



Identification of Factors Associated With Colorectal Cancer Risk and Survival

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**IDENTIFICATION OF FACTORS ASSOCIATED WITH COLORECTAL CANCER
RISK AND SURVIVAL**

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The Harvard T.H. Chan School of Public Health
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Identification of Factors Associated with Colorectal Cancer Risk and Survival

Abstract

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in the United States. In this dissertation, we examined several factors possibly associated with CRC risk or survival among participants from the Nurses' Health Study and the Health Professionals Follow-Up Study, using Cox proportional hazards models.

Chapter 1 examined fructose and sucrose intake in relation to CRC risk and mortality. We followed 86,323 women and 46,380 men for up to 32 years and found that fructose and sucrose intake was not associated with CRC risk or mortality (all $P_{\text{trend}} \geq 0.22$), though separate analyses by gender and tumor location suggested a trend toward a positive association of fructose and sucrose intake with risk of proximal colon cancer in men ($P_{\text{trend}} = 0.05$ and 0.06 , respectively).

Chapter 2 examined the influence of pre-existing diabetes on survival among 2,604 patients with non-metastatic CRC. Diabetes was not associated with overall survival during the first 5 years after CRC diagnosis. Beyond 5 years, patients with diabetes experienced elevated overall mortality, compared to those without diabetes. The hazard ratios (HRs) for death were 1.01 (95% confidence interval [CI], 0.78-1.32), 1.51 (95% CI, 1.07-2.13) and 2.58 (95% CI, 1.79-3.73) during 0-5, >5-10, and >10 years after CRC diagnosis, respectively. Regarding cause-specific mortality, patients with diabetes had increased mortality from non-CRC cancers (HR, 2.03; 95% CI, 1.28-3.23) and cardiovascular disease (HR, 1.98; 95% CI, 1.23-3.20).

Chapter 3 examined the relationship between plasma vitamin D binding protein (VDBP), bioavailable or free 25-hydroxyvitamin D [25(OH)D], and CRC survival. Among 604 CRC patients with prediagnostic blood samples, higher VDBP levels were associated with a significant improvement in overall and CRC-specific survival ($P_{\text{trend}} = 0.005$ and 0.02 , respectively). However, no association with overall or CRC-specific survival was observed for bioavailable or free 25(OH)D levels.

In conclusion, we found little evidence for an association of fructose and sucrose intake with CRC risk or mortality. Diabetes was associated with increased long-term mortality among patients with non-metastatic CRC, especially mortality from non-CRC cancers and cardiovascular disease. Higher prediagnostic plasma VDBP levels were associated with increased overall and CRC-specific survival.

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Introduction

Colorectal cancer (CRC) is the third most common cancer in both men and women and the second leading cause of cancer-related deaths in the United States (1). Among CRC patients, only 39% are diagnosed at a local stage with a 5-year survival rate of 90%; the survival rates decline to 71% and 14% for the regional and distant stage, respectively (2). This dissertation focuses on the association of fructose and sucrose intake with CRC risk and mortality, the influence of type 2 diabetes (from here on described as “diabetes”) on CRC survival, as well as the relationship between plasma vitamin D binding protein (VDBP), bioavailable or free 25-hydroxyvitamin D [25(OH)D], and CRC survival.

Insulin resistance and hyperinsulinemia may play important roles in the development and progression of CRC. Higher circulating levels of insulin and C-peptide have been associated with increased risk of CRC (3-6). Diet can affect insulin levels, especially among individuals with established insulin resistance. We previously found that higher dietary glycemic indices and insulin indices were associated with decreased survival among CRC patients (7-9). However, it is unclear whether long-term consumption of a high-sugar diet can increase CRC risk and mortality.

Diabetes is known to increase risk of developing CRC. In a meta-analysis of 15 studies, individuals with diabetes had a relative risk for CRC of 1.30 (95% confidence interval [CI], 1.20-1.40), compared to those without diabetes (10). Insulin resistance and hyperinsulinemia have been proposed to be one of the underlying mechanisms. In contrast, the influence of diabetes on CRC survival remains unclear. Among a limited number of studies, the results are mixed: most, but not all, studies found an association between diabetes and decreased overall survival (11). Furthermore, it is unclear whether the observed reduction in survival was primarily explained by increased mortality from the CRC itself or from other diseases (e.g., cardiovascular disease) (12-14). Understanding the role of diabetes in CRC survival could provide important implications for clinical practice and stratification in clinical trials.

Vitamin D is hypothesized to play a role in the development and progression of CRC. VDBP, also known as the Group-specific component, is the major vitamin D carrier protein. About 88% of 25(OH)D is bound to VDBP, while 12% of 25(OH)D is loosely bound to albumin, leaving very little as the free form (15,

16). The “free hormone hypothesis” postulates that the bound fraction of 25(OH)D is not available to target cells for signaling and gene regulation, suggesting that free 25(OH)D and albumin-bound 25(OH)D [collectively referred to as bioavailable 25(OH)D], which can dissociate during tissue perfusion, may be more biologically active. Although the link between higher total vitamin D levels and improved survival among CRC patients has been well documented (17), the relationship between plasma VDBP, bioavailable or free 25(OH)D, and CRC survival remains unknown.

In the following chapters, we sought to address these gaps in the literature. We utilized two ongoing prospective US cohorts with data on diet, lifestyle, biomarkers, and diagnoses of major chronic diseases. Over 170,000 participants had been followed for up to three decades with a high follow-up rate of more than 90% in both cohorts. In Chapter 1, we examined the association of fructose and intake with CRC risk and mortality among all participants from the two cohorts. In Chapter 2, we examined the association between pre-existing diabetes and survival among patients with non-metastatic CRC. In Chapter 3, we examined the association of prediagnostic plasma levels of VDBP, bioavailable 25(OH)D, and free 25(OH)D with CRC survival.

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Chapter 1 Sugar intake and colorectal cancer risk and mortality

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Abstract

Background: Although hyperinsulinemia plays a role in colorectal carcinogenesis, it is unclear whether long-term consumption of a high-sugar diet can increase colorectal cancer (CRC) risk and mortality.

Methods: In two prospective US cohorts, we followed 86,323 women and 46,380 men who were free of cancer and diabetes at baseline and identified 3,094 incident CRCs and 1,104 deaths from CRC during up to 32 years of follow-up. We examined the association of fructose and sucrose intake with CRC risk and mortality, using multivariable-adjusted Cox proportional hazards models.

Results: Fructose and sucrose intake was not associated with CRC risk in the combined cohorts ($P_{\text{trend}} = 0.81$ and 0.97 , respectively). The pooled multivariable-adjusted relative risks of CRC for the highest versus lowest quintile were 0.98 (95% CI, 0.86-1.12) and 1.01 (95% CI, 0.89-1.15) for fructose and sucrose intake, respectively. Separate analyses by gender and tumor location suggested a positive association of fructose and sucrose intake with risk of proximal colon cancer in men, but the associations did not reach statistical significance ($P_{\text{trend}} = 0.05$ and 0.06 , respectively). In addition, fructose and sucrose intake was not associated with CRC mortality in the combined cohorts ($P_{\text{trend}} = 0.71$ and 0.22 , respectively).

Conclusions: In this large prospective study, fructose and sucrose intake was not associated with CRC risk or mortality.

Introduction

Fructose is a natural simple sugar found in many foods like fruits and honey. Fructose is often made into high fructose corn syrup (most often 45% glucose and 55% fructose) added to soft drinks and processed foods. Fructose has a very low glycemic index and is more readily metabolized to lipid in the liver than glucose. When fructose is attached to glucose, it forms the disaccharide sucrose (table sugar), another common added sugar. The 2015-2020 Dietary Guidelines for Americans recommended that added sugars be limited to less than 10% of total daily calories (1). However, in the National Health and Nutrition Examination Survey 2011-2012, added sugars accounted for 17% of total daily calories among adult Americans (2), indicating excessive intake of added sugars in the US population.

Insulin resistance and hyperinsulinemia may play important roles in the development and progression of colorectal cancer (CRC). Higher circulating levels of insulin and C-peptide have been associated with increased risk of CRC (3-6). Diet can affect insulin levels, especially among individuals with established insulin resistance. We previously found that higher dietary glycemic indices and insulin indices were associated with decreased survival among CRC patients (7-9). However, it is unclear whether long-term consumption of a high-sugar diet can increase CRC risk and mortality.

Epidemiological data on sugar intake and CRC risk are sparse (10). Fourteen years ago, Michaud et al. published their findings in the Nurses' Health Study (NHS) and the Health Professionals Follow-Up Study (HPFS) that higher intake of fructose and sucrose was associated with increased risk of CRC in men but not in women (11). The differential association by gender is intriguing but requires further exploration. In addition, no studies have examined fructose and sucrose intake in relation to CRC mortality, an indicator that reflects the influence on CRC incidence, aggressiveness at presentation, and patient survival. In order to better understand the association of fructose and sucrose intake with CRC risk and mortality, we conducted an updated analysis of these two prospective cohorts with 12 more years of follow-up and about 1,300 more CRC patients than the previous study by Michaud et al..

Methods

Study population

In 1976, NHS was initiated when 121,700 US female registered nurses aged 30 to 55 years responded to a mailed questionnaire on risk factors for cancer and cardiovascular disease and medical history (12, 13). In 1986, HPFS was established when 51,529 US male dentists, optometrists, osteopaths, podiatrists, pharmacists, and veterinarians aged 40 to 75 years completed a mailed questionnaire on health-related behaviors and medical history (14). Since baseline, follow-up questionnaires have been sent to participants every two years requesting an update on potential risk factors and new cancer and disease diagnoses. This study was approved by the Human Research Committee at the Brigham and Women's Hospital and the Harvard T.H. Chan School of Public Health. Completion and return of questionnaires were considered informed consent.

Identification of CRC patients

On each follow-up questionnaire, participants were asked to report specified medical conditions including CRC that were diagnosed within the previous two years. Whenever a participant (or next of kin for decedents) reported a diagnosis of CRC, we asked permission to obtain hospital records and pathology reports. For nonrespondents, the National Death Index was used to ascertain any diagnosis of CRC that caused death or was a contributing cause of death, and we asked permission from next of kin for decedents to obtain medical records. A physician blinded to exposure status reviewed these records to confirm the diagnosis and record information on histology, location of primary tumor, and cancer stage. We estimate that 96- 97% of patients were identified through these methods (15, 16).

Mortality assessment

Ascertainment of deaths included review of the National Death Index and reporting by next of kin for decedents or the US Postal Service, and interrogation of names of persistent nonrespondents in the

National Death Index (17). These methods have been shown to identify more than 98% of deaths (18). Cause of death was assigned by physicians via review of death certificates and medical records.

Dietary assessment

Dietary intake was assessed via self-administered semiquantitative food frequency questionnaires (FFQs) in 1980, 1984, 1986, and every four years thereafter in NHS and every four years beginning in 1986 in HPFS. On each FFQ, participants were asked to report their average frequency of intake over the previous year for a specified serving size of each food. Individual nutrient intake was calculated by multiplying the frequency of each food by its nutrient content (obtained from the US Department of Agriculture (19) and supplemented with information from manufactures) and then summing contributions from all foods. The nutrient values were then adjusted for total energy intake. Fructose intake was calculated as free fructose intake plus half sucrose intake, because the disaccharide sucrose is digested rapidly in the small intestine into glucose and fructose.

The validity of the FFQ has been evaluated in a subset of participants who also completed multiple 1-week diet records (20-22). In NHS, the Pearson correlation coefficient between the FFQ and the diet records was 0.54 for sucrose, but was not examined for fructose. However, we observed reasonable correlation coefficients for individual high-fructose foods as follows: 0.84 for sugar-sweetened cola, 0.56 for fruit punch, 0.79 for bananas, 0.80 for apples, 0.74 for oranges, and 0.84 for orange or grapefruit juice (20, 21). Similar correlations were seen in HPFS (22).

Assessment of non-dietary factors

Height, weight, smoking history, diabetes history, and menopausal status and postmenopausal hormone use (in NHS only) were initially reported at study enrollment. Aspirin use and physical activity were first asked in 1980 and 1986, respectively, in NHS and in 1986 in HPFS. Endoscopy history was first asked in 1988 in both cohorts. During follow-up, these factors have been updated every two years.

Family history of CRC was ascertained in 1982, 1988, 1992, 1996, 2000, 2004, and 2008 in NHS and in 1986, 1990, 1992, 1996, and 2008 in HPFS.

Statistical analyses

We excluded participants who left an extensive number of blank items in the FFQ or had implausibly high or low energy intake (<600 or >3,500 kcal/d for women; <800 or >4,200 kcal/d for men). We also excluded participants who had previously diagnosed cancer (other than nonmelanoma skin cancer) or diabetes at baseline, which left 86,323 women and 46,380 men eligible.

One outcome was CRC risk, with person-years accrued from the return date of the baseline FFQ (1980 in NHS; 1986 in HPFS) until the date of CRC diagnosis, death, or last follow-up (June 2012 in NHS; January 2012 in HPFS), whichever came first. Another outcome was CRC mortality, with person-years accrued from the return date of the baseline FFQ until the date of death or last follow-up (the same as above), whichever came first.

Cox proportional hazards models were used to examine the association of fructose and sucrose intake with CRC risk and mortality. To control as finely as possible for confounding by age, calendar time, and a possible interaction between these two time scales, we stratified the models jointly by age (in months) and 2-year questionnaire cycle. To better reflect long-term habits and to minimize random error in reporting of dietary intake, we calculated the cumulative average of fructose and sucrose intake since the baseline FFQ until the beginning of each 2-year questionnaire cycle. Because diabetics usually limit their sugar intake, we excluded FFQs returned after diabetes diagnosis. We also excluded FFQs returned after CRC diagnosis to make the exposure reflect pre-diagnosis diet.

We then categorized fructose and sucrose intake into quintiles, with cutoffs determined separately for each cohort, and combined data from the two cohorts for pooled analysis. Relative risks (RRs) and 95% confidence intervals (CIs) were calculated by comparing quintiles 2-5 with the lowest quintile as the reference group. Tests for trend were performed by entering in the model the median value for each quintile as a continuous variable, with statistical significance evaluated by the Wald test. We adjusted for

known and suspected risk factors for CRC and potential confounders, including gender, race, family history of CRC, body mass index (BMI), physical activity, pack-years of smoking, alcohol intake, regular aspirin use, endoscopy history, menopausal status and postmenopausal hormone use, total energy intake, red meat intake, processed meat intake, and energy-adjusted intake of dietary fiber, calcium, and folate. Diabetes history was not included in the model, because it may lie within the causal pathway of sugar intake and CRC. However, adjusting for this variable or censoring participants with diabetes during follow-up did not alter the results.

We also examined whether the association between sugar intake and CRC risk varied by potential effect modifiers. Interactions were assessed by entering in the model the product of the quintile-specific median of sugar intake and the stratification variable (either binary or continuous), evaluated by the likelihood ratio test (one degree of freedom). All analyses were performed with SAS 9.4 statistical package. All *P* values were two sided.

Results

During follow-up, we identified a total of 3,094 (1,771 in NHS; 1,323 in HPFS) incident CRCs and 1,104 (659 in NHS; 445 in HPFS) deaths from CRC. Age-standardized characteristics of person-years by quintile of fructose intake are demonstrated in Table 1.1. Female and male participants in the highest quintile had average fructose intake accounting for 15.0% and 13.9% of total daily calories, respectively. Participants with higher fructose intake were less likely to smoke, had a lower BMI and were more physically active, and consumed higher levels of carbohydrates, folate, fruits, fruit juice and sugar-sweetened beverages and lower levels of protein, total fat, red meat, processed meat and alcohol. Other characteristics did not vary remarkably across quintiles of fructose intake.

