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Calcium intake and risk of colorectal cancer according to expression status of calcium-sensing receptor (CASR)

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Use of standardized official symbols: We use HUGO (Human Genome Organisation)-approved official symbols for genes and gene products, including CASR and VDR, all of which are described at www.genenames.org Gene names are italicized, and gene product names are non-italicized.

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Abstract

Objective—Although evidence suggests an inverse association between calcium intake and the risk of colorectal cancer, the mechanisms remain unclear. The calcium sensing receptor (CASR) is expressed abundantly in normal colonic epithelium and may influence carcinogenesis. We hypothesized that calcium intake might be associated with a lower risk of CASR-positive, but not CASR-negative, colorectal cancer.

Design—We assessed tumor CASR protein expression using immunohistochemistry in 779 incident colon and rectal cancer cases that developed among 136,249 individuals in the Nurses' Health Study and Health Professionals Follow-Up Study. Duplication method Cox proportional hazards regression analysis was used to assess associations of calcium intake with incidence of colorectal adenocarcinoma subtypes by CASR status.

Results—Total calcium intake was inversely associated with the risk of developing colorectal cancer [P_{trend}=0.01, comparing ≥1200 versus <600 mg/day: multivariable hazard ratio (HR)=0.75, 95% confidence interval (CI), 0.60 to 0.95]. For the same comparison, higher total calcium intake was associated with a lower risk of CASR-positive tumors (P_{trend}=0.003, multivariable HR=0.67, 95% CI, 0.51 to 0.86) but not with CASR-negative tumors (P_{trend} =0.67, multivariable HR=1.15, 95% CI, 0.75 to 1.78; $P_{heterogeneity}$ = 0.06 between the CASR subtypes). The stronger inverse associations of calcium intake with CASR-positive but not CASR-negative tumors generally appeared consistent regardless of sex, tumor location, and source of calcium.

Conclusions—Our molecular pathological epidemiology data suggest a causal relationship between higher calcium intake and lower colorectal cancer risk, and a potential role of CASR in mediating anti-neoplastic effect of calcium.

Keywords

calcium; calcium-sensing receptor; cancer epidemiology; cancer prevention; cohort study; colon cancer; diet; etiologic heterogeneity; molecular pathological epidemiology; rectal cancer; tumor microenvironment

Introduction

Colorectal cancer is the third most commonly diagnosed cancer both in the US[1] and worldwide.[2] Calcium intake has been associated with a lower risk of colorectal cancer.[3–6] We have demonstrated that each 300 mg/day increase in total calcium intake was associated with an approximately 8% decreased risk of colorectal cancer both in cohort studies[5] and a meta-analysis.[6] Although not all epidemiological studies found such associations, the World Cancer Research Fund and American Institute for Cancer Research consider the association between dietary calcium and lower risk of colorectal cancer as "probable".[7] Most studies have examined total colorectal cancer, but this cancer comprises a heterogeneous group of diseases in which each tumor arises and behaves in a unique fashion due to its distinctive genetic and epigenetic background.[8,9] The tumor suppressive potential of calcium in colon may thus differ by specific tumor molecular subtype.

Experimental studies suggest that calcium may exert anti-carcinogenic effects through down-regulating cellular proliferation and increasing differentiation and apoptosis.[10,11] The mechanisms behind these effects are not well established, but the extracellular calcium-sensing receptor (CASR)[12,13] might play a role. CASR is a calcium-binding G protein-coupled receptor and expressed abundantly in normal colonic epithelium. The CASR has been identified as a key molecule in maintaining systemic calcium homeostasis through actions on the parathyroid gland, kidney, small intestine, and bone.[14] Despite a growing body of evidence for the role of calcium in colorectal cancer, no studies to date have examined whether the inverse association between calcium intake and colorectal cancer risk differs according to expression level of CASR in the tumor. We hypothesized that calcium intake is associated with a reduced risk of colorectal carcinomas that over-express CASR, but not tumors with no or weak expression of CASR.

To test this hypothesis, we prospectively investigated the association of a long-term calcium intake with colorectal cancer risk according to tumor CASR expression within two large U.S. nationwide cohorts, in which an association has been found between calcium intake and a reduced risk of overall colorectal cancer.[5,15,16] In secondary analyses, we examined the potential differential association by CASR expression according to sex, intake sources of calcium, anatomic subsites, and tumor molecular characteristic classified by the vitamin D receptor (VDR) expression.

Methods

Study population

We conducted this study by utilizing data from the Nurses' Health Study (NHS) and Health Professionals Follow-Up Study (HPFS). Details for the two cohorts have been described

elsewhere.[517–19] In brief, NHS is a prospective cohort with 121,700 U.S. female registered nurses aged 30 to 55 years who completed their baseline survey in 1976. HPFS began in 1986 and enrolled 51,529 U.S. male professionals aged 40 to 75 years at entry. Biennial questionnaires were used to update demographics, lifestyle factors, medical history, and identify newly-diagnosed cancers, with follow-up rates over 90% in each cohort. The study protocol was approved by the institutional review boards of the Brigham and Women's Hospital and Harvard T.H. Chan School of Public Health. A flow chart showing how the study population for analysis was developed is presented in Supplementary Figure 1. After excluding participants with a history of cancer (except for non-melanoma skin cancer), polyposis syndrome, ulcerative colitis/Crohn's disease, implausible energy intakes at baseline (< 600 or > 3,500 kcal/day for women, or < 800 or > 4,200 kcal/day men), or with no reports of calcium intake at baseline, a total of 136,249 participants (88,509 women and 47,740 men) were included in the final analysis.

