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Original Research Article

Prediagnostic serum calcium concentrations and risk of colorectal cancer development in 2 large European prospective cohorts

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Abbreviations: AMORIS, Apolipoprotein-related MOrtality RISk; CRC, colorectal cancer; EPIC, European Prospective Investigation into Cancer and Nutrition; NSAID, nonsteroidal anti-inflammatory drug; UK-BB, United Kingdom Biobank; RCT, randomized controlled trial; TXRF, total reflection X-ray fluorescence spectrometry.

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A B S T R A C T

Background: Higher dietary calcium consumption is associated with lower colorectal cancer (CRC) risk. However, little data are available on the association between circulating calcium concentrations and CRC risk.

Objectives: To explore the association between circulating calcium concentrations and CRC risk using data from 2 large European prospective cohort studies.

Methods: Conditional logistic regression models were used to calculate multivariable-adjusted ORs and 95% CIs in case-control studies nested within the European Prospective Investigation into Cancer and Nutrition (EPIC; *n*-cases = 947, *n*-controls = 947) and the UK Biobank (UK-BB; *n*-cases = 2759, *n*-controls = 12,021) cohorts.

Results: In EPIC, nonalbumin-adjusted total serum calcium (a proxy of free calcium) was not associated with CRC (OR: 0.94; 95% CI: 0.85, 1.03; modeled as continuous variable, per 1 mg/dL increase), colon cancer (OR: 0.93; 95% CI: 0.82, 1.05) or rectal cancer (OR: 1.01; 95% CI: 0.84, 1.20) risk in the multivariable adjusted model. In the UK-BB, serum ionized calcium (free calcium, most active form) was inversely associated with the risk of CRC (OR: 0.85; 95% CI: 0.76, 0.95; per 1 mg/dL) and colon cancer (OR: 0.78; 95% CI: 0.68, 0.90), but not rectal cancer (OR: 1.02; 95% CI: 0.83, 1.24) in multivariable adjusted models. Meta-analysis of EPIC and UK-BB CRC risk estimates showed an inverse risk association for CRC in the multivariable adjusted model (OR: 0.90; 95%CI: 0.84, 0.97). In analyses by quintiles, in both cohorts, higher levels of serum calcium were associated with reduced CRC risk (EPIC: $OR_{Q5vs.Q1}$: 0.69; 95% CI: 0.47, 1.00; *P*-trend = 0.03; UK-BB: $OR_{Q5vs.Q1}$: 0.82; 95% CI: 0.72, 0.94; *P*-trend < 0.01). Analyses by anatomical subsite showed an inverse cancer risk association in the colon (EPIC: $OR_{Q5vs.Q1}$: 0.63, 95% CI: 0.39, 1.02; *P*-trend = 0.05; UK-BB: $OR_{Q5vs.Q1}$: 0.75; 95% CI: 0.64, 0.88; *P*-trend < 0.01) but not the rectum.

Conclusions: In UK-BB, higher serum ionized calcium levels were inversely associated with CRC, but the risk was restricted to the colon. Total serum calcium showed a null association in EPIC. Additional prospective studies in other populations are needed to better investigate these associations.

Keywords: cancer, cohort, colorectal, risk, serum calcium

Introduction

Colorectal cancer (CRC) is the third most common malignancy worldwide [1]. Factors such as physical inactivity, body fatness, tobacco smoking, and alcohol intake, as well as high consumption of red and/or processed meats and limited consumption of whole grains, dietary fiber, and dairy products are associated with higher risk of CRC development [2]. The observed inverse associations between dairy products and CRC risk have been mostly attributed to their content of calcium, with epidemiological studies usually reporting high dietary calcium consumption or intake of calcium supplements to be related to lower CRC risk [2]. Two recent umbrella reviews of meta-analyses of findings from prospective cohort studies show inverse risk associations between higher dietary calcium consumption and CRC [3,4]. However, it is unclear if or to what extent circulating calcium levels may be associated with CRC development.

Calcium is one of the most abundant elements in the human body. In healthy populations, the amount of dietary calcium absorbed is relatively constant with minor fluctuations [5]. Intestinal absorption of dietary calcium at normal intake levels is largely by active transport, with approximately one-third of ingested calcium being absorbed in healthy adults [5], suggesting that the majority of ingested calcium reaches the colorectum. Serum calcium homeostasis is tightly regulated in all aspects from intestinal absorption of dietary calcium to fluxes with skeletal calcium (the dominant body calcium storage) and renal excretion via intricate feedback loops by PTH, vitamin D, and their receptors, and is only marginally influenced by dietary calcium intake levels except in situations of very low intakes, deficiency, or disease [5,6]. Calcium plays an essential role in bone mineralization, but also has a broad range of functions across body systems [5]. Within the colonic milieu, dietary calcium may bind to bile acids hence potentially reducing their carcinogenic and tumor promoting properties on colonic tissues [7], whereas intracellular calcium, derived largely from circulating calcium, has a stronger role in inhibiting proliferation and promoting differentiation and apoptosis of normal and neoplastic colorectal epithelium [8]. Circulating calcium exists either bound to albumin or in an ionized (free) form, in roughly equal partitions, along with a small percentage of complexed calcium [9], together referred to as total serum calcium. Ionized calcium is recognized as the physiologically relevant parameter and is responsible for the intra- and extracellular functions of the mineral, largely by interacting with calcium-specific receptors. In the absence of direct measures of ionized calcium, circulating levels can be estimated through formulas that correct total serum calcium measures with albumin concentrations [9], although some recent findings suggest that total serum calcium not corrected for albumin is an adequate reflection of calcium status and of free calcium levels [10].

We have previously shown CRC risk associations for some components of calcium homeostasis, namely PTH [11], vitamin D [12], and genetic variation in the vitamin D and calcium-sensing receptors, which are present in intestinal tissues [13]. To date, few studies have assessed the association between circulating calcium levels and CRC risk. Findings from a Swedish prospective study show a positive association between circulating albumin-adjusted total serum calcium concentrations and CRC risk (HR: 1.04, 95% CI: 1.01, 1.07) [14]. Conversely, a recent Mendelian randomization study of people of European descent with over 58,000 CRC cases found little evidence of an association between genetically predicted concentrations of calcium and CRC risk [15].

Given the paucity of data, particularly from prospective cohort studies, we investigated the association between circulating calcium concentrations and CRC risk in 2 case-control studies nested within EPIC and the UK-BB cohorts; 2 large, multicenter, international prospective cohort studies that together comprise over 1 million participants with detailed dietary, lifestyle, and biological specimens collected prediagnostically.

Methods

European Prospective Investigation into Cancer and Nutrition (EPIC)

Study population and data collection

EPIC is a large, multicenter prospective cohort study designed to investigate the associations between diet, lifestyle, genetic and

environmental factors, and the incidence of cancers and other chronic diseases. Over 520,000 participants aged 25–70 y were recruited between 1992 and 2000 in 23 centers in Europe (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the UK). Upon recruitment, standardized lifestyle data, anthropometric data, and blood samples were collected. Diet over the previous 12 mo was assessed using validated country-specific questionnaires [16]. Blood samples are stored at the International Agency for Research on Cancer (IARC-WHO) at -196°C in liquid nitrogen for all countries except Denmark (-150°C, nitrogen vapor) and Sweden (-80°C, freezers). Detailed information on the study design, methods, and rationale of the EPIC study design has been previously reported [16, 17]. All study participants provided written informed consent. Ethical approval for this study was obtained by the IARC review board and local participating centers.

Case ascertainment

Incident cancer cases were identified by record linkage (Denmark, Italy, the Netherlands, Spain, and the UK) or other methods such as pathology registries, health insurance records, and active communication with study participants or next of kin (France, Germany). The latest dates of complete information for CRC incidence in the cases with available serum calcium biomarker data applicable to the current study ranged between June 2002 and 2003, depending on the subcohort.

