A prospective study of intakes of zinc and heme iron and colorectal cancer risk in men and women

Xuehong Zhang · Edward L. Giovannucci · Stephanie A. Smith-Warner · Kana Wu · Charles S. Fuchs · Michael Pollak · Walter C. Willett · Jing Ma

Abstract Although laboratory studies linked zinc and heme iron to colorectal cancer, epidemiologic evidence is limited. We prospectively examined these associations in the Nurses' Health Study and Health Professionals Follow-up Study. We used Cox proportional hazards regression analyses to calculate cohort-specific relative risks (RRs) and pooled results using a fixed-effects model. We documented 2,114 incident colorectal cancer cases during up to 22 years of follow-up. Compared highest to lowest quintile of dietary zinc intake, the pooled multivariable RRs (95% CIs) were 0.86 (0.73, 1.02) for colorectal cancer, 0.92 (0.76, 1.11) for colon cancer, and 0.68 (0.47, 0.99) for rectal cancer. The significant inverse association between dietary zinc intake and risk of rectal cancer was mainly driven by data in women, although the difference in the sex-specific results was not statistically significant. For the same comparison, the pooled multivariable RRs (95% CIs) for heme iron were 1.10 (0.93, 1.30) for colorectal cancer, 1.06 (0.88, 1.29) for colon cancer, and 1.20 (0.83, 1.75) for rectal cancer. These associations were not significantly modified by alcohol consumption, body mass index, physical activity, menopausal status, or postmenopausal hormone use. Total zinc intake, total iron intake, dietary iron intake, and zinc or iron supplement uses were largely not associated with colorectal cancer risk. Our study does not support strong roles of zinc and heme iron intake in colorectal cancer risk; however, a suggestive inverse association of dietary zinc intake with rectal cancer risk in women requires further study.

Keywords Colorectal cancer · Zinc · Iron · Heme iron · Cohort study

X. Zhang (✉) · E. L. Giovannucci · C. S. Fuchs · W. C. Willett · J. Ma
Channing Laboratory at Landmark Center (West Wing), Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, 401 Park Drive, Boston, MA 02115, USA
e-mail: xuehong.zhang@channing.harvard.edu

E. L. Giovannucci · S. A. Smith-Warner · K. Wu · W. C. Willett
Department of Nutrition, Harvard School of Public Health, Boston, MA, USA

E. L. Giovannucci · S. A. Smith-Warner · W. C. Willett
Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA

C. S. Fuchs
Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA

M. Pollak
Department of Oncology, McGill University and Lady Davis Research Institute, 3999 Rue Côte Sainte Catherine, Montreal, QC H3T 1E2, Canada

Introduction

Colorectal cancer is the third most common type of cancer in both men and women worldwide [1]. The positive association reported in most studies between insulin/C-peptide [2, 3] levels and type 2 diabetes [4] and colorectal cancer risk suggests a possible role of hyperinsulinemia, insulin resistance, and insulin-like growth factor 1 (IGF-1) in colorectal carcinogenesis [3, 5]. Zinc, an essential trace element with antioxidant properties, is vital for various cellular functions, and as early as 1930, zinc was shown as an integral element of the insulin crystalline structure [6]. Subsequently, rodent models showed that zinc supplementation attenuated hyperinsulinemia in *db/db*

mice [7]. In addition, zinc supplementation for 4 weeks significantly improved insulin sensitivity in obese women [8]. More recently, zinc intake was inversely associated with risk of type 2 diabetes among women in the Nurses' Health Study [9]. Despite the potential beneficial effect of zinc on risk of colorectal cancer, the hypothesis that dietary zinc intake is associated with a lower risk of colorectal cancer has been examined in only two prospective studies in women; one study reported a significant [10] inverse association, whereas the other found a null [11] association between dietary zinc intake and colon cancer risk. In the former study, the inverse association was markedly stronger among alcohol drinkers [10].

In contrast to zinc, redox active iron is a pro-oxidant that may influence colorectal carcinogenesis by forming reactive oxygen species [12]. However, epidemiological studies of colorectal cancer risk in relation to iron intake or markers of body iron stores have been mixed [10, 11, 13–19]. Emerging evidence suggests that heme iron may play a more important role in colorectal carcinogenesis than other forms of iron [