participants with valid BQ, DQX and no cancer history, of whom 620 were incident CRC cases and 49,356 were CRC free participants (Figure 3.1).

8. Statistical Analysis

The incidence of colorectal adenoma and metachronous adenoma were only followed up within the intervention arm of the PLCO. The information on CRC incidence was abstracted within both intervention and control arms. Thus, participants included in this study were from both intervention and control arms of the PLCO trial.

8.1 Power analysis

The main purpose of the project A was to investigate the association between calcium intake and the risk of various stages of colorectal neoplasia (incident and metachronous adenoma, and incident CRC). Among these three longitudinal outcomes, the number of

events is lowest for CRC incidence (n=749). Since the number of events is a major determinant of power in the survival analysis, the power analysis was conducted on the uses the CRC incidence. We expect power will be larger for other outcomes such as incidence and recurrence of adenoma with more events for similar HR's.

This power analysis was based on the following parameters: 29,288 experimental subjects (whose calcium intake is higher than 1135 magnesium per day (median)), 29,288 control subjects (whose calcium intake is no more than 1135 magnesium per day), an accrual interval of 1,095.75 days, and additional follow-up after the accrual interval of 4,748 days. An estimated median survival time of the control group is 230,115.5 days. If the true hazard ratio of control subjects relative to experiment subjects is 1.25, we will be able to reject the null hypothesis that the experimental and control survival curves are equal with a power of 0.893. The type I error probability associated with this test of the null hypothesis is 0.05. The power analysis was done using the PS software. **Table 3.1** shows the detailed alternatives to this power analysis.

Table 3.1 Study power for main effect of Ca intake in relationship to CRC in Aim 1											
Median survival time (days)*	Hazard Ratio (Relative Risk)										
	1.05	1.10	1.15	1.20	1.21	1.22	1.23	1.24	1.25	1.30	
230115.5	0.113	0.294	0.537	0.754	0.788	0.820	0.847	0.872	0.893	0.961	
226463	0.115	0.297	0.543	0.761	0.795	0.826	0.853	0.877	0.898	0.964	
175328	0.134	0.367	0.651	0.857	0.885	0.908	0.927	0.943	0.956	0.989	
138803	0.156	0.444	0.751	0.925	0.944	0.958	0.970	0.978	0.984	0.998	
102270	0.195	0.561	0.865	0.977	0.985	0.990	0.994	0.996	0.998	1.000	
65745	0.274	0.747	0.967	0.998	0.999	1.000	1.000	1.000	1.000	1.000	
29220	0.518	0.972	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	

*: This median survival time for controls is estimated using a formula of $t \times \log(1/2) / \log(p)$; i.e.: t is the observed maximal follow-up time of 4,748 days at which $p = 0.98864$ is the probably that a control subject survived.

8.2 The list of potential co-variables

Continuous variables included age at randomization, body mass index (BMI), total intakes of calcium, calories, copper, folic acid, iron, magnesium, phosphorous, vitamins A, B₆, B₁₂, C, D, and E, zinc. Categorical variables contained aspirin use (yes, no), cigarette smoking status (never, current cigarette smoker, and former cigarette smoker), drinker (yes, no), education (<8 years, 8-11 years, 12 years or completed high school, post-high school training other than college, some college, college graduate, and postgraduate), family history of CRC (yes, no), race (white, black, Hispanic, Asian, pacific islander, American Indian), sex (female, male), vigorous physical activity (none, <1, 1, 2, 3, ≥4 h/week). Due to the small count in certain groups, education status was reorganized to less than high school, 12 years high school, post-high school or some college, and college graduate or more; race was categorized into white, black, Asian and others. Other necessary data transformation of variables followed the rule of non-violation of assumptions of proportional hazards. Potential confounding factors was selected based on biological plausibility, literature reports and ≥10% change in relative risks²⁹⁷.

8.3 Effect modifiers

It was hypothesized that the association between calcium intake and the risk of colorectal neoplasia differs by sex, race and the levels of Ca:Mg ratio, magnesium and vitamin D. Thus, these factors were considered as possible effect modifiers. Likelihood ratio tests, comparing the differences in log-likelihoods for interaction terms in the full model and no interaction terms in the reduced model, were used to assess the

significance (<0.05) of interactions. For the outcome of CRC incidence, Wald test was conducted to assess interactions.

8.4 Analytical methods

Summary statistics for both continuous (mean \pm standard deviation) and categorical variables (count and percent) were used to describe study populations. Chi-square tests for categorical variables, t-tests and generalized linear models for continuous variables were conducted to evaluate differences in the distribution of potential confounding factors between different categories of calcium intake. Person-years for CRC incidence were calculated from the date of randomization to the date of the most recently completed end point follow-up questionnaire, the date of CRC diagnosis, death, or end of follow-up, whichever came first ³¹.

For incident and metachronous adenoma, the occurrence of disease as a case or control rather than time to event was considered; thus, unconditional logistic regression models were used to calculate odds ratios (OR) and 95% confidence intervals (95%CI) to estimate the associations after adjusting for covariates. Hazard ratios (HR) and 95%CI for incident CRC were estimated using Cox proportional hazard models. Potential confounding factors were selected based on biological plausibility, literature reports and/or $\geq 10\%$ change in relative risks ²⁹⁷. Confounding factors evaluated included age, sex, race, education, recruitment site, family history of CRC, body mass index, smoking status, alcohol consumption, exercise and daily intakes of total energy, vitamin D and magnesium. Tests for trend across categories were performed in

regression models by assigning the score j to the j th level of the variable selected. An *a priori* stratified analysis by Ca:Mg intake ratio was conducted. For metachronous adenoma and CRC incidence in the intervention arm, stratified analyses on baseline adenoma characteristics (e.g. advanced and/or synchronous adenoma) and subsites were performed. All tests were 2-sided and statistical significance level was set at 0.05. Statistical analyses were performed using SAS statistical software (version 9.4; SAS Institute, Cary, NC).

B. The Personalized Prevention of CRC Trial (PPCCT, project B&C)

1. Study Population

Adenoma and/or hyperplastic polyp cases aged from 40 to 85 years were recruited from two major sources: 1) Two existing large adenoma studies (Tennessee Colorectal Polyp Study (TCPS) and Tennessee-Indiana Adenoma Recurrence Study (TIARS)) in which participants were diagnosed between 2003 to 2010 and agreed to be contacted for future studies; and 2) Patients with colorectal adenoma and/or hyperplastic polyp diagnosed from 2008 to 2012 at Vanderbilt University Hospital. Participants had a calcium intake ≥ 700 mg/day and < 2000 mg/day measured with two 24-hour dietary recalls. In addition, the Ca:Mg intake ratio of participants was greater than 2.6. A list of exclusion criteria was applied: a history of colectomy, inflammatory bowel disease, any organ transplantation, cancer other than non-melanoma skin cancer, gastric bypass, chronic renal diseases and hepatic cirrhosis, chronic ischemic heart disease, diarrhea, type I diabetes mellitus, pituitary dwarfism; current use of lithium carbonate therapy,

blood anticoagulant drugs, digoxin and licorice; without contact information and informed consent.

2. Enrollment

Trained research staff reviewed the medical record of potential participants. If participants were eligible, an introductory letter signed by the parent study principal investigator and another introductory letter signed by the principal investigator and the gastroenterologist described the study and invited them to participate. A few days after the letter was mailed, the trained research staff of the Survey Research Shared Resource called the potential participant to provide more detailed information about the study, answered questions about the study, and to see if they may be interested in participating. During the telephone conversation, a series of yes/no screening questions were administered to determine eligibility based on the exclusion criteria. If the person was eligible, implied verbal consent was obtained to conduct the baseline interview survey and 24-hour dietary recalls prior to the first in-person clinic visit (CV#1). For eligible participants from Vanderbilt University Hospital and other sources, implied verbal consent was obtained to collect a mouthwash sample for genotype assay and then to conduct two 24 hour dietary recalls and a baseline interview survey. Eligible participants were scheduled for three clinic visits.

3. Randomization and study design

The project of 2&3 are nested in the parent PPCCT study. The PPCCT is a 2x2 factorial design (magnesium treatment and *TRPM7* genotype) which enrolled 250 participants, of which 239 completely finished the study and 11 subjects finished part of the study

before withdrawing. The primary endpoints of the PPCCT were the expression of carcinogenesis biomarkers in colorectal mucosa. Within each group of individuals with different *TRPM7* genotypes (GG vs GA/AA), a permuted-block randomization algorithm was used to allocate the subjects into either the treatment or placebo arm.

4. Dietary Ca:Mg intake assessment, intervention and clinic visit

Two 24-hour dietary recall assessments were used to estimate the baseline Ca:Mg intake ratio. The recalls were based on the Minnesota Nutrient Data System (NDS) and were administered by the Survey Research Shared Resource. Information on the participant's use of medications, nutritional supplements, and other conditions was collected. Based on a participant's baseline Ca:Mg intake ratio, the participant was assigned to a customized dose that would reduce the Ca:Mg intake ratio to less than 2.6 but above 2.0. Identical-appearing placebos were made to match magnesium capsules.

The intervention period was designed to be 12 weeks. Eligible participants were scheduled for three clinic visits (week 1, 6 and 12). Before each clinic visit, participants received a study package, which included an instruction booklet, a Food Amounts booklet, clinic visit questionnaire forms, and diet, health, and probiotics questionnaires. Information on the participant's use of medications, nutritional supplements, and other conditions were collected. Rectal biopsy, blood, and urine biospecimens were collected and processed in each clinic visit. Anthropometric measurements (blood pressures,

weight, height, waist and hip circumference) were measured at least twice in each clinic visit.

5. Biomarkers assay

As of April, 2014, 150 participants had finished the trial and their samples were assayed for a lipid profile and uric acid. Blood was collected from a forearm vein at each clinic visit after participants had fasted for at least 8 hours. The blood was clotted and immediately centrifuged to separate serum, which was rapidly cooled and frozen at -80°C before biochemistry analysis. The 150 pairs of pre- and post-treatment serum samples of participants who finished the trial were sent to the Vanderbilt Lipid Laboratory for assay.

The four lipid biomarkers (LDL-c, HDL-c, total cholesterol, and triglycerides) as well as uric acid were assayed by the Vanderbilt Lipid Laboratory, which is standardized by the Centers for Disease Control and Prevention for lipid analysis²⁹⁸. Total cholesterol, HDL-c, triglycerides, and uric acid were measured using the Reagents of ACE® Cholesterol (#SA1010), HDL-c (#SA1038), triglycerides (#SA1023), and uric acid (#SA1025) respectively²⁹⁸. The detailed assay procedure can be referred to the appropriate Alfa Wassermann Diagnostic Technologies, LLC Clinical Chemistry Systems Operator's Manual. When triglycerides was less than or equal to 400 mg/dl, the LDL-c was calculated by subtracting HDL-c and one-fifth of triglycerides from the total cholesterol. Otherwise, the direct LDL-c was directly measured using the ACE LDL-c Reagent (#SA1040)²⁹⁸. Based on values measured from quality control samples, intra-assay and

inter-assay variation coefficients were 1.28% and 4.03% for LDL-c; 0.63% and 2.19% for HDL-c; 0.67% and 5.12% for triglycerides; 0.75% and 2.06% for total cholesterol, 1.72% and 7.64% for uric acid.

6. Statistical power

The estimate of power is focused on the primary endpoint of LDL-c and uric acid. The conservative estimate of power is based on McNemar's test. The paired fourfold table of

the McNemar's test was

presented in **Table 3.2**. Our

pilot data shows that 38% at

		Post-treatment	
		Normal	Abnormal
Pre-treatment	Normal	p_{00}	p_{01}
	Abnormal	p_{10}	p_{11}

baseline had abnormal LDL-c, and after 12 weeks of treatment 25% had abnormal LDL-c. The same pattern was found for uric acid in the pilot data. One third with abnormal LDL-c at baseline lowered their LDL-c to normal range, and none of those with normal LDL-c at baseline became abnormal after 12 weeks of treatment. In the biomarker assay, we have measured 150 pairs (150 subjects before and after treatment) of serum samples. We assumed that the balance of treatment and placebo was 1:1. Thus, we had 75 pairs of LDL-c and uric acid measures in the treatment group. With a 0.05 two-sided significance level, a sample size of 75 will have 95% power to detect a difference in proportions of 0.125 ($p_{10} - p_{01}$) when the proportion of discordant pairs is expected to be 0.135 ($p_{10} + p_{01}$). **Table 3.3** contains estimated power for McNemar's test of equality of paired proportions. We do not expect a significant change of biomarkers in the placebo arm, thus we have an equal number ($n=75$) of subjects in the placebo arm.

	1	2	3	4	5	6
Test significance level, α	0.050	0.050	0.050	0.050	0.050	0.050
Difference in proportions, $p_{10} - p_{01}$	0.125	0.115	0.105	0.095	0.085	0.075
Proportions of discordant pairs, $p_{10} + p_{01}$	0.135	0.125	0.115	0.105	0.095	0.085
Power	0.95	0.92	0.88	0.83	0.75	0.66

7. Analytic methods

The parent PPCCT is still blinded for primary outcomes; thus, an independent statistician outside of the study team conducted the statistical analyses for the current study. Summary statistics for lipid biomarkers as continuous variables (mean \pm standard deviation, median, and inter quartile range) was derived for each randomization group. The distributions of continuous outcomes were assessed for normality by using the Kolmogorov-Smirnov test. Both within-group change and between-group comparisons were evaluated. Paired t-test (normal) or the Wilcoxon-signed rank test (non-normal) was conducted to evaluate whether pre- and post-treatment values were different within each group (treatment or placebo). Either the parametric two-sample t-test or the non-parametric Wilcoxon Rank Sum test was performed to evaluate between-group differences. Generalized linear regression analysis was performed to examine the effect of magnesium treatment on lipid profile while adjusting for baseline lipid biomarkers. All participants had a baseline Ca:Mg ratio ≥ 2.6 . Participants were thus classified into three groups based on the concordance between the baseline Ca:Mg ratio and the Ca:Mg ratio measured from the FFQ (the median interval to baseline was around 5.0 years): long-term high ratio (FFQ Ca:Mg ratio ≥ 2.6), recent high ratio (FFQ Ca:Mg ratio < 2.6), and unknown ratio (Ca:Mg ratio from the FFQ is unmeasured). Stratified analyses were conducted by the categories of the change in the Ca:Mg ratio for both long-term and recent high. The statistical significance was 0.05 using two-sided test.

The data analysis used software R and SAS[®] (version 9.4; SAS Institute, Cary, North Carolina).

Chapter 4. Results

A. Calcium: magnesium intake ratio and colorectal carcinogenesis, results from the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial

Characteristics of the selected population

The distribution of selected demographic characteristics and potential confounders in cases and controls for incident and metachronous adenoma are shown in **Table 4.1**.

Compared to controls, adenoma cases were more likely to be male, smokers, physically inactive, and had higher body mass index, higher intake of energy, but lower intakes of calcium, magnesium and vitamin D. Compared to participants without metachronous adenoma, metachronous adenoma cases were also more likely to be male and obese, to have family history of CRC, higher intake of total energy but lower intake of calcium. Metachronous adenoma cases were less likely to be white.

The selected basic characteristics in the intervention and control arms for the study of incident CRC are compared in **Table 4.2**. For both intervention and control arms, compared to the lower calcium intake (<600mg/day), participants with higher calcium intake were less likely to be male, but more likely to be white, never smokers, aspirin users, physically active, and had lower BMI, higher education achievement and family history of CRC , and higher intakes of energy, magnesium and vitamin D.

Incident colorectal adenoma

There was no significant association between calcium intake and risk of incident colorectal adenoma (**Table 4.3**). However, compared to the lowest category of calcium

intake, higher intakes of calcium were associated with a suggestive reduced risk of advanced and/or synchronous adenoma (P for trend, 0.05). In the stratified analysis Ca:Mg ratios, the inverse association between calcium intake and advanced and/or synchronous adenoma primarily appeared in participants with Ca:Mg ratios ranging from 1.7 to 2.5 (P for trend, 0.05). No significant association was observed when the Ca:Mg ratio was above 2.5.

Metachronous (recurrent) adenoma

We found calcium intake was not related to risk of metachronous adenoma (**Table 4.4**). We also did not find significant associations in the stratified analysis by Ca:Mg intake ratio (**Table 4.4**), location of adenoma (i.e. distal or proximal, data not shown), and baseline adenoma characteristics (i.e. advanced/synchronous adenoma) (**Supplement Table 4.7**). This null association was similar for advanced and/or metachronous adenoma (**Table 4.4**). The Ca:Mg ratio stratified analysis showed null results as well.

