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Calcium and Milk Product Intakes and Risk for Colorectal Neoplasia
and Other Health Outcomes

By

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Calcium and Milk Product Intakes and Risk for Colorectal Neoplasia and Other Health Outcomes

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Abstract

Calcium and Milk Product Intakes and Risk for Colorectal Neoplasia and Other Health Outcomes

By Caroline Y. Um

There is extensive evidence that calcium and calcium-rich foods, such as milk products, are associated with lower risk of colorectal neoplasms, and supplemental calcium reduced sporadic colorectal adenoma recurrence in two of three large randomized controlled trials. However, clinical trials suggest that milk product consumption may increase circulating insulin-like growth factor 1 (IGF-1) levels, and cross-sectional studies reported positive associations of milk consumption with IGF-1 concentrations. IGF-1 concentrations were also positively associated with risk of colorectal cancer. However, there is no evidence on the effect of supplemental calcium on IGF-1, and there is inconsistent evidence on the associations of calcium and milk product intakes with risk for all-cause and cause-specific mortality.

The purpose of this dissertation was to further clarify the role of calcium and milk products in colorectal neoplasia and other health outcomes. The specific research aims were to: 1) investigate associations of calcium and milk products with risk for colorectal adenomatous polyps; 2) investigate associations of the non-calcium component of milk products with risk for adenomas and mortality; and 3) investigate the association of calcium and milk products with risk for all-cause and cause-specific mortality. These aims were addressed by analyzing data from three colonoscopy-based case-control studies of incident, sporadic colorectal adenoma, a clinical trial of calcium supplementation in adenoma patients, and a national population-based prospective cohort study.

We found an inverse association of dietary calcium with risk of adenomas, consistent with previous literature. We found no appreciable effects of 1.0 or 2.0g of daily supplemental calcium relative to placebo on levels of IGF-1, IGFBP-3, or the IGF-1:IGFBP-3 molar ratio. IGF-1 and IGFBP-3 concentrations were inversely associated with age and were lower in women, which is consistent with most previous literature. Our estimation of milk product residuals is a novel modification of the energy adjustment method that can be utilized to estimate the non-calcium/non-fat component of milk, and our analyses suggest that milk fat may be directly associated with all-cause mortality, the non-calcium/non-fat, IGF-1-containing component of milk may be directly associated with cancer mortality, and calcium intake independent of milk product intake may not be associated with mortality.

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CHAPTER 1. INTRODUCTION AND BACKGROUND

INTRODUCTION

Colorectal cancer is highly correlated with the Western diet and lifestyle, with an estimated 50% of colorectal cancer cases in the United States thought to be preventable by healthy diets and physical activity.[1] Migration studies indicate the importance of environmental factors, particularly diet and physical activity, in the etiology of this disease that has a higher incidence in developed regions.[2] While incidence rates appear to be stabilizing in developed countries, rates are now rapidly rising in less-developed countries undergoing economic transitions.[3]

Colorectal cancer continues to be the second leading cause of cancer deaths among men and women combined in the United States.[4] Although the association between diet and cancer, in general and with colorectal cancer, was briefly suggested in early cancer studies, it is a relatively recent area of research that began gaining interest with the publication of "Diet, Nutrition, and Cancer" by the National Academy of Sciences in 1982.[5] The Third Expert Report of colorectal cancer published by the World Cancer Research Fund currently lists calcium, milk, vitamin D, dietary fiber, and fruit and vegetable intake as dietary factors that may lower risk of colorectal cancer while red and processed meats and alcohol are dietary factors that increase risk.[1]

There is strong plausibility that calcium reduces the risk for colorectal neoplasia, as evidenced by consistent findings from clinical trials and observational studies.[6,7] Additionally, milk products, which are a major source of dietary calcium in the American diet, may similarly reduce the risk for colorectal neoplasia as also evidenced by numerous studies.[8]

As our understanding of the mechanisms by which calcium and milk potentially reduce the risk for colorectal neoplasia continues to expand, there is recent concern regarding constituents of milk-based products that may adversely affect the risk for colorectal neoplasia and other chronic diseases. Though milk products are rich in calcium, which likely reduces risk for colorectal neoplasia, there is a variety of milk products with different amounts of protein, fat, and

other nutrients that may affect the risk for colorectal neoplasia otherwise. Additionally, there is little evidence regarding associations of calcium and milk product intakes with risk for mortality. The few studies that investigated this association reported conflicting associations of calcium consumption with cardiovascular disease-related and cancer mortality.

Therefore, my objective of this dissertation is to further clarify the role of calcium and milk products in colorectal neoplasia and other health outcomes. The specific research aims are to: 1) investigate associations of calcium and milk products with risk for colorectal adenomatous polyps; 2) test the effects of calcium on circulating growth factors; 3) investigate associations of the non-calcium component of milk products with risk for adenomatous polyps and mortality; and 4) investigate associations of calcium and milk products with risk for all-cause and cause-specific mortality. These aims will be addressed by analyzing data from three previously conducted colonoscopy-based case-control studies of incident, sporadic colorectal adenoma, a clinical trial of calcium supplementation in adenoma patients, and a national population-based prospective cohort study.

BACKGROUND

Colorectal Cancer

Colorectal cancer incidence and mortality rates appear to be stabilizing in many developed countries, such as the United States, but these rates are rapidly increasing in countries that have experienced rapid economic growth in recent years.[3] Evidence from these countries, in addition to migration studies, indicate the importance of environmental and lifestyle factors in the etiology, and thus the prevention, of colorectal cancer.[2,9] Among these modifiable risk factors, various dietary components have been tested in clinical trials of colorectal neoplasia, including calcium,[10] antioxidant micronutrients,[11] dietary fiber,[12] fruit and vegetable intake,[13] folic acid,[14] and nonsteroidal anti-inflammatory drugs (NSAIDs).[15-17] However, only calcium intake and NSAIDs were found to protect against colorectal neoplasms.

Colorectal neoplasia originates from colonic or rectal epithelial cells, where cells normally undergo rapid daily turnover. As a result, countless DNA damage "hits" occur in these cells, but normal genomic repair systems, such as the mismatch repair gene, are able to repair the damage. If the damage cannot be repaired, the affected cell normally undergoes programmed cell death, or apoptosis. If damaged DNA is not repaired, the cell does not undergo apoptosis, and the damaged DNA is passed to the daughter cell, and thus, by definition, a mutation.

In colorectal carcinogenesis, accumulation of mutations occurs over a long period of time, and the sporadic (non-Mendelian inherited) form of this disease primarily occurs in older adults. These mutations lead to hyper-proliferation of normal colonic epithelial cells, which then lead to the development of an adenomatous polyp, or adenoma, which are well-established precursors to colorectal cancer.

The chromosome instability pathway is one established pathway in colon carcinogenesis that leads to the development of most sporadic colorectal neoplasia. Mutation of the adenomatous polyposis coli (*APC*) tumor suppressor gene is considered to be the usual first step in this pathway as carcinogenesis would be unlikely without a mutation in this gene.[18] Inactivating *APC* gene mutations prevent binding of the APC protein to beta-catenin, which increases levels of free beta-catenin causing up-regulation of the Wnt-signaling pathway and consequently, unregulated growth and decreased apoptosis and differentiation of cells.[18] *APC* gene mutations cause Familial Adenomatous Polyposis (FAP), which is characterized by the development of hundreds of adenomas in the colon and rectum at a young age, which, if left untreated, almost inevitably progress to colorectal cancer.[19]

A mutation in the *K-ras* oncogene that occurs prior to an *APC* gene mutation often leads to early dysplasia or hyperplastic polyps. However, if a *K-ras* mutation occurs after an *APC* mutation, the lesion is likely to progress to cancer.[20] *K-ras* mutations affect regulation of mitosis, cell differentiation, and apoptosis.[21] The *p53* tumor suppressor gene maintains genomic stability by regulating the cell cycle to allow time for DNA repair and induction of apoptosis if DNA

damage is too extensive.[22] Mutations that cause the loss of *p53* function transform non-invasive cancers into invasive malignancies.

The microsatellite instability pathway is the primary pathway of autosomal dominantly inherited Lynch Syndrome, or Hereditary Non-Polyposis Colon Cancer (HNPCC), and is caused by mutations in genes of the mismatch repair system. Impairment of this repair system leads to an accumulation of single base pair mismatches or short repeated DNA sequences (i.e., microsatellites).[23] Similar to FAP, HNPCC is also characterized by early onset of disease but is associated with tumor development beyond the colon, including in the stomach, ovary, small intestine, brain, and skin.[24]

Calcium

Calcium is an essential nutrient, necessary for maintenance of healthy bones and teeth, in addition to normal muscle function, nerve transmission, blood clotting, and blood pressure.[25] In the United States, dietary calcium intakes have continually increased in all age groups.[26] Supplemental calcium use has also increased, but primarily among white and Mexican-American women 60 years of age or older.[27]

Calcium levels are tightly regulated by the actions of parathyroid hormone and calcitonin, which are released from the parathyroid and thyroid glands, respectively. 1,25-dihydroxyvitamin D is also needed for calcium homeostasis by aiding in calcium absorption in the intestine, bone resorption, and renal reabsorption of calcium.[28]

There is strong biological plausibility for protection against colorectal neoplasms by calcium intake.[29-31] Calcium is hypothesized to reduce the risk for neoplasms by binding with secondary bile acids and free fatty acids in the colon, reducing epithelial cell exposure to their damaging effects.[29,32] Bile acids form micelles and solubilize lipids; however, excess amounts of bile acids are metabolized by colonic bacteria to form hydrophobic secondary bile acids. These secondary bile acids are damaging to cell DNA and membranes,[33] which may also lead to

production of reactive oxygen species (ROS). Thus, via bile acid binding, calcium prevents the production of ROS. In addition, calcium directly inhibits proliferation and induces differentiation of colonic epithelial cells.[34-36]

Results from a major randomized, placebo-controlled clinical trial also support a protective effect of calcium against the recurrence of colorectal neoplasms. In the Calcium Polyp Prevention Study of patients with previous adenomas, the adjusted relative risk for adenoma recurrence among the 1.2 g calcium treatment group compared to placebo was statistically significantly lower after a 1 or 4 year study period (relative risk [RR] 0.85; 95% confidence interval [CI] 0.74-0.98).[10] Five years after the intervention phase of this trial ended, the estimated decrease in adenoma recurrence was even greater (odds ratio [OR] 0.63; 95% CI 0.46-0.87).[37]

Associations of calcium with colorectal neoplasms from observational studies also consistently support a protective role for calcium. Of 46 observational studies of calcium and risk for colorectal cancer (20 prospective cohort and 26 case-control studies), 35 (76%) found inverse associations, of which 13 were statistically significant. The most recent meta-analysis of 15 cohort studies reported an RR of 0.92 (95% CI 0.89-0.95) per 300 mg daily increase in total calcium intake with risk for colorectal cancer.[6] Supplemental calcium intakes were also associated with a statistically significant lower risk for colorectal cancer (RR 0.91 [95% CI 0.86-0.98] per 300 mg daily increase in intake).

Similarly, in 6 observational studies (6 prospective cohort and 10 case-control studies) of colorectal adenoma, 15 (94%) reported inverse associations, of which 4 were statistically significant. In the most recent meta-analysis of 14 prospective and retrospective studies, total calcium was associated with statistically significant lower risk for adenoma (RR 0.93 [95% 0.90-0.97] per 300 mg daily increase).[38] Additionally, supplemental calcium intake, included in 4 studies, was associated with a statistically significant lower risk for adenoma, although the association was closer to null (RR 0.96; 95% CI 0.93-0.99).

Milk Products

Milk products are a major source of dietary calcium in the average American diet, and the current Dietary Guidelines recommend 3 cup equivalents of dairy products daily for adults.[39] However, overall milk consumption has steadily declined since the 1970s among all age groups, though lower fat milk intakes have increased while whole milk has decreased.[40]

Similar to those of calcium, observational studies of total milk product intakes with risk for colorectal cancer and adenomas are relatively consistent. In a recent meta-analysis of 15 cohort studies, consumption of non-fermented milk was statistically significantly inversely associated with risk for colorectal cancer (RR 0.85; 95% CI 0.77-0.93) among both men and women, while no associations were found with intakes of cheese or fermented milk.[8] An additional meta-analysis of 12 cohort studies found a statistically significant inverse association of total milk products with risk of colorectal cancer (RR 0.81; 95% CI 0.74-0.90) upon comparison of high and low categories of intake.[41] In the same meta-analysis, a dose-response analysis of 10 cohort studies yielded a statistically significant 17% lower risk of colorectal cancer per 400 gram daily increase in milk product consumption. There have been fewer observational studies and no meta-analyses of associations with adenoma. Of the three most recent prospective cohort studies,[42,43] only one reported an inverse association with risk for adenoma.[44] This analysis of French women in the E3N-European Prospective Investigation into Cancer and Nutrition (EPIC) prospective cohort study reported a 20% lower risk of adenoma with higher calcium intakes (95% CI 0.62-1.03; $p_{trend} = 0.04$), 14% lower risk with dairy calcium (95% CI 0.67-1.11; $p_{trend} = 0.04$), 20% lower risk with total milk products (95% CI 0.62-1.05; $p_{trend} = 0.04$), but no association with milk consumption alone.

Though milk products are calcium-rich foods, "conventionally-produced" (referred to as "conventional" hereinafter) milk also contains significant levels of insulin-like growth factor-1 (IGF-1). IGF-1 is a peptide primarily produced in the liver and is regulated by growth hormone produced in the pituitary gland. Binding of IGF-1 to its primary receptor, insulin-like growth

factor-1 receptor (IGF-1R), initiates an intracellular signaling pathway that stimulates cell growth and proliferation and inhibits apoptosis. Circulating IGF-1 can stimulate growth in almost every type of cell in the body. Most circulating IGF-1 is bound to binding proteins to prolong its half-life, with at least 75% bound to IGF binding protein-3 (IGFBP-3).[45] There are six binding proteins (IGFBP-1 – IGFBP-6), including IGFBP-2, which is also implicated in carcinogenesis, but its binding affinity is greater for insulin-like growth factor-2 (IGF-2) than for IGF-1.[46] IGFBP-3 is the most abundant IGF-1 binding protein, to which it binds in a 1 to 1 molar ratio. There is little evidence that IGFBP-3 may have an independent role in carcinogenesis, especially since no cancer has been attributed to any IGFBP mutations.[47] However, the molar ratio of IGF-1 to IGFBP-3 is currently the most widely used measure of circulating and bioavailable IGF-1. Normal levels of IGF-1 vary widely, but, in general, levels normally increase during phases of growth, such as puberty and pregnancy, and decrease with age.[48] Abnormal levels are observed in disorders of growth, such as dwarfism and acromegaly.

IGF-1 is normally present in milk since cows also produce growth hormone and have similar IGF-1 regulatory pathways. However, higher IGF-1 levels have been discovered in milk produced from cows administered bovine somatotropin (bST) hormone.[49] Use of this recombinant hormone was first approved by the US Food and Drug Administration (FDA) in 1993[50] to increase milk production by mimicking the actions of growth hormone to upregulate the insulin-like growth factor system.[51] Conventional milk produced from these cows were shown to contain higher levels of IGF-1 in comparison to bST-free and organic milks.[49] It was also reported that IGF-1 is not destroyed by milk processing methods, as IGF-1 levels remained unchanged after homogenization and pasteurization.[52] Additional animal studies previously detected radioactively-labeled IGF-I from consumption of conventional milk in circulation,[53] and the single known human trial of milk consumption reported a statistically significant positive association with serum IGF-1 levels, which was not observed with meat consumption.[54] However, it is important to note that this trial was conducted in 8 year old boys who are likely to

have higher, fluctuating levels of IGF-1 given their developmental stage. Nonetheless, the positive correlation between milk and IGF-1 was consistently found across several observational studies that reported positive associations of both calcium and milk product intake with circulating IGF-1 levels.[52,55-57]

Given the pro-proliferation/pro-growth effects of IGF-1, it is plausible that high levels of serum IGF-1 may increase risk for certain cancers, such as breast, prostate, and colorectal.[58] Supporting this plausibility is that numerous studies reported statistically significantly higher levels of IGF-1 and IGFBP-3 in patients with adenomatous polyps and colorectal cancer,[55,59-63] breast,[64,65] and prostate cancers.[66,67]

IGF-1 levels in the body fluctuate in response to hormonal control mechanisms, stimuli, and pathologic conditions, but various regulatory mechanisms act to maintain levels within a normal range.[68] IGF-1R is overexpressed in certain cancer cells, and hypothesized to be a critical factor for independent cell growth.[69] Therefore, higher circulating levels of IGF-1 result in greater binding to IGF-1R, which increases the likelihood of tumor development. Diet and lifestyle factors appear to be important regulators of IGF-1 levels,[70] and thus, consumption of foods, such as milk products, that contain higher levels of IGF-1 may subsequently alter circulating levels in the body. In a systematic review of 13 cross-sectional studies, 10 studies reported a statistically significant positive correlation of milk consumption with IGF-1, and 3 of 12 studies reported a statistically significant positive correlation between total dairy products and IGF-1. Correlations with IGFBP-3 were less evident, with only 2 of 11 studies reporting statistically significant positive correlations with milk.[52]

Interestingly, to our knowledge, the only clinical trials that examined the effect of supplemental calcium on circulating levels of IGF-1 were studies of bone health conducted among postmenopausal women and teenage girls. In two of the trials, no effect of calcium supplementation on IGF-1 concentrations was found after follow-up periods of 6 months and 2 years among postmenopausal women.[71,72] The third trial, conducted among 18 Japanese teenage

girls, did not report treatment effects of calcium supplementation on circulating IGF-1 levels.[73] By our calculations based on the data provided in the manuscript, the estimated absolute treatment effect was 82.6 ng/mL and the relative treatment effect was 1.16 ng/mL for the calcium supplementation group compared to placebo after the 8-month follow-up period. Based on the reported p-values for the within-treatment group changes, it is likely that the treatment effect would be statistically significant since IGF-1 levels increased in the calcium supplementation group and decreased in the placebo group. However, it is important to note that teenage girls would be expected to have higher and fluctuating levels of IGF-1 during this growth and developmental phase.

The fourth trial that investigated IGF-1 levels, an intervention trial of bone health among Greek postmenopausal women, tested the effects of 600 mg daily supplemental calcium vs. 3 daily servings of low-fat dairy products fortified with calcium and vitamin D_3 (providing approximately 1,200 mg dietary calcium daily) vs. their usual diet.[74] After 5 and 12 months, there were no differences in percent changes in IGF-1 among the 3 treatment groups.

There is little information on a potential molecular mechanism by which supplemental calcium may alter circulating levels of IGF-1. The available evidence from animal studies suggests that low serum levels of calcium may activate the IGF signaling pathway in calcium-transporting epithelial cells to stimulate proliferation of these cells, which contain Na^+-K^+-ATPase transporters needed for calcium absorption.[75] Under normal calcium levels, this IGF signaling appears to be suppressed.

Although milk product consumption may be correlated with IGF-1 levels and each of these factors are independently associated with risk of colorectal cancer, to our knowledge, only two observational studies investigated associations of calcium and milk product intakes with IGF-1 levels in relation to risk for colorectal cancer. In a case-control study nested in the prospective Physicians' Health Study, higher intakes of low-/non-fat milk and dairy calcium were associated with statistically significantly higher levels of IGF-1, IGFBP-3, and the IGF-1:IGFBP-3 molar

ratio.[55] The molar ratio was also positively associated with risk of colorectal cancer ($p_{trend} = 0.01$). However, those in the highest tertile of the IGF-1:IGFBP-3 molar ratio and also in the highest tertiles of low-/non-fat milk or dairy calcium intakes were at no or lower risk of colorectal cancer, respectively. Interestingly, the highest risk of colorectal cancer was among those in the highest tertile of the IGF-1:IGFBP-3 molar ratio but the lowest tertile of milk intake.

In contrast, in a case-control study nested in the European Prospective Investigation into Cancer and Nutrition cohort, IGF-1 was not associated with risk of colorectal cancer while IGFBP-3 was associated with a 14% higher risk, though non-statistically significant.[76] The association of IGF-1 (per 100 ng/mL increase) with risk for colorectal cancer, in analyses stratified by milk or dairy calcium intakes, was statistically significantly higher only among those in the lowest tertiles of milk or dairy calcium. Given the results of these studies, the association of IGF-1 with risk for colorectal cancer according to different levels of milk product and dairy calcium intakes is unclear.

It is important to note that in studies of milk products conducted outside of the United States, the use of bST is unknown since milk production regulations may differ among these countries. Thus, studies of milk products conducted in other countries may or may not contain elevated levels of IGF-1.

Calcium, Milk Products, and Mortality

Global patterns of colorectal cancer mortality reflect changes in incidence that are rapidly rising in less-developed countries undergoing economic transitions.[3] In the United States, colorectal cancer incidence and mortality appear to be stabilizing among black and white men and women. Still, it is estimated that over 49,000 deaths due to colorectal cancer will occur this year.[77] Though there is consistent evidence supporting the inverse association of calcium and milk products with risk for colorectal neoplasms, there is less evidence on the association of these factors with colorectal or overall cancer mortality.

In the most recent meta-analysis of 22 prospective cohort studies of calcium and cancer mortality, total calcium was associated with 16% and 49% higher risk of all-cause and cancer mortality, respectively, though the findings were not statistically significant.[78] However, dietary calcium was associated with a non-statistically significant 11% and 6% lower risk of all-cause and cancer mortality, respectively. Supplemental calcium was associated with a non-statistically significant 22% higher risk of cancer mortality but a statistically significant 9% lower risk of all-cause mortality. Since the publication of this meta-analysis, an additional analysis of the Cancer Prevention Study-II (CPS-II) Nutrition Cohort reported a statistically significant 28% lower risk of colorectal cancer-specific mortality with total calcium intake but no association with all-cause mortality.[79] Dietary calcium was inversely associated with both all-cause and colorectal cancer mortality while supplemental calcium was associated with a higher risk of all-cause and lower risk of colorectal cancer, though none of the associations was statistically significant. Additionally, total dairy and milk intakes were associated with a borderline 25% lower and statistically significant 28% lower risk of colorectal cancer mortality, respectively. In an additional recent analysis of the European Investigation into Cancer and Nutrition cohort, pre-diagnostic intakes of dietary calcium were not associated with risk of colorectal cancer-specific mortality.[80] In contrast to the findings of the CPS-II study, total dairy and milk intakes were associated with non-statistically significant 17% and 21% higher risk of colorectal cancer mortality, respectively.

Calcium and dairy products may affect the risk of colorectal cancer and other chronic diseases via several plausible mechanisms. Calcium binds bile acids and free fatty acids in the gut, decreasing fat absorption, potentially lowering cholesterol and reducing damage to colonic epithelial cells.[29,32] However, consumption of calcium and milk products may both positively and negatively influence the risk for cardiovascular disease via other mechanisms of action. Calcium is essential for normal cardiac and vascular smooth muscle function, in addition to the activation of platelets for coagulation.[81,82] However, high levels of calcium may increase coronary artery

calcification, especially among those with reduced renal function.[83] Clinical trials of calcium supplementation, with or without vitamin D, reported favorable changes in blood lipids but no appreciable effects on blood pressure,[84,85] whereas trials of dairy products reported no changes in blood lipid profiles or blood pressure.[86,87]

In the previously mentioned meta-analysis of 22 prospective cohort studies, total, dietary, and supplemental calcium were not associated with risk of cardiovascular disease mortality.[78] In another review, it was noted that 8 out of 11 prospective cohort studies reported positive, statistically significant associations of serum calcium levels and risk of myocardial infarction or coronary heart disease.[88] Although supplemental calcium was previously associated with lower risk of cardiovascular disease mortality, two recent secondary analyses of two previously conducted randomized controlled trials of calcium supplementation suggested that calcium supplementation may increase risk for myocardial infarction and related deaths among postmenopausal women.[89,90]

The evidence on milk product consumption in relation to cardiovascular disease outcomes and mortality is similarly inconsistent. In the most recent meta-analysis of 17 total prospective cohort studies, there was no association of milk intake with all-cause mortality per 200 mL daily increase, though significant heterogeneity was reported ($p = 0.001$) among 8 pooled studies on milk consumption.[91] Six of the 17 studies were pooled to investigate coronary heart disease risk, but there was no association with milk intake. Only total cardiovascular disease risk was statistically significantly inversely associated with milk consumption (RR 0.94; 95% CI 0.89-0.99) in pooled analysis of 4 of the 17 studies. The authors of this meta-analysis did not specify whether milk intakes were in reference to total or specific types of milk. Upon review of the 17 included studies, only one study specified whole and low-fat milks as exposures in association with stroke risk.[92] Separate meta-analyses were conducted for the low-fat and whole milk results of the study; and similar inverse associations with risk for stroke were reported, though not statistically significant.

Since the latter meta-analysis, 2 additional studies of milk product consumption and cardiovascular outcomes were published. A cross-sectional analysis of 162 overweight or obese adults yielded inverse associations of total dairy products, reduced fat milk, and dairy calcium with adiposity measures (BMI, percent body fat, waist circumference), but positive associations of whole milk and dairy fat intakes with the same outcomes.[93] A population-based cohort study of total dairy product intake found no association with risk of coronary heart disease or stroke.[94] An additional meta-analysis of 11 prospective studies published in the same year reported an inverse association between dietary calcium and risk for stroke; however, heterogeneity among all studies was reported ($p < 0.001$).[95] After stratifying by dietary calcium intakes, a statistically significant 16% lower risk of stroke was reported among those with low dietary calcium intakes (< 700 mg/day). Dairy calcium was associated with a statistically significant 22% lower risk for stroke, but there was no non-dairy calcium-stroke association.

Although there has been much progress in reducing colorectal cancer incidence and mortality, largely attributable to improved screening and treatment methods in the United States, cancer remains a leading cause of death in the United States and worldwide, second only to cardiovascular disease.[96,97] There are consistent data on inverse associations of calcium and milk products with risk for colorectal cancer, but there is inconsistent evidence on the potential role of calcium and milk products in other cancer- and cardiovascular disease-related deaths.

Gaps in the Literature Addressed by this Dissertation

Although research in colorectal cancer etiology and prevention has greatly progressed, there remains a need to further investigate specific dietary factors, specifically calcium and milk products, which potentially modulate the risk of colorectal cancer and mortality. Therefore, the objectives of my dissertation are to address these specific gaps in the literature: 1) calcium and IGF-1 are independently associated with risk for colorectal cancer, and dietary calcium may be associated with IGF-1 levels, but there is no evidence on the effect of supplemental calcium on

IGF-1; and 2) there is inconsistent evidence on the association of calcium and milk products with risk for all-cause and cause-specific mortality.

There is extensive evidence that calcium and calcium-rich foods are associated with lower risk of colorectal cancer, but in contrast, specific milk components, such as IGF-1, are associated with higher risk of colorectal cancer. Dietary and dairy calcium are highly correlated with IGF-1, but the effect of supplemental calcium on IGF-1 is unknown. Current evidence on the association of calcium and milk products with risk for different cancer- and cardiovascular disease-related deaths are inconsistent. In this dissertation, the results of investigations of associations of calcium and milk products with risk for colorectal neoplasia and mortality and the effect of calcium supplementation on IGF-1 are discussed.

DISSERTATION RESEARCH PLAN

Objectives, Specific Aims, and Study Hypotheses

The projects in this dissertation are to investigate associations of calcium and milk products with risk of colorectal adenomatous polyps; associations of the non-calcium component of milk products with risk for colorectal polyps and all-cause and cause-specific mortality; the effects of supplemental calcium on circulating IGF-1, IGFBP-3, and their molar ratio; and associations of calcium and milk products with risk of all-cause and cause-specific mortality. The specific aims are:

Aim 1: Investigate associations of calcium and milk product intakes with risk of incident, sporadic adenomatous polyps using data from three previously conducted colonoscopy-based case-control studies of incident, sporadic colorectal adenoma (pooled n = 807 cases and 2,185 controls): the Minnesota Cancer Prevention Research Unit Case-Control Study (CPRU) and the Markers of Adenomatous Polyps studies I and II (MAP I, II).

Hypothesis: Higher intakes of calcium and milk are associated with lower risk for incident, sporadic adenomatous polyps.

Aim 2: In sporadic colorectal adenoma patients, **a)** test the effect of supplemental calcium on circulating IGF-1, IGFBP-3, and the molar ratio of IGF-1 to IGFBP-3, and **b)** investigate cross-sectional associations of milk product intakes with circulating IGF-1 and IGFBP-3 levels using stored blood samples and data from the Calcium and Colorectal Epithelial Cell Proliferation (Calcium CECP) trial (n = 193), a previously conducted randomized, double-blinded, placebo-controlled, 3-arm parallel group clinical trial comparing two dose levels of calcium (1g/day and 2g/day) with placebo.