Cases were subjects who developed primary, first-incident, histologically confirmed CRC between recruitment and the latest date of complete information. The ICD-10 and the Second Revision of the International Classification of Disease for Oncology were used to code cancer incidence. Tumors of the cecum, appendix, ascending colon, hepatic flexure, transverse colon, splenic flexure, descending and sigmoid colon (C18.0–C18.7) and overlapping tumors (C18.8 and C18.9) were characterized as colon cancers. Tumors occurring at the rectosigmoid junction (C19) or rectum (C20) were defined as rectal cancers. Anal canal cancers (C21) were excluded. Colon and rectal cancer cases combined were defined as CRC cases.

Nested case-control study design

Detailed description of the study design has been reported previously [18]. Briefly, the current study was based on data from all centers except Norway and Sweden (serum samples were not available for these centers) and Greece (data not available). Participants with a prior cancer diagnosis at any site (except for nonmelanoma skin cancer) or with missing serum calcium levels were not included in the analyses. For every case, a control was selected from all cohort members alive and free of cancer (incidence density sampling) and matched by study center of recruitment, sex, age at blood collection, time of blood collection, and fasting status; females were also matched by menopausal status. Premenopausal females were additionally matched by the phase of menstrual cycle while postmenopausal females were additionally matched by current use of menopausal hormonal therapy. After exclusion of participants with missing biomarker measurements in either the case or the matched control, 1894 individuals [CRC cases: 947 (585 colon and 362 rectal cancers); matched controls: 947] were used in the current analyses. A participant flowchart is shown in Figure 1.

Serum calcium measurements

Total serum calcium levels were determined from the emission spectrograms recorded during analyses of serum selenium levels by total reflection X-ray fluorescence spectrometry (TXRF; Pico-foxTM S2, Bruker Nano GmbH), as previously reported [18]. Case-control status was blinded during the analyses for quality control. Samples were measured in duplicate and the mean concentration values, the SD and the CV were calculated. Duplicate samples with differences in concentration varying more than 10% were measured again.

Additional validation and accuracy experiments for the serum calcium measurements were conducted. To this end, calcium values in samples of certified reference serum (article no. 201405, Seronorm™ Trace Elements Serum L-1) were analyzed, and the TXRF analysis yielded calcium concentration values within the reference range of the standard material, with interassay CV of 3.9%. Next, tests with spiked serum samples were conducted wherein 5 different amounts of 10 mM $CaCl_2$ were added to set volumes of the certified reference sample and calcium levels were then assessed in each sample by TXRF. The additional calcium spiked into the serum samples was recovered with $<10\%$ deviation from the theoretical value. Finally, a dilution test was conducted, wherein the certified reference serum of known concentration was diluted by H_2O 2, 4, and 8 times, i.e., to 50%, 25%, and 12.5% (vol:vol), yielding the expected concentrations within 10% of theoretical values. The quantification of serum calcium proved hereby to be linear across 1 order of magnitude [i.e., within the measured concentration range from as low as 25 mg/L (dilution test) to as high as 200 mg/l (spiking test)]. In EPIC, serum albumin levels are unavailable and hence correction or adjustment for serum albumin concentrations was not feasible.

UK Biobank (UK-BB)

Study population and data collection

The UK-BB cohort is a large, population-based prospective study. Over 500,000 participants were assessed throughout the UK between 2006 and 2010, covering a variety of settings to provide socioeconomic, ethnic, and residential heterogeneity. The UK-BB has approval from the North West Multicentre Research Ethics Committee, the National Information Governance Board for Health and Social Care in England and Wales, and the Community Health Index Advisory Group in Scotland. In addition, an independent Ethics and Governance Council was formed in 2004 to oversee the UK-BB's continuous adherence to the Ethics and Governance Framework that was developed for the study (http://www.ukbiobank.ac.uk/ethics/). Detailed information on the study design, methods, and rationale of the cohort have been previously reported [19]. Upon recruitment, the participants provided medical, dietary, and lifestyle data, including information on alcohol use, smoking status, physical activity, education, reproductive history, hormone use, and previous illnesses. Nonfasting blood samples were collected from all participants at recruitment and from a subset of approximately 20,000 participants who reattended the assessment center between 2012 and 2013 for a repeat assessment visit. This research has been conducted using the UK-BB resource under application number 25897.

Case ascertainment

Cohort participants were followed using record linkage, and incident cases were identified through national cancer registries. Complete follow-up was available through March 31, 2016 for England and Wales and October 31, 2015 for Scotland. First primary incident CRC were coded according to the ICD-10 (https://biobank.ndph.ox.ac.uk/crystal/crystal/docs/CancerLinkage.pdf).

Nested case-control study design

In the UK-BB, controls were selected from the full cohort of individuals who were alive and free of cancer (except nonmelanoma skin

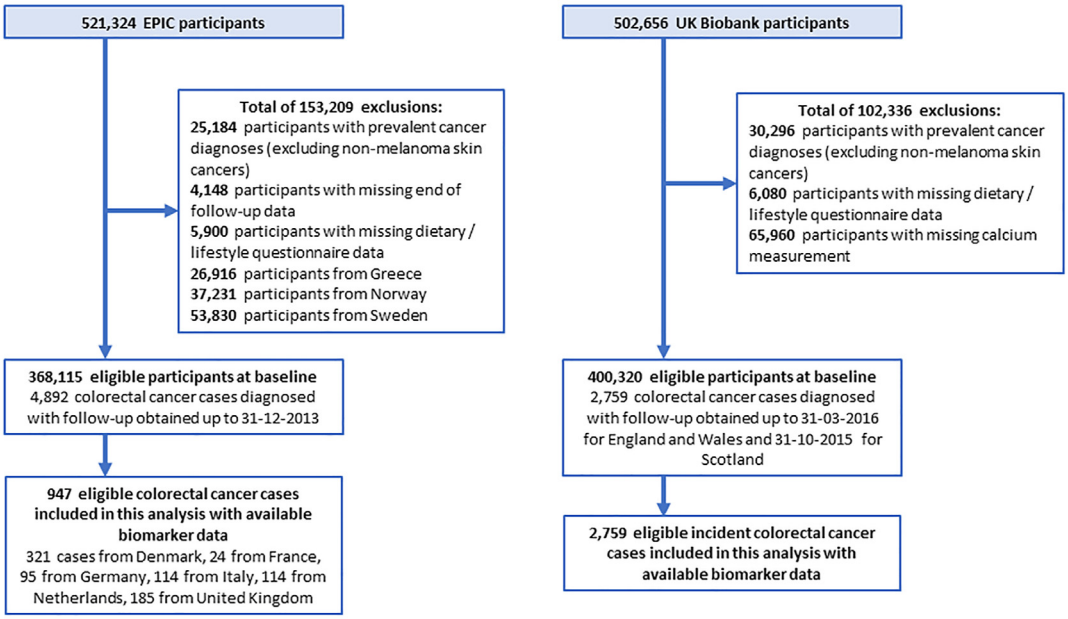

Figure 1. Participant flowcharts for the European Prospective Investigation into Cancer and Nutrition (EPIC) and United Kingdom Biobank (UK-BB) cohorts.

cancer, ICD-10 C44) at the time of diagnosis of the cases, using incidence density sampling. Five controls were matched to each CRC case by age at recruitment (5-y categories), sex, region, and follow-up time since baseline blood collection. Thus, the final analysis included 2,759 incident CRC cases matched to 12,021 controls. UK-BB participants were excluded if they had missing serum calcium levels. A participant flowchart is shown in Figure 1.