Fructose and sucrose intake was not associated with CRC risk in the combined cohorts ($P_{\text{trend}} = 0.81$ and 0.97, respectively; Table 1.2). The pooled multivariable-adjusted RRs of CRC for the highest versus lowest quintile were 0.98 (95% CI, 0.86-1.12) and 1.01 (95% CI, 0.89-1.15) for fructose and sucrose intake, respectively. The unadjusted RRs are shown in Table S1.1, indicating no association either. However,

separate analyses by gender and tumor location suggested a positive association of fructose and sucrose intake with risk of proximal colon cancer in men, but the associations did not reach statistical significance ($P_{\text{trend}} = 0.05$ and 0.06 , respectively; Table 1.3). To examine whether sugars from non-fruit sources have a greater detrimental effect, we additionally adjusted for total fruit intake, but the association remained null (data not shown).

We observed no association of fructose and sucrose intake with CRC mortality ($P_{\text{trend}} = 0.71$ and 0.22 , respectively; Table 1.4). The pooled multivariable-adjusted RRs of CRC mortality for the highest versus lowest quintile were 1.00 (95% CI, 0.81 - 1.23) and 0.87 (95% CI, 0.71 - 1.07) for fructose and sucrose intake, respectively. Separate analyses by gender and tumor location revealed no material difference in the associations (Table 1.4 and Table S1.2).

We evaluated whether the association between sugar intake and CRC risk varied by potential effect modifiers (Table 1.5). No significant interactions were observed for age, BMI, alcohol intake, dietary fiber intake, and fruit intake (all $P_{\text{interaction}} \geq 0.13$). However, higher intake of fructose and sucrose was associated with decreased risk of CRC among participants in the highest tertile of physical activity ($P_{\text{interaction}} = 0.02$ and 0.07 , respectively).

Discussion

In this large prospective study, women and men had similar fructose intake in terms of percentage of total daily calories, with an average of 14-15% in the highest quintile. We found little evidence for an overall association of fructose and sucrose intake with CRC risk or mortality. However, separate analyses by gender and tumor location suggested a trend toward a positive association of fructose and sucrose intake with risk of proximal colon cancer in men.

The observed lack of association is in line with most previous studies that examined sugar intake in relation to CRC risk. In a meta-analysis of six cohort studies, the summary RR was 1.05 (95% CI, 0.87 - 1.27) for fructose and 1.01 (95% CI, 0.87 - 1.16) for sucrose, comparing high versus low intake (10). In HPFS, higher intake of fructose and sucrose was previously found to be associated with increased risk

of CRC (11). Those analyses included 1,809 (1,113 in NHS; 696 in HPFS) CRC patients, whereas the current study included 3,094 CRC patients.

There could be several explanations for the overall null association of sugar intake with CRC risk and mortality. First, whether sugar intake contributes to chronic hyperglycemia and hyperinsulinemia may depend on the underlying insulin resistance of the individual. In our cohorts, postprandial insulin response to a meal, as represented by dietary insulin indices, was not associated with biomarkers of glycemic control such as HbA1c and C-peptide (23). Moreover, these indices have been found to be associated with CRC survival but not with CRC risk (7, 24). Taken together, these data suggest that a high-sugar diet may not cause hyperinsulinemia among healthy individuals and have limited impact on the natural history of CRC before presentation. Second, the effects of high-sugar foods may depend on which other foods they displace in an isocaloric setting, since many animal products also enhance insulin exposure. To maintain energy balance, individuals who eat less sugar have to eat more protein and/or fat, and these macronutrients may have a stronger effect on chronic insulin exposure. Third, CRC is a multifactorial disorder with over 10 risk factors, including several dietary factors (25). As shown in Table 1, participants with higher sugar intake were less likely to possess other risk factors for CRC, which may overwhelm the effect of sugar intake. Although we rigorously adjusted for these factors, residual confounding remained a possibility as with any observational study.

The physiological effects of sugars may depend on which foods they are contained in. As a major source of fructose and sucrose, fruits are rich in health-promoting micronutrients and bioactive compounds, which may offset the adverse effect of sugars. In contrast, added sugars in beverages and processed foods may have a greater detrimental effect than sugars from fruits and other natural sources due to higher rates of absorption and metabolism. A previous study found that processed-food snacks, compared to whole-food snacks, led to a wider fluctuation in plasma glucose levels as well as a greater area under the plasma insulin curve (26). To investigate whether results may differ by source of sugar, we additionally adjusted for fruit intake and continued to observe no association between sugar intake and

CRC risk, indicating that sugars from non-fruit sources (mostly added sugars) may also not affect CRC development.

The strengths of this study include the prospective design that precludes recall bias, large sample size, long follow-up period, repeated measures of diet, and detailed data on many potential confounders. With a large number of CRC patients, we had sufficient power to analyze the data by gender and tumor location.

Limitations of this study deserve further consideration. First, dietary intake was self-reported by participants. However, we previously showed that the FFQ used in our study had reasonable validity compared to diet records (20-22). In addition, the use of multiple FFQs over time to calculate the cumulative average intake can reduce random measurement error. Second, we were not able to examine associations for sugar intake from different food sources. Finally, our study participants are predominantly individuals of European descent, so our findings may not be generalized to populations of other races.

In summary, our data suggest that long-term consumption of a high-sugar diet may not play a role in CRC development and mortality. Future studies should examine sugar intake from different food sources and individual high-sugar foods such as sugar-sweetened beverages to provide a better understanding for the role of sugar intake in colorectal carcinogenesis.

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Tables

Table 1.1: Age-standardized characteristics of person-years by quintile of fructose intake

Characteristic	Female			Male		
	Quintile 1	Quintile 3	Quintile 5	Quintile 1	Quintile 3	Quintile 5
% of total daily calories from fructose, mean (SD)	6.3 (1.3)	9.9 (0.5)	15.0 (3.1)	5.8 (1.2)	9.4 (0.5)	13.9 (2.1)
Age, years, mean (SD)	59.9 (10.7)	60.8 (11.3)	61.2 (11.8)	62.9 (10.4)	64.3 (11.2)	64.6 (11.6)
White, %	98.5	98.2	94.9	92.0	91.6	88.1
Family history of colorectal cancer, %	12.8	13.6	12.8	11.6	12.1	12.6
Body mass index, kg/m ² , mean (SD)	25.5 (4.7)	25.2 (4.5)	25.1 (4.6)	26.3 (3.6)	25.8 (3.3)	25.4 (3.4)
Physical activity, MET-h/wk, mean (SD)	14.1 (16.0)	16.5 (17.5)	17.2 (20.2)	27.5 (26.6)	31.3 (28.4)	32.4 (31.9)
Pack-years of smoking, mean (SD)	19 (23)	12 (18)	11 (18)	18 (21)	11 (17)	9 (16)
Alcohol intake, g/d, mean (SD)	12 (14)	5 (7)	3 (6)	21 (19)	10 (11)	5 (8)
Regular aspirin use, ≥ 2 times/wk, %	39.5	39.7	38.8	45.7	46.2	44.6
Endoscopy history, %	35.8	37.2	33.8	53.3	57.4	55.9
Diabetes history, %	6.9	4.6	6.4	7.3	5.0	5.1
Postmenopausal, %	81.1	81.4	81.7	-	-	-
Current postmenopausal hormone use, %*	29.4	29.8	27.2	-	-	-
Dietary intake, mean (SD)						
Energy, kcal/d	1,648 (436)	1,701 (429)	1,657 (467)	1,956 (557)	1,999 (546)	1,952 (572)
Carbohydrate, energy-adjusted, g/d	150 (30)	182 (23)	209 (24)	202 (32)	244 (25)	282 (30)
Protein, energy-adjusted, g/d	79 (12)	75 (10)	67 (11)	96 (15)	91 (12)	83 (13)
Fat, energy-adjusted, g/d	69 (12)	62 (9)	55 (9)	76 (13)	70 (11)	62 (11)
Dietary fiber, energy-adjusted, g/d	14 (4)	17 (4)	17 (6)	19 (5)	22 (6)	24 (8)
Calcium, energy-adjusted, mg/d	907 (357)	959 (348)	874 (366)	917 (388)	946 (363)	909 (383)
Folate, energy-adjusted, μ g/d	355 (209)	398 (191)	407 (223)	495 (244)	549 (246)	574 (272)
Red meat, servings/d	1.3 (0.7)	1.1 (0.6)	0.9 (0.6)	1.4 (0.8)	1.1 (0.7)	0.8 (0.6)
Processed meat, servings/d	0.7 (0.6)	0.7 (0.5)	0.6 (0.5)	0.4 (0.4)	0.3 (0.3)	0.2 (0.3)
Fruit, servings/d	1.3 (0.7)	2.3 (0.9)	2.9 (1.6)	1.4 (0.8)	2.5 (1.1)	3.5 (1.9)
Fruit juice, servings/wk	2.9 (2.7)	5.0 (3.5)	6.8 (5.6)	2.3 (2.3)	4.2 (3.5)	7.0 (6.9)
Sugar-sweetened beverages, servings/wk	0.7 (1.1)	1.7 (2.0)	6.0 (7.0)	0.8 (1.2)	2.0 (2.4)	5.4 (6.2)

Abbreviations: SD, standard deviation; MET, metabolic equivalent

*Proportions calculated in postmenopausal women.

Table 1.2: Multivariable-adjusted relative risks for the association of fructose and sucrose intake with colorectal, colon, and rectal cancer risk*

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	<i>P</i> _{trend}
Fructose intake						
Female						
Person-years	504,330	505,938	505,948	505,662	503,909	
Colorectal cancer						
No. of events	355	373	349	348	346	
RR (95% CI)	Referent	1.05 (0.91-1.23)	0.97 (0.83-1.13)	0.95 (0.80-1.11)	0.91 (0.77-1.08)	0.13
Colon cancer						
No. of events	268	279	262	266	261	
RR (95% CI)	Referent	1.03 (0.86-1.23)	0.95 (0.79-1.14)	0.94 (0.78-1.14)	0.91 (0.75-1.10)	0.15
Rectal cancer						
No. of events	76	87	79	73	78	
RR (95% CI)	Referent	1.20 (0.87-1.66)	1.08 (0.77-1.51)	0.98 (0.69-1.39)	0.99 (0.69-1.43)	0.77
Male						
Person-years	204,238	204,744	204,799	204,785	203,962	
Colorectal cancer						
No. of events	288	260	246	274	255	
RR (95% CI)	Referent	1.01 (0.85-1.21)	0.97 (0.80-1.16)	1.11 (0.92-1.34)	1.07 (0.88-1.31)	0.30
Colon cancer						
No. of events	184	161	157	186	169	
RR (95% CI)	Referent	0.98 (0.79-1.23)	0.98 (0.78-1.24)	1.20 (0.95-1.52)	1.17 (0.91-1.50)	0.08
Rectal cancer						
No. of events	64	58	52	50	46	
RR (95% CI)	Referent	0.97 (0.66-1.40)	0.84 (0.56-1.25)	0.83 (0.54-1.25)	0.71 (0.46-1.11)	0.12
Combined						
Colorectal cancer	Referent	1.04 (0.93-1.16)	0.97 (0.86-1.09)	1.02 (0.90-1.15)	0.98 (0.86-1.12)	0.81
Colon cancer	Referent	1.02 (0.89-1.16)	0.96 (0.83-1.11)	1.04 (0.90-1.20)	1.00 (0.86-1.16)	0.89
Rectal cancer	Referent	1.10 (0.86-1.40)	0.98 (0.76-1.26)	0.92 (0.70-1.20)	0.89 (0.67-1.17)	0.23
Sucrose intake						
Female						
Person-years	504,316	505,744	505,808	505,759	504,161	
Colorectal cancer						
No. of events	355	350	394	331	341	
RR (95% CI)	Referent	1.00 (0.86-1.17)	1.12 (0.96-1.31)	0.91 (0.78-1.07)	0.90 (0.76-1.07)	0.10
Colon cancer						
No. of events	266	260	309	244	257	
RR (95% CI)	Referent	0.99 (0.83-1.18)	1.17 (0.98-1.39)	0.90 (0.74-1.08)	0.91 (0.75-1.11)	0.17
Rectal cancer						
No. of events	78	81	77	81	76	
RR (95% CI)	Referent	1.06 (0.77-1.47)	1.02 (0.73-1.43)	1.03 (0.74-1.44)	0.90 (0.63-1.29)	0.53
Male						
Person-years	204,311	205,032	204,672	204,798	203,716	
Colorectal cancer						
No. of events	268	257	269	254	275	
RR (95% CI)	Referent	1.04 (0.87-1.24)	1.13 (0.95-1.36)	1.08 (0.90-1.31)	1.13 (0.93-1.37)	0.20
Colon cancer						
No. of events	164	178	161	167	187	
RR (95% CI)	Referent	1.18 (0.95-1.47)	1.12 (0.89-1.41)	1.17 (0.93-1.49)	1.28 (1.01-1.63)	0.06
Rectal cancer						
No. of events	68	40	61	56	45	
RR (95% CI)	Referent	0.62 (0.41-0.93)	0.96 (0.66-1.40)	0.89 (0.60-1.31)	0.66 (0.43-1.01)	0.24

Table 1.2 (Continued): Multivariable-adjusted relative risks for the association of fructose and sucrose intake with colorectal, colon, and rectal cancer risk*

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	<i>P</i> _{trend}
Combined						
Colorectal cancer	Referent	1.03 (0.92-1.16)	1.14 (1.01-1.28)	0.99 (0.88-1.12)	1.01 (0.89-1.15)	0.97
Colon cancer	Referent	1.08 (0.94-1.24)	1.16 (1.01-1.33)	1.00 (0.87-1.16)	1.06 (0.91-1.23)	0.70
Rectal cancer	Referent	0.86 (0.67-1.11)	0.99 (0.77-1.26)	0.97 (0.76-1.26)	0.81 (0.62-1.06)	0.25

Abbreviations: CI, confidence interval; RR, relative risk

*Models stratified by age (in months) and 2-year questionnaire cycle and adjusted for gender (in the combined analyses), race (white, non-white, unknown), family history of colorectal cancer, body mass index (<25.0, 25.0-29.9, 30.0-34.9, ≥35.0 kg/m²), physical activity (quintiles), pack-years of smoking (0, 1-4, 5-19, 20-39, ≥40), alcohol intake (0, 0.1-4.9, 5.0-9.9, 10.0-14.9, 15.0-29.9, ≥30.0 g/d), regular aspirin use (<2, ≥2 times/wk), endoscopy history, menopausal status and postmenopausal hormone use (premenopausal, never, past and current users of postmenopausal hormone, unknown), total energy intake (quintiles), red meat intake (quintiles), processed meat intake (quintiles), and energy-adjusted intake of dietary fiber (quintiles), calcium (quintiles), and folate (quintiles).