Hypothesis: a) Supplemental calcium decreases IGF-1 and IGFBP-3 levels relative to placebo; and b) higher intakes of milk products are associated with lower, but the non-calcium component of milk products (as estimated by residuals) with higher circulating levels of IGF-1.

Aim 3: Investigate associations of calcium and milk product intakes with risk of all-cause and cause-specific mortality using baseline and mortality data from the prospective REasons for Geographic and Racial Differences in Stroke (REGARDS) study (n = 30,239) in black and white men and women 45 years of age or older.

Hypothesis: Higher intakes of calcium and milk products are associated with lower risk for all-cause and cause-specific mortality.

Methods for Aim 1

For the pooled analysis of the CPRU, MAP I, and II studies, adults between 35 to 74 years of age, with no known genetic syndromes associated with colonic neoplasia, and no history of inflammatory bowel disease, cancer (excluding non-melanoma skin cancer), or colorectal adenoma were recruited from large private-practice gastroenterology groups. In the CPRU study,

additional controls were recruited from patients being screened for colon cancer using flexible sigmoidoscopy in the same community gastroenterology practices as for the colonoscopy-based controls, and from the general population in the Minneapolis-St. Paul metropolitan region as previously described in detail.[98] For the endoscopy-based participants, all self-reported data, which included medical, lifestyle, and dietary history, were obtained before case or control status was determined.

The characteristics of the cases and controls were compared using the Pearson's chi square and Fisher's exact tests for categorical variables and the Student *t* test for continuous variables. Appropriate transformations were made for any continuous variable as needed to meet normality assumptions.

Multivariable unconditional logistic regression, which is the standard method for assessing associations of risk factors with a dichotomous outcome in case-control studies, was used to calculate odds ratios (OR) and their 95% confidence intervals (Cis) as measures of associations of calcium and milk product intakes with incident, sporadic colorectal adenomas. For these analyses, calcium and milk product intakes was categorized as quintiles of intake (based on the study- and sex-specific distributions among the controls), or other categories of intake if sample sizes did not allow for quintiles. Selection of potential confounding variables was based on biological plausibility, previous literature, consideration of directed acyclic graphs, and the effects of inclusion/exclusion of the variables from the models on the estimated associations of the primary exposure variables with the outcome. Selection of potential effect modifying variables was based on biological plausibility and previous literature, and included study, age, sex, body mass index (BMI), oxidative balance score (OBS), family history of colorectal cancer, and NSAID/aspirin use. The non-calcium component of milk products, as an indirect indicator of IGF-1 intake from milk products, was calculated as the residuals of linear regression models in which total milk products and non-fat milk were the dependent variables and dietary calcium was the independent variable. This method is modeled after the nutrient residual (energy-adjustment)

model, which is commonly used to examine associations of calorie-bearing macronutrients, such as fat, with various outcomes.[99] Using this method, the residuals from fat-energy linear regression model represent fat other than its energy content. Using this concept, total milk product intake was adjusted for dietary calcium to examine the non-calcium component of milk products, and non-fat milk was adjusted for dietary calcium to examine the non-calcium, non-fat component of milk. The estimated residuals were categorized into quintiles and used as a primary independent variable in the logistic regression models, similar to the other primary exposure variables of interest. Tests for trend were based on the sex-specific median of each category of calcium, milk product intake, and milk product residual. Effect modification was assessed by conducting stratified analyses. Where indicated, the statistical significance of potential multiplicative interactions was assessed by including interaction terms in the logistic regression models.

Sensitivity analyses included 1) re-categorization of supplemental use for less than 2 years as no supplemental calcium use and 2) separate analyses of the CPRU and MAP studies to analyze potential differences in findings before and after the FDA approved bST use in conventional milk production in 1993.

A two-sided P value < 0.05 was considered statistically significant. All statistical tests were conducted using SAS version 9.4 software (SAS Institute Inc., Cary, North Carolina).

Methods for Aim 2

We used data and blood samples from the Calcium CECP study, a randomized, double-blind, placebo-controlled, 3-arm parallel group clinical trial, to examine the effect of calcium supplementation on circulating levels of IGF-1 and IGFBP-3. In this study, adults between 30 to 74 years of age in general good health with a history of at least one pathology-confirmed sporadic colon or rectal adenoma within the past 5 years were recruited. Exclusion criteria included

contraindications to calcium supplementation or rectal biopsy procedures; further details of the study were previously published.[100]

Following the baseline visit, eligible participants (n = 193) were randomly assigned to one of the following three groups: 1) a placebo control group (n = 66), 2) a calcium supplement group (n = 64) taking 1.0 g elemental supplemental calcium as calcium carbonate in two equal divided doses twice daily with food, and 3) a calcium supplement group (n = 63) taking 2.0 g elemental supplemental calcium as calcium carbonate in two equal divided doses twice daily with food over 6 months. The supplement and placebo pills, prepared by SmithKline Beecham, Pittsburgh, PA, were identical in size, appearance, and taste.

Circulating levels of IGF-1 and IGFBP-3 were measured using solid-phase sandwich Enzyme-linked Immunoassay (ELISA) and quantitative Western Ligand blot (qWLB), respectively. Of the original 193 participants, 189 baseline and 175 follow-up samples were available for analysis and were quantified by Ligandis laboratory (Ligandis GbR, Gülzow, Germany) using double analysis. Thirty-five blinded duplicate quality control samples were also included with the participants' samples.

The baseline characteristics of the subjects between treatment groups (placebo vs. 1 g vs. 2 g calcium) were compared using chi-square or Fisher's exact tests for categorical variables and analysis of variance (ANOVA) for continuous variables. Appropriate transformations were made for any continuous variable as needed to meet normality assumptions.

Treatment effects of supplemental calcium on IGF-1, IGFBP-3, and the molar ratio of IGF-1 to IGFBP-3 from baseline to 4-months follow-up across the treatment groups were compared using a repeated-measures mixed linear effects model using SAS Institute's Mixed Procedure. This procedure is an advanced method of conducting analysis of covariance/linear models in order to account for correlated data from repeated measurements on the study participants. The procedure also allows specification of random and fixed effects in the model and retention of baseline and follow-up measures from individuals on whom one or more

measurements is missing. The model also generates a relative treatment effect, defined as ([treatment group follow-up mean] / [treatment group baseline mean]) / ([placebo group follow-up mean] / [placebo group baseline mean]). The dependent variables in the models were the growth factors (transformed if necessary to meet normality assumptions), and the model included as predictors the intercept, follow-up visit effects (baseline and 4-month follow-up), treatment groups, treatment group and follow-up visit effect interactions, and others as appropriate. The molar ratio of IGF-1 to IGFBP-3 was calculated prior to any necessary log-transformations using the mass to molar conversion of 1 ng/mL IGF-1 = 0.130 nM for IGF-1 and 1 ng/mL IGFBP-3 = 0.036 nM IGFBP-3.

The associations of baseline concentrations of IGF-1, IGFBP-3, and the IGF-1:IGFBP-3 molar ratio with demographic, lifestyle, and dietary characteristics of participants were also investigated using generalized linear models. Of particular interest was dairy product intake, especially the non-calcium and non-fat component of milk as an indirect measure of the IGF-1 component of milk. To estimate this, the residuals obtained from the linear regression models of total dairy and non-fat milk with dietary calcium intake were determined. This method is modeled after the energy adjustment residual method,[99] as previously described in Aim 1, with the dependent variables being total milk products and non-fat milk and the independent variable being dietary calcium. Participant characteristics, calcium and milk product intakes, and residuals were categorized as sex-specific high and low groups or other relevant categories, and least squares means and standard errors were obtained from the generalized linear models, adjusting for covariates as appropriate. A two-sided P value < 0.05 was considered statistically significant. All statistical tests were conducted using SAS version 9.4 software (SAS Institute Inc., Cary, North Carolina).

Methods for Aim 3

In the REGARDS study, 30,239 adults 45 years of age or older were enrolled from January 2003 to October 2007. Exclusion criteria included race other than white or African-American, active treatment for cancer, medical conditions preventing long-term participation, cognitive impairment based on the telephone interview, residence in or on a waiting list for a nursing home, and inability to speak English. Detailed methodology of the study was previously published.[101] The final cohort included 58% women and 42% blacks. As of November 2014, 7,565 (25%) total deaths were recorded.

The baseline characteristics of the subjects according to categories of the primary exposures variables (e.g., total calcium intake) were compared using chi-square tests for categorical variables and analysis of variance for continuous variables. Appropriate transformations were made for any continuous variable as needed to meet normality assumptions.

Cox proportional hazards regression, which is a standard method for analyzing data from prospective cohort studies with variable lengths of follow-up and dichotomous outcomes, was used to calculate multivariable-adjusted hazard ratios (HR) and 95% CIs to assess associations of baseline categories of calcium and milk product intakes with all-cause and cause-specific mortality rates. Sex-specific quintiles of calcium and milk products in the whole analytic cohort were determined, with the lowest categories of consumption as reference. Potential confounding variables were selected on the basis of biological plausibility, statistical significance, consideration of directed acyclic graphs, and whether inclusion of the variable changed the adjusted HR for the primary exposure variable by ≥ 10%. Proportional hazards assumptions were tested using Log-Log survival curves, Schoenfeld residuals, and extended Cox models for each exposure and potential covariate. To assess potential effect modification, stratified analyses were conducted for all-cause and cause-specific mortality. Where indicated, the statistical significance of potential multiplicative interactions was assessed by including interaction terms in the Cox hazards regression models.

Sensitivity analyses included exclusion of those who died within 1 or 2 years of enrollment, and those with comorbidities (cardiovascular disease, diabetes, or cancer) at baseline.

A two-sided *P* value < 0.05 was considered statistically significant, and all statistical tests were conducted using SAS version 9.4 software (SAS Institute Inc., Cary, North Carolina).

Significance and Impact of the Study

These specific aims are innovative in several respects. First, although there is substantial literature on calcium and milk products, few studies included both in comprehensive analyses of their associations with risk for incident, sporadic adenomatous polyps. Second, Aim 2 provides further evidence on the effect of supplemental calcium on serum IGF-1 and IGFBP-3 levels. To our knowledge, whether calcium can affect IGF-1 and IGFBP-3 has only been tested in postmenopausal women and teenage girls in association with bone health. Thus, our aim of testing the efficacy and dose-response of calcium supplementation on serum IGF-1 and IGFBP-3 among adenoma patients is innovative. The third innovation is the estimation of the IGF-1 content in milk products. To our knowledge, the projects in this dissertation are the first studies to use the residuals of linear regression models of milk product intakes with dietary calcium intakes to represent the non-calcium components of milk, including IGF-1. Last, to further investigate the role of calcium in reducing the risk for disease and mortality, this is the first analysis of calcium and milk product intake in association with all-cause and cause-specific mortality using data from the REasons for Geographic and Racial Differences in Stroke (REGARDS) study.

CHAPTER 2. Associations of Calcium and Milk Product Intakes with Incident, Sporadic Colorectal Adenomas

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ABSTRACT

Calcium intake has been consistently, modestly associated with lower risk of colorectal cancer and adenomas, and supplemental calcium reduced adenoma recurrence in large randomized controlled trials. Milk products are the major source of dietary calcium in the US, but their associations with colorectal neoplasms are unclear. Data were pooled from three colonoscopy-based case-control studies of incident, sporadic colorectal adenoma (n=807 cases, 2,185 controls) conducted between 1991 and 2002, and analyzed using multivariable unconditional logistic regression. As an indirect indicator of milk-derived IGF-1 intake, the non-calcium component of milk was determined as the residuals from linear regression models of milk products with dietary calcium intake. For total, dietary, and supplemental calcium intakes, the multivariable-adjusted odds ratios (ORs) comparing the highest to the lowest intake quintiles were 0.94 (95% confidence interval [CI] 0.69-1.30; P_{trend}=0.43), 0.86 (95% CI 0.62-1.20; P_{trend}=0.62), and 0.99 (95% CI 0.77-1.27; P_{trend}=0.79), respectively. The corresponding ORs for consumption of total milk products, total milk, non-fat milk, total milk product residuals, and non-fat milk residuals were, respectively, 0.99 (95% CI 0.74-1.34; P_{trend}=0.84), 0.90 (95% CI 0.68-1.19; P_{trend}=0.32), 0.92 (95% CI 0.70-1.19; P_{trend}=0.33), 0.94 (95% CI 0.71-1.24; P_{trend}=0.33), and 0.95 (95% CI 0.69-1.31; P_{trend}=0.98). For those who consumed any whole milk relative to those who consumed none, the OR was 1.15 (95% CI 0.89-1.49). These results are consistent with previous findings of modestly lower risk with higher dietary calcium intakes, but suggest that neither specific nor all milk products combined may be associated with risk for sporadic colorectal adenoma.

INTRODUCTION

Colorectal cancer is the second leading cause of cancer deaths among men and women combined in the United States.[4] Various lifestyle and dietary factors have been found to be important risk factors for colorectal cancer and adenomatous polyps (adenomas), including calcium intake, which has been consistently, albeit modestly, associated with lower risk.[6,102,103]

A major proportion of calcium intake in the average American diet is from milk products, which include, but not exclusively, milk, cheese, and fermented milk products. Milk product consumption has been fairly consistently associated with lower risk for colorectal cancer and adenomas, though the high fat content of some products, such as certain high fat cheeses, was hypothesized to increase risk by increasing bile acid excretion.[104] However, studies of high-fat milk products and cheese consumption with risk for colorectal cancer have been inconsistent.[8]

Although greater milk product consumption increases dietary calcium intake, it simultaneously contributes to increased intake of insulin-like growth factor 1 (IGF-1). "Conventionally-produced" milk contains significant levels of IGF-1 due to the use of bovine somatotropin hormone.[49] Calcium and milk consumption have both been positively associated with levels of IGF-1 and its binding proteins, such as IGF binding protein 3 (IGFBP-3).[52] However, higher circulating levels of IGF-1 and IGFBP-3 have also been associated with certain cancers, including breast, prostate, and colorectal.[55,59,60,64] Therefore, there is a need to investigate factors that may increase or decrease levels of these growth factors, particularly those related to dietary intake which may be more easily modified. It is unclear whether higher levels of IGF-1 and IGF binding proteins specifically due to calcium and milk product consumption are associated with risk for colorectal cancer and adenomas.

Therefore, our aims in the study reported herein were to investigate associations of total calcium intake and total milk product consumption with risk for incident, sporadic colorectal adenomas, and to analyze the association of the non-calcium component of milk separately, as an indirect measure of IGF-1 in milk, with risk for adenoma.

MATERIALS AND METHODS

Study Population

Data for this study were pooled from three colonoscopy-based case-control studies of incident, sporadic colorectal adenoma. The protocols for the Minnesota Cancer Prevention Research Unit Case-Control Study (CPRU; 1991-1994) and the Markers of Adenomatous Polyps studies I (MAP I; North Carolina, 1995-1997)[105] and II (MAP II; South Carolina, 2002)[98] were previously published. Analyses using the pooled data were also previously published.[106,107]

Briefly, the studies, conducted by the same principal investigator in three states, recruited participants from patients scheduled for outpatient, elective colonoscopy by community gastroenterology practices using identical participant recruitment and data collection protocols. In the CPRU study, additional controls were recruited from patients being screened for colon cancer using flexible sigmoidoscopy in the same community practices as the colonoscopy-based controls, and from the general population in the Minneapolis-St. Paul metropolitan region as previously described in detail.[98] For the endoscopy-based participants, all self-reported data, including medical, lifestyle, and dietary history, were obtained before case or control status was determined.

The same eligibility criteria were used for all studies, which included English-speaking participants 35 to 74 years of age, with no known genetic syndromes associated with colonic neoplasia, and no history of inflammatory bowel disease, cancer (excluding non-melanoma skin cancer), or colorectal adenoma. Cases were defined as patients with first ever pathology-confirmed adenoma(s) at endoscopy while controls were those with no history of adenoma and no adenomatous or hyperplastic polyps found at colonoscopy (all studies) or sigmoidoscopy (CPRU), and the CPRU community controls who reported no history of colorectal neoplasms. For our pooled analysis, all cases were combined into one case group, and all control groups were combined into one control group.

The initial sample sizes of each study were: 574 cases and 707 endoscopy, 538 sigmoidoscopy, and 550 community controls combined as one control group for CPRU; 184 cases and 236 controls for MAP I; and 49 cases and 154 controls for MAP II. Participants were excluded from this pooled analysis if their total energy intake estimated from the self-reported Willett semi-quantitative food frequency questionnaire (FFQ) was >6,000 or <600 kilocalories daily, or if ≥10% of their FFQ data was missing. The final sample size for this pooled case-control study was 787 incident, sporadic adenoma cases and 2,033 controls.

Dietary Assessment

From the above noted FFQ, calcium intake was assessed as total, dietary, and supplemental intake, and milk product consumption was assessed as individual and total milk products. Total milk products included milk, creams, ice cream and sherbet, fermented dairy products, cheeses, and butter. Milk intake was categorized as whole and non-/low-fat milk.

Statistical Analyses

The characteristics of the cases and the controls were compared using the Pearson's chi square and Fisher's exact tests for categorical variables and the Student *t* test for continuous variables.

In this study, calcium and milk product variables were the exposure variables of interest. Calcium (total and dietary) and milk product intakes (total and non-fat milk) were categorized into quintiles based on the study- and sex-specific distributions among the control group. Whole milk was dichotomized as any or no intake, and supplemental calcium intake was categorized into three groups (none and according to the median dose among those who did take supplemental calcium). To examine a possible association of the non-calcium component of milk products with adenomas, the residuals from the linear regression models of total milk products and non-fat milk with dietary calcium intake were determined. This method was modeled after the energy

adjustment residual method[99] with the dependent variables being total milk products and non-fat milk products and the independent variable being dietary calcium. These residuals, as an indirect indicator of IGF-1 intake, were also categorized into study- and sex-specific quintiles.

Multivariable unconditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) for the categorized exposure variables of interest with incident, sporadic colorectal adenomas. In addition, the associations of calcium and milk intakes with various categories of adenomas were examined, and associations were stratified by potential effect-modifying variables which were dichotomized according to the study- and sex-specific medians of the controls.

Confounding factors were assessed using the following criteria: 1) biological plausibility, 2) whether the variable of interest was associated with the outcome and exposure, 3) *a priori* information on established risk factors for colorectal adenoma, and 4) whether the logistic regression coefficient of the primary exposure variable changed by > 10% after adding the potential confounder to the model. Initial models were adjusted for study, age, and sex, while full models were adjusted for study, age, sex, total energy intake (continuous), regular aspirin or nonsteroidal anti-inflammatory drug (NSAID) use (yes/no; defined as ≥ once per week), oxidative balance score (OBS; continuous), family history of colorectal cancer in a first-degree relative, total fat intake (continuous; adjusted for total energy), supplemental calcium intake (continuous; in total milk products and milk models), and dietary calcium intake (continuous; in total milk product and milk residual models). The OBS was created using a previously described equal weight method[107] and is comprised of lifestyle (physical activity, smoking, alcohol intake, and obesity measures [body mass index, BMI; waist-to-hip ratio, WHR]) and dietary variables (total carotene, lutein, lycopene, vitamins C and E, linoleic and linolenic acid, flavonoid, glucosinolate, dietary iron, and saturated fat).

We also conducted several sensitivity analyses. All analyses were repeated separately by study to analyze possible differences before and after US Food and Drug Administration (FDA)

approval of somatotropin use in conventional milk production in 1993. Specifically, the CPRU study, which began in 1991, was analyzed independently of the two MAP studies, which began after 1993. We also considered potential confounding by the OBS components individually rather than combined into the OBS. Finally, we assessed designating participants who reported taking supplemental calcium for less than 2 years as non-supplemental calcium users.

The lowest category of each exposure variable was used as the referent category, and a test for trend was calculated using the sex-specific median of each category of the exposure variable. All statistical tests were conducted using SAS version 9.3 software (SAS Institute Inc., Cary, North Carolina). All tests were 2-sided, and P values < 0.05 were considered statistically significant.

RESULTS

Selected characteristics of the study participants are presented in Table 2.1. Cases were more likely to be male, current smokers, and not regularly take an NSAID. On average, cases consumed greater total energy and total fat but less total calcium, particularly from supplemental calcium. Cases were also, on average, 4 years older and had a slightly higher BMI and WHR. Among the cases, 31% had more than one adenoma at colonoscopy, 27% had at least one adenoma >1.0 cm in diameter, and the largest or most advanced adenoma was pedunculated in 22%, villous or tubulovillous in 23%, and in the right colon in 14%.

Dietary calcium intake was associated with a 14% lower risk of incident, sporadic colorectal adenomas upon comparison of the highest versus lowest quintiles of intake after multivariable adjustment, although the finding was not statistically significant (Table 2.2). The separate findings for total and supplemental calcium were closer to the null and not statistically significant.

The estimated associations of total milk products, total milk, and non-fat milk consumption with adenoma were slightly inverse and not statistically significant (Table 2.3).

Consumption of any whole milk was associated with a non-statistically significant 15% higher risk for colorectal adenoma after multivariable adjustment. There was a suggestion of a U-shaped association of total milk product residuals with adenoma, with approximately 30-40% statistically significant lower risk among those in the third and fourth quintiles after multivariable adjustment, whereas the findings for non-fat milk residuals were null. We also assessed associations of milk products and milk product residuals separately for the CPRU and MAP studies (Appendix Tables 2.5 – 2.8) to examine potential differences before and after the FDA approved somatotropin use in 1993,[50] but there was little difference in the associations for adenoma risk by study period (early 1990s vs. late 1990s-early 2000s).

Possible differences in the associations of calcium and milk product intakes with adenoma according to various demographic, lifestyle, and dietary risk factors were also examined. Of particular interest was NSAID use, which may particularly strongly mask modest associations of dietary factors with colorectal neoplasms. Among those who did not regularly take NSAIDs, the estimated associations were slightly more inverse for total and dietary calcium, total milk products, total milk, non-fat milk, and total milk product residuals (Tables A2 and A3). There were no strong or consistent patterns to indicate effect modification by other demographic or lifestyle factors (Appendix Table 2.9).

We also examined whether the investigated associations differed according to adenoma characteristics. Based on the largest adenoma, total calcium was more strongly inversely associated with adenomas of more advanced characteristics (larger size, more dysplasia) or located in the distal colon (Table 2.4).

In sensitivity analyses, we found no evidence of important differences in our findings according to the separate CPRU and MAP studies, when the OBS components were included in the models individually rather than combined into the OBS, nor when we re-categorized participants who reported taking supplemental calcium for less than 2 years as non-supplemental calcium users.

We analyzed associations of calcium/magnesium and calcium/phosphorus ratios and of calcium according to magnesium and phosphorus intakes since some data suggest that magnesium and phosphorus intakes may influence the effects of calcium by competing for absorption and transport.[108,109] Similarly, since calcium and vitamin D are closely metabolically linked, we repeated all analyses according to serum levels of 25-hydroxyvitamin D_3 (25[OH]D_3) among those with available measures (n = 613 cases, 751 controls [we previously reported that serum 25(OH)D_3 levels were inversely associated with adenoma in this same pooled population[106]]). However, there were no strong, clear patterns or statistically significant findings observed in any of the associations (Appendix Table 2.10).

DISCUSSION

The results from this study, although mostly not statistically significant, are consistent with previous extensive literature that higher calcium intakes are associated with modestly lower risk for incident, sporadic colorectal adenoma. We found no evidence that milk product intake was associated with risk for adenoma except for a suggestion of a U-shaped association with total milk product residuals. Also, we found no evidence that the results for milk product residuals differed among the studies that were conducted before or after the approval of bovine somatotropin.

Calcium is hypothesized to reduce risk for colorectal cancer and adenomas by binding with secondary bile acids and free fatty acids in the colon and subsequently reducing epithelial cell exposure to their damaging effects and lowering the risk for colorectal cancer and adenomas.[29,32] Additionally, calcium directly inhibits proliferation and induces differentiation of colonic epithelial cells.[35,36]

Consistent associations of calcium intake with colorectal cancer and adenomas were reported in numerous epidemiologic studies. Of 46 observational studies of calcium and colorectal cancer (20 prospective cohort and 26 case-control studies), 35 (76%) found inverse

associations, of which 13 were statistically significant. In addition, several meta-analyses reported on the association between calcium and colorectal cancer.[6,7,110,111] From a recent pooled analysis of 17 cohort studies on total or dietary calcium intake in relation to incident colorectal cancer, a summary relative risk (RR) of 0.77 (95% CI (0.71-0.81) was reported.[111] A pooled analysis of 13 case-control studies reported a similar summary OR of 0.77 (95% CI 0.71-0.84), although there was substantial heterogeneity of unidentified source(s). The most recent meta-analysis of 15 cohort studies on total and dietary calcium intake reported a summary RR of 0.92 (95% CI 0.89-0.95) per 300 mg per day increase in calcium intake.[6]

Fewer studies investigated the calcium-colorectal adenoma association but support the results of colorectal cancer studies. Among 16 observational studies (6 prospective cohort and 10 case-control studies), 15 (94%) reported inverse associations, of which 4 were statistically significant. Additionally, in a meta-analysis of 7 prospective studies, a statistically significant 13% lower risk of adenomas was reported among those in the highest relative to the lowest levels of total calcium intake.[38] In the same meta-analysis, 8 additional prospective studies were pooled in a dose-response analysis in which a statistically significant 5% lower risk of adenomas per 300 mg daily increase in total calcium intake was reported. Analysis of 3 of these 8 studies also found a statistically significant 4% lower risk for adenoma per 300 mg daily increase in supplemental calcium intake. Although we found no substantial inverse association with total or supplemental calcium, we also found dietary calcium intake to be modestly, inversely associated with risk for adenomas.

Milk products are a major source of dietary calcium in the US. However, some milk products also contain a high percentage of total and saturated fat, which may offset the inverse association from calcium. Although consumption of dietary fats stimulate the release of bile acids, epidemiological studies, in general, do not support the hypothesis that total fat intake increases colorectal cancer risk.[112]

Due to the high calcium content of milk products, observational studies also examined associations of milk product intakes with colorectal cancer risk. We reviewed 56 observational studies (28 prospective cohort and 28 case-control studies) that included either total or specific milk products, with or without calcium. Of 23 studies that included total milk products, 19 (83%) reported inverse associations, with 6 being statistically significant. Of 36 studies that included milk, 24 (67%) reported inverse associations, with 8 being statistically significant. Several meta-analyses of milk products and colorectal cancer were also conducted.[8,41,104,110,111,113] In a recent meta-analysis of 15 cohorts, only non-fermented milk was statistically significantly inversely associated with colorectal cancer (summary RR 0.85, 95% CI 0.77-0.93).[8] In another recent meta-analysis of 12 studies, a statistically significant inverse association of total milk products with colorectal cancer was found (summary RR 0.81, 95% CI 0.74-0.90).[41] In the same meta-analysis, a dose-response analysis of 10 cohort studies yielded a statistically significant 17% lower risk of colorectal cancer per 400 gram daily increase in milk product consumption.

Fewer studies examined the milk product-adenoma associations, and to our knowledge, only one systematic review of milk product consumption and adenoma risk was conducted.[114] In this review of 11 case-control and 2 cohort studies, there was no association between milk product consumption and risk for adenoma. Since this review, three additional observational studies of milk product-adenoma associations have been conducted, with only one reporting an inverse association.[44] This study of French women from the E3N-EPIC prospective cohort study included 1,933 participants who completed dietary questionnaires between 1993 and 1995 and were diagnosed with a colorectal polyp by December 1997. The RRs for total milk product and milk consumption were 0.80 (95% CI 0.62-1.05; p_{trend}=0.04) and 0.93 (95% CI 0.73-1.19; p_{trend}=0.36), respectively.

Conventional milk products contain substantial amounts of IGF-1 compared to organic,[49] and thus may increase levels of circulating IGF-1, as reported in a recent meta-analysis in which 10 of 13 cross-sectional studies observed a statistically significant positive correlation between

milk consumption and IGF-1. However, only 3 of 12 studies reported statistically significant positive correlations between total milk products and IGF-1.[52] Higher levels of IGF-1 are present in conventional milk products due to bovine somatotropin hormone use, which upregulates the insulin-like growth factor system to increase milk production, and subsequently increases cell proliferation, decreases differentiation, and inhibits apoptosis.[51,58] Positive associations were reported between IGF-1 and risk for colorectal, breast, prostate, and liver cancers,[61-63,65-67,115,116] and a pooled analysis of 16 prospective nested case-control and 3 case-control studies reported a statistically significant 25% higher risk for colorectal cancer among those in the highest categories of circulating IGF-1.[117]

Although milk products and IGF-1 may be independently associated with colorectal cancer risk, to our knowledge, only one study included milk intake in their analyses of IGF-1 and colorectal cancer. In this nested case-control study of 596 colorectal cancer cases, IGF-1 was positively but not statistically significantly associated with colorectal cancer upon comparison of the highest to lowest IGF-1 quintiles (OR 1.11, 95% CI 0.83-1.48).[76] However, within the lowest category of milk intake, there was a statistically significant higher risk for colorectal cancer (OR 1.43, 95% CI 1.10-1.85) per 100 ng/mL increase in IGF-1.

