Vitamin D, magnesium, calcium, and their interaction in relation to colorectal cancer recurrence and all-cause mortality

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ABSTRACT

Background: Higher concentrations of 25-hydroxyvitamin D_3 [25(OH)D_3] at diagnosis are associated with a lower mortality risk in colorectal cancer (CRC) patients. However, magnesium and calcium are important in vitamin D metabolism.

Objectives: We aimed to investigate 25(OH)D_3, magnesium, or calcium and their interaction among patients with CRC in relation to recurrence and all-cause mortality.

Methods: The study population included 1169 newly diagnosed stage I–III CRC patients from 2 prospective cohorts. Associations between 25(OH)D_3 concentrations, magnesium or calcium intake through diet and/or supplements at diagnosis, and recurrence and all-cause mortality were evaluated using multivariable Cox proportional hazard models. The interaction between 25(OH)D_3 and magnesium or calcium was assessed by investigating *1*) joint compared with separate effects, using a single reference category; and *2*) the effect estimates of 1 factor across strata of another.

Results: Serum 25(OH)D_3, calcium, and magnesium, alone and their interactions, were not associated with recurrence. Serum 25(OH)D_3 concentrations seemed to be associated with all-cause mortality. An inverse association between magnesium intake ($HR_{Q3\ vs.\ Q1}$: 0.55; 95% CI: 0.32, 0.95 and $HR_{Q4\ vs.\ Q1}$: 0.65; 95% CI: 0.35, 1.21), but not calcium intake, and all-cause mortality was observed. When investigating the interaction between 25(OH)D_3 and magnesium, we observed the lowest risk of all-cause mortality in patients with sufficient vitamin D concentrations (≥50 nmol/L) and a high magnesium intake (median split) (HR: 0.53; 95% CI: 0.31, 0.89) compared with patients who were vitamin D deficient (<50 nmol/L) and had a low magnesium intake. No interactions between calcium and vitamin D in relation to all-cause mortality were observed.

Conclusions: Our findings suggest that the presence of an adequate status of 25(OH)D_3 in combination with an adequate magnesium intake is essential in lowering the risk of mortality in CRC patients, yet the underlying mechanism should be studied. In addition, diet and lifestyle intervention studies are needed to confirm our findings. The COLON study was registered at clinicaltrials.gov as NCT03191110. The EnCoRe study was registered at trialregister.nl as NTR7099. *Am J Clin Nutr* 2020;111:1007–1017.

Keywords: colorectal cancer patients, 25(OH)D_3, interactions, magnesium, calcium, recurrence, all-cause mortality

Introduction

Evidence is accumulating that circulating vitamin D concentrations are inversely associated with mortality in colorectal

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Because the data consist of identifying cohort information, some access restrictions apply, and therefore they cannot be made publicly available. Data will be shared with permission, from the acting committee of the COLON Study. Requests for data can be sent to Dr. Fränzel van Duijnhoven, Division of Human Nutrition and Health, Wageningen University & Research, Netherlands (e-mail: franzel.vanduijnhoven@wur.nl).

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Abbreviations used: COLON, COlorectal cancer: Longitudinal, Observational study on Nutritional and lifestyle factors that may influence colorectal tumour recurrence, survival and quality of life; CRC, colorectal cancer; DCRA, Dutch Colorectal Audit; EnCoRe, Energy for life after ColoRectal cancer; RERI, relative excess risk due to interaction; 25(OH)D_3, 25-hydroxyvitamin D_3.

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cancer (CRC) patients (1–11). Recently, Maalmi et al. (12) performed a meta-analysis, including 11 original studies with a total of 7718 CRC patients. Pooled effect estimates comparing the highest with the lowest category of circulating 25-hydroxyvitamin D [25(OH)D_3] showed an HR of 0.68 (95% CI: 0.55, 0.85) for all-cause mortality and 0.67 (95% CI: 0.57, 0.78) for CRC-specific mortality (12). Although recurrence of the disease is a concern for CRC survivors (13) and a contributor to morbidity and mortality in CRC survivors (14), the association between 25(OH)D_3 concentrations and CRC recurrence has hardly been reported so far.

Magnesium plays crucial roles in several biochemical processes involved in the synthesis and metabolism of vitamin D (15). The enzymatic conversion of 25(OH)D_3 to 1,25(OH)D_3, the active form of vitamin D, is magnesium dependent (16, 17). Vitamin D–resistant rickets, in which patients do not respond to vitamin D supplementation, could be reversed by magnesium supplementation (18). In addition, a previous cohort study in the general population observed a stronger inverse association between 25(OH)D_3 concentrations and all-cause mortality in participants with a high magnesium intake (median >264 mg/d) than in participants with a low magnesium intake (<264 mg/d) (15). When investigating magnesium alone, a borderline statistically significant inverse association between magnesium intake and all-cause mortality was observed in a meta-analysis of 6 prospective cohort studies among the general population ($HR_{highest\ vs.\ lowest}$: 0.88; 95% CI: 0.76, 1.01). Whether magnesium, alone or in interaction with vitamin D, is also beneficial for patients with CRC is unknown.

Besides magnesium, calcium is also involved in vitamin D metabolism. A low calcium intake causes a high turnover of vitamin D metabolites, resulting in vitamin D deficiency, whereas a high calcium intake is vitamin D sparing (19). Previously, a high postdiagnostic calcium intake was associated with a lower risk of all-cause mortality in CRC patients (20, 21). Moreover, 3 previous randomized controlled trials in patients with colorectal adenomas showed a reduced adenoma recurrence with high-dose calcium supplementation (pooled RR: 0.80; 95% CI: 0.68, 0.93) (22). On the contrary, another large randomized controlled trial observed no associations between high-dose calcium and/or vitamin D supplementation and the risk of recurrent adenomas and even a higher risk of sessile serrated adenomas (23, 24). Until now, however, it is unknown whether calcium intake is associated with CRC recurrence, especially in interaction with vitamin D concentrations.

The aim of our study was to investigate our hypothesis that higher vitamin D concentrations, magnesium intake, and calcium intake, at diagnosis, are associated with a lower risk of recurrence and all-cause mortality in CRC patients. Beyond that, given the importance of magnesium and calcium in vitamin D metabolism, the interaction between vitamin D concentrations and magnesium intake or calcium intake in relation to CRC recurrence and all-cause mortality was investigated.