Table 1.3: Multivariable-adjusted relative risks for the association of fructose and sucrose intake with colon cancer risk by tumor location*

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	<i>P</i> _{trend}
Fructose intake						
Female						
Proximal colon						
No. of events	161	174	168	164	161	
RR (95% CI)	Referent	1.07 (0.85-1.33)	1.02 (0.81-1.29)	0.97 (0.76-1.23)	0.95 (0.74-1.21)	0.37
Distal colon						
No. of events	107	105	94	102	100	
RR (95% CI)	Referent	0.98 (0.74-1.30)	0.84 (0.63-1.14)	0.91 (0.68-1.23)	0.85 (0.63-1.16)	0.23
Male						
Proximal colon						
No. of events	84	76	91	108	87	
RR (95% CI)	Referent	0.98 (0.71-1.36)	1.18 (0.86-1.64)	1.43 (1.04-1.99)	1.25 (0.87-1.78)	0.05
Distal colon						
No. of events	92	80	65	73	70	
RR (95% CI)	Referent	1.02 (0.75-1.41)	0.86 (0.61-1.22)	1.01 (0.71-1.43)	1.03 (0.71-1.49)	0.91
Combined						
Proximal colon	Referent	1.05 (0.87-1.26)	1.07 (0.89-1.30)	1.12 (0.93-1.36)	1.05 (0.86-1.28)	0.44
Distal colon	Referent	0.99 (0.80-1.21)	0.85 (0.68-1.07)	0.94 (0.75-1.18)	0.91 (0.71-1.15)	0.33
Sucrose intake						
Female						
Proximal colon						
No. of events	157	160	202	151	158	
RR (95% CI)	Referent	1.04 (0.83-1.30)	1.30 (1.04-1.62)	0.94 (0.74-1.20)	0.97 (0.76-1.25)	0.45
Distal colon						
No. of events	109	100	107	93	99	
RR (95% CI)	Referent	0.93 (0.70-1.23)	0.98 (0.74-1.30)	0.83 (0.61-1.11)	0.83 (0.61-1.13)	0.21
Male						
Proximal colon						
No. of events	70	103	78	94	101	
RR (95% CI)	Referent	1.58 (1.15-2.16)	1.23 (0.87-1.73)	1.45 (1.04-2.03)	1.53 (1.08-2.16)	0.06
Distal colon						
No. of events	84	72	79	68	77	
RR (95% CI)	Referent	0.94 (0.68-1.31)	1.11 (0.80-1.55)	1.00 (0.70-1.41)	1.11 (0.78-1.58)	0.51
Combined						
Proximal colon	Referent	1.21 (1.00-1.45)	1.28 (1.07-1.54)	1.11 (0.91-1.34)	1.16 (0.95-1.41)	0.38
Distal colon	Referent	0.96 (0.78-1.19)	1.05 (0.85-1.30)	0.90 (0.72-1.13)	0.95 (0.76-1.20)	0.67

Abbreviations: CI, confidence interval; RR, relative risk

*Models stratified by age (in months) and 2-year questionnaire cycle and adjusted for gender (in the combined analyses), race (white, non-white, unknown), family history of colorectal cancer, body mass index (<25.0, 25.0-29.9, 30.0-34.9, ≥35.0 kg/m²), physical activity (quintiles), pack-years of smoking (0, 1-4, 5-19, 20-39, ≥40), alcohol intake (0, 0.1-4.9, 5.0-9.9, 10.0-14.9, 15.0-29.9, ≥30.0 g/d), regular aspirin use (<2, ≥2 times/wk), endoscopy history, menopausal status and postmenopausal hormone use (premenopausal, never, past and current users of postmenopausal hormone, unknown), total energy intake (quintiles), red meat intake (quintiles), processed meat intake (quintiles), and energy-adjusted intake of dietary fiber (quintiles), calcium (quintiles), and folate (quintiles).

Table 1.4: Multivariable-adjusted relative risks for the association of fructose and sucrose intake with colorectal, colon, and rectal cancer mortality*

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	<i>P</i> _{trend}
Fructose intake						
Female						
Person-years	511,357	512,437	512,579	512,550	511,680	
Colorectal cancer						
No. of events	144	129	124	118	144	
RR (95% CI)	Referent	0.91 (0.71-1.17)	0.88 (0.68-1.14)	0.82 (0.63-1.07)	0.95 (0.73-1.25)	0.74
Colon cancer						
No. of events	100	92	90	86	102	
RR (95% CI)	Referent	0.89 (0.66-1.19)	0.86 (0.64-1.17)	0.81 (0.59-1.11)	0.94 (0.68-1.28)	0.64
Rectal cancer						
No. of events	36	31	28	24	35	
RR (95% CI)	Referent	1.02 (0.61-1.68)	0.93 (0.55-1.60)	0.77 (0.43-1.36)	1.01 (0.59-1.75)	0.99
Male						
Person-years	208,806	209,356	209,500	209,623	209,208	
Colorectal cancer						
No. of events	104	77	81	85	98	
RR (95% CI)	Referent	0.82 (0.60-1.12)	0.85 (0.62-1.16)	0.89 (0.64-1.23)	1.08 (0.77-1.51)	0.45
Colon cancer						
No. of events	71	44	54	54	68	
RR (95% CI)	Referent	0.68 (0.46-1.00)	0.82 (0.56-1.22)	0.83 (0.55-1.24)	1.15 (0.76-1.72)	0.29
Rectal cancer						
No. of events	22	19	19	18	18	
RR (95% CI)	Referent	0.91 (0.47-1.76)	0.85 (0.43-1.67)	0.78 (0.38-1.61)	0.72 (0.34-1.53)	0.41
Combined						
Colorectal cancer	Referent	0.87 (0.71-1.05)	0.86 (0.71-1.05)	0.85 (0.69-1.04)	1.00 (0.81-1.23)	0.71
Colon cancer	Referent	0.80 (0.63-1.01)	0.85 (0.67-1.08)	0.82 (0.64-1.05)	1.01 (0.79-1.30)	0.63
Rectal cancer	Referent	0.96 (0.65-1.43)	0.89 (0.59-1.35)	0.76 (0.49-1.19)	0.88 (0.57-1.37)	0.56
Sucrose intake						
Female						
Person-years	511,406	512,256	512,546	512,586	511,809	
Colorectal cancer						
No. of events	145	123	140	119	132	
RR (95% CI)	Referent	0.87 (0.68-1.11)	0.97 (0.76-1.25)	0.79 (0.61-1.03)	0.82 (0.63-1.07)	0.12
Colon cancer						
No. of events	99	86	111	82	92	
RR (95% CI)	Referent	1.02 (0.61-1.68)	0.93 (0.55-1.60)	0.77 (0.43-1.36)	1.01 (0.59-1.75)	0.99
Rectal cancer						
No. of events	38	29	23	31	33	
RR (95% CI)	Referent	0.90 (0.54-1.49)	0.71 (0.41-1.23)	0.91 (0.54-1.53)	0.84 (0.49-1.45)	0.65
Male						
Person-years	208,783	209,442	209,460	209,670	209,139	
Colorectal cancer						
No. of events	98	77	90	83	97	
RR (95% CI)	Referent	0.83 (0.61-1.14)	0.95 (0.70-1.28)	0.85 (0.62-1.17)	0.91 (0.66-1.26)	0.64
Colon cancer						
No. of events	58	63	51	55	64	
RR (95% CI)	Referent	1.20 (0.83-1.73)	0.91 (0.61-1.36)	0.98 (0.66-1.47)	1.07 (0.71-1.61)	0.95
Rectal cancer						
No. of events	25	10	23	21	17	
RR (95% CI)	Referent	0.38 (0.18-0.81)	0.89 (0.48-1.65)	0.74 (0.39-1.41)	0.49 (0.24-1.00)	0.19

Table 1.4 (Continued): Multivariable-adjusted relative risks for the association of fructose and sucrose intake with colorectal, colon, and rectal cancer mortality*

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	<i>P</i> _{trend}
Combined						
Colorectal cancer	Referent	0.85 (0.70-1.03)	0.96 (0.80-1.17)	0.81 (0.66-1.00)	0.87 (0.71-1.07)	0.22
Colon cancer	Referent	0.96 (0.77-1.21)	1.02 (0.81-1.29)	0.84 (0.65-1.07)	0.92 (0.71-1.18)	0.37
Rectal cancer	Referent	0.66 (0.44-0.99)	0.78 (0.52-1.16)	0.83 (0.55-1.24)	0.69 (0.45-1.05)	0.24

Abbreviations: CI, confidence interval; RR, relative risk

*Models stratified by age (in months) and 2-year questionnaire cycle and adjusted for gender (in the combined analyses), race (white, non-white, unknown), family history of colorectal cancer, body mass index (<25.0, 25.0-29.9, 30.0-34.9, ≥35.0 kg/m²), physical activity (quintiles), pack-years of smoking (0, 1-4, 5-19, 20-39, ≥40), alcohol intake (0, 0.1-4.9, 5.0-9.9, 10.0-14.9, 15.0-29.9, ≥30.0 g/d), regular aspirin use (<2, ≥2 times/wk), endoscopy history, menopausal status and postmenopausal hormone use (premenopausal, never, past and current users of postmenopausal hormone, unknown), total energy intake (quintiles), red meat intake (quintiles), processed meat intake (quintiles), and energy-adjusted intake of dietary fiber (quintiles), calcium (quintiles), and folate (quintiles).

Table 1.5: Multivariable-adjusted relative risks for the association of fructose and sucrose intake with colorectal cancer risk, stratified by covariates

	No. of events	Relative risk (95% confidence interval) in the combined cohorts*					P_{trend}	$P_{\text{interaction}}$
		Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5		
Fructose intake								
Age								0.43
<70 years	1,773	Referent	1.05 (0.91-1.22)	1.05 (0.90-1.23)	1.06 (0.90-1.24)	1.00 (0.85-1.19)	0.91	
≥70 years	1,321	Referent	1.05 (0.88-1.26)	0.95 (0.79-1.15)	1.07 (0.89-1.30)	1.08 (0.89-1.32)	0.41	
Body mass index								0.73
<25.0 kg/m ²	1,369	Referent	1.03 (0.86-1.24)	0.98 (0.81-1.18)	1.13 (0.94-1.36)	0.99 (0.81-1.20)	0.98	
25.0-29.9 kg/m ²	1,250	Referent	1.03 (0.86-1.23)	0.99 (0.82-1.19)	0.91 (0.75-1.11)	0.91 (0.74-1.12)	0.34	
≥30.0 kg/m ²	470	Referent	1.08 (0.81-1.43)	0.89 (0.65-1.21)	0.98 (0.71-1.36)	1.08 (0.79-1.50)	0.71	
Physical activity								0.02
Tertile 1 (median: 4 MET-h/wk)	1,068	Referent	1.04 (0.85-1.26)	0.97 (0.79-1.19)	1.22 (0.99-1.49)	1.10 (0.89-1.37)	0.10	
Tertile 2 (median: 13 MET-h/wk)	980	Referent	0.96 (0.78-1.18)	0.92 (0.74-1.14)	0.97 (0.78-1.21)	0.93 (0.74-1.18)	0.64	
Tertile 3 (median: 35 MET-h/wk)	905	Referent	1.04 (0.83-1.29)	0.96 (0.76-1.20)	0.85 (0.67-1.08)	0.80 (0.62-1.03)	0.02	
Alcohol intake								0.29
<1 drink/d	2,371	Referent	1.04 (0.90-1.20)	0.96 (0.84-1.12)	1.02 (0.88-1.18)	0.95 (0.82-1.11)	0.40	
1-<2 drinks/d	410	Referent	1.02 (0.78-1.34)	0.93 (0.68-1.26)	0.84 (0.59-1.21)	1.08 (0.72-1.61)	0.80	
≥2 drinks/d	313	Referent	1.03 (0.75-1.41)	1.04 (0.70-1.53)	1.03 (0.63-1.68)	1.20 (0.64-2.26)	0.36	
Dietary fiber intake, energy-adjusted								0.90
<20 g/d	1,932	Referent	1.01 (0.89-1.16)	0.98 (0.85-1.13)	0.99 (0.85-1.15)	0.91 (0.77-1.07)	0.29	
≥20 g/d	1,162	Referent	1.17 (0.93-1.47)	1.03 (0.82-1.30)	1.15 (0.91-1.45)	1.14 (0.89-1.45)	0.38	
Fruit intake								0.34
<2 servings/wk	1,320	Referent	1.06 (0.92-1.23)	0.90 (0.75-1.08)	1.09 (0.90-1.32)	1.00 (0.81-1.24)	0.88	
≥2 servings/wk	1,774	Referent	1.11 (0.90-1.38)	1.09 (0.88-1.35)	1.08 (0.87-1.35)	1.05 (0.84-1.32)	0.87	
Sucrose intake								
Age								0.37
<70 years	1,773	Referent	1.12 (0.96-1.30)	1.22 (1.05-1.42)	1.14 (0.97-1.33)	1.13 (0.96-1.34)	0.15	
≥70 years	1,321	Referent	0.96 (0.80-1.15)	1.12 (0.94-1.34)	0.93 (0.77-1.13)	1.06 (0.87-1.28)	0.66	
Body mass index								0.71
<25.0 kg/m ²	1,369	Referent	1.04 (0.87-1.25)	1.18 (0.99-1.41)	0.95 (0.79-1.15)	1.11 (0.92-1.34)	0.60	
25.0-29.9 kg/m ²	1,250	Referent	1.04 (0.87-1.25)	1.14 (0.95-1.37)	1.02 (0.84-1.23)	0.94 (0.77-1.15)	0.68	
≥30.0 kg/m ²	470	Referent	0.91 (0.68-1.22)	1.02 (0.76-1.36)	1.02 (0.74-1.39)	0.86 (0.61-1.20)	0.58	
Physical activity								0.07
Tertile 1 (median: 4 MET-h/wk)	1,068	Referent	1.13 (0.92-1.37)	1.11 (0.90-1.36)	1.23 (1.00-1.51)	1.06 (0.86-1.31)	0.36	
Tertile 2 (median: 13 MET-h/wk)	980	Referent	1.11 (0.90-1.37)	1.08 (0.88-1.34)	1.06 (0.85-1.32)	1.09 (0.87-1.38)	0.59	
Tertile 3 (median: 35 MET-h/wk)	905	Referent	0.81 (0.66-1.00)	1.09 (0.89-1.34)	0.67 (0.53-0.84)	0.80 (0.63-1.01)	0.07	

Table 1.5 (Continued): Multivariable-adjusted relative risks for the association of fructose and sucrose intake with colorectal cancer risk, stratified by covariates

	No. of events	Relative risk (95% confidence interval) in the combined cohorts*					<i>P</i> _{trend}	<i>P</i> _{interaction}
		Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5		
Alcohol intake								0.34
<1 drink/d	2,371	Referent	1.12 (0.97-1.30)	1.18 (1.03-1.36)	1.04 (0.90-1.20)	1.02 (0.88-1.18)	0.44	
1-<2 drinks/d	410	Referent	0.77 (0.59-1.02)	0.92 (0.68-1.23)	0.75 (0.53-1.06)	0.94 (0.64-1.37)	0.45	
≥2 drinks/d	313	Referent	0.97 (0.71-1.33)	1.53 (1.07-2.17)	1.02 (0.61-1.68)	1.25 (0.73-2.15)	0.15	
Dietary fiber intake, energy-adjusted								0.93
<20 g/d	1,932	Referent	1.03 (0.90-1.19)	1.14 (0.98-1.32)	0.97 (0.83-1.13)	0.96 (0.82-1.13)	0.48	
≥20 g/d	1,162	Referent	1.04 (0.85-1.27)	1.14 (0.94-1.39)	0.99 (0.81-1.22)	1.04 (0.84-1.30)	0.80	
Fruit intake								0.13
<2 servings/wk	1,320	Referent	1.03 (0.88-1.22)	1.09 (0.92-1.29)	1.01 (0.84-1.21)	0.98 (0.82-1.19)	0.93	
≥2 servings/wk	1,774	Referent	1.05 (0.88-1.25)	1.19 (1.00-1.41)	1.01 (0.84-1.20)	1.07 (0.89-1.29)	0.74	

Abbreviation: MET, metabolic equivalent

*Models stratified by age (in months) and 2-year questionnaire cycle and adjusted for gender, race (white, non-white, unknown), family history of colorectal cancer, body mass index (<25.0, 25.0-29.9, 30.0-34.9, ≥35.0 kg/m²), physical activity (quintiles), pack-years of smoking (0, 1-4, 5-19, 20-39, ≥40), alcohol intake (0, 0.1-4.9, 5.0-9.9, 10.0-14.9, 15.0-29.9, ≥30.0 g/d), regular aspirin use (<2, ≥2 times/wk), endoscopy history, menopausal status and postmenopausal hormone use (premenopausal, never, past and current users of postmenopausal hormone, unknown), total energy intake (quintiles), red meat intake (quintiles), processed meat intake (quintiles), and energy-adjusted intake of dietary fiber (quintiles), calcium (quintiles), and folate (quintiles), excluding the stratification variable.