CRC incidence

We found calcium intake was significantly related to a reduced risk of CRC (P for trend, 0.03) (**Table 4.5**). This significant inverse association was primarily present for distal CRC (P for trend, <0.01) but not for proximal colon cancer. In the analysis stratified by the Ca:Mg intake ratio, we found the inverse association was only statistically significant in participants whose Ca:Mg ratio ranged from 1.7 to 2.5 (P for trend, 0.04). There was a significant interaction between calcium intake and the Ca:Mg ratio in relation to distal CRC (P for interaction, <0.01).

Stratified analysis by treatment

We found, in the control arm without regular endoscopy, the significant association between calcium intake and risk of incident distal CRC remained (P for trend, <0.01 , **Table 4.6**) and this inverse association appeared to be primarily in those with Ca:Mg intake ratios between 1.7 and 2.5, although this was not statistically significant.

However, in the intervention arm with endoscopy screening, the inverse association between calcium intake and risk of incident distal CRC was of borderline significance (P for trend, 0.06, **Table 4.6**). In further analysis stratified by feature of the baseline adenomas, we found among participants with advanced/synchronous adenoma at baseline, higher calcium intake was inversely associated with risk of incident CRC (P for trend, 0.04) in the intervention arm (**Supplement Table 4.7**). Comparable data on were not available in the control arm.

Table 4.1 Selected descriptive characteristics of adenoma cases and controls

Characteristics	Adenoma			Metachronous adenoma		
	Cases (N=1147)	Controls (N=17929)	<i>P</i> value	Cases (N=855)	Controls (N=886)	<i>P</i> value
Age (years), Mean ± SD	62.1±5.3	62.3±5.2	0.13	62.9±4.8	62.7±5.2	0.41
Sex, %						
Men	67.5	55.9	<.0001	72.4	60.0	<.0001
Race, %						
White	90.6	88.9	0.07	93.0	96.1	0.009
Education, %						
College or higher	38.7	39.5	0.38	38.0	35.7	0.68
Smoking status, %						
Never smoker	42.6	53.4		34.5	37.5	
Former smoker	47.3	41.4		52.1	49.3	
Current smoker	10.1	5.2	<.0001	13.4	13.2	0.42
Alcohol consumption, %	81.1	79.1	0.10	84.0	83.1	0.61
Family History of CRC, %	10.5	9.1	0.13	13.2	13.7	0.79
Aspirin use, %	48.4	46.8	0.30	44.7	43.9	0.74
Physically inactive, %	16.6	12.0	<.0001	15.1	15.2	0.93
Body mass index ≥ 30, %	25.3	21.9	0.003	27.9	23.7	0.02
Daily nutrients intake, Mean ± SE						
Total energy (kcal) ¹	2187.0±23.6	2086.5±6.0	<.0001	2185.5±27.6	2088.9±27.1	0.01
Total calcium (mg) ²	1160.5±15.8	1255.1±4.0	<.0001	1119.0±16.6	1196.8±16.3	0.0008
Total magnesium (mg) ²	431.6±3.1	444.4±0.8	<.0001	431.4±3.7	439.4±3.6	0.12
Vitamin D (mcg/day) ²	10.8±0.3	12.1±0.1	<.0001	10.4±0.3	11.0±0.3	0.09

¹ Least squares mean value, SE

² Least squares mean value, SE, adjusting for total energy

Table 4.2 Selected descriptive characteristics of participants by calcium intake (mg/day)

Characteristics	Intervention arm					Control arm				
	< 600	600-1200	1200-1600	≥1600	<i>P</i>	< 600	600-1200	1200-1600	≥1600	<i>P</i>
N	7323	24475	12354	14383		12678	21185	9584	6487	
No. of CRC cases	100	315	128	154		174	242	99	63	
Person-years	80222	273937	138958	161286		142777	241744	110113	74392	
Age (years)										
Mean ¹	62.6	62.7	62.6	62.6		62.6	62.5	62.5	62.5	
SE	0.06	0.03	0.05	0.04	0.12	0.05	0.04	0.05	0.07	<.0001
Sex (men), %	53.7	58.6	50.2	39.2	<.0001	55.9	52.2	36.1	34.0	<.0001
Race (white), %	81.3	90.2	93.1	94.7	<.0001	85.2	91.9	93.4	95.7	<.0001
Education, %										
College or higher	48.5	56.9	59.3	61.1	<.0001	54.0	57.9	59.3	61.1	<.0001
Smoking status, %										
Never smoker	42.4	44.5	47.9	52.2		44.0	47.1	50.6	51.6	
Former smoker	44.0	44.8	43.3	40.5		44.4	43.9	41.3	40.9	
Current smoker	13.6	10.7	8.8	7.3	<.0001	11.6	9.0	8.1	7.5	<.0001
Alcohol consumption, %	75.6	78.7	77.8	76.2	<.0001	72.8	73.8	72.5	69.7	<.0001
Family history of CRC, %	10.3	10.9	10.5	11.0	0.0038	9.9	10.3	10.2	10.4	0.61
Aspirin use, %	43.0	46.4	49.1	49.3	<.0001	45.2	47.7	47.5	48.5	<.0001
Body mass index ≥ 30, %	25.1	24.1	24.3	22.7	<.0001	24.5	23.8	21.2	22.3	<.0001
Total energy (kcal) ²										
Mean ¹	1340.8	1913.4	2236.7	2477.5		1296.0	1781.0	1867.9	2217.1	
SE	8.4	4.6	6.5	6.0	<.0001	6.0	4.6	6.9	8.4	<.0001
Total magnesium (mg) ²										
Mean ³	351.4	410.3	454.3	500.7		324.3	364.9	391.1	432.3	
SE	1.2	0.6	0.9	0.8	<.0001	0.8	0.6	0.9	1.1	<.0001
Vitamin D (mcg/day)										
Mean ²	4.0	9.1	13.8	19.3		6.6	9.0	10.9	13.8	
SE	0.09	0.07	0.05	0.07	<.0001	0.05	0.03	0.05	0.06	<.0001

¹ Least squares mean value, SE² Least squares mean value, SE, adjusting for total energy

Table 4.3 ORs and 95% CIs¹ for colorectal adenoma incidence by calcium intake

Ca Intake (mg/day)	Any adenoma		Advanced and/or synchronous adenoma	
	Cases	OR (95% CI)	Cases	OR (95% CI)
All				
<600	139	0.89 (0.72-1.12)	55	1.17 (0.82-1.68)
600-1200	545	1.00 (Ref)	192	1.00 (Ref)
1200-1600	219	0.82 (0.68-0.97)	70	0.71 (0.52-0.96)
≥1600	244	0.84 (0.69-1.03)	83	0.80 (0.58-1.11)
P_{trend}^2		0.17		0.05
Ca:Mg ratio is between 1.7 to 2.5				
<600	64	0.91 (0.66-1.26)	31	1.50 (0.92-2.47)
600-1200	284	1.00 (Ref)	93	1.00 (Ref)
1200-1600	62	0.72 (0.51-1.02)	19	0.60 (0.33-1.08)
≥1600	35	0.91 (0.55-1.51)	11	0.64 (0.27-1.54)
P_{trend}^2		0.61		0.05
Ca:Mg ratio is above 2.5				
<600	13	0.86 (0.47-1.60)	2	0.45 (0.11-1.88)
600-1200	190	1.00 (Ref)	63	1.00 (Ref)
1200-1600	154	0.81 (0.64-1.03)	50	0.74 (0.50-1.12)
≥1600	207	0.81 (0.62-1.06)	72	0.77 (0.49-1.22)
P_{trend}^2		0.16		0.43
$P_{\text{interaction}}^3$		0.94		0.11

¹ Adjusted for age (continuous), sex, BMI (<25, 25-30, ≥30), education (less than high school, 12 years or completed high school, post high school training other than college, some college, college graduate, postgraduate), race (white, black, asian or others), family history of CRC (yes or no), cigarette (never smoked cigarettes, current or former), hours spent in vigorous activities (less than 1 hour/week, 1 hour/week, 2 hours/week, 3 hours/week, 4+ hours/week), and total energy and vitamin D intake

² Assigned the score j to the jth level of calcium intake and evaluated the significance of Wald test

³ Estimated the full model with interaction term of calcium intake and Ca:Mg ratio and without this term in reduce model using likelihood ratio test

Table 4.4 ORs and 95% CIs¹ for metachronous colorectal adenoma incidence by calcium intake

Calcium Intake (mg/day)	Any metachronous adenoma		Advanced and/or metachronous adenoma	
	Cases	OR (95% CI)	Cases	OR (95% CI)
All				
<600	124	1.23 (0.87-1.73)	65	1.45 (0.96-2.19)
600-1200	393	1.00 (Ref)	198	1.00 (Ref)
1200-1600	190	1.02 (0.77-1.34)	96	0.97 (0.69-1.38)
≥1600	148	0.83 (0.60-1.15)	81	0.92 (0.61-1.37)
P_{trend}^2		0.15		0.21
Ca:Mg ratio is between 1.7 to 2.5				
<600	63	1.33 (0.82-2.17)	32	1.44 (0.80-2.62)
600-1200	194	1.00 (Ref)	91	1.00 (Ref)
1200-1600	53	0.90 (0.52-1.56)	24	1.03 (0.51-2.07)
≥1600	22	2.34 (0.82-6.68)	11	3.32 (0.95-11.64)
P_{trend}^2		0.89		0.90
Ca:Mg ratio is above 2.5				
<600	9	0.85 (0.32-2.27)	4	0.76 (0.22-2.59)
600-1200	137	1.00 (Ref)	74	1.00 (Ref)
1200-1600	133	1.02 (0.69-1.50)	70	0.86 (0.53-1.38)
≥1600	124	0.73 (0.47-1.13)	68	0.65 (0.37-1.13)
P_{trend}^2		0.21		0.18
$P_{\text{interaction}}^3$		0.53		0.40

¹ Adjusted for age (continuous), sex, BMI (<25, 25-30, ≥30), education (less than high school, 12 years or completed high school, post high school training other than college, some college, college graduate, postgraduate), race (white, black, asian or others), family history of CRC (yes or no), cigarette (never smoked cigarettes, current or former), hours spent in vigorous activities (less than 1 hour/week, 1 hour/week, 2 hours/week, 3 hours/week, 4+ hours/week), and total energy, magnesium and vitamin D intake

² Assigned the score j to the jth level of calcium intake and evaluated the significance of Wald test

³ Estimated the full model with interaction term of calcium intake and Ca:Mg ratio and without this term in reduce model using likelihood ratio test

Table 4.5 HRs and 95% CIs¹ for CRC incidence by calcium intake

Calcium Intake (mg/day)	CRC incidence		Proximal CRC		Distal CRC	
	Cases	HR (95% CI)	Cases	HR (95% CI)	Cases	HR (95% CI)
All						
<600	274	1.12 (0.95-1.33)	133	0.94 (0.74-1.19)	141	1.38 (1.08-1.76)
600-1200	557	1.00 (Ref)	302	1.00 (Ref)	254	1.00 (Ref)
1200-1600	227	0.85 (0.72-1.01)	134	0.94 (0.75-1.17)	93	0.75 (0.58-0.97)
≥1600	217	0.89 (0.73-1.07)	121	0.97 (0.75-1.26)	92	0.75 (0.56-1.01)
<i>P</i> _{trend} ²		0.03		0.99		<0.01
Ca:Mg ratio is between 1.7 to 2.5						
<600	101	1.12 (0.85-1.47)	50	0.95 (0.65-1.39)	51	1.34 (0.90-1.99)
600-1200	269	1.00 (Ref)	144	1.00 (Ref)	124	1.00 (Ref)
1200-1600	51	0.70 (0.49-1.01)	28	0.80 (0.48-1.33)	23	0.62 (0.37-1.06)
≥1600	21	0.89 (0.51-1.55)	11	1.19 (0.56-2.54)	9	0.59 (0.25-1.39)
<i>P</i> _{trend} ²		0.17		0.94		0.04
Ca:Mg ratio is above 2.5						
<600	25	1.16 (0.75-1.79)	10	0.78 (0.41-1.51)	15	1.76 (0.98-3.14)
600-1200	213	1.00 (Ref)	124	1.00 (Ref)	89	1.00 (Ref)
1200-1600	174	0.91 (0.73-1.13)	105	0.97 (0.73-1.29)	69	0.83 (0.59-1.17)
≥1600	195	0.93 (0.73-1.20)	109	0.97 (0.70-1.35)	83	0.84 (0.57-1.24)
<i>P</i> _{trend} ²		0.41		0.93		0.12
<i>P</i> _{interaction} ³		0.08		0.64		<0.01

¹ Adjusted for arm, age (continuous), sex, BMI (<25, 25-30, ≥30), education (less than high school, 12 years or completed high school, post high school training other than college, some college, college graduate, postgraduate), race (white, black, asian or others), family history of CRC (yes or no), cigarette (never smoked cigarettes, current or former), hours spent in vigorous activities (less than 1 hour/week, 1 hour/week, 2 hours/week, 3 hours/week, 4+ hours/week), and total energy and vitamin D intake

² Assigned the score *j* to the *j*th level of calcium intake and evaluated the significance of Wald test

³ Estimated the full model with interaction term of calcium intake and Ca:Mg ratio and without this term in reduce model using likelihood ratio test

Table 4.6 HRs and 95% CIs¹ for CRC incidence by arms

Calcium Intake (mg/day)	Distal CRC in the intervention arm		Distal CRC in the control arm	
	Cases	HR (95% CI)	Cases	HR (95% CI)
All				
<600	52	1.42 (0.97-2.07)	89	1.43 (1.03-1.98)
600-1200	135	1.00 (Ref)	119	1.00 (Ref)
1200-1600	51	0.74 (0.52-1.06)	42	0.78 (0.54-1.13)
≥1600	62	0.85 (0.58-1.25)	30	0.71 (0.45-1.12)
<i>P</i> _{trend}		0.06		<0.01
Ca:Mg ratio is between 1.7 to 2.5				
<600	26	1.31 (0.77-2.25)	25	1.42 (0.80-2.53)
600-1200	71	1.00 (Ref)	53	1.00 (Ref)
1200-1600	15	0.68 (0.35-1.32)	8	0.58 (0.24-1.41)
≥1600	7	0.71 (0.25-1.99)	2	0.55 (0.11-2.65)
<i>P</i> _{trend}		0.19		0.11
Ca:Mg ratio is above 2.5				
<600	7	1.63 (0.72-3.69)	8	2.03 (0.88-4.65)
600-1200	51	1.00 (Ref)	38	1.00 (Ref)
1200-1600	36	0.74 (0.46-1.18)	33	0.96 (0.58-1.57)
≥1600	55	0.82 (0.49-1.36)	28	0.86 (0.47-1.60)
<i>P</i> _{trend}		0.25		0.29
<i>P</i> _{interaction}		0.03		0.07

¹ Adjusted for age (continuous), sex, BMI (<25, 25-30, ≥30), education (less than high school, 12 years or completed high school, post high school training other than college, some college, college graduate, postgraduate), race (white, black, asian or others), family history of CRC (yes or no), cigarette (never smoked cigarettes, current or former), and total energy and vitamin D intake

² Assigned the score *j* to the *j*th level of calcium intake and evaluated the significance of Wald test

³ Estimated interaction term of calcium intake and Ca:Mg ratio in Wald test

Supplemental Table 4.7 ORs¹ and 95% CIs for additional outcomes by calcium intake

Ca Intake (mg/day)	Metachronous adenoma		CRC in intervention arm	
	Cases	OR (95% CI)	Cases	HR (95% CI)
No advanced/synchronous adenoma at baseline				
<600	37	1.92 (1.02-3.59)	73	0.98 (0.74-1.30)
600 -1200	98	1.00 (Ref)	245	1.00 (Ref)
1200 -1600	46	0.97 (0.57-1.66)	107	0.86 (0.67-1.10)
≥1600	41	0.97 (0.52-1.81)	133	0.99 (0.76-1.28)
<i>P</i> _{trend} ²		0.26		0.80
Has advanced/synchronous adenoma at baseline				
<600	87	1.03 (0.70-1.52)	21	1.46 (0.83-2.59)
600 -1200	295	1.00 (Ref)	51	1.00 (Ref)
1200 -1600	144	1.01 (0.73-1.40)	14	0.52 (0.26-1.00)
≥1600	107	0.78 (0.54-1.14)	17	0.66 (0.34-1.30)
<i>P</i> _{trend} ²		0.28		0.04
<i>P</i> _{interaction} ³		0.55 ³		0.09 ⁴

¹ Adjusted for age (continuous), sex, BMI (<25, 25-30, ≥30), education (less than high school, 12 years or completed high school, post high school training other than college, some college, college graduate, postgraduate), race (white, black, asian or others), family history of CRC (yes or no), cigarette (never smoked cigarettes, current or former), hours spent in vigorous activities (less than 1 hour/week, 1 hour/week, 2 hours/week, 3 hours/week, 4+ hours/week), and total energy and vitamin D intake

² Assigned the score *j* to the *j*th level of calcium intake and evaluated the significance of Wald test

³ Estimated the full model with interaction term of calcium intake and Ca:Mg ratio and without this term in reduce model using likelihood ratio test

B. Ca:Mg Ratio and lipid profile, a randomized clinical trial

The participants from the magnesium supplementation arm did not significantly differ from those from the placebo arm on age, sex, body mass index, smoking, drinking, physical activity, education, anti-hypertensive and oral hypoglycemic medication use. A higher percentage of participants used lipid-modulating drugs in the placebo arm compared to the magnesium treatment arm (**Table 4.8**). The Kolmogorov-Smirnov test showed that the distribution of four lipid biomarkers was not normal (data not shown).