Methods

Study design

The designs of the COLON (COlorectal cancer: Longitudinal, Observational study on Nutritional and lifestyle factors that may influence colorectal tumour recurrence, survival and quality of life) study (25) (NCT03191110) and the EnCoRe (Energy for life after ColoRectal cancer) study (26) (NTR7099) have been described elsewhere. Briefly, newly diagnosed CRC patients were recruited directly after diagnosis in 14 hospitals and were followed during and after treatment from 2010 (COLON) or 2012 (EnCoRe) onwards. Men and women >18 y of age were eligible. In the COLON study, patients with stage I–IV CRC were eligible. In the EnCoRe study, patients with stage IV disease were not recruited. Non–Dutch speaking patients, and those with (partial) bowel resection, chronic inflammatory bowel disease, hereditary CRC syndromes (e.g., Lynch syndrome, Familial Adenomatous Polyposis, Peutz-Jegher), dementia, or another mental condition obstructing participation were excluded in both studies. The COLON study was approved by the Committee on Research involving Human Subjects, region Arnhem-Nijmegen, Netherlands (2009-349). The EnCoRe study was approved by the Medical Ethics Committee of the University Hospital Maastricht and Maastricht University, Netherlands (METC 11-3-075). All patients provided signed informed consent.

Blood samples were available for 1169 patients, 71% of all recruited participants. Patients with stage IV disease ($n = 90$) or with unknown stage ($n = 37$) were excluded from the analyses (**Figure 1**).

Blood collection and measurement of 25(OH)D_3 concentrations

For the COLON study, blood samples were obtained in the hospital at diagnosis. In 93% of the patients included in these analyses, blood was collected before the start of treatment. For the EnCoRe study, blood samples at diagnosis were obtained in the hospital or by a research assistant during a home visit before the start of treatment. For both studies, blood samples were collected in a serum tube, centrifuged (at 1300 × g at 4°C for 15 minutes in the COLON study and at 1800 × g at 20°C for 10 minutes in the EnCoRe study), and aliquots were immediately stored at −80°C until further analysis.

For both cohorts, serum 25(OH)D_3 concentrations were measured by isotope-dilution LC–tandem MS in Canisius Wilhelmina Hospital, Nijmegen, Netherlands (27). The interassay CVs were 7.4%, 4.0%, and 3.1% at 25(OH)D_3 concentrations of 36.0, 88, and 124 nmol/L, respectively. Serum 25(OH)D_3 is the main circulating form of vitamin D and generally considered the most reliable measurement of an individual's vitamin D status (28).

Data collection

Habitual dietary intake in the month (COLON study) or year (EnCoRe study) preceding diagnosis was assessed using an extended semiquantitative FFQ. The validated FFQ used in the COLON study consists of 204 items. The FFQ used in the EnCoRe study consists of 253 items and was recently validated for macro- and micronutrients (29). Dietary intake of vitamin D, magnesium, and calcium was calculated for each food item based on frequency of intake, number of portions, and portion size, as well as the type of product (e.g., whole grain or brown bread). Mean daily vitamin D (μg/d), magnesium

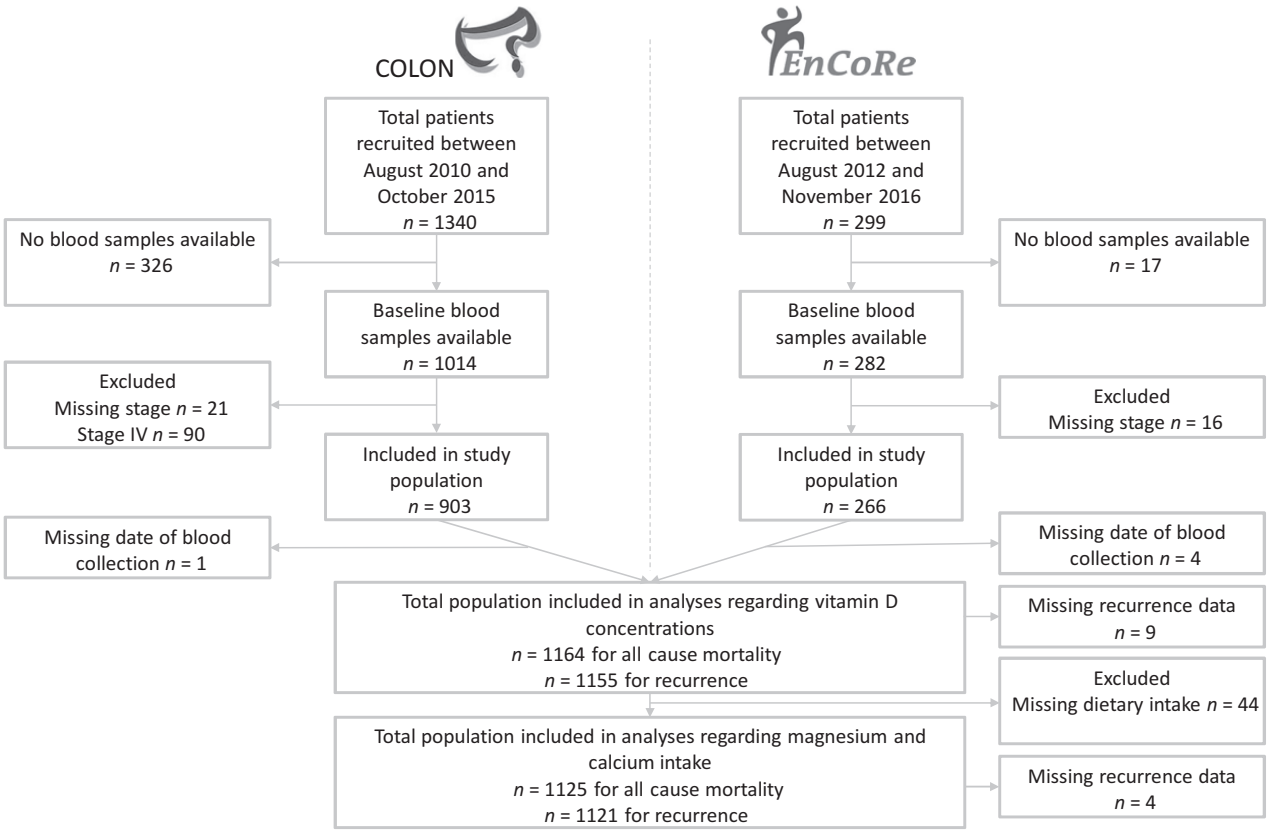

FIGURE 1 Flowchart representing patient selection for the current study. COLON, COlorectal cancer: Longitudinal, Observational study on Nutritional and lifestyle factors that may influence colorectal tumour recurrence, survival and quality of life; EnCoRe, Energy for life after ColoRectal cancer.