Supplementary tables

Table S1.1: Unadjusted relative risks for the association of fructose and sucrose intake with colorectal cancer risk and mortality

	Relative risk (95% confidence interval) in the combined cohorts*					<i>P</i> _{trend}
	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	
Risk						
Fructose intake						
Colorectal cancer	Referent	0.94 (0.84-1.05)	0.84 (0.75-0.94)	0.85 (0.76-0.95)	0.82 (0.73-0.91)	0.62
Colon cancer	Referent	0.92 (0.81-1.05)	0.84 (0.73-0.96)	0.88 (0.77-1.00)	0.83 (0.73-0.95)	0.25
Rectal cancer	Referent	1.00 (0.79-1.26)	0.86 (0.68-1.10)	0.79 (0.62-1.01)	0.79 (0.62-1.01)	0.33
Sucrose intake						
Colorectal cancer	Referent	0.94 (0.84-1.05)	1.00 (0.90-1.12)	0.86 (0.77-0.96)	0.88 (0.79-0.99)	0.98
Colon cancer	Referent	0.98 (0.86-1.12)	1.03 (0.90-1.17)	0.88 (0.77-1.00)	0.92 (0.81-1.06)	0.66
Rectal cancer	Referent	0.80 (0.63-1.02)	0.89 (0.71-1.13)	0.87 (0.69-1.10)	0.75 (0.59-0.96)	0.37
Mortality						
Fructose intake						
Colorectal cancer	Referent	0.78 (0.65-0.94)	0.74 (0.61-0.89)	0.70 (0.58-0.84)	0.82 (0.68-0.98)	0.35
Colon cancer	Referent	0.74 (0.59-0.93)	0.75 (0.60-0.94)	0.69 (0.55-0.86)	0.83 (0.67-1.03)	0.35
Rectal cancer	Referent	0.82 (0.56-1.19)	0.73 (0.50-1.08)	0.62 (0.42-0.93)	0.77 (0.53-1.13)	0.25
Sucrose intake						
Colorectal cancer	Referent	0.78 (0.65-0.94)	0.87 (0.72-1.04)	0.73 (0.61-0.88)	0.80 (0.67-0.96)	0.16
Colon cancer	Referent	0.90 (0.72-1.12)	0.94 (0.75-1.17)	0.76 (0.61-0.96)	0.85 (0.68-1.06)	0.19
Rectal cancer	Referent	0.59 (0.39-0.87)	0.68 (0.46-0.99)	0.74 (0.51-1.07)	0.68 (0.47-0.99)	0.25

*Models stratified by age (in months) and 2-year questionnaire cycle.

Table S1.2: Multivariable-adjusted relative risks for the association of fructose and sucrose intake with colon cancer mortality by tumor location*

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	<i>P</i> _{trend}
Fructose intake						
Female						
Proximal colon						
No. of events	60	59	53	49	55	
RR (95% CI)	Referent	0.99 (0.68-1.44)	0.89 (0.60-1.32)	0.81 (0.53-1.22)	0.93 (0.61-1.41)	0.57
Distal colon						
No. of events	40	33	37	37	47	
RR (95% CI)	Referent	0.75 (0.47-1.22)	0.82 (0.51-1.33)	0.80 (0.49-1.30)	0.94 (0.58-1.52)	0.92
Male						
Proximal colon						
No. of events	33	16	33	30	36	
RR (95% CI)	Referent	0.51 (0.27-0.94)	1.11 (0.65-1.88)	0.99 (0.56-1.73)	1.30 (0.73-2.31)	0.10
Distal colon						
No. of events	31	23	20	20	23	
RR (95% CI)	Referent	0.81 (0.46-1.43)	0.66 (0.36-1.22)	0.69 (0.37-1.31)	0.90 (0.47-1.73)	0.65
Combined						
Proximal colon	Referent	0.81 (0.59-1.11)	0.95 (0.69-1.30)	0.87 (0.63-1.22)	1.05 (0.75-1.47)	0.40
Distal colon	Referent	0.78 (0.54-1.13)	0.78 (0.54-1.13)	0.76 (0.52-1.12)	0.94 (0.64-1.38)	0.70
Sucrose intake						
Female						
Proximal colon						
No. of events	59	53	65	48	51	
RR (95% CI)	Referent	0.92 (0.63-1.35)	1.12 (0.77-1.62)	0.80 (0.53-1.20)	0.83 (0.55-1.27)	0.32
Distal colon						
No. of events	40	33	46	34	41	
RR (95% CI)	Referent	0.76 (0.47-1.22)	1.00 (0.64-1.57)	0.71 (0.43-1.16)	0.77 (0.47-1.25)	0.28
Male						
Proximal colon						
No. of events	21	38	28	28	33	
RR (95% CI)	Referent	1.91 (1.10-3.32)	1.43 (0.79-2.62)	1.35 (0.73-2.50)	1.48 (0.79-2.76)	0.60
Distal colon						
No. of events	26	25	19	23	24	
RR (95% CI)	Referent	1.05 (0.59-1.85)	0.71 (0.38-1.34)	0.94 (0.51-1.74)	0.89 (0.47-1.67)	0.61
Combined						
Proximal colon	Referent	1.17 (0.86-1.59)	1.17 (0.86-1.61)	0.94 (0.67-1.31)	1.02 (0.72-1.44)	0.82
Distal colon	Referent	0.88 (0.61-1.26)	0.94 (0.66-1.35)	0.80 (0.55-1.17)	0.84 (0.57-1.24)	0.33

Abbreviations: CI, confidence interval; RR, relative risk

*Models stratified by age (in months) and 2-year questionnaire cycle and adjusted for gender (in the combined analyses), race (white, non-white, unknown), family history of colorectal cancer, body mass index (<25.0, 25.0-29.9, 30.0-34.9, ≥35.0 kg/m²), physical activity (quintiles), pack-years of smoking (0, 1-4, 5-19, 20-39, ≥40), alcohol intake (0, 0.1-4.9, 5.0-9.9, 10.0-14.9, 15.0-29.9, ≥30.0 g/d), regular aspirin use (<2, ≥2 times/wk), endoscopy history, menopausal status and postmenopausal hormone use (premenopausal, never, past and current users of postmenopausal hormone, unknown), total energy intake (quintiles), red meat intake (quintiles), processed meat intake (quintiles), and energy-adjusted intake of dietary fiber (quintiles), calcium (quintiles), and folate (quintiles).

Chapter 2 Pre-existing type 2 diabetes and survival among patients with non-metastatic colorectal cancer

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Abstract

Background: Type 2 diabetes is known to increase risk of developing colorectal cancer (CRC). However, the influence of pre-existing diabetes on survival among CRC patients remains unclear.

Methods: We analyzed survival by diabetes status at cancer diagnosis among 2,604 participants with non-metastatic CRC diagnosed between 1976 and 2012 from the Nurses' Health Study and the Health Professionals Follow-Up Study. Cox proportional hazards models were used to estimate hazard ratios (HRs) for overall mortality and cause-specific mortality, adjusted for other prognostic markers and potential confounders.

Results: There were 1,230 deaths by the end of follow-up, including 583 deaths from CRC, 185 deaths from non-CRC cancers, and 141 deaths from cardiovascular disease. Median survival times were 10 years and 17 years for patients with and without diabetes, respectively. However, diabetes was not associated with overall survival during the first 5 years after CRC diagnosis. Beyond 5 years, patients with diabetes experienced elevated overall mortality, compared to those without diabetes. The HRs for death were 1.01 (95% confidence interval [CI], 0.78-1.32), 1.51 (95% CI, 1.07-2.13) and 2.58 (95% CI, 1.79-3.73) during 0-5, >5-10, and >10 years after CRC diagnosis, respectively. Regarding cause-specific mortality, patients with diabetes had increased mortality from non-CRC cancers (HR, 2.03; 95% CI, 1.28-3.23) and cardiovascular disease (HR, 1.98; 95% CI, 1.23-3.20), but not from CRC (HR, 1.00; 95% CI, 0.74-1.35).

Conclusions: Among patients with non-metastatic CRC, diabetes was associated with increased long-term mortality, especially mortality from non-CRC cancers and cardiovascular disease.

Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related death in the United States (1). In the US alone, around 50,630 deaths are expected in 2018 (1). Among CRC patients, only 39% are diagnosed at a local stage with a 5-year survival rate of 90%; the survival rates decline to 71% and 14% for the regional and distant stage, respectively (2). Aside from disease stage, few other markers have been used to predict a patient's survival in clinical settings.

Type 2 diabetes (from now on referred to as “diabetes”) is known to increase risk of developing CRC. In a meta-analysis of 15 studies, individuals with diabetes had a relative risk for CRC of 1.30 (95% CI, 1.20-1.40), compared to those without diabetes (3). Insulin resistance and hyperinsulinemia have been proposed to be one of the underlying mechanisms. In contrast, the influence of diabetes on CRC survival remains unclear. Among a limited number of studies, the results are mixed: most, but not all, studies found an association between diabetes and decreased overall survival (4). Furthermore, it is unclear whether the observed reduction in survival was primarily explained by increased mortality from the CRC itself or from other diseases (e.g., cardiovascular disease) (5-7). Understanding the role of diabetes in CRC survival could provide important implications for clinical practice and stratification in clinical trials.

CRC is a heterogeneous disease with diverse genetic and epigenetic alterations in tumor cells. Thus, the effect of diabetes on CRC survival could vary according to tumor molecular markers, particularly those in the insulin signaling pathway. These molecular markers include two major insulin receptor substrates (IRS1 and IRS2) and downstream signaling molecules such as *KRAS* and *PIK3CA*. Fatty acid synthase (FASN) is a key enzyme that converts excess carbohydrate into fatty acids (8, 9) and is often overexpressed in cancer cells, which is hypothesized to be a selection mechanism for cancer cells to achieve a growth or survival advantage (8). In addition, the association between diabetes and CRC survival could depend on the patient's degree of insulin resistance, as measured by circulating biomarkers such as adiponectin, C-peptide, and insulin-like growth factor binding protein (IGFBP) 1.

In this study, we examined the association between pre-existing diabetes and survival among participants diagnosed with non-metastatic CRC from two prospective US cohorts. We also examined

whether the association varied across strata of tumor molecular markers and prediagnostic circulating biomarkers of insulin resistance.

Methods

Study population

The Nurses' Health Study (NHS) was initiated in 1976 when 121,700 US female nurses aged 30-55 years completed a mailed questionnaire describing demographics, lifestyle choices, and medical history (10, 11). The Health Professionals Follow-Up Study (HPFS) was initiated in 1986 when 51,529 US men aged 40-75 years working in health professions completed a mailed questionnaire on health-related behaviors and medical history (12). Participants have updated information through biennial follow-up questionnaires. A high follow-up rate of more than 90% was achieved in both cohorts.

Participants with pathologically confirmed non-metastatic CRC diagnosed between 1976 and 2012 were included in this study. Patients were excluded if had reported any cancer (other than nonmelanoma skin cancer) prior to CRC diagnosis. When a participant (or next of kin for decedents) reported a diagnosis of CRC on a follow-up questionnaire, we asked permission to obtain hospital records and pathology reports. For nonrespondents, the National Death Index was used to ascertain any diagnosis of CRC that contributed to death; we then asked permission from next of kin for decedents to obtain medical records. Blinded study physicians reviewed these records to confirm the diagnosis and record information on important tumor characteristics. We estimate that 96-97% of patients were identified through these methods (13, 14).

This study was approved by the Human Research Committee at the Brigham and Women's Hospital and the Harvard T.H. Chan School of Public Health. Completion and return of questionnaires were considered informed consent.

Mortality assessment

Patients were observed until death or last follow-up dates (June 2012 in NHS; January 2012 in HPFS), whichever came first. Ascertainment of deaths included reporting by family or postal authorities, and

interrogation of names of persistent nonrespondents in the National Death Index (15). More than 98% of deaths were identified by these methods (16). Cause of death was assigned by study physicians blinded to other data.

Assessment of diabetes

On the baseline and all follow-up questionnaires, participants were asked if they had ever been diagnosed with diabetes by a physician. To verify the diagnosis, a supplementary questionnaire was sent later to obtain details on the date of diagnosis, symptoms, diagnostic tests, and treatment. In both cohorts, the validity of the supplementary questionnaire has been established by review of medical records (17, 18). In this study, diabetes status was determined from patient report on biennial questionnaires together with the supplementary questionnaire. Of 257 patients with diabetes included in the current analysis, 206 (80%) had the supplementary questionnaire allowing confirmation.

Assessment of circulating biomarkers of insulin resistance

In NHS, blood samples were collected from 32,826 women and returned in a mailed blood collection kit by overnight courier in 1989 and 1990. In HPFS, a total of 18,225 men returned a mailed blood collection kit by overnight courier in 1993 through 1995. More than 95% of samples were received within 26 hours of blood collection. Blood samples were centrifuged on arrival and separated into plasma, white blood cells, and red blood cells.

Plasma adiponectin was measured by enzyme-linked immunosorbent assay (ELISA) (ALPCO Diagnostics). Plasma C-peptide and IGFBP 1 were measured by ELISA with reagents from Diagnostic Systems Laboratory in the laboratory of Dr. Michael Pollak (McGill University, Montreal, Canada). The mean intra-assay coefficients of variation for these biomarkers were all <13%.

Assessment of tumor molecular markers

Paraffin-embedded tissue blocks were collected from hospitals and were reviewed by one of the authors (S.O.).

Sequencing of *KRAS* and *PIK3CA*: DNA was extracted from paraffin-embedded tumor tissue. Polymerase chain reaction and pyrosequencing targeted for *KRAS* (codons 12, 13, 61, and 146) (19, 20) and *PIK3CA* (exons 9 and 20) (21, 22) were performed as previously described.

Immunohistochemistry for IRS1, IRS2, and FASN: Methods of immunohistochemical methods and representative images have been described in previous studies as follows: IRS1 (23), IRS2 (23), and FASN (24, 25). Expression levels were graded by one pathologist, and a selected group of >100 patients was independently reviewed by another pathologist to assess reproducibility. Both pathologists were blinded to other data. We confirmed reasonable agreement between the two pathologists, with κ coefficients of 0.69, 0.77, and 0.57 for IRS1, IRS2, and FASN, respectively (all $P < 0.001$) (23-26).

Covariates

Cancer stage, grade of tumor differentiation, location of primary tumor, and year of diagnosis (as a surrogate for treatment) were extracted from medical records. Body mass index (BMI), physical activity, pack-years of smoking, and alcohol intake were evaluated as the cumulative average from baseline to CRC diagnosis. Regular aspirin users were defined as participants who had reported regular aspirin use (i.e., ≥ 2 times/week) in at least half of the questionnaires returned before CRC diagnosis.

Statistical analyses

Follow-up time was calculated from date of CRC diagnosis to date of death or date of last follow-up (June 2012 in NHS; January 2012 in HPFS), whichever came first. Participants without diabetes were the main referent group for all analyses. Survival curves by diabetes status were generated by the Kaplan-Meier method, with statistical significance evaluated by the log-rank test. Cox proportional hazards models were used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for death from any cause (primary

outcome), death from CRC, death from non-CRC cancers, and death from cardiovascular disease. To test the proportional hazards assumption, we evaluated the cross product of diabetes status and time. The assumption was met for cause-specific survival but not overall survival, which was addressed by including an interaction term between diabetes status and time in the models, allowing calculation of HRs for different follow-up periods (0-5, >5-10, and >10 years).