We found magnesium treatment did not significantly affect the lipid profile, including LDL-c, HDL-c, triglycerides, and total cholesterol (**Table 4.9**). Further, compared to the placebo arm, the treatment arm also did not significantly affect lipid profile. We further conducted analysis among participants with long-term high Ca:Mg ratios measured by two 24-hour dietary recalls and the previously administered FFQ (**Table 4.10**). We found magnesium treatment led to a significant increase of HDL-c level by 5 mg/dl (P value, <0.001), but no changes were found for LDL-c, triglycerides, and total cholesterol within the magnesium treatment group. In the placebo arm, LDL-c significantly decreased (P value, 0.044). Compared to the placebo arm, magnesium treatment significantly increased HDL-c (P value, 0.004) and total cholesterol (P value, 0.017) even after controlling for controlling for baseline lipid profile. After Bonferroni multiple comparison correction, only the effect on HDL-c remained statistically significant.

Finally, we conducted analysis among participants with recent high Ca:Mg ratio measures (i.e. >2.6 from two 24-hour dietary recalls, but <2.6 from FFQ conducted

earlier) (**Table 4.11**). We found within the magnesium treatment arm, magnesium treatment significantly reduced HDL-c by 3 mg/dl (*P* value, 0.025), but not others. In the placebo arm, levels of triglycerides significantly reduced (*P* value, 0.043). Compared to the placebo arm, magnesium treatment reduced HDL-c (*P* value, 0.099), but increased triglycerides (*P* value, 0.054) at borderline significance. After adjusting for baseline lipid profile, the effect on HDL-c still remained of borderline significance (*P* value, 0.055), although the significance disappeared after Bonferroni correction.

Table 4.8 Descriptive characteristics of the included population

	Magnesium (N=76)	Placebo (N=74)	P value
Age	60.4±8.2	62.0±7.8	0.303 ¹
Sex - male (%)	47 (62%)	41 (55%)	0.423 ²
Body mass index (BMI)	29.5±6.2	29.9±6.1	0.533 ¹
Waist to hip ratio	0.97±0.06	0.97±0.05	0.5 ¹
TRPM7 Genotype - GG (%)	61 (80%)	56 (76%)	0.519 ²
Smoking status (%)			0.293 ²
Never	35 (47%)	44 (59%)	
Ever	33 (44%)	25 (34%)	
Current	7 (9%)	5 (7%)	
Drinking status (%)			0.078 ²
Never	21 (28%)	32 (43%)	
Ever	20 (26%)	11 (15%)	
Current	35 (46%)	31 (42%)	
Physically active in ≥ 2 days per week (%)	57 (75%)	60 (81%)	0.369 ²
Education under college (%)	7 (9%)	8 (11%)	0.744 ²
Lipid-modulating medication use - yes (%)	18 (24%)	32 (43%)	0.011 ²
Oral hypoglycemic drug use - yes (%)	3 (4%)	6 (8%)	0.283 ²
Anti-hypertensive drug use - yes (%)	33 (43%)	35 (47%)	0.634 ²
Ca:Mg ratio measured by FFQ			0.813 ²
High (≥2.6)	35 (46%)	33 (45%)	
Low (<2.6)	21 (28%)	18 (24%)	
Not available	20 (26%)	23 (31%)	
Baseline Ca:Mg ratio	3.5 (3.0-4.0)	3.6 (3.0-4.1)	0.875 ¹

For continuous variables: X±SD, median (the lower quartile-the upper quartile)

Tests used: ¹Wilcoxon test; ²Pearson test

Table 4.9 Within and between group comparison for lipid biomarkers among all participants

	N	Magnesium (N=76)				Placebo (N=74)				Between group	
		Week 0	Week 12	Differences	P ¹	Week 0	Week 12	Differences	P ¹	P ²	P ³
LDL-c	150	115 (95-153) 126±51	117 (92-150) 124±45	-5 (-15-10.2) -1.8±23.6	0.320	118 (94-140) 120±37	108 (88-142) 117±40	-4.5 (-17.2-13.5) -2.7±28	0.197	0.73	0.58
HDL-c	150	54 (44-71) 62±27	56 (44-72) 62±24	1.5 (-4-6) 0.1±10.1	0.403	56 (47-73) 61±20	55 (46-71) 60±19	-1 (-5-5) -0.7±9.2	0.627	0.38	0.53
TG	150	113 (82-168) 135±81	114 (83-164) 142±96	5 (-17-42.8) 6.6±57.4	0.238	119 (77-156) 133±75	110 (79-157) 133±78	-3.5 (-28.8-15.3) -0.9±50.3	0.575	0.23	0.38
TC	150	199 (164-255) 214±73	201 (168-250) 213±62	0.5 (-14.8-16) -0.9±33	0.943	199 (173-234) 207±50	196 (169-228) 203±52	-5.5 (-18.8-16.3) -4.0±37.2	0.278	0.40	0.37

a (b-c) represent the median a, the lower quartile b, and the upper quartile c for continuous variables

x ± s represents $\bar{X} \pm SD$

LDL-c: low-density lipoprotein cholesterol, HDL-c: high-density lipoprotein cholesterol, TG: triglycerides, TC: total cholesterol

N is the number of non-missing values

P¹: the P value for within group differences using Wilcoxon signed rank test

P²: the P value for between group differences using Wilcoxon rank-sum test

P³: the P value for between group comparison while adjusting for baseline level for each biomarker in the generalized linear model

Table 4.10 Within and between group comparison for lipid biomarkers among long-term high ratio participants

	N	Magnesium (N=35)				Placebo (N=33)				Between group	
		Week 0	Week 12	Differences	P ¹	Week 0	Week 12	Differences	P ¹	P ²	P ³
LDL-c	68	108 (86-140) 112±48	112 (90-139) 115±41	1 (-12-21) 2.5±27.3	0.473	120 (87-135) 117±37	104 (86-128) 108±35	-11 (-23-8) -8.8±21.8	0.044	0.035	0.066
HDL-c	68	53 (43-63) 54±19	56 (43-69) 58±20	5 (-0.5-8.5) 4.4±6.7	<0.001	49 (47-60) 54±12	50 (46-59) 53±12	-2 (-5-5) -0.8±7.6	0.555	0.004	0.004
TG	68	107 (68-192) 142±99	114 (84-154) 151±118	8 (-12.5-39) 9±59.8	0.207	124 (84-162) 143±83	100 (77-201) 144±91	-5 (-33-16) 0.7±58.1	0.668	0.270	0.569
TC	68	181 (152-236) 195±67	190 (165-244) 202±55	10 (-7.5-28) 7.5±33.1	0.118	195 (172-216) 200±49	186 (165-206) 189±45	-12 (-37-10) -10.2±30.7	0.072	0.019	0.017

a (b-c) represent the median a, the lower quartile b, and the upper quartile c for continuous variables

x ± s represents $\bar{X} \pm SD$

LDL-c: low-density lipoprotein cholesterol, HDL-c: high-density lipoprotein cholesterol, TG: triglycerides, TC: total cholesterol

N is the number of non-missing values

P¹: the P value for within group differences using Wilcoxon signed rank test

P²: the P value for between group differences using Wilcoxon rank-sum test

P³: the P value for between group comparison while adjusting for baseline level for each biomarker in the generalized linear model

Table 4.11 Within and between group comparison for lipid biomarkers among recent high ratio participants

	N	Magnesium (N=21)				Placebo (N=19)				Between group	
		Week 0	Week 12	Differences	P ¹	Week 0	Week 12	Differences	P ¹	P ²	P ³
LDL-c	39	110 (96-131) 112±32	107 (85-133) 110±32	-7 (-11-5) -1.9±17.2	0.244	110 (88-137) 114±39	102 (81-139) 110±38	-4.5 (-10.5-3) -4.4±16.6	0.246	0.98	0.675
HDL-c	39	52 (44-64) 55±14	50 (44-60) 53±12	-3 (-6-2) -2.7±4.8	0.025	48 (44-64) 56±17	50 (42-70) 57±19	-1 (-3.5-5.8) 1.3±7.7	0.861	0.099	0.055
TG	39	110 (81-154) 116±50	116 (77-163) 122±58	5 (-8-45) 6±43.5	0.444	131 (98-149) 132±52	109 (77-122) 111±48	-13.5 (-39.5-2.2) -20.3±43.1	0.043	0.054	0.115
TC	39	188 (162-227) 190±42	184 (164-217) 187±39	0 (-14-8) -3.3±19	0.432	195 (169-217) 196±47	188(157-211) 189±44	-4.5 (-17-2) -7.2±24.8	0.133	0.47	0.685

a (b-c) represent the median a, the lower quartile b, and the upper quartile c for continuous variables

x ± s represents $\bar{X} \pm 1SD$

LDL-c: low-density lipoprotein cholesterol, HDL-c: high-density lipoprotein cholesterol, TG: triglycerides, TC: total cholesterol

N is the number of non-missing values

P¹: the P value for within group differences using Wilcoxon signed rank test

P²: the P value for between group differences using Wilcoxon rank-sum test

P³: the P value for between group comparison while adjusting for baseline level for each biomarker in the generalized linear model

C. Ca:Mg ratio and uric acid, a randomized clinical trial

Overall, no significant difference in the change of uric acid from baseline to week 12 was observed in the magnesium treatment arm (**Table 4.12**). Among subjects with a long-term high Ca:Mg ratio, uric acid was lowered by 0.4 mg/dl within the placebo arm (*P* value, 0.011), which further resulted in a significant difference between magnesium treatment arm and placebo arm (*P* value, 0.026). However, this reduction is not significant after we adjusted for baseline uric acid (*P* value, 0.057).

Table 4.12 Within and between group comparison for uric acid levels

Magnesium					Placebo					Between group	
N	Week 0	Week 12	Differences	<i>P</i> ¹	N	Week 0	Week 12	Differences	<i>P</i> ¹	<i>P</i> ²	<i>P</i> ³
All participants											
76	6.1 (4.6-6.5) 6.0±1.6	5.8 (4.9-6.8) 6.0±1.6	0 (-0.6-0.5) 0±0.9	0.886	74	5.9 (4.9-7.1) 6.2±1.6	5.7 (4.9-6.8) 6.0±1.6	-0.1 (-0.8-0.5) -0.1±1.0	0.29	0.44	0.457
Long-term high ratio participants											
35	5.5 (4.3-6.4) 5.5±1.3	5.5 (4.7-6.5) 5.6±1.4	0.1 (-0.6-0.6) 0.1±0.9	0.568	33	5.8 (4.8-7.3) 6.1±1.5	5.7 (4.9-6.2) 5.7±1.4	-0.4 (-1.0-0.2) -0.4±0.8	0.011	0.026	0.057
Recent high ratio participants											
21	6.3 (5.1-6.5) 6.1±1.3	6.0 (4.8-6.8) 6.0±1.4	0 (-0.4-0.3) -0.1±0.7	0.711	18	6.0 (5.0-6.7) 6.1±2.0	5.5 (4.8-6.6) 5.9±1.5	-0.1 (-0.4-0.5) -0.2±1.2	0.896	0.99	0.739

a (b-c) represent the median a, the lower quartile b, and the upper quartile c for continuous variables

$x \pm s$ represents $\bar{X} \pm SD$

N is the number of non-missing values

*P*¹: the *P* value for within group differences using Wilcoxon signed rank test

*P*²: the *P* value for between group differences using Wilcoxon rank-sum test

*P*³: the *P* value for between group comparison while adjusting for baseline level for each biomarker in the generalized linear model

Chapter 5. Discussion and summary

A. Project A-discussion

In the current study, we found higher intakes of calcium, compared to low calcium intake (<600 magnesium/day), were not related to a reduced risk of incident adenoma, but were associated with a suggestive reduced risk for advanced/synchronous adenomas. The inverse association primarily appeared in subjects with Ca:Mg intake ratios between 1.7 and 2.5. Although calcium intake was not related to risk of metachronous adenoma regardless of initial adenoma characteristic and Ca:Mg intake ratio, we found higher intakes of calcium were associated with a reduced risk of incident distal CRC. Further, the inverse association with incident distal CRC was also primarily present in those with Ca:Mg intake ratios ranging from 1.7 to 2.5. Moreover, we found the inverse association was only significant among control arm without regular endoscopy, and became weaker in the intervention arm with regular endoscopy. We found the inverse association was only significant for those with baseline advanced/synchronous adenomas. To our knowledge, this is the first study to examine whether regular endoscopy use modifies the association between calcium and incident CRC risk.

Both cohort studies and earlier intervention trials relatively consistently found high calcium intake or calcium supplementation was related to moderately reduced risks of adenoma, metachronous adenoma and CRC ^{18–20,22,23,25,299,300}. However, results from a very recent large-scale randomized clinical trial (WHI) do not support an effect of calcium plus vitamin D supplementation on the incidence of CRC after 7 years of follow-

up ¹⁵. Very recently, Baron et al reported from a large scale randomized trial that calcium supplementation did not reduce risk of colorectal metachronous adenoma ¹⁶.

It is possible the follow-up time in the WHI was not sufficiently long to observe an effect. CRC is believed to arise, in the overwhelming majority of cases, from adenomas via the well-established adenoma-carcinoma sequence ³⁰¹. Our findings from the current study may provide possible explanations for the inconsistent results ^{125,126} in the previous studies on the effect of calcium on the colorectal carcinogenesis ¹⁷⁻²⁰. Earlier studies hypothesized that the chemoprevention effect of calcium intake on CRC may primarily exert in its early stages (adenoma) ¹⁵. Our findings are consistent with previous epidemiologic data ^{21,22} indicating higher calcium intake may only inhibit early colorectal carcinogenesis at the stage of incident adenoma ^{17-20,300} and the association may be stronger for prevention of advanced adenoma, a premalignant lesion for CRC ⁴, than other types of adenoma or polyps ³⁰². The possibility is consistent with the observation that the magnitude of reduction in overall CRC risk associated with high calcium intake is similar to the reduction in adenoma risk.

We did not find calcium intake was related to risk of metachronous colorectal adenoma in the current study. However, the sample size, and thus statistical power, is smaller for this outcome than for the analyses of incident adenoma and incident cancer. Although earlier randomized trials found calcium supplementation reduced risk of colorectal metachronous adenoma ²³, a recent trial did not find calcium supplementation reduced risk of metachronous colorectal adenoma ¹⁶. As such, other underlying factors may

account for the inconsistency between these randomized trials such as the change of the Ca:Mg intake ratio over the time. The Ca:Mg intake ratio has increased from about 2.6³⁰³ during the period the earlier trials^{23,299} were conducted to over 3 when the recent randomized trial was conducted¹⁶.

We found in the current study that the Ca:Mg intake ratio modifies the association between calcium intake and development of adenoma and CRC . We also found the significant inverse associations between calcium intake and advanced/synchronous adenomas, or distal CRC may primarily appear in those with Ca:Mg intake ratios between 1.7 and 2.5. We reported earlier that the dietary intake ratio of Ca:Mg modified the association between intakes of calcium and magnesium in relation to prevalent colorectal adenoma¹⁵⁴. In a subsequent randomized clinical trial, calcium supplementation only reduced risk of metachronous colorectal adenoma when the baseline Ca:Mg was below 2.63²⁶. Very recently, we found that Ca:Mg ratio modified the associations between intakes of calcium and magnesium and risk of esophageal neoplasia²⁷. Consistent with our finding, one case-control study conducted in Belgians reported that a high calcium intake with a low magnesium intake was associated with increased risk of bladder cancer¹⁴⁴. In studies conducted in East Asian populations with a low Ca:Mg intake ratio (a median around 1.7), we found the association between intakes of calcium and magnesium with the risk of total, cardiovascular and/or cancer mortalities were modified by Ca:Mg ratio, but not by calcium or magnesium intake alone²⁸. Thus, the balance between calcium and magnesium intake is an important factor to

consider in the investigation of associations between intakes of calcium and magnesium and cancer development.