(mg/d), and calcium (g/d) intakes were calculated by adding all items containing the respective nutrient using data from the 2011 Dutch food composition tables (30). In the COLON study, supplement use was assessed by a dietary supplement questionnaire developed by the Division of Human Nutrition and Health of Wageningen University & Research (25). The dietary supplement questionnaire provided at the time of diagnosis contained questions on use of multivitamin/mineral supplements and on the dosage and frequency of their intake. In the EnCoRe study, supplement use was assessed in detail by a research dietitian during a home visit, using standardized forms, to record type and brand name of supplements, as well as frequency and duration of use, dosage, and ingredients (recorded from the package if necessary). For both studies, supplement use was defined as using supplements at least once a week for ≥1 mo during the preceding year. In addition, vitamins or minerals that were used once a month, but contained a high dose to cover the intake for a longer period (e.g., D-CURE 25.000 IE Cholecalciferol supplementation), were also classified as supplement use. Supplement dosage per day was calculated using frequency of intake (e.g., once a week, every day), number of supplements, and dosage per supplement. Total intake of vitamin D, magnesium, or calcium was calculated by summing dietary intake and intake from dietary supplements.

Information on demographics (age, gender, education) and smoking habits was obtained using self-administered questionnaires in both cohorts at the same time as the blood samples were collected. Information on height, weight, and waist and hip circumference was collected using self-administered questionnaires in the COLON study. In the EnCoRe study, these measurements were performed by trained research dietitians during home visits. Physical activity was assessed using the Short QUestionnaire to ASsess Health-enhancing physical activity (SQUASH) in both cohorts (31).

Clinical data, such as stage of disease, tumor location (colon/rectum), date of start of treatment, type of treatment (surgery, neo-adjuvant/adjuvant chemotherapy, radiation therapy), and presence of comorbidities (among others: diabetes, endocrine disorders, cardiovascular, gastrointestinal), were derived from the Dutch ColoRectal Audit (DCRA) (COLON) and hospital records (EnCoRe). The DCRA is a nationwide audit initiated by the Association of Surgeons from the Netherlands to monitor, evaluate, and improve CRC care (32).

Study endpoints

Information on recurrence was collected from medical records by the Dutch Cancer Registration. Recurrence is defined as a loco-regional recurrence or distant metastasis. Information on all-cause mortality was gathered from linkage with the Municipal Personal Record Database.

Follow-up time for recurrence was calculated starting from the date of blood collection until the date of recurrence or until the date recurrence status was updated (February 2018 for the COLON study and March 2018 for the EnCoRe study) or until the

date of end of follow-up, whichever came first. Follow-up time for all-cause mortality was defined starting from the date of blood collection until the date of death, the last date vital status was updated (25 June, 2019 for the COLON study and 20 May, 2019 for the EnCoRe study), or the date of end of follow-up, whichever came first.

Data analyses

Patient characteristics at diagnosis were described as numbers with percentages or medians with IQRs for the total study population and stratified by vitamin D status [deficiency = serum 25(OH)D_3 < 50 nmol/L and sufficiency = serum 25(OH)D_3 ≥ 50 nmol/L] (33). Patients with missing data in the main exposure variables (n = 343 for vitamin D concentrations and n = 44 for dietary intake) were excluded from analyses (Figure 1). Descriptive statistics were used to assess differences in characteristics between patients with missing exposure data and those without missing exposure data. Correlations between magnesium, calcium, and vitamin D intake and concentrations were assessed using Pearson correlation coefficients.

The associations between serum 25(OH)D_3 concentrations and CRC recurrence as well as all-cause mortality were assessed using multivariable Cox proportional hazard models. Serum 25(OH)D_3 concentrations were entered in the model continuously per 10 nmol/L and based on clinically defined cutoffs (33) [severely deficient: <30 nmol/L; deficient: 30–49 nmol/L; sufficient: 50–74 nmol/L (reference); optimal: ≥75 nmol/L].

The association between magnesium and calcium intake and CRC recurrence and all-cause mortality was also examined using multivariable Cox proportional hazard models. Cohort-specific quartiles of intake were calculated, because slightly different FFQs were used in the 2 cohorts. To test for linearity among quartiles of magnesium and calcium intake, P values for trend were calculated by including the quartiles as a continuous variable in the model. Analyses were performed for dietary intake of magnesium and calcium as well as for total intake (diet and supplements).

First a crude model, including only the main exposure of interest and the outcome, was performed. Second, based on the literature, the following covariates were added to the multivariable models investigating the association between vitamin D, magnesium, and calcium individually: age (continuous), sex (male/female), stage (I, II, III), tumor location (colon/rectal), BMI (continuous), moderate-to-vigorous physical activity (continuous; h/wk), season of blood collection (spring, summer, autumn, winter; only in the model for vitamin D), total energy intake (quartiles) (12, 15), and cohort. In addition, other potential confounders were tested and included in the model when the HR changed by >10%. Smoking, education level, having comorbidities at diagnosis (yes/no), the use of statins (yes/no), use of proton pump inhibitors (yes/no), and alcohol intake (g/d) did not influence the HR and were thus not included in the models. Finally, in a third model we also added the nutrients involved in vitamin D metabolism, to get more insight into how each nutrient individually, independently of the others, was associated with recurrence and mortality. Thus, magnesium (quartiles) and calcium (quartiles) intake were added to the models of vitamin D. Calcium and vitamin D concentrations were added to the models of magnesium. Magnesium and vitamin D concentrations were added to the models of calcium.

Log-transformed curves were used for visual inspection of the assumption for the Cox proportional hazard model. No strong evidence of nonparallelism of the log-log curves was observed.

We investigated interaction using 2 different methods, as recommended by Knol and VanderWeele (34), *1*) by investigating the joint compared with separate effects of 25(OH)D_3 concentrations and magnesium or calcium intake using 1 reference category (vitamin D deficient as well as low magnesium or low calcium intake), and *2*) by investigating the effect estimate of 1 factor across strata of another factor. A median split was used to define high and low magnesium and calcium intakes. Both additive and multiplicative interactions were investigated. Interaction on the additive scale was investigated, because assessing additive rather than multiplicative interaction can help determine which subgroups would benefit most from an increase in vitamin D and/or magnesium or calcium (34, 35). To investigate interaction on an additive scale, the relative excess risk due to interaction (RERI) was calculated: RERI = $HR_{vitD-Mg-}$ − $HR_{vitD+Mg-}$ − $HR_{vitD-Mg+}$ + 1 (35). Thus, the HR found for the combined exposure (vitamin D ≥ 50 nmol/L and Mg above the median) was compared with the HRs for each of the 2 exposures alone. Because the RERI was developed for risk factors rather than preventive factors, the group with the lowest risk was used as the reference category (i.e., vitamin D concentrations ≥ 50 nmol/L and magnesium and calcium intakes above the median). A RERI of 0 means no additive interaction, a RERI < 0 a negative additive interaction, and a RERI > 0 a positive additive interaction. The P value for multiplicative interaction was calculated by adding vitamin D status and magnesium or calcium intake as well as the product term of vitamin D status × magnesium or calcium intake to the model.