Although we found no evidence of an association between milk products and adenoma, the findings for total milk product residuals, representing the non-calcium component of milk products and an indirect measure of IGF-1, were unexpectedly U-shaped. The potential reasons for this finding are unclear but may be due to chance, especially given the generally low intake of milk products in this study population. However, the use of residuals as the non-calcium/non-fat component of milk products and an indirect measure of IGF-1 is a novel approach that adds to the limited evidence on milk products and IGF-1 and risk for colorectal adenoma.

Because regular use of aspirin and other NSAIDs has been strongly and consistently inversely associated with risk for colorectal neoplasia,[17,118-120] we assessed whether regular use of these medications may have masked associations of calcium and milk products. We observed

stronger inverse associations for total and dietary calcium, total milk products, total milk, and non-fat milk. In our review of previous studies on calcium and risk for colorectal cancer, only 7 stratified by regular aspirin and/or NSAID use. Of those, 4 reported stronger inverse associations between calcium intake and risk for colorectal cancer or adenoma among regular users of aspirin and/or NSAIDs,[121-124] 2 reported stronger inverse associations among those who did not take NSAIDs,[10,105] and 1 reported no difference in association.[125] Taken together, these studies provide no consistent evidence that regular NSAID use so overwhelms any protective effect of calcium that a calcium-colorectal neoplasm association is undetectable among those who regularly take NSAIDs.

Although we found no evidence of confounding or interactions with magnesium or vitamin D, these nutrients were previously independently inversely associated with risk for colorectal neoplasia, similar to calcium. Magnesium is involved in DNA synthesis, cell proliferation and apoptosis, and defenses against oxidative stress which may influence colorectal carcinogenesis.[126] Though studied less extensively than calcium, a meta-analysis of 8 prospective studies reported 11% lower risk for colorectal cancer (RR 0.89, 95% CI 0.79-1.00) for those in the highest to lowest categories of magnesium intake.[127] However, magnesium may be antagonistic to calcium and compete for the same binding sites on plasma proteins,[128] although clinical trials have reported conflicting evidence.[129,130] Several observational studies analyzed the calcium to magnesium ratio and calcium intakes stratified by levels of magnesium to account for this potential interaction, but the findings were inconsistent.[108,131,132] Vitamin D has been more consistently inversely associated with risk for colorectal neoplasia with proposed mechanisms involving detoxification of secondary bile acids, direct effects on the cell cycle, growth factor signaling, and immunomodulation.[133-136] A recent meta-analysis reported inverse associations between circulating 25(OH)D and dietary vitamin D intake with incident and recurrent colorectal adenomas.[137] However, there is limited evidence on a possible combined effect of vitamin D and calcium on risk for colorectal neoplasia. Two large cohort studies reported positive interactions

between calcium and vitamin D,[138,139] although a separate analysis of the Nurses' Health Study revealed no significant interaction.[123] In a large randomized trial, calcium supplementation reduced colorectal adenoma recurrence (RR 0.71, 95% CI 0.57-0.89) only among those with baseline 25(OH)D levels above the median.[140] Further evidence from studies of biomarkers of risk for colorectal cancer supports that calcium and vitamin D, separately and in combination, reduces cell proliferation, induces differentiation, and promotes apoptosis.[100,141] Last, phosphorus has been studied in context with calcium because of its hypothesized antagonistic effects in reducing intestinal absorption of calcium,[142] and similar to our findings, there was no evidence of a calcium-phosphorus interaction in relation to colorectal neoplasms.[108,143,144]

Our study had several limitations and strengths. First, there was a limited number of participants who consumed supplemental calcium or whole milk. Second, we assumed that participants consumed conventional rather than organic milk products, as issue that may not have been as relevant to the CPRU study, which was conducted prior to the approval of bovine somatotropin use. Nevertheless, it may be important to include this distinction on future dietary questionnaires. Additional limitations of this study include the general limitations of case-control studies (e.g., inability to assess temporality) and food frequency questionnaires (e.g., measurement error). Finally, most study participants were white and more likely to be recommended for routine colonoscopy, thus limiting the generalizability of our findings. One of the main strengths of this study was the use of milk product residuals to examine possible effects of the non-calcium/non-fat component of milk. Although the results did not support our hypothesis, to our knowledge, the use of residuals as an indirect measure of IGF in milk products has not been previously utilized. Additional strengths of this study include the comprehensive analysis of total, dietary, and supplemental calcium intake as well as total and specific milk product consumption.

Overall, in this pooled case-control study, our findings of a modestly lower risk for colorectal adenoma with higher intakes of calcium are consistent with those of previous studies.

We introduced a novel method of analyzing the non-calcium and non-fat component of milk products as an indirect measure of IGF-1 intake, and contrary to our hypotheses, found a statistically significant U-shaped association of this indirect IGF-1 measure with adenomas. This latter finding, which may have been due to chance, should be assessed in future studies.

TABLES

Table 2.1. Selected characteristics of participants in pooled case-control study of incident, sporadic colorectal adenomas

	Cases (n = 787)	**Controls** (n =2,033)	***P* value**
Demographics			
Age (years)	58.1 (9.2)	54.5 (10.9)	<0.0001
Male, %	61.3	43.0	<0.0001
White, %	94.7	96.3	0.05
Family history, %[b]	16.9	17.7	0.62
Lifestyle Factors			
College education or higher, %	28.5	31.8	0.10
Current smoker, %	24.2	14.1	<0.0001
Current drinker, 1-6 drinks/week, %	22.8	20.9	<0.0001
Body mass index (kg/m^2)	27.5 (5.2)	26.8 (5.0)	0.001
Waist-to-hip ratio	0.9 (0.2)	0.9 (0.2)	<0.0001
Physical activity (MET-hrs/week)	37.4 (39.4)	35.9 (34.9)	0.42
Take NSAID/aspirin, %[c]	35.4	41.3	0.004
HRT use (% females)	35.5	37.4	0.56
Dietary Factors			
Total energy (kcal/day)	2,069 (767)	1,985 (713)	0.01
Total fat (% total kcals)	46.4 (29.0)	35.7 (19.9)	<0.0001
Dietary fiber (g/day)	21.8 (9.4)	21.9 (10.0)	0.71
Total calcium (mg/day)	913.2 (509.5)	971.4 (523.4)	0.01
Dietary calcium	808.3 (431.3)	821.5 (433.2)	0.47
Supplemental calcium	94.8 (272.8)	129.9 (314.6)	0.003
Total magnesium (mg/day)	322.3 (120.4)	324.2 (125.2)	0.70
Total milk product (servings/day)	2.5 (1.8)	2.4 (1.8)	0.89
High fat dairy	1.4 (1.5)	1.3 (1.4)	0.08
Low fat dairy	1.1 (1.2)	1.2 (1.2)	0.05
Cheese	0.6 (0.7)	0.6 (0.6)	0.82
Fermented dairy	0.7 (0.8)	0.8 (0.7)	0.06
Total milk	0.9 (1.1)	1.0 (1.1)	0.37
Whole milk	0.1 (0.3)	0.1 (0.3)	0.27
Non-fat milk	0.9 (1.0)	0.9 (1.1)	0.22
Total fruit (servings/day)	2.3 (1.8)	2.6 (1.9)	0.0002
Total vegetable (servings/day)	3.7 (2.3)	3.7 (2.4)	0.62
Total meat (servings/day)	1.9 (1.3)	1.7 (1.1)	0.0001
Red meat	0.7 (0.5)	0.6 (0.5)	0.01
Processed meat	0.4 (0.5)	0.3 (0.4)	<0.0001

Abbreviations: MET, metabolic equivalents of task; NSAID, nonsteroidal anti-inflammatory drug; HRT, hormone replacement therapy.

[a] Values presented are mean (standard deviation) unless otherwise specified.

[b] Family history of colorectal cancer in a first-degree relative.

[c] Regularly take aspirin or NSAID ≥ once per week.

Table 2.2. Multivariable-adjusted associations of calcium intake with incident, sporadic colorectal adenomas

Quintiles	No. of Cases/ Controls (n=787/ 2,033)	Initial Model[a]		Full Model[b]		Among Non-Regular Users of Aspirin and NSAIDs No. of Cases/ Controls (n=508/ 1,187)	Full Model[c]	
		OR	95% CI	OR	95% CI		OR	95% CI
Total Calcium								
1	164/405	1.00 (ref)		1.00 (ref)		122/249	1.00 (ref)	
2	164/407	0.96	0.74, 1.26	0.98	0.74, 1.29	104/238	0.88	0.63, 1.24
3	167/409	1.00	0.77, 1.30	1.00	0.75, 1.33	106/234	0.93	0.65, 1.32
4	132/406	0.76	0.58, 1.01	0.75	0.55, 1.02	78/244	0.64	0.44, 0.93
5	160/406	0.94	0.72, 1.23	0.94	0.69, 1.30	98/222	0.91	0.61, 1.35
P_{trend}[d]		0.27		0.43			0.38	
Dietary Calcium								
1	167/404	1.00 (ref)		1.00 (ref)		124/243	1.00 (ref)	
2	152/408	0.89	0.68, 1.16	0.90	0.68, 1.19	91/228	0.75	0.53, 1.07
3	161/409	0.94	0.72, 1.23	0.92	0.69, 1.23	105/242	0.82	0.58, 1.17
4	148/407	0.90	0.68, 1.18	0.87	0.64, 1.17	89/234	0.75	0.51, 1.09
5	159/405	0.95	0.73, 1.25	0.86	0.62, 1.20	99/240	0.77	0.51, 1.17
P_{trend}[d]		0.88		0.62			0.35	
Supplemental Calcium[e]								
1	580/1,419	1.00 (ref)		1.00 (ref)		387/888	1.00 (ref)	
2	94/286	0.82	0.63, 1.06	0.86	0.65, 1.12	57/137	0.97	0.69, 1.38
3	113/328	0.86	0.67, 1.10	0.99	0.77, 1.27	64/162	1.02	0.73, 1.42
P_{trend}[d]		0.15		0.79			0.97	

Abbreviations: NSAIDs, non-steroidal anti-inflammatory drugs; OR, odds ratio; 95% CI, 95% confidence interval.

[a] Adjusted for study, age, and sex.

[b] Adjusted for study, age, sex, oxidative balance score, family history of colorectal cancer in first-degree relative, regular use of aspirin or nonsteroidal anti-inflammatory drugs, total energy intake, total fat intake (energy-adjusted).

[c] Among non-regular users of aspirin and NSAIDs, adjusted for study, age, sex, oxidative balance score, family history of colorectal cancer in first-degree relative, total energy intake, total fat intake (energy-adjusted).

[d] P_{trend} calculated using sex-specific median of each quintile (for total and dietary calcium) or tertile (for supplemental) of calcium intake as a continuous variable.

[e] Supplemental calcium intake categorized as three groups (none and according to the median dose among those who did take supplemental calcium) due to small sample size.

Table 2.3. Multivariable-adjusted associations of milk product consumption with incident, sporadic colorectal adenomas

Quintiles	No. of Cases/ Controls (n=787/ 2,033)	Initial Model[a]		Full Model[b,c]		Among Non-Regular Users of Aspirin and NSAIDs No. of Cases/ Controls (n=508/ 1,187)	Full Model[d,e]	
		OR	95% CI	OR	95% CI		OR	95% CI
Total Milk Products								
1	148/392	1.00 (ref)		1.00 (ref)		109/228	1.00 (ref)	
2	173/420	1.06	0.81, 1.38	1.02	0.78, 1.35	114/256	0.88	0.63, 1.22
3	145/393	1.04	0.79, 1.38	1.01	0.76, 1.35	93/213	0.93	0.65, 1.33
4	153/414	0.97	0.74, 1.28	0.94	0.71, 1.25	89/244	0.74	0.52, 1.06
5	168/414	1.11	0.85, 1.46	0.99	0.74, 1.34	103/246	0.84	0.58, 1.22
P_{trend}[f]		0.63		0.84			0.27	
Total Milk								
1	140/366	1.00 (ref)		1.00 (ref)		97/217	1.00 (ref)	
2	84/258	1.01	0.73, 1.40	1.00	0.71, 1.39	48/162	0.73	0.48, 1.11
3	238/596	1.07	0.82, 1.38	1.06	0.82, 1.38	162/343	1.02	0.74, 1.40
4	152/329	1.07	0.79, 1.44	1.06	0.78, 1.45	87/163	1.12	0.76, 1.66
5	173/484	0.95	0.73, 1.25	0.90	0.68, 1.19	114/302	0.85	0.60, 1.20
P_{trend}[f]		0.47		0.32			0.64	
Whole Milk[g]								
1	661/1,818	1.00 (ref)		1.00 (ref)		434/1,050	1.00 (ref)	
2	126/215	1.26	0.96, 1.62	1.15	0.89, 1.49	74/137	1.01	0.73, 1.40
Non-fat Milk								
1	183/417	1.00 (ref)		1.00 (ref)		125/247	1.00 (ref)	
2	81/220	1.03	0.75, 1.43	1.07	0.77, 1.48	51/141	0.88	0.59, 1.32
3	117/337	1.08	0.81, 1.45	1.14	0.85, 1.53	75/194	0.99	0.69, 1.43
4	205/558	0.95	0.74, 1.21	0.99	0.77, 1.28	136/301	1.02	0.75, 1.41
5	201/501	0.92	0.72, 1.18	0.92	0.70, 1.19	121/304	0.83	0.60, 1.15
P_{trend}[f]		0.48		0.33			0.31	
Total Milk Product Residuals								
1	177/402	1.00 (ref)		1.00 (ref)		111/227	1.00 (ref)	
2	167/408	0.92	0.71, 1.20	0.84	0.63, 1.11	120/238	0.83	0.58, 1.17
3	126/408	0.70	0.53, 0.92	0.61	0.45, 0.82	89/242	0.55	0.37, 0.80
4	130/409	0.74	0.56, 0.98	0.67	0.50, 0.90	81/234	0.58	0.40, 0.85
5	187/406	1.09	0.84, 1.42	0.94	0.71, 1.24	107/246	0.71	0.50, 1.02
P_{trend}[f]		0.40		0.98			0.10	
Non-fat Milk Residuals								
1	154/401	1.00 (ref)		1.00 (ref)		88/235	1.00 (ref)	
2	141/408	0.83	0.63, 1.10	0.86	0.64, 1.15	86/226	0.87	0.60, 1.27
3	177/408	1.09	0.84, 1.43	1.15	0.86, 1.55	123/232	1.42	0.98, 2.06
4	154/408	0.93	0.71, 1.22	0.96	0.70, 1.31	105/256	1.02	0.69, 1.50
5	161/408	0.92	0.70, 1.21	0.95	0.69, 1.31	106/238	1.06	0.71, 1.59
P_{trend}[f]		0.69		0.69			0.76	

Abbreviations: NSAIDs, non-steroidal anti-inflammatory drugs; OR, odds ratio; 95% CI, 95% confidence interval.

[a] Adjusted for study, age, and sex.

[b] For Total Milk Products, Total Milk, Whole Milk, and Non-fat Milk, adjusted for study, age, sex, oxidative balance score, family history of colorectal cancer in first-degree relative, regular use of aspirin or nonsteroidal anti-inflammatory drugs, total energy intake, total fat intake (energy-adjusted), supplemental calcium intake.

[c] For Total Milk Product Residuals and Non-fat Milk Residuals, adjusted for study, age, sex, oxidative balance score, family history of colorectal cancer in first-degree relative, regular use of aspirin or nonsteroidal anti-inflammatory drugs, total energy intake, total fat intake (energy-adjusted), dietary calcium intake.

[d] For non-regular users of aspirin and NSAIDs in Total Milk Products, Total Milk, Whole Milk, and Non-fat Milk models, adjusted for study, age, sex, oxidative balance score, family history of colorectal cancer in first-degree relative, total energy intake, total fat intake (energy-adjusted), supplemental calcium intake.

[e] For non-regular users of aspirin and NSAIDs in Total Milk Product Residuals and Non-fat Milk Residuals models, adjusted for study, age, sex, oxidative balance score, family history of colorectal cancer in first-degree relative, total energy intake, total fat intake (energy-adjusted), dietary calcium intake.

[f] P_{trend} calculated using sex-specific median of each quintile of calcium intake as a continuous variable.

[g] Whole milk consumption categorized as dichotomous due to small sample size.

Table 2.4. Multivariable-adjusted associations of total calcium with incident, sporadic colorectal adenomas by characteristics of the largest adenoma

		Full Model[a]					
	Quintiles	No. of Cases/ Controls	OR	95% CI	No. of Cases/ Controls	OR	95% CI
Atypia		**Mild**			**More Severe**		
	1	86/406	1.00 (ref)		90/406	1.00 (ref)	
	2	96/407	1.11	0.79, 1.57	83/407	0.94	0.66, 1.33
	3	88/407	1.05	0.72, 1.51	57/407	0.64	0.43, 0.95
	4	63/407	0.75	0.51, 1.12	65/407	0.70	0.47, 1.04
	5	83/406	1.05	0.70, 1.57	62/406	0.68	0.44, 1.05
Location		**Proximal**[b]			**Distal**[c]		
	1	22/406	1.00 (ref)		154/406	1.00 (ref)	
	2	34/407	1.54	0.86, 2.76	143/407	0.93	0.70, 1.24
	3	25/407	1.16	0.61, 2.21	120/407	0.58	0.58, 1.07
	4	23/407	1.02	0.53, 1.99	104/407	0.67	0.49, 0.93
	5	21/406	1.07	0.52, 2.21	123/406	0.81	0.58, 1.13
Shape		**Pedunculated**			**Sessile**		
	1	55/406	1.00 (ref)		94/406	1.00 (ref)	
	2	43/407	0.75	0.48, 1.18	107/407	1.12	0.80, 1.57
	3	38/407	0.66	0.41, 1.08	84/407	0.91	0.63, 1.31
	4	32/407	0.55	0.33, 0.92	70/407	0.77	0.52, 1.12
	5	28/406	0.47	0.27, 0.84	88/406	1.01	0.68, 1.49
Size		**< 1 cm**			**≥ 1 cm**		
	1	116/406	1.00 (ref)		65/406	1.00 (ref)	
	2	123/407	1.11	0.81, 1.51	59/407	0.88	0.59, 1.32
	3	107/407	0.97	0.70, 1.36	42/407	0.65	0.41, 1.02
	4	84/407	0.77	0.54, 1.09	45/407	0.68	0.43, 1.07
	5	108/406	1.05	0.73, 1.51	38/406	0.57	0.34, 0.95
Subtype		**Tubular**			**Villous/Tubulovillous**		
	1	129/406	1.00 (ref)		46/406	1.00 (ref)	
	2	135/407	1.07	0.79, 1.45	43/407	0.91	0.57, 1.45
	3	103/407	0.84	0.61, 1.17	41/407	0.84	0.51, 1.37
	4	88/407	0.73	0.52, 1.03	40/407	0.79	0.48, 1.30
	5	113/406	0.97	0.68, 1.39	32/406	0.61	0.34, 1.08

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

[a] Adjusted for study, age, sex, oxidative balance score, family history of colorectal cancer in first-degree relative, regular use of aspirin or nonsteroidal anti-inflammatory drugs, total energy intake, total fat intake (energy-adjusted).

[b] Cecum, ascending colon, hepatic flexure

[c] Transverse colon, splenic flexure, descending colon, sigmoid colon, rectum

CHAPTER 3. Circulating insulin-like growth factor-1 and insulin-like growth factor binding protein-3: Correlates and responses to calcium supplementation in colorectal adenoma patients

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ABSTRACT

Previous studies suggested a direct association of circulating insulin-like growth factor 1 (IGF-1) concentrations with colorectal cancer risk, and insulin-like growth factor binding protein-3 (IGFBP-3) is one of the most abundantly expressed binding proteins in various cancers. Calcium intakes, primarily from food, have been directly associated with circulating IGF-1 concentrations. Whether supplemental calcium affects circulating IGF-1 and IGFBP-3 concentrations is unknown. Therefore, we tested the effects of 1.0 and 2.0 g of supplemental elemental calcium (as calcium carbonate) daily over four months on IGF-1 and IGFBP-3 concentrations in colorectal adenoma patients in a randomized, double-blinded, placebo-controlled trial (n = 193). We also assessed cross-sectional associations of the growth factor biomarkers with participants' baseline characteristics.

We found no appreciable effect of calcium relative to placebo on circulating concentrations of IGF-1, IGFBP-3, or the IGF-1:IGFBP-3 molar ratio. However, mean IGF-1 concentrations were 11.1% higher in those with greater milk intakes ($p = 0.05$), and mean IGF-1 and IGFBP-3 concentrations were, respectively, 18.0% ($p = 0.003$) and 16.5% ($p = 0.01$) higher in men and were monotonically lower with increasing age (both $p = 0.01$). IGFBP-3 concentrations were 17.7% higher among those with higher relative to no alcohol consumption ($p = 0.04$), but 13.0% lower among those in the upper relative to the lowest tertile of total milk product residuals ($p = 0.04$).

While these results support previous findings that IGF-1 concentrations are higher with greater milk intakes, and IGF-1 and IGFBP-3 concentrations differ according to sex and age, they provide no evidence to suggest that supplemental calcium has an appreciable effect on circulating levels of IGF-1, IGFBP-3, or the IGF-1:IGFBP-3 molar ratio in sporadic colorectal adenoma patients.

INTRODUCTION

Circulating insulin-like growth factor 1 (IGF-1) concentrations were directly associated with risk for colorectal, breast, and prostate cancers in previous observational studies.[59,60,62,145] IGF-1 is an essential growth factor, critical for normal growth and maintenance of homeostasis, but may potentially act as a potent mitogen, anti-apoptotic peptide, and promoter of angiogenesis when overexpressed.[146] Circulating IGF-1 is predominantly bound to insulin-like growth factor binding protein 3 (IGFBP-3) to increase stability and regulate distribution to tissues.[46] There is no consistent evidence that IGFBP-3 levels are associated with risk of colorectal cancer, but levels may be an indicator of bioavailable IGF-1.

Colorectal cancer is the second leading cause of cancer deaths among men and women combined in the United States.[4] Calcium has been consistently associated with lower risk of colorectal neoplasms,[6,38,110,111] and supplemental calcium reduced sporadic colorectal adenoma recurrence in two of three large randomized controlled trials.[10,147,148] The hypothesized mechanisms of action are through the binding of secondary bile acids and free fatty acids in the colon, which reduces epithelial cell exposure to their damaging effects,[29,32] and by directly inhibiting proliferation and inducing differentiation of colonic epithelial cells.[35,36]

Milk products are a common source of dietary calcium in the average American diet, and tend to be inversely associated with risk of colorectal cancer, similar to calcium.[41,110] However, clinical trials suggest that milk product consumption may increase circulating IGF-1 levels, and cross-sectional studies reported positive associations of milk consumption with IGF-1 concentrations.[52,57] This may be attributable to higher levels of IGF-1 measured in milk produced from cows administered bovine somatotropin (bST) hormone, in comparison to bST-free or organically produced milks.[49] However, calcium may also be obtained from supplemental sources, and to our knowledge, whether supplemental calcium affects circulating IGF-1 and insulin-like growth factor binding protein-3 (IGFBP-3) concentrations is unclear and has only been tested in clinical trials of postmenopausal women and teenage girls in association with bone

health. Thus, we tested the effects of 1.0 and 2.0 g of supplemental elemental calcium on IGF-1 and IGFBP-3 concentrations in colorectal adenoma patients in a randomized, double-blinded, placebo-controlled trial.

MATERIALS AND METHODS

Participant Population

For this investigation, we used data and stored blood samples from a randomized, double-blinded, placebo-controlled, three-arm, parallel-group clinical trial of calcium supplementation conducted in Minneapolis-St. Paul, Minnesota from 1990 - 1993. The recruitment methods and study protocol of this trial were previously described.[149,150] Briefly, patients 30-74 years of age with a history of a pathology confirmed colorectal adenomatous polyp within the previous 5 years were recruited from a large, private gastroenterology practice. Eligible participants were in good health and must have consumed a western-style diet that included meat and no more than 1,100 mg of calcium daily. Additional exclusion criteria included current use of calcium supplements; contraindications to calcium supplementation or rectal biopsies; clinical conditions, dietary habits, or medication that would otherwise affect safety, adherence, or interpretation of the study results; or less than 80% adherence to a study pill-taking in a 4-week placebo run-in trial.

Clinical Trial Protocol

As previously described,[149] of the patients who were invited for an eligibility visit and subsequently completed the placebo run-trial, 193 were randomized. Participants underwent a baseline visit and were randomly assigned to one of three treatments groups: a placebo control group (n = 66) and 1.0 g (n = 64) and 2.0 g (n = 63) elemental calcium supplementation groups. Randomized group assignment was blinded to all participants, study personnel, and laboratory staff. The calcium carbonate and placebo tablets (prepared by SmithKline Beecham, Pittsburgh,

PA) were administered twice daily with meals, and all pills were identical in size, appearance, and taste.

Medical history, medication use, and information on demographic, dietary, and lifestyle factors were collected at the eligibility and baseline visits via self-administered questionnaires. Usual dietary intakes over the previous 12 months were collected using a 153-item Willett semi-quantitative food-frequency questionnaire (FFQ). The treatment period was 6 months with follow-up visits at 1, 2, 4, and 6 months. Participants were instructed to follow their usual diets, and pill-taking adherence was assessed at each follow-up visit by interview, questionnaire, and pill count. Non-fasting venous blood samples were collected only at the baseline and 4-month follow-up visits. Participants were comfortably seated in a chair for 5 minutes with both feet on the floor before venipuncture. Blood was drawn in to pre-chilled Vacutainer tubes and immediately placed on ice and shielded from light. The blood was immediately processed, plasma and serum were aliquoted into separate cryopreservation tubes, air was displaced with nitrogen, and the aliquots were immediately placed in a -80^{o}C freezer for storage. During the course of the trial duplicate blood samples were collected on 35 participant visits as blinded quality control samples.

Laboratory Protocol

IGFBP-2 and IGFBP-3 concentrations were measured at Ligandis laboratory (Ligandis GbR, Gülzow, Germany) in April 2014 using quantitative Western Ligand blot to ensure detection of intact IGFBPs. Briefly, serum samples and serial dilutions of recombinant human IGFBP standards (R&D Systems, Wiesbaden, Germany) in artificial serum matrix were diluted 1:20 and boiled in sample buffer [312.5 mM Tris (pH 6.8), 50% (w/v) glycerol, 5 mM EDTA (pH 8), 1% (w/v) SDS and 0.02% bromophenol blue] for 5 minutes. Proteins were separated by 12% SDS-PAGE followed by the transfer onto a polvinylidene fluoride membrane (Millipore, Bedfore, USA). The blots were blocked and then incubated with biotin-labeled human IGF-II

(1:500; BioIGF2-10; ibt-systems, Binzwangen, Germany). The binding proteins were detected by using enhanced chemiluminescence using Luminata™ Forte (Millipore, Bedfore, USA). Bands were visualized on a KODAK Image Station 4000MM (Molecular Imaging Systems, Carestream Health, New Haven USA) and quantified by using Gelanalyzer2010a software. Signal intensities were corrected for background and quantified using human recombinant standards as calibrators on each blot (R&D Systems, Wiesbaden-Nordenstadt, Germany). Curve fitting was performed by using a four parametric nonlinear regression (HILL equation) of each separate IGFBP. The calculation of the IGFBP concentrations in serum was performed using the software GraphPad Prism6 and corrected for dilution and volume/lane of each sample.

IGF-1 was also measured at Ligandis laboratory in October 2015 using the solid-phase sandwich enzyme-linked immunoassay (Mediagnost). Briefly, serum samples and recombinant human standards were added to wells pre-coated with human IGF-1 monoclonal antibody and incubated. Unbound material was removed by washing, and streptavidin-horseradish peroxidase (HRP) conjugated anti-human IGF-1 antibody was added. Wells were washed again to remove unbound streptavidin-HRP, and a substrate solution reactive with HRP was added. After incubation, stop solution was added, and absorbance was measured using an automated ELISA reader.