Serum calcium measurements

During recruitment, several biological samples were collected. Various preservatives, anticoagulants, and clot accelerators were used to allow the widest possible range of assays. Ionized serum calcium measurements were based on calcium ions (Ca^{2+}) reacting with Arsenazo III (2,2'-[1,8-dihydroxy-3,6-disulphonaphthylene-2,7-bisazo]-bisbenzenear-sonic acid) to form an intense purple colored complex. In this method, the absorbance of the Ca-Arsenazo III complex was measured bichromatically at 660/700 nm. The resulting increase in absorbance of the reaction mixture is directly proportional to the calcium concentration in the sample [20].

Statistical analyses in EPIC and UK-BB

Baseline categorical data were expressed as percentages and continuous data as mean and SD.

For EPIC data, we used conditional logistic regression with multivariable adjustment to estimate ORs and 95% CIs to assess the association between total serum calcium levels and risk of CRC development. A three-stage tiered approach to confounder adjustment was adopted: **Model 1** was conditioned only by the matching factors (study center of recruitment, sex, age at blood collection, time of blood collection, and fasting status; females were also matched by menopausal status); **Model 2** was additionally adjusted for *a priori* determined confounders including smoking status/duration/intensity [never smokers, current smoker (1-15 cig/d; 16-25 cig/d; 26+ cig/d; pipe/cigar/occasional), former smokers (quit $\leq$ 10 y; quit 11–20 y; quit 20+ y), missing/unknown], physical activity (sex-specific categories of metabolic equivalents; inactive, moderately inactive, moderately active, active, missing [21]), highest level of attained education (none/not specified, primary school completed, technical/professional school, secondary school, higher education including university degree), BMI (kg/m^2), and alcohol intake (g/d); **Model 3**, the full model, was further adjusted for total dietary energy consumption (kcal/d) and intake of total dietary fiber (g/d), total fruits, nuts and seeds (g/d), total vegetables (g/d), and red and processed meats (g/d). This adjustment strategy was adopted because of the various lifestyle, dietary, and metabolic factors that may affect both calcium intake and circulating concentrations as well as risk of CRC. Additional adjustments for dairy product consumption and height, as well as circulating PTH and vitamin D levels did not alter the findings and were thus not included in the final statistical models. To assess potential reverse causality, a sensitivity analysis was conducted on Model 3, excluding CRC cases diagnosed $\leq$ 2 y from baseline.

For UK-BB data, we applied conditional logistic regression with multivariable adjustment to assess the association between serum ionized calcium levels and risk of CRC development. The results were presented as ORs and their corresponding 95% CIs. A 4-stage tiered approach to confounder adjustment was adopted, using as similar a list of confounders as possible to those used in analyses on the EPIC cohort: **Model 1** was conditioned on matching factors [sex, age at recruitment (5-y intervals), and recruitment region]; **Model 2** was additionally adjusted for smoking status/intensity, physical activity, highest level of attained education, BMI (kg/m^2), and alcohol intake; **Model 3**, was further adjusted for fruit and vegetable consumption and red and processed meat intake. The level of detail in the available UK-BB dietary information does not allow for calculation of total energy consumption, which was thus not considered as part of Model 3. In UK-BB, existing information on important factors related to CRC [i.e., use of nonsteroidal anti-inflammatory drugs (NSAIDs) and aspirin, colorectal screening attendance, and first-degree family history for CRC] allowed us to extend our assessment of CRC risk. Thus, we included **Model 4**, which was equivalent to Model 3 but additionally adjusted for these important factors.

For each model in each cohort, serum calcium concentrations were assessed as (i) continuous variable (per one mg/dL increase in serum calcium concentration) and (ii) categorized in quintiles. In both studies,

Table 1
Baseline characteristics of first incident colorectal cancer cases and their matched controls in the EPIC nested case-control study

General Characteristics[1]	Colorectal Cancer Cases	Matched Controls
N	947	947
Females, %	52.7	52.7
Age at blood collection, y	58.6 (7.0)	58.5 (7.0)
Education, %		
Primary	37.9	41.4
Technical/professional school	25.3	26.6
Secondary	15.2	12.7
University degree	18.1	17.1
Unknown	3.5	2.2
Smoking status and intensity of smoking, %		
Never	36.8	39.3
Current, 1–15 cigarettes/d	11.1	11.2
Current, 16–25 cigarettes/d	6.6	6.8
Current, 26+ cigarettes/d	1.6	0.8
Current, pipe/cigar	10.0	7.6
Former, quit less than 10 y	9.1	8.5
Former, quit 11–20 y	10.1	9.3
Former, quit 20+ y	12.1	13.5
Unknown	2.6	3.0
Physical activity, %		
Inactive	16.2	12.8
Moderately inactive	28.8	29.7
Moderately active	44.0	43.4
Active	10.5	12.7
Unknown	0.5	1.5
Among females, %		
Premenopausal	10.6	11.2
Ever use of menopausal hormonal therapy	42.4	46.5
Ever use of oral contraceptives	23.2	24.6
Baseline measurements		
BMI, kg/m^2	26.7 (4.4)	26.3 (3.8)
Waist circumference, cm	90.2 (13.2)	88.3 (12.4)
Baseline dietary intakes[2]		
Total energy, kcal/d	2179.5 (731.1)	2162.6 (624.8)
Alcohol, g/d	17.9 (22.3)	16.0 (20.0)
Calcium, mg/d	1010.0 (417.2)	1055.0 (419.1)
Total fiber, g/ d	23.1 (7.9)	23.9 (8.0)
Total fruits, nuts, and seeds, g/d	219.9 (171.2)	241.0 (180.7)
Total vegetables, g/d	183.2 (111.3)	191.7 (121.0)
Red meat, g/d	59.3 (41.3)	58.3 (40.6)
Processed meat, g/d	36.2 (52.1)	32.6 (28.7)
Dairy products, g/d	334.0 (255.1)	367.6 (259.0)
Baseline serum concentration		
Calcium, mg/dL	10.4 (1.2)	10.5 (1.2)

[1] Characteristics are reported as mean (SD) unless otherwise specified.
[2] Two cases with missing dietary information did not contribute to the baseline dietary intakes. Abbreviations: BMI, body mass index

Table 2
Baseline characteristics of first incident CRC cases and matched controls in UK-BB

General characteristics[1]	Colorectal Cancer Cases	Matched Controls
N	2759	12021
Females, %	42.2	41.7
Age at recruitment, y	60.7 (6.6)	60.7 (6.5)
Education, %		
None of the below	21.3	21.9
National examination at age 16 y (CSEs/O-levels/GCSEs or equivalent)	23.3	22.8
National examination at ages 17/18 y or equivalent (NVQ/HND/HNC/A-levels/AS-levels or equivalent)	15.5	15.9
Other professional qualifications	26.8	27.5
College or university degree	11.5	10.8
Missing/prefer not to answer	1.6	1.2
Smoking status and intensity of smoking, %		
Never	45.8	51.6
Former	43.5	38.8
Current < 15 cigarettes/d	2.7	2.6
Current 15+ cigarettes/d	3.9	3.5
Current, unknown intensity	3.6	3.0
Missing/prefer not to answer	0.6	0.5
Alcohol consumption status, %		
Never	7.1	7.5
Special occasions only	9.9	10.2
1–3 times/mo	9.2	10.0
1–2 times/wk	24.7	25.2
3–4 times/wk	23.3	23.9
Daily or almost daily	25.5	23.1
Missing/prefer not to answer	0.3	0.1
Physical activity, %		
<10 MET h/wk	22.5	22.2
10–<20 MET h/wk	17.9	16.4
20–<40 MET h/wk	22.0	22.7
40–<60 MET h/wk	11.9	12.7
60+ MET h/wk	22.2	22.7
Unknown	3.5	3.4
Family history for colorectal cancer, %		
Yes	14.2	11.8
No	83.5	85.9
Unknown	2.3	2.3
Baseline measurements		
BMI, kg/m^2	27.9 (4.6)	27.5 (4.4)
Waist circumference, cm	93.8 (13.5)	92.2 (12.9)
Red and processed meat consumption, %		
< 2 times/wk	10.6	12.2
2.00–2.99 times/wk	26.3	28.3
3.00–3.99 times/wk	15.1	14.8
More than 4 times/wk	46.9	43.3
Unknown	1.2	1.4
Baseline serum concentration		
Calcium, mg/dL	9.5 (0.4)	9.5 (0.4)