Assessments of calcium intake and other dietary factors

Details on assessments of calcium intake as well as other dietary factors has been described previously.[515] Briefly, self-reported dietary information was collected at baseline (1980 for the NHS; and 1986 for the HPFS) and updated almost every 4 years thereafter using a validated[20–22] semiquantitative food frequency questionnaire (FFQ) for each cohort. Additionally, we collected information on calcium supplements and multivitamin use in each biennial questionnaire. We used the composition database from the U.S. Department of Agriculture to calculate nutrient intake by multiplying the frequency of each food consumed by the nutrient content per serving of that food, and summing across all foods and beverages.[515] We calculated an individual's total calcium intake by summing calcium from dietary sources including fortified foods plus supplements. The calcium from dairy sources alone was calculated by summing the contributions of all dairy products and food items containing dairy products. Calcium from non-dairy sources was calculated by subtracting dairy calcium intake from dietary calcium intake.[515] In the NHS, dairy products contributed 30% to 35% (relatively stable over follow-up), calcium supplement use contributed about 25% (in early 1990s) to 35% (in 2000s), and multivitamin use contributed about 3% (in early 1990s) to 8% (in 2000) in total calcium intake. In the HPFS, dairy products contributed 34% to 45%, and multivitamin use contributed about 3% (in early 1990s) to 8% (in 2000s).[5] The FFQs used in this study have been validated among 127 men from the HPFS[20] and among 173 women from the NHS.[22] The energy-adjusted correlation coefficients of total calcium intake comparing the FFQ and the average of multiple 1-week diet records (four for women and two for men) were 0.61 for men[20] and 0.63 for women.[22] The correlation coefficients for dietary calcium intake were 0.60 for men[20] and 0.70 for women.[22]

Assessment of covariates

Potential colorectal cancer risk factors including height, adult body weight, physical activity (METs-hours/week), cigarette smoking, sigmoidoscopy/colonoscopy screening, family history of colorectal cancer, aspirin use, and menopausal status and use of menopausal hormones, were collected in the baseline and updated in biennial follow-up questionnaires. As mentioned above, dietary factors including intakes of alcohol, vitamin D, folate, red meat

and processed meat were assessed at baseline and updated every 4 years using a validated FFQ.[20,21]

Ascertainment of colorectal cancer cases

Participants from the two cohorts were asked for written permission to obtain medical records and pathological reports if they reported colorectal cancer on biennial questionnaires. We searched state vital statistics records, the National Death Index, to identify additional unreported cancer deaths. For all deaths attributable to colorectal cancer, we requested permission from next-of-kin to review medical records. All possible cancer cases were further confirmed through review of medical and pathological records by a study physician who was blinded to exposure data, and information on tumor anatomic location, stage and histologic type was also retrieved. Incident colorectal cancer cases were defined as a primary tumor with International Classification of Diseases-9 codes of 153 and 154. Outcome data through June 1, 2012 for the NHS, and January 31, 2012 for the HPFS, were used for the present analysis. During an average follow-up period of 30.4 years in the NHS and 23.2 years in the HPFS, 779 incident colorectal cancer cases with available tumor CASR expression data were documented.

Immunohistochemistry for CASR expression and VDR expression

As previously described,[23] we constructed tissue microarrays (TMA)[24] from colorectal cancer blocks, and conducted immunohistochemistry for CASR and VDR expression. Tumor CASR immunohistochemistry analysis was limited to 779 colorectal cancer cases (461 from the NHS and 318 from the HPFS) with available TMA for the immunohistochemistry (see Supplementary Figure 1).

For CASR immunohistochemistry, tissue sections were deparaffinized, rehydrated, and heated in a microwave for 15 minutes in Antigen Retrieval Citra Solution, pH 6 (BioGenex Laboratories, San Ramon, CA, USA). Sections were incubated with Dual Endogenous Enzyme Block (Dako, Glostrup, Denmark), followed by the treatment with Protein Block Serum-Free (Dako). Slides were then incubated for 1 hour at room temperature with a rabbit polyclonal anti-CASR antibody (ab137408; Abcam, Cambridge, MA, USA; dilution, 1:100). The primary antibody was visualized using EnVision+ System-HRP (Dako) with diaminobenzidine, and counterstained with hematoxylin. Sections processed with the replacement of primary antibody by Tris-buffered saline were used as a negative control.