In addition to the Ca:Mg intake ratio, we found the inverse association between calcium intake and risk of incident distal CRC was stronger in the control arm compared to the intervention arm with multiple flexible sigmoidoscopies. These findings suggested the association between calcium intake and colorectal carcinogenesis may be attenuated by endoscopy screening and the removal of polyps with an endoscopy, which is the most effective prevention strategy to reduce the morbidity and mortality of CRC ²⁹.

The protective association with higher calcium intake was mainly in distal CRC, with approximately 30-50% reduced risk. This finding is consistent with a meta-analysis of 17 cohort studies, in which the protective effect of calcium intake was more evident for distal colon cancer compared with proximal colon tumors (33% vs 25% of risk reduction) ¹². Previous etiologic studies indicated proximal and distal sections differ in physiological functions, fecal composition and transit, as well as suggested that environmental factors such as diet mainly affect distal colon carcinogenesis ³⁰⁴.

No previous study has prospectively evaluated the associations of calcium intake with incident adenoma, metachronous adenoma, and incident cancer in the same cohort using the same food frequency questionnaire, which minimizes misclassification that can occur when combining dietary estimates from multiple studies with different food

frequency questionnaire instruments. The PLCO screening trial provides a unique and unparalleled opportunity to prospectively and concurrently evaluate these associations. One weakness is that the PLCO is a sigmoidoscopy-based randomized trial, which only screened distal colorectum. Thus, incident adenoma cases mainly included adenomas from the left side of the bowel although metachronous adenoma and incident CRC cases included cases from both proximal and distal colorectum. However, it is interesting that recent reports found that colonoscopy was linked to reduced deaths from distal colorectum, but not the right ^{30,32} while similarly a recent report from the PLCO observed sigmoidoscopic screenings only reduced distal, but not proximal CRC mortality ³¹. Further, sigmoidoscopic screenings also significantly reduced both distal and proximal CRC incidence in the PLCO ³¹. Thus, the PLCO provides a unique opportunity to test whether calcium intake confers additional protection against proximal and distal CRC among those receiving sigmoidoscopic screening(s). Similar to other nutritional epidemiological studies using food frequency questionnaire, there are possibly non-differential measurement errors which may bias the results to the null.

In conclusion, higher calcium intake may primarily be related to reduced risks of incident advanced/synchronous adenoma and incident distal CRC among subjects with Ca:Mg intake ratios between 1.7 and 2.5, or participants without regular endoscopy and those with advanced/multiple adenomas at baseline.

B. Project B-discussion

In this study, we found overall magnesium supplementation did not affect lipid profile. However, among individuals whose Ca:Mg intake ratios were consistently higher than 2.6, reducing the Ca:Mg ratio through magnesium treatment significantly led to a beneficial effect on HDL-c (i.e. an increase by 5 mg/dl). This change remained significant after adjusting for baseline HDL-c and multiple comparisons. Conversely, among subjects with a Ca:Mg intake ratio which varied over time (i.e. <2.6 from FFQ, but >2.6 in two 24-hour dietary recalls), reducing the Ca:Mg ratio through magnesium treatment may have reduced HDL-c, although the change was not significant after correcting for multiple comparison.

Although not consistent ^{315,316}, earlier clinical trials indicated an increase of HDL-c by 3 to 4 mg/dl with oral magnesium supplementation among diabetic patients ³¹¹. Similarly, a more recent meta-analysis of clinical trials among participants with diabetes found magnesium significantly increased HDL-c by around 3 mg/dl, and had no effect on LDL-c, triglycerides, and total cholesterol ²¹². Consistent with these studies, our results also suggested null effects of magnesium treatment on LDL-c, triglycerides, and total cholesterol, although previous clinical trials ^{310,311} generated inconsistent results. These findings are biologically plausible since both patients with type 2 diabetes ³¹² and individuals with high Ca:Mg intakes ratio (i.e. ratios >2.6) ^{17,26,27} are at high risk of magnesium deficiency. On the other hand, we previously found among those with very low Ca:Mg intake ratios (i.e. ratio<1.7), high intakes of magnesium were related to an increased risk of total mortality or mortality due to cardiovascular disease ²⁸. This may

explain in our study further reducing the Ca:Mg ratio via magnesium supplementation may even decrease HDL-c among those with inconsistent ratios measured by FFQ (i.e. ratio<2.6).

It is possible that previous trials of magnesium supplementation enrolled subjects with both high and low Ca:Mg ratios; the high doses of magnesium treatment generally used in these trials may have led to a very low Ca:Mg ratio (<1.7), particularly for those with a low ratio at baseline ²¹². The modifying effect of the Ca:Mg ratio may explain the inconsistent results in previous clinical trials of magnesium supplementations. Likewise, the findings for dietary and supplement calcium ^{232,233} in relation to cardiovascular disease have also been inconsistent. It is likely that dietary sources of calcium are usually accompanied by high dietary magnesium intake, and this, in turn, leads to a moderate Ca:Mg ratio from dietary sources. In contrast, calcium supplementation alone substantially increases the Ca:Mg ratio. This may explain the differential effects of calcium from dietary ^{234,235} and supplemental sources ^{232,233} on cardiovascular disease risk and mortality. Some recent randomized trials found high calcium supplementation led to increased risk of myocardial infarction ³⁰⁸. Therefore, instead of considering absolute calcium and magnesium alone, the Ca:Mg ratio is another critical factor in lipid metabolism as well as other physiologic activities.

The body's calcium and magnesium status is an integrated function of oral intake, intestinal absorption, renal reabsorption and skeletal remodeling ¹⁰¹. Calcium and magnesium have common food sources and are considered to be “metabolically

interactive” as well ¹⁴¹. Calcium and magnesium may share the same homeostatic regulation system ⁴⁵ primarily through absorption in the intestine and reabsorption in the kidneys to maintain the balance of Ca:Mg ^{45,46}. Nevertheless, calcium and magnesium compete with each other in (re)absorption and transport ⁴⁷⁻⁵¹. A high calcium intake reduces absorption of both magnesium and calcium ¹⁴⁵ whereas moderate magnesium deprivation results in negative magnesium balance but increased calcium retention ¹⁴⁶. In addition, magnesium supplementation increased urinary calcium excretion if calcium intake was less than 800 mg/day ¹⁴⁸, suggesting that magnesium may suppress calcium reabsorption when Ca:Mg intake ratio is very low ²⁸. Therefore, the Ca:Mg intake ratio may have an important role in regulating calcium and magnesium (re)absorption. A high Ca:Mg intake ratio implies relatively high calcium but low magnesium intake; thus, magnesium supplementation is essential for lowering the already-high calcium status among participants with a high Ca:Mg ratio.

Although potential mechanisms of magnesium deficiency on the development of cardiovascular disease is not completely understood, animal studies have suggested that magnesium intake is highly related to atherogenic process ³¹⁷. Specifically, magnesium affects apolipoproteins and enzymes involved in lipid metabolism ³¹⁷. The enzyme activity of lecithin cholesterol acyltransferase (LCAT) ²⁴⁰, lipoprotein lipase (LPL) ²⁰², and 2-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase ²⁴⁴, requires the involvement of magnesium ²⁴¹. In the pathway of cholesterol biosynthesis, HMG-CoA reductase is a rate-limiting enzyme to transform HMG-CoA to mevalonate ²⁴⁴. This conversion process could be affected by statins and regulated by magnesium ²⁴⁴.

Magnesium supplementation among participants with a long-term high Ca:Mg ratio may their improve magnesium deficiency or insufficiency and subsequently change the overall cholesterol biosynthesis process. Therefore, we observed a significant increase of HDL-c.

Participants in this study are otherwise healthy volunteers with previous diagnoses of hyperplastic polyps or colorectal adenomas, which is different from those reported in previous studies ^{318–320}. The baseline levels of lipid profile for the participants are close to the normal range. In this study, the average of HDL-c is around 55 mg/dl and LDL-c is around 115 mg/dl. Thus, a 5 mg/dl increase in HDL-c may be more beneficial than a decrease in LDL-c among this group of participants, which has very significant implications for the prevention of cardiovascular disease. It is possible that the “regression to the mean” effect exists. Thus, we further adjusted for baseline level of lipid profile in final models. Serum calcium and magnesium is strictly regulated by the homeostasis system ²³¹. Thus, serum calcium and magnesium are not sensitive markers of total body calcium and magnesium status. In our study, dietary intake and supplemental use of calcium and magnesium were used as a surrogate of total body calcium and magnesium status. However, these measurements have not taken into account gastrointestinal absorption.

In conclusion, reducing the high Ca:Mg ratio through magnesium supplementation may increase HDL-c in participants with long-term high Ca:Mg intake ratios, but may have a detrimental effect among subjects with Ca:Mg ratios <2.6 or not remaining stable. If this

finding is confirmed, it provides a novel and inexpensive approach for the prevention of cardiovascular disease.

C. Project C-discussion

In this randomized trial, reducing Ca:Mg ratios through magnesium supplementation did not affect concentrations of serum uric acid. Previously, we found the Ca:Mg intake ratio modified the associations between intakes of calcium and magnesium and risk of gastrointestinal neoplasia^{17,26,27}, total, cardiovascular disease and cancer mortalities²⁸. No previous studies have investigated the effect of the Ca:Mg ratio on uric acid. To our knowledge, this is the first clinical trial to investigate whether reducing the Ca:Mg intake ratio through magnesium supplementation affected uric acid. Our findings indicated the Ca:Mg ratio may not affect risk of cancer or cardiovascular disease by modifying uric acid. Two previous studies found that magnesium status was correlated with uric acid status^{275,322}. However, these are cross-sectional studies which are difficult to determine their temporal relationships. It is possible that uric acid is correlated with lipid profiles, inflammation biomarkers and other pathways. Future studies should evaluate whether the Ca:Mg ratio may affect pathways such as lipid profiles³²³ as well as inflammatory responses^{324,325}. We also cannot exclude the possibility that the null result in the current study is due to a small sample size so future larger studies may be needed.

D. Summary, conclusions and future directions

CRC, a worldwide health burden, is the 3rd most common incident cancer and the 3rd leading cause of cancer death in the United States⁵⁵. A few modifiable risk factors were

associated with colorectal carcinogenesis. Although it is inconsistent, good behavioral factors which seem to decrease risk include being physically active, eating healthy, keeping an optimal body mass index (18.5 to 25), abstinence from smoking, no to light alcohol consumption, hormonal replacement therapy for postmenopausal women, regular endoscopy screening to remove polyps, aspirin ^{326,327} and other non-steroidal anti-inflammatory drugs ⁸⁸ would potentially prevent against CRC ⁶⁷.

Calcium, a chemopreventive nutrient, was suggested to reduce the risk of incident adenoma ¹⁷⁻²⁰, metachronous adenoma ²³⁻²⁵ and cancer ^{21,22}. The Ca:Mg intake ratio was suggested to modify the associations between intakes of calcium and magnesium and risk of gastrointestinal neoplasia ^{17,26,27}, and total, cardiovascular disease and cancer mortalities ²⁸. Previous studies also indicated that a ratio between 1.7 and 2.6 is potentially the range needed for calcium intake to be protective against these outcomes ^{17,26-28}. It is plausible that calcium intake and the Ca:Mg intake ratio play an essential role in the development of CRC .

We evaluated the associations between calcium intake and the risks of incident colorectal adenoma (1,147 cases), metachronous adenoma (855 cases) and incident CRC (697 and 578 cases in intervention and control arms, respectively) among 108,563 PLCO participants aged 55 to 74 years. Compared to low calcium intake (<600 mg/day), higher intakes of calcium were not related to a reduced risk of incident or metachronous colorectal adenoma, but were related to a suggestive reduced risk of advanced and/or synchronous adenomas and to a significantly reduced risk of CRC, especially for distal

CRC. The inverse association was primarily apparent in participants whose Ca:Mg ratios ranged from 1.7 to 2.5. In addition, the statistically significant association between calcium intake and risk of incident distal CRC appeared to be primarily present in the control arm without regular endoscopy.

The 150 pairs of pre- and post-treatment serum samples of participants in the PPCCT study who finished the trial were sent to the Vanderbilt Lipid Laboratory assaying of lipid panel and uric acid. Overall, magnesium treatment did not significantly affect the levels of the lipid profile including LDL-c, HDL-c, triglycerides, and total cholesterol. Among participants with a long-term high Ca:Mg ratio, magnesium supplementation led to a statically significant increase of HDL-c level by 5 mg/dl. No significant difference in the change of uric acid from baseline to week 12 was observed in the magnesium treatment arm.

This dissertation suggests higher calcium intake may primarily be related to reduced risks of incident advanced and/or synchronous adenoma as well as incident distal CRC among subjects with Ca:Mg intake ratios between 1.7 and 2.5, or participants without regular endoscopy and those with advanced and/or synchronous adenomas at baseline. Thus, this study adds additional evidence to understand associations between calcium intake and risk of CRC development at various stages. Previous studies suggested that the association between intakes of calcium and magnesium with the risk of total, cardiovascular and/or cancer mortalities were modified by Ca:Mg ratio, but not by calcium or magnesium intake alone ²⁸. In this study, the potential modifying effect of

Ca:Mg intake ratios was further suggested. It is possible that lack of considering Ca:Mg balance may explain some of the inconsistency in previous studies on either calcium or magnesium intake. Moreover, the association between calcium intake and risk of CRC appears to be stronger in individuals without endoscopic screenings. To our best knowledge, this is the first study to examine whether regular endoscopy use modifies the association between calcium intake and incident CRC risk.

Recent studies indicate that Ca:Mg intake ratio may modify the associations between intakes of calcium and magnesium and risk of gastrointestinal neoplasia ^{17,26,27}, and total, cardiovascular disease and cancer mortalities ²⁸. This study found that reducing a high Ca:Mg ratio through magnesium supplementation may increase HDL-c in participants with long-term high Ca:Mg intake ratios. A meta-analysis of nine randomized trials conducted among type 2 diabetic patients, individuals at high risk of magnesium deficiency ³¹², showed that oral magnesium supplementation significantly, but weakly, increased HDL-c by 0.08 mmol/l, and had no effect on LDL-c and triglycerides ²¹². Our study is consistent with this meta-analysis and found a stronger effect of magnesium supplement when the Ca:Mg ratio is considered. These human findings are consistent with the evidence from animal studies indicating that calcium-adequate and magnesium-deficient diets lead to increases in heart lipid peroxidation, plasma levels of triglycerides, as well as inflammatory markers; whereas both calcium-deficient and magnesium-deficient diets normalize the levels of inflammatory markers, lipid peroxidation and plasma triglycerides concentration ^{52,53}.

Both metabolic syndrome and deficits of calcium or magnesium are potential risk factors of colorectal neoplasia. However, no studies have examined whether the Ca:Mg ratio may have a role in uric acid metabolism nor whether uric acid is an intermediate between the association of the Ca:Mg ratio and colorectal neoplasia. Our findings indicated the Ca:Mg ratio may not affect risk of cancer or cardiovascular disease by modifying uric acid. Future studies should evaluate whether the Ca:Mg ratio may affect biomarkers such as lipid profiles³²³ and uric acid. We also cannot exclude the possibility that the null result for uric acid in the current study is due to a small sample size so future larger studies may be needed.

REFERENCES

1. Fact Sheets by Population. Available at:
http://globocan.iarc.fr/Pages/fact_sheets_population.aspx. (Accessed: 2nd April 2015)
2. Cancer of the Colon and Rectum - SEER Stat Fact Sheets. Available at:
<http://seer.cancer.gov/statfacts/html/colorect.html>. (Accessed: 13th February 2016)
3. *Global Cancer Facts & Figure 2nd Edition*. (American Cancer Society, 2011).
4. Strum, W. B. Colorectal Adenomas. *N. Engl. J. Med.* **374**, 1065–1075 (2016).
5. Tanaka, T. Colorectal carcinogenesis: Review of human and experimental animal studies. *J. Carcinog.* **8**, (2009).
6. Bostick, R. M. *et al.* Relation of calcium, vitamin D, and dairy food intake to incidence of colon cancer among older women. The Iowa Women's Health Study. *Am. J. Epidemiol.* **137**, 1302–1317 (1993).
7. Flood, A. *et al.* Calcium from diet and supplements is associated with reduced risk of colorectal cancer in a prospective cohort of women. *Cancer Epidemiol. Biomark. Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol.* **14**, 126–132 (2005).
8. Larsson, S. C., Bergkvist, L., Rutegard, J., Giovannucci, E. & Wolk, A. Calcium and dairy food intakes are inversely associated with colorectal cancer risk in the Cohort of Swedish Men. *Am J Clin Nutr* **83**, 667–73; quiz 728–9 (2006).
9. Shin, A. *et al.* Dietary intake of calcium, fiber and other micronutrients in relation to colorectal cancer risk: Results from the Shanghai Women's Health Study. *Int J Cancer* **119**, 2938–42 (2006).
10. Oh, K., Willett, W. C., Wu, K., Fuchs, C. S. & Giovannucci, E. L. Calcium and vitamin D intakes in relation to risk of distal colorectal adenoma in women. *Am. J. Epidemiol.* **165**, 1178–1186 (2007).
11. Ishihara, J., Inoue, M., Iwasaki, M., Sasazuki, S. & Tsugane, S. Dietary calcium, vitamin D, and the risk of colorectal cancer. *Am J Clin Nutr* **88**, 1576–83 (2008).