To examine whether diabetes status can predict survival independent of tumor characteristics, the models were stratified by gender and cancer stage and adjusted for age at diagnosis, grade of tumor differentiation, location of primary tumor, and year of diagnosis. To examine whether the association was confounded by lifestyle factors, we additionally adjusted for BMI, physical activity, smoking status, alcohol intake, and regular aspirin use, which was considered to be the main model.

Tests of interaction between diabetes and covariates were performed by entering their cross product in the model, evaluated by the likelihood ratio test. Age at diagnosis, BMI, cancer stage, grade of tumor differentiation, location of primary tumor, and levels of C-peptide, IGFBP 1, and adiponectin were examined as potential effect modifiers for overall survival. To address violation of the proportional hazards assumption and for ease of report, we censored patients as we followed them for up to 5 years and reported the HRs for 5-year overall mortality. In addition, *KRAS*, *PIK3CA*, *IRS1*, *IRS2*, and *FASN* were examined as potential effect modifiers for CRC-specific survival. Because we examined five tumor markers, the alpha level for statistical significance was adjusted to 0.01 (0.05/5), consistent with our prior studies (27). All analyses were performed with SAS 9.4 statistical package. All *P* values were two sided.

Results

Among 2,604 patients with non-metastatic CRC, there were 1,230 deaths, 583 of which were due to CRC (47%). CRC accounted for 74%, 34%, and 13% of deaths during 0-5, >5-10, and >10 years after cancer diagnosis, respectively (Table S2.1). Non-CRC causes of death included other malignancies (15%), cardiovascular disease (12%), neurologic disorders (5%), pulmonary disorders (4%), cerebrovascular disease (4%), and other or unknown reasons (12%). The median follow-up time among patients who were

alive was 11.6 years. Baseline characteristics according to diabetes status are shown in Table 2.1. In general, patients with diabetes were older and more likely to be male, had a greater BMI and were less physically active, consumed less alcohol, and were less likely to be current smokers and more likely to be regular aspirin users.

The association between diabetes and overall survival was examined among men and women separately and combined (Table 2.2 and Figure 2.1). We noted similar results with and without adjustment for lifestyle factors. In the combined cohorts, median survival times were 10 years and 17 years for patients with and without diabetes, respectively. However, diabetes was not associated with overall survival during the first 5 years after CRC diagnosis (HR, 1.01; 95% CI, 0.78-1.32). Beyond 5 years, patients with diabetes experienced elevated overall mortality, compared to those without diabetes. The HRs for death were 1.51 (95% CI, 1.07-2.13) and 2.58 (95% CI, 1.79-3.73) during >5-10 and >10 years after CRC diagnosis, respectively. The association between diabetes and overall survival did not differ significantly by gender (all $P_{\text{heterogeneity}} \geq 0.10$).

The null association between diabetes and overall survival during the first 5 years could be due to the relatively shorter duration of diabetes. Therefore, we examined the association between duration of diabetes at cancer diagnosis and 5-year overall survival (Table S2.2). Compared to patients without diabetes, the HRs for 5-year overall mortality were 0.77 (95% CI, 0.45-1.32) for patients with diabetes ≤ 5 years, 1.04 (95% CI, 0.64-1.70) for diabetes >5-10 years, and 1.42 (95% CI, 0.99-2.04) for diabetes >10 years.

The null association between diabetes and 5-year overall mortality did not differ significantly by age at diagnosis, BMI, or cancer stage (all $P_{\text{interaction}} \geq 0.43$; Table 2.3). However, the association appeared more apparent among patients with well-to-moderately differentiated tumors (HR, 1.32; 95% CI, 0.96-1.80; $P_{\text{interaction}} = 0.04$) and among those with rectal cancer (HR, 1.69; 95% CI, 1.03-2.76; $P_{\text{interaction}} = 0.07$). Further analyses revealed that the increased mortality in these strata was explained by non-CRC causes of death (data not shown). We did not observe significant interactions between diabetes and levels of adiponectin, C-peptide, or IGFBP 1 (all $P_{\text{interaction}} \geq 0.22$).

Regarding cause-specific mortality, CRC patients with diabetes had increased mortality from non-CRC cancers (HR, 2.03; 95% CI, 1.28-3.23) and cardiovascular disease (HR, 1.98; 95% CI, 1.23-3.20), compared to those without diabetes (Table 2.4). However, we did not observe increased CRC-specific mortality associated with diabetes (HR, 1.00; 95% CI, 0.74-1.35; Table 2.4 and Figure 2.1). The null association between diabetes and CRC-specific mortality was not modified by *KRAS*, *PIK3CA*, *IRS1*, *IRS2*, or *FASN* (all $P_{\text{interaction}} \geq 0.04$; Table 2.5).

Discussion

In this patient population drawn from two prospective cohorts, we observed that pre-existing diabetes was associated with decreased long-term overall survival among patients with non-metastatic CRC, translating to a 7-year shorter median survival. The excess mortality observed in those patients might relate to non-CRC cancers and cardiovascular disease rather than CRC.

To date, a number of studies have evaluated the association between diabetes and CRC survival, but most of them were hospital-based with relatively small sample sizes (4). Only a few large population-based cohort studies have been conducted, with mixed results (5-7, 28). Among 2,278 patients from the Cancer Prevention Study-II Nutrition Cohort, CRC patients with diabetes were at higher risk of overall mortality (HR, 1.53; 95% CI, 1.23-1.83), CRC-specific mortality (HR, 1.29; 95% CI, 0.98-1.70), and cardiovascular disease-specific mortality (HR, 2.16; 95% CI, 1.44-3.24) (6). In a study of 61,213 older patients documented in the US Surveillance Epidemiology and End Results database linked with Medicare claims data, diabetes was associated with increased overall mortality (HR, 1.20; 95% CI, 1.17-1.23), but not with CRC-specific mortality (HR, 0.99; 95% CI, 0.95-1.03) (5). Similar findings were reported by a Scottish study of 19,505 CRC patients, in which diabetes was associated with poorer long-term survival due to higher mortality from non-cancer causes (7). However, among 3,913 patients within the Multiethnic Cohort Study in California and Hawaii, diabetes was associated with neither overall (HR, 0.99; 95% CI, 0.95-1.03) nor CRC-specific mortality (HR, 1.11; 95% CI, 0.98-1.27) (28).

Insulin resistance and hyperinsulinemia have been linked to colorectal carcinogenesis. Obesity (29),

physical inactivity (30), Western dietary pattern (31), and higher dietary insulin indices (27, 32) have been associated with cancer recurrence, progression, and death among CRC patients. In the current study, we observed that diabetes was associated with overall but not CRC-specific survival, which is consistent with our prior finding in the same cohorts that higher plasma C-peptide (a marker of long-term insulin secretion) levels were associated with increased overall mortality, but not with CRC-specific mortality (33). In addition, the null association between diabetes and CRC-specific mortality was not modified by tumor markers including *KRAS*, *PIK3CA*, *IRS1*, *IRS2*, and *FASN*.

Diabetes was not associated with overall survival during the first 5 years after CRC diagnosis. One possible explanation is that duration of diabetes in this period may not be sufficient to elicit an observable reduction in survival. Indeed, we examined 5-year survival by duration of diabetes and detected increased mortality among patients with diabetes >10 years. Another factor is that deaths from CRC tend to be clustered at early follow-up. In this population, CRC accounted for as much as 74% of deaths during the first 5 years after CRC diagnosis. Because diabetes was not associated with CRC-specific mortality, any effect of diabetes on other causes of death would be diluted by the high rate of death from CRC.

We observed that patients with diabetes had significantly increased mortality from non-CRC cancers. Diabetes is a risk factor for several types of non-CRC cancers (e.g., liver, pancreas, endometrium, breast, bladder) (34). Furthermore, recent studies suggest that diabetes is associated with decreased survival after cancer diagnosis (35-38). However, the two-fold higher mortality from non-CRC cancers that was observed among CRC patients in the current study was substantially greater than the magnitude seen in more general populations (39, 40). Regardless of the underlying mechanism, our findings suggest that prevention of non-CRC cancers should be an important concern for these patients.

Consistent with previous reports (6, 7), we observed a two-fold higher mortality from cardiovascular disease among CRC patients with diabetes. Diabetes and cardiovascular disease are closely linked, since risk factors for cardiovascular disease (e.g., obesity, hypertension, hyperglycemia, dyslipidemia) are common among diabetics (41). Cardiovascular disease is the leading cause of death among diabetics, and reducing risk factors for cardiovascular disease is a critical part of diabetes management. While the current

study included CRC patients diagnosed as early as 1970s, recent studies demonstrate that over the past decades, cardiovascular disease mortality among diabetics has declined substantially due to effective treatment and care for diabetes (42, 43).

This study has several strengths, including the prospective design, long follow-up time, high follow-up rates, confirmation of self-reported disease, availability of plasma biomarkers and tumor markers, and detailed data on tumor characteristics and lifestyle factors. Limitations of our study also require consideration. Among study participants, CRC treatment may have varied by diabetes status, and we could not control for differences in treatment since this information was not systematically collected. However, 68% of patients in this study had stage I or II disease, for which surgery alone is generally considered the standard of care (44). Our study participants were predominantly of European descent, and additional studies in other populations are warranted.

Our study suggests that among patients with non-metastatic CRC, diabetes was associated with increased long-term mortality, especially mortality from non-CRC cancers and cardiovascular disease. These findings highlight the importance of cardioprotection and cancer prevention for these patients.

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Tables/Figure

Table 2.1: Characteristics of patients with non-metastatic colorectal cancer by diabetes status at cancer diagnosis

Characteristic	No diabetes	Diabetes
No. of patients	2,347	257
Age at diagnosis, years, mean (SD)	67.6 (9.7)	71.3 (8.4)
Gender, No. (%)		
Female	1,574 (67.1)	163 (63.4)
Male	773 (32.9)	94 (36.6)
Race, No. (%)		
White	2,240 (95.4)	246 (95.7)
Black	37 (1.6)	6 (2.3)
Other	32 (1.4)	3 (1.2)
Unknown	38 (1.6)	2 (0.8)
Body mass index, kg/m ² , mean (SD)	25.5 (4.1)	29.4 (5.0)
Physical activity, MET-h/wk, mean (SD)	21 (21)	16 (15)
Pack-years of smoking, mean (SD)	17 (22)	20 (24)
Current smokers, No. (%)	289 (12.3)	17 (6.6)
Alcohol intake, g/d, median (range)	9 (13)	6 (10)
Regular aspirin use, No. (%)	703 (30.0)	111 (43.2)
Cancer stage, No. (%)		
I	812 (34.6)	82 (31.9)
II	791 (33.7)	85 (33.1)
III	744 (31.7)	90 (35.0)
Grade of tumor differentiation, No. (%)		
Well differentiated	356 (15.2)	29 (11.3)
Moderately differentiated	1,408 (60.0)	159 (61.9)
Poorly differentiated	348 (14.8)	46 (17.9)
Unknown	235 (10.0)	23 (8.9)
Location of primary tumor, No. (%)		
Proximal colon	1,023 (43.6)	128 (49.8)
Distal colon	772 (32.9)	74 (28.8)
Rectum	531 (22.6)	52 (20.2)
Unknown	21 (0.9)	3 (1.2)
Median overall survival by cancer stage, years		
I	23	12
II	17	10
III	9	8

Abbreviations: SD, standard deviation; MET, metabolic equivalent

Table 2.2: Hazard ratios for overall mortality by diabetes status at cancer diagnosis, stratified by follow-up period

	NHS		HPFS		Combined	
	No diabetes	Diabetes	No diabetes	Diabetes	No diabetes	Diabetes
No. of patients	1,574	163	773	94	2,347	257
Median overall survival, years	19	12	13	9	17	10
Follow-up period						
0-5 years						
No. of events	351	42	176	23	527	65
Age-adjusted HR (95% CI)	Referent	1.12 (0.81-1.54)	Referent	0.93 (0.60-1.43)	Referent	1.03 (0.80-1.34)
Multivariable-adjusted HR (95% CI)*	Referent	1.20 (0.87-1.66)	Referent	0.78 (0.50-1.22)	Referent	1.04 (0.80-1.35)
Multivariable-adjusted HR (95% CI)†	Referent	1.18 (0.85-1.65)	Referent	0.74 (0.47-1.15)	Referent	1.01 (0.78-1.32)
>5-≤10 years						
No. of events	166	19	97	19	263	38
Age-adjusted HR (95% CI)	Referent	1.38 (0.86-2.22)	Referent	1.78 (1.09-2.92)	Referent	1.58 (1.12-2.22)
Multivariable-adjusted HR (95% CI)*	Referent	1.40 (0.87-2.25)	Referent	1.57 (0.94-2.60)	Referent	1.55 (1.10-2.18)
Multivariable-adjusted HR (95% CI)†	Referent	1.39 (0.86-2.25)	Referent	1.49 (0.89-2.48)	Referent	1.51 (1.07-2.13)
>10 years						
No. of events	191	23	112	11	303	34
Age-adjusted HR (95% CI)	Referent	2.41 (1.56-3.72)	Referent	2.92 (1.55-5.49)	Referent	2.37 (1.66-3.39)
Multivariable-adjusted HR (95% CI)*	Referent	2.43 (1.57-3.77)	Referent	2.78 (1.44-5.37)	Referent	2.54 (1.77-3.66)
Multivariable-adjusted HR (95% CI)†	Referent	2.56 (1.64-4.01)	Referent	2.53 (1.30-4.92)	Referent	2.58 (1.79-3.73)

Abbreviations: CI, confidence interval; HPFS, Health Professionals Follow-Up Study; HR, hazard ratio; NHS, Nurses' Health Study

*Stratified by gender (in the combined analyses) and cancer stage (I to III) and adjusted for age at diagnosis (continuous), race (white, non-white, unknown), grade of tumor differentiation (well differentiated, moderately differentiated, poorly differentiated, unknown), location of primary tumor (proximal colon, distal colon, rectum, unknown), and year of diagnosis (1976-1989, 1990-1999, 2000-2012).

†Additionally adjusted for body mass index (continuous), physical activity (continuous), smoking status (never, past, current), alcohol intake (continuous), and regular aspirin use (yes, no).

Table 2.3: Hazard ratios for 5-year overall mortality by diabetes status at cancer diagnosis, stratified by potential effect modifiers

Subgroup	No. of patients	No. of events	HR (95% CI)*	<i>P</i> _{interaction}
Age				0.43
<70 years	1,474	284	0.99 (0.62-1.56)	
≥70 years	1,130	308	1.23 (0.89-1.71)	
Body mass index				0.56
<25.0 kg/m ²	1,206	276	0.92 (0.51-1.65)	
25.0-29.9 kg/m ²	1,022	228	1.08 (0.72-1.63)	
≥30.0 kg/m ²	376	88	1.36 (0.86-2.16)	
Cancer stage				0.92
I	894	103	0.99 (0.49-1.97)	
II	876	155	1.17 (0.72-1.91)	
III	834	334	1.08 (0.76-1.54)	
Grade of tumor differentiation				0.04
Well/moderately differentiated	1,952	409	1.32 (0.96-1.80)	
Poorly differentiated	394	129	0.66 (0.34-1.27)	
Location of primary tumor				0.07
Colon	1,997	421	0.97 (0.70-1.34)	
Rectum	583	158	1.69 (1.03-2.76)	
Adiponectin, ng/mL†				0.22
<6,619	228	51	0.85 (0.37-1.97)	
≥6,619	229	42	0.25 (0.03-1.88)	
C-peptide, ng/mL†				0.46
<2.15	192	35	0.41 (0.09-1.77)	
≥2.15	193	45	0.78 (0.28-2.13)	
IGFBP-1, ng/mL†				0.28
<20.6	168	41	1.32 (0.38-4.55)	
≥20.6	169	29	0.46 (0.10-2.05)	

Abbreviations: CI, confidence interval; HR, hazard ratio; IGFBP-1, Insulin-like growth factor-binding protein 1

*Comparing patients with diabetes to those without diabetes, stratified by gender and cancer stage (I to III) and adjusted for age at diagnosis (continuous), race (white, non-white, unknown), grade of tumor differentiation (well differentiated, moderately differentiated, poorly differentiated, unknown), location of primary tumor (proximal colon, distal colon, rectum, unknown), year of diagnosis (1976-1989, 1990-1999, 2000-2012), body mass index (continuous), physical activity (continuous), smoking status (never, past, current), alcohol intake (continuous), and regular aspirin use (yes, no).