Immunohistochemical assessment for CASR was interpreted by a pathologist (Y.M.) who was unaware of any information concerning the colorectal cancer cases. Tumor CASR expression was scored as 0 (no/minimal staining), 1 (weak staining), 2 (moderately intense staining), and 3 (intense staining) based on the staining intensity in colorectal carcinoma cells according to previously reported criteria.[25] Tumors were classified as CASR–positive (for overexpression) if the score ranged from 2 to 3, while tumors were classified as CASR–negative if the score ranged from 0 to 1, to retain statistical power in subgroup analysis of each stratum of CASR subtypes. Despite the lack of a widely accepted, standardized classification scheme for CASR expression in colorectal cancer, the CASR intensity scoring system used in this study was similar to other previous studies.[26–28] CASR expression levels

(ranging from 0 to 3) in selected tumors (N = 118) were independently examined by a second pathologist (Z.R.Q.), and the concordance between the two pathologists was reasonable with a weighted κ of 0.71 (95% CI, 0.61 to 0.82). Methods for measuring the VDR expression in tumor have been reported previously.[29]

Statistical analysis

Age-adjusted and multivariable-adjusted cohort-specific hazard ratios (HRs) and 95% confidence intervals (CIs) for each colorectal cancer subtype (CASR-positive and CASR-negative cancer) were calculated using the duplication method Cox proportional hazards regression model,[30] which was stratified simultaneously by age (in months) and year of questionnaire return (every two years since baseline questionnaire). Person-years of follow-up were calculated from the date of baseline questionnaire return to the date of diagnosis of colorectal cancer, date of death, loss to follow-up, or the end of follow-up (June 1, 2012 for the NHS, and January 31, 2012 for the HPFS), whichever came first. We used the energy-adjusted[31] cumulative average intake of total calcium as reported on all available questionnaires up to the start of each 4-year follow-up interval as main exposure[5] to minimize within-person variation and to better reflect long-term intake. Likewise, cumulative average for covariates was used when appropriate. The covariates as well as their categorizations in the multivariable models were as indicated in Table 2 (see footnotes). When applicable, total calcium and covariates were modeled as time-varying variables allowing for potential changes over follow-up periods. For variables with missing (generally 2–3%), we assigned a separate “missing” indicator variable and included those participants in the multivariate Cox models. We found no violation of proportionality in testing of the proportional hazard assumption. The trend tests were conducted using the median of each category of calcium intake as a continuous variable, and *P* value for trend was calculated using a Wald test.

To maximize the statistical power, we combined the data from two cohorts to detect the association of calcium intake and risk of colorectal cancer according to CASR expression since we did not observe any statistical significant heterogeneity between cohorts ($P_{\text{heterogeneity}}$ for sex=0.43). We examined the statistical significance of the difference in association according to the cancer subtype using the likelihood ratio test that compared the model fit that allows separate associations by different tumor CASR expression status with the model fit that assumed a common effect.

In secondary analysis, the influence of calcium intake on colorectal cancer risk according to CASR expression stratified by sex was examined. We also considered separately the intake source of calcium and anatomic subsites. Considering that vitamin D plays an important role in calcium absorption and homeostasis,[32] we evaluated the association between calcium intake and risk of colorectal cancer according to joint classification of tumor CASR and VDR expression status. All analysis were performed using the SAS software (SAS Institute, Version 9.2, Cary, NC), and a two-sided *P* value of 0.05 was considered statistically significant.

Results

Baseline characteristics of study participants

Among the 88,510 eligible women and 47,740 eligible men reporting baseline calcium intake, participants with lower total calcium intake were more likely to be current smokers, consumed more alcohol, and tended to have higher intake of red meat, processed meat, and fat, but less vitamin D, folate, and phosphorous consumption (Table 1). In NHS, women who consumed less calcium were slightly less physically active compared to those with higher calcium intake. In HPFS, men with lower calcium intake were less likely to have a history of sigmoidoscopy/endoscopy. In addition, the characteristics of cases with available CASR expression data were similar to those without CASR expression data (Supplementary Table 1).

Calcium intake and colorectal cancer subtype classified by tumor CASR expression

In the current analysis, we documented 779 (461 cases in NHS and 318 cases in HPFS) incident colorectal cancer cases with available tissue for analysis of CASR expression. Among these 779 tumors, 629 (80.7%) had moderate or intense CASR expression, while 150 (19.3%) had absent or weak CASR expression. Consistent with our previous study,[5] total calcium intake was significantly associated with decreased risk of colorectal cancer (P_{trend}=0.01) (Table 2).

We found that the inverse association with high total calcium intake appeared to be restricted to CASR-positive tumors (P_{trend}=0.003, comparing ≥1200 versus <600 mg/day: multivariable HR=0.67, 95% CI, 0.51 to 0.86). No significant association with total calcium intake was observed for CASR-negative tumor (P_{trend}=0.67, same comparison: multivariable HR=1.15, 95% CI = 0.75 to 1.78, $P_{heterogeneity}$ for subtypes=0.06, Table 2). When stratified by sex, results were generally consistent between women and men (Table 2). Likewise, a stronger inverse association in CASR-positive but not CASR-negative tumors was observed for both dietary calcium ($P_{heterogeneity}$ for subtypes=0.07) and dairy calcium intake ($P_{heterogeneity}$ for subtype=0.03) (Table 3). A similar pattern was suggested for calcium supplement, although there was no statistically significant heterogeneity across the CASR subtypes ($P_{heterogeneity}$ for subtypes=0.71) (Table 3).