12. Huncharek, M., Muscat, J. & Kupelnick, B. Colorectal cancer risk and dietary intake of calcium, vitamin D, and dairy products: a meta-analysis of 26,335 cases from 60 observational studies. *Nutr Cancer* **61**, 47–69 (2009).
13. Baron, J. A. *et al.* Calcium Supplements for the Prevention of Colorectal Adenomas. *N. Engl. J. Med.* **340**, 101–107 (1999).
14. Gonzalez, C. A. & Riboli, E. Diet and cancer prevention: Contributions from the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Eur. J. Cancer Oxf. Engl. 1990* **46**, 2555–2562 (2010).
15. Wactawski-Wende, J. *et al.* Calcium plus vitamin D supplementation and the risk of colorectal cancer. *N Engl J Med* **354**, 684–96 (2006).
16. Baron, J. A. *et al.* A Trial of Calcium and Vitamin D for the Prevention of Colorectal Adenomas. *N. Engl. J. Med.* **373**, 1519–1530 (2015).
17. 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* **86**, 743–51 (2007).
18. Kesse, E., Boutron-Ruault, M. C., Norat, T., Riboli, E. & Clavel-Chapelon, F. Dietary calcium, phosphorus, vitamin D, dairy products and the risk of colorectal adenoma and cancer among French women of the E3N-EPIC prospective study. *Int J Cancer* **117**, 137–44 (2005).
19. Martinez, M. E., McPherson, R. S., Annegers, J. F. & Levin, B. Association of diet and colorectal adenomatous polyps: dietary fiber, calcium, and total fat. *Epidemiology* **7**, 264–8 (1996).
20. Boutron, M. C. *et al.* Calcium, phosphorus, vitamin D, dairy products and colorectal carcinogenesis: a French case--control study. *Br. J. Cancer* **74**, 145–151 (1996).
21. Zhang, X. & Giovannucci, E. Calcium, vitamin D and colorectal cancer chemoprevention. *Best Pract. Res. Clin. Gastroenterol.* **25**, 485–494 (2011).

22. Cho, E. *et al.* Dairy foods, calcium, and colorectal cancer: a pooled analysis of 10 cohort studies. *J Natl Cancer Inst* **96**, 1015–22 (2004).
23. Baron, J. A. *et al.* Calcium supplements for the prevention of colorectal adenomas. Calcium Polyp Prevention Study Group. *N. Engl. J. Med.* **340**, 101–7 (1999).
24. Bonithon-Kopp, C., Kronborg, O., Giacosa, A., Rath, U. & Faivre, J. Calcium and fibre supplementation in prevention of colorectal adenoma recurrence: a randomised intervention trial. European Cancer Prevention Organisation Study Group. *Lancet* **356**, 1300–6 (2000).
25. Grau, M. V. *et al.* Vitamin D, calcium supplementation, and colorectal adenomas: results of a randomized trial. *J. Natl. Cancer Inst.* **95**, 1765–71 (2003).
26. Dai, Q. *et al.* Calcium, Magnesium, and Colorectal Cancer. *Epidemiology* **23**, 504–505 (2012).
27. Dai, Q. *et al.* Dietary magnesium, calcium:magnesium ratio and risk of reflux oesophagitis, Barrett's oesophagus and oesophageal adenocarcinoma: a population-based case-control study. *Br. J. Nutr.* **115**, 342–350 (2016).
28. Dai, Q. *et al.* Modifying effect of calcium/magnesium intake ratio and mortality: a population-based cohort study. *BMJ Open* **3**, (2013).
29. Espey, D. K. *et al.* Annual report to the nation on the status of cancer, 1975-2004, featuring cancer in American Indians and Alaska Natives. *Cancer* **110**, 2119–52 (2007).
30. Baxter, N. N. *et al.* Association of colonoscopy and death from colorectal cancer. *Ann Intern Med* **150**, 1–8 (2009).
31. Schoen, R. E. *et al.* Colorectal-Cancer Incidence and Mortality with Screening Flexible Sigmoidoscopy. *N. Engl. J. Med.* **366**, 2345–2357 (2012).
32. Nishihara, R. *et al.* Long-term colorectal-cancer incidence and mortality after lower endoscopy. *N. Engl. J. Med.* **369**, 1095–1105 (2013).

33. Yamaji, Y. *et al.* Incidence and recurrence rates of colorectal adenomas estimated by annually repeated colonoscopies on asymptomatic Japanese. *Gut* **53**, 568–572 (2004).
34. Hu, F. B. *et al.* Prospective study of adult onset diabetes mellitus (type 2) and risk of colorectal cancer in women. *J. Natl. Cancer Inst.* **91**, 542–547 (1999).
35. Wideroff, L. *et al.* Cancer incidence in a population-based cohort of patients hospitalized with diabetes mellitus in Denmark. *J. Natl. Cancer Inst.* **89**, 1360–1365 (1997).
36. Marugame, T. *et al.* Relation of impaired glucose tolerance and diabetes mellitus to colorectal adenomas in Japan. *Cancer Causes Control CCC* **13**, 917–921 (2002).
37. Nishii, T. *et al.* Glucose intolerance, plasma insulin levels, and colon adenomas in Japanese men. *Jpn. J. Cancer Res. Gann* **92**, 836–840 (2001).
38. Pais, R., Silaghi, H., Silaghi, A.-C., Rusu, M.-L. & Dumitrascu, D.-L. Metabolic syndrome and risk of subsequent colorectal cancer. *World J. Gastroenterol. WJG* **15**, 5141–5148 (2009).
39. Moghaddam, A. A., Woodward, M. & Huxley, R. Obesity and risk of colorectal cancer: a meta-analysis of 31 studies with 70,000 events. *Cancer Epidemiol. Biomark. Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol.* **16**, 2533–2547 (2007).
40. Tsilidis, K. K. *et al.* Metabolic syndrome components and colorectal adenoma in the CLUE II cohort. *Cancer Causes Control CCC* **21**, 1–10 (2010).
41. Rayssiguier, Y., Durlach, J., Gueux, E., Rock, E. & Mazur, A. Magnesium and ageing. I. Experimental data: importance of oxidative damage. *Magnes. Res. Off. Organ Int. Soc. Dev. Res. Magnes.* **6**, 369–378 (1993).
42. Grossman, C. *et al.* Serum uric acid is associated with coronary artery calcification. *J. Clin. Hypertens. Greenwich Conn* **16**, 424–428 (2014).
43. Gouri, A. *et al.* Serum uric acid level and cardiovascular risks in hemodialysis patients: an Algerian cohort study. *Clin. Lab.* **60**, 751–758 (2014).

44. Chavan, V. U., Ramavataram, D., Patel, P. A. & Rupani, M. P. Evaluation of serum magnesium, lipid profile and various biochemical parameters as risk factors of cardiovascular diseases in patients with rheumatoid arthritis. *J. Clin. Diagn. Res. JCDR* **9**, BC01–05 (2015).
45. Brown, E. M. & MacLeod, R. J. Extracellular calcium sensing and extracellular calcium signaling. *Physiol Rev* **81**, 239–297 (2001).
46. Hardwick, L. L., Jones, M. R., Brautbar, N. & Lee, D. B. Magnesium absorption: mechanisms and the influence of vitamin D, calcium and phosphate. *J Nutr* **121**, 13–23 (1991).
47. Iseri, L. T. & French, J. H. Magnesium: nature's physiologic calcium blocker. *Am Heart J* **108**, 188–93 (1984).
48. Flatman, P. W. Mechanisms of magnesium transport. *Annu Rev Physiol* **53**, 259–71 (1991).
49. Hartwig, A. Role of magnesium in genomic stability. *Mutat Res* **475**, 113–21 (2001).
50. Tashiro, M., Konishi, M., Iwamoto, T., Shigekawa, M. & Kurihara, S. Transport of magnesium by two isoforms of the Na⁺-Ca²⁺ exchanger expressed in CCL39 fibroblasts. *Pflugers Arch* **440**, 819–27 (2000).
51. Lyu, R. M., Smith, L. & Smith, J. B. Sodium-calcium exchange in renal epithelial cells: dependence on cell sodium and competitive inhibition by magnesium. *J Membr Biol* **124**, 73–83 (1991).
52. Bussiere, F. I., Gueux, E., Rock, E., Mazur, A. & Rayssiguier, Y. Protective effect of calcium deficiency on the inflammatory response in magnesium-deficient rats. *Eur. J. Nutr.* **41**, 197–202 (2002).
53. Ziegler, D. Type 2 diabetes as an inflammatory cardiovascular disorder. *Curr Mol Med* **5**, 309–22 (2005).
54. Fact Sheets by Cancer. Available at: http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx. (Accessed: 2nd April 2015)

55. Siegel, R., Desantis, C. & Jemal, A. Colorectal cancer statistics, 2014. *CA. Cancer J. Clin.* **64**, 104–117 (2014).
56. Cancer of the Colon and Rectum - SEER Stat Fact Sheets. Available at:
<http://seer.cancer.gov/statfacts/html/colorect.html>. (Accessed: 10th October 2014)
57. Cancer of the Colon and Rectum - SEER Stat Fact Sheets.
58. Sobin, L. H., Gospodarowicz, M. K. & Wittekind, C. *TNM Classification of Malignant Tumours, 7th Edition*. (UICC, Wiley-Blackwell, 2009).
59. Nawa, T. *et al.* Differences between right- and left-sided colon cancer in patient characteristics, cancer morphology and histology. *J. Gastroenterol. Hepatol.* **23**, 418–423 (2008).
60. Matanoski, G., Tao, X. G., Almon, L., Adade, A. A. & Davies-Cole, J. O. Demographics and tumor characteristics of colorectal cancers in the United States, 1998-2001. *Cancer* **107**, 1112–1120 (2006).
61. Iacopetta, B. Are there two sides to colorectal cancer? *Int. J. Cancer J. Int. Cancer* **101**, 403–408 (2002).
62. Ferlay, J. *et al.* Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Available at:
http://s3.amazonaws.com/academia.edu.documents/42372408/Estimates_of_worldwide_burden_of_cancer_20160208-19580-1ie5atq.pdf?AWSAccessKeyId=AKIAJ56TQJRTWSMTNPEA&Expires=1475712520&Signature=Z6UDnVy46e67o2JsJluL8F9MuQI%3D&response-content-disposition=inline%3B%20filename%3DEstimates_of_worldwide_burden_of_cancer.pdf. (Accessed: 5th October 2016)
63. Kim, S.-E. *et al.* Sex- and gender-specific disparities in colorectal cancer risk. *World J. Gastroenterol. WJG* **21**, 5167–5175 (2015).

64. Pal, S. K. & Hurria, A. Impact of age, sex, and comorbidity on cancer therapy and disease progression. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* **28**, 4086–4093 (2010).
65. Gao, R.-N., Neutel, C. I. & Wai, E. Gender differences in colorectal cancer incidence, mortality, hospitalizations and surgical procedures in Canada. *J. Public Health* **30**, 194–201 (2008).
66. Murphy, G. *et al.* Sex Disparities in Colorectal Cancer Incidence by Anatomic Subsite, Race and Age. *Int. J. Cancer J. Int. Cancer* **128**, 1668–1675 (2011).
67. *Colorectal Cancer Facts & Figures 2011-2013*. (American Cancer Society, 2011).
68. How is colorectal cancer staged? Available at:
<http://www.cancer.org/cancer/colonandrectumcancer/detailedguide/colorectal-cancer-staged>. (Accessed: 29th April 2015)
69. Definition and Facts for Colon Polyps. Available at: <http://www.niddk.nih.gov/health-information/health-topics/digestive-diseases/colon-polyps/Pages/definition-facts.aspx>. (Accessed: 30th June 2016)
70. Morson, B. The Polyp-cancer Sequence in the Large Bowel. *Proc. R. Soc. Med.* **67**, 451 (1974).
71. Schofield, P. F. & Jones, D. J. ABC of colorectal diseases. Colorectal neoplasia--I: Benign colonic tumours. *BMJ* **304**, 1498–1500 (1992).
72. Jackman, R. J. & Mayo, C. W. The adenoma-carcinoma sequence in cancer of the colon. *Surg. Gynecol. Obstet.* **93**, 327–330 (1951).
73. Clark, J. C. *et al.* Prevalence of polyps in an autopsy series from areas with varying incidence of large-bowel cancer. *Int. J. Cancer J. Int. Cancer* **36**, 179–186 (1985).
74. Winawer, S. J. *et al.* Prevention of colorectal cancer by colonoscopic polypectomy. The National Polyp Study Workgroup. *N. Engl. J. Med.* **329**, 1977–1981 (1993).
75. Zauber, A. G. *et al.* Colonoscopic polypectomy and long-term prevention of colorectal-cancer deaths. *N. Engl. J. Med.* **366**, 687–696 (2012).

76. Hurlstone, D. P. The detection of flat and depressed colorectal lesions: which endoscopic imaging approach? *Gastroenterology* **135**, 338–43 (2008).
77. Corley, D. A., Levin, T. R. & Doubeni, C. A. Adenoma detection rate and risk of colorectal cancer and death. *N. Engl. J. Med.* **370**, 2541 (2014).
78. Heitman, S. J. *et al.* Prevalence of Adenomas and Colorectal Cancer in Average Risk Individuals: A Systematic Review and Meta-analysis. *Clin. Gastroenterol. Hepatol.* **7**, 1272–1278 (2009).
79. Bond, J. H. Polyp guideline: diagnosis, treatment, and surveillance for patients with colorectal polyps. Practice Parameters Committee of the American College of Gastroenterology. *Am. J. Gastroenterol.* **95**, 3053–3063 (2000).
80. Lieberman, D. A. *et al.* Low rate of large polyps (>9 mm) within 10 years after an adequate baseline colonoscopy with no polyps. *Gastroenterology* **147**, 343–350 (2014).
81. van Enckevort, C. C. G. *et al.* Predictors of colorectal neoplasia after polypectomy: based on initial and consecutive findings. *Neth. J. Med.* **72**, 139–145 (2014).
82. Fleming, M., Ravula, S., Tatishchev, S. F. & Wang, H. L. Colorectal carcinoma: Pathologic aspects. *J. Gastrointest. Oncol.* **3**, 153–173 (2012).
83. Tziris, N. *et al.* Synchronous and metachronous adenocarcinomas of the large intestine. *Hippokratia* **12**, 150–152 (2008).
84. Villavicencio, R. T. & Rex, D. K. Colonic adenomas: prevalence and incidence rates, growth rates, and miss rates at colonoscopy. *Semin. Gastrointest. Dis.* **11**, 185–193 (2000).
85. Viel, J.-F., Studer, J.-M., Ottignon, Y., Hirsch, J.-P. & Franche-Comté Polyp Surveillance Study Group. Predictors of colorectal polyp recurrence after the first polypectomy in private practice settings: a cohort study. *PloS One* **7**, e50990 (2012).
86. Markowitz, S. DNA repair defects inactivate tumor suppressor genes and induce hereditary and sporadic colon cancers. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* **18**, 75S–80S (2000).