†Cutpoints chosen based on median values.

Table 2.4: Hazard ratios for cause-specific mortality by diabetes status at cancer diagnosis

	NHS		HPFS		Combined	
	No diabetes	Diabetes	No diabetes	Diabetes	No diabetes	Diabetes
No. of patients	1,574	163	773	94	2,347	257
CRC						
No. of events	379	32	152	20	531	52
Age-adjusted HR (95% CI)	Referent	0.96 (0.67-1.38)	Referent	1.12 (0.70-1.80)	Referent	1.01 (0.76-1.35)
Multivariable-adjusted HR (95% CI)*	Referent	1.03 (0.71-1.48)	Referent	0.90 (0.55-1.47)	Referent	1.00 (0.75-1.34)
Multivariable-adjusted HR (95% CI)†	Referent	1.04 (0.71-1.53)	Referent	0.86 (0.52-1.41)	Referent	1.00 (0.74-1.35)
Non-CRC cancers						
No. of events	111	18	50	6	161	24
Age-adjusted HR (95% CI)	Referent	2.10 (1.27-3.47)	Referent	1.38 (0.59-3.27)	Referent	1.86 (1.20-2.87)
Multivariable-adjusted HR (95% CI)*	Referent	2.10 (1.27-3.49)	Referent	1.40 (0.57-3.41)	Referent	1.89 (1.22-2.94)
Multivariable-adjusted HR (95% CI)†	Referent	2.27 (1.31-3.94)	Referent	1.49 (0.60-3.69)	Referent	2.03 (1.28-3.23)
Cardiovascular disease						
No. of events	42	9	76	14	118	23
Age-adjusted HR (95% CI)	Referent	2.63 (1.27-5.45)	Referent	2.18 (1.21-3.94)	Referent	2.23 (1.42-3.52)
Multivariable-adjusted HR (95% CI)*	Referent	2.67 (1.28-5.55)	Referent	2.21 (1.20-4.09)	Referent	2.37 (1.49-3.80)
Multivariable-adjusted HR (95% CI)†	Referent	2.01 (0.91-4.45)	Referent	1.98 (1.06-3.69)	Referent	1.98 (1.23-3.20)

Abbreviations: CI, confidence interval; CRC, colorectal cancer; HPFS, Health Professionals Follow-Up Study; HR, hazard ratio; NHS, Nurses' Health Study

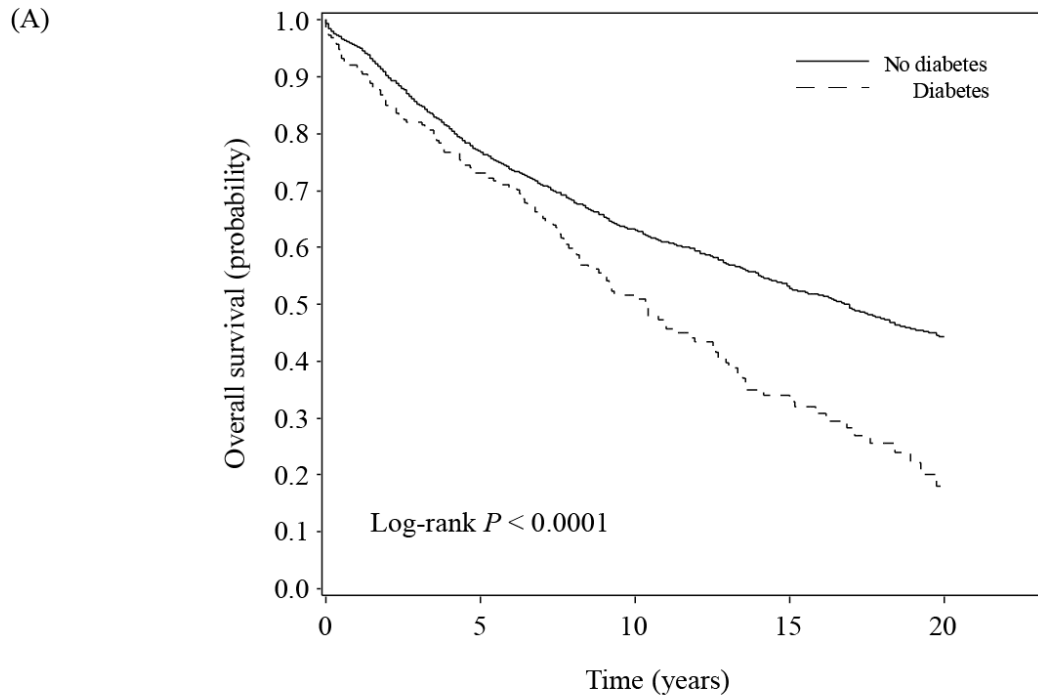
*Stratified by gender (in the combined analyses) and cancer stage (I to III) and adjusted for age at diagnosis (continuous), race (white, non-white, unknown), grade of tumor differentiation (well differentiated, moderately differentiated, poorly differentiated, unknown), location of primary tumor (proximal colon, distal colon, rectum, unknown), and year of diagnosis (1976-1989, 1990-1999, 2000-2012).

†Additionally adjusted for body mass index (continuous), physical activity (continuous), smoking status (never, past, current), alcohol intake (continuous), and regular aspirin use (yes, no).

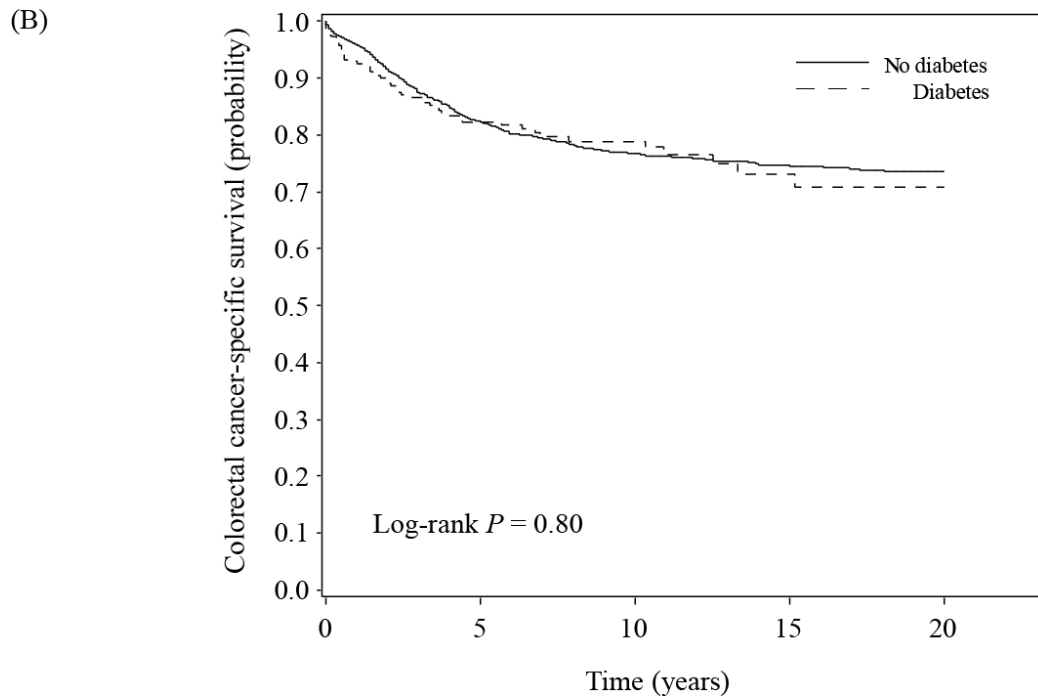
Table 2.5: Hazard ratios for colorectal cancer-specific mortality by diabetes status at cancer diagnosis, stratified by tumor molecular markers

Subgroup	No. of patients	No. of events	HR (95% CI)*	$P_{interaction}$
<i>KRAS</i> mutation				0.78
Wild-type	741	125	0.94 (0.50-1.75)	
Mutant	408	94	1.07 (0.53-2.17)	
<i>PIK3CA</i> mutation				0.76
Wild-type	893	176	1.07 (0.64-1.79)	
Mutant	170	29	0.88 (0.26-2.98)	
Cytoplasmic IRS1 expression				0.50
-	410	88	1.06 (0.50-2.23)	
+	174	31	0.61 (0.14-2.72)	
Cytoplasmic IRS2 expression				0.04
-	396	82	1.51 (0.72-3.15)	
+	187	37	0.38 (0.11-1.33)	
Cytoplasmic FASN expression				0.94
-	330	68	1.03 (0.42-2.50)	
+	533	106	0.99 (0.48-2.02)	

Abbreviations: CI, confidence interval; FASN, fatty acid synthase; HR, hazard ratio; IRS, insulin receptor substrate
 *Comparing patients with diabetes to those without diabetes, stratified by gender and cancer stage (I to III) and adjusted for age at diagnosis (continuous), race (white, non-white, unknown), grade of tumor differentiation (well differentiated, moderately differentiated, poorly differentiated, unknown), location of primary tumor (proximal colon, distal colon, rectum, unknown), year of diagnosis (1976-1989, 1990-1999, 2000-2012), body mass index (continuous), physical activity (continuous), smoking status (never, past, current), alcohol intake (continuous), and regular aspirin use (yes, no).



Number at risk	0	5	10	15	20
No diabetes	2347	1622	1084	634	337
Diabetes	257	155	74	34	9



Number at risk	0	5	10	15	20
No diabetes	2347	1622	1084	634	337
Diabetes	257	155	74	34	9

Figure 2.1: Survival curves for (A) overall and (B) colorectal cancer-specific survival by diabetes status at cancer diagnosis

Supplementary tables

Table S2.1: Number of cause-specific deaths during each follow-up period

Cause of death	Follow-up period		
	0-5 years	>5-10 years	>10 years
CRC, No. (%)	436 (73.6)	102 (33.9)	45 (13.4)
Non-CRC cancers, No. (%)	58 (9.8)	63 (20.9)	64 (19.0)
Cardiovascular disease, No. (%)	32 (5.4)	49 (16.3)	65 (19.3)
Neurologic disorders, No. (%)	10 (1.7)	16 (5.3)	36 (10.7)
Pulmonary disorders, No. (%)	8 (1.4)	18 (6.0)	29 (8.6)
Cerebrovascular disease, No. (%)	13 (2.2)	15 (5.0)	22 (6.5)
Other or unknown, No. (%)	35 (5.9)	38 (12.6)	76 (22.6)

Abbreviation: CRC, colorectal cancer

Table S2.2: Hazard ratios for 5-year overall mortality by duration of diabetes at cancer diagnosis

	Duration of diabetes at cancer diagnosis			
	No diabetes	≤5 years	>5 - 10 years	>10 years
No. of patients	2,347	79	72	106
No. of events during the first 5 years	527	14	17	34
Age-adjusted HR (95% CI)	Referent	0.73 (0.43-1.25)	1.07 (0.66-1.73)	1.46 (1.03-2.08)
Multivariable-adjusted HR (95% CI)*	Referent	0.75 (0.44-1.27)	1.03 (0.63-1.67)	1.39 (0.97-1.97)
Multivariable-adjusted HR (95% CI)†	Referent	0.77 (0.45-1.32)	1.04 (0.64-1.70)	1.42 (0.99-2.04)

Abbreviations: CI, confidence interval; HR, hazard ratio

*Stratified by gender and cancer stage (I to III) and adjusted for age at diagnosis (continuous), race (white, non-white, unknown), grade of tumor differentiation (well differentiated, moderately differentiated, poorly differentiated, unknown), location of primary tumor (proximal colon, distal colon, rectum, unknown), and year of diagnosis (1976-1989, 1990-1999, 2000-2012).

†Additionally adjusted for body mass index (continuous), physical activity (continuous), smoking status (never, past, current), alcohol intake (continuous), and regular aspirin use (yes, no).

Chapter 3 Prediagnostic circulating concentrations of vitamin D binding protein and survival among colorectal cancer patients

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Abstract

Background: Higher plasma levels of total 25-hydroxyvitamin D [25(OH)D] are associated with an improvement in overall survival among colorectal cancer (CRC) patients, but the relationship between plasma vitamin D binding protein (VDBP), bioavailable or free 25(OH)D, and CRC survival remains unknown.

Methods: In two large prospective US cohorts, we examined the association of prediagnostic plasma levels of VDBP, bioavailable 25(OH)D, and free 25(OH)D with survival among 604 participants diagnosed with CRC between 1991 and 2011. Plasma 25(OH)D and VDBP were directly measured, whereas bioavailable and free 25(OH)D were calculated using a validated formula based on total 25(OH)D, VDBP, and albumin levels, as well as the genotype-specific affinity of VDBP. Cox proportional hazards models were used to estimate hazard ratios (HRs) for overall and CRC-specific mortality, adjusted for other prognostic markers and potential confounders.

Results: Higher VDBP levels were associated with a significant improvement in overall and CRC-specific survival ($P_{\text{trend}} = 0.005$ and 0.02 , respectively). Compared to patients in the lowest quartile, those in the highest quartile of VDBP had a multivariable-adjusted HR of 0.61 (95% confidence interval [CI], 0.42-0.89) for overall mortality and 0.56 (95% CI, 0.35-0.92) for CRC-specific mortality. The results remained unchanged after further adjustment for total 25(OH)D levels. However, no association with overall or CRC-specific mortality was observed for bioavailable or free 25(OH)D levels.

Conclusions: Among CRC patients, higher prediagnostic plasma VDBP levels were associated with improved overall and CRC-specific survival. The clinical utility of VDBP as a prognostic marker warrants further exploration, as well as research into underlying mechanisms of action.

Introduction

Vitamin D is hypothesized to play a role in the development and progression of colorectal cancer (CRC). Colon cancer cells express vitamin D receptor (VDR) (1, 2) and 1- α -hydroxylase (3), which converts the main circulating form of vitamin D, 25-hydroxyvitamin D [25(OH)D], into the active metabolite, calcitriol [1,25(OH)₂D]. Binding of 1,25(OH)₂D to VDR leads to multiple anti-cancer effects, including increased cell differentiation and apoptosis (4, 5) and reduced proliferation (6), angiogenesis (7, 8), and metastasis (9, 10). Previous studies have shown that higher total vitamin D levels are associated with improved survival among CRC patients (11-16).

Vitamin D binding protein (VDBP), also known as the Group-specific component, is the major vitamin D carrier protein. About 88% of 25(OH)D is bound to VDBP, while 12% of 25(OH)D is loosely bound to albumin, leaving very little as the free form (17, 18). The “free hormone hypothesis” postulates that the bound fraction of 25(OH)D is not available to target cells for signaling and gene regulation, suggesting that free 25(OH)D and albumin-bound 25(OH)D, which can dissociate during tissue perfusion, may be more biologically active. Unfortunately, most current laboratory assays of 25(OH)D do not differentiate between the bound and free forms of 25(OH)D. However, free 25(OH)D and albumin-bound 25(OH)D levels [collectively referred to as bioavailable 25(OH)D] can be calculated using a validated formula based on total 25(OH)D, VDBP, and albumin levels, as well as the genotype-specific affinity of VDBP (17).

Although the link between higher total vitamin D levels and improved survival among CRC patients has been well documented (19), the relationship between plasma VDBP, bioavailable and free 25(OH)D, and CRC survival remains unknown. In this study, we prospectively assessed the association between plasma levels of these biomarkers and survival among CRC patients from two large prospective cohorts, the Nurses' Health Study (NHS) and the Health Professionals Follow-Up Study (HPFS).