In secondary analysis, the differential associations between calcium intake and cancer risk according to CASR expression status were consistent across tumor anatomic subsite (Supplementary Table 2), although the heterogeneity test did not reach statistical significance. Furthermore, the differential associations appeared slightly stronger in VDR positive tumors but not in VDR negative cancers (Supplementary Table 3).

Discussion

We tested the hypothesis that the inverse association between calcium intake and the risk of colorectal cancer might differ by tumor CASR expression status. The hypothesis was based on the combination of two different lines of evidence. Firstly, high calcium intake has been associated with a lower risk of colorectal cancer in the majority of epidemiological studies.[3–7] Secondly, the CASR might have tumor suppressive roles in the colon;[33–35] and

extracellular ionized calcium is the primary agonist of CASR.[3637] We found that high calcium intake appeared to be associated with the lower risk of CASR–positive colorectal adenocarcinomas, but not with the CASR–negative tumors.

The observed differential association between calcium and colorectal cancer by CASR expression status has biological support. The CASR is expressed abundantly in normal colonic epithelium and progressively downregulated or lost during the process of colorectal carcinogenesis.[38] Calcium may activate certain signaling pathways that are involved in regulating the balance between proliferation, differentiation, and apoptosis of colonic epithelial cells via CASR through the promotion of E-cadherin expression, the suppression of beta-catenin/T cell factor activation,[3536] as well as the activation of p38 mitogen-activated protein kinase cascade.[363940] Additionally, previous studies indicated that CASR could mediate the proliferative effects of low intestinal calcium concentration.[3441] Therefore, the possible beneficial effects of calcium in colorectal carcinogenesis may be impaired or lost[1013] due to the decreased CASR expressions in the tumors.[4243]

Although most epidemiological studies have suggested a lower risk of colorectal cancer and adenoma associated with higher calcium intake, whether this association represents causality or confounding remains unsettled. A large randomized controlled trial involving 36,282 postmenopausal women found no significant benefit of 1000 mg/day of elemental calcium plus 400 IU of vitamin D3 on colorectal cancer risk during an average of 7 years of follow-up.[44] However, several design limitations in this trial[44] could have partly explained the negative findings, including the generally high calcium intake in the study population, relatively poor compliance and short duration. Another randomized controlled trial examined the association of supplementation with 1200 mg/day of calcium carbonate and 1000 IU/day vitamin D with adenomas recurrence among 2,259 patients who underwent at least one colorectal adenoma resection before enrolment, with follow-up duration of 3 to 5 years, and that study also yielded a null association.[45] However, unadjusted confounders,[46] short surveillance periods,[4647] and the narrow scope of study population [47] within this trial may have influenced its conclusion. The assessment of the association of calcium with colorectal cancer subtypes characterized by molecular pathological features may not only improve the understanding for the potential causal relationship between calcium and colorectal cancer, but also give hints about the molecular mechanisms by which calcium may exert its chemopreventive functions in colorectal carcinogenesis. The observed subtype-specific difference in the association between CASR-positive and CASR-negative colorectal adenocarcinomas provide evidence that calcium may causally decrease colorectal cancer risk.

Our findings of the potentially differential associations were robust. The stronger inverse association for the CASR–positive but not CASR–negative tumors was observed for all sources of calcium intake and generally consistent across the anatomic subsites. However, we previously found that in our cohorts higher calcium intake appeared only associated with lower risk of distal colon cancer.[515] Whether differential proportions of CASR-positive and CASR-negative cancers across subsites contribute to apparently stronger associations for calcium with distal colon cancer require larger study sizes.

Interestingly, although not statistically significant, we found that the differential association of calcium and colorectal cancer by CASR expression status appeared slightly stronger in VDR-positive tumors than in VDR-negative tumors, implying potential cross talk between the CASR and the vitamin D system in prevention of colorectal cancer. According to a review,[48] the active vitamin D metabolite, 1,25-dihydroxyvitamin D_3, bound to VDR can induce translation of the CASR; on the other hand, the amount and activity of the CASR might affect 1,25-dihydroxyvitamin D_3 signaling. The complexity of the cross-talk between the CASR and the vitamin D system appeared go beyond affecting expression mutually[48] and needs further investigations.

Strengths of the current study include prospective cohort design with large sample size, long follow-up periods for validated colorectal cancer outcomes, and high follow-up rate of over 90% in each cohort. In addition, taking into account the repeated measures of calcium and other covariates during the follow-up periods may strengthen the association. Lastly, we utilized the MPE approach,[849] which enabled us to investigate etiologic heterogeneity according to tumor molecular features, and provide evidence in support of differential association of calcium intake and colorectal cancer risk.