In a sensitivity analysis, patients who donated blood after the start of treatment (n = 76) were excluded.

Statistical analyses were performed in SAS version 9.4 (SAS Institute). P values <0.05 were considered statistically significant.

Results

In total, 1169 CRC patients recruited between August 2010 and November 2016 with stage I–III CRC were included in the present analyses (Figure 1): 903 (77%) from the COLON study and 266 (23%) from the EnCoRe study. Median age was 67.0 [IQR: 61.7–72.9] y and 418 (36%) participants were women (**Table 1**). Two-thirds of the patients had colon cancer. Almost half of the patients had stage III disease. Around 25% of the patients used vitamin D–containing supplements, 21% used calcium-containing supplements, and 19% used magnesium-containing supplements. Patients who had sufficient vitamin D concentrations (≥50 nmol/L) were more often women, were more physically active, and more often used vitamin D, calcium, and magnesium supplements than patients who had deficient concentrations (<50 nmol/L) (Table 1).

TABLE 1 Baseline characteristics of stage I–III colorectal cancer patients, overall and stratified by vitamin D status[1]

	Total population ($n = 1169$)	Deficient [25(OH)D < 50 nmol/L] ($n = 500$)	Sufficient [25(OH)D ≥ 50 nmol/L] ($n = 669$)
Serum 25(OH)D_3 concentrations, nmol/L	54.5 [39.8–70.2]	37.3 [28.4–43.9]	67.4 [58.9–79.6]
Season of blood collection[2]			
Spring	295 (25)	162 (33)	133 (20)
Summer	327 (28)	69 (14)	258 (39)
Autumn	268 (23)	103 (21)	165 (25)
Winter	274 (24)	164 (33)	110 (17)
Unknown, *n*	5	2	3
Age, y	67.0 [61.7–72.9]	67.0 [60.9–74.4]	66.9 [62.3–72.4]
Gender, female	418 (36)	164 (33)	254 (38)
BMI, kg/m^2	26.3 [24.1–29.3]	26.8 [24.2–29.8]	26.1 [24.1–29.0]
Unknown, *n*	2	0	2
Education[3]			
Low	542 (49)	225 (48)	317 (49)
Medium	260 (23)	105 (22)	155 (24)
High	314 (28)	142 (30)	172 (27)
Unknown, *n*	53	28	25
Smoking habits			
Current	141 (12)	58 (12)	83 (13)
Former	665 (59)	278 (57)	387 (59)
Never	329 (29)	148 (31)	181 (28)
Unknown, *n*	34	16	18
Physical activity,[4] h/wk	10.5 [5.0–19.5]	8.7 [4.0–17.5]	12.0 [5.9–20.5]
Unknown, *n*	36	16	20
Dietary intake			
Vitamin D, μg/d	3.1 [2.2–4.2]	3.1 [2.2–4.0]	3.2 [2.3–4.3]
Calcium, mg/d	861 [639–1094]	862 [630–1109]	859 [648–1087]
Magnesium, mg/d	318 [257–384]	316 [252–381]	321 [259–387]
Alcohol, g/d	8.1 [0.8–20.5]	6.1 [0.4–19.4]	8.9 [1.3–20.7]
Unknown, *n*	44	20	24
Supplement use, yes			
Vitamin D	289 (25)	76 (16)	213 (33)
Calcium	238 (21)	80 (16)	158 (24)
Magnesium	226 (19)	81 (16)	145 (22)
Type of cancer			
Colon	768 (66)	320 (64)	448 (67)
Rectum	401 (34)	180 (36)	221 (33)
Tumor stage			
I	312 (27)	115 (23)	197 (29)
II	346 (30)	148 (30)	198 (30)
III	511 (44)	237 (47)	274 (41)
Comorbidities			
Yes	285 (71)	361 (72)	464 (70)
Unknown, *n*	8	1	7

[1] Values are median [IQR] or *n* (%) unless otherwise indicated. 25(OH)D_3, 25-hydroxyvitamin D_3.
[2] Spring: March–May; summer: June–August; autumn: September–November; winter: December–February.
[3] Low education was defined as primary school and lower general secondary education; medium as lower vocational training and higher general secondary education; high as high vocational training and university.
[4] Activities with a Metabolic Equivalent score ≥ 3 were defined as moderate-to-vigorous physical activity.

No differences were observed between patients who donated blood and patients who did not. Patients for whom no dietary data were available seemed to be slightly older and more often had advanced disease and comorbidities (data not shown). Magnesium intake and calcium intake were moderately correlated ($r = 0.6$). A moderate correlation between vitamin D intake and magnesium or calcium intake was observed ($r = 0.4$). Vitamin D concentrations were not linearly correlated with magnesium or calcium intake ($r = 0.1$).

During a median follow-up of 3.5 [IQR: 2.5–4.7] y for recurrence and 4.7 [IQR: 4.0–6.2] y for all-cause mortality, 155 recurrences and 191 deaths occurred. Almost half (42%) of the patients died after a recurrence. The total follow-up time was 4084 y for recurrence and 5769 y for all-cause mortality.

Circulating concentrations of 25(OH)D_3 and CRC recurrence and all-cause mortality

No association between 25(OH)D_3 concentrations at diagnosis and CRC recurrence was observed (**Table 2**). Severe vitamin D deficiency (<30 nmol/L) compared with sufficient concentrations (50–74 nmol/L) tended to be associated with a higher risk of all-cause mortality (HR: 1.46; 95% CI: 0.92, 2.32; *P*-trend = 0.08).