Abbreviations: BMI, body mass index; cm, centimetre; cigarettes/d, cigarettes per day; h/wk, hours per week; kg/m2, kilograms per meter squared; MET, metabolic equivalent of task; mg/dL, milligrams per decilitre; N, number; SD, standard deviation; times/mo, times per month; times/wk, times per week; y, year.
[1] Characteristics are reported as mean (SD) unless otherwise specified.

quintile categorization was based on the distribution of serum calcium concentrations in the controls. In analyses by quintile, the reference category was set as the first quintile. The normality of the distribution of the serum calcium was assessed by visual inspection of the histogram. *P* values for trend were calculated using the median value of the quintiles in the models. Subgroup analyses were performed for the anatomical subsites of colon and rectum as well as by males and females separately, given potential sex-specific differences in dietary patterns and calcium consumption.

Model 3 adjusted OR and 95% CI of continuous serum calcium concentrations for EPIC and UK-BB were also meta-analyzed using both fixed (common-effect inverse-variance method) and random (inverse-variance model with DerSimonian-Laird method) effect models calculated using the "Metan" command in Stata [22].

Additionally, the UK-BB models were corrected for regression dilution using regression dilution ratios obtained from 12,692 participants

Table 3
The association of serum calcium levels and risk of colorectal, colon, and rectal cancers in the overall EPIC nested case-control study

Serum Calcium, mg/dL	Colorectal Cancer				Colon Cancer				Rectal Cancer			
	Cases/ Controls	OR (95% CI)			Cases/ Controls	OR (95% CI)			Cases/ Controls	OR (95% CI)		
		Model 1[1]	Model 2[2]	Model 3[3]		Model 1[1]	Model 2[2]	Model 3[3]		Model 1[1]	Model 2[2]	Model 3[3]
Continuous	947[4]/947	0.96 (0.87, 1.05)	0.94 (0.86, 1.04)	0.94 (0.85, 1.03)	585[4]/585	0.94 (0.83, 1.05)	0.93 (0.83, 1.05)	0.93 (0.82, 1.05)	362[4]/362	1.00 (0.85, 1.17)	1.00 (0.84, 1.19)	1.01 (0.84, 1.20)
Quintiles												
<9.5	195/189	Ref.	Ref.	Ref.	120/107	Ref.	Ref.	Ref.	75/82	Ref.	Ref.	Ref.
9.5-10.1	210/189	1.05 (0.78, 1.41)	1.05 (0.77, 1.42)	0.99 (0.73, 1.35)	132/123	0.91 (0.63, 1.32)	0.92 (0.62, 1.35)	0.85 (0.57, 1.27)	78/66	1.33 (0.81, 2.18)	1.45 (0.85, 2.45)	1.37 (0.80, 2.35)
10.1-10.7	193/190	0.94 (0.69, 1.28)	0.92 (0.67, 1.28)	0.89 (0.64, 1.23)	124/121	0.85 (0.57, 1.26)	0.79 (0.52, 1.20)	0.76 (0.49, 1.16)	69/69	1.12 (0.67, 1.88)	1.24 (0.72, 2.13)	1.18 (0.67, 2.05)
10.7-11.4	184/189	0.87 (0.62, 1.22)	0.86 (0.61, 1.22)	0.79 (0.56, 1.13)	117/126	0.73 (0.48, 1.12)	0.73 (0.47, 1.15)	0.67 (0.42, 1.05)	67/63	1.16 (0.67, 2.01)	1.21 (0.67, 2.18)	1.11 (0.61, 2.04)
>11.4	165/190	0.76 (0.53, 1.08)	0.73 (0.50, 1.05)	0.69 (0.47, 1.00)	92/108	0.66 (0.42, 1.04)	0.66 (0.41, 1.07)	0.63 (0.39, 1.02)	73/82	0.95 (0.54, 1.69)	0.96 (0.52, 1.80)	0.91 (0.48, 1.72)
P-trend		0.07	0.05	0.03		0.05	0.06	0.05		0.64	0.62	0.50

[1] Model based on matching factors only

[2] Model based on matching factors plus further adjustment for smoking status/duration/intensity, BMI (kg/m^2), total physical activity, highest level of attained education and total alcohol intake

[3] Model based on matching factors, smoking status/duration/intensity, BMI (kg/m^2), total physical activity, highest level of attained education and total alcohol intake (Model 2), further adjusted for total energy, intake of red and processed meat, fruit, vegetable and fiber (g/d).

[4] Two cases (1 colon and 1 rectal cancer case) with missing dietary information are not included in Model 3. Conditional logistic regression was used to analyze the data. Abbreviations: OR= Odds-ratio, CI: Confidence interval.

Table 4
The association of serum calcium levels and risk of colorectal, colon, and rectal cancers in the overall UK Biobank nested case-control study

Serum Calcium	Colorectal Cancer					Cases/ Controls	Colon Cancer				Rectal Cancer				
	Cases/ Controls	OR (95% CI)					OR (95% CI)				Cases/ Controls	OR (95% CI)			
		Model 1[1]	Model 2[2]	Model 3[3]	Model 4[4]		Model 1[1]	Model 2[2]	Model 3[3]	Model 4[4]		Model 1[1]	Model 2[2]	Model 3[3]	Model 4[4]
Continuous, mg/dL	2759/ 12,021	0.84 (0.75, 0.94)	0.85 (0.75, 0.95)	0.85 (0.76, 0.95)	0.85 (0.76, 0.95)	1880/ 8001	0.77 (0.67, 0.89)	0.78 (0.68, 0.89)	0.78 (0.68, 0.90)	0.77 (0.67, 0.89)	958/4160	1.00 (0.82, 1.21)	1.01 (0.82, 1.23)	1.02 (0.83, 1.24)	1.03 (0.84, 1.25)
Regression dilution ratio corrected, mg/ dL	2759/ 12,021	0.69 (0.51, 0.87)	0.68 (0.52, 0.88)	0.68 (0.52, 0.90)	0.69 (0.52, 0.90)	1880/ 8001	0.55 (0.40, 0.76)	0.55 (0.40, 0.77)	0.56 (0.40, 0.77)	0.55 (0.40, 0.77)	958/4160	0.99 (0.63, 1.57)	1.01 (0.64, 1.61)	1.04 (0.65, 1.65)	1.06 (0.67, 1.69)
Quintiles															
<9.22 mg/dL	640/2417	Ref.	Ref.	Ref.	Ref.	449/1576	Ref.	Ref.	Ref.	Ref.	211/869	Ref.	Ref.	Ref.	Ref.
9.22–9.41 mg/dL	544/2399	0.85 (0.75, 0.97)	0.86 (0.76, 0.98)	0.85 (0.75, 0.97)	0.85 (0.75, 0.97)	375/1599	0.82 (0.70, 0.95)	0.82 (0.70, 0.95)	0.81 (0.69, 0.94)	0.80 (0.69, 0.94)	186/826	0.93 (0.75, 1.16)	0.94 (0.75, 1.17)	0.93 (0.75, 1.17)	0.93 (0.75, 1.17)
9.41–9.58 mg/dL	527/2413	0.82 (0.72, 0.94)	0.82 (0.72, 0.94)	0.83 (0.73, 0.94)	0.83 (0.73, 0.94)	353/1581	0.78 (0.67, 0.92)	0.79 (0.67, 0.92)	0.79 (0.67, 0.92)	0.78 (0.67, 0.92)	190/860	0.90 (0.73, 1.13)	0.91 (0.73, 1.13)	0.93 (0.74, 1.16)	0.93 (0.74, 1.16)
9.59–9.80 mg/dL	524/2398	0.82 (0.72, 0.94)	0.82 (0.72, 0.94)	0.82 (0.72, 0.94)	0.82 (0.72, 0.93)	346/1611	0.75 (0.64, 0.88)	0.75 (0.64, 0.88)	0.75 (0.64, 0.87)	0.73 (0.63, 0.86)	191/815	0.97 (0.78, 1.21)	0.96 (0.77, 1.20)	0.98 (0.78, 1.22)	0.98 (0.78, 1.22)
>9.80 mg/dL	524/2394	0.82 (0.72, 0.93)	0.82 (0.72, 0.94)	0.82 (0.72, 0.94)	0.82 (0.72, 0.94)	357/1634	0.75 (0.64, 0.88)	0.75 (0.64, 0.88)	0.75 (0.64, 0.88)	0.75 (0.64, 0.88)	180/790	0.94 (0.75, 1.17)	0.95 (0.76, 1.19)	0.95 (0.76, 1.19)	0.96 (0.77, 1.21)
P-trend		<0.01	<0.01	<0.01	<0.01		<0.01	<0.01	<0.01	<0.01		0.68	0.73	0.80	0.86