However, our study has several limitations. First, residual confounding cannot be totally ruled out although our detailed data resources enabled us to adjust for a variety of potential confounders including known and suspected risk factors for CRC. Nonetheless, age-adjusted results were similar to multivariable-adjusted results. Second, although the parent cohorts are large overall, we had limited number of cancer cases with available tumor CASR expression; therefore, future MPE studies with more available tumor tissues are needed to replicate our findings. Third, the exclusion of cancer cases without tissue specimen may introduce potential selection bias. However, cases with tumor CASR data were comparable to all eligible cases with regard to a number of demographic, diet and lifestyle factors. Fourth, our findings were likely to be influenced by the potential misclassification of CASR expression status. This misclassification could be non-differential and would bias the results toward the null (i.e. non-significant difference in the association by CASR expression status) in our cohorts. However, a blinded and independent assessment of CASR expression was performed, and we confirmed a substantial interobserver agreement between the two pathologists. Lastly, our study population consisted mainly of Caucasian U.S. health professionals and the results may not be generalizable to other ethnic groups. Nonetheless, the association between calcium intake and lower risk of colorectal cancer and adenoma has been observed with little heterogeneity across diverse populations.[650]

In summary, we observed that calcium intake is inversely associated with the risk of CASR-positive, but not with CASR-negative, colorectal cancer. Our finding supported the hypothesis that the CASR may partially mediate the anti-carcinogenic effect of calcium in the colon, and highlighted the potential use of CASR as a molecular marker for colorectal cancer. Future studies with more available tumor specimens are needed to confirm these findings and better understand the related mechanisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CASR	calcium sensing receptor
CI	confidence interval
FFQ	food frequency questionnaire
HPFS	Health Professionals Follow-Up Study
HR	hazard ratio
MET	metabolic equivalent task
MPE	molecular pathological epidemiology
NHS	Nurses' Health Study
TMA	tissue microarrays
VDR	vitamin D receptor

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Significance of this study

What is already known on this subject?

- High calcium intake is associated with lower risk of colorectal cancer in most epidemiological studies.
- The calcium-sensing receptor (CASR) is variably expressed in the lumen and acts as a master regulator of systemic calcium homeostasis.
- The CASR is suggested to mediate the antiproliferative effects of calcium in colon.

What are the new findings?

- Calcium intake was inversely associated with the risk of CASR-positive, but not with CASR-negative colorectal cancer.
- The possible differential associations between calcium and cancer risk according to tumor CASR expression persisted regardless of sex, source of calcium intake, and tumor location.

How might it impact on clinical practice in the foreseeable future?

- Our findings provide further support for a role of calcium in colorectal carcinogenesis and may help identify sub-populations particularly susceptible to prevention of CRC through increased calcium intake via diet or supplementation.

Table 1

Baseline characteristics of participants by frequency of total calcium intake in the Nurses' Health Study (1980) and Health Professionals Follow-up Study (1986)

	Total calcium intake (mg/day)			
	<800	**800–999**	**1000–1199**	**≥1200**
Women (Nurses' Health Study)				
No.	58,428	14,733	8,326	7,022
Age, years *	46.6(7.1)	46.8(7.3)	46.7(7.4)	47.1(7.4)
White, %	97.1	98.3	98.4	98.2
Body mass index, kg/m²	24.0(4.2)	24.1(4.1)	24.2(4.1)	24.4(4.4)
Activity, MET-hours/week	13.2(18.8)	15.2(21.5)	15.3(21.0)	16.2(26.4)
Family history of colorectal cancer, %	7.8	7.9	7.9	7.7
Regular aspirin use (2 or more tablets/week), %	33.3	32.7	31.4	30.6
Past smoking, %	26.8	29.2	28.9	28.5
Current smoking, %	30.1	26.4	26.0	26.1
Multivitamin use, %	30.7	37.9	40.6	45.0
History of sigmoidoscopy/endoscopy, %	10.0	10.0	10.6	10.4
Postmenopausal status, %	44.6	44.2	43.9	44.8
Postmenopausal hormone use, %	18.4	18.8	19.3	19.2
Total energy intake, kcal/day	1562(501)	1565(518)	1602(481)	1568(497)
Dietary calcium intake, mg/day	554(143)	883(71)	1078(88)	1376(263)
Dairy calcium intake, mg/day	295(138)	595(113)	791(132)	1082(287)
Supplemental calcium intake, mg/day ¶	46(73)	144(140)	228(189)	436(325)
Alcohol, g/day	7.1(11.4)	5.5(8.8)	4.8(8.1)	3.9(7.2)
Total folate intake, μg/day	333(233)	399(253)	417(261)	502(504)
Total vitamin D, IU/day	267(234)	377(252)	451(268)	606(488)
Red meat, servings/week	2.9(2.2)	2.1(1.7)	1.9(1.5)	1.5(1.4)
Processed meat, servings/week	1.3(1.9)	1.0(1.6)	0.9(1.6)	0.7(1.2)
Total phosphorous, mg/day	1030(175)	1262(164.4)	1403(169)	1636(242)
Total fat, g/day	72.0(13.8)	66.9(12.8)	64.9(12.8)	61.9(13.6)
Total fiber, g/day	16.3(6.0)	18.1(6.9)	17.9(6.9)	17.6(7.4)
Men (Health Professionals Follow-up study)				
No.	24,637	9,049	5,328	8,726
Age, years *	54.0(9.7)	54.5(9.9)	54.6(9.9)	55.8(9.8)
White, %	94.7	96.7	97.0	97.3
Body mass index, kg/m²	25.6(3.3)	25.5(3.2)	25.4(3.3)	25.4(3.3)
Activity, MET-hours/week	19.7(28.0)	22.3(31.7)	21.8(31.5)	22.6(30.5)
Family history of colorectal cancer, %	8.5	8.3	8.6	8.5
Regular aspirin use (2 or more tablets/week), %	28.0	30.1	31.0	31.3
Past smoking, %	43.1	40.9	40.5	39.6
Current smoking, %	10.9	8.2	8.9	8.1