TABLE 2 Association of serum 25-hydroxyvitamin D_3 concentrations at diagnosis with CRC recurrence and all-cause mortality in CRC patients[1]

	Continuous per 10 nmol/L	Severely deficient (<30 nmol/L)	Deficient (30–49 nmol/L)	Sufficient (50–74 nmol/L)	Optimal (≥75 nmol/L)	*P*-trend[2]
CRC recurrence						
n/Events	1155/155	146/21	352/47	444/61	223/26	
Events/1000 person-years	37	43	38	38	34	
Model 1						
HR (95% CI)	0.97 (0.91, 1.05)	1.10 (0.67, 1.81)	0.99 (0.68, 1.44)	1.0 (ref)	0.88 (0.56, 1.39)	0.50
Model 2						
HR (95% CI)	0.98 (0.90, 1.07)	1.18 (0.68, 2.04)	1.09 (0.72, 1.63)	1.0 (ref)	1.07 (0.66, 1.73)	0.69
Model 3						
HR (95% CI)	0.98 (0.90, 1.07)	1.19 (0.69, 2.06)	1.10 (0.73, 1.65)	1.0 (ref)	1.04 (0.64, 1.69)	0.62
All-cause mortality						
n/Events	1164/191	146/33	354/56	446/70	223/32	
Events/1000 person-years	33	46	32	31	29	
Model 1						
HR (95% CI)	0.95 (0.89, 1.01)	1.47 (0.97, 2.22)	1.02 (0.72, 1.45)	1.0 (ref)	0.93 (0.61, 1.42)	0.10
Model 2						
HR (95% CI)	0.93 (0.86, 1.00)	1.46 (0.92, 2.32)	1.06 (0.72, 1.55)	1.0 (ref)	0.87 (0.55, 1.36)	0.08
Model 3						
HR (95% CI)	0.94 (0.87, 1.01)	1.39 (0.87, 2.21)	1.04 (0.71, 1.52)	1.0 (ref)	0.89 (0.56, 1.40)	0.14

[1]Model 1: crude Cox proportional hazard model. Model 2: adjusted for age, sex, stage, BMI, physical activity (moderate to vigorous; h/wk), tumor location, season of blood collection, cohort, and total energy intake. Model 3: as model 2 and further adjusted for total magnesium and calcium intake. CRC, colorectal cancer.

[2]*P*-trend values were calculated by including categories of vitamin D status (severely deficient, deficient, sufficient, optimal) as a continuous variable in the model.

Magnesium or calcium intake and CRC recurrence and all-cause mortality

No associations between dietary as well as total magnesium intake and CRC recurrence were found (**Table 3**). An inverse association between magnesium intake (dietary as well as total intake) and all-cause mortality was found ($HR_{Q3\ vs.\ Q1}$: 0.48; 95% CI: 0.29, 0.82 and $HR_{Q4\ vs.\ Q1}$: 0.55; 95% CI: 0.31, 0.98; *P*-trend = 0.02 for total intake). After adjustment for 25(OH)D_3 concentrations and calcium intake, this association was attenuated ($HR_{Q3\ vs.\ Q1}$: 0.55; 95% CI: 0.32, 0.95 and $HR_{Q4\ vs.\ Q1}$: 0.65; 95% CI: 0.35, 1.21; *P*-trend = 0.11 for total intake) (Table 3).

No associations between dietary as well as total calcium intake and CRC recurrence were observed (**Table 4**). An inverse association between total, but not dietary, calcium intake and all-cause mortality was observed ($HR_{Q4\ vs.\ Q1}$: 0.58; 95% CI: 0.34, 0.98; *P*-trend = 0.07). However, after adjustment for magnesium and 25(OH)D_3 concentrations, this association was attenuated and no longer statistically significant ($HR_{Q4\ vs.\ Q1}$: 0.70; 95% CI: 0.40, 1.21; *P*-trend = 0.27).

The interaction between 25(OH)D_3 concentrations and magnesium or calcium intake in relation to CRC recurrence and all-cause mortality

Vitamin D and magnesium.

For CRC recurrence, no interaction between 25(OH)D_3 concentrations and magnesium intake was observed (**Table 5**). In contrast, the risk of all-cause mortality was lowest in patients who had sufficient concentrations of 25(OH)D_3 (≥50 nmol/L) and a high magnesium intake (≥322 mg/d for COLON and ≥383 mg/d for EnCoRe) (HR: 0.53; 95% CI: 0.31, 0.89) compared with patients with deficient 25(OH)D_3 concentrations and a low magnesium intake. Borderline statistically significant multiplicative (*P* = 0.06) and additive (RERI: 0.27; 95% CI: −0.08, 0.61) interactions were observed.

When analyzing the association for magnesium across strata of vitamin D status, the association between magnesium and all-cause mortality was statistically significant in patients who had sufficient vitamin D concentrations (HR: 0.43; 95% CI: 0.25, 0.76), whereas no association was observed in patients who had deficient vitamin D concentrations (HR: 0.94; 95% CI: 0.52, 1.69). When analyzing the association for vitamin D across strata of magnesium intake, the association between vitamin D concentrations and all-cause mortality was stronger in patients with a high magnesium intake (HR: 0.69; 95% CI: 0.42, 1.18) than in patients with a low magnesium intake (HR: 0.98; 95% CI: 0.65, 1.49), but observed associations were not statistically significant.

Vitamin D and calcium.

No interactions between calcium and vitamin D with respect to CRC recurrence and all-cause mortality were observed (**Table 6**).

Similar results were observed when excluding patients who donated blood after the start of treatment (data not shown).

Discussion

No associations between serum 25(OH)D_3 concentrations and magnesium or calcium intake and CRC recurrence were observed in the current study. Lower vitamin D concentrations appear to be associated with a higher risk of all-cause mortality.

TABLE 3 Association of dietary and total magnesium intakes at diagnosis with recurrence and all-cause mortality in CRC patients[1]