[1] Model based on matching factors (sex, age category, region).
[2] Model with further adjustment for smoking status/duration/intensity, BMI (kg/m^2), total physical activity, highest level of attained education, and total alcohol intake.
[3] Model further adjusted for frequency of red and processed meat intake and fruit and vegetable intake.
[4] Model further adjusted for regular nonsteroidal anti-inflammatory drug/aspirin use, colorectal cancer screening attendance, first-degree family history for colorectal cancer. Missing information in dietary variables resulted in slightly lower counts for Models 3 and 4. Conditional logistic regression was used to analyze the data. Abbreviations: CI: Confidence interval, OR = Odds ratio.

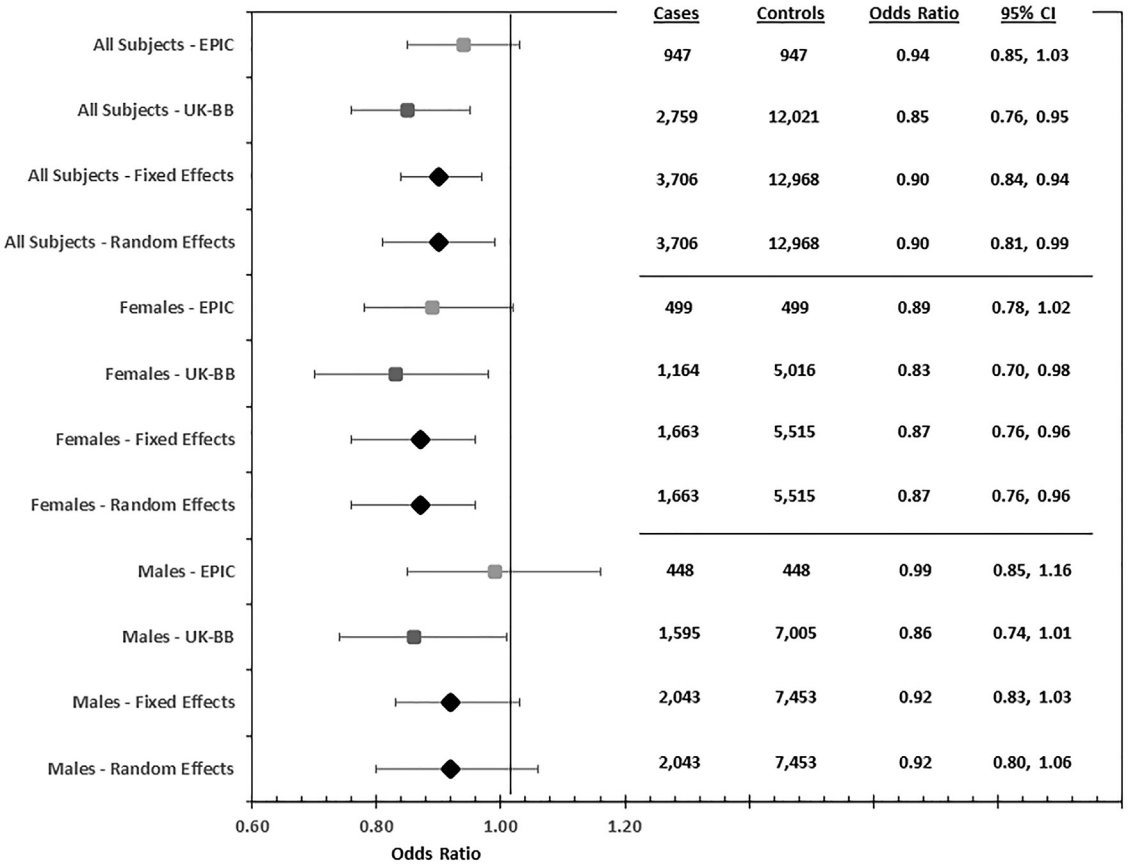

Figure 2. Meta-analysis of the European Prospective Investigation into Cancer and Nutrition (EPIC) and United Kingdom Biobank (UK-BB) findings on the association of circulating serum calcium concentrations and CRC risk in all subjects, females and males. Light gray squares represent ORs from EPIC, dark gray from UK-BB, and black diamonds the meta-analyzed risk estimate from both cohorts. Findings are presented for all subjects (i.e., females and males together) in each cohort, as well as for females and males separately. Fixed effects and random effects refer to the meta-analysis model utilized. Numbers of cases and matched controls included in each analysis are provided, along with point estimate and 95% CIs for each analysis.

with repeated serum ionized calcium measurements collected a median of 4 y after baseline. Measurement error and within-person variability using single measures at baseline may lead to biased estimation of risk (i.e., regression dilution bias) [23]. In order to provide more precise and generalizable risk estimates, ORs for trend were estimated per absolute increase in ionized calcium concentrations, with correction for regression dilution bias using the McMahon-Peto method [23,24].

All statistical analyses were conducted using SAS version 9.4 (SAS Institute) and Stata version 13 (StataCorp). All statistical tests were 2-sided and P values <0.05 were considered statistically significant.

Results

Description of the study populations and baseline characteristics are shown in Table 1 (EPIC) and Table 2 (UK-BB). In EPIC, cases were less likely to be physically active and on average had lower fruit and vegetable intake and higher red and processed meat intake compared to the controls. In the UK-BB, cases reported more frequently being former smokers, having a family history of CRC, and consuming more red and processed meat compared to the controls. No differences in serum calcium concentration were observed between cases and controls in either study.