Methods

Study population

In 1976, NHS was initiated when 121,700 US female registered nurses aged 30 to 55 years responded to a mailed questionnaire on risk factors for cancer and cardiovascular disease (20, 21). Blood samples were collected from 32,826 NHS participants between 1989 and 1990. In 1986, HPFS was established when 51,529 US male dentists, optometrists, osteopaths, podiatrists, pharmacists, and veterinarians aged 40 to 75 years completed a mailed questionnaire on health-related behaviors and medical history (22). Blood samples were collected from 18,225 HPFS participants between 1993 and 1995. In both cohorts, participants receive biennial questionnaires to update information on nondietary exposures and medical diagnoses, with dietary exposures updated every four years. A high follow-up rate of more than 90% was achieved in both cohorts.

When a participant (or next of kin for decedents) reported a diagnosis of CRC on a follow-up questionnaire, we asked permission to obtain hospital records and pathology reports. For nonrespondents, the National Death Index was used to ascertain any diagnosis of CRC that contributed to death, and we asked permission from next of kin for decedents to obtain medical records. Blinded study physicians then reviewed these records to confirm the diagnosis and record information on important tumor characteristics. We estimate that 96- 97% of patients were identified through these methods (23, 24).

Participants from these two cohorts who had pathologically confirmed CRC diagnosed after the date of blood collection through December 2011 were eligible for this study. Patients were excluded if they were non-white (due to the inefficacy of the VDBP assay in African Americans) or had reported any cancer (other than nonmelanoma skin cancer) prior to CRC diagnosis. To minimize any bias associated with presence of occult cancer, we excluded 67 patients who were diagnosed with CRC within 2 years after blood collection, leaving 604 patients for analysis. Two patients had available VDBP levels but not total 25(OH)D levels and were included only in the analyses of VDBP.

This study was approved by the Human Research Committee at the Brigham and Women's Hospital and the Harvard T.H. Chan School of Public Health. Completion and return of questionnaires were considered informed consent.

Mortality assessment

Patients were observed until death or last follow-up dates (June 2012 in NHS; January 2012 in HPFS), whichever came first. Ascertainment of deaths included reporting by family or postal authorities, and interrogation of names of persistent nonrespondents in the National Death Index (25). More than 98% of deaths were identified by these methods (23, 26). Cause of death was assigned by study physicians blinded to other data.

Measurement of total 25(OH)D, VDBP, albumin, and GC genotypes

Blood samples were collected in tubes with heparin and shipped by overnight courier in chilled containers. On receipt, bloods were centrifuged, aliquoted, and stored in continuously-monitored liquid nitrogen freezers at -130°C or below. More than 95% of the blood samples arrived in our laboratory within 26 hours of phlebotomy.

Plasma total 25(OH)D was measured in the laboratory of Dr. Bruce Hollis (The Medical University of South Carolina, Charleston, SC) and Heartland Assays (Ames, IA) by radioimmunoassay (27). Plasma VDBP was measured at Heartland Assays by a monoclonal antibody-based, enzyme-linked immunosorbent assay (ELISA) (R&D Systems). We standardized 25(OH)D and VDBP levels by batch to account for batch-to-batch variation (28). Plasma albumin was measured by a colorimetric assay (Roche Diagnostics) in the laboratory of Dr. Nader Rifai (Children's Hospital, Boston, MA). The mean intra-assay coefficients of variation for total 25(OH)D, VDBP, and albumin were 13.5%, 12.7%, and 3.0%, respectively.

VDBP binding affinity is influenced by its gene (*GC*) haplotype (25). There are three common *GC* haplotypes (GC1F, GC1S, GC2) determined by two single-nucleotide polymorphisms (SNPs), rs4588 and

rs7041. We successfully genotyped these SNPs using the TaqMan OpenArray SNP Genotyping Platform (Applied Biosystems) among 548 patients.

Calculation of bioavailable and free 25(OH)D

We calculated bioavailable and free 25(OH)D using the following equations (17):

$$\text{Free 25(OH)D} = \frac{\text{Total 25(OH)D}}{1 + K_{a\text{Albumin}} \times \text{Albumin} + K_{a\text{VDBP}} \times \text{VDBP}}$$

$$\text{Bioavailable 25(OH)D} = \text{Free 25(OH)D} + \text{Free 25(OH)D} \times \text{Albumin} \times K_{a\text{Albumin}}$$

where $K_{a\text{Albumin}}$ is the binding affinity of albumin for 25(OH)D (6×10^5), $K_{a\text{VDBP}}$ is the genotype-specific VDBP binding affinity (Table S3.1) (29), and all units are mol/L. For those with unknown genotypes, $K_{a\text{VDBP}}$ was imputed as the mean VDBP binding affinity in this population.

Covariates

Cancer stage, grade of tumor differentiation, location of primary tumor, and year of diagnosis (as a surrogate for treatment) were extracted from medical records. Body mass index (BMI), physical activity, and dietary and supplemental vitamin D intake were obtained from the questionnaire before blood collection.

Statistical analyses

The three vitamin D-related biomarkers were categorized into quartiles and evaluated as exposures: VDBP, bioavailable 25(OH)D, and free 25(OH)D. Follow-up time was calculated from CRC diagnosis to death or last follow-up dates (June 2012 in NHS; January 2012 in HPFS), whichever came first. Cox proportional hazards models were used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for death from any cause (primary outcome) and death as a result of CRC (secondary outcome). In multivariable analyses, the models were stratified by gender, cancer stage, and grade of tumor differentiation and adjusted for other prognostic factors, including age at diagnosis, BMI, physical activity,

year of diagnosis, and location of primary tumor. We additionally adjusted for season of blood collection when the exposure was bioavailable or free 25(OH)D. Test for trend was performed using the median value of each quartile as a continuous variable. Interactions between vitamin D-related biomarkers and potential effect modifiers were assessed by entering in the model the cross product of the quartile-specific median of the biomarker and the stratification variable, evaluated by the likelihood ratio test. The Cox models were tested for and met the proportional hazards assumption. All analyses were performed with SAS software, version 9.4. All *P*-values were two sided.

Results

Among 604 CRC patients, there were 279 deaths, 177 of which were due to CRC (63%). Non-CRC causes of death included cardiovascular disease (9%), other malignancies (8%), neurologic disorders (6%), cerebrovascular disease (4%), pulmonary disorders (3%), and other or unknown reasons (7%). The median time of follow-up among patients who were still alive was 10.0 years.

Plasma samples were collected at a median of 9.4 years before diagnosis, and the mean VDBP level was 250 $\mu\text{g/mL}$. Patient characteristics were well balanced by quartile of VDBP (Table 3.1), except that patients with higher VDBP levels tended to have a lower BMI. The mean bioavailable and free 25(OH) levels were 10.6 nmol/L and 26.1 pmol/L, respectively, and these two biomarkers were highly correlated ($r = 0.98$; $P < 0.0001$; Table S3.2). Patient characteristics by quartile of bioavailable (Table 3.1) and free 25(OH)D (Table S3.3) were very similar, and patients with higher bioavailable or free 25(OH)D levels were more likely to be physically active. Total 25(OH)D levels were modestly correlated with VDBP levels ($r = 0.15$; $P < 0.001$) and moderately correlated with bioavailable 25(OH)D levels ($r = 0.54$; $P < 0.0001$) (Table S3.2). In addition, bioavailable 25(OH)D levels were negatively correlated with VDBP levels ($r = -0.56$; $P < 0.0001$) and positively correlated with albumin levels ($r = 0.18$; $P < 0.0001$) (Table S3.2).

Higher VDBP levels were significantly associated with decreased overall and CRC-specific mortality ($P_{\text{trend}} = 0.005$ and 0.02 , respectively) (Table 3.2). Compared to patients in the lowest quartile, those in the highest quartile of VDBP had a multivariable-adjusted HR of 0.61 (95% CI, 0.42-0.89) for overall mortality

and 0.56 (95% CI, 0.35-0.92) for CRC-specific mortality. The HRs were not materially changed after further adjustment for total 25(OH)D levels. To further address concerns about the possible influence of occult cancer on VDBP levels, we performed sensitivity analyses by excluding patients who developed CRC within 3, 4, and 5 years after blood collection, respectively. Although statistical power was diminished, the association between VDBP levels and CRC survival remained largely unchanged (data not shown). In contrast, no association with overall or CRC-specific survival was observed for bioavailable or free 25(OH)D levels (Table 3.3).

We examined the association of VDBP levels with overall and CRC-specific mortality across strata of potential effect modifiers, and did not detect any significant interactions. The relationship between VDBP levels and survival remained largely unchanged across most subgroups, including age at diagnosis, time between blood collection and cancer diagnosis, gender, BMI, cancer stage, grade of tumor differentiation, year of diagnosis, and total 25(OH)D levels (Figure 3.1). However, the improvement in CRC-specific survival associated with higher VDBP levels appeared more apparent among patients with rectal cancer (HR, 0.09; 95% CI, 0.01-0.76) than colon cancer (HR, 0.72; 95% CI, 0.41-1.26; $P_{\text{interaction}} = 0.06$).

Discussion

We found that CRC patients who had prediagnostic plasma VDBP in the highest quartile had a significant improvement in overall and CRC-specific survival that was independent of total 25(OH)D levels. However, no association with CRC survival was observed for plasma bioavailable or free 25(OH)D. To our knowledge, no previous studies have examined the association between VDBP levels and CRC survival.

The findings of this study are biologically plausible. As the major carrier protein of 25(OH)D, VDBP can prolong the half-life of 25(OH)D and boost its anti-cancer effects. In addition, VDBP has several important biological functions related to carcinogenesis. First, VDBP serves as an actin scavenger that binds to actin released after tissue injury and cell death, thereby preventing vascular occlusion and organ dysfunction (30). Second, in immune responses, VDBP is deglycosylated into VDBP-macrophage

activating factor, a potent anti-angiogenic and anti-tumorigenic agent (31-34). Third, VDBP has an anti-inflammatory effect by directing neutrophils to sites of inflammation (neutrophil chemotaxis) (35, 36).

A number of studies have reported that higher vitamin D levels are associated with improved CRC survival (11-16), including previous studies in NHS and HPFS (11, 13), whereas neither bioavailable nor free 25(OH)D levels were associated with CRC survival in the current study. This finding is consistent with a previous study in NHS and HPFS, in which total 25(OH)D levels, but not bioavailable or free 25(OH)D levels, were inversely associated with CRC risk (37). Together, these data do not support the “free hormone hypothesis” regarding the role of 25(OH)D in colorectal carcinogenesis and suggest that there exists an alternative internalization mechanism of 25(OH)D. Recent experimental data have shown that the 25(OH)D-VDBP complex can, in fact, be taken up and transported into cells by the endocytic receptor megalin (38), which is known to be expressed in several absorptive epithelia including that of the colon (39-41). Further studies to investigate the presence and role of megalin in colorectal carcinogenesis are desired.

This study has several strengths, including the prospective design, long follow-up time, high follow-up rate, and detailed data on many potential confounders. In addition, the comprehensive measurement of total 25(OH)D, VDBP, albumin, and *GC* genotypes allowed us to examine the role of various vitamin D-related biomarkers in CRC survival.

Several limitations of our study deserve comment. We used a single measurement of 25(OH)D and VDBP from plasma samples collected years before diagnosis. However, VDBP levels are very stable over much of the life course (42), and previous studies have shown a good correlation for repeated measures of circulating 25(OH)D up to 14 years apart (43-45). We did not directly measure bioavailable or free 25(OH)D, but calculated free 25(OH)D has been shown to be reasonably correlated with directly measured free 25(OH)D (46). It has been suggested that a monoclonal antibody-based ELISA cannot correctly measure VDBP in African Americans (46, 47), which consequently introduces a critical flaw into the calculation of bioavailable and free 25(OH)D. To reduce this concern, we excluded non-white patients in the analyses. Finally, information on treatment regimens was not systematically collected in our cohorts.

However, treatment programs were unlikely to have varied by VDBP levels years before diagnosis. Furthermore, we adjusted for year of diagnosis to control for any secular trends in CRC treatment.

In conclusion, higher prediagnostic plasma VDBP levels were associated with decreased overall and CRC-specific mortality among CRC patients. However, bioavailable or free 25(OH)D levels were not associated with CRC survival. Additional efforts to understand the mechanisms through which the vitamin D pathway influences colorectal carcinogenesis and cancer progression are warranted.

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Tables/Figure

Table 3.1: Baseline characteristics among colorectal cancer patients by quartile of plasma vitamin D binding protein and bioavailable 25-hydroxyvitamin D

Characteristic	Vitamin D binding protein				Bioavailable 25-hydroxyvitamin D			
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	Quartile 1	Quartile 2	Quartile 3	Quartile 4
No. of patients	151	151	151	151	150	151	151	150
Age at blood collection, years, mean (SD)	61.0 (7.6)	62.6 (8.2)	61.3 (8.5)	60.5 (8.3)	60.4 (8.2)	61.6 (8.0)	61.9 (8.4)	61.4 (8.2)
Age at diagnosis, years, mean (SD)	71.2 (7.7)	72.0 (8.5)	71.3 (8.5)	70.7 (8.7)	71.5 (8.7)	71.3 (8.0)	71.5 (8.6)	70.8 (8.1)
Time between blood collection and cancer diagnosis, years, mean (SD)	10.2 (4.8)	9.4 (4.7)	10.0 (4.9)	10.2 (5.0)	11.1 (5.0)	9.6 (4.4)	9.5 (5.1)	9.5 (4.8)
Gender, No. (%)								
Female	99 (65.6)	80 (53.0)	80 (53.0)	99 (65.6)	106 (70.7)	78 (51.7)	85 (56.3)	87 (58.0)
Male	52 (34.4)	71 (47.0)	71 (47.0)	52 (34.4)	44 (29.3)	73 (48.3)	66 (43.7)	63 (42.0)
Body mass index, kg/m ² , mean (SD)	27.0 (5.1)	25.8 (3.6)	25.6 (4.3)	25.2 (3.7)	26.0 (4.8)	26.0 (4.0)	26.1 (4.5)	25.4 (3.8)
Physical activity, MET-h/wk, mean (SD)	24.0 (32.9)	26.2 (32.6)	22.2 (24.7)	25.3 (32.8)	20.3 (30.0)	24.2 (30.1)	23.4 (24.4)	30.0 (37.7)
Total vitamin D intake, energy-adjusted, IU/d, mean (SD) ^a	374 (267)	386 (287)	341 (218)	406 (317)	313 (235)	411 (285)	405 (306)	369 (257)
Year of diagnosis, No. (%)								
1991-2000	72 (47.7)	75 (49.7)	65 (43.0)	67 (44.4)	57 (38.0)	69 (45.7)	80 (53.0)	73 (48.7)
2001-2011	79 (52.3)	76 (50.3)	86 (57.0)	84 (55.6)	93 (62.0)	82 (54.3)	71 (47.0)	77 (51.3)
Cancer stage, No. (%)								
I	34 (22.5)	45 (29.8)	39 (25.8)	41 (27.2)	41 (27.3)	39 (25.8)	41 (27.2)	38 (25.3)
II	41 (27.2)	37 (24.5)	33 (21.9)	40 (26.5)	44 (29.3)	32 (21.2)	40 (26.5)	34 (22.7)
III	37 (24.5)	34 (22.5)	40 (26.5)	40 (26.5)	35 (23.3)	37 (24.5)	38 (25.2)	41 (27.3)
IV	19 (12.6)	20 (13.2)	25 (16.6)	15 (9.9)	17 (11.3)	24 (15.9)	19 (12.6)	18 (12.0)
Unknown	20 (13.2)	15 (9.9)	14 (9.3)	15 (9.9)	13 (8.7)	19 (12.6)	13 (8.6)	19 (12.7)
Grade of tumor differentiation, No. (%)								
Well differentiated	19 (12.6)	11 (7.3)	17 (11.3)	17 (11.3)	22 (14.7)	9 (6.0)	15 (9.9)	17 (11.3)
Moderately differentiated	87 (57.6)	92 (60.9)	84 (55.6)	88 (58.3)	81 (54.0)	98 (64.9)	89 (58.9)	82 (54.7)
Poorly differentiated	24 (15.9)	19 (12.6)	19 (12.6)	24 (15.9)	29 (19.3)	15 (9.9)	20 (13.2)	22 (14.7)
Unknown	21 (13.9)	29 (19.2)	31 (20.5)	22 (14.6)	18 (12.0)	29 (19.2)	27 (17.9)	29 (19.3)
Location of primary tumor, No. (%)								
Proximal colon	68 (45.0)	57 (37.7)	74 (49.0)	72 (47.7)	70 (46.7)	59 (39.1)	73 (48.3)	67 (44.7)
Distal colon	45 (29.8)	42 (27.8)	34 (22.5)	43 (28.5)	38 (25.3)	46 (30.5)	42 (27.8)	38 (25.3)
Rectum	30 (19.9)	39 (25.8)	31 (20.5)	29 (19.2)	34 (22.7)	33 (21.9)	28 (18.5)	34 (22.7)
Unknown	8 (5.3)	13 (8.6)	12 (7.9)	7 (4.6)	8 (5.3)	13 (8.6)	8 (5.3)	11 (7.3)