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P*-trend
Dietary magnesium intake					
CRC recurrence					
n/Events	280/34	281/36	280/35	280/40	
Events/1000 person-years	35	35	35	43	
Model 1					
HR (95% CI)	1.0 (ref)	1.02 (0.64, 1.63)	1.01 (0.63, 1.61)	1.20 (0.76, 1.89)	0.47
Model 2					
HR (95% CI)	1.0 (ref)	1.19 (0.72, 1.97)	1.10 (0.61, 2.01)	1.38 (0.66, 2.87)	0.70
Model 3					
HR (95% CI)	1.0 (ref)	1.25 (0.74, 2.08)	1.20 (0.64, 2.26)	1.56 (0.71, 3.46)	0.40
All-cause mortality					
n/Events	280/56	282/42	282/34	281/42	
Events/1000 person-years	41	29	24	31	
Model 1					
HR (95% CI)	1.0 (ref)	0.71 (0.48, 1.06)	0.59 (0.38, 0.90)	0.76 (0.51, 1.13)	0.10
Model 2					
HR (95% CI)	1.0 (ref)	0.65 (0.42, 1.00)	0.46 (0.26, 0.79)	0.51 (0.26, 0.98)	0.04
Model 3					
HR (95% CI)	1.0 (ref)	0.69 (0.44, 1.09)	0.52 (0.29, 0.93)	0.59 (0.29, 1.20)	0.15
Total magnesium intake (diet and supplements)					
CRC recurrence					
n/Events	280/34	281/34	280/37	280/40	
Events/1000 person-years	35	33	38	42	
Model 1					
HR (95% CI)	1.0 (ref)	0.96 (0.60, 1.55)	1.07 (0.67, 1.70)	1.18 (0.75, 1.86)	0.42
Model 2					
HR (95% CI)	1.0 (ref)	1.15 (0.69, 1.90)	1.20 (0.69, 2.11)	1.39 (0.77, 2.53)	0.24
Model 3					
HR (95% CI)	1.0 (ref)	1.19 (0.72, 1.99)	1.32 (0.74, 2.36)	1.57 (0.84, 2.92)	0.13
All-cause mortality					
n/Events	280/55	282/46	282/36	281/37	
Events/1000 person-years	41	33	26	27	
Model 1					
HR (95% CI)	1.0 (ref)	0.80 (0.54, 1.18)	0.63 (0.41, 0.95)	0.66 (0.40, 1.00)	0.02
Model 2					
HR (95% CI)	1.0 (ref)	0.77 (0.50, 1.19)	0.48 (0.29, 0.82)	0.55 (0.31, 0.98)	0.02
Model 3					
HR (95% CI)	1.0 (ref)	0.83 (0.53, 1.28)	0.55 (0.32, 0.95)	0.65 (0.35, 1.21)	0.11

[1]Quartiles of intake were cohort-specific. Dietary intake of magnesium: COLON quartile 1: <246 mg/d; quartile 2: 246–305 mg/d; quartile 3: 306–371 mg/d; quartile 4: >371 mg/d; EnCoRe quartile 1: <300 mg/d; quartile 2: 300–364 mg/d; quartile 3: 364–429 mg/d; quartile 4: >429 mg/d. Total intake of magnesium: COLON quartile 1: <258 mg/d; quartile 2: 258–322 mg/d; quartile 3: 323–398 mg/d; quartile 4: >398 mg/d; EnCoRe quartile 1: <315 mg/d; quartile 2: 315–383 mg/d; quartile 3: 384–463 mg/d; quartile 4: >464 mg/d. Model 1: crude Cox proportional hazard model. Model 2: adjusted for age, sex, stage, BMI, physical activity (moderate to vigorous; h/wk), tumor location, cohort, and total energy intake. Model 3: as model 2 and further adjusted for dietary calcium and vitamin D concentrations for the dietary intake models and total calcium and vitamin D concentrations for the total intake (diet and supplements) models. *P* values for trend were calculated by including the quartiles as a continuous variable in the model. COLON, COlorectal cancer: Longitudinal, Observational study on Nutritional and lifestyle factors that may influence colorectal tumour recurrence, survival and quality of life; CRC, colorectal cancer; EnCoRe, Energy for life after ColoRectal cancer.

An inverse association between magnesium intake, but not calcium intake, and all-cause mortality was observed. All-cause mortality was lowest in patients with sufficient vitamin D concentrations in combination with a high magnesium intake.

Severe vitamin D deficiency compared with sufficient vitamin D concentrations was statistically nonsignificantly associated with a higher risk of all-cause mortality in our study. A recent meta-analysis including 11 studies among 7718 CRC patients observed a similar, but statistically significant, association between 25(OH)D concentrations and all-cause mortality (12). However, previous studies did not take magnesium intake into account, whereas we found an attenuated association after correction for magnesium intake. Magnesium is essential in the conversion of $25(OH)D_3$ to the active form of vitamin D, $1,25(OH)D_3$ (15), and could potentially strengthen the association between vitamin D and outcomes.

In the present study, we observed a statistically significant lower risk of all-cause mortality for quartile 3 of magnesium intake (∼300–400 mg/d), but not for quartile 4 (∼>400 mg/d), than for quartile 1 (∼<250 mg/d). As far as we know, this association has not been reported before in CRC patients. In the general population, a dose-response meta-analysis showed an inverse nonlinear association between dietary magnesium

TABLE 4 Association of dietary and total calcium intakes at diagnosis with recurrence and all-cause mortality in CRC patients[1]

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P*-trend
Dietary calcium intake					
CRC recurrence					
n/Events	280/36	281/42	280/32	280/35	
Events/1000 person-years	38	43	33	34	
Model 1					
HR (95% CI)	1.0 (ref)	1.16 (0.74, 1.81)	0.87 (0.54, 1.40)	0.92 (0.57, 1.46)	0.46
Model 2					
HR (95% CI)	1.0 (ref)	1.20 (0.76, 1.90)	0.93 (0.56, 1.55)	0.95 (0.55, 1.65)	0.66
Model 3					
HR (95% CI)	1.0 (ref)	1.17 (0.74, 1.86)	0.87 (0.52, 1.48)	0.86 (0.48, 1.54)	0.39
All-cause mortality					
n/Events	280/47	282/46	282/44	281/37	
Events/1000 person-years	34	33	31	26	
Model 1					
HR (95% CI)	1.0 (ref)	0.97 (0.65, 1.45)	0.93 (0.61, 1.40)	0.74 (0.48,1.14)	0.17
Model 2					
HR (95% CI)	1.0 (ref)	0.97 (0.64, 1.48)	0.86 (0.55, 1.35)	0.66 (0.40, 1.11)	0.31
Model 3					
HR (95% CI)	1.0 (ref)	1.02 (0.67, 1.57)	0.97 (0.61, 1.54)	0.76 (0.44, 1.32)	0.52
Total calcium intake (diet and supplements)					
CRC recurrence					
n/Events	280/38	281/39	280/36	280/32	
Events/1000 person-years	41	40	37	31	
Model 1					
HR (95% CI)	1.0 (ref)	0.99 (0.63, 1.44)	0.91 (0.58, 1.44)	0.78 (0.49, 1.25)	0.28
Model 2					
HR (95% CI)	1.0 (ref)	0.99 (0.62, 1.56)	0.95 (0.58, 1.55)	0.79 (0.46, 1.38)	0.44
Model 3					
HR (95% CI)	1.0 (ref)	0.95 (0.60, 1.50)	0.88 (0.53, 1.46)	0.71 (0.40, 1.27)	0.25
All-cause mortality					
n/Events	280/47	282/46	282/44	281/37	
Events/1000 person-years	36	31	36	23	
Model 1					
HR (95% CI)	1.0 (ref)	0.86 (0.57, 1.30)	0.99 (0.66, 1.47)	0.63 (0.40, 0.98)	0.09
Model 2					
HR (95% CI)	1.0 (ref)	0.86 (0.57, 1.32)	0.88 (0.57, 1.36)	0.58 (0.34, 0.98)	0.07
Model 3					
HR (95% CI)	1.0 (ref)	0.97 (0.63, 1.49)	1.06 (0.66, 1.67)	0.70 (0.40, 1.21)	0.27