The overall associations between serum calcium concentration (in continuous models) and CRC risk are shown in Table 3 (EPIC) and Table 4 (UK-BB). In EPIC, total serum calcium was not associated with risk of CRC (OR: 0.94; 95% CI: 0.85, 1.03; per 1 mg/dL increase), colon cancer (OR: 0.93; 95% CI: 0.82, 1.05; per 1 mg/dL increase) or rectal cancer (OR: 1.01; 95% CI: 0.84, 1.20; per 1 mg/dL increase) in Model 3, the fully adjusted model. In the UK-BB, serum ionized calcium was inversely associated with the risk of CRC (OR: 0.85; 95% CI: 0.76, 0.95; per 1 mg/dL increase) and colon cancer (OR: 0.78; 95% CI: 0.68, 0.90; per 1 mg/dL increase), but not rectal cancer (OR: 1.02; 95% CI: 0.83, 1.24). These associations were unchanged following additional adjustment for other important confounding factors for CRC, available only in UK-BB data and not in EPIC (i.e., regular NSAID/aspirin use, CRC screening attendance, first-degree family history for CRC; Model 4). In EPIC, exclusion of CRC cases diagnosed ≤ 2 y from baseline did not modify the findings (Model 3). Meta-analyses of the obtained risk estimates of serum calcium concentration and CRC risk in EPIC and UK-BB together (Model 3, calcium as a continuous variable) showed an inverse overall risk association for CRC (fixed effect OR: 0.90; 95% CI: 0.84, 0.97; per 1 mg/dL increase; $I^2 = 44.3\%$; P value for Cochran's Q measure of heterogeneity $= 0.180$; random effect OR: 0.90; 95% CI: 0.81, 0.99; $I^2 = 44.3\%$; P value for Cochran's Q measure of heterogeneity $= 0.180$) (Figure 2). The meta-analyzed risk association appeared stronger in females (fixed effect OR: 0.87; 95% CI: 0.78, 0.96; $I^2 = 0.0\%$; P value for Cochran's Q measure of heterogeneity $= 0.525$; random effect OR: 0.87; 95% CI: 0.78, 0.96; $I^2 = 0.0\%$; P value for Cochran's Q measure of heterogeneity $= 0.525$) than in males (fixed

Table 5
The sex-specific associations of serum calcium levels and risk of colorectal, colon, and rectal cancers in the EPIC nested case-control study

Serum Calcium	Females				Males			
	Cases/ Controls	OR (95% CI)			Cases/ Controls	OR (95% CI)		
		Model 1[1]	Model 2[2]	Model 3[3]		Model 1[1]	Model 2[2]	Model 3[3]
Colorectal cancer								
Continuous, mg/ dL	499/499	0.92 (0.81, 1.04)	0.90 (0.79, 1.02)	0.89 (0.78, 1.02)	448[4]/448	1.01 (0.88, 1.16)	1.00 (0.86, 1.16)	0.99 (0.85, 1.16)
Quintiles								
<9.5	107/110	Ref.	Ref.	Ref.	88/79	Ref.	Ref.	Ref.
9.5–10.1	113/92	1.24 (0.83, 1.85)	1.28 (0.84, 1.97)	1.27 (0.82, 1.96)	97/97	0.86 (0.56, 1.34)	0.82 (0.52, 1.31)	0.73 (0.45, 1.18)
10.1–10.7	103/98	1.01 (0.66, 1.55)	1.01 (0.64, 1.59)	0.96 (0.60, 1.53)	90/92	0.83 (0.52, 1.32)	0.79 (0.48, 1.31)	0.74 (0.44, 1.23)
10.7–11.4	92/100	0.86 (0.54, 1.37)	0.83 (0.51, 1.35)	0.78 (0.47, 1.29)	92/89	0.85 (0.52, 1.39)	0.82 (0.48, 1.39)	0.71 (0.41, 1.22)
>11.4	84/99	0.78 (0.49, 1.26)	0.77 (0.46, 1.26)	0.74 (0.44, 1.22)	81/91	0.72 (0.42, 1.22)	0.66 (0.37, 1.17)	0.60 (0.33, 1.08)
P-trend		0.14	0.12	0.09		0.26	0.21	0.13
Colon cancer								
Continuous, mg/ dL	322/322	0.91 (0.78, 1.06)	0.89 (0.76, 1.05)	0.89 (0.76, 1.06)	263[4]/263	0.97 (0.81, 1.16)	0.97 (0.80, 1.19)	0.96 (0.78, 1.17)
Quintiles								
<9.5	67/65	Ref.	Ref.	Ref.	53/42	Ref.	Ref.	Ref.
9.5–10.1	74/63	1.11 (0.68, 1.80)	1.09 (0.64, 1.84)	1.06 (0.61, 1.83)	58/60	0.70 (0.39, 1.25)	0.69 (0.37, 1.29)	0.57 (0.30, 1.10)
10.1–10.7	69/66	0.94 (0.56, 1.58)	0.89 (0.51, 1.55)	0.83 (0.47, 1.48)	55/55	0.71 (0.38, 1.32)	0.59 (0.30, 1.16)	0.51 (0.25, 1.04)
10.7–11.4	64/68	0.82 (0.47, 1.43)	0.73 (0.40, 1.34)	0.68 (0.36, 1.28)	53/58	0.61 (0.32, 1.19)	0.62 (0.30, 1.28)	0.50 (0.23, 1.06)
>11.4	48/60	0.67 (0.37, 1.22)	0.68 (0.36, 1.30)	0.71 (0.36, 1.38)	44/48	0.61 (0.31, 1.22)	0.53 (0.24, 1.16)	0.46 (0.21, 1.01)
P-trend		0.13	0.16	0.19		0.20	0.15	0.09
Rectal cancer								
Continuous, mg/ dL	177/177	0.93 (0.74, 1.16)	0.86 (0.67, 1.11)	0.84 (0.65, 1.08)	185[4]/185	1.08 (0.85, 1.36)	1.06 (0.80, 1.40)	1.09 (0.80, 1.48)
Quintiles								
<9.5	40/45	Ref.	Ref.	Ref.	35/37	Ref.	Ref.	Ref.
9.5–10.1	39/29	1.63 (0.78, 3.39)	2.11 (0.91, 4.93)	2.08 (0.87, 4.97)	39/37	1.14 (0.57, 2.26)	1.08 (0.47, 2.50)	0.90 (0.37, 2.18)
10.1–10.7	34/32	1.15 (0.53, 2.50)	1.36 (0.58, 3.17)	1.38 (0.57, 3.38)	35/37	1.03 (0.51, 2.09)	1.24 (0.52, 2.96)	1.13 (0.45, 2.80)
10.7–11.4	28/32	0.94 (0.41, 2.15)	0.96 (0.38, 2.43)	1.02 (0.39, 2.65)	39/31	1.30 (0.61, 2.75)	1.26 (0.51, 3.13)	1.02 (0.38, 2.72)
>11.4	36/39	1.03 (0.47, 2.28)	0.88 (0.36, 2.16)	0.79 (0.31, 2.02)	37/43	0.88 (0.38, 2.02)	0.78 (0.27, 2.27)	0.65 (0.21, 2.03)
P-trend		0.66	0.28	0.19		0.84	0.77	0.57

[1] Model based on matching factors only.
[2] Model based on matching factors plus further adjustment for smoking status/duration/intensity, BMI (kg/m^2), total physical activity, highest level of attained education and total alcohol intake.
[3] Model based on matching factors, smoking status/duration/intensity, BMI (kg/m^2), total physical activity, highest level of attained education and total alcohol intake (Model 2), further adjusted for total energy, intake of red and processed meat, fruit, vegetable and fiber (g/d).
[4] Two males (1 colon and 1 rectal cancer case) with missing dietary information are not included in Model 3. Conditional logistic regression was used to analyze the data. Abbreviations: OR= Odds-ratio, CI: Confidence interval.

effect OR: 0.92; 95% CI: 0.83, 1.03; I^2 = 36.5%; *P* value for Cochran's Q measure of heterogeneity = 0.21; random effect OR: 0.92; 95% CI: 0.80, 1.06; I^2 = 36.5%; *P* value for Cochran's Q measure of heterogeneity = 0.210) (Figure 2).

For both EPIC and UK-BB, categorical models showed an inverse association between serum calcium concentrations and risk of CRC comparing the highest to the lowest concentration categories (EPIC: $OR_{Q5vs.Q1}$: 0.69; 95% CI: 0.47, 1.00; *P*-trend = 0.03, Table 3; UK-BB: $OR_{Q5vs.Q1}$: 0.82; 95% CI: 0.72, 0.94; *P*-trend < 0.01, Tables 3 and 4). For both cohorts, the inverse cancer risk associations were restricted to the colon (EPIC: $OR_{Q5vs.Q1}$: 0.63; 95% CI: 0.39, 1.02; *P*-trend = 0.05; UK-BB: $OR_{Q5vs.Q1}$: 0.75; 95% CI: 0.64, 0.88; *P*-trend < 0.01), with null associations in the rectum (Tables 3 and 4). In UK-BB, the observed CRC risk associations were similar when further adjusted for additional relevant adjustment factors for CRC (Model 4). A broadly similar pattern of cancer risk associations was observed in subgroup analyses stratified by sex (Tables 5 and 6) compared to findings by males and females together, but differences by subgroup were not statistically heterogeneous. The association appeared stronger in females and limited to the colon.