87. Endoscopy, A. S. for G. Colorectal Polyps. (2014). Available at:
<http://www.asge.org/press/press.aspx?id=8142>. (Accessed: 14th April 2015)
88. Rothwell, P. M. *et al.* Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. *Lancet* **376**, 1741–50 (2010).
89. WCRF & AICR. *World Cancer Research Fund / American Institute for Cancer Research. Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective.* (AICR, 2007).
90. Giovannucci, E. Diet, body weight, and colorectal cancer: a summary of the epidemiologic evidence. *J. Womens Health* **12**, 173–82 (2003).
91. Gaziano, J. M. *et al.* Vitamins E and C in the prevention of prostate and total cancer in men: the Physicians' Health Study II randomized controlled trial. *JAMA* **301**, 52–62 (2009).
92. Benito, E., Cabeza, E., Moreno, V., Obrador, A. & Bosch, F. X. Diet and colorectal adenomas: a case-control study in Majorca. *Int. J. Cancer J. Int. Cancer* **55**, 213–219 (1993).
93. Potter, J. D., Slattery, M. L., Bostick, R. M. & Gapstur, S. M. Colon cancer: a review of the epidemiology. *Epidemiol. Rev.* **15**, 499–545 (1993).
94. Giovannucci, E. *et al.* Folate, methionine, and alcohol intake and risk of colorectal adenoma. *J. Natl. Cancer Inst.* **85**, 875–884 (1993).
95. Ulrich, C. M. & Potter, J. D. Folate and cancer--timing is everything. *JAMA* **297**, 2408–9 (2007).
96. Chung, M. *et al.* Vitamin D and calcium: a systematic review of health outcomes. *Evid. ReportTechnology Assess.* 1–420 (2009).
97. Cross, H. S. & Peterlik, M. Vitamin D, calcium, and cancer. *Anticancer Res* **29**, 3685 (2009).
98. Garland, C. *et al.* Dietary vitamin D and calcium and risk of colorectal cancer: a 19-year prospective study in men. *Lancet* **1**, 307–9 (1985).

99. Giovannucci, E. Calcium plus vitamin D and the risk of colorectal cancer. *N. Engl. J. Med.* **354**, 2287–8; author reply 2287–8 (2006).
100. Calcium and calcium supplements: Achieving the right balance - Mayo Clinic. Available at: <http://www.mayoclinic.org/healthy-living/nutrition-and-healthy-eating/in-depth/calcium-supplements/art-20047097>. (Accessed: 26th December 2014)
101. Peacock, M. Calcium Metabolism in Health and Disease. *Clin. J. Am. Soc. Nephrol.* **5**, S23–S30 (2010).
102. Catterall, W. A. Voltage-Gated Calcium Channels. *Cold Spring Harb. Perspect. Biol.* **3**, a003947 (2011).
103. Prevarskaya, N., Ouadid-Ahidouch, H., Skryma, R. & Shuba, Y. Remodelling of Ca²⁺ transport in cancer: how it contributes to cancer hallmarks? *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **369**, 20130097 (2014).
104. Pence, B. C. Role of calcium in colon cancer prevention: experimental and clinical studies. *Mutat Res* **290**, 87–95 (1993).
105. Peters, U. *et al.* Vitamin D, calcium, and vitamin D receptor polymorphism in colorectal adenomas. *Cancer Epidemiol. Biomark. Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol.* **10**, 1267–74 (2001).
106. Fedirko, V. *et al.* Effects of vitamin d and calcium on proliferation and differentiation in normal colon mucosa: a randomized clinical trial. *Cancer Epidemiol Biomark. Prev* **18**, 2933–41 (2009).
107. Fedirko, V. *et al.* Effects of vitamin D and calcium supplementation on markers of apoptosis in normal colon mucosa: a randomized, double-blind, placebo-controlled clinical trial. *Cancer Prev Res Phila* **2**, 213–23 (2009).
108. Newmark, H. L., Wargovich, M. J. & Bruce, W. R. Colon cancer and dietary fat, phosphate, and calcium: a hypothesis. *J Natl Cancer Inst* **72**, 1323–5 (1984).

109. Rowland, G. W., Schwartz, G. G., John, E. M. & Ingles, S. A. Protective effects of low calcium intake and low calcium absorption vitamin D receptor genotype in the California Collaborative Prostate Cancer Study. *Cancer Epidemiol Biomark. Prev* **22**, 16–24 (2013).
110. Flood, A. *et al.* Calcium from diet and supplements is associated with reduced risk of colorectal cancer in a prospective cohort of women. *Cancer Epidemiol Biomark. Prev* **14**, 126–32 (2005).
111. D'Souza-Li, L. The calcium-sensing receptor and related diseases. *Arq. Bras. Endocrinol. Amp Metabol.* **50**, 628–639 (2006).
112. Peterlik, M., Kallay, E. & Cross, H. S. Calcium nutrition and extracellular calcium sensing: relevance for the pathogenesis of osteoporosis, cancer and cardiovascular diseases. *Nutrients* **5**, 302–27 (2013).
113. Hendy, G. N., D'Souza-Li, L., Yang, B., Canaff, L. & Cole, D. E. Mutations of the calcium-sensing receptor (CASR) in familial hypocalciuric hypercalcemia, neonatal severe hyperparathyroidism, and autosomal dominant hypocalcemia. *Hum. Mutat.* **16**, 281–296 (2000).
114. Toka, H. R. *et al.* Deficiency of the calcium-sensing receptor in the kidney causes parathyroid hormone-independent hypocalciuria. *J Am Soc Nephrol* **23**, 1879–90 (2012).
115. Speer, G. *et al.* Calcium-sensing receptor A986S polymorphism in human rectal cancer. *Int. J. Colorectal Dis.* **17**, 20–24 (2002).
116. Peters, U. *et al.* Association of genetic variants in the calcium-sensing receptor with risk of colorectal adenoma. *Cancer Epidemiol. Biomark. Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol.* **13**, 2181–2186 (2004).
117. Bácsi, K. *et al.* Effects of the lactase 13910 C/T and calcium-sensor receptor A986S G/T gene polymorphisms on the incidence and recurrence of colorectal cancer in Hungarian population. *BMC Cancer* **8**, 317 (2008).

118. Jacobs, E. T. *et al.* Genetic variation in the retinoid X receptor and calcium-sensing receptor and risk of colorectal cancer in the Colon Cancer Family Registry. *Carcinogenesis* **31**, 1412–1416 (2010).
119. Hibler, E. A., Hu, C., Jurutka, P. W., Martinez, M. E. & Jacobs, E. T. Polymorphic variation in the GC and CASR genes and associations with vitamin D metabolite concentration and metachronous colorectal neoplasia. *Cancer Epidemiol. Biomark. Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol.* **21**, 368–375 (2012).
120. Jenab, M. *et al.* Vitamin D receptor and calcium sensing receptor polymorphisms and the risk of colorectal cancer in European populations. *Cancer Epidemiol. Biomark. Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol.* **18**, 2485–2491 (2009).
121. Fedirko, V. *et al.* Prediagnostic 25-hydroxyvitamin D, VDR and CASR polymorphisms, and survival in patients with colorectal cancer in western European populations. *Cancer Epidemiol. Biomark. Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol.* **21**, 582–593 (2012).
122. Kim, K.-Z. *et al.* Association between CASR polymorphisms, calcium intake, and colorectal cancer risk. *PloS One* **8**, e59628 (2013).
123. Mahmoudi, T. *et al.* Parathyroid hormone gene rs6256 and calcium sensing receptor gene rs1801725 variants are not associated with susceptibility to colorectal cancer in Iran. *Asian Pac. J. Cancer Prev. APJCP* **15**, 6035–6039 (2014).
124. Jeong, S. *et al.* Genetic polymorphisms of CASR and cancer risk: evidence from meta-analysis and HuGE review. *OncoTargets Ther.* **9**, 655–669 (2016).
125. Li, K., Kaaks, R., Linseisen, J. & Rohrmann, S. Dietary calcium and magnesium intake in relation to cancer incidence and mortality in a German prospective cohort (EPIC-Heidelberg). *Cancer Causes Control* **22**, 1375–82 (2011).
126. Carroll, C. *et al.* Supplemental calcium in the chemoprevention of colorectal cancer: a systematic review and meta-analysis. *Clin. Ther.* **32**, 789–803 (2010).

127. Grau, M. V. *et al.* Prolonged effect of calcium supplementation on risk of colorectal adenomas in a randomized trial. *J. Natl. Cancer Inst.* **99**, 129–36 (2007).
128. Bolland, M. J., Grey, A., Gamble, G. D. & Reid, I. R. Calcium and vitamin D supplements and health outcomes: a reanalysis of the Women’s Health Initiative (WHI) limited-access data set. *Am J Clin Nutr* **94**, 1144–9 (2011).
129. Rude, R. *Magnesium*. In: Ross AC, Caballero B, Cousins RJ, Tucker KL, Ziegler TR, eds. *Modern Nutrition in Health and Disease*. (Lippincott Williams & Wilkins, 2012).
130. Swaminathan, R. Magnesium Metabolism and its Disorders. *Clin. Biochem. Rev.* **24**, 47–66 (2003).
131. Moshfegh, A., Goldman, J., Ahuja, J., Rhodes, D. & LaComb, R. *What We Eat in America, NHANES 2005-2006: Usual Nutrient Intakes from Food and Water Compared to 1997 Dietary Reference Intakes for Vitamin D, Calcium, Phosphorus, and Magnesium*. (U.S. Department of Agriculture, Agricultural Research Service, 2009).
132. Nadler, J. L. *et al.* Magnesium deficiency produces insulin resistance and increased thromboxane synthesis. *Hypertension* **21**, 1024–1029 (1993).
133. Moslehi, N., Vafa, M., Rahimi-Foroushani, A. & Golestan, B. Effects of oral magnesium supplementation on inflammatory markers in middle-aged overweight women. *J. Res. Med. Sci. Off. J. Isfahan Univ. Med. Sci.* **17**, 607–614 (2012).
134. Larsson, S. C., Bergkvist, L. & Wolk, A. Magnesium intake in relation to risk of colorectal cancer in women. *JAMA* **293**, 86–9 (2005).
135. Yang, C. Y., Chiu, H. F., Chiu, J. F., Tsai, S. S. & Cheng, M. F. Calcium and magnesium in drinking water and risk of death from colon cancer. *Jpn. J. Cancer Res. Gann* **88**, 928–933 (1997).
136. Yang, C. Y. & Chiu, H. F. Calcium and magnesium in drinking water and risk of death from rectal cancer. *Int. J. Cancer J. Int. Cancer* **77**, 528–532 (1998).

137. Folsom, A. R. & Hong, C. P. Magnesium intake and reduced risk of colon cancer in a prospective study of women. *Am J Epidemiol* **163**, 232–5 (2006).
138. Ma, E. *et al.* High dietary intake of magnesium may decrease risk of colorectal cancer in Japanese men. *J Nutr* **140**, 779–85 (2010).
139. Zhang, X. *et al.* Magnesium intake, plasma C-peptide, and colorectal cancer incidence in US women: a 28-year follow-up study. *Br J Cancer* **106**, 1335–41 (2012).
140. Lin, J. *et al.* Total magnesium intake and colorectal cancer incidence in women. *Cancer Epidemiol Biomark. Prev* **15**, 2006–9 (2006).
141. Cohen, S. M. Role of urinary physiology and chemistry in bladder carcinogenesis. *Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc.* **33**, 715–730 (1995).
142. Nihei, T. Adenosine Triphosphatase activated by Magnesium and Superprecipitation of Myosin B. *Nature* **215**, 886–887 (1967).
143. Rardon, D. & Fisch, C. *Electrolytes and the Heart*. (McGraw-Hill Book Co, 1990).
144. Brinkman, M. T. *et al.* Dietary intake of micronutrients and the risk of developing bladder cancer: results from the Belgian case-control study on bladder cancer risk. *Cancer Causes Control* **22**, 469–78 (2011).
145. Norman, D. A. *et al.* Jejunal and ileal adaptation to alterations in dietary calcium: changes in calcium and magnesium absorption and pathogenetic role of parathyroid hormone and 1,25-dihydroxyvitamin D. *J. Clin. Invest.* **67**, 1599–603 (1981).
146. Nielsen, F. H., Milne, D. B., Gallagher, S., Johnson, L. & Hoverson, B. Moderate magnesium deprivation results in calcium retention and altered potassium and phosphorus excretion by postmenopausal women. *Magnes. Res. Off. Organ Int. Soc. Dev. Res. Magnes.* **20**, 19–31 (2007).
147. Smith, J. B., Cragoe, E. J., Jr. & Smith, L. Na⁺/Ca²⁺ antiport in cultured arterial smooth muscle cells. Inhibition by magnesium and other divalent cations. *J. Biol. Chem.* **262**, 11988–94 (1987).

148. Spencer, H., Fuller, H., Norris, C. & Williams, D. Effect of magnesium on the intestinal absorption of calcium in man. *J Am Coll Nutr* **13**, 485–92 (1994).
149. Dai, Q. *et al.* Blood magnesium, and the interaction with calcium, on the risk of high-grade prostate cancer. *PLoS One* **6**, e18237 (2011).
150. Manolio, T. A. *et al.* Correlates of fasting insulin levels in young adults: the CARDIA study. *J Clin Epidemiol* **44**, 571–8 (1991).
151. Hans, C. P., Chaudhary, D. P. & Bansal, D. D. Effect of magnesium supplementation on oxidative stress in alloxanic diabetic rats. *Magnes Res* **16**, 13–9 (2003).
152. Lamprecht, S. A. & Lipkin, M. Cellular mechanisms of calcium and vitamin D in the inhibition of colorectal carcinogenesis. *Ann N Acad Sci* **952**, 73–87 (2001).
153. Lamprecht, S. A. & Lipkin, M. Chemoprevention of colon cancer by calcium, vitamin D and folate: molecular mechanisms. *Nat Rev Cancer* **3**, 601–14 (2003).
154. Sun, Y. *et al.* Increase in Serum Ca²⁺/Mg²⁺ Ratio Promotes Proliferation of Prostate Cancer Cells by Activating TRPM7 Channels. *J Biol Chem* **288**, 255–63 (2013).
155. Ervin, R. B., Wang, C. Y., Wright, J. D. & Kennedy-Stephenson, J. Dietary intake of selected minerals for the United States population: 1999-2000. *Adv Data* 1–5 (2004).
156. Cai, H. *et al.* Variation in nutrient intakes among women in Shanghai, China. *Eur J Clin Nutr* **58**, 1604–11 (2004).
157. Holick, M. F. Calcium plus vitamin D and the risk of colorectal cancer. *N. Engl. J. Med.* **354**, 2287–8; author reply 2287–8 (2006).
158. Martinez, M. E. & Jacobs, E. T. Calcium supplementation and prevention of colorectal neoplasia: lessons from clinical trials. *J Natl Cancer Inst* **99**, 99–100 (2007).
159. Kesse, E. *et al.* Dairy products, calcium and phosphorus intake, and the risk of prostate cancer: results of the French prospective SU.VI.MAX (Supplementation en Vitamines et Mineraux Antioxydants) study. *Br J Nutr* **95**, 539–45 (2006).

160. Slattery, M. L. *et al.* Associations between BMI, energy intake, energy expenditure, VDR genotype and colon and rectal cancers (United States). *Cancer Causes Control CCC* **15**, 863–72 (2004).
161. Slattery, M. L. *et al.* Dietary calcium, vitamin D, VDR genotypes and colorectal cancer. *Int. J. Cancer J. Int. Cancer* **111**, 750–6 (2004).
162. Rogers, A. C., Hanly, A. M., Collins, D., Baird, A. W. & Winter, D. C. Review Article: Loss of the Calcium-Sensing Receptor in Colonic Epithelium is a Key Event in the Pathogenesis of Colon Cancer. *Clin Colorectal Cancer* (2011). doi:10.1016/j.clcc.2011.04.003
163. Miller, E. A. *et al.* Calcium, dietary, and lifestyle factors in the prevention of colorectal adenomas. *Cancer* **109**, 510–7 (2007).
164. Zhu, X. *et al.* Calcium Intake and Ion Transporter Genetic Polymorphisms Interact in Human Colorectal Neoplasia Risk in a 2-Phase Study. *J. Nutr.* (2014). doi:10.3945/jn.114.196709
165. Vastag, B. Nutrients for prevention: negative trials send researchers back to drawing board. *J Natl Cancer Inst* **101**, 446–8, 451 (2009).
166. Klein, E. A. *et al.* Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *JAMA* **306**, 1549–56 (2011).
167. Skinner, H. G. & Schwartz, G. G. A prospective study of total and ionized serum calcium and fatal prostate cancer. *Cancer Epidemiol Biomark. Prev* **18**, 575–8 (2009).
168. Schwartz, G. G. & Skinner, H. G. A prospective study of total and ionized serum calcium and time to fatal prostate cancer. *Cancer Epidemiol Biomark. Prev* **21**, 1768–73 (2012).
169. Skinner, H. G. & Schwartz, G. G. Serum calcium and incident and fatal prostate cancer in the National Health and Nutrition Examination Survey. *Cancer Epidemiol Biomark. Prev* **17**, 2302–5 (2008).
170. Chan, J. M. *et al.* Dairy products, calcium, and prostate cancer risk in the Physicians' Health Study. *Am J Clin Nutr* **74**, 549–54 (2001).