[1]Quartiles of intake were cohort specific. Dietary intake of calcium: COLON quartile 1: <642 g/d; quartile 2: 642–855 g/d; quartile 3: 856–1088 g/d; quartile 4: >1088 g/d; EnCoRe quartile 1: <656 g/d; quartile 2: 656–875 g/d; quartile 3: 876–1144 g/d; quartile 4: >1144 g/d. Total intake of calcium: COLON quartile 1: <669 g/d; quartile 2: 669–888 g/d; quartile 3: 889–1137 g/d; quartile 4: >1137 g/d; EnCoRe quartile 1: <673 g/d; quartile 2: 673–930 g/d; quartile 3: 930–1230 g/d; quartile 4: >1230 g/d. Model 1: crude Cox proportional hazard model. Model 2: adjusted for age, sex, stage, BMI, physical activity (moderate to vigorous; h/wk), tumor location, cohort, and total energy intake. Model 3: as model 2 and further adjusted for dietary magnesium and $25(OH)D_3$ concentrations for the dietary intake models and total magnesium and $25(OH)D_3$ concentrations for the total intake (diet and supplements) models. *P* values for trend were calculated by including the quartiles as a continuous variable in the model. COLON, COlorectal cancer: Longitudinal, Observational study on Nutritional and lifestyle factors that may influence colorectal tumour recurrence, survival and quality of life; CRC, colorectal cancer; EnCoRe, Energy for life after ColoRectal cancer; $25(OH)D_3$, 25-hydroxyvitamin D_3.

intake and the risk of all-cause mortality (36). However, in this meta-analysis results were not adjusted for vitamin D concentrations, whereas the results of our study showed that this is important. Furthermore, a possible explanation for the observation that we found a lower risk of all-cause mortality for quartile 3 of magnesium intake, but not for quartile 4, is the interaction between magnesium and vitamin D. Findings of a recent randomized controlled trial with magnesium supplementation indicate that excessive magnesium intake >400 mg/d may actually reduce $25(OH)D_3$ concentrations (37). Although increasing dietary magnesium intake until an optimum of ~400 mg/d appears to reduce all-cause mortality, the HRs for the association between magnesium intake and recurrence were >1. However, the CIs were wide and no trend over quartiles of intake was observed. Thus, although a high magnesium intake seems beneficial in relation to all-cause mortality, this may not be true with respect to recurrence.

A high calcium intake at diagnosis was inversely associated with all-cause mortality in CRC patients; however, after correcting for magnesium the HR for the association between calcium and all-cause mortality attenuated from 0.58 to 0.70. Results of previous studies (20, 21) suggest an inverse association between high postdiagnostic calcium intake and all-cause mortality in CRC survivors. However, these previous studies did not correct

TABLE 5 Interaction of vitamin D concentrations with total magnesium intake in relation to CRC recurrence and all-cause mortality in CRC patients[1]

Total magnesium intake	Deficient $25(OH)D_3$ concentrations (<50 nmol/L)		Sufficient $25(OH)D_3$ concentrations (≥50 nmol/L)		HR (95% CI) for $25(OH)D_3$ concentrations within strata of magnesium intake
	n/Events; Events/1000 person-years	HR (95% CI)	*n*/Events; Events/1000 person-years	HR (95% CI)	
CRC recurrence					
Low magnesium intake	248/36; 42	1.0 (ref)	314/33; 29	0.77 (0.47, 1.26)	0.78 (0.47, 1.29)
High magnesium intake	230/29; 37	0.99 (0.56, 1.77)	329/47; 41	1.07 (0.63, 1.84)	0.98 (0.58, 1.67)
HR (95% CI) for magnesium within strata of $25(OH)D_3$ concentrations		1.08 (0.56, 2.09)		1.20 (0.67, 2.12)	
P for multiplicative interaction = 0.6[2]					
RERI (95% CI) = −0.01 (−0.20, 0.18)[3]					
All-cause mortality					
Low magnesium intake	249/47; 39	1.0 (ref)	314/55; 36	1.03 (0.68, 1.54)	0.98 (0.65, 1.49)
High magnesium intake	231/36; 30	0.82 (0.49, 1.38)	331/36; 22	0.53 (0.31, 0.89)	0.69 (0.42, 1.18)
HR (95% CI) for magnesium within strata of $25(OH)D_3$ concentrations		0.94 (0.52, 1.69)		0.43 (0.25, 0.76)	
P for multiplicative interaction = 0.06[2]					
RERI (95% CI) = 0.27 (−0.08, 0.61)[3]					

[1]Analyzed with a Cox proportional hazard model adjusted for age, sex, BMI, physical activity (moderate to vigorous; h/wk), stage, tumor location, season of blood collection, total calcium intake, total energy intake, and cohort. Vitamin D deficient: <50 nmol/L; vitamin D sufficient: ≥50 nmol/L. High and low intakes of magnesium were determined based on the median. For total magnesium intake the median was 322 mg/d (COLON) and 383 mg/d (EnCoRe). CRC, colorectal cancer; RERI, relative excess risk due to interaction; $25(OH)D_3$, 25-hydroxyvitamin D_3.

[2]The *P* for multiplicative interaction was calculated by adding vitamin D status, magnesium intake, as well as vitamin D status × magnesium to the model, adjusted for the aforementioned confounders.

[3]To investigate interaction on an additive scale the RERI was calculated, adjusted for the aforementioned confounders. For example, the RERI for joint effects of vitamin D status and magnesium was calculated as $HR_{vitD-Mg-} - HR_{vitD+Mg-} - HR_{vitD-Mg+} + 1$.

for magnesium intake. Although it seems that the association between calcium and mortality is partly caused by magnesium, it should be noted that magnesium and calcium intake are correlated ($r = 0.6$), thus the effect of magnesium and calcium can probably not be disentangled completely. Of note, albeit not statistically significantly, a high calcium intake appears to be associated with a lower risk of recurrence as well. Therefore, based on our data, we can carefully conclude that calcium is at least not harmful for CRC patients.