Table 6
The sex-specific associations of serum calcium levels and risk of colorectal, colon, and rectal cancers in the UK Biobank nested case-control study

Serum Calcium	Females					Males				
	Cases/ Controls	OR (95% CI)				Cases/ Controls	OR (95% CI)			
		Model 1[1]	Model 2[2]	Model 3[3]	Model 4[4]		Model 1[1]	Model 2[2]	Model 3[3]	Model 4[4]
Colorectal cancer										
Continuous, mg/dL	1164/5016	0.82 (0.69, 0.97)	0.83 (0.69, 0.98)	0.83 (0.70, 0.98)	0.83 (0.70, 0.98)	1595/7005	0.86 (0.74, 1.00)	0.86 (0.74, 1.01)	0.86 (0.74, 1.01)	0.87 (0.74, 1.02)
Regression dilution ratio corrected, mg/dL	1164/5016	0.63 (0.42, 0.94)	0.64 (0.43, 0.96)	0.65 (0.43, 0.96)	0.64 (0.43, 0.96)	1595/7005	0.70 (0.49, 1.00)	0.71 (0.49, 1.01)	0.71 (0.49, 1.02)	0.72 (0.50, 1.04)
Quintiles										
<9.22 mg/dL	217/795	Ref.	Ref.	Ref.	Ref.	423/1622	Ref.	Ref.	Ref.	Ref.
9.22–9.41 mg/dL	222/929	0.88 (0.71, 1.08)	0.88 (0.71, 1.09)	0.87 (0.71, 1.08)	0.87 (0.71, 1.08)	322/1470	0.84 (0.71, 0.99)	0.85 (0.73, 1.00)	0.85 (0.72, 1.00)	0.85 (0.72, 1.00)
9.41–9.58 mg/dL	220/966	0.84 (0.68, 1.03)	0.84 (0.68, 1.03)	0.84 (0.68, 1.04)	0.84 (0.68, 1.04)	307/1447	0.81 (0.69, 0.96)	0.82 (0.70, 0.97)	0.83 (0.70, 0.98)	0.83 (0.70, 0.98)
9.59–9.80 mg/dL	235/1068	0.82 (0.66, 1.00)	0.82 (0.67, 1.01)	0.82 (0.67, 1.01)	0.82 (0.66, 1.01)	289/1330	0.83 (0.70, 0.98)	0.83 (0.70, 0.98)	0.83 (0.70, 0.98)	0.83 (0.70, 0.98)
>9.80 mg/dL	270/1258	0.78 (0.64, 0.96)	0.79 (0.64, 0.96)	0.79 (0.64, 0.97)	0.79 (0.64, 0.97)	254/1136	0.85 (0.72, 1.01)	0.86 (0.72, 1.03)	0.86 (0.72, 1.03)	0.87 (0.73, 1.04)
P-trend		0.02	0.02	0.03	0.02		0.05	0.06	0.07	0.08
Colon cancer										
Continuous, mg/dL	857/3687	0.76 (0.62, 0.93)	0.77 (0.63, 0.94)	0.77 (0.63, 0.95)	0.77 (0.63, 0.95)	987/4350	0.78 (0.64, 0.94)	0.78 (0.64, 0.94)	0.78 (0.64, 0.94)	0.77 (0.63, 0.94)
Regression dilution ratio corrected, mg/dL	857/3687	0.53 (0.33, 0.85)	0.54 (0.34, 0.86)	0.55 (0.34, 0.88)	0.55 (0.34, 0.88)	987/4350	0.56 (0.36, 0.88)	0.56 (0.35, 0.88)	0.56 (0.35, 0.88)	0.55 (0.35, 0.86)
Quintiles										
<9.22 mg/dL	168/572	Ref.	Ref.	Ref.	Ref.	273/1012	Ref.	Ref.	Ref.	Ref.
9.22–9.41 mg/dL	159/672	0.83 (0.65, 1.06)	0.84 (0.66, 1.07)	0.83 (0.65, 1.06)	0.83 (0.65, 1.06)	205/938	0.81 (0.66, 0.98)	0.81 (0.67, 1.00)	0.81 (0.66, 0.99)	0.80 (0.65, 0.98)
9.41–9.58 mg/dL	163/700	0.82 (0.64, 1.05)	0.82 (0.64, 1.05)	0.82 (0.64, 1.05)	0.83 (0.65, 1.06)	189/882	0.76 (0.61, 0.93)	0.77 (0.62, 0.95)	0.77 (0.62, 0.95)	0.76 (0.61, 0.94)
9.59–9.80 mg/dL	175/795	0.78 (0.62, 0.99)	0.78 (0.62, 1.00)	0.79 (0.62, 1.00)	0.78 (0.61, 1.00)	162/825	0.72 (0.58, 0.89)	0.71 (0.58, 0.89)	0.71 (0.58, 0.89)	0.70 (0.56, 0.87)
>9.80 mg/dL	192/948	0.71 (0.56, 0.89)	0.71 (0.56, 0.90)	0.72 (0.56, 0.91)	0.71 (0.56, 0.90)	158/693	0.81 (0.65, 1.01)	0.82 (0.66, 1.02)	0.81 (0.65, 1.02)	0.81 (0.65, 1.01)
P-trend		<0.01	<0.01	<0.01	<0.01		0.02	0.02	0.02	0.02
Rectal cancer										
Continuous, mg/dL	316/1377	1.01 (0.73, 1.39)	1.02 (0.73, 1.42)	1.01 (0.73, 1.40)	1.00 (0.71, 1.39)	632/2793	0.99 (0.77, 1.27)	1.01 (0.78, 1.29)	1.02 (0.79, 1.31)	1.03 (0.80, 1.33)
Regression dilution ratio corrected, mg/dL	316/1377	1.02 (0.48, 2.16)	1.05 (0.49, 2.25)	1.02 (0.48, 2.20)	0.99 (0.46, 2.14)	632/2793	0.97 (0.55, 1.73)	1.01 (0.57, 1.82)	1.04 (0.58, 1.87)	1.07 (0.59, 1.93)
Quintiles										
<9.22 mg/dL	51/228	Ref.	Ref.	Ref.	Ref.	155/646	Ref.	Ref.	Ref.	Ref.
9.22–9.41 mg/dL	63/268	1.04 (0.69, 1.58)	1.07 (0.70, 1.64)	1.06 (0.69, 1.61)	1.04 (0.68, 1.59)	122/559	0.89 (0.68, 1.15)	0.91 (0.70, 1.19)	0.91 (0.70, 1.19)	0.92 (0.70, 1.20)
9.41–9.58 mg/dL	59/275	0.97 (0.65, 1.46)	0.97 (0.64, 1.47)	0.97 (0.64, 1.47)	0.96 (0.63, 1.45)	125/591	0.88 (0.68, 1.14)	0.89 (0.69, 1.16)	0.91 (0.70, 1.19)	0.92 (0.70, 1.19)
9.59–9.80 mg/dL	62/286	0.93 (0.62, 1.41)	0.92 (0.61, 1.41)	0.92 (0.61, 1.42)	0.90 (0.59, 1.38)	130/528	1.00 (0.77, 1.30)	1.01 (0.77, 1.32)	1.03 (0.79, 1.34)	1.03 (0.79, 1.35)
>9.80 mg/dL	81/320	1.08 (0.73, 1.59)	1.11 (0.74, 1.65)	1.10 (0.73, 1.63)	1.08 (0.72, 1.62)	100/469	0.86 (0.65, 1.14)	0.88 (0.67, 1.17)	0.88 (0.66, 1.17)	0.89 (0.67, 1.18)
P-trend		0.80	0.77	0.78	0.82		0.49	0.56	0.61	0.65

Abbreviations: CI: Confidence interval, OR= Odds ratio.