171. Tseng, M., Breslow, R. A., Graubard, B. I. & Ziegler, R. G. Dairy, calcium, and vitamin D intakes and prostate cancer risk in the National Health and Nutrition Examination Epidemiologic Follow-up Study cohort. *Am J Clin Nutr* **81**, 1147–54 (2005).
172. Rodriguez, C. *et al.* Calcium, dairy products, and risk of prostate cancer in a prospective cohort of United States men. *Cancer Epidemiol Biomark. Prev* **12**, 597–603 (2003).
173. Giovannucci, E., Liu, Y., Stampfer, M. J. & Willett, W. C. A prospective study of calcium intake and incident and fatal prostate cancer. *Cancer Epidemiol Biomark. Prev* **15**, 203–10 (2006).
174. Mitrou, P. N. *et al.* A prospective study of dietary calcium, dairy products and prostate cancer risk (Finland). *Int J Cancer* **120**, 2466–73 (2007).
175. Pyo, J. H. *et al.* Fasting blood sugar and serum triglyceride as the risk factors of colorectal adenoma in korean population receiving screening colonoscopy. *Clin. Nutr. Res.* **2**, 34–41 (2013).
176. Lin, C.-C. *et al.* Hypertension is an important predictor of recurrent colorectal adenoma after screening colonoscopy with adenoma polypectomy. *J. Chin. Med. Assoc. JCMA* **77**, 508–512 (2014).
177. doi:10.1097/MCG.0b013e3182688c15
178. Morita, T. *et al.* The metabolic syndrome is associated with increased risk of colorectal adenoma development: the Self-Defense Forces health study. *Asian Pac. J. Cancer Prev. APJCP* **6**, 485–489 (2005).
179. How to Understand a Lipid Panel. *Forever Health* Available at: <http://www.foreverhealth.com/blood-tests/how-to-understand-a-lipid-panel/>. (Accessed: 25th December 2014)
180. Voloshyna, I., Hai, O., Littlefield, M. J., Carsons, S. & Reiss, A. B. Resveratrol mediates anti-atherogenic effects on cholesterol flux in human macrophages and endothelium via PPAR γ and adenosine. *Eur. J. Pharmacol.* **698**, 299–309 (2013).

181. Miller, G. J. & Miller, N. E. Plasma-high-density-lipoprotein concentration and development of ischaemic heart-disease. *Lancet* **1**, 16–19 (1975).
182. Glomset, J. A. Physiological role of lecithin-cholesterol acyltransferase. *Am. J. Clin. Nutr.* **23**, 1129–1136 (1970).
183. CDC - DHDSP - Heart Disease - Coronary Artery Disease. Available at: http://www.cdc.gov/heartdisease/coronary_ad.htm. (Accessed: 4th September 2014)
184. Murphy, S. L., Xu, J. & Kochanek, K. D. *Deaths: Final data for 2010*. (Natl Vital Stat Rep., 2013).
185. Albrink, M. J., Meigs, J. W. & Man, E. B. Serum lipids, hypertension and coronary artery disease. *Am. J. Med.* **31**, 4–23 (1961).
186. Gofman, J. W., Young, W. & Tandy, R. Ischemic heart disease, atherosclerosis, and longevity. *Circulation* **34**, 679–697 (1966).
187. Grundy, S. M. *et al.* Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. *Circulation* **110**, 227–239 (2004).
188. LaRosa, J. C., He, J. & Vupputuri, S. Effect of statins on risk of coronary disease: a meta-analysis of randomized controlled trials. *JAMA* **282**, 2340–2346 (1999).
189. Cholesterol levels: What numbers should you aim for? - Mayo Clinic. Available at: <http://www.mayoclinic.org/diseases-conditions/high-blood-cholesterol/in-depth/cholesterol-levels/art-20048245>. (Accessed: 25th December 2014)
190. Lisak, M., Demarin, V., Trkanjec, Z. & Basić-Kes, V. Hypertriglyceridemia as a possible independent risk factor for stroke. *Acta Clin. Croat.* **52**, 458–463 (2013).
191. Gordon, T., Castelli, W. P., Hjortland, M. C., Kannel, W. B. & Dawber, T. R. High density lipoprotein as a protective factor against coronary heart disease: The Framingham study. *Am. J. Med.* **62**, 707–714 (1977).
192. Barter, P. *et al.* HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. *N. Engl. J. Med.* **357**, 1301–1310 (2007).

193. Assmann, G., Cullen, P. & Schulte, H. Simple scoring scheme for calculating the risk of acute coronary events based on the 10-year follow-up of the prospective cardiovascular Münster (PROCAM) study. *Circulation* **105**, 310–315 (2002).
194. Emerging Risk Factors Collaboration *et al.* Major lipids, apolipoproteins, and risk of vascular disease. *JAMA* **302**, 1993–2000 (2009).
195. Hoogeveen, R. C. *et al.* Evaluation of Lp[a] and other independent risk factors for CHD in Asian Indians and their USA counterparts. *J. Lipid Res.* **42**, 631–638 (2001).
196. Gordon, D. J. *et al.* High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation* **79**, 8–15 (1989).
197. Kochanek, K. D., Xu, J., Murphy, S. L., Mininõ, A. M. & Kung, H.-C. *Deaths: final data for 2009.* (2011).
198. Coronary Heart Disease Risk Factors - NHLBI, NIH. Available at: <http://www.nhlbi.nih.gov/health/health-topics/topics/hd/atrisk>. (Accessed: 28th January 2015)
199. Wijeyesundera, H. C. *et al.* Association of temporal trends in risk factors and treatment uptake with coronary heart disease mortality, 1994-2005. *JAMA* **303**, 1841–1847 (2010).
200. Prevalence of coronary heart disease--United States, 2006-2010. *MMWR Morb Mortal Wkly Rep* **60**, 1377–81 (2011).
201. Fruchart, J.-C. *et al.* Residual macrovascular risk in 2013: what have we learned? *Cardiovasc. Diabetol.* **13**, 26 (2014).
202. Rayssiguier, Y., Gueux, E. & Weiser, D. Effect of magnesium deficiency on lipid metabolism in rats fed a high carbohydrate diet. *J. Nutr.* **111**, 1876–1883 (1981).
203. Rayssiguier, Y. Magnesium, lipids and vascular diseases. Experimental evidence in animal models. *Magnesium* **5**, 182–190 (1986).
204. Inagaki, O. *et al.* Effect of magnesium deficiency on lipid metabolism in uremic rats. *Nephron* **55**, 176–180 (1990).

205. Olatunji, L. A. & Soladoye, A. O. Increased magnesium intake prevents hyperlipidemia and insulin resistance and reduces lipid peroxidation in fructose-fed rats. *Pathophysiology* **14**, 11–15 (2007).
206. Rasmussen, H. S. *et al.* Influence of magnesium substitution therapy on blood lipid composition in patients with ischemic heart disease. A double-blind, placebo controlled study. *Arch Intern Med* **149**, 1050–3 (1989).
207. Hadjistavri, L. S. *et al.* Beneficial effects of oral magnesium supplementation on insulin sensitivity and serum lipid profile. *Med Sci Monit* **16**, CR307–312 (2010).
208. Mooren, F. C. *et al.* Oral magnesium supplementation reduces insulin resistance in non-diabetic subjects - a double-blind, placebo-controlled, randomized trial. *Diabetes Obes Metab* **13**, 281–4 (2011).
209. Chacko, S. A. *et al.* Magnesium supplementation, metabolic and inflammatory markers, and global genomic and proteomic profiling: a randomized, double-blind, controlled, crossover trial in overweight individuals. *Am J Clin Nutr* **93**, 463–73 (2011).
210. Petersen, B., Christiansen, C. & Hansen, P. F. Treatment of hypercholesterolaemia and hypertriglyceridaemia with magnesium. *Acta Med. Scand.* **200**, 59–61 (1976).
211. Marken, P. A., Weart, C. W., Carson, D. S., Gums, J. G. & Lopes-Virella, M. F. Effects of magnesium oxide on the lipid profile of healthy volunteers. *Atherosclerosis* **77**, 37–42 (1989).
212. Song, Y., He, K., Levitan, E. B., Manson, J. E. & Liu, S. Effects of oral magnesium supplementation on glycaemic control in Type 2 diabetes: a meta-analysis of randomized double-blind controlled trials. *Diabet Med* **23**, 1050–6 (2006).
213. Lal, J., Vasudev, K., Kela, A. K. & Jain, S. K. Effect of oral magnesium supplementation on the lipid profile and blood glucose of patients with type 2 diabetes mellitus. *J. Assoc. Physicians India* **51**, 37–42 (2003).

214. Ma, J. *et al.* Associations of serum and dietary magnesium with cardiovascular disease, hypertension, diabetes, insulin, and carotid arterial wall thickness: the ARIC study. Atherosclerosis Risk in Communities Study. *J Clin Epidemiol* **48**, 927–40 (1995).
215. Song, Y., Manson, J. E., Buring, J. E. & Liu, S. Dietary magnesium intake in relation to plasma insulin levels and risk of type 2 diabetes in women. *Diabetes Care* **27**, 59–65 (2004).
216. Zhang, W., Iso, H., Ohira, T., Date, C. & Tamakoshi, A. Associations of dietary magnesium intake with mortality from cardiovascular disease: the JACC study. *Atherosclerosis* **221**, 587–95 (2012).
217. Liao, F., Folsom, A. R. & Brancati, F. L. Is low magnesium concentration a risk factor for coronary heart disease? The Atherosclerosis Risk in Communities (ARIC) Study. *Am Heart J* **136**, 480–90 (1998).
218. Joosten, M. M. *et al.* Urinary and plasma magnesium and risk of ischemic heart disease. *Am J Clin Nutr* **97**, 1299–306 (2013).
219. Khan, A. M. *et al.* Lack of association between serum magnesium and the risks of hypertension and cardiovascular disease. *Am Heart J* **160**, 715–20 (2010).
220. Song, Y. *et al.* Dietary magnesium intake and risk of cardiovascular disease among women. *Am J Cardiol* **96**, 1135–41 (2005).
221. Song, Y., Li, T. Y., Dam, R. M. van, Manson, J. E. & Hu, F. B. Magnesium intake and plasma concentrations of markers of systemic inflammation and endothelial dysfunction in women. *Am. J. Clin. Nutr.* **85**, 1068–1074 (2007).
222. Ridker, P. M., Rifai, N., Rose, L., Buring, J. E. & Cook, N. R. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N. Engl. J. Med.* **347**, 1557–1565 (2002).
223. Wang, G. *et al.* A Prospective Follow-up Study of the Relationship between C-Reactive Protein and Human Cancer Risk in the Chinese Kailuan Female Cohort. *Cancer Epidemiol.*

- Biomark. Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol.* (2014).
doi:10.1158/1055-9965.EPI-14-1112
224. Lumachi, F. *et al.* Preoperative Serum C-Reactive Protein and its Prognostic Significance in Patients with Stage III-IV Colorectal Cancer. *Anticancer Res.* **34**, 7263–7266 (2014).
225. Chiuve, S. E. *et al.* Dietary and plasma magnesium and risk of coronary heart disease among women. *J Am Heart Assoc* **2**, e000114 (2013).
226. Guerrero-Romero, F. & Rodríguez-Morán, M. Low serum magnesium levels and metabolic syndrome. *Acta Diabetol.* **39**, 209–213 (2002).
227. Petersen, B., Schroll, M., Christiansen, C. & Transbol, I. Serum and erythrocyte magnesium in normal elderly danish people. Relationship to blood pressure and serum lipids. *Acta Med. Scand.* **201**, 31–34 (1977).
228. Bersohn, I. & Oelofse, P. J. CORRELATION OF SERUM-MAGNESIUM AND SERUM-CHOLESTEROL LEVELS IN SOUTH AFRICAN BANTU AND EUROPEAN SUBJECTS. *The Lancet* **269**, 1020–1021 (1957).
229. Brown, D. F., McGANDY, R. B., Gillie, E. & Doyle, J. T. Magnesium-lipid relations in health and in patients with myocardial infarction. *Lancet* **2**, 933–935 (1958).
230. Ford, E. S. & Bowman, B. A. Serum and red blood cell folate concentrations, race, and education: findings from the third National Health and Nutrition Examination Survey. *Am J Clin Nutr* **69**, 476–81 (1999).
231. Elin, R. J. Magnesium: the fifth but forgotten electrolyte. *Am. J. Clin. Pathol.* **102**, 616–622 (1994).
232. Bolland, M. J., Grey, A., Avenell, A., Gamble, G. D. & Reid, I. R. Calcium supplements with or without vitamin D and risk of cardiovascular events: reanalysis of the Women’s Health Initiative limited access dataset and meta-analysis. *BMJ* **342**, d2040 (2011).
233. Bolland, M. J. *et al.* Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis. *BMJ* **341**, c3691 (2010).

234. Wang, L., Manson, J. E. & Sesso, H. D. Calcium Intake and Risk of Cardiovascular Disease: A Review of Prospective Studies and Randomized Clinical Trials. *Am. J. Cardiovasc. Drugs Drugs Devices Interv.* (2012). doi:10.2165/11595400-000000000-00000
235. Kaluza, J. *et al.* Dietary calcium and magnesium intake and mortality: a prospective study of men. *Am. J. Epidemiol.* **171**, 801–807 (2010).
236. Chai, W., Cooney, R. V., Franke, A. A. & Bostick, R. M. Effects of calcium and vitamin D supplementation on blood pressure and serum lipids and carotenoids: a randomized, double-blind, placebo-controlled, clinical trial. *Ann. Epidemiol.* **23**, 564–570 (2013).
237. Goto, K. *et al.* Magnesium deficiency detected by intravenous loading test in variant angina pectoris. *Am. J. Cardiol.* **65**, 709–712 (1990).
238. Cohen, L., Laor, A. & Kitzes, R. Serum magnesium in propranolol-treated patients with acute myocardial infarction. *Magnesium* **3**, 138–144 (1984).
239. Purvis, J. R. & Movahed, A. Magnesium disorders and cardiovascular diseases. *Clin. Cardiol.* **15**, 556–568 (1992).
240. Itoh, K., Kawasaka, T. & Nakamura, M. The effects of high oral magnesium supplementation on blood pressure, serum lipids and related variables in apparently healthy Japanese subjects. *Br. J. Nutr.* **78**, 737–750 (1997).
241. Inoue, I. [Lipid metabolism and magnesium]. *Clin. Calcium* **15**, 65–76 (2005).
242. Paolisso, G. *et al.* Improved insulin response and action by chronic magnesium administration in aged NIDDM subjects. *Diabetes Care* **12**, 265–269 (1989).
243. Rayssiguier, Y. *et al.* Effect of magnesium deficiency on post-heparin lipase activity and tissue lipoprotein lipase in the rat. *Lipids* **26**, 182–186 (1991).
244. Rosanoff, A. & Seelig, M. S. Comparison of mechanism and functional effects of magnesium and statin pharmaceuticals. *J. Am. Coll. Nutr.* **23**, 501S–505S (2004).
245. Kang, D.-H. & Ha, S.-K. Uric Acid Puzzle: Dual Role as Anti-oxidant and Pro-oxidant. *Electrolyte Blood Press. E BP* **12**, 1–6 (2014).

246. Lipkowitz, M. S. Regulation of Uric Acid Excretion by the Kidney. *Curr. Rheumatol. Rep.* **14**, 179–188 (2012).
247. Angelopoulos, T. J. *et al.* The effect of high-fructose corn syrup consumption on triglycerides and uric acid. *J. Nutr.* **139**, 1242S–1245S (2009).
248. Emmerson, B. T. Effect of oral fructose on urate production. *Ann. Rheum. Dis.* **33**, 276–280 (1974).
249. Purine: List of Foods High and Low in Purine Content. Available at: <http://www.dietaryfiberfood.com/purine-and-uric-acid/purines-food-and-gout.php>. (Accessed: 27th October 2014)
250. Symptoms and Diagnosis, Causes, Managing Elevated Uric Acid Levels Naturally, High Uric Acid |. *My Wellness*
251. Diuretic-Related Side Effects: Development and Treatment: Hyperuricemia. Available at: http://www.medscape.com/viewarticle/489521_7. (Accessed: 27th October 2014)
252. Umeki, S. *et al.* Oral zinc therapy normalizes serum uric acid level in Wilson's disease patients. *Am. J. Med. Sci.* **292**, 289–292 (1986).
253. Ames, B. N., Cathcart, R., Schwiers, E. & Hochstein, P. Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. *Proc. Natl. Acad. Sci. U. S. A.* **78**, 6858–6862 (1981).
254. Sundström, J. *et al.* Relations of serum uric acid to longitudinal blood pressure tracking and hypertension incidence. *Hypertension* **45**, 28–33 (2005).
255. Shankar, A., Klein, R., Klein, B. E. K. & Nieto, F. J. The association between serum uric acid level and long-term incidence of hypertension: Population-based cohort study. *J. Hum. Hypertens.* **20**, 937–945 (2006).
256. Johnson, R. J., Feig, D. I., Herrera-Acosta, J. & Kang, D.-H. Resurrection of Uric Acid as a Causal Risk Factor in Essential. *Hypertension* **45**, 18–20 (2005).