The lowest risk of all-cause mortality was found in patients who had both high vitamin D concentrations and a high magnesium intake. In addition, the association between vitamin D concentrations and all-cause mortality is only present in those with a high magnesium intake. This is in line with previous research in the general population, showing a stronger association between vitamin D concentrations and all-cause mortality in those with a higher magnesium intake (15). Thus, if the observed associations are causal, the presence of an adequate status of both nutrients is essential in lowering the risk of all-cause mortality. Considering the importance of magnesium for the enzymatic conversion of vitamin D into its active form (15, 37), magnesium might be crucial in maintaining a sufficient vitamin D status (15, 16, 37). The active form of vitamin D is hypothesized to have beneficial effects on cancer prognosis (38). There are also indications that vitamin D influences CRC mortality by modulation of immune and inflammatory responses (39). In addition, magnesium deficiency is associated with chronic low-grade inflammation (40). Because vitamin D and magnesium are both suggested to influence systemic inflammation (39, 40), it is tempting to speculate that vitamin D and magnesium contribute to a lowered inflammatory status via shared mechanisms, possibly resulting in better survival rates.

In our study among stage I–III patients, no associations between $25(OH)D_3$, magnesium, or calcium and CRC recurrence were observed. One previous study investigating the association between $25(OH)D_3$ concentrations and CRC recurrence reported a strong inverse association (HR: 0.37; 95% CI: 0.18, 0.84) (8). However, this study population consisted of CRC patients with liver metastasis (stage IV), which is a very specific population at high risk of recurrences. Although we did not observe an association between $25(OH)D_3$ concentrations, magnesium and calcium intake, and CRC recurrence, this should be further investigated before a solid conclusion can be drawn.

Because the presence of an adequate status of both magnesium and vitamin D seems to be essential in lowering the risk of all-cause mortality, attention should be paid to both vitamin D concentrations as well as magnesium intake. However, more data are needed to draw firm conclusions and provide practical guidance. At first, the underlying mechanisms explaining the interaction between magnesium and vitamin D in attenuating all-cause mortality should be further unraveled. Second, diet and lifestyle intervention studies should investigate whether an increase in magnesium intake and vitamin D concentrations results in better CRC prognosis. In these studies, the influence of different sources of magnesium (diet or supplements) and vitamin D (sunlight exposure or diet and supplements) should also be investigated.

The present study had some limitations. First, we did not analyze concentrations of magnesium and calcium. Blood concentrations of these minerals are tightly regulated and <1%

TABLE 6 Interaction of vitamin D concentrations with total calcium intake in relation to CRC recurrence and all-cause mortality in CRC patients[1]

Total calcium intake	Deficient 25(OH)D_3 concentrations (<50 nmol/L)		Sufficient 25(OH)D_3 concentrations (≥50 nmol/L)		HR (95% CI) for 25(OH)D_3 concentrations within strata of calcium intake
	n/Events; Events/1000 person-years	HR (95% CI)	*n*/Events; Events/1000 person-years	HR (95% CI)	
CRC recurrence					
Low calcium intake	244/38; 47	1.0 (ref)	316/39; 36	0.74 (0.46, 1.18)	0.77 (0.47, 1.26)
High calcium intake	234/27; 33	0.65 (0.37, 1.13)	327/41; 34	0.74 (0.44, 1.26)	1.11 (0.65, 1.91)
HR (95% CI) for calcium within strata of 25(OH)D_3 concentrations		0.77 (0.42, 1.40)		1.04 (0.61, 1.77)	
P for multiplicative interaction = 0.1[2]					
RERI (95% CI) = 0.05 (−0.13, 0.22)[3]					
All-cause mortality					
Low calcium intake	245/46; 39	1.0 (ref)	317/46; 30	0.80 (0.52, 1.23)	0.79 (0.50, 1.25)
High calcium intake	235/37; 32	0.87 (0.53, 1.42)	328/45; 27	0.78 (0.48, 1.27)	0.99 (0.62, 1.59)
HR (95% CI) for calcium within strata of 25(OH)D_3 concentrations		0.82 (0.48, 1.40)		1.16 (0.70, 1.92)	
P for multiplicative interaction = 0.5[2]					
RERI (95% CI) = 0.06 (−0.01, 0.14)[3]					

[1] Analyzed with a Cox proportional hazard model adjusted for age, sex, BMI, physical activity (moderate to vigorous; h/wk), stage, tumor location, season of blood collection, dietary magnesium intake, total energy intake, and cohort. Vitamin D deficient: <50 nmol/L; vitamin D sufficient: ≥50 nmol/L. High and low intakes of calcium were determined based on the median. For total calcium intake the median was 888 mg/d (COLON) and 930 mg/d (EnCoRe). CRC, colorectal cancer; RERI, relative excess risk due to interaction; 25(OH)D_3, 25-hydroxyvitamin D_3.

[2] The *P* for multiplicative interaction was calculated by adding vitamin D status, calcium intake, as well as vitamin D status × calcium intake to the model, adjusted for the aforementioned confounders.

[3] To investigate interaction on an additive scale the RERI was calculated, adjusted for the aforementioned confounders. For example, the RERI for joint effects of vitamin D status and magnesium was calculated as $HR_{vitD-Ca-} - HR_{vitD+Ca-} - HR_{vitD-Ca+} + 1$.

of the total body magnesium and calcium is circulating (41, 42), thus measuring magnesium and calcium blood concentrations would not likely have resulted in more information (16). Second, the number of events was relatively low in our study population ($n = 155$ for recurrence; $n = 191$ for mortality), which limits the power to detect statistically significant associations, especially in the interaction analyses. Nonetheless, a significant interaction between magnesium and vitamin D was observed for all-cause mortality. Third, it could be that participants of our study are relatively health conscious, which probably led to an attenuation of the real effect. Furthermore, we had no data available about the cause of death. Therefore, we were not able to perform analyses with disease-specific mortality as an outcome. Finally, results of this study can only be generalized to the Western population. The present study also had some important strengths. First, to the best of our knowledge this study was the first to investigate 25(OH)D_3 concentrations and magnesium and calcium intakes, individually and jointly, in relation to CRC recurrence and all-cause mortality. Second, we could investigate the influence of total magnesium and calcium intakes, because we obtained information about dietary as well as supplemental intakes. Finally, because of the availability of detailed data on diet and other clinical and lifestyle factors, we could adjust for the most relevant confounders, although residual confounding can never be fully excluded.

To conclude, we observed that 25(OH)D_3 and magnesium may work synergistically in decreasing the risk of all-cause mortality in CRC patients. Although our results should be confirmed in diet and lifestyle intervention studies, our findings could contribute to improving recommendations regarding magnesium and vitamin D intake for newly diagnosed CRC patients.

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