[1] Model based on matching factors (sex, age category, region).

[2] Model with further adjustment for smoking status/duration/intensity, BMI (kg/m^2), total physical activity, highest level of attained education, and total alcohol intake.

[3] Model further adjusted for frequency of red and processed meat intake and fruit and vegetable intake.

[4] Model further adjusted for regular nonsteroidal anti-inflammatory drug/aspirin use, colorectal cancer screening attendance, first-degree family history for colorectal cancer. Missing information in dietary variables resulted in slightly lower counts for Models 3 and 4. Conditional logistic regression was used to analyze the data.

In UK-BB, using existing repeat calcium measurements collected a median of 4 y after baseline in 12,692 participants, we computed the intraclass correlation with the baseline values [males and females combined = 0.41 (95% CI: 0.40, 0.43); males = 0.38 (95% CI: 0.36, 0.40); females = 0.43 (95% CI: 0.41, 0.45)].

Discussion

Our observations, derived from 2 large European prospective cohort studies, indicate that higher levels of serum calcium are associated with reduced risk of CRC development. In EPIC, measures of nonalbumin-

adjusted total serum calcium were not associated with CRC risk. In UK-BB, measures of serum ionized calcium demonstrated inverse CRC risk associations of approximately similar magnitude to those observed in EPIC but were largely statistically significant. This may be due to differences in study power, varying design aspects between the 2 cohorts, or that ionized calcium was measured in UK-BB, whereas in EPIC we relied on measures of total serum calcium. Nevertheless, meta-analysis of the EPIC and UK-BB data together showed a statistically significant overall inverse CRC risk association that was generally more pronounced in females than in males.

Ionized serum calcium is recognized as the most physiologically pertinent measure of calcium status, but total serum calcium is often measured in clinical biochemistry laboratories as a suitable approximation of ionized calcium levels in healthy populations. However, the clinical utility of total serum calcium in some patient groups has been questioned, leading to the calculation of albumin-adjusted total serum calcium levels, a practice which is also under debate for its own clinical utility [10]. One of the few studies that has analyzed the association between circulating blood calcium concentrations and CRC risk is the Swedish AMORIS (Apolipoprotein-related MOrtality RISk) study [14]. In continuous models of albumin-corrected total serum calcium, they observed a statistically significant, slightly elevated risk of colorectal and colon cancers (per 1 SD increase of calcium), whereas the risk association for rectal cancer was of similar magnitude but not statistically significant [14]. In analyses by sex, they observed higher risk associations in both males and females in the colon, rectum and colorectum, although point estimates for females were marginally higher and statistically significant for colon cancer and CRC than those in males [14]. We can speculate that apparent sex-differences may be due to potential disparities in calcium metabolism between males and females and by age [25], whereas differences by anatomical subsite may be related to calcium availability within the colonic milieu and its precipitation of bile acids and similar potentially carcinogenic compounds. The difference in the direction of the association between the AMORIS study [14] and our findings in UK-BB and EPIC may be due to study design in terms of the degree of adjustment for confounding variables. The AMORIS study utilized a case-cohort approach, adjusting only for sex, socioeconomic status, and an index of comorbidities [14], whereas our analyses in both EPIC and UK-BB cohorts were nested case-control analyses with more detailed confounder adjustment. Nevertheless, the diversity of findings in these 3 cohorts suggests that additional analyses in other prospective studies and other populations are necessary.

Inconsistent findings have also been observed from Mendelian randomization studies of genetically predicted serum calcium concentrations in people of European descent. One study assessed 2 different genetic instruments of serum calcium concentrations with risk of CRC in 58,221 cases and 67,694 controls, observing a statistically nonsignificant inverse association with 1 instrument and a null association with a second, more detailed instrument [15], while a second phenome-wide Mendelian randomization study on UK-BB data observed a nonstatistically significant positive CRC risk association [26]. A similar nonstatistically significant positive CRC risk association was also observed in 26,397 CRC cases and 41,481 controls (not including UK-BB data) [27].

A possible explanatory mechanism for our findings is through the calcium-related regulation of PTH. A decrease in serum calcium level activates calcium-sensing receptors in the parathyroid gland leading to increased PTH biosynthesis and higher serum levels [5]. Elevated PTH levels have been suggested to be cancer promoting [28]. In the EPIC cohort, higher PTH concentration has been associated with higher CRC risk in males [11]. However, adjustment of the EPIC data for either PTH or circulating vitamin D levels did not attenuate our observations.

Our study has several strengths. First, the inclusion of 2 large, well characterized cohort studies with prospective study designs, i.e., the prediagnostic collection of epidemiological data and biospecimens. The latter allowed us to minimize the bias of reverse causation and to reduce any potential interference with hypercalcemia of malignancy, a common finding in people with advanced cancer [29], although this is thought to be rare in CRC [30]. Additionally, the detailed information collection at baseline in both cohorts enabled adjustment for important lifestyle factors that may influence CRC development. Nevertheless, the study also has a few notable limitations. Measurements of ionized calcium, which are thought to be of greater clinical and physiological relevance than total serum calcium, were not available in the EPIC cohort, and the lack of data on blood albumin concentration in EPIC did not allow the calculation of albumin-corrected calcium levels. EPIC participants were in apparent health at recruitment and it is likely that for the majority, measures of total serum calcium can be regarded as good estimates of calcium homeostasis [31]. Another limitation is that the serum calcium measurements were based on one time point, at blood drawn at recruitment. We assume that the measures are reflective of long-term serum calcium levels. In the UK-BB, serial serum calcium measures exist for a small proportion of participants. These repeated measures in the same individuals allowed us to apply regression dilution methods to correct the UK-BB data for this potential bias. However, the correlations between the 2 measures were modest, suggesting likely variations of serum calcium levels over time.

In summary, we found that higher serum ionized calcium levels are inversely associated with risk of colon but not rectal cancer in the UK-BB while higher total serum calcium was not associated with either cancer in EPIC. These observations contrast with findings from the AMORIS cohort that suggest positive colon cancer risk associations. Thus, further studies are needed to verify our findings and to investigate underlying mechanisms between serum calcium concentrations and development of colon and rectal cancers and whether these common clinical biochemistry measures may show utility in risk assessment or stratification for these common malignancies.

Conflicts of interest

The authors' responsibilities were as follows—DJH, MJ, MJG, LS: conceived and managed study and generated the data; NK, NM, MJ: analyzed the data; NK, NM, DJH, MJ, LS: wrote the paper; MJG, HF, EKA, AJC, ER, TK, KEB, KP: reviewed paper and interpretation of results; QS, VS, SR, EW, AT, AO, KO, MCB-R, FRM, YM-S, RK, MBS, RT, SP, GM, AP, CS, JWGD, GS, AH, CL, AG, M-JS, M-DC, EA, PA, BvG, BG, DA: commented on the analysis and interpretation of the findings; and all authors: read and approved the final manuscript. The authors report no conflicts of interest.

IARC Disclosure Statement

Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer/World Health Organization.

Data availability

For information on how to submit an application for gaining access to EPIC data and/or biospecimens, please follow the instructions at http://epic.iarc.fr/access/index.php. The UK Biobank is an open access resource and bona fide researchers can apply to use the UK Biobank dataset by registering and applying at http://ukbiobank.ac.uk/register-apply/.

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