257. Fang, J. & Alderman, M. H. Serum uric acid and cardiovascular mortality the NHANES I epidemiologic follow-up study, 1971-1992. National Health and Nutrition Examination Survey. *JAMA* **283**, 2404–2410 (2000).
258. Klein R *et al.* Serum uric acid: Its relationship to coronary heart disease risk factors and cardiovascular disease, evans county, georgia. *Arch. Intern. Med.* **132**, 401–410 (1973).
259. Mangge, H., Pilz, S., Haj-Yahya, S. & Almer, G. Uric Acid Indicates a High Cardiovascular Risk Profile but Is Not Closely Associated With Insulin Resistance in Obese Adolescents. *Diabetes Care* **31**, e21–e21 (2008).
260. Brand, F. N., Mcgee, D. L., Kannel, W. B., Stokes, J. & Castelli, W. P. Hyperuricemia as a Risk Factor of Coronary Heart Disease: The Framingham Study. *Am. J. Epidemiol.* **121**, 11–18 (1985).
261. Chien, K.-L. *et al.* Plasma uric acid and the risk of type 2 diabetes in a Chinese community. *Clin. Chem.* **54**, 310–316 (2008).
262. Tomita, M. *et al.* Does hyperuricemia affect mortality? A prospective cohort study of Japanese male workers. *J. Epidemiol. Jpn. Epidemiol. Assoc.* **10**, 403–409 (2000).
263. Iseki, K. *et al.* Significance of hyperuricemia on the early detection of renal failure in a cohort of screened subjects. *Hypertens. Res. Off. J. Jpn. Soc. Hypertens.* **24**, 691–697 (2001).
264. Iseki, K. *et al.* Significance of hyperuricemia as a risk factor for developing ESRD in a screened cohort. *Am. J. Kidney Dis. Off. J. Natl. Kidney Found.* **44**, 642–650 (2004).
265. Feig, D. I., Kang, D.-H. & Johnson, R. J. Uric Acid and Cardiovascular Risk. *N. Engl. J. Med.* **359**, 1811–1821 (2008).
266. Ashtari, F., Bahar, M., Aghaei, M. & Zahed, A. Serum uric acid level in patients with relapsing-remitting multiple sclerosis. *J. Clin. Neurosci.* **20**, 676–678 (2013).
267. Sotgiu, S. *et al.* Serum uric acid and multiple sclerosis. *Neurol. Sci. Off. J. Ital. Neurol. Soc. Ital. Soc. Clin. Neurophysiol.* **23**, 183–188 (2002).

268. Cappuccio FP, Strazzullo P, Farinaro E & Trevisan M. Uric acid metabolism and tubular sodium handling: Results from a population-based study. *JAMA* **270**, 354–359 (1993).
269. Herman, J. & Goldbourt, U. URIC ACID AND DIABETES: OBSERVATIONS IN A POPULATION STUDY. *The Lancet* **320**, 240–243 (1982).
270. Nan, H., Dong, Y., Gao, W., Tuomilehto, J. & Qiao, Q. Diabetes associated with a low serum uric acid level in a general Chinese population. *Diabetes Res. Clin. Pract.* **76**, 68–74 (2007).
271. Dehghan, A., Hoek, M. van, Sijbrands, E. J. G., Hofman, A. & Witteman, J. C. M. High Serum Uric Acid as a Novel Risk Factor for Type 2 Diabetes. *Diabetes Care* **31**, 361–362 (2008).
272. Kramer, C. K., von Mühlen, D., Jassal, S. K. & Barrett-Connor, E. Serum uric acid levels improve prediction of incident type 2 diabetes in individuals with impaired fasting glucose: the Rancho Bernardo Study. *Diabetes Care* **32**, 1272–1273 (2009).
273. Taniguchi, Y. *et al.* Serum uric acid and the risk for hypertension and Type 2 diabetes in Japanese men: The Osaka Health Survey. *J. Hypertens.* **19**, 1209–1215 (2001).
274. Oda, E., Kawai, R., Sukumaran, V. & Watanabe, K. Uric acid is positively associated with metabolic syndrome but negatively associated with diabetes in Japanese men. *Intern. Med. Tokyo Jpn.* **48**, 1785–1791 (2009).
275. Håglin, L., Bäckman, L. & Törnkvist, B. A structural equation model for assessment of links between changes in serum triglycerides, -urate, and -glucose and changes in serum calcium, -magnesium and -phosphate in type 2 diabetes and non-diabetes metabolism. *Cardiovasc. Diabetol.* **10**, 116 (2011).
276. Nakagawa, T. *et al.* A causal role for uric acid in fructose-induced metabolic syndrome. *Am. J. Physiol. Renal Physiol.* **290**, F625–631 (2006).
277. Heinig, M. & Johnson, R. J. Role of uric acid in hypertension, renal disease, and metabolic syndrome. *Cleve. Clin. J. Med.* **73**, 1059–1064 (2006).

278. Yang, T. *et al.* Uric acid level as a risk marker for metabolic syndrome: A Chinese cohort study. *Atherosclerosis* **220**, 525–531 (2012).
279. Liu, Z. & Ho, S. C. The association of serum C-reactive protein, uric acid and magnesium with insulin resistance in Chinese postmenopausal women with prediabetes or early untreated diabetes. *Maturitas* **70**, 176–181 (2011).
280. Viazzi, F. *et al.* Serum uric acid and its relationship with metabolic syndrome and cardiovascular risk profile in patients with hypertension: insights from the I-DEMAND study. *Nutr. Metab. Cardiovasc. Dis. NMCD* **24**, 921–927 (2014).
281. Grayson, P. C., Kim, S. Y., LaValley, M. & Choi, H. K. Hyperuricemia and incident hypertension: a systematic review and meta-analysis. *Arthritis Care Res.* **63**, 102–110 (2011).
282. Niskanen, L. K. *et al.* Uric acid level as a risk factor for cardiovascular and all-cause mortality in middle-aged men: a prospective cohort study. *Arch. Intern. Med.* **164**, 1546–1551 (2004).
283. Alderman, M. H., Cohen, H., Madhavan, S. & Kivlighn, S. Serum uric acid and cardiovascular events in successfully treated hypertensive patients. *Hypertension* **34**, 144–150 (1999).
284. Niskanen, L. *et al.* Serum uric acid as a harbinger of metabolic outcome in subjects with impaired glucose tolerance: the Finnish Diabetes Prevention Study. *Diabetes Care* **29**, 709–711 (2006).
285. Wheeler, J. G., Juzwishin, K. D. M., Eiriksdottir, G., Gudnason, V. & Danesh, J. Serum uric acid and coronary heart disease in 9,458 incident cases and 155,084 controls: prospective study and meta-analysis. *PLoS Med.* **2**, e76 (2005).
286. Wannamethee, S. G., Shaper, A. G. & Whincup, P. H. Serum urate and the risk of major coronary heart disease events. *Heart Br. Card. Soc.* **78**, 147–153 (1997).

287. Moriarity, J. T., Folsom, A. R., Iribarren, C., Nieto, F. J. & Rosamond, W. D. Serum uric acid and risk of coronary heart disease: Atherosclerosis Risk in Communities (ARIC) Study. *Ann. Epidemiol.* **10**, 136–143 (2000).
288. Culleton, B. F., Larson, M. G., Kannel, W. B. & Levy, D. Serum uric acid and risk for cardiovascular disease and death: the Framingham Heart Study. *Ann. Intern. Med.* **131**, 7–13 (1999).
289. Hasson, M. A. *et al.* Design and evolution of the data management systems in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial. *Control Clin Trials* **21**, 329S–348S (2000).
290. Prorok, P. C. *et al.* Design of the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial. *Control Clin Trials* **21**, 273S–309S (2000).
291. *PLCO DIETARY USER GUIDE. 2* (CDAS, 2013).
292. Subar, A. F. *et al.* Evaluation of alternative approaches to assign nutrient values to food groups in food frequency questionnaires. *Am J Epidemiol* **152**, 279–86 (2000).
293. Ahn, J. *et al.* Dairy products, calcium intake, and risk of prostate cancer in the prostate, lung, colorectal, and ovarian cancer screening trial. *Cancer Epidemiol Biomark. Prev* **16**, 2623–30 (2007).
294. *Centers for Disease Control and Prevention: the Third National Health and Nutrition Examination Survey (NHANES III 1988–94) Reference Manuals and Reports.* (National Center for Health Statistics, 1996).
295. CDSA. PLCO. Available at:
https://biometry.nci.nih.gov/cdas/plco/learn/data_collected/screening/colorectal/.
296. Kitahara, C. M. *et al.* Prospective investigation of body mass index, colorectal adenoma, and colorectal cancer in the prostate, lung, colorectal, and ovarian cancer screening trial. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* **31**, 2450–2459 (2013).

297. Rothman, K. J., Lash, T. L., Greenland, S. & 19 contributors. *Modern Epidemiology*. (Lippincott, Williams, & Wilkins).
298. Lee, S.-A. *et al.* Stability and Reliability of Plasma Level of Lipid Biomarkers and Their Correlation with Dietary Fat Intake. *Dis. Markers* **24**, 73–79 (2008).
299. Bonithon-Kopp, C., Kronborg, O., Giacosa, A., R ath, U. & Faivre, J. Calcium and fibre supplementation in prevention of colorectal adenoma recurrence: a randomised intervention trial. European Cancer Prevention Organisation Study Group. *Lancet Lond. Engl.* **356**, 1300–1306 (2000).
300. Kampman, E. *et al.* Calcium, vitamin D, dairy foods, and the occurrence of colorectal adenomas among men and women in two prospective studies. *Am. J. Epidemiol.* **139**, 16–29 (1994).
301. Kronborg, O. & Fenger, C. Clinical evidence for the adenoma-carcinoma sequence. *Eur J Cancer Prev* **8 Suppl 1**, S73–86 (1999).
302. Wallace, K. *et al.* Effect of calcium supplementation on the risk of large bowel polyps. *J Natl Cancer Inst* **96**, 921–5 (2004).
303. Rosanoff, A., Dai, Q. & Shapses, S. A. Essential Nutrient Interactions: Does Low or Suboptimal Magnesium Status Interact with Vitamin D and/or Calcium Status? *Adv. Nutr. Int. Rev. J.* **7**, 25–43 (2016).
304. McMichael, A. J. & Potter, J. D. Host factors in carcinogenesis: certain bile-acid metabolic profiles that selectively increase the risk of proximal colon cancer. *J. Natl. Cancer Inst.* **75**, 185–191 (1985).
305. On Geographical Relationship between the Chemical Nature of River Water and Death-rate. Available at: <http://www.mgwater.com/story.shtml>. (Accessed: 12th December 2015)
306. Monarca, S., Donato, F., Zerbini, I., Calderon, R. L. & Craun, G. F. Review of epidemiological studies on drinking water hardness and cardiovascular diseases. *Eur. J.*

- Cardiovasc. Prev. Rehabil. Off. J. Eur. Soc. Cardiol. Work. Groups Epidemiol. Prev. Card. Rehabil. Exerc. Physiol.* **13**, 495–506 (2006).
307. Reid, I. R. *et al.* Effects of calcium supplementation on lipids, blood pressure, and body composition in healthy older men: a randomized controlled trial. *Am. J. Clin. Nutr.* **91**, 131–139 (2010).
308. Bolland, M. J. *et al.* Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis. *BMJ* **341**, c3691 (2010).
309. Song, Y., He, K., Levitan, E. B., Manson, J. E. & Liu, S. Effects of oral magnesium supplementation on glycaemic control in Type 2 diabetes: a meta-analysis of randomized double-blind controlled trials. *Diabet. Med. J. Br. Diabet. Assoc.* **23**, 1050–1056 (2006).
310. Singh, R. B., Rastogi, S. S., Mani, U. V., Seth, J. & Devi, L. Does dietary magnesium modulate blood lipids? *Biol. Trace Elem. Res.* **30**, 59–64 (1991).
311. Corica, F. *et al.* Effects of oral magnesium supplementation on plasma lipid concentrations in patients with non-insulin-dependent diabetes mellitus. *Magnes. Res. Off. Organ Int. Soc. Dev. Res. Magnes.* **7**, 43–47 (1994).
312. Tosiello, L. Hypomagnesemia and diabetes mellitus. A review of clinical implications. *Arch. Intern. Med.* **156**, 1143–1148 (1996).
313. Reid, I. R. Effects of calcium supplementation on circulating lipids: potential pharmacoeconomic implications. *Drugs Aging* **21**, 7–17 (2004).
314. Islam, M. Z., Shamim, A. A., Akhtaruzzaman, M., Kärkkäinen, M. & Lamberg-Allardt, C. Effect of vitamin D, calcium and multiple micronutrients supplementation on lipid profile in pre-menopausal Bangladeshi garment factory workers with hypovitaminosis D. *J. Health Popul. Nutr.* **32**, 687–695 (2014).
315. Eriksson, J. & Kohvakka, A. Magnesium and ascorbic acid supplementation in diabetes mellitus. *Ann. Nutr. Metab.* **39**, 217–223 (1995).

316. Rodríguez-Morán, M. & Guerrero-Romero, F. Oral magnesium supplementation improves insulin sensitivity and metabolic control in type 2 diabetic subjects: a randomized double-blind controlled trial. *Diabetes Care* **26**, 1147–1152 (2003).
317. Altura, B. T. *et al.* Magnesium dietary intake modulates blood lipid levels and atherogenesis. *Proc. Natl. Acad. Sci. U. S. A.* **87**, 1840–1844 (1990).
318. Kirsten, R. *et al.* Magnesium pyridoxal 5-phosphate glutamate reduces hyperlipidaemia in patients with chronic renal insufficiency. *Eur. J. Clin. Pharmacol.* **34**, 133–137 (1988).
319. Song, Y., He, K., Levitan, E. B., Manson, J. E. & Liu, S. Effects of oral magnesium supplementation on glycaemic control in Type 2 diabetes: a meta-analysis of randomized double-blind controlled trials. *Diabet. Med. J. Br. Diabet. Assoc.* **23**, 1050–1056 (2006).
320. Ziniewicz, H. K., Gesteiro, E., González-Muñoz, M. J., Bastida, S. & Sánchez-Muniz, F. J. RELATIONSHIPS BETWEEN SERUM CALCIUM AND MAGNESIUM LEVELS AND LIPOPROTEINS, HOMOCYSTEINE AND INSULIN RESISTANCE/SENSITIVITY MARKERS AT BIRTH. *Nutr. Hosp.* **31**, 278–285 (2014).
321. Fini, M. A., Elias, A., Johnson, R. J. & Wright, R. M. Contribution of uric acid to cancer risk, recurrence, and mortality. *Clin. Transl. Med.* **1**, 16 (2012).
322. Zeng, C. *et al.* Association between low serum magnesium concentration and hyperuricemia. *Magnes. Res. Off. Organ Int. Soc. Dev. Res. Magnes.* **28**, 56–63 (2015).
323. Ziniewicz, H. K., Gesteiro, E., González-Muñoz, M. J., Bastida, S. & Sánchez-Muniz, F. J. Relationships between serum calcium and magnesium levels and lipoproteins, homocysteine and insulin resistance/sensitivity markers at birth. *Nutr. Hosp.* **31**, 278–285 (2015).
324. Huang, J.-H., Tsai, L.-C., Chang, Y.-C. & Cheng, F.-C. High or low calcium intake increases cardiovascular disease risks in older patients with type 2 diabetes. *Cardiovasc. Diabetol.* **13**, (2014).

325. Rosanoff, A. Rising Ca:Mg intake ratio from food in USA Adults: a concern? *Magnes. Res.* **23**, S181–193 (2010).
326. Seshasai, S. R. *et al.* Effect of aspirin on vascular and nonvascular outcomes: meta-analysis of randomized controlled trials. *Arch Intern Med* **172**, 209–16 (2012).
327. Mora, S. Aspirin therapy in primary prevention: comment on ‘effect of aspirin on vascular and nonvascular outcomes’. *Arch Intern Med* **172**, 217–8 (2012).