All samples were measured in duplicate, and technicians were blinded to treatment assignment. The between- and within-plate coefficients of variation (CVs) for IGF-1 were < 6.8% and < 6.7%, respectively, and the CVs for low and high quality control IGFBP-3 samples were 19.2% and 14.9%, respectively. All but two of the 35 blinded duplicate quality control samples were measured in different IGF-1 plates or IGFBP-2 and 3 batches than the samples to which they were paired; for these duplicate pairs, the CVs for IGF-1 and IGFBP-3 were 14.7% and 22.7%, respectively. IGFBP-2 concentrations were below the limit of detection in 39 samples; the CVs for low and high quality control IGFBP-2 samples were 21.4% and 6.7%, respectively; and the CV for the duplicate pairs was 53%. Based on the large number of samples

with values below the lower limit of detection and the large CV for the duplicate pairs, we excluded IGFBP-2 from subsequent data analyses.

The analytical range of IGFBP-3, defined by the highest and lowest concentration of recombinant standard used, was 159 – 10,000 ng/mL. The lower limits of quantification was 962.37 ng for IGFBP-3, and the analytical sensitivity for IGF-1 was 0.09 ng/mL.

Statistical Analysis

Baseline characteristics of the treatment groups were compared using chi-square or Fisher's exact tests for categorical variables and analysis of variance (ANOVA) for continuous variables.

Of the initial 193 participants, blood samples for measurement of growth factors were available for 189 participants at baseline and 175 at follow-up. Primary analysis was based on the original treatment group assignment at randomization, regardless of adherence (intent-to-treat). IGF-1 and IGFBP-3 values were natural logarithm-transformed before statistical testing to improve normality. Mean growth factor parameters were calculated for each treatment group at baseline and 4 months follow-up. The molar ratio of IGF-1 to IGFBP-3 was calculated prior to ln-transformation using the mass to molar conversion of 1 ng/mL IGF-1 = 0.130 nM for IGF-1 and 1 ng/mL IGFBP-3 = 0.036 nM IGFBP-3.

Treatment effects were estimated using a repeated-measures mixed linear model implemented using SAS Institute's Mixed Procedure (PROC MIXED) (SAS version 9.4 software; SAS Institute Inc., Cary, North Carolina). The model included visit, treatment groups, and a treatment group by visit interaction term. Because it was necessary to natural log transform the growth factor values, the visit-specific treatment effects for each growth factor were estimated in the log scale based on geometric means. Thus, the relative treatment effects, defined as ([treatment group follow-up mean] / [treatment group baseline mean]) / ([placebo follow-up mean] / [placebo baseline mean]), were obtained from the Mixed model. We also manually

calculated the absolute treatment effect, defined as the "difference of differences," or ([treatment group follow-up mean] - [treatment group baseline mean]) - ([placebo follow-up mean] - [placebo baseline mean]), using the geometric means for each treatment group.

We also conducted a cross-sectional analysis using generalized linear models to investigate associations of participants' baseline characteristics with IGF-1 and IGFBP-3 concentrations. Of particular interest was dairy product intakes, especially the non-calcium (and non-fat) component of milk, which contains IGF-1 as discussed above. To estimate the latter, we used residuals from linear regression models of total dairy and non-fat milk (as the dependent variables) with dietary calcium intake (as the independent variable). This method was modeled after the energy adjustment residual method.[99] Least squares means and standard errors were obtained from the generalized linear models, adjusting for covariates as appropriate. A two-sided *P* value of < 0.05 was considered statistically significant, and the analyses were performed using SAS version 9.4 software (SAS Institute Inc., Cary, North Carolina).

RESULTS

At baseline, the mean age of the 193 study participants was 59 years, 63% were men, and 99% were white. Participants also consumed an average of 800 mg of dietary calcium and 2.2 servings of dairy products daily. The treatment groups did not differ significantly on demographic, lifestyle, or dietary characteristics (Table 3.1). Adherence to follow-up visit attendance averaged 95.3% and also did not differ among treatment groups. In each group, more than 98% of participants took 80% or more of their pills, and the mean percentage of pills taken was 97%.

Since IGF-1 and IGFBP-3 values were natural logarithm-transformed, geometric mean concentrations at baseline and 4-months follow-up are shown in Table 3.2. The mean concentrations of IGF-1, IGFBP-3, and the IGF-1:IGFBP-3 molar ratio did not differ among treatment groups. There were no appreciable effects of either 1g or 2g calcium supplementation

on any of the growth factor concentrations. We also stratified by age, sex, BMI, NSAID or aspirin use, smoking status, alcohol use, physical activity level, total energy intake, and dietary calcium intake, but the estimated treatment effects were similarly null across the strata (Appendix Table 3.4).

To gain potential insight into whether the null treatment effects were valid or possibly due to the age of the blood samples, we analyzed baseline associations of participant characteristics with IGF-1, IGFBP-3, and the IGF-1:IGFBP-3 molar ratio (Table 3.3). IGF-1 and IGFBP-3 concentrations were 14.9% ($p = 0.02$) and 16.5% ($p = 0.01$) higher in men, respectively, and both were lower with increasing age ($p = 0.02$ and $p = 0.01$, respectively). IGF-1 concentrations were also 11.1% higher among participants in the higher total milk category ($p = 0.05$), but only an estimated 6.1% higher among those in the upper relative to the lowest tertile of non-fat milk residuals ($p = 0.39$). IGFBP-3 concentrations were 17.7% higher among participants consuming 7 or more alcoholic drinks per week compared to those consuming none ($p = 0.04$); however, concentrations were not higher among those with moderate consumption. IGFBP-3 concentrations were also 13.0% lower among those in the upper tertile of total milk product residuals ($p = 0.04$), and IGF-1 was an estimated 7.3% lower among those in the highest relative to the lowest tertile of total milk product residuals ($p = 0.28$).

DISCUSSION

In this analysis of a dose-response trial of calcium supplementation, we found no appreciable effects of either 1 or 2g elemental calcium daily on circulating concentrations of IGF-1, IGFBP-3, or the IGF-1:IGFBP-3 molar ratio in sporadic colorectal adenoma patients over a 4-month treatment period. However, consistent with previous findings, IGF-1 concentrations were higher with greater milk intakes, and IGF-1 and IGFBP-3 concentrations were lower in females and inversely associated with age.

To our knowledge, the effect of calcium supplementation on IGF-1 and IGFBP-3 concentrations among adenoma patients was not previously tested in a clinical trial. Four previously conducted placebo-controlled trials of calcium supplementation and bone health investigated changes in IGF-1 levels, although as secondary outcomes of interest. In two of the trials, both in postmenopausal women, no effect of calcium supplementation on IGF-1 concentrations was found after follow-up periods of 6 months and 2 years.[71,72] In the third trial among 18 Japanese teenage girls over 8 months, only within-treatment group changes in IGF-1 levels were reported.[73] However, by our calculations, there was an estimated approximately 16% increase in IGF-1 levels in the calcium supplementation group relative to the placebo group. Based on the reported p-values, the estimated treatment effect was likely statistically significant since the IGF-1 levels statistically significantly increased in the calcium supplementation group and decreased in the placebo group. However, it is important to note that teenage girls are expected to have higher and fluctuating levels of IGF-1 during this growth and developmental phase.

The fourth trial that investigated IGF-1 levels, an intervention trial of bone health among Greek postmenopausal women, tested the effects of 600 mg supplemental calcium vs. 3 daily servings of low-fat dairy products fortified with calcium and vitamin D_3 (providing approximately 1,200 mg dietary calcium daily) vs. usual diet.[74] After 5 and 12 months, there were no differences in percent changes in IGF-1 among the 3 treatment groups. Although there is no epidemiological evidence of an association of supplemental calcium with circulating IGF-1 or IGFBP-3 concentrations, a cross-sectional study of men and women 20 years of age or older reported a positive association of serum calcium levels with circulating IGF-1 and IGFBP-3 levels.[151] However, there was no association with the IGF-1:IGFBP-3 molar ratio.

There is consistent, though limited, evidence that milk consumption may increase circulating IGF-1 levels. In a clinical trial conducted in adult men and women, there was a statistically significant increase in IGF-1 concentrations among those randomized to 3 servings of

non-fat or low-fat milk daily for 12 weeks relative to those randomized to no milk consumption.[152] Positive associations of milk consumption and IGF-1 concentrations were also reported in observational studies,[55-57,153,154] though associations of milk consumption with IGFBP-3 levels were primarily null. The findings across the few studies that reported an association of milk consumption with the IGF-1:IGFBP-3 molar ratio were inconsistent.[55,154,155]

Our findings of differences in IGF-1 and IGFBP-3 concentrations according to sex and age are consistent with those of some, but not all, observational studies. Our observed inverse association of IGF-1 and IGFBP-3 concentrations with age is consistent with previous studies,[153,154,156-158] and lower concentrations of IGF-1 were consistently reported in women.[154-156] However, the association of IGFBP-3 levels with sex is inconsistent among these studies. Our findings also suggest that IGFBP-3 levels may be positively associated with alcohol consumption. In an 8-week crossover trial among postmenopausal women (n = 53), IGFBP-3 concentrations increased with consumption of one but not 2 alcoholic drinks daily.[159] However, the association is unclear as limited observational studies reported inconsistent associations of IGFBP-3 concentrations with alcohol consumption.[56,160] Our findings also suggest an inverse association of IGFBP-3 concentrations with total milk product residuals, representing the non-calcium component of milk products and an indirect measure of IGF-1. This would suggest higher concentrations of unbound (bioavailable) IGF-1 in the upper tertile of residuals, but IGF-1 concentrations were similarly inversely associated, although not statistically significantly so. However, given that this study was conducted from 1990 to 1993, milk consumed in our study population may not have been produced using bovine somatotropin hormone, which was approved for use by the Food and Drug Administration in 1993[50] and is hypothesized to increase levels of IGF-1 in milk products. Thus, this is a potential reason why total milk product and non-fat milk residuals, were not positively associated with circulating IGF-1 levels.

To our knowledge, this is the first report on the effect of calcium supplementation on circulating levels of IGF-1 and IGFBP-3 among adenoma patients, and a strength of the study

was the randomized, double-blind, controlled, dose-response design. However, there were also several limitations in this study. First, the blood samples collected at the baseline and follow-up visits were non-fasting samples. It is possible that circulating IGF-1 levels may be influenced by dietary sources of IGF-1, which are primarily animal products. Additionally, the time of day at which blood is collected may affect levels of IGF-1 and IGFBP-3.[161] Therefore, in future studies, it may be important to collect blood samples fasting and at a consistent time of day. Additionally, although numerous studies similarly examined the IGF-1:IGFBP-3 molar ratio as an indicator of IGF-1 bioavailability, it is interesting to note that, to our knowledge, this ratio has never been experimentally validated as a reflection of IGF-1 bioavailability. Over 75% of the circulating (endocrine) form of IGF-1 is bound to IGFBP-3 in a 1:1 molar ratio;[46] thus, the IGF-1:IGFBP-3 molar ratio is currently the most widely used method of estimating IGF-1 bioavailability. However, experimental validation of this molar ratio is needed.

Although we found no appreciable effects of calcium supplementation on circulating levels of IGF-1, IGFBP-3, or the IGF-1:IGFBP-3 molar ratio among patients with previous sporadic adenoma, our results support the existing evidence that IGF-1 and IGFBP-3 concentrations are lower in females and inversely associated with age. Additionally, our results suggest a potential direct association of milk consumption with circulating IGF-1 levels. Further investigations are needed to investigate potential modifiable dietary and lifestyle factors that may impact the insulin-like growth factor system, which in turn may modulate risk of colorectal neoplasia.

TABLES

Table 3.1. Selected baseline characteristics of study participants by treatment group[a]

	Treatment Group			
	Placebo (n = 64)	**1 g Calcium (n = 62)**	**2 g Calcium (n = 63)**	***P* value[b]**
Demographics				
Age, years	59.6 (9.1)	60.3 (9.0)	57.9 (10.4)	0.36
Male, %	65.6	61.3	61.9	0.86
White, %	98.4	100.0	100.0	1.00
Family history of colon cancer, %[c]	26.6	24.2	30.2	0.75
Lifestyle Factors				
College education or higher, %	34.4	17.7	33.3	0.07
Current smokers, %	20.3	16.1	23.8	0.56
Alcohol, grams/day	11.1 (19.5)	13.7 (20.4)	7.7 (13.1)	0.19
Body mass index, kg/m^2	28.8 (4.4)	28.6 (5.7)	27.8 (4.6)	0.51
Physical activity, MET-hrs/week[d]	7.6 (12.9)	10.5 (15.4)	6.1 (11.6)	0.18
NSAID or aspirin use, %	26.6	33.9	25.4	0.52
HRT, % females	22.7	33.3	41.7	0.39
Dietary Intakes				
Total energy, kcal/day				
Males	2,312 (795)	2,061 (611)	2,244 (623)	0.26
Females	1,700 (461)	1,852 (619)	1,868 (589)	0.55
Total Fat, g/day	70.6 (29.2)	67.3 (26.5)	76.5 (26.7)	0.18
Dietary fiber, g/day	23.3 (9.4)	21.3 (6.5)	21.5 (8.9)	0.34
Total calcium, mg/day	884.6 (343.6)	785.5 (369.4)	855.2 (416.2)	0.33
Dietary calcium	850.8 (331.1)	736.8 (349.9)	811.0 (349.7)	0.17
Supplemental calcium	33.8 (71.5)	48.7 (119.9)	44.1 (185.2)	0.81
Total vitamin D, IU/day	334.0 (242.5)	297.5 (271.1)	314.3 (206.7)	0.70
Red meat, servings/day	0.6 (0.7)	0.7 (0.8)	0.7 (0.4)	0.89
Processed meat, servings/day	0.4 (0.5)	0.3 (0.4)	0.4 (0.6)	0.54
Total fruit & vegetable, servings/day	5.3 (2.4)	5.2 (2.2)	5.0 (2.9)	0.74
Total dairy, servings/day	2.3 (1.1)	2.2 (1.4)	2.2 (1.5)	0.75
High fat dairy	1.1 (1.0)	1.3 (1.2)	1.1 (1.1)	0.43
Low fat dairy	1.3 (1.0)	0.9 (0.9)	1.1 (1.0)	0.17
Cheese	0.6 (0.5)	0.6 (0.4)	0.7 (0.7)	0.71
Fermented dairy	0.7 (0.6)	0.8 (0.5)	0.8 (0.8)	0.61
Whole milk	1.1 (0.9)	0.8 (0.9)	0.9 (0.9)	0.23
Non-fat milk	0.03 (0.14)	0.09 (0.44)	0.03 (0.13)	0.39

Abbreviations: MET, metabolic equivalents of task; NSAID, non-steroidal anti-inflammatory drug; HRT, hormone replacement therapy.

[a] Values presented are mean (standard deviation) unless otherwise specified.

[b] *P* value calculated from Chi-square or Fisher's exact test for categorical variables and analysis of variance for continuous variables.

[c] Family history of a first-degree relative with colorectal cancer.

[d] Physical activity calculated as MET-hrs per week of moderate and vigorous activity.

Table 3.2. Changes in growth factor concentrations among colorectal adenoma patients in response to calcium supplementation

	Baseline[a]			Follow-up[a]			Relative Treatment Effect[b]			Absolute Treatment Effect[c]
Growth Factor	**n**	**Mean**	**SE**	**n**	**Mean**	**SE**	**Mean**	**SE**	***P***	
IGF-1										
Placebo	64	108.1	1.1	60	104.1	1.1				
1 g calcium	62	109.9	1.0	58	109.9	1.0	1.02	1.05	0.63	3.97
2 g calcium	63	109.1	1.0	57	110.5	1.1	1.03	1.05	0.49	5.34
IGFBP-3										
Placebo	64	3,104.6	1.0	60	3,104.8	1.1				
1 g calcium	62	3,327.9	1.0	58	3,415.0	1.1	1.03	1.05	0.57	86.96
2 g calcium	63	3,133.1	1.1	57	3,184.0	1.1	1.00	1.05	0.93	50.80
IGF-1 : IGFBP-3										
Placebo	64	0.13	1.1	60	0.12	1.1				
1 g calcium	62	0.12	1.1	58	0.12	1.1	1.00	1.07	0.98	0.002
2 g calcium	63	0.13	1.0	57	0.13	1.1	1.03	1.07	0.64	0.01

Abbreviations: SE, standard error; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; IGF-1:IGFBP-3, molar ratio of IGF-1 to IGFBP-3.

[a] Geometric means

[b] Relative treatment effect from SAS Institute's Mixed Procedure (SAS version 9.4; SAS Institute, Cary, NC) defined as [(treatment group follow-up mean) / (treatment group baseline mean)] / [(placebo follow-up mean) / (placebo baseline mean)].

[c] Absolute treatment effect calculated as [(treatment group follow-up mean) - (treatment group baseline mean)] - [(placebo follow-up mean) - (placebo baseline mean)].

Table 3.3. Mean baseline concentrations of growth factors by demographic, lifestyle, and dietary factors[a]

			IGF-1			IGFBP-3			IGF-1:IGFBP-3		
		N	Mean	SE	*P*[b]	Mean	SE	*P*[b]	Mean	SE	*P*[b]
Sex	Male	117	115.9	1.0		3,371.6	1.0		0.12	1.04	
	Female	69	98.2	1.0	0.003	2,893.2	1.0	0.01	0.12	1.05	0.85
Age, years	< 55	60	117.5	1.0		3,540.6	1.0		0.12	1.06	
	55 - 64	70	103.7	1.0		2,930.9	1.0		0.13	1.05	
	≥ 65	56	99.3	1.0	0.01	2,946.0	1.0	0.01	0.12	1.06	0.83
BMI, kg/m²	< 25	49	114.0	1.1		2,944.7	1.1		0.14	1.06	
	25 - 29.99	73	105.8	1.0		3,275.9	1.0		0.12	1.05	
	> 30	64	101.9	1.0	0.11	3,117.6	1.0	0.53	0.12	1.05	0.06
NSAID or aspirin use	Yes	54	101.3	1.1		2,930.7	1.1		0.12	1.06	
	No	132	109.0	1.0	0.21	3,207.0	1.0	0.13	0.12	1.04	0.81
Alcohol	None	54	103.6	1.0		3,048.5	1.0		0.12	1.06	
	1-6 drinks/wk	87	105.5	1.0		2,986.4	1.0		0.13	1.05	
	7+ drinks/wk	45	114.3	1.1	0.20	3,586.9	1.1	0.04	0.12	1.07	0.52
Total energy, kcal/day	Low	92	104.0	1.0		3,032.8	1.0		0.12	1.05	
	High	94	109.2	1.0	0.36	3,214.7	1.0	0.28	0.12	1.05	0.88
Total fat	Low	92	111.1	1.0		3,216.6	1.0		0.12	1.05	
	High	94	103.2	1.0	0.27	3,060.6	1.0	0.47	0.12	1.05	0.76
Red or processed meat	Low	91	111.1	1.0		3,124.3	1.0		0.13	1.05	
	High	95	103.0	1.0	0.17	3,140.7	1.0	0.93	0.12	1.05	0.21
Total calcium	Low	92	105.9	1.0		3,256.0	1.0		0.12	1.05	
	High	94	107.7	1.0	0.78	3,024.3	1.0	0.21	0.13	1.05	0.19
Dietary calcium	Low	92	105.6	1.0		3,239.5	1.0		0.12	1.05	
	High	94	107.9	1.0	0.71	3,038.9	1.0	0.29	0.13	1.05	0.22
Total milk products	Low	92	107.3	1.0		3,179.5	1.0		0.12	1.05	
	High	94	106.3	1.0	0.87	3,091.7	1.0	0.63	0.12	1.05	0.78
Total milk	Low	100	100.6	1.0		3,077.2	1.0		0.12	1.05	
	High	86	111.8	1.0	0.05	3,178.3	1.0	0.56	0.13	1.04	0.25
OBS	Low	93	104.5	1.0		2,997.0	1.0		0.13	1.05	
	High	93	108.9	1.0	0.44	3,253.8	1.0	0.12	0.12	1.05	0.51
Milk product residuals, tertiles	1	62	109.1	1.0		3,302.5	1.0		0.12	1.06	
	2	62	109.7	1.0		3,239.7	1.0		0.12	1.06	
	3	62	101.7	1.0	0.28	2,874.5	1.0	0.04	0.13	1.06	0.37
Non-fat milk residuals, tertiles	1	62	98.8	1.1		3,092.1	1.1		0.12	1.06	
	2	62	118.1	1.0		3,396.9	1.0		0.13	1.06	
	3	62	104.8	1.1	0.39	2,923.5	1.1	0.55	0.13	1.06	0.21

Abbreviations: IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; IGF-1 : IGFBP-3, molar ratio of IGF-1 to IGFBP-3; SE, standard error; NSAID, non-steroidal anti-inflammatory drug; OBS, oxidative balance score.

[a] Models for all variables except sex were adjusted for sex (M/F). Models for all variables except age were adjusted for age (continuous). Models for all variables except BMI, obesity, and OBS were adjusted for BMI (continuous). Models for dietary variables also adjusted for total energy intake (continuous). Models for Total Dairy, Total milk, whole, and skim milks also adjusted for supplemental calcium (continuous). Models for Total Dairy Residuals and Skim Residuals also adjusted for dietary calcium (continuous).

[b] *P* values from analysis of variance.

CHAPTER 4. Associations of calcium and dairy products with all-cause and cause-specific mortality: REasons for Geographic and Racial Differences in Stroke (REGARDS) Prospective Cohort Study

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ABSTRACT

Associations of calcium and dairy product intakes with risk for cardiovascular disease and cancer mortality are controversial. We analyzed data from the REasons for Geographic and Racial Differences in Stroke (REGARDS) study, a prospective cohort study of 30,239 black and white American adults enrolled from 2003 to 2007, to investigate associations of calcium and dairy product intakes (including the non-calcium/non-fat, insulin-like growth factor-1 (IGF-1)-containing component of dairy products, assessed as the residuals from linear regression models of non-fat milk with dietary calcium), with risk of all-cause, cardiovascular disease (CVD), and cancer mortality.

For those in the upper fifth relative to the lowest quantile of intakes, notable adjusted hazards ratios (HR) (95% confidence intervals [CI]) for all-cause mortality were 1.13 (CI 0.95-1.35; P_{trend} 0.004) for whole milk, 0.75 (CI 0.61-0.93; P_{trend} 0.001) for non-fat milk, and 0.81 (CI 0.66-1.00; P_{trend} 0.0001) for non-fat milk residuals; for CVD mortality the corresponding HRs were 0.80 (CI 0.55-1.16; P_{trend} 0.80), 0.72 (CI 0.49-1.05; P_{trend} 0.06), and 0.71 (CI 0.49-1.03; P_{trend} 0.09); and for cancer mortality they were 1.56 (CI 1.17-2.08; P_{trend} 0.006), 0.89 (CI 0.62-1.28; P_{trend} 0.86), and 1.11 (CI 0.79-1.55; P_{trend} 0.99). Total, dietary, and supplemental calcium were not associated with all-cause, cardiovascular, or cancer mortality.

These results suggest that milk fat may be directly associated with all-cause mortality, the non-calcium/non-fat, IGF-1-containing component of milk may be directly associated with cancer mortality, and calcium intake independent of milk product intake may not be associated with mortality.

INTRODUCTION

Calcium is an essential nutrient required for many processes, including bone health, cardiac and vascular smooth muscle function, nerve transmission, and enzyme secretion. Calcium concentrations are tightly maintained in the body and sources are both dietary and supplemental. Dietary Reference Intakes (DRI) in adults over age 50 is 1,000 mg for males and 1,200 mg for females until the age of 70, at which point the overall recommendation for males and females is 1,200 mg.[77] Dairy products are one of the leading sources of dietary calcium in the average American diet, and the recently released 2015 Dietary Guidelines recommends consumption of 3 cups of milk products a day for adults.[162]

Recent reviews and meta-analyses reported inverse associations between calcium and dairy products with risk for colorectal cancer and metabolic syndrome,[6,8,163] which suggests that higher intakes, within reasonable limits, may reduce the risk of cardiovascular disease and cancer mortality. Heart disease and cancer are the two leading causes of mortality in the United States and worldwide,[96,97] and calcium supplements are commonly recommended, especially among women and the elderly. However, recent reports have raised concern of a potential increase in risk for myocardial infarction and related deaths among older postmenopausal women taking calcium supplements.[89,90] Observational studies also reported higher risk of prostate cancer associated with intakes of dietary and dairy calcium and dairy products but not with supplemental or non-dairy calcium, suggesting a potential association with insulin-like growth factor 1 (IGF-1), which was positively associated with intake of milk and dairy products.[164]

In general, evidence from numerous clinical trials and observational studies on calcium and dairy products with risk for cardiovascular disease mortality are conflicting, and very few studies reported associations with risk for cancer mortality. Thus, the purpose of this analysis was to investigate associations of total, dietary, and supplemental calcium and milk products (including the non-calcium/non-fat, insulin-like growth factor-1 (IGF-1)-containing component of dairy products) with risk of all-cause, cardiovascular disease-specific, and cancer-specific

mortality in the prospective REasons for Geographic and Racial Differences in Stroke (REGARDS) study.

MATERIALS AND METHODS

Study Population

The REGARDS study is a national prospective cohort study designed to recruit and follow equal numbers of black and white men and women. Details on the study population and objectives were described previously.[101,165] Briefly, between January 2003 and October 2007, participants aged 45 years and older were recruited from the continental United States with an oversampling of blacks and persons residing in the "Stroke Belt," a region of high stroke mortality defined as encompassing Alabama, Arkansas, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, and Tennessee. Potential participants were first contacted via mail and then telephone by trained interviewers to determine eligibility. Exclusion criteria included race other than black or white, active treatment for cancer, medical conditions that would prevent long-term participation, cognitive impairment judged by the telephone interviewer, residence in or inclusion on a waiting list for a nursing home, or inability to communicate in English. The study was approved by the Institutional Review Boards at all participating institutions. Verbal and written informed consent were obtained from all participants.

Computer-assisted telephone interviews, in-home visits, and self-administered questionnaires left with participants to complete after the in-home visit were used to collect data. Medical history, including risk factors, and demographics were collected via telephone interview while blood and urine samples, anthropometrics, and additional risk factor data were collected during in-home visits. Self-administered questionnaires included the Block 98 Food Frequency Questionnaire (FFQ) and a family history questionnaire.

Dietary Assessment

Dietary intake was assessed using a self-administered Block FFQ (www.Nutritionquest.com), which contains over 150 multiple-choice questions based on 107 food items and was validated for most nutrients and in different populations.[166,167] Participants were provided with pictures to assist in identifying portion sizes. Relevant questions that were used to create variables of interest for this analysis included those to assess intakes of calcium supplements and multivitamins, dairy products (milk, cream, fermented dairy products, ice cream, butter, cheeses), and other non-dairy, calcium-containing foods (e.g., fortified orange juice).

Outcome Assessment

Participants and their proxies were contacted via telephone at 6-month intervals to ascertain hospitalizations and deaths, for which medical records were also obtained. For reported deaths, interviews with next of kin or other proxy were conducted to collect information relevant to the death. Deaths were confirmed through death certificates and the National Death Index. Two adjudicators independently reviewed reported deaths, using baseline participant clinical characteristics, proxy interviews, death certificates, and if available, medical records for hospitalizations occurring within 30 days of the death to determine the cause of the participant's death. The Social Security and National Death Indices were searched for participants who were reported as lost to follow-up. For our analyses, all-cause and cause-specific (cardiovascular and cancer) mortalities were the primary outcomes. Cardiovascular disease (CVD) mortality included deaths from coronary heart disease, myocardial infarction, stroke, heart failure, other cardiac, and other cardiovascular causes. Deaths from specific types of cancers were not collected.

Statistical Analyses

Since dietary data obtained from food frequency questionnaires are generally analyzed as ranked values,[166] sex-specific quintiles of calcium and dairy product intakes were used in this

analysis, with the lowest categories used as reference. For supplemental calcium and whole, low-fat, and non-fat milks, the reference category was no consumption and quartiles of consumption were used as the remaining 4 categories. Low-fat milk consumption included 1% and 2% milks. The characteristics of the study population at baseline were summarized by quintiles of total calcium and total dairy intakes, and chi-square tests for categorical variables and analysis of variance for continuous variables were used to assess differences across groups.

Total follow-up time was calculated as the time between the first interview date and the date of death, last follow-up date, or December 31, 2012, whichever came first. To examine the non-calcium component of milk, residuals from the linear regression models of whole and low-fat milk intakes with dietary calcium intake were determined. This method was modeled after the energy adjustment residual method[99] with the dependent variable being total dairy products and the independent variable being dietary calcium. To examine the non-calcium/non-fat component of milk, also as an indirect measure of the IGF-1 component of milk, the residuals of non-fat milk were also determined with non-fat milk as the dependent variable and dietary calcium as the independent variable. The residual variables were also categorized into no consumption as the reference category and the remaining 4 categories created as sex-specific quartiles.