Table 3.1 (Continued): Baseline characteristics among colorectal cancer patients by quartile of plasma vitamin D binding protein and bioavailable 25-hydroxyvitamin D

Characteristic	Vitamin D binding protein				Bioavailable 25-hydroxyvitamin D			
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Season of blood collection, No. (%)								
Summer (June, July, August)	46 (30.5)	46 (30.5)	49 (32.5)	52 (34.4)	36 (24.0)	52 (34.4)	50 (33.1)	54 (36.0)
Fall (September, October, November)	39 (25.8)	50 (33.1)	40 (26.5)	43 (28.5)	31 (20.7)	42 (27.8)	53 (35.1)	45 (30.0)
Winter (December, January, February)	35 (23.2)	24 (15.9)	28 (18.5)	26 (17.2)	44 (29.3)	26 (17.2)	16 (10.6)	27 (18.0)
Spring (March, April, May)	31 (20.5)	31 (20.5)	34 (22.5)	30 (19.9)	39 (26.0)	31 (20.5)	32 (21.2)	24 (16.0)

Abbreviations: SD, standard deviation; MET, metabolic equivalent

Table 3.2: Hazard ratios for overall and colorectal cancer-specific mortality among colorectal cancer patients by quartile of plasma vitamin D binding protein

	Vitamin D binding protein				<i>P</i> _{trend}
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
Median (range), µg/mL	145 (39-200)	231 (201-257)	286 (257-330)	388 (331-639)	
No. of patients	151	151	151	151	
Overall mortality					
No. of events	74	80	67	58	
Age-adjusted HR (95% CI)	Referent	0.94 (0.68-1.29)	0.88 (0.63-1.22)	0.68 (0.48-0.96)	0.02
Multivariable-adjusted HR (95% CI)*	Referent	0.84 (0.59-1.19)	0.58 (0.40-0.84)	0.61 (0.42-0.89)	0.005
Colorectal cancer-specific mortality					
No. of events	46	45	51	35	
Age-adjusted HR (95% CI)	Referent	0.91 (0.60-1.37)	1.11 (0.74-1.65)	0.69 (0.45-1.08)	0.17
Multivariable-adjusted HR (95% CI)*	Referent	0.79 (0.50-1.25)	0.65 (0.41-1.03)	0.56 (0.35-0.92)	0.02

Abbreviations: CI, confidence interval; HR, hazard ratio

*Stratified by gender, cancer stage (I to IV or unknown), and grade of tumor differentiation (well differentiated, moderately differentiated, poorly differentiated, unknown) and adjusted for age at diagnosis (continuous), body mass index (continuous), physical activity (continuous), year of diagnosis (1991-2000, 2001-2011), and location of primary tumor (proximal colon, distal colon, rectum, unknown).

Table 3.3: Hazard ratios for overall and colorectal cancer-specific mortality among colorectal cancer patients by quartile of plasma bioavailable or free 25-hydroxyvitamin D

	Bioavailable or free 25-hydroxyvitamin D				<i>P</i> _{trend}
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
Bioavailable 25-hydroxyvitamin D					
Median (range), nmol/L	5.6 (1.5-6.9)	7.9 (6.9-9.4)	11.0 (9.4-13.3)	15.9 (13.4-56.7)	
No. of patients	150	151	151	150	
Overall mortality					
No. of events	59	77	71	70	
Age- and season-adjusted HR (95% CI)	Referent	1.23 (0.87-1.73)	1.11 (0.78-1.58)	1.16 (0.81-1.65)	0.67
Multivariable-adjusted HR (95% CI)*	Referent	1.06 (0.73-1.54)	1.13 (0.77-1.65)	1.14 (0.77-1.67)	0.51
Colorectal cancer-specific mortality					
No. of events	38	44	44	50	
Age- and season-adjusted HR (95% CI)	Referent	1.10 (0.71-1.71)	1.10 (0.71-1.71)	1.28 (0.84-1.97)	0.26
Multivariable-adjusted HR (95% CI)*	Referent	0.95 (0.59-1.53)	1.09 (0.67-1.77)	1.33 (0.83-2.13)	0.15
Free 25-hydroxyvitamin D					
Median (range), pmol/L	14.1 (4.1-16.8)	19.5 (16.9-22.7)	27.0 (22.7-31.8)	39.0 (31.9-142.3)	
No. of patients	150	151	151	150	
Overall mortality					
No. of events	58	77	71	71	
Age- and season-adjusted HR (95% CI)	Referent	1.29 (0.92-1.83)	1.17 (0.82-1.66)	1.24 (0.87-1.76)	0.45
Multivariable-adjusted HR (95% CI)*	Referent	1.14 (0.78-1.67)	1.13 (0.77-1.65)	1.27 (0.86-1.88)	0.27
Colorectal cancer-specific mortality					
No. of events	40	42	45	49	
Age- and season-adjusted HR (95% CI)	Referent	1.00 (0.65-1.56)	1.09 (0.71-1.68)	1.21 (0.80-1.85)	0.30
Multivariable-adjusted HR (95% CI)*	Referent	0.88 (0.54-1.44)	1.01 (0.62-1.63)	1.36 (0.84-2.20)	0.11

Abbreviations: CI, confidence interval; HR, hazard ratio

*Stratified by gender, cancer stage (I to IV or unknown), and grade of tumor differentiation (well differentiated, moderately differentiated, poorly differentiated, unknown) and adjusted for age at diagnosis (continuous), body mass index (continuous), physical activity (continuous), year of diagnosis (1991-2000, 2001-2011), location of primary tumor (proximal colon, distal colon, rectum, unknown), and season of blood collection (summer, fall, winter, spring).

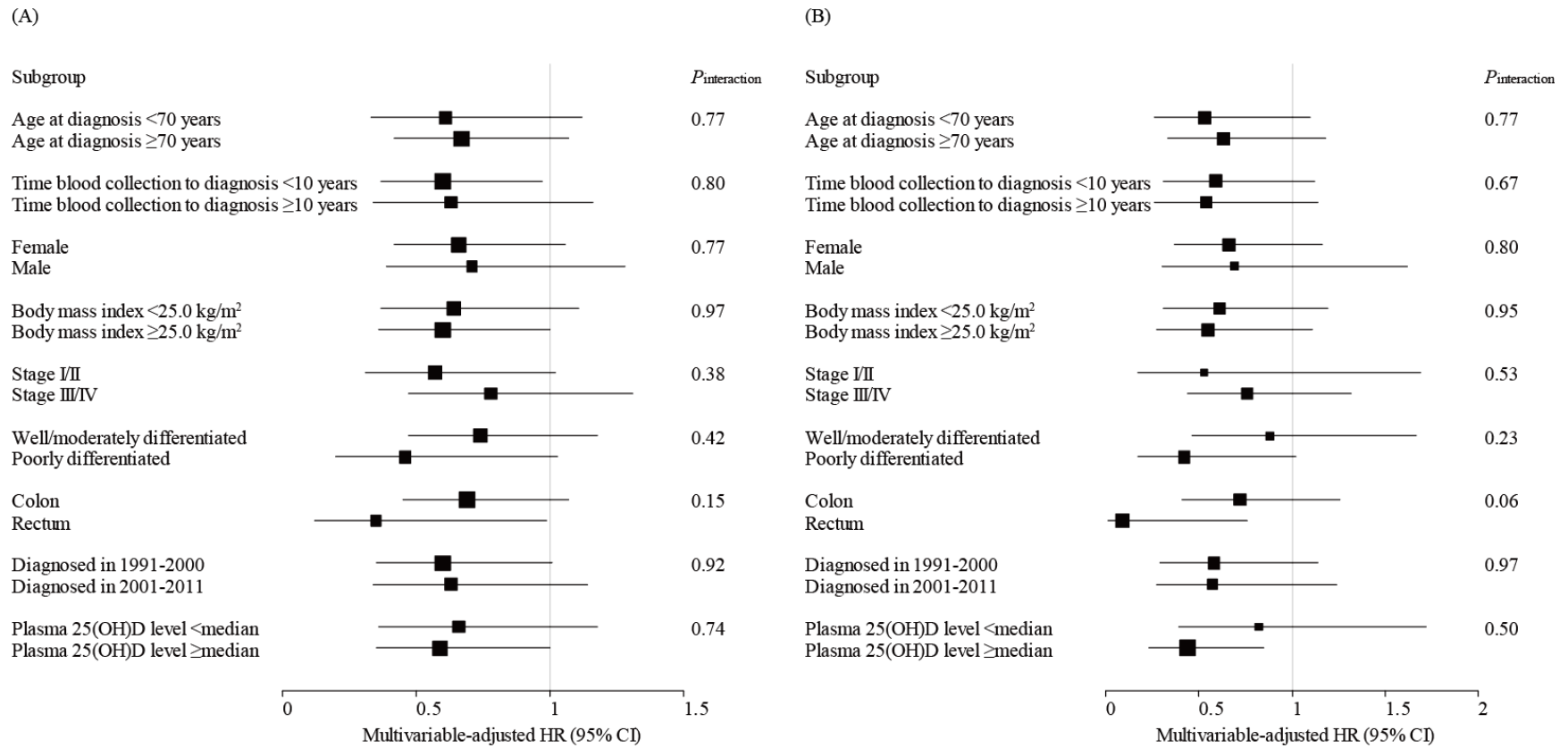


Figure 3.1: Multivariable-adjusted hazard ratios for (A) overall and (B) colorectal cancer-specific mortality comparing the highest to the lowest quartile of vitamin D binding protein, stratified by covariates

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; HR, hazard ratio

Supplementary tables

Table S3.1: Genotype-specific affinity of vitamin D binding protein

VDBP phenotype	rs7041	rs4588	Binding affinity ($\times 10^8$)
GC1F-1F*	TT	CC	11.2
GC1S-1S*	GG	CC	6
GC2-2*	TT	AA	3.6
GC1F-1S†	TG	CC	8.6
GC1F-2†	TT	AC	7.4
GC1S-2†	TG	AC	4.8

Abbreviation: VDBP, vitamin D binding protein

*Binding affinity for homozygous phenotypes was obtained from literature.

†Binding affinity for heterozygous phenotypes was calculated as the mean affinity of the corresponding homozygous phenotypes, assuming that all the alleles are co-dominant.

Table S3.2: Age-adjusted Spearman correlation coefficients between vitamin D-related biomarkers among colorectal cancer patients

	Total 25(OH)D	Bioavailable 25(OH)D	Free 25(OH)D	VDBP	Albumin
Total 25(OH)D	1	0.54*	0.54*	0.15†	0.05‡
Bioavailable 25(OH)D		1	0.98*	-0.56*	0.18*
Free 25(OH)D			1	-0.58*	0.04‡
VDBP				1	0.03‡
Albumin					1

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; VDBP, vitamin D binding protein

* $P < 0.0001$

† $P < 0.001$

‡ $P > 0.05$

Table S3.3: Baseline characteristics among colorectal cancer patients by quartile of plasma free 25-hydroxyvitamin D

Characteristic	Free 25-hydroxyvitamin D			
	Quartile 1	Quartile 2	Quartile 3	Quartile 4
No. of patients	150	151	151	150
Age at blood collection, years, mean (SD)	60.8 (7.8)	61.6 (8.5)	61.6 (8.3)	61.3 (8.2)
Age at diagnosis, years, mean (SD)	71.5 (8.5)	71.1 (8.4)	71.3 (8.3)	71.3 (8.2)
Time between blood collection and cancer diagnosis, years, mean (SD)	10.6 (4.8)	9.4 (4.6)	9.7 (5.0)	10.0 (5.0)
Gender, No. (%)				
Female	98 (65.3)	80 (53.0)	85 (56.3)	93 (62.0)
Male	52 (34.7)	71 (47.0)	66 (43.7)	57 (38.0)
Body mass index, kg/m ² , mean (SD)	25.8 (4.6)	26.1 (3.9)	26.6 (4.6)	25.2 (3.9)
Physical activity, MET-h/wk, mean (SD)	20.6 (29.4)	24.7 (30.0)	22.0 (24.8)	30.6 (37.6)
Total vitamin D intake, energy-adjusted, IU/d, mean (SD)	333 (236)	384 (296)	401 (297)	383 (262)
Year of diagnosis, No. (%)				
1991-2000	60 (40.0)	75 (49.7)	76 (50.3)	68 (45.3)
2001-2011	90 (60.0)	76 (50.3)	75 (49.7)	82 (54.7)
Cancer stage, No. (%)				
I	42 (28.0)	42 (27.8)	38 (25.2)	37 (24.7)
II	42 (28.0)	35 (23.2)	37 (24.5)	36 (24.0)
III	37 (24.7)	35 (23.2)	37 (24.5)	42 (28.0)
IV	17 (11.3)	22 (14.6)	23 (15.2)	16 (10.7)
Unknown	12 (8.0)	17 (11.3)	16 (10.6)	19 (12.7)
Grade of tumor differentiation, No. (%)				
Well differentiated	20 (13.3)	14 (9.3)	10 (6.6)	19 (12.7)
Moderately differentiated	84 (56.0)	97 (64.2)	88 (58.3)	81 (54.0)
Poorly differentiated	29 (19.3)	13 (8.6)	21 (13.9)	23 (15.3)
Unknown	17 (11.3)	27 (17.9)	32 (21.2)	27 (18.0)
Location of primary tumor, No. (%)				
Proximal colon	64 (42.7)	68 (45.0)	67 (44.4)	70 (46.7)
Distal colon	40 (26.7)	45 (29.8)	42 (27.8)	37 (24.7)
Rectum	39 (26.0)	26 (17.2)	30 (19.9)	34 (22.7)
Unknown	7 (4.7)	12 (7.9)	12 (7.9)	9 (6.0)
Season of blood collection, No. (%)				
Summer (June, July, August)	37 (24.7)	52 (34.4)	49 (32.5)	54 (36.0)
Fall (September, October, November)	29 (19.3)	48 (31.8)	48 (31.8)	46 (30.7)
Winter (December, January, February)	43 (28.7)	26 (17.2)	18 (11.9)	26 (17.3)
Spring (March, April, May)	41 (27.3)	25 (16.6)	36 (23.8)	24 (16.0)

Abbreviations: SD, standard deviation; MET, metabolic equivalent