	Total calcium intake (mg/day)			
	<800	**800–999**	**1000–1199**	**≥1200**
Multivitamin use, %	54.5	62.0	67.3	74.2
History of sigmoidoscopy/colonoscopy, %	25.2	26.9	26.7	27.1
Total energy intake, kcal/day	1978(620)	1956(632)	2111(631)	1958(583)
Dietary calcium intake, mg/day	603(119)	845(114)	982(189)	1180(394)
Dairy calcium intake, mg/day	289(118)	506(136)	643(206)	838(409)
Supplemental calcium intake, mg/day	15(44)	52(103)	118(180)	423(550)
Alcohol, g/day	13.4(17.1)	9.6(13.3)	9.9(14.2)	8.2(12.1)
Total folate intake, μg/day	418(222)	496(251)	528(286)	612(363)
Total vitamin D, IU/day	309(250)	407(279)	488(290)	637(371)
Red meat, servings/week	2.0(1.7)	1.6(1.5)	1.7(1.5)	1.4(1.4)
Processed meat, servings/week	1.3(1.9)	1.1(1.8)	1.2(1.9)	1.0(1.7)
Total phosphorous, mg/day	1257(175)	1427(168)	1516(187)	1678(289)
Total fat, g/day	72.8(13.9)	70.2(13.7)	70.6(13.8)	68.6(14.5)
Total fiber, g/day	20.2(6.5)	22.3(7.1)	21.6(7.6)	21.8(7.9)

Values are means (SD) or percentages and are standardized to the age distribution of the study population.

*Value is not age adjusted.

¶1986 value was used.

Table 2

Total calcium intake and risk of colorectal cancer according to tumor expression of CASR in the Nurses' Health Study (1980–2012) and Health Professionals Follow-up Study (1986–2012)

	Total calcium intake (mg/day)					
	<800	**800–999**	**1000–1199**	**≥1200**	P_{trend}*	$P_{heterogeneity}$¶
NHS						
Total colorectal cancer						
Person-years (n=2,602,872)	1,054,538	562,138	439,473	546,723		
No. cases (n=461)	187	110	77	87		
Age-adjusted HR (95% CI)	1 (ref)	0.92 (0.72 to 1.16)	0.77 (0.59 to 1.01)	0.66 (0.51 to 0.85)	0.0009	
Multivariable HR (95%CI)§	1 (ref)	0.93 (0.73 to 1.19)	0.82 (0.62 to 1.09)	0.76 (0.57 to 1.02)	0.05	
CASR						
No/weak						
No. cases (n=92)	35	22	12	23		
Age-adjusted HR (95% CI)	1 (ref)	0.98 (0.57 to 1.69)	0.63 (0.33 to 1.23)	0.89 (0.52 to 1.54)	0.50	0.35
Multivariable HR (95% CI)§	1 (ref)	0.99 (0.57 to 1.70)	0.65 (0.33 to 1.28)	1.05 (0.59 to 1.84)	0.89	0.34
Moderate/intense						
No. cases (n=369)	152	88	65	64		
Age-adjusted HR (95% CI)	1 (ref)	0.90 (0.69 to 1.17)	0.81 (0.60 to 1.08)	0.60 (0.45 to 0.81)	0.0008	
Multivariable HR (95% CI)§	1 (ref)	0.89 (0.68 to 1.18)	0.84 (0.62 to 1.16)	0.70 (0.50 to 0.98)	0.04	
HPFS						
Total colorectal cancer						
Person-years (n=1,060,107)	452,625	247,228	156,710	203,544		
No. cases (n=318)	152	64	48	54		
Age-adjusted HR (95% CI)	1 (ref)	0.71 (0.52 to 0.95)	0.84 (0.60 to 1.16)	0.68 (0.50 to 0.94)	0.02	
Multivariable HR (95%CI)§	1 (ref)	0.78 (0.57 to 1.06)	0.98 (0.70 to 1.39)	0.86 (0.61 to 1.21)	0.48	
CASR						
No/weak						
No.cases (n=58)	24	10	8	16		
Age-adjusted HR (95% CI)	1 (ref)	0.69 (0.33 to 1.45)	0.77 (0.34 to 1.74)	1.26 (0.66 to 2.41)	0.52	0.06