Cox proportional hazards models were used to calculate multivariable-adjusted hazard ratios (HR) and 95% confidence intervals (CI) for the associations of quintiles of calcium and dairy product intakes with all-cause and cause-specific mortalities. Age was used as the time variable in all models. Potential confounders were selected on the basis of biological plausibility, statistical significance, and whether inclusion of the variable changed the adjusted HR for the primary exposure variable by ≥ 10%. The fully adjusted model included age (continuous), sex (male/female), race (black/white), region (Belt, Buckle, Non-belt), body mass index (kg/m^2; continuous), smoking status (never, past, current), alcohol intake (none, moderate, heavy), physical activity (none, 1-3 times/week, 4+ times/week), nonsteroidal anti-inflammatory drug and aspirin use (yes/no), hormone replacement therapy use in females (yes/no), college education or

higher (yes/no), annual income categories (refused, < $20,000, $20,000-$34,000, $35,000-$74,000; ≥ $75,000), total energy intake (kcal), fruit and vegetable servings/day, processed and red meat servings/day, and a dietary oxidative balance score (OBS). The dietary OBS was calculated using a previously described equal weight method[107] and is comprised of anti- (carotene, lutein, lycopene, vitamin C and E, omega-3 fatty acids, flavonoids) and pro-oxidant (dietary iron, omega-6 fatty acids, saturated fat) nutrients. Proportional hazards assumptions were tested using Log-Log survival curves, Schoenfeld residuals, and extended Cox models for each exposure and potential covariate. Linear trends across quintiles of calcium and dairy product intakes were assessed by including quintile- and sex-specific median values as a continuous variable in regression models.

To assess potential effect modification, stratified analyses were conducted for all-cause and cause-specific mortalities by age (< / ≥ 65 years), sex, race, region, BMI (sex-specific medians; males < / ≥ 27.8 kg/m^2; females < / ≥ 28.5 kg/m^2), smoking status, alcohol intake, NSAID and aspirin use, total energy intake (sex-specific medians; males < / ≥ 1,744.4 kcal/day; females </ ≥ 1,460.5 kcal/day), total fat intake (sex-specific medians; males < / ≥ 71.5 g/day; females </ ≥ 59.5 g/day), and supplemental calcium use (yes / no).

Various sensitivity analyses were also conducted. Models were further adjusted for baseline comorbidities (self-reported history of cardiovascular disease, diabetes, or cancer). We also excluded deaths that occurred within 1 or 2 years of enrollment to assess the potential influence of pre-morbid health conditions. Additionally, we excluded participants based on age limits of 75, 80, and 90 years. Participants who were at the age limit or older at baseline were excluded, and for those who reached the limit during follow-up, follow-up time was calculated as the period between baseline and the point at which the age limit was reached. Finally, supplemental calcium users who reported taking supplements for less than 1 year were categorized as non-supplemental calcium users.

A two-sided *P* value of < 0.05 was considered statistically significant. All statistical analyses were performed using SAS version 9.4 software (SAS Institute Inc., Cary, North Carolina).

RESULTS

Of the original 30,239 participants enrolled in the REGARDS study, we excluded 8,812 with missing data on ≥ 15% of the FFQ questions or who reported implausible energy intakes (< 800 or > 5,000 kcal/day for men and < 500 or > 4,500 kcal/day for women). The overall median baseline total calcium intake was 839.8 mg/day, with higher intakes among whites (67%) compared to blacks (33%). Dietary and supplemental calcium, as well as total dairy product intakes were higher among whites ($P < 0.0001$ for all variables).

Characteristics of the participants at baseline are summarized by total calcium and by total dairy product quintiles (Table 4.1). Those in the highest quintiles of total calcium and dairy intake, on average, were older and had a lower BMI, and were more likely to currently smoke, have a college education or higher, participate in physical activity at least once a week, and regularly take aspirin or NSAIDs. Women in the highest quintiles were also more likely to take hormone replacement therapy. Those with the highest intakes of total calcium were less likely to have a history of cardiovascular disease or diabetes and more likely have a higher annual household income. Additionally, those in the highest quintiles of total calcium and dairy intake had higher mean intakes of total energy, dietary fiber, supplemental calcium, total vitamin D, total meat, and fruits and vegetables.

Upon comparison of the highest to the lowest quintiles of intake, total calcium was not associated with risk for all-cause or cancer mortality but was associated with a non-statistically significant 9% lower risk for CVD mortality in multivariable-adjusted models (Table 4.2**)**. Total calcium was also associated with borderline statistically significant 23% higher risk of all non-cardiovascular/non-cancer-related mortality. Dietary and supplemental calcium were not

associated with all-cause, CVD, or cancer mortality, but supplemental calcium was associated with statistically significant 22% higher risk for non-cardiovascular-/non-cancer-related mortality.

Total dairy products were not associated with all-cause or cause-specific mortality (Table 4.3). However, as noted in Table 4.4, for those in the upper relative to the lowest categories of intakes, whole milk was associated with 13% higher risk of all-cause mortality (point estimate not statistically significant, but $p_{trend} = 0.004$), 20% lower risk for CVD mortality (not statistically significant), and statistically significant 56% higher risk for all cancer mortality, but was not associated with other causes of death combined. Low-fat milks were associated with 8% higher risk for all-cause mortality, 20% higher risk for CVD mortality, and 14% higher risk for other mortalities, but was associated with 11% lower risk for cancer mortality (all associations not statistically significant). On the other hand, non-fat milk was statistically significantly associated with 25% lower risk for all-cause mortality, non-statistically significant 28% lower risk for CVD mortality, non-statistically significant 11% lower risk for cancer mortality, and statistically significant 31% lower risk for other causes of death.

As shown in Table 4.5, for those in the upper relative to the lowest quintiles, residuals of whole/low-fat milks were associated with borderline statistically significant 11% higher risk of all-cause mortality, 17% higher risk of CVD mortality (not statistically significant), and 16% higher risk of cancer mortality (not statistically significant). However, non-fat milk residuals were associated with borderline statistically significant 19% lower risk for all-cause mortality, non- statistically significant 29% lower risk for CVD mortality, non-statistically significant 11% higher risk of cancer mortality, and statistically significant 30% lower risk for other mortalities.

Because previous evidence suggested that supplemental calcium use, especially without concomitant supplemental vitamin D, in postmenopausal women may increase risk for myocardial infarction and related deaths,[89,90] we conducted an additional analysis of calcium supplement use among pre- and postmenopausal females. However, we found no evidence of

higher risk for all-cause or cause-specific mortality among postmenopausal women who took calcium supplements, with or without vitamin D (Appendix Table 4.6).

Analyses stratified by various participant characteristics were repeated for all exposures, but there were no strong or consistent patterns to suggest effect modification (Appendix Table 4.7). In sensitivity analyses, further adjustment for comorbidities at baseline (Appendix Table 4.8 – 4.9), exclusion of deaths that occurred within 1 or 2 years after enrollment, re-classification of supplemental calcium use for less than 1 year as no supplemental calcium use, and exclusions and follow-up time adjustments based on age limits of 75, 80, and 90 years of age did not materially change any of the associations (data not shown).

DISCUSSION

The results from this study of a national cohort of black and white adults, suggest that milk containing fat may be associated with higher risk of all-cause and cardiovascular disease mortality, while the non-calcium, non-fat component of milk, as an indirect measure of the IGF-1 component of milk, may be associated with higher risk of cancer mortality. Also, we found no evidence that total, dietary, or supplemental calcium intakes were associated with all-cause or cardiovascular- or cancer-specific mortality, but that supplemental calcium intake may be directly associated with risk for other non-cardiovascular/non-cancer mortality. This pattern of findings suggests that milk fat may be directly associated with all-cause mortality, the non-calcium/non-fat, IGF-1 containing component of milk may be directly associated with cancer mortality, and calcium intake independent of milk product intake may not be associated with mortality.

Calcium and dairy products may affect risk of cardiovascular disease and cancer by various mechanisms. Calcium binds bile acids and free fatty acids in the gut, decreasing fat absorption, potentially lowering cholesterol, and reducing damage to gut epithelial cells, which decreases the risk for colorectal cancer.[29,32] Calcium is also essential for normal cardiac and vascular smooth muscle function and is needed for platelet activation and coagulation.[81,82]

However, high levels of calcium may also increase coronary artery calcification, especially among those with reduced renal function.[83] Milk products are a major source of dietary calcium, so the mechanisms of action are expected to be similar, although there are other nutrients with different potential mechanisms in milk. Clinical trials of calcium supplementation, with or without vitamin D, also reported favorable changes in blood lipids but no appreciable effects on blood pressure.[84,85] Clinical trials of dairy products reported no changes in blood lipid profiles or blood pressure with non-fat milk consumption.[86,87]

The most recent meta-analysis of 1 nested case-control study and 21 prospective cohort studies reported inconsistent associations of total, dietary, and supplemental calcium intakes with risk of mortality. Total calcium was associated with higher risk of all-cause, cardiovascular disease, and cancer mortality, while dietary calcium was associated with lower risks.[78] Supplemental calcium was associated with higher risk of cancer mortality but lower risk of cardiovascular disease and all-cause mortality. Among these associations, only that of supplemental calcium with all-cause mortality risk was statistically significant. An additional meta-analysis of 8 prospective cohort studies with a weighted mean follow-up time of 13.4 years reported a 13% higher risk of CVD mortality associated with higher baseline levels of serum calcium.[88]

Associations of dairy products with cardiovascular disease and mortality are also inconsistent. In the most recent meta-analysis of 8 studies, there was no association of milk intake with risk of all-cause mortality per 200 mL daily increase.[91] However, there was significant heterogeneity in these analyses ($p = 0.001$). Milk intake was only statistically significantly associated with lower risk of cardiovascular disease. Since the publication of this meta-analysis, 2 additional studies of dairy product consumption and cardiovascular disease outcomes were published. In a cross-sectional analysis of 162 overweight or obese adults, total dairy products, reduced fat milk, and dairy calcium were inversely associated with BMI, percent body fat, and waist circumference, while whole milk and dairy fat intakes were positively

associated.[93] A population-based cohort study of total dairy product intake found no associations with risk of coronary heart disease or stroke.[94]

The fat content of dairy products was hypothesized to increase risk for cardiovascular disease, but the most recent review of high-fat dairy product consumption and cardiovascular outcomes reported inconsistent associations involving total high-fat dairy products and whole milk.[168] Our results suggest that milk fat intake may be associated with risk of cardiovascular disease mortality since whole and low-fat milks were associated with higher risk of all-cause and cause-specific mortality while non-fat milk was associated with lower risk. This suggestion was also supported by the positive associations that remained of whole and low-fat milk residuals, representing the non-calcium, fat-containing component of milk, with risk for all-cause, cardiovascular disease, and cancer mortalities. Taken together, these findings suggest that bovine milk fats may increase risk of mortality, but the possibility our observed associations being due to chance or uncontrolled/residual confounding cannot be ruled out.

In our analyses, low- and non-fat milk intakes were inversely associated with risk for cancer mortality. This association may be attributable to the calcium component of milk since whole/low-fat milk residuals and non-fat milk residuals, representing the non-calcium and non-fat components of milk, were associated with higher risk of cancer mortality. These residuals are also an indirect measure of the IGF-1 component of milk, which is consistent with previous studies that reported positive associations of IGF-1 levels with risk for breast, prostate, and colorectal cancers. In the most recent meta-analysis of IGF-1 with risk of cancer, IGF-1 was associated with 93% higher risk of pre-menopausal breast cancer, 83% higher risk of prostate cancer, and 58% higher risk of colorectal cancer, all statistically significant.[60] Additionally, the most recent meta-analysis of calcium, milk products, and the risk for prostate cancer reported positive associations of dietary and dairy calcium, total milk products, and total milk with risk for prostate cancer.[169] Supplemental and non-dairy calcium were not associated with risk for prostate cancer, which supports the hypothesis that the positive association with risk for prostate cancer

may be due to a non-calcium component of milk products. The potential association of IGF-1 with higher risk for cancers is plausible since many tumor cells both produce IGF-1 and overexpress the IGF-1 receptor, which then leads to increased cell survival and tumor growth.[70] However, it will be important to further investigate specific cancer mortalities because although IGF-1 was previously associated with higher risk of cancers and dietary calcium was associated with higher risk of prostate cancer, total calcium was previously associated with lower risk of all-cause and colorectal cancer-specific mortality.[79] Therefore, the association of calcium, dairy products, and the IGF-1 component of milk with cancer mortality may vary by cancer type.

Our results do not suggest an association of supplemental calcium intake with higher risk of CVD mortality among postmenopausal women. Previous studies reported associations of higher intakes of supplemental calcium with lower risk of CVD mortality in postmenopausal women,[170,171] but recent trials and observational studies suggested conflicting associations.[89,90] The most recent meta-analysis of postmenopausal women from 18 randomized controlled trials reported no effect of calcium supplementation, with or without vitamin D, on the risk of coronary heart disease, all-cause mortality, or other cardiovascular disease outcomes.[172]

There were several limitations to our study. As mentioned, data on cancer-specific mortalities were unavailable, and would need to be assessed in future studies since associations with calcium and dairy products may differ by cancer type. Self-reported food frequency questionnaires may also not accurately capture all sources of each nutrient; however, the Block 98 is a standardized and validated FFQ, and categories of calcium and dairy product intakes were used in this analysis, rather than absolute values. Additionally, we could not distinguish specific milk type consumption (conventionally-produced vs. organic) from the data collected by the Block FFQ. This information may be important to collect in future studies since IGF-1 concentrations were higher in conventionally-produced compared to organic milk due to the use of bovine somatotropin hormone.[49] Finally, covariate information and dietary data were available

only at baseline. However, although dietary patterns may change, many other covariates are demographic factors, such as race, sex, and region, which are unlikely to change over time.

Strengths of this analysis include the use of a novel approach in estimating total dairy and non-fat milk residuals to investigate the associations of non-calcium and non-fat components of dairy products with mortality. In addition, this study population included sufficient numbers of black and white men and women to allow stratified analyses by race and sex. Finally, all mortalities and causes of death were adjudicated by clinicians using death certificates, medical records, and interviews with proxies and family members.

In summary, our results suggest that milk fat may be directly associated with all-cause mortality, the non-calcium/non-fat, IGF-1 containing component of milk may be directly associated with cancer mortality, and calcium intake independent of milk product intake may not be associated with mortality. Further study of the fat and non-calcium/non-fat components of milk and dairy products are needed to clarify our findings. Finally, our results, taken together with previous literature, suggest that the current dietary recommendations for the consumption dairy products as part of a balanced diet that may reduce the risk of chronic diseases[39] may be dependent on the fat and IGF-1 composition of these products.

TABLES

Table 4.1. Selected characteristics of REGARDS participants at baseline by quintiles of total calcium and total dairy products[a]

	Total Calcium				Total Dairy Products			
	Quintile 1 (n = 4,284)	Quintile 3 (n = 4,285)	Quintile 5 (n = 4,285)	*P* value[b]	Quintile 1 (n = 3,439)	Quintile 3 (n = 4,256)	Quintile 5 (n = 4,438)	*P* value[b]
Demographics								
Age, years	63.8 (9.1)	64.6 (9.6)	66.6 (9.2)	<0.0001	64.4 (8.8)	64.6 (9.2)	65.4 (9.7)	<0.0001
Male, %	44.1	44.1	44.1	1.00	58.4	42.0	41.8	<0.0001
White, %	49.6	66.5	81.6	<0.0001	47.5	68.5	80.8	<0.0001
Region, Stroke Belt,[c] %	31.1	31.2	32.8	<0.0001	33.7	34.3	33.7	<0.0001
Medical history,[d] %								
Heart disease	17.3	17.2	16.5	0.05	18.8	16.1	17.6	0.0002
Diabetes	23.7	20.3	15.3	<0.0001	20.8	19.1	19.8	0.39
Cancer	7.6	8.6	11.6	<0.0001	9.1	8.0	10.6	<0.0001
College graduate or higher, %	28.5	38.5	44.9	<0.0001	32.2	38.1	40.9	<0.0001
Annual income $35,000+, %	43.0	49.0	50.8	<0.0001	43.1	50.5	48.6	<0.0001
Lifestyle Factors								
Current smokers, %	41.7	46.1	49.2	<0.0001	39.1	44.9	48.7	<0.0001
Alcohol, 0-14 M, 0-7 F (drinks/week), %	30.3	36.0	38.4	<0.0001	33.4	35.9	36.0	<0.0001
Body mass index, kg/m^2	30.0 (6.5)	29.3 (6.4)	28.1 (5.9)	<0.0001	29.2 (5.9)	29.3 (6.3)	28.8 (6.3)	<0.0001
Physical activity, 1+ times/week, %	25.8	29.1	35.8	<0.0001	27.2	29.4	33.0	<0.0001
NSAID or aspirin use, %	47.2	51.1	57.8	<0.0001	49.1	53.8	53.9	<0.0001
HRT, % females	49.8	57.7	67.0	<0.0001	22.7	34.8	35.7	<0.0001
Dietary Intakes								
Total energy, kcal/day	1,211 (401)	1,853 (682)	2,043 (779)	<0.0001	1,440 (629)	1,691.8 (679.1)	2,048.9 (752.7)	<0.0001
Total fat, % total energy	37.4 (8.5)	37.6 (7.7)	36.3 (7.5)	<0.0001	37.2 (8.7)	38.0 (7.6)	35.9 (7.6)	<0.0001
Dietary fiber, g/day	10.3 (4.7)	17.0 (7.7)	20.8 (0.1)	<0.0001	13.4 (7.9	15.9 (8.0)	19.0 (9.4)	<0.0001
Total calcium, mg/day	342.9 (90.2)	886.9 (163.3)	1,896.3 (351.1)	<0.0001	602.3 (435.5)	927.8 (480.1)	1,533.8 (570.1)	<0.0001
Dietary calcium	315.5 (90.3)	719.3 (210.4)	999.3 (425.0)	<0.0001	350.8 (174.0)	580.6 (180.4)	1,132.9 (333.2)	<0.0001
Supplemental calcium	27.4 (52.5)	167.6 (244.9)	897.0 (386.2)	<0.0001	251.5 (398.4)	347.3 (448.3)	400.9 (471.9)	<0.0001
Total vitamin D, IU/day	153.3 (154.9)	331.5 (203.1)	495.1 (223.7)	<0.0001	218.8 (193.6)	297.3 (194.9)	481.7 (245.2)	<0.0001
Total meat, servings/day	1.5 (0.8)	2.1 (1.3)	2.2 (1.3)	<0.0001	1.8 (1.2)	2.0 (1.2)	2.1 (1.3)	<0.0001
Total fruit and vegetable, servings/day	1.94 (1.33)	3.15 (2.07)	3.83 (2.65)	<0.0001	2.49 (2.09)	2.98 (2.11)	3.37 (2.38)	<0.0001
Total dairy, servings/day	2.3 (1.8)	8.1 (4.8)	13.0 (8.6)	<0.0001	0.9 (0.7)	5.5 (0.9)	17.8 (5.9)	<0.0001
Whole milk, servings/week	0.3 (0.8)	0.7 (2.4)	0.9 (3.9)	<0.0001	0.1 (0.2)	0.5 (1.3)	1.6 (4.9)	<0.0001
Reduced/low-fat milk, servings/week	1.2 (2.3)	4.6 (7.5)	6.0 (11.7)	<0.0001	0.3 (0.6)	3.0 (3.8)	8.9 (13.8)	<0.0001
Skim milk, servings/week	0.1 (0.6)	1.2 (3.3)	3.2 (6.4)	<0.0001	0.03 (0.2)	0.6 (1.4)	4.3 (7.2)	<0.0001

Abbreviations: NSAID, non-steroidal anti-inflammatory drug; HRT, hormone replacement therapy.

[a] Values presented are mean (standard deviation) unless otherwise specified.
[b] *P* values from Chi-square test for categorical variables and analysis of variance for continuous variables.
[c] Stroke Belt defined as Alabama, Arkansas, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, and Tennessee; otherwise, live in other continental US states.
[d] Medical history of heart disease including self-reported myocardial infarction, coronary bypass grafting, cardiac bypass, angioplasty, stenting, or evidence of myocardial infarction via electrocardiogram; self-reported diabetes; history of any type of cancer.

Table 4.2. Associations of calcium intakes with all-cause and cause-specific mortality, REGARDS

	Quintiles					
	1	2	3	4	5	
Total Calcium	**(n = 4,284)**	**(n = 4,287)**	**(n = 4,285)**	**(n = 4,286)**	**(n = 4,285)**	P_{trend}[a]
All-cause						
No. of deaths	648	585	578	527	628	
Unadjusted HR (95% CI)	1.00	0.86 (0.77, 0.96)	0.79 (0.71, 0.89)	0.71 (0.63, 0.79)	0.74 (0.66, 0.82)	0.0001
Fully adjusted HR (95% CI)[b]	1.00	0.94 (0.84, 1.06)	0.96 (0.85, 1.08)	0.89 (0.79, 1.02)	1.04 (0.91, 1.18)	0.86
Cardiovascular disease[c]						
No. of deaths	224	179	193	172	189	
Unadjusted HR (95% CI)	1.00	0.76 (0.63, 0.93)	0.76 (0.63, 0.92)	0.67 (0.55, 0.81)	0.63 (0.52, 0.77)	0.002
Fully adjusted HR (95% CI)[b]	1.00	0.85 (0.70, 1.05)	0.93 (0.76, 1.15)	0.87 (0.69, 1.08)	0.91 (0.73, 1.15)	0.41
Cancer						
No. of deaths	184	180	160	157	173	
Unadjusted HR (95% CI)	1.00	0.94 (0.76, 1.15)	0.79 (0.64, 0.98)	0.74 (0.60, 0.91)	0.74 (0.60, 0.91)	0.02
Fully adjusted HR (95% CI)[b]	1.00	1.00 (0.81, 1.23)	0.91 (0.72, 1.14)	0.88 (0.69, 1.12)	0.97 (0.76, 1.23)	0.66
Other						
No. of deaths	240	226	225	198	266	
Unadjusted HR (95% CI)	1.00	0.90 (0.75, 1.08)	0.82 (0.69, 0.99)	0.72 (0.59, 0.87)	0.83 (0.70, 0.99)	0.40
Fully adjusted HR (95% CI)[b]	1.00	0.99 (0.82, 1.19)	1.03 (0.84, 1.25)	0.93 (0.76, 1.15)	1.23 (1.00, 1.51)	0.13
Dietary Calcium	**(n = 4,282)**	**(n = 4,285)**	**(n = 4,289)**	**(n = 4,286)**	**(n = 4,285)**	
All-causes						
No. of deaths	635	588	571	591	581	
Unadjusted HR (95% CI)	1.00	0.91 (0.81, 1.02)	0.85 (0.76, 0.95)	0.85 (0.76, 0.95)	0.80 (0.72, 0.90)	0.58
Fully adjusted HR (95% CI)[b]	1.00	0.98 (0.88, 1.10)	0.95 (0.84, 1.07)	1.00 (0.88, 1.14)	0.98 (0.85, 1.13)	0.97
Cardiovascular disease[c]						
No. of deaths	219	192	170	187	189	
Unadjusted HR (95% CI)	1.00	0.87 (0.71, 1.05)	0.74 (0.60, 0.90)	0.78 (0.64, 0.95)	0.76 (0.62, 0.92)	0.93
Fully adjusted HR (95% CI)[b]	1.00	0.97 (0.79, 1.18)	0.86 (0.69, 1.06)	0.97 (0.78, 1.22)	0.99 (0.77, 1.28)	0.87
Cancer						
No. of deaths	174	181	179	156	164	
Unadjusted HR (95% CI)	1.00	1.01 (0.82, 1.24)	0.97 (0.79, 1.20)	0.83 (0.67, 1.03)	0.85 (0.69, 1.05)	0.94
Fully adjusted HR (95% CI)[b]	1.00	1.05 (0.85, 1.30)	1.03 (0.82, 1.29)	0.92 (0.72, 1.17)	0.94 (0.71, 1.23)	0.44
Other						
No. of deaths	242	215	222	248	228	
Unadjusted HR (95% CI)	1.00	0.87 (0.72, 1.04)	0.86 (0.72, 1.03)	0.93 (0.78, 1.11)	0.82 (0.68, 0.98)	0.32
Fully adjusted HR (95% CI)[b]	1.00	0.95 (0.78, 1.15)	0.96 (0.79, 1.16)	1.10 (0.90, 1.35)	1.01 (0.80, 1.27)	0.50

Table 4.2. (cont.) Associations of calcium intakes with all-cause and cause-specific mortality, REGARDS

	Quintiles					
Supplemental Calcium[d]	**1 (n = 8,114)**	**2 (n = 3,360)**	**3 (n = 4,208)**	**4 (n = 1,999)**	**5 (n = 3,746)**	
All-causes						
No. of deaths	1,230	411	582	226	517	
Unadjusted HR (95% CI)	1.00	0.80 (0.72, 0.90)	0.82 (0.74, 0.90)	0.64 (0.56, 0.74)	0.72 (0.65, 0.80)	<0.0001
Fully adjusted HR (95% CI)[b]	1.00	1.00 (0.89, 1.12)	0.94 (0.85, 1.04)	0.92 (0.79, 1.07)	1.05 (0.94, 1.17)	0.96
Cardiovascular disease[c]						
No. of deaths	399	134	195	76	153	
Unadjusted HR (95% CI)	1.00	0.81 (0.66, 0.98)	0.85 (0.72, 1.01)	0.67 (0.52, 0.85)	0.65 (0.54, 0.79)	0.001
Fully adjusted HR (95% CI)[b]	1.00	1.05 (0.86, 1.29)	0.95 (0.80, 1.14)	0.97 (0.75, 1.26)	0.95 (0.78, 1.16)	0.32
Cancer						
No. of deaths	371	114	163	62	144	
Unadjusted HR (95% CI)	1.00	0.74 (0.60, 0.91)	0.76 (0.63, 0.91)	0.59 (0.45, 0.77)	0.67 (0.55, 0.81)	0.002
Fully adjusted HR (95% CI)[b]	1.00	0.91 (0.73, 1.13)	0.85 (0.70, 1.03)	0.84 (0.63, 1.10)	0.95 (0.78, 1.17)	0.48
Other						
No. of deaths	460	163	224	88	220	
Unadjusted HR (95% CI)	1.00	0.85 (0.71, 1.01)	0.84 (0.72, 0.99)	0.66 (0.53, 0.83)	0.81 (0.69, 0.95)	0.28
Fully adjusted HR (95% CI)[b]	1.00	1.04 (0.86, 1.25)	1.00 (0.85, 1.18)	0.95 (0.75, 1.20)	1.22 (1.03, 1.45)	0.09

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

[a] P_{trend} calculated using sex-specific medians of each quintile.

[b] Adjusted for age, sex, race, region, body mass index, smoking, alcohol, physical activity, non-steroidal anti-inflammatory drug and aspirin use, hormone replacement therapy use (females), education, annual income, supplemental calcium, total energy intake, fruit and vegetable intake, processed and red meat intake, dietary oxidative balance score

[c] Cardiovascular disease mortality includes deaths due to myocardial infarction, stroke, heart failure, sudden death, other cardiac causes, and other cardiovascular non-cardiac causes.

[d] Supplemental calcium analyzed as 5 categories of intake with no intake as the reference category.

Table 4.3. Associations of total dairy intakes with all-cause and cause-specific mortality, REGARDS

	Quintiles					
Total Dairy Products	**1 (n = 3,715)**	**2 (n = 4,592)**	**3 (n = 4,256)**	**4 (n = 4,219)**	**5 (n = 4,645)**	P_{trend}[a]
All-causes						
No. of deaths	512	648	572	563	671	
Unadjusted HR (95% CI)	1.00	0.95 (0.85, 1.07)	0.91 (0.81, 1.02)	0.81 (0.72, 0.92)	0.87 (0.77, 0.97)	0.09
Fully adjusted HR (95% CI)[b]	1.00	0.97 (0.86, 1.09)	1.01 (0.90, 1.15)	0.93 (0.82, 1.06)	1.05 (0.93, 1.19)	0.39
Cardiovascular disease[c]						
No. of deaths	171	201	200	181	204	
Unadjusted HR (95% CI)	1.00	0.88 (0.72, 1.08)	0.95 (0.77, 1.17)	0.78 (0.63, 0.96)	0.78 (0.64, 0.96)	0.92
Fully adjusted HR (95% CI)[b]	1.00	0.90 (0.73, 1.11)	1.11 (0.90, 1.37)	0.93 (0.74, 1.16)	1.03 (0.82, 1.29)	0.75
Cancer						
No. of deaths	145	193	171	155	190	
Unadjusted HR (95% CI)	1.00	1.00 (0.80, 1.24)	0.96 (0.77, 1.20)	0.81 (0.64, 1.01)	0.89 (0.72, 1.11)	0.64
Fully adjusted HR (95% CI)[b]	1.00	1.01 (0.81, 1.25)	1.03 (0.82, 1.30)	0.90 (0.71, 1.14)	1.02 (0.80, 1.29)	0.85
Other						
No. of deaths	196	254	201	227	277	
Unadjusted HR (95% CI)	1.00	0.98 (0.81, 1.18)	0.83 (0.68, 1.01)	0.85 (0.70, 1.02)	0.92 (0.77, 1.11)	0.04
Fully adjusted HR (95% CI)[b]	1.00	1.02 (0.84, 1.23)	0.92 (0.75, 1.13)	0.97 (0.79, 1.19)	1.10 (0.90, 1.35)	0.22

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

[a] P_{trend} calculated using sex-specific medians of each quintile.

[b] Adjusted for age, sex, race, region, body mass index, smoking, alcohol, physical activity, non-steroidal anti-inflammatory drug and aspirin use, hormone replacement therapy use (females), education, annual income, supplemental calcium, total energy intake, fruit and vegetable intake, processed and red meat intake, dietary oxidative balance score

[c] Cardiovascular disease mortality includes deaths due to myocardial infarction, stroke, heart failure, sudden death, other cardiac causes, and other cardiovascular non-cardiac causes.

Table 4.4. Associations of milk intakes with all-cause and cause-specific mortality, REGARDS

	Categories[a]					
	1	2	3	4	5	P_{trend}[b]
Whole Milk	**(n = 18,429)**	**(n = 738)**	**(n = 760)**	**(n = 748)**	**(n = 752)**	
All-causes						
No. of deaths	2,429	136	125	136	140	
Unadjusted HR (95% CI)	1.00	1.78 (1.50, 2.12)	1.44 (1.21, 1.73)	1.48 (1.25, 1.76)	1.40 (1.18, 1.66)	<0.001
Fully adjusted HR (95% CI)[c]	1.00	1.25 (1.05, 1.50)	1.05 (0.87, 1.26)	1.05 (0.88, 1.25)	1.13 (0.95, 1.35)	0.004
Cardiovascular disease[d]						
No. of deaths	806	40	34	45	32	
Unadjusted HR (95% CI)	1.00	1.56 (1.15, 2.17)	1.19 (0.85, 1.68)	1.48 (1.10, 2.00)	0.95 (0.67, 1.36)	0.38
Fully adjusted HR (95% CI)[c]	1.00	1.09 (0.78, 1.52)	0.84 (0.59, 1.20)	1.09 (0.80, 1.48)	0.80 (0.55, 1.16)	0.80
Cancer						
No. of deaths	689	35	41	34	55	
Unadjusted HR (95% CI)	1.00	1.61 (1.14, 2.26)	1.62 (1.18, 2.23)	1.29 (0.91, 1.82)	2.00 (1.52, 2.63)	<0.001
Fully adjusted HR (95% CI)[c]	1.00	1.14 (0.81, 1.62)	1.20 (0.87, 1.65)	0.87 (0.62, 1.24)	1.56 (1.17, 2.08)	0.006
Other						
No. of deaths	934	61	50	57	53	
Unadjusted HR (95% CI)	1.00	2.09 (1.61, 2.71)	1.52 (1.15, 2.02)	1.63 (1.25, 2.13)	1.35 (1.02, 1.79)	0.001
Fully adjusted HR (95% CI)[c]	1.00	1.50 (1.14, 1.96)	1.11 (0.83, 1.49)	1.16 (0.88, 1.53)	1.07 (0.80, 1.43)	0.05
Low-Fat Milk	**(n = 9,114)**	**(n = 3,074)**	**(n = 3,087)**	**(n = 3,074)**	**(n = 3,078)**	
All-causes						
No. of deaths	1,236	387	414	434	495	
Unadjusted HR (95% CI)	1.00	1.10 (0.98, 1.23)	0.94 (0.84, 1.05)	0.98 (0.88, 1.09)	1.01 (0.91, 1.12)	0.002
Fully adjusted HR (95% CI)[c]	1.00	1.02 (0.91, 1.14)	0.95 (0.85, 1.06)	1.00 (0.90, 1.12)	1.08 (0.97, 1.12)	0.65
Cardiovascular disease[c]						
No. of deaths	369	140	139	147	162	
Unadjusted HR (95% CI)	1.00	1.35 (1.12, 1.65)	1.04 (0.86, 1.27)	1.11 (0.91, 1.34)	1.09 (0.91, 1.31)	0.004
Fully adjusted HR (95% CI)[c]	1.00	1.24 (1.02, 1.51)	1.04 (0.85, 1.26)	1.14 (0.94, 1.38)	1.20 (0.99, 1.45)	0.12
Cancer						
No. of deaths	372	108	123	135	116	
Unadjusted HR (95% CI)	1.00	0.98 (0.79, 1.22)	0.95 (0.77, 1.16)	1.02 (0.84, 1.25)	0.82 (0.66, 1.01)	0.93
Fully adjusted HR (95% CI)[c]	1.00	0.95 (0.76, 1.18)	0.99 (0.81, 1.22)	1.07 (0.88, 1.31)	0.89 (0.72, 1.10)	0.33
Other						
No. of deaths	495	139	152	152	217	
Unadjusted HR (95% CI)	1.00	1.00 (0.83, 1.21)	0.85 (0.71, 1.02)	0.85 (0.71, 1.01)	1.08 (0.92, 1.26)	0.03
Fully adjusted HR (95% CI)[c]	1.00	0.91 (0.75, 1.10)	0.85 (0.71, 1.03)	0.85 (0.71, 1.02)	1.14 (0.97, 1.34)	0.97

Table 4.4. (cont.) Associations of milk intakes with all-cause and cause-specific mortality, REGARDS

	Categories[a]					
	1	2	3	4	5	P_{trend}[b]
Whole Milk	**(n = 18,429)**	**(n = 738)**	**(n = 760)**	**(n = 748)**	**(n = 752)**	
Non-Fat Milk	**(n = 17,749)**	**(n = 917)**	**(n = 920)**	**(n = 906)**	**(n = 935)**	
All-causes						
No. of deaths	2,575	98	92	110	91	
Unadjusted HR (95% CI)	1.00	0.78 (0.64, 0.96)	0.60 (0.49, 0.74)	0.74 (0.61, 0.90)	0.56 (0.46, 0.69)	<0.001
Fully adjusted HR (95% CI)[c]	1.00	0.93 (0.76, 1.14)	0.76 (0.61, 0.94)	1.01 (0.83, 1.22)	0.75 (0.61, 0.93)	0.001
Cardiovascular disease[c]						
No. of deaths	829	36	26	38	28	
Unadjusted HR (95% CI)	1.00	0.91 (0.65, 1.27)	0.53 (0.36, 0.78)	0.80 (0.58, 1.10)	0.53 (0.37, 0.78)	0.01
Fully adjusted HR (95% CI)[c]	1.00	1.02 (0.73, 1.43)	0.65 (0.44, 0.97)	1.05 (0.75, 1.46)	0.72 (0.49, 1.05)	0.06
Cancer						
No. of deaths	735	22	26	40	31	
Unadjusted HR (95% CI)	1.00	0.60 (0.39, 0.91)	0.60 (0.41, 0.89)	0.93 (0.68, 1.28)	0.68 (0.47, 0.97)	0.22
Fully adjusted HR (95% CI)[c]	1.00	0.73 (0.47, 1.11)	0.75 (0.50, 1.12)	1.25 (0.90, 1.73)	0.89 (0.62, 1.28)	0.86
Other						
No. of deaths	1,011	40	40	32	32	
Unadjusted HR (95% CI)	1.00	0.82 (0.60, 1.13)	0.66 (0.48, 0.91)	0.55 (0.39, 0.78)	0.50 (0.35, 0.72)	<0.001
Fully adjusted HR (95% CI)[c]	1.00	0.99 (0.72, 1.37)	0.86 (0.62, 1.18)	0.78 (0.54, 1.11)	0.69 (0.48, 0.98)	0.007

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

[a] Five categories of milk intake with no intake as reference category and remaining 4 categories created as sex-specific quartiles of intake.

[b] P_{trend} calculated using sex-specific medians of each quintile.

[c] Adjusted for age, sex, race, region, body mass index, smoking, alcohol, physical activity, non-steroidal anti-inflammatory drug and aspirin use, hormone replacement therapy use (females), education, annual income, supplemental calcium, total energy intake, fruit and vegetable intake, processed and red meat intake, dietary oxidative balance score

[d] Cardiovascular disease mortality includes deaths due to myocardial infarction, stroke, heart failure, sudden death, other cardiac causes, and other cardiovascular non-cardiac cause

Table 4.5. Associations of milk residuals with all-cause and cause-specific mortality, REGARDS

Whole/Low-Fat Milk Residuals	Quintiles					
	1 (n = 6,116)	2 (n = 3,827)	3 (n = 3,828)	4 (n = 3,828)	5 (n = 3,828)	P_{trend}[a]
All-causes						
No. of deaths	699	523	508	580	656	
Unadjusted HR (95% CI)	1.00	1.38 (1.23, 1.55)	1.25 (1.12, 1.41)	1.33 (1.19, 1.49)	1.32 (1.18, 1.46)	<0.0001
Fully adjusted HR (95% CI)[b]	1.00	1.16 (1.02, 1.31)	1.03 (0.92, 1.16)	1.11 (0.99, 1.24)	1.11 (0.99, 1.25)	0.03
Cardiovascular disease[c]						
No. of deaths	218	179	147	204	209	
Unadjusted HR (95% CI)	1.00	1.53 (1.25, 1.86)	1.17 (0.95, 1.44)	1.49 (1.23, 1.80)	1.33 (1.10, 1.61)	<0.0001
Fully adjusted HR (95% CI)[b]	1.00	1.34 (1.08, 1.65)	0.98 (0.79, 1.21)	1.23 (1.01, 1.50)	1.17 (0.95, 1.43)	0.10
Cancer						
No. of deaths	207	152	149	160	186	
Unadjusted HR (95% CI)	1.00	1.34 (1.09, 1.66)	1.24 (1.00, 1.53)	1.26 (1.03, 1.55)	1.32 (1.09, 1.61)	0.0004
Fully adjusted HR (95% CI)[b]	1.00	1.13 (0.90, 1.42)	1.03 (0.83, 1.27)	1.09 (0.88, 1.34)	1.16 (0.94, 1.44)	0.33
Other						
No. of deaths	274	192	212	216	261	
Unadjusted HR (95% CI)	1.00	1.29 (1.07, 1.56)	1.34 (1.12, 1.60)	1.26 (1.05, 1.50)	1.30 (1.10, 1.54)	<0.0001
Fully adjusted HR (95% CI)[b]	1.00	1.04 (0.85, 1.27)	1.09 (0.91, 1.31)	1.03 (0.86, 1.24)	1.05 (0.87, 1.25)	0.50
Non-fat Milk Residuals	**(n = 17,749)**	**(n = 918)**	**(n = 920)**	**(n = 920)**	**(n = 920)**	
All-causes						
No. of deaths	2,575	93	88	103	107	
Unadjusted HR (95% CI)	1.00	0.68 (0.55, 0.83)	0.66 (0.53, 0.81)	0.67 (0.55, 0.82)	0.65 (0.54, 0.79)	<0.0001
Fully adjusted HR (95% CI)[b]	1.00	0.86 (0.70, 1.07)	0.83 (0.67, 1.04)	0.86 (0.70, 1.05)	0.81 (0.66, 1.00)	0.0001
Cardiovascular disease[c]						
No. of deaths	829	27	27	43	31	
Unadjusted HR (95% CI)	1.00	0.61 (0.42, 0.89)	0.63 (0.43, 0.93)	0.88 (0.64, 1.19)	0.58 (0.41, 0.84)	0.02
Fully adjusted HR (95% CI)[b]	1.00	0.75 (0.50, 1.11)	0.79 (0.54, 1.17)	1.07 (0.78, 1.46)	0.71 (0.49, 1.03)	0.09
Cancer						
No. of deaths	735	23	24	32	40	
Unadjusted HR (95% CI)	1.00	0.58 (0.39, 0.88)	0.61 (0.40, 0.91)	0.74 (0.52, 1.05)	0.86 (0.63, 1.18)	0.19
Fully adjusted HR (95% CI)[b]	1.00	0.74 (0.48, 1.13)	0.74 (0.49, 1.13)	0.96 (0.67, 1.38)	1.11 (0.79, 1.55)	0.99
Other						
No. of deaths	1,011	43	37	28	36	
Unadjusted HR (95% CI)	1.00	0.80 (0.59, 1.08)	0.71 (0.51, 0.99)	0.46 (0.32, 0.67)	0.56 (0.40, 0.78)	<0.0001
Fully adjusted HR (95% CI)[b]	1.00	1.06 (0.78, 1.46)	0.95 (0.68, 1.33)	0.59 (0.40, 0.86)	0.70 (0.49, 0.98)	0.002

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

[a] P_{trend} calculated using sex-specific medians of each quintile.

[b] Adjusted for age, sex, race, region, body mass index, smoking, alcohol, physical activity, non-steroidal anti-inflammatory drug and aspirin use, hormone replacement therapy use (females), education, annual income, dietary calcium, total energy intake, fruit and vegetable intake, processed and red meat intake, dietary oxidative balance score

[c] Cardiovascular disease mortality includes deaths due to myocardial infarction, stroke, heart failure, sudden death, other cardiac causes, and other cardiovascular non-cardiac causes.

CHAPTER 5. DISCUSSION AND FUTURE DIRECTIONS

Summary and Public Health Implications

The goals of this dissertation were to investigate associations of calcium and milk product intakes with risk for colorectal neoplasms and mortality and to test the effects of calcium supplementation on circulating levels of insulin-like growth factor-1 (IGF-1) and insulin-like growth factor binding protein-3 (IGFBP-3).

In the first aim, data from 3 previously conducted case-control studies of incident, sporadic adenoma were pooled to analyze associations of calcium and milk product intakes with risk for adenoma. Although most of our results were not statistically significant, they were interpreted based on biological plausibility consistency with findings from previous studies. Thus, our finding of a modest inverse association of dietary calcium intake with risk of adenoma is consistent with previous literature that calcium is associated with lower risk of colorectal neoplasia. We also analyzed the non-calcium/non-fat component of milk using a novel approach of estimating the residuals of total milk products and non-fat milk adjusted for dietary calcium. There was suggestion of a potential inverse association of the total milk product residuals with risk for adenoma that warrants further investigation.

In the second aim, we conducted an analysis of the first clinical trial to test the effects of calcium supplementation on circulating levels of IGF-1 and IGFBP-3 in patients with a previous history of adenoma. We found no appreciable effects of 1.0 or 2.0 g of daily supplemental calcium relative to placebo on levels of IGF-1, IGFBP-3, or the IGF-1:IGFBP-3 molar ratio. IGF-1 and IGFBP-3 concentrations were inversely associated with age and were lower in women, which is consistent with most previous literature. Our results also support previous findings that IGF-1 concentrations are higher with greater milk intakes.

In the third aim, data from a large national prospective cohort study, REasons for Geographic and Racial Differences in Stroke (REGARDS), were used to investigate associations of calcium and milk product intakes with risk of all-cause and cause-specific mortality. There

was no evidence that calcium was associated with all-cause or cause-specific mortality. However, whole and low-fat milk intakes and whole and low-fat milk residuals (as the non-calcium, fat-containing component of milk) were associated with higher risk for all-cause and cardiovascular disease mortality, while non-fat milk and the non-fat milk residuals (as the non-calcium/non-fat component of milk) were associated with lower risk for all-cause and cardiovascular disease mortality but higher risk for cancer mortality. Therefore, these patterns suggest that milk fats may be associated with higher risk of cardiovascular disease-related mortality, and that the non-calcium, non-fat portion of milk, potentially containing IGF-1, may be associated with higher risk of cancer mortality. We additionally investigated the association of calcium supplementation among postmenopausal women with risk of cardiovascular disease mortality, but our results do not support the findings of higher risk of mortality reported in previous studies.[89,90]

Overall, the results from this dissertation add to the existing literature on calcium and milk products as modifiable dietary risk factors of colorectal neoplasia. Our results strongly suggest that there is no effect of supplemental calcium on circulating IGF-1 and IGFBP-3 concentrations, but the association of IGF-1 from milk products with risk of adenoma is unclear. Our estimation of milk product residuals is a novel modification of a commonly used energy adjustment method that can be utilized to estimate the non-calcium/non-fat component of milk when serum measures are unavailable. Our analyses of these residuals provide additional evidence supporting a potential association of the fat component of milk with risk for cardiovascular disease-related mortality and of the non-calcium, non-fat component of milk, as an indirect measure of IGF-1 with risk for cancer-related mortality that warrants further investigation.

Future Directions

In the pooled analysis of 3 previous case-control studies, consistent with previously reported studies, we found evidence for a modest inverse association of dietary calcium with risk for colorectal adenoma. However, we found no evidence of a milk products-adenoma association. Because of the limitation of our study in respect to the limitations of commonly used food frequency questionnaires, especially regarding calcium and milk product intakes, I propose a new case-control or prospective cohort study in which the food frequency questionnaire would be customized to collect more detailed, accurate information on intakes of specific types of milk and milk-based products (e.g., conventional vs. organic, brand names, etc.) and all potential sources of calcium (e.g., supplements, antacids, and non-dairy calcium-fortified foods). Detailed assessment of milk product intakes are needed to address the greater variety of milk products available (e.g., conventional, rBST-free, and organic) relative to the time periods of the previous studies. Additionally, in a case-control study or clinical trial, I would like to collect blood samples and colon biopsies at the time of colonoscopy, which would allow us to measure circulating and tissue concentrations of IGF-1 and IGFBP-3. To our knowledge, only one study previously investigated the correlation of circulating and colon tissue levels of IGF-1.[173] In this study of men and postmenopausal women (n = 48), circulating IGF-1 levels were not correlated with colorectal mRNA expression of IGF-1 and IGFBP-3. However, it is unclear if mRNA expression of IGF-1 and IGFBP-3 correlates with actual tissue concentrations of these growth factors, and therefore further investigation of the correlation between circulating and tissue concentrations of IGF-1 and IGFBP-3 is warranted. Such a study would also allow us to investigate the association of dietary and lifestyle factors with IGF-1 concentrations in persons with and without colorectal adenoma.

Our results strongly suggest that calcium supplementation does not have an appreciable effect on circulating levels of IGF-1 or IGFBP-3; however, our study, taken in context with previous studies, suggests that a clinical trial to further investigate the potential effects of

different types of milk, possibly with a supplemental calcium arm, on IGF-1 and IGFBP-3 levels is justified. A feeding trial would be most ideal to test the effect of both calcium and milk intakes on IGF-1 and IGFBP-3 levels; however, this study design would be expensive and challenging for both investigators and participants. Thus, an alternative would be to conduct a 6-week dietary intervention trial among healthy adult participants without a history of adenoma that would include the following 4 treatment groups: 1.2 g supplemental calcium daily, 3 servings of non-fat bovine somatotropin (bST)-free milk daily, 3 servings of non-fat conventional milk daily, and a usual diet group as a control. All groups would be advised to maintain their baseline dietary intakes of other non-milk, calcium-containing foods. The two milk treatment groups would be provided with milk to ensure consistency in the fat content and brand, and instructed to consume 3 servings (1 serving = 1 cup or 8 fluid ounces) daily. Since we did not observe a difference in the 1.0 g and 2.0 g calcium supplementation groups in our previous trial, this supplementation group would receive the 1.2 g dose of elemental calcium daily successfully used in a previous colorectal adenoma recurrence trial.[10] Additionally, because previous studies suggested that IGF-1 and IGFBP-3 levels may vary diurnally and with physical activity[157,174,175], unlike in our trial, blood samples should be collected fasting and at the same time of day for all participants at baseline and follow-up. By conducting this trial, we will be able to test the effect of supplemental calcium, milk intakes, and the use of bST in milk production on circulating levels of IGF-1 and IGFBP-3. It would also be beneficial to measure the IGF-1 and IGFBP-3 concentrations in each type of milk to compare levels between milk with and without bST and to assess the association with serum levels of these growth factors.

Finally, I propose further investigation of the association of calcium and milk product intakes with cardiovascular disease- and cancer-related mortality in the REGARDS and the Iowa Women's Health Study (IWHS) cohorts. Because there may be differences by cancer type, it will be necessary to investigate the specific cancer mortalities individually. If possible, linkage of the REGARDS study data with state cancer registries would allow us to identify the specific cancer-

related deaths (such linkage is already available in the IWHS). Additionally, since our results suggested a potential direct association of the fat component of milk with risk for mortality, additional dietary information should be collected, including specific types of milk and other high- and low-fat milk products. It would also be desirable to collect dietary information not only at baseline but also at additional follow-up visits to monitor changes in dietary intakes. Alternatives would be to start a new prospective cohort study or add on calcium and milk product questionnaires to relatively new cohort studies. A cohort study would recruit a nationally representative sample with a racially diverse representation of equal proportions of men and women since calcium and milk product consumption patterns are likely to vary by race (whites, blacks, Hispanics, and Asians) and sex.

The findings reported in this dissertation do not provide evidence against the current 2015 Dietary Guidelines, which recommend the consumption of calcium and milk products as part of a healthy dietary pattern that may reduce the risk of chronic diseases.[39] However, our data suggest that the risk of cardiovascular disease- and cancer-related mortality may depend, at least to some extent, on the fat and IGF-1 content of milk products. Overall, further investigation, through the new studies described above and others, is needed to clarify the role of calcium and milk products as modifiable dietary factors that may modulate the risk of colorectal neoplasia and mortality.

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APPENDIX

Table 2.5. Multivariable-adjusted associations of calcium intake with incident, sporadic colorectal adenomas in Minnesota Cancer Prevention Research Unit Case-Control Study (CPRU; 1991-1994)

Quintiles	No. of Cases/ Controls (n=564/ 1,737)	Initial Model[a]		Full Model[b]		Among Non-Regular Users of Aspirin and NSAIDs: No. of Cases/ Controls (n=399/ 1,064)	Full Model[c]	
		OR	95% CI	OR	95% CI		OR	95% CI
Total Calcium								
1	120/347	1.00 (ref)		1.00 (ref)		92/224	1.00 (ref)	
2	110/347	0.88	0.65, 1.20	0.92	0.67, 1.26	78/212	0.90	0.62, 1.31
3	124/349	0.99	0.74, 1.34	1.05	0.76, 1.45	85/209	0.99	0.67, 1.45
4	90/347	0.71	0.52, 0.98	0.74	0.53, 1.05	63/218	0.70	0.46, 1.05
5	120/347	0.97	0.72, 1.31	1.03	0.72, 1.47	81/201	1.02	0.66, 1.58
Dietary Calcium								
1	120/347	1.00 (ref)		1.00 (ref)		93/223	1.00 (ref)	
2	112/347	0.89	0.66, 1.21	0.94	0.68, 1.28	69/203	0.81	0.55, 1.20
3	114/349	0.93	0.68, 1.25	0.95	0.68, 1.32	82/213	0.94	0.64, 1.39
4	109/347	0.90	0.67, 1.23	0.91	0.65, 1.28	73/214	0.84	0.56, 1.26
5	109/347	0.91	0.67, 1.23	0.86	0.59, 1.26	82/211	0.98	0.63, 1.53
Supplemental Calcium[d]								
1	425/1,265	1.00 (ref)		1.00 (ref)		311/810	1.00 (ref)	
2	58/215	0.90	0.65, 1.24	0.91	0.66, 1.25	40/112	0.99	0.66, 1.47
3	81/257	1.01	0.77, 1.34	1.15	0.86, 1.53	48/142	1.03	0.71, 1.49

Abbreviations: NSAIDs, non-steroidal anti-inflammatory drugs; OR, odds ratio; 95% CI, 95% confidence interval.

[a] Adjusted for study, age, and sex.

[b] Adjusted for study, age, sex, oxidative balance score, family history of colorectal cancer in first-degree relative, regular use of aspirin or nonsteroidal anti-inflammatory drugs, total energy intake, total fat intake (energy-adjusted).

[c] Among non-regular users of aspirin and NSAIDs, adjusted for study, age, sex, oxidative balance score, family history of colorectal cancer in first-degree relative, total energy intake, total fat intake (energy-adjusted).

[d] Supplemental calcium intake categorized as three groups (none and according to the median dose among those who did take supplemental calcium) due to small sample size.

Table 2.6. Multivariable-adjusted associations of milk product consumption with incident, sporadic colorectal adenomas in Minnesota Cancer Prevention Research Unit Case-Control Study (CPRU; 1991-1994)

Quintiles	No. of Cases/ Controls (n=564/ 1,737)	Initial Model[a]		Full Model[b,c]		Among Non-Regular Users of Aspirin and NSAIDs		
						No. of Cases/ Controls (n=399/ 1,064)	Full Model[d,e]	
		OR	95% CI	OR	95% CI		OR	95% CI
Total Milk Products								
1	110/332	1.00 (ref)		1.00 (ref)		86/205	1.00 (ref)	
2	114/361	0.96	0.71, 1.31	0.95	0.70, 1.30	77/230	0.79	0.55, 1.15
3	109/339	1.03	0.75, 1.40	1.04	0.75, 1.43	77/189	1.02	0.69, 1.50
4	111/352	0.96	0.70, 1.30	0.94	0.68, 1.30	73/218	0.78	0.53, 1.15
5	120/353	1.09	0.80, 1.47	1.00	0.71, 1.41	86/222	0.92	0.62, 1.38
Total Milk								
1	97/320	1.00 (ref)		1.00 (ref)		69/195	1.00 (ref)	
2	61/218	1.15	0.79, 1.67	1.12	0.77, 1.64	39/138	0.93	0.59, 1.49
3	209/560	1.12	0.84, 1.48	1.11	0.83, 1.48	146/333	1.07	0.76, 1.52
4	58/211	1.26	0.85, 1.86	1.27	0.85, 1.89	41/121	1.31	0.81, 2.12
5	139/428	1.04	0.77, 1.42	0.98	0.72, 1.35	104/277	1.01	0.69, 1.47
Whole Milk[f]								
1	504/1,575	1.00 (ref)		1.00 (ref)		360/952	1.00 (ref)	
2	60/162	1.06	0.77, 1.47	1.02	0.73, 1.41	39/112	0.87	0.59, 1.30
Non-fat Milk								
1	93/332	1.00 (ref)		1.00 (ref)		70/205	1.00 (ref)	
2	68/192	1.25	0.87, 1.81	1.27	0.88, 1.84	43/127	1.01	0.64, 1.58
3	105/325	1.22	0.88, 1.69	1.24	0.89, 1.72	70/189	1.11	0.74, 1.64
4	164/474	1.18	0.87, 1.58	1.21	0.90, 1.64	116/275	1.18	0.82, 1.69
5	134/414	1.09	0.80, 1.48	1.04	0.76, 1.43	100/268	1.02	0.70, 1.49
Total Milk Product Residuals								
1	125/346	1.00 (ref)		1.00 (ref)		72/205	1.00 (ref)	
2	126/348	0.97	0.72, 1.30	0.85	0.62, 1.16	58/198	0.87	0.60, 1.27
3	83/349	0.67	0.49, 0.92	0.56	0.39, 0.79	95/218	0.53	0.35, 0.81
4	93/346	0.76	0.55, 1.04	0.65	0.46, 0.92	84/229	0.52	0.34, 0.79
5	137/348	1.14	0.85, 1.53	0.94	0.69, 1.30	90/214	0.73	0.49, 1.08
Non-fat Milk Residuals								
1	110/345	1.00 (ref)		1.00 (ref)		91/209	1.00 (ref)	
2	94/347	0.81	0.59, 1.12	0.84	0.60, 1.17	96/205	0.77	0.51, 1.18
3	131/350	1.16	0.86, 1.57	1.16	0.83, 1.62	63/211	1.23	0.82, 1.84
4	116/347	1.02	0.75, 1.38	0.98	0.69, 1.39	60/215	0.97	0.63, 1.49
5	113/348	0.96	0.71, 1.31	0.90	0.62, 1.30	89/224	1.04	0.67, 1.63

Abbreviations: NSAIDs, non-steroidal anti-inflammatory drugs; OR, odds ratio; 95% CI, 95% confidence interval.

[a] Adjusted for study, age, and sex.

[b] For Total Milk Products, Total Milk, Whole Milk, and Non-fat Milk, adjusted for study, age, sex, oxidative balance score, family history of colorectal cancer in first-degree relative, regular use of aspirin or nonsteroidal anti-inflammatory drugs, total energy intake, total fat intake (energy-adjusted), supplemental calcium intake.

[c] For Total Milk Product Residuals and Non-fat Milk Residuals, adjusted for study, age, sex, oxidative balance score, family history of colorectal cancer in first-degree relative, regular use of aspirin or nonsteroidal anti-inflammatory drugs, total energy intake, total fat intake (energy-adjusted), dietary calcium intake.

[d] For non-regular users of aspirin and NSAIDs in Total Milk Products, Total Milk, Whole Milk, and Non-fat Milk models, adjusted for study, age, sex, oxidative balance score, family history of colorectal cancer in first-degree relative, total energy intake, total fat intake (energy-adjusted), supplemental calcium intake.

[e] For non-regular users of aspirin and NSAIDs in Total Milk Product Residuals and Non-fat Milk Residuals models, adjusted for study, age, sex, oxidative balance score, family history of colorectal cancer in first-degree relative, total energy intake, total fat intake (energy-adjusted), dietary calcium intake.

[f] Whole milk consumption categorized as dichotomous due to small sample size.

Table 2.7. Multivariable-adjusted associations of calcium intake with incident, sporadic colorectal adenomas in the Markers of Adenomatous Polyps studies I (MAP I; North Carolina, 1995-1997) and II (MAP II; South Carolina, 2002)

Quintiles	No. of Cases/ Controls	Initial Model[a]		Full Model[b]		Among Non-Regular Users of Aspirin and NSAIDs		
						No. of Cases/ Controls	Full Model[c]	
	(n=223/ 296)	OR	95% CI	OR	95% CI	(n=109/ 123)	OR	95% CI
Total Calcium								
1	44/58	1.00 (ref)		1.00 (ref)		30/25	1.00 (ref)	
2	54/60	1.34	0.76, 2.38	1.26	0.68, 2.32	26/26	0.76	0.33, 1.80
3	43/60	1.08	0.60, 1.95	1.00	0.52, 1.91	21/25	0.90	0.35, 2.30
4	42/59	0.99	0.55, 1.80	0.81	0.40, 1.61	15/26	0.45	0.16, 1.26
5	40/59	0.89	0.49, 1.63	0.81	0.40, 1.66	17/21	0.58	0.20, 1.71
Dietary Calcium								
1	47/57	1.00 (ref)		1.00 (ref)		31/20	1.00 (ref)	
2	40/61	0.90	0.50, 1.62	0.87	0.47, 1.64	22/25	0.54	0.22, 1.37
3	47/60	1.03	0.58, 1.84	0.89	0.47, 1.68	23/29	0.41	0.16, 1.08
4	39/60	0.89	0.49, 1.60	0.73	0.36, 1.48	16/20	0.36	0.12, 1.10
5	50/58	1.21	0.68, 2.16	0.91	0.44, 1.89	17/29	0.26	0.08, 0.80
Supplemental Calcium[d]								
1	155/154	1.00 (ref)		1.00 (ref)		76/78	1.00 (ref)	
2	36/71	0.68	0.42, 1.11	0.82	0.49, 1.39	17/25	1.14	0.51, 2.56
3	32/71	0.57	0.34, 0.95	0.79	0.45, 1.39	16/20	1.29	0.51, 3.26

Abbreviations: NSAIDs, non-steroidal anti-inflammatory drugs; OR, odds ratio; 95% CI, 95% confidence interval.

[a] Adjusted for study, age, and sex.

[b] Adjusted for study, age, sex, oxidative balance score, family history of colorectal cancer in first-degree relative, regular use of aspirin or nonsteroidal anti-inflammatory drugs, total energy intake, total fat intake (energy-adjusted).

[c] Among non-regular users of aspirin and NSAIDs, adjusted for study, age, sex, oxidative balance score, family history of colorectal cancer in first-degree relative, total energy intake, total fat intake (energy-adjusted).

[d] Supplemental calcium intake categorized as three groups (none and according to the median dose among those who did take supplemental calcium) due to small sample size.

Table 2.8. Multivariable-adjusted associations of milk product consumption with incident, sporadic colorectal adenomas in the Markers of Adenomatous Polyps studies I (MAP I; North Carolina, 1995-1997) and II (MAP II; South Carolina, 2002)

Quintiles	No. of Cases/ Controls (n=223/ 296)	Initial Model[a]		Full Model[b,c]		Among Non-Regular Users of Aspirin and NSAIDs		
						No. of Cases/ Controls (n=109/ 123)	Full Model[d,e]	
		OR	95% CI	OR	95% CI		OR	95% CI
Total Milk Products								
1	38/60	1.00 (ref)		1.00 (ref)		23/23	1.00 (ref)	
2	59/59	1.39	0.78, 2.47	1.37	0.74, 2.54	37/26	1.44	0.57, 3.61
3	36/54	1.09	0.59, 2.03	1.00	0.51, 1.94	16/24	0.51	0.19, 1.42
4	42/62	1.03	0.57, 1.88	0.99	0.52, 1.91	16/26	0.54	0.19, 1.54
5	48/61	1.21	0.67, 2.18	0.99	0.50, 1.93	17/24	0.47	0.16, 1.37
Total Milk								
1	43/46	1.00 (ref)		1.00 (ref)		28/22	1.00 (ref)	
2	23/40	0.63	0.31, 1.27	0.63	0.30, 1.33	9/24	0.21	0.07, 0.64
3	29/36	1.07	0.54, 2.11	1.02	0.49, 2.13	16/10	0.97	0.32, 2.88
4	94/118	0.74	0.44, 1.26	0.72	0.41, 1.27	46/42	0.60	0.26, 1.39
5	34/56	0.67	0.35, 1.28	0.63	0.32, 1.27	10/25	0.31	0.11, 0.93
Whole Milk[f]								
1	157/243	1.00 (ref)		1.00 (ref)		74/98	1.00 (ref)	
2	66/53	1.72	1.11, 2.67	1.54	0.96, 2.48	35/25	1.51	0.78, 2.91
Non-fat Milk								
1	90/85	1.00 (ref)		1.00 (ref)		55/42	1.00 (ref)	
2	13/28	0.66	0.31, 1.41	0.61	0.27, 1.38	8/14	0.52	0.17, 1.60
3	12/12	0.94	0.37, 2.41	1.30	0.47, 3.62	5/5	0.67	0.17, 2.68
4	41/84	0.55	0.33, 0.91	0.57	0.33, 0.98	20/26	0.66	0.29, 1.49
5	67/87	0.68	0.43, 1.09	0.66	0.40, 1.09	21/36	0.39	0.18, 0.84
Total Milk Product Residuals								
1	52/56	1.00 (ref)		1.00 (ref)		20/18	1.00 (ref)	
2	41/60	0.76	0.42, 1.36	0.70	0.37, 1.31	24/33	0.44	0.16, 1.22
3	43/59	0.75	0.42, 1.34	0.74	0.39, 1.40	26/31	0.47	0.16, 1.38
4	37/63	0.68	0.38, 1.23	0.67	0.35, 1.27	21/19	0.77	0.25, 2.37
5	50/58	0.91	0.52, 1.61	0.84	0.45, 1.56	18/22	0.39	0.14, 1.12
Non-fat Milk Residuals								
1	44/56	1.00 (ref)		1.00 (ref)		16/30	1.00 (ref)	
2	47/61	0.81	0.45, 1.45	0.95	0.49, 1.83	28/28	1.52	0.56, 4.11
3	46/58	0.81	0.45, 1.46	1.03	0.51, 2.06	28/14	2.90	0.96, 8.76
4	38/61	0.61	0.33, 1.12	0.75	0.36, 1.56	21/27	1.16	0.37, 3.62
5	48/60	0.72	0.40, 1.30	1.16	0.56, 2.38	16/24	1.43	0.46, 4.45

Abbreviations: NSAIDs, non-steroidal anti-inflammatory drugs; OR, odds ratio; 95% CI, 95% confidence interval.

[a] Adjusted for study, age, and sex.

[b] For Total Milk Products, Total Milk, Whole Milk, and Non-fat Milk, adjusted for study, age, sex, oxidative balance score, family history of colorectal cancer in first-degree relative, regular use of aspirin or nonsteroidal anti-inflammatory drugs, total energy intake, total fat intake (energy-adjusted), supplemental calcium intake.

[c] For Total Milk Product Residuals and Non-fat Milk Residuals, adjusted for study, age, sex, oxidative balance score, family history of colorectal cancer in first-degree relative, regular use of aspirin or nonsteroidal anti-inflammatory drugs, total energy intake, total fat intake (energy-adjusted), dietary calcium intake.

[d] For non-regular users of aspirin and NSAIDs in Total Milk Products, Total Milk, Whole Milk, and Non-fat Milk models, adjusted for study, age, sex, oxidative balance score, family history of colorectal cancer in first-degree relative, total energy intake, total fat intake (energy-adjusted), supplemental calcium intake.

[e] For non-regular users of aspirin and NSAIDs in Total Milk Product Residuals and Non-fat Milk Residuals models, adjusted for study, age, sex, oxidative balance score, family history of colorectal cancer in first-degree relative, total energy intake, total fat intake (energy-adjusted), dietary calcium intake.

[f] Whole milk consumption categorized as dichotomous due to small sample size.

Table 2.9. Stratified multivariable-adjusted associations of total calcium with incident, sporadic colorectal adenomas

	Total Calcium Quintiles	No. of Cases/ Controls	Initial Model[a]			Full Model[b]		
			OR	Lower 95% CI	Upper 95% CI	OR	Lower 95% CI	Upper 95% CI
Age								
High	1	108/179	1.00 (ref)			1.00 (ref)		
	2	107/198	1.01	0.75	1.38	0.96	0.68	1.36
	3	99/198	0.99	0.72	1.35	0.91	0.63	1.31
	4	83/203	0.86	0.62	1.18	0.72	0.49	1.05
	5	94/212	1.01	0.74	1.39	0.85	0.57	1.28
Low	1	73/224	1.00 (ref)			1.00 (ref)		
	2	73/206	1.06	0.74	1.52	1.09	0.74	1.61
	3	51/207	0.76	0.51	1.12	0.77	0.49	1.19
	4	45/201	0.69	0.46	1.03	0.69	0.44	1.09
	5	53/192	0.86	0.58	1.26	0.86	0.53	1.41
Sex								
Male	1	105/173	1.00 (ref)			1.00 (ref)		
	2	110/174	1.11	0.82	1.51	1.02	0.71	1.48
	3	90/174	0.93	0.68	1.29	0.85	0.57	1.27
	4	81/174	0.87	0.63	1.21	0.81	0.54	1.22
	5	96/174	1.07	0.78	1.48	0.87	0.56	1.35
Female	1	76/230	1.00 (ref)			1.00 (ref)		
	2	70/230	0.94	0.66	1.35	1.07	0.71	1.59
	3	60/231	0.85	0.58	1.23	0.91	0.60	1.40
	4	47/230	0.68	0.46	1.01	0.66	0.42	1.04
	5	51/230	0.79	0.54	1.16	0.86	0.54	1.36
Body Mass Index, kg/m^2								
High	1	99/194	1.00 (ref)			1.00 (ref)		
	2	100/213	1.02	0.74	1.39	0.99	0.71	1.38
	3	88/198	0.95	0.69	1.31	0.92	0.64	1.31
	4	74/198	0.81	0.58	1.13	0.76	0.52	1.10
	5	63/196	0.71	0.50	1.01	0.66	0.44	0.99
Low	1	82/209	1.00 (ref)			1.00 (ref)		
	2	80/191	1.07	0.76	1.52	1.08	0.75	1.55
	3	62/207	0.83	0.57	1.19	0.80	0.54	1.20
	4	54/206	0.77	0.53	1.12	0.71	0.47	1.08
	5	84/208	1.28	0.91	1.81	1.18	0.77	1.80
Family History								
Yes	1	29/66	1.00 (ref)			1.00 (ref)		
	2	35/66	1.28	0.76	2.14	1.49	0.77	2.89
	3	27/75	1.01	0.58	1.75	1.03	0.50	2.13
	4	16/84	0.62	0.33	1.17	0.52	0.24	1.15
	5	26/67	1.11	0.63	1.94	1.50	0.65	3.50

Table 2.9. (cont.) Stratified multivariable-adjusted associations of total calcium with incident, sporadic colorectal adenomas

	Total Calcium Quintiles	No. of Cases/ Controls	Initial Model[a]			Full Model[b]		
			OR	Lower 95% CI	Upper 95% CI	OR	Lower 95% CI	Upper 95% CI
Family History (cont.)								
No	1	152/337	1.00 (ref)			1.00 (ref)		
	2	145/338	0.99	0.76	1.30	0.97	0.72	1.31
	3	123/330	0.88	0.66	1.16	0.86	0.62	1.18
	4	112/320	0.83	0.62	1.10	0.80	0.58	1.12
	5	121/337	0.93	0.70	1.23	0.80	0.56	1.13
Oxidative Balance Score								
High	1	60/157	1.00 (ref)			1.00 (ref)		
	2	67/185	1.18	0.80	1.73	1.12	0.74	1.68
	3	60/233	1.10	0.74	1.62	0.92	0.60	1.42
	4	65/211	1.27	0.86	1.86	1.01	0.66	1.56
	5	69/220	1.39	0.95	2.04	0.97	0.61	1.54
Low	1	121/246	1.00 (ref)			1.00 (ref)		
	2	113/219	0.97	0.72	1.30	1.04	0.74	1.45
	3	90/172	0.80	0.59	1.09	1.00	0.69	1.45
	4	63/193	0.57	0.40	0.79	0.64	0.43	0.96
	5	78/184	0.75	0.54	1.03	0.81	0.53	1.24
Total Energy Intake, kcal								
High	1	28/62	1.00 (ref)			1.00 (ref)		
	2	73/161	2.87	1.80	4.58	1.95	1.19	3.19
	3	93/240	3.83	2.43	6.05	2.04	1.25	3.33
	4	82/236	3.56	2.24	5.65	1.77	1.07	2.92
	5	119/313	5.53	3.53	8.66	1.99	1.19	3.33
Low	1	153/341	1.00 (ref)			1.00 (ref)		
	2	107/243	0.72	0.54	0.95	1.24	0.90	1.71
	3	57/165	0.39	0.28	0.55	0.96	0.66	1.41
	4	46/168	0.32	0.23	0.47	0.75	0.50	1.13
	5	28/91	0.21	0.13	0.32	0.73	0.45	1.18
Total Fat Intake, g								
High	1	54/101	1.00 (ref)			1.00 (ref)		
	2	87/187	1.70	1.17	2.48	1.23	0.82	1.85
	3	90/231	1.87	1.29	2.72	1.08	0.71	1.64
	4	78/225	1.68	1.15	2.46	0.95	0.62	1.47
	5	113/267	2.57	1.79	3.70	1.13	0.73	1.77
Low	1	127/302	1.00 (ref)			1.00 (ref)		
	2	93/217	0.76	0.56	1.03	1.20	0.85	1.69
	3	60/174	0.50	0.36	0.70	1.08	0.73	1.59
	4	50/179	0.43	0.30	0.61	0.84	0.56	1.27
	5	34/137	0.31	0.20	0.46	0.87	0.54	1.40

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

[a] Adjusted for study.

[b] Adjusted for study, age, sex, total energy intake, oxidative balance score, family history of colorectal cancer in first-degree relative, regular use of aspirin or nonsteroidal anti-inflammatory drugs, total fat intake (energy-adjusted). Stratification variable was excluded from the model.

Table 2.10. Multivariable-adjusted associations of magnesium and phosphorus intakes with incident, sporadic colorectal adenomas

Quintiles	No. of Cases/ Controls (n=787/ 2,033)	Initial Model[a]			Full Model[b]		
		OR	Lower 95% CI	Upper 95% CI	OR	Lower 95% CI	Upper 95% CI
Total Magnesium							
1	156/406	1.00 (ref)			1.00 (ref)		
2	170/407	1.10	0.84	1.44	1.10	0.82	1.46
3	155/407	1.07	0.81	1.40	1.07	0.78	1.46
4	171/407	1.10	0.84	1.44	1.10	0.77	1.56
5	135/406	0.88	0.66	1.16	0.88	0.55	1.39
	P_{trend}[c]	0.58			0.56		
Total Phosphorus							
1	159/406	1.00 (ref)			1.00 (ref)		
2	169/407	1.06	0.82	1.38	1.05	0.78	1.39
3	148/407	0.97	0.74	1.26	0.98	0.71	1.35
4	165/407	1.11	0.86	1.45	1.12	0.78	1.62
5	146/406	0.99	0.76	1.30	0.93	0.56	1.55
	P_{trend}[c]	0.13			0.99		
Calcium:Magnesium							
1	190/406	1.00 (ref)			1.00 (ref)		
2	166/406	0.93	0.72	1.20	0.94	0.72	1.22
3	137/408	0.80	0.62	1.05	0.83	0.63	1.09
4	154/406	0.92	0.71	1.19	0.89	0.68	1.16
5	140/407	0.84	0.65	1.10	0.82	0.62	1.08
	P_{trend}[c]	0.0028			0.19		
Calcium + Magnesium							
1	187/406	1.00 (ref)			1.00 (ref)		
2	170/407	0.93	0.72	1.20	0.90	0.69	1.18
3	159/407	0.90	0.70	1.17	0.87	0.65	1.16
4	124/407	0.73	0.55	0.95	0.67	0.49	0.91
5	147/406	0.90	0.69	1.17	0.82	0.59	1.15
	P_{trend}[c]	0.06			0.10		
Calcium:Phosphorus							
1	187/404	1.00 (ref)			1.00 (ref)		
2	164/408	0.94	0.73	1.22	0.96	0.73	1.25
3	162/407	0.98	0.76	1.27	1.01	0.77	1.33
4	150/407	0.96	0.74	1.25	0.93	0.71	1.23
5	124/407	0.76	0.58	0.99	0.78	0.58	1.03
	P_{trend}[c]	<0.0001			0.21		
Magnesium:Phosphorus							
1	159/405	1.00 (ref)			1.00 (ref)		
2	148/408	0.92	0.70	1.20	0.94	0.71	1.25
3	182/406	1.18	0.91	1.52	1.22	0.93	1.61
4	137/407	0.85	0.65	1.12	0.93	0.70	1.25
5	161/407	0.96	0.74	1.25	1.08	0.81	1.44
	P_{trend}[c]	0.16			0.68		

Table 2.10. (cont.) Multivariable-adjusted associations of magnesium and phosphorus intakes with incident, sporadic colorectal adenomas

Quintiles	No. of Cases/ Controls (n=787/ 2,033)	Initial Model[a]			Full Model[b]		
		OR	Lower 95% CI	Upper 95% CI	OR	Lower 95% CI	Upper 95% CI
(Calcium + Magnesium) : Phosphorus							
1	186/405	1.00 (ref)			1.00 (ref)		
2	143/407	0.84	0.65	1.10	0.85	0.64	1.12
3	183/407	1.12	0.87	1.45	1.12	0.86	1.46
4	153/407	0.98	0.75	1.27	0.96	0.73	1.27
5	122/407	0.75	0.57	0.98	0.78	0.58	1.04
	P_{trend}[c]	<0.0001			0.28		

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

[a] Adjusted for study, age, and sex.

[b] For Magnesium: adjusted for study, age, sex, total energy intake, oxidative balance score, family history of colorectal cancer in first-degree relative, regular use of aspirin or nonsteroidal anti-inflammatory drugs, total fat intake (energy-adjusted), total calcium intake, total phosphorus intake.
For Phosphorus: Adjusted for study, age, sex, total energy intake, oxidative balance score, family history of colorectal cancer in first-degree relative, regular use of aspirin or nonsteroidal anti-inflammatory drugs, total fat intake (energy-adjusted), total calcium intake
For Calcium:Magnesium, Calcium + Magnesium, Calcium:Phosphorus, Magnesium:Phosphorus, and (Calcium+Magnesium):Phosphorus: Adjusted for study, age, sex, total energy intake, oxidative balance score, family history of colorectal cancer in first-degree relative, regular use of aspirin or nonsteroidal anti-inflammatory drugs, total fat intake (energy-adjusted)

[c] P_{trend} calculated using sex-specific median of each quintile of calcium intake as a continuous variable.

Table 3.4. Stratified relative treatment effects of growth factor concentrations among colorectal adenoma patients in response to calcium supplementation

	Relative Treatment Effect[a]			Relative Treatment Effect[a]		
	Mean	SE	*P*	Mean	SE	*P*
Sex	**Male**			**Female**		
IGF-1						
Placebo						
1 g calcium	1.05	1.05	0.43	0.97	1.09	0.76
2 g calcium	1.04	1.06	0.50	1.02	1.09	0.77
IGFBP-3						
Placebo						
1 g calcium	1.03	1.07	0.70	1.03	1.08	0.73
2 g calcium	1.04	1.07	0.58	0.95	1.08	0.52
IGF-1 : IGFBP-3						
Placebo						
1 g calcium	1.02	1.09	0.79	0.95	1.12	0.67
2 g calcium	1.01	1.09	0.95	1.07	1.12	0.52
Age, years	**< 60**			**≥ 60**		
IGF-1						
Placebo						
1 g calcium	1.08	1.07	0.26	0.98	0.81	0.68
2 g calcium	1.04	1.06	0.66	1.04	1.07	0.58
IGFBP-3						
Placebo						
1 g calcium	1.01	1.07	0.91	1.04	1.07	0.55
2 g calcium	1.02	1.06	0.80	0.99	1.08	0.92
IGF-1 : IGFBP-3						
Placebo						
1 g calcium	1.07	1.09	0.42	0.94	1.11	0.57
2 g calcium	1.01	1.08	0.88	1.05	1.11	0.66
Body Mass Index, kg/m^2	**< 30**			**≥ 30**		
IGF-1						
Placebo						
1 g calcium	1.01	1.08	0.91	1.03	1.06	0.65
2 g calcium	0.94	1.08	0.40	1.07	1.06	0.26
IGFBP-3						
Placebo						
1 g calcium	1.08	1.06	0.23	0.94	1.08	0.43
2 g calcium	1.02	1.06	0.76	0.99	1.08	0.90
IGF-1 : IGFBP-3						
Placebo						
1 g calcium	0.96	1.09	0.60	1.06	1.12	0.60
2 g calcium	1.06	1.09	0.51	0.94	1.13	0.62

Table 3.4. (cont.) Stratified relative treatment effects of growth factor concentrations among colorectal adenoma patients in response to calcium supplementation

	Relative Treatment Effect[a]			Relative Treatment Effect[a]		
	Mean	**SE**	***P***	**Mean**	**SE**	***P***
NSAID/Aspirin use	**Yes**			**No**		
IGF-1						
Placebo						
1 g calcium	0.97	1.08	0.70	1.04	1.06	0.46
2 g calcium	1.03	1.09	0.74	1.03	1.06	0.55
IGFBP-3						
Placebo						
1 g calcium	1.11	1.08	0.19	0.99	1.06	0.91
2 g calcium	0.98	1.09	0.82	1.01	1.06	0.87
IGF-1 : IGFBP-3						
Placebo						
1 g calcium	0.88	1.12	0.27	1.05	1.09	0.53
2 g calcium	1.06	1.13	0.64	1.03	1.08	0.74
Current Smoker	**Yes**			**No**		
IGF-1						
Placebo						
1 g calcium	1.21	1.14	0.17	1.00	1.05	0.99
2 g calcium	1.01	1.13	0.93	1.03	1.05	0.51
IGFBP-3						
Placebo						
1 g calcium	1.12	1.13	0.36	1.02	1.05	0.78
2 g calcium	1.07	1.11	0.53	0.99	1.06	0.81
IGF-1 : IGFBP-3						
Placebo						
1 g calcium	1.08	1.21	0.68	0.99	1.07	0.87
2 g calcium	0.95	1.19	0.75	1.05	1.08	0.49
Consume Alcohol	**Yes**			**No**		
IGF-1						
Placebo						
1 g calcium	1.19	1.15	0.21	1.00	1.05	0.94
2 g calcium	0.97	1.13	0.79	1.05	1.05	0.37
IGFBP-3						
Placebo						
1 g calcium	1.12	1.13	0.33	1.01	1.06	0.80
2 g calcium	1.06	1.11	0.58	0.99	1.06	0.83
IGF-1 : IGFBP-3						
Placebo						
1 g calcium	1.05	1.20	0.78	1.00	1.07	0.95
2 g calcium	0.92	1.18	0.61	1.06	1.08	0.41

Table 3.4. (cont.) Stratified relative treatment effects of growth factor concentrations among colorectal adenoma patients in response to calcium supplementation

	Relative Treatment Effect[a]			Relative Treatment Effect[a]		
	Mean	**SE**	***P***	**Mean**	**SE**	***P***
Physical Activity[c]	**High**			**Moderate**		
IGF-1						
Placebo						
1 g calcium	1.06	1.07	0.41	0.94	1.11	0.52
2 g calcium	1.05	1.08	0.54	1.01	1.10	0.93
IGFBP-3						
Placebo						
1 g calcium	1.01	1.07	0.92	0.77	0.57	0.09
2 g calcium	1.12	1.08	0.15	0.91	0.68	0.53
IGF-1 : IGFBP-3						
Placebo						
1 g calcium	1.05	1.09	0.55	0.96	1.15	0.77
2 g calcium	0.94	1.10	0.53	1.11	1.14	0.42
Physical Activity[c]	**Low**					
IGF-1						
Placebo						
1 g calcium	1.06	1.08	0.48			
2 g calcium	1.03	1.08	0.69			
IGFBP-3						
Placebo						
1 g calcium	1.08	1.09	0.39			
2 g calcium	1.02	1.09	0.80			
IGF-1 : IGFBP-3						
Placebo						
1 g calcium	0.98	1.12	0.88			
2 g calcium	1.01	1.12	0.95			
Total Energy Intake[b]	**High**			**Low**		
IGF-1						
Placebo						
1 g calcium	0.99	1.07	0.93	1.06	1.07	0.45
2 g calcium	0.99	1.06	0.87	1.09	1.08	0.27
IGFBP-3						
Placebo						
1 g calcium	1.08	1.06	0.18	0.97	1.08	0.75
2 g calcium	1.04	1.05	0.44	0.96	1.09	0.64
IGF-1 : IGFBP-3						
Placebo						
1 g calcium	0.92	1.09	0.32	1.08	1.11	0.47
2 g calcium	0.95	1.08	0.53	1.13	1.12	0.29

Table 3.4. (cont.) Stratified relative treatment effects of growth factor concentrations among colorectal adenoma patients in response to calcium supplementation

	Relative Treatment Effect[a]			Relative Treatment Effect[a]		
	Mean	**SE**	***P***	**Mean**	**SE**	***P***
Total Calcium Intake[b]	**High**			**Low**		
IGF-1						
Placebo						
1 g calcium	0.98	1.07	0.81	1.04	1.07	0.54
2 g calcium	1.07	1.06	0.29	1.00	1.08	0.99
IGFBP-3						
Placebo						
1 g calcium	1.06	1.06	0.39	0.99	1.08	0.86
2 g calcium	1.06	1.06	0.35	0.94	1.09	0.46
IGF-1 : IGFBP-3						
Placebo						
1 g calcium	0.94	1.09	0.45	1.06	1.11	0.61
2 g calcium	1.02	1.08	0.85	1.07	1.12	0.57
Dietary Calcium Intake[b]	**High**			**Low**		
IGF-1						
Placebo						
1 g calcium	0.97	1.07	0.61	1.07	1.07	0.32
2 g calcium	1.04	1.06	0.56	1.03	1.08	0.66
IGFBP-3						
Placebo						
1 g calcium	1.04	1.06	0.57	1.00	1.08	0.95
2 g calcium	1.06	1.06	0.31	0.94	1.09	0.46
IGF-1 : IGFBP-3						
Placebo						
1 g calcium	0.94	1.09	0.44	1.07	1.11	0.55
2 g calcium	0.98	1.08	0.80	1.11	1.12	0.40
Supplemental Calcium Use	**Yes**			**No**		
IGF-1						
Placebo						
1 g calcium	0.92	1.10	0.42	1.05	1.06	0.41
2 g calcium	1.04	1.11	0.68	1.03	1.05	0.57
IGFBP-3						
Placebo						
1 g calcium	1.31	1.11	0.01	0.96	1.05	0.45
2 g calcium	1.06	1.11	0.59	0.99	1.05	0.88
IGF-1 : IGFBP-3						
Placebo						
1 g calcium	0.71	1.15	0.02	1.09	1.08	0.25
2 g calcium	0.99	1.16	0.94	1.04	1.08	0.57

Abbreviations: SE, standard error; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; IGF-1:IGFBP-3, molar ratio of IGF-1 to IGFBP-3; NSAID, non-steroidal anti-inflammatory drug.

[a] Relative treatment effect from SAS Institute's Mixed Procedure (SAS version 9.4; SAS Institute, Cary, NC) defined as [(treatment group follow-up mean) / (treatment group baseline mean)] / [(placebo follow-up mean) / (placebo baseline mean)].

[b] High/low categories determined using sex-specific medians.

[c] High, moderate, and low categories determined using sex-specific tertiles of physical activity.

Table 4.6. Stratified associations of supplemental calcium and vitamin D use with all-cause and cause-specific mortality among women by menopausal status, REGARDS

Pre-menopause	No Calcium or vitamin D[a] 1 (n = 414)	Calcium, no vitamin D 2 (n = 126)	Vitamin D, no calcium 3 (n = 10)	Calcium + vitamin D 3 (n = 556)
All-cause				
No. of deaths	18	9	1	30
Unadjusted HR (95% CI)	1.00	1.26 (0.55, 2.87)	2.08 (0.27, 16.26)	0.80 (0.44, 1.45)
Fully adjusted HR (95% CI)[b]	1.00	2.12 (0.83, 5.42)	1.30 (0.07, 24.63)	1.18 (0.61, 2.29)
Cardiovascular disease[c]				
No. of deaths	5	6	1	8
Unadjusted HR (95% CI)	1.00	3.87 (1.12, 13.36)	6.99 (0.72, 67.87)	0.70 (0.22, 2.21)
Fully adjusted HR (95% CI)[b]	1.00	25.65 (1.87, 351.89)	0.18 (0.00, 391.91)	1.00 (0.17, 5.86)
Cancer				
No. of deaths	7	1	0	10
Unadjusted HR (95% CI)	1.00	0.28 (0.03, 2.30)	--	0.73 (0.27, 1.95)
Fully adjusted HR (95% CI)[b]	1.00	0.25 (0.02, 2.55)	--	0.84 (0.29, 2.46)
Other				
No. of deaths	6	2	0	12
Unadjusted HR (95% CI)	1.00	0.88 (0.17, 4.51)	--	0.99 (0.37, 2.68)
Fully adjusted HR (95% CI)[b]	1.00	2.55 (0.38, 16.93)	--	1.59 (0.46, 5.53)

Post-menopause	No Calcium or vitamin D[a] 1 (n = 3,196)	Calcium, no vitamin D 2 (n = 1,642)	Vitamin D, no calcium 3 (n = 56)	Calcium + vitamin D 3 (n = 5,699)
All-causes				
No. of deaths	389	154	6	594
Unadjusted HR (95% CI)	1.00	0.65 (0.54, 0.78)	0.70 (0.31, 1.56)	0.72 (0.64, 0.82)
Fully adjusted HR (95% CI)[b]	1.00	0.82 (0.68, 0.99)	0.74 (0.33, 1.66)	0.91 (0.79, 1.04)
Cardiovascular disease[c]				
No. of deaths	111	47	4	183
Unadjusted HR (95% CI)	1.00	0.70 (0.50, 0.99)	1.62 (0.60, 4.40)	0.78 (0.62, 0.99)
Fully adjusted HR (95% CI)[b]	1.00	0.92 (0.65, 1.30)	1.85 (0.68, 5.08)	1.03 (0.80, 1.31)
Cancer				
No. of deaths	112	41	1	172
Unadjusted HR (95% CI)	1.00	0.62 (0.43, 0.88)	0.41 (0.06, 2.96)	0.73 (0.58, 0.93)
Fully adjusted HR (95% CI)[b]	1.00	0.72 (0.50, 1.04)	0.45 (0.06, 3.25)	0.86 (0.67, 1.10)
Other				
No. of deaths	166	66	1	239
Unadjusted HR (95% CI)	1.00	0.64 (0.48, 0.85)	0.27 (0.04, 1.93)	0.67 (0.55, 0.82)
Fully adjusted HR (95% CI)[b]	1.00	0.82 (0.61, 1.11)	0.28 (0.04, 2.00)	0.86 (0.70, 1.06)

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

[a] No supplemental calcium or vitamin D group designated as reference group.

[b] Adjusted for age, race, region, body mass index, smoking, alcohol, physical activity, non-steroidal anti-inflammatory drug and aspirin use, hormone replacement therapy use, education, annual income, total energy intake, fruit and vegetable intake, processed and red meat intake, dietary oxidative balance score

[c] Cardiovascular disease mortality includes deaths due to myocardial infarction, stroke, heart failure, sudden death, other cardiac causes, and other cardiovascular non-cardiac causes.

Table 4.7. Stratified associations of total calcium intake with all-cause and cause-specific mortality by various characteristics, REGARDS

	Total Calcium Quintiles					Total Calcium Quintiles				
SEX	***MALES***					***FEMALES***				
	1 (n = 1,890)	**2 (n = 1,893)**	**3 (n = 1,891)**	**4 (n = 1,892)**	**5 (n = 1,891)**	**1 (n = 2,394)**	**2 (n = 2,394)**	**3 (n = 2,394)**	**4 (n = 2,394)**	**5 (n = 2,394)**
All-cause										
No. of deaths	360	324	343	310	395	288	261	235	217	233
Adjusted HR	1.00	0.94	1.03	0.94	1.18	1.00	0.94	0.87	0.85	0.84
(95% CI)[a]		(0.80, 1.09)	(0.88, 1.20)	(0.79, 1.11)	(1.00, 1.39)		(0.79, 1.12)	(0.72, 1.05)	(0.70, 1.02)	(0.68, 1.04)
CVD disease[b]										
No. of deaths	124	110	113	100	127	100	69	80	72	62
Adjusted HR	1.00	0.90	0.94	0.83	1.07	1.00	0.78	0.90	0.89	0.73
(95% CI)[a]		(0.69, 1.17)	(0.71, 1.23)	(0.62, 1.12)	(0.81, 1.43)		(0.57, 1.08)	(0.65, 1.26)	(0.64, 1.24)	(0.50, 1.08)
Cancer										
No. of deaths	109	98	104	89	107	75	82	56	68	66
Adjusted HR	1.00	0.94	1.01	0.84	1.04	1.00	1.07	0.76	0.90	0.81
(95% CI)[a]		(0.71, 1.25)	(0.76, 1.35)	(0.61, 1.16)	(0.76, 1.42)		(0.77, 1.49)	(0.52, 1.11)	(0.63, 1.29)	(0.54, 1.20)
RACE	***WHITE***					***BLACK***				
	(n = 2,126)	**(n = 2,576)**	**(n = 2,850)**	**(n = 3,209)**	**(n = 3,498)**	**(n = 2,158)**	**(n = 1,711)**	**(n = 1,435)**	**(n = 1,077)**	**(n = 787)**
All-causes										
No. of deaths	331	351	1,372	388	512	317	234	206	139	116
Adjusted HR	1.00	0.89	0.87	0.82	0.97	1.00	1.01	1.12	1.00	1.07
(95% CI)[a]		(0.76, 1.03)	(0.74, 1.02)	(0.70, 0.97)	(0.83, 1.14)		(0.85, 1.21)	(0.92, 1.35)	(0.80, 1.24)	(0.84, 1.37)
CVD disease[b]										
No. of deaths	114	119	121	131	144	110	60	72	41	45
Adjusted HR	1.00	0.87	0.81	0.82	0.77	1.00	0.81	1.21	0.92	1.41
(95% CI)[a]		(0.67, 1.14)	(0.62, 1.06)	(0.62, 1.07)	(0.58, 1.03)		(0.58, 1.12)	(0.87, 1.67)	(0.63, 1.35)	(0.95, 2.11)
Cancer										
No. of deaths	104	105	104	114	140	80	75	56	43	33
Adjusted HR	1.00	0.86	0.78	0.77	0.89	1.00	1.17	1.08	1.05	0.99
(95% CI)[a]		(0.65, 1.14)	(0.59, 1.05)	(0.58, 1.04)	(0.66, 1.19)		(0.84, 1.62)	(0.74, 1.56)	(0.70, 1.58)	(0.62, 1.58)

Table 4.7. (cont.) Stratified associations of total calcium intake with all-cause and cause-specific mortality by various characteristics, REGARDS

REGION[c]	**Total Calcium Quintiles**					**Total Calcium Quintiles**				
	BELT **(n = 1,504)**	**(n = 1,501)**	**(n = 1,465)**	**(n = 1,492)**	**(n = 1,407)**	***BUCKLE*** **(n = 1,057)**	**(n = 995)**	**(n = 929)**	**(n = 894)**	**(n = 807)**
All-causes										
No. of deaths	227	207	198	185	220	138	122	118	99	119
Adjusted HR	1.00	1.00	0.93	0.83	1.06	1.00	0.92	0.96	0.81	1.04
(95% CI)[a]		(0.83, 1.22)	(0.75, 1.14)	(0.67, 1.03)	(0.85, 1.32)		(0.71, 1.18)	(0.73, 1.25)	(0.60, 1.08)	(0.78, 1.39)
CVD disease[b]										
No. of deaths	72	55	73	57	62	50	43	40	38	37
Adjusted HR	1.00	0.86	1.05	0.81	0.94	1.00	0.92	0.91	0.84	0.84
(95% CI)[a]		(0.60, 1.24)	(0.73, 1.50)	(0.55, 1.20)	(0.63, 1.41)		(0.60, 1.40)	(0.57, 1.44)	(0.52, 1.36)	(0.50, 1.39)
Cancer										
No. of deaths	64	60	47	51	61	36	34	35	29	35
Adjusted HR	1.00	1.00	0.78	0.75	1.00	1.00	0.99	1.14	0.97	1.31
(95% CI)[a]		(0.70, 1.45)	(0.52, 1.16)	(0.50, 1.14)	(0.66, 1.53)		(0.61, 1.63)	(0.69, 1.90)	(0.57, 1.67)	(0.76, 2.26)
REGION[c]	***NON-BELT*** **(n = 1,723)**	**(n = 1,791)**	**(n = 1,891)**	**(n = 1,900)**						
All-causes										
No. of deaths	283	256	262	243						
Adjusted HR	1.00	0.92	0.98	0.98	1.02					
(95% CI)[a]		(0.78, 1.10)	(0.81, 1.17)	(0.81, 1.18)	(0.84, 1.24)					
CVD disease[b]										
No. of deaths	102	81	80	77						
Adjusted HR	1.00	0.81	0.86	0.88	0.88					
(95% CI)[a]		(0.60, 1.09)	(0.62, 1.18)	(0.64, 1.23)	(0.63, 1.23)					
Cancer										
No. of deaths	84	86	78	77						
Adjusted HR	1.00	1.01	0.93	0.93	0.83					
(95% CI)[a]		(0.74, 1.38)	(0.66, 1.29)	(0.66, 1.31)	(0.58, 1.19)					

Table 4.7. (cont.) Stratified associations of total calcium intake with all-cause and cause-specific mortality by various characteristics, REGARDS

	Total Calcium Quintiles					Total Calcium Quintiles				
BODY MASS INDEX	***LOW***[d] **(n = 1,912)**	**(n = 1,936)**	**(n = 2,111)**	**(n = 2,307)**	**(n = 2,483)**	***HIGH*** **(n = 2,372)**	**(n = 2,351)**	**(n = 2,174)**	**(n = 1,979)**	**(n = 1,802)**
All-causes										
No. of deaths	326	291	318	299	381	322	294	260	228	247
Adjusted HR	1.00	0.96	0.96	0.92	1.07	1.00	0.96	0.95	0.85	1.01
(95% CI)[a]		(0.82, 1.13)	(0.81, 1.13)	(0.78, 1.10)	(0.90, 1.27)		(0.81, 1.13)	(0.80, 1.14)	(0.70, 1.02)	(0.83, 1.23)
CVD disease[b]										
No. of deaths	112	77	113	83	105	112	102	80	89	84
Adjusted HR	1.00	0.77	1.01	0.77	0.86	1.00	0.95	0.83	0.93	0.94
(95% CI)[a]		(0.57,1.03)	(0.76, 1.34)	(0.56, 1.05)	(0.63, 1.17)		(0.72, 1.25)	(0.61, 1.13)	(0.68, 1.27)	(0.68, 1.32)
Cancer										
No. of deaths	104	101	84	93	105	80	79	76	64	68
Adjusted HR	1.00	0.99	0.75	0.80	0.82	1.00	1.08	1.19	1.05	1.25
(95% CI)[a]		(0.74, 1.31)	(0.55, 1.02)	(0.58, 1.09)	(0.60, 1.14)		(0.78, 1.49)	(0.84, 1.68)	(0.73, 1.51)	(0.86, 1.82)
NSAID/ASPIRIN USE	***NO*** **(n = 2,264)**	**(n = 2,121)**	**(n = 2,097)**	**(n = 1,873)**	**(n = 1,807)**	***YES*** **(n = 2,020)**	**(n = 2,166)**	**(n = 2,188)**	**(n = 2,413)**	**(n = 2,478)**
All-causes										
No. of deaths	330	239	249	217	245	318	346	329	310	383
Adjusted HR	1.00	0.83	0.87	0.92	0.98	1.00	1.04	1.04	0.89	1.09
(95% CI)[a]		(0.70, 0.99)	(0.73, 1.05)	(0.76, 1.12)	(0.80, 1.19)		(0.89, 1.22)	(0.88, 1.22)	(0.75, 1.05)	(0.92, 1.30)
CVD disease[b]										
No. of deaths	99	59	85	65	64	125	120	108	107	125
Adjusted HR	1.00	0.68	0.95	0.89	0.81	1.00	0.97	0.90	0.86	0.98
(95% CI)[a]		(0.49, 0.95)	(0.69, 1.32)	(0.63, 1.27)	(0.55, 1.17)		(0.75, 1.26)	(0.69, 1.19)	(0.64, 1.14)	(0.74, 1.31)
Cancer										
No. of deaths	104	87	72	67	83	80	93	88	90	90
Adjusted HR	1.00	0.95	0.79	0.83	1.02	1.00	1.06	1.04	0.93	0.93
(95% CI)[a]		(0.70, 1.27)	(0.57, 1.09)	(0.59, 1.17)	(0.72, 1.43)		(0.78, 1.45)	(0.76, 1.44)	(0.66, 1.29)	(0.65, 1.31)

Table 4.7. (cont.) Stratified associations of total calcium intake with all-cause and cause-specific mortality by various characteristics, REGARDS

	Total Calcium Quintiles					Total Calcium Quintiles				
TOTAL ENERGY INTAKE	***LOW[d]*** **(n = 3,573)**	**(n = 2,286)**	**(n = 1,674)**	**(n = 1,860)**	**(n = 1,321)**	***HIGH*** **(n = 711)**	**(n = 2,001)**	**(n = 2,611)**	**(n = 2,426)**	**(n = 2,964)**
All-causes										
No. of deaths	545	332	245	228	226	103	253	333	299	402
Adjusted HR	1.00	0.93	0.96	0.96	1.05	1.00	0.91	0.89	0.78	0.95
(95% CI)[a]		(0.81, 1.07)	(0.82, 1.12)	(0.82, 1.13)	(0.88, 1.24)		(0.72, 1.15)	(0.71, 1.12)	(0.62, 0.99)	(0.76, 1.20)
CVD disease[b]										
No. of deaths	191	109	91	80	61	33	70	102	92	128
Adjusted HR	1.00	0.84	0.96	0.95	0.76	1.00	0.78	0.79	0.69	0.88
(95% CI)[a]		(0.66, 1.07)	(0.74, 1.24)	(0.72, 1.24)	(0.56, 1.03)		(0.51, 1.18)	(0.53, 1.18)	(0.46, 1.05)	(0.59, 1.33)
Cancer										
No. of deaths	152	93	62	59	66	32	87	98	98	107
Adjusted HR	1.00	0.96	0.86	0.85	1.10	1.00	1.04	0.91	0.88	0.87
(95% CI)[a]		(0.74, 1.25)	(0.63, 1.16)	(0.62, 1.17)	(0.81, 1.50)		(0.69, 1.56)	(0.61, 1.38)	(0.58, 1.34)	(0.57, 1.32)
TOTAL FAT INTAKE	***LOW[d]*** **(n = 3,618)**	**(n = 2,517)**	**(n = 2,100)**	**(n = 2,277)**	**(n = 1,922)**	***HIGH*** **(n = 666)**	**(n = 1,770)**	**(n = 2,185)**	**(n = 2,009)**	**(n = 2,363)**
All-causes										
No. of deaths	538	370	290	269	301	110	215	288	258	327
Adjusted HR	1.00	1.01	0.98	0.98	1.07	1.00	0.82	0.87	0.74	0.93
(95% CI)[a]		(0.88, 1.16)	(0.84, 1.15)	(0.83, 1.14)	(0.90, 1.25)		(0.65, 1.03)	(0.69, 1.09)	(0.58, 0.95)	(0.72, 1.18)
CVD disease[b]										
No. of deaths	182	118	107	94	84	42	61	86	78	105
Adjusted HR	1.00	0.97	1.09	1.05	0.91	1.00	0.57	0.60	0.52	0.67
(95% CI)[a]		(0.76, 1.23)	(0.84, 1.41)	(0.80, 1.38)	(0.68, 1.23)		(0.38, 0.86)	(0.41, 0.90)	(0.34, 0.79)	(0.45, 1.02)
Cancer										
No. of deaths	151	106	74	72	83	33	74	86	85	90
Adjusted HR	1.00	1.03	0.87	0.88	1.02	1.00	0.97	0.94	0.87	0.90
(95% CI)[a]		(0.79, 1.33)	(0.64, 1.16)	(0.65, 1.19)	(0.75, 1.39)		(0.64, 1.47)	(0.62, 1.44)	(0.56, 1.35)	(0.58, 1.42)

Table 4.7. (cont.) Stratified associations of total calcium intake with all-cause and cause-specific mortality by various characteristics, REGARDS

	Total Calcium Quintiles					Total Calcium Quintiles				
SUPPLEMENTAL CALCIUM USE	***NO*** **(n = 3,166)**	**(n = 2,214)**	**(n = 1,603)**	**(n = 861)**	**(n = 270)**	***YES*** **(n = 1,118)**	**(n = 2,073)**	**(n = 2,682)**	**(n = 3,425)**	**(n = 4,015)**
All-causes										
No. of deaths	494	300	241	141	54	154	285	337	386	574
Adjusted HR	1.00	0.91	0.99	0.91	1.31	1.00	1.02	1.00	0.95	1.07
(95% CI)[a]		(0.78, 1.06)	(0.82, 1.18)	(0.72, 1.14)	(0.94, 1.82)		(0.84, 1.25)	(0.82, 1.22)	(0.78, 1.16)	(0.88, 1.31)
CVD disease[b]										
No. of deaths	169	91	76	42	21	55	88	117	130	168
Adjusted HR	1.00	0.83	0.94	0.82	1.62	1.00	0.89	0.96	0.91	0.86
(95% CI)[a]		(0.63, 1.09)	(0.68, 1.29)	(0.54, 1.24)	(0.94, 2.79)		(0.63, 1.26)	(0.68, 1.34)	(0.65, 1.27)	(0.61, 1.21)
Cancer										
No. of deaths	144	92	71	49	15	40	88	89	108	158
Adjusted HR	1.00	0.88	0.87	0.86	0.92	1.00	1.25	1.06	1.05	1.23
(95% CI)[a]		(0.66, 1.16)	(0.62, 1.21)	(0.57, 1.29)	(0.49, 1.71)		(0.85, 1.83)	(0.72, 1.56)	(0.71, 1.54)	(0.84, 1.81)

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval; CVD, cardiovascular disease.

[a] Adjusted for age, sex, race, region, body mass index, smoking, alcohol, physical activity, non-steroidal anti-inflammatory drug and aspirin use, hormone replacement therapy use, education, annual income, total energy intake, fruit and vegetable intake, processed and red meat intake, dietary oxidative balance score. Stratification variable was excluded from the model.

[b] Cardiovascular disease mortality includes deaths due to myocardial infarction, stroke, heart failure, sudden death, other cardiac causes, and other cardiovascular non-cardiac causes.

[c] Stroke Belt defined as Alabama, Arkansas, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, and Tennessee; Buckle defined as Georgia, North Carolina, and South Carolina; otherwise, live in other continental US states.

[d] Low/high categories created using sex-specific medians.

Table 4.8. Associations of calcium intakes with all-cause and cause-specific mortality, adjusted for co-morbidities, REGARDS

	Quintiles				
	1	2	3	4	5
Total Calcium	**(n = 4,284)**	**(n = 4,287)**	**(n = 4,285)**	**(n = 4,286)**	**(n = 4,285)**
All-cause					
No. of deaths	648	585	578	527	628
Adjusted HR (95% CI)[a]	1.00	0.92 (0.82, 1.04)	0.93 (0.83, 1.05)	0.89 (0.78, 1.01)	1.03 (0.91, 1.17)
Cardiovascular disease[b]					
No. of deaths	224	179	193	172	189
Adjusted HR (95% CI)[a]	1.00	0.84 (0.68, 1.03)	0.91 (0.74, 1.12)	0.87 (0.70, 1.08)	0.93 (0.74, 1.17)
Cancer					
No. of deaths	184	180	160	157	173
Adjusted HR (95% CI)[a]	1.00	0.99 (0.80, 1.22)	0.89 (0.71, 1.12)	0.86 (0.68, 1.09)	0.93 (0.73, 1.19)
Other					
No. of deaths	240	226	225	198	266
Adjusted HR (95% CI)[a]	1.00	0.96 (0.80, 1.16)	1.00 (0.82, 1.22)	0.92 (0.75, 1.14)	1.21 (0.99, 1.49)
Dietary Calcium	**(n = 4,282)**	**(n = 4,285)**	**(n = 4,289)**	**(n = 4,286)**	**(n = 4,285)**
All-causes					
No. of deaths	635	588	571	591	581
Adjusted HR (95% CI)[a]	1.00	0.96 (0.86, 1.08)	0.93 (0.82, 1.05)	0.96 (0.85, 1.09)	0.93 (0.80, 1.07)
Cardiovascular disease[c]					
No. of deaths	219	192	170	187	189
Adjusted HR (95% CI)[a]	1.00	0.94 (0.77, 1.15)	0.82 (0.66, 1.02)	0.92 (0.74, 1.15)	0.92 (0.71, 1.18)
Cancer					
No. of deaths	174	181	179	156	164
Adjusted HR (95% CI)[a]	1.00	1.05 (0.85, 1.31)	1.03 (0.83, 1.30)	0.89 (0.69, 1.14)	0.92 (0.70, 1.21)
Other					
No. of deaths	242	215	222	248	228
Adjusted HR (95% CI)[a]	1.00	0.93 (0.77, 1.12)	0.94 (0.77, 1.14)	1.06 (0.86, 1.29)	0.95 (0.75, 1.20)
Supplemental Calcium[c]	**(n = 8,114)**	**(n = 3,360)**	**(n = 4,208)**	**(n = 1,999)**	**(n = 3,746)**
All-causes					
No. of deaths	1,230	411	582	226	517
Adjusted HR (95% CI)[a]	1.00	1.02 (0.90, 1.14)	0.97 (0.88, 1.07)	0.97 (0.84, 1.12)	1.09 (0.98, 1.22)
Cardiovascular disease[c]					
No. of deaths	399	134	195	76	153
Adjusted HR (95% CI)[a]	1.00	1.09 (0.89, 1.33)	1.01 (0.85, 1.20)	1.05 (0.82, 1.36)	1.04 (0.85, 1.26)
Cancer					
No. of deaths	371	114	163	62	144
Adjusted HR (95% CI)[a]	1.00	0.92 (0.74, 1.14)	0.85 (0.71, 1.03)	0.84 (0.64, 1.11)	0.94 (0.77, 1.16)
Other					
No. of deaths	460	163	224	88	220
Adjusted HR (95% CI)[a]	1.00	1.05 (0.87, 1.26)	1.03 (0.87, 1.21)	1.00 (0.79, 1.26)	1.27 (1.07, 1.51)

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

[a] Adjusted for age, sex, race, region, body mass index, smoking, alcohol, physical activity, non-steroidal anti-inflammatory drug and aspirin use, hormone replacement therapy use, education, annual income, total energy intake, fruit and vegetable intake, processed and red meat intake, dietary oxidative balance score, history of cardiovascular disease, diabetes, or cancer diagnosis.

[b] Cardiovascular disease mortality includes deaths due to myocardial infarction, stroke, heart failure, sudden death, other cardiac causes, and other cardiovascular non-cardiac causes.

[c] Supplemental calcium analyzed as 5 categories of intake with no intake as the reference category and remaining 4 categories created as sex-specific quartiles of intake.

Table 4.9. Associations of milk intakes with all-cause and cause-specific mortality, adjusted for co-morbidities, REGARDS

	5 Categories[a]				
	1	2	3	4	5
Whole Milk	**(n = 18,429)**	**(n = 738)**	**(n = 760)**	**(n = 748)**	**(n = 752)**
All-causes					
No. of deaths	2,429	136	125	136	140
Adjusted HR (95% CI)[a]	1.00	1.34 (1.12, 1.61)	1.11 (0.93, 1.34)	1.11 (0.93, 1.33)	1.14 (0.95, 1.36)
Cardiovascular disease[c]					
No. of deaths	806	40	34	45	32
Adjusted HR (95% CI)[a]	1.00	1.28 (0.92, 1.78)	0.94 (0.66, 1.34)	1.19 (0.87, 1.62)	0.80 (0.55, 1.16)
Cancer					
No. of deaths	689	35	41	34	55
Adjusted HR (95% CI)[a]	1.00	1.15 (0.81, 1.63)	1.22 (0.89, 1.68)	0.91 (0.64, 1.29)	1.57 (1.18, 2.10)
Other					
No. of deaths	934	61	50	57	53
Adjusted HR (95% CI)[a]	1.00	1.59 (1.21, 2.08)	1.19 (0.89, 1.59)	1.23 (0.93, 1.61)	1.09 (0.82, 1.45)
Low-Fat Milk	**(n = 9,114)**	**(n = 3,074)**	**(n = 3,087)**	**(n = 3,074)**	**(n = 3,078)**
All-causes					
No. of deaths	1,236	387	414	434	495
Adjusted HR (95% CI)[a]	1.00	1.01 (0.89, 1.15)	1.00 (0.89, 1.13)	1.00 (0.89, 1.13)	1.10 (0.98, 1.23)
Cardiovascular disease[c]					
No. of deaths	369	140	139	147	162
Adjusted HR (95% CI)[a]	1.00	1.22 (0.98, 1.51)	1.11 (0.90, 1.36)	1.10 (0.90, 1.35)	1.23 (1.01, 1.49)
Cancer					
No. of deaths	372	108	123	135	116
Adjusted HR (95% CI)[a]	1.00	1.03 (0.82, 1.30)	1.15 (0.93, 1.42)	1.15 (0.93, 1.42)	0.98 (0.79, 1.23)
Other					
No. of deaths	495	139	152	152	217
Adjusted HR (95% CI)[a]	1.00	0.84 (0.68, 1.04)	0.82 (0.67, 1.00)	0.83 (0.69, 1.01)	1.08 (0.91, 1.28)
Non-Fat Milk	**(n = 17,749)**	**(n = 917)**	**(n = 920)**	**(n = 906)**	**(n = 935)**
All-causes					
No. of deaths	2,575	98	92	110	91
Adjusted HR (95% CI)[a]	1.00	0.92 (0.75, 1.13)	0.75 (0.60, 0.92)	1.01 (0.83, 1.22)	0.75 (0.61, 0.93)
Cardiovascular disease[c]					
No. of deaths	829	36	26	38	28
Adjusted HR (95% CI)[a]	1.00	0.98 (0.70, 1.37)	0.63 (0.43, 0.94)	1.04 (0.74, 1.44)	0.72 (0.49, 1.05)
Cancer					
No. of deaths	735	22	26	40	31
Adjusted HR (95% CI)[a]	1.00	0.74 (0.48, 1.14)	0.75 (0.50, 1.12)	1.26 (0.91, 1.74)	0.89 (0.62, 1.28)
Other					
No. of deaths	1,011	40	40	32	32
Adjusted HR (95% CI)[a]	1.00	0.99 (0.72, 1.37)	0.85 (0.62, 1.18)	0.79 (0.55, 1.13)	0.68 (0.48, 0.98)

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

[a] Five categories of milk intake with no intake as reference category and remaining 4 categories created as sex-specific quartiles of intake.

[b] Adjusted for age, sex, race, region, body mass index, smoking, alcohol, physical activity, non-steroidal anti-inflammatory drug and aspirin use, hormone replacement therapy use, education, annual income, total energy intake, fruit and vegetable intake, processed and red meat intake, dietary oxidative balance score, supplemental calcium, history of cardiovascular disease, diabetes, or cancer diagnosis.

[c] Cardiovascular disease mortality includes deaths due to myocardial infarction, stroke, heart failure, sudden death, other cardiac causes, and other cardiovascular non-cardiac causes.