	Total calcium intake (mg/day)					
	<800	**800–999**	**1000–1199**	**≥1200**	P_{trend}*	$P_{heterogeneity}$¶
Multivariable HR (95% CI)§	1 (ref)	0.68 (0.32 to 1.43)	0.81 (0.36 to 1.85)	1.37 (0.70 to 2.68)	0.37	0.06
Moderate/intense						
No.cases (n=260)	128	54	40	38		
Age-adjusted HR (95% CI)	1 (ref)	0.71 (0.51 to 0.98)	0.85 (0.59 to 1.22)	0.58 (0.40 to 0.83)	0.004	
Multivariable HR (95% CI)§	1 (ref)	0.71 (0.51 to 0.99)	0.86 (0.59 to 1.26)	0.64 (0.43 to 0.95)	0.04	
Pooled						
Total colorectal cancer						
Person-years (n= 3,662,980)	1,507,164	809,366	596,183	750,267		
No. cases (n=779)	339	174	125	141		
Age-adjusted HR (95% CI)	1 (ref)	0.83 (0.69 to 0.99)	0.79 (0.64 to 0.98)	0.67 (0.55 to 0.81)	<0.0001	
Multivariable HR (95%CI)§	1 (ref)	0.82 (0.68 to 1.00)	0.82 (0.65 to 1.02)	0.75 (0.60 to 0.95)	0.01	
CASR						
No/weak						
No. cases (n=150)	59	32	20	39		
Age-adjusted HR (95% CI)	1 (ref)	0.87 (0.56 to 1.35)	0.68 (0.41 to 1.14)	1.02 (0.67 to 1.54)	0.88	0.06
Multivariable HR (95% CI)§	1 (ref)	0.87 (0.56 to 1.35)	0.71 (0.42 to 1.20)	1.15 (0.75 to 1.78)	0.67	0.06
Moderate/intense						
No. cases (n=629)	280	142	105	102		
Age-adjusted HR (95% CI)	1 (ref)	0.82 (0.67 to 1.00)	0.82 (0.65 to 1.03)	0.59 (0.47 to 0.74)	<0.0001	
Multivariable HR (95% CI)§	1 (ref)	0.81 (0.66 to 1.01)	0.84 (0.66 to 1.07)	0.67 (0.51 to 0.86)	0.003	

CI, confidence interval; HR, hazard ratio.

Duplication-method Cox proportional cause-specific hazards regression for competing risks data was used to compute HRs and 95% CIs.

All analyses were stratified by age (in month), year of questionnaire return and sex as well as restricted to cases with CASR data.

*Linear trend test using the median intake of each category.

¶The likelihood ratio test was used to test for the heterogeneity of the association between total calcium intake and colorectal cancer risk by CASR expression.

§Multivariable hazard ratios were adjusted for age (in month), race (Caucasian vs. non-Caucasian), adult BMI (< 25, 25 – < 27.5, 27.5 – < 30, or ≥ 30 kg/m^2), smoking (0, 1–10, or > 10 pack-years), history of colorectal cancer in a parent or sibling (yes or no), history of sigmoidoscopy/colonoscopy (yes or no), physical activity (< 3, 3 – < 27, ≥ 27 MET-hours/week), regular aspirin use (yes, no), alcohol consumption (0 -< 5, 5 – < 15, or ≥ 15 g/day), total intake of vitamin D, folate, red meat and processed meat (all in tertiles).

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Table 3

Intake of dietary calcium, dairy calcium and calcium supplement and risk of colorectal cancer according to tumor CASR expression in the Nurses' Health Study (1980–2012) and Health Professionals Follow-up Study (1986–2012)

	Dietary calcium intake (mg/day)					
	<600	**600–749**	**750–899**	**≥900**	P_{trend}*	$P_{heterogeneity}$¶
Total colorectal cancer						
Person-years(n=3,662,980)	1,006,102	1,018,371	769,798	868,709		
No. cases (n=779)	211	230	168	170		
Age-adjusted HR (95% CI)	1 (ref)	0.96 (0.80 to 1.16)	0.90 (0.73 to 1.10)	0.78 (0.64 to 0.96)	0.01	
Multivariable HR (95%CI) §	1 (ref)	0.97 (0.80 to 1.18)	0.92 (0.74 to 1.14)	0.83 (0.66 to 1.04)	0.07	
CASR						
No/weak						
No. cases (n=150)	42	31	31	46		
Age-adjusted HR (95% CI)	1 (ref)	0.63 (0.40 to 1.01)	0.81 (0.50 to 1.30)	1.02 (0.66 to 1.56)	0.58	0.06
Multivariable HR (95% CI)§	1 (ref)	0.64 (0.40 to 1.02)	0.82 (0.51 to 1.33)	1.07 (0.69 to 1.66)	0.46	0.07
Moderate/intense						
No. cases (n=629)	169	199	137	124		
Age-adjusted HR (95% CI)	1 (ref)	1.05 (0.85 to 1.29)	0.92 (0.73 to 1.15)	0.72 (0.57 to 0.91)	0.002	
Multivariable HR (95% CI)§	1 (ref)	1.05 (0.85 to 1.30)	0.94 (0.74 to 1.20)	0.76 (0.59 to 0.98)	0.02	
	Dairy calcium intake (mg/day)					
	<300	**300–499**	**500–699**	**≥700**		
Total colorectal cancer						
Person-years(n=3,662,980)	1,045,048	1,372,023	749,943	495,965		
No. cases (n=779)	232	296	151	100		
Age-adjusted HR (95% CI)	1 (ref)	0.93 (0.78 to 1.11)	0.86 (0.70 to 1.05)	0.84 (0.66 to 1.06)	0.08	
Multivariable HR (95%CI) §	1 (ref)	0.94 (0.78 to 1.12)	0.88 (0.71 to 1.09)	0.89 (0.68 to 1.15)	0.26	
CASR						
No/weak						

	Dietary calcium intake (mg/day)					
	<600	**600–749**	**750–899**	**≥900**	P_{trend} *	$P_{heterogeneity}$ ¶
No. cases (n=150)	42	53	23	32		
Age-adjusted HR (95% CI)	1 (ref)	0.89 (0.59 to 1.34)	0.70 (0.42 to 1.16)	1.41 (0.88 to 2.24)	0.27	0.04
Multivariable HR (95% CI)§	1 (ref)	0.89 (0.59 to 1.34)	0.71 (0.42 to 1.19)	1.50 (0.93 to 2.43)	0.19	0.03
Moderate/intense						
No. cases (n=629)	190	243	128	68		
Age-adjusted HR (95% CI)	1 (ref)	0.94 (0.78 to 1.14)	0.89 (0.71 to 1.12)	0.70 (0.53 to 0.93)	0.01	
Multivariable HR (95% CI)§	1 (ref)	0.95 (0.78 to 1.15)	0.92 (0.73 to 1.16)	0.74 (0.55 to 1.00)	0.06	
	Calcium supplement (mg/day)					
	<200	**200–299**	**300–499**	**≥500**		
Total colorectal cancer						
Person-years(n=3,662,980)	2,505,419	321,401	428,882	407,278		
No. cases (n=779)	529	85	101	64		
Age-adjusted HR (95% CI)	1 (ref)	1.11 (0.88 to 1.41)	1.01 (0.81 to 1.26)	0.63 (0.48 to 0.82)	0.006	
Multivariable HR (95%CI)§	1 (ref)	1.20 (0.94 to 1.52)	1.14 (0.90 to 1.43)	0.76 (0.58 to 1.01)	0.31	
CASR						
No/weak						
No. cases (n=150)	100	14	22	14		
Age-adjusted HR (95% CI)	1 (ref)	0.87 (0.49 to 1.56)	1.12 (0.69 to 1.82)	0.70 (0.39 to 1.25)	0.36	0.73
Multivariable HR (95% CI)§	1 (ref)	0.94 (0.52 to 1.68)	1.27 (0.78 to 2.08)	0.86 (0.48 to 1.54)	0.89	0.71
Moderate/intense						
No. cases (n=629)	429	71	79	50		
Age-adjusted HR (95% CI)	1 (ref)	1.17 (0.91 to 1.52)	0.98 (0.76 to 1.25)	0.61 (0.45 to 0.83)	0.01	
Multivariable HR (95% CI)§	1 (ref)	1.27 (0.98 to 1.65)	1.10 (0.86 to 1.42)	0.74 (0.54 to 1.01)	0.28	

CI, confidence interval; HR, hazard ratio.

Duplication-method Cox proportional cause-specific hazards regression for competing risks data was used to compute HRs and 95% CIs.

All analyses were stratified by age (in month), year of questionnaire return and sex, as well as restricted to cases with CASR data.

*Linear trend test using the median intake of each category.

[¶] The likelihood ratio test was used to test for the heterogeneity of the association between calcium intake and colorectal cancer risk by expression of CASR.

[§] Multivariable hazard ratios were adjusted for age (in month), race (Caucasian vs. non-Caucasian), adult BMI (< 25, 25 – < 27.5, 27.5 – < 30, or ≥ 30 kg/m^2), smoking (0, 1–10, or > 10 pack-years), history of colorectal cancer in a parent or sibling (yes or no), history of sigmoidoscopy/colonoscopy (yes or no), physical activity (< 3, 3 – < 27, ≥ 27 MET-hours/week), regular aspirin use (yes, no), alcohol consumption (0 – < 5, 5 – < 15, or ≥ 15 g/day), total intake of vitamin D, folate, red meat and processed meat (all in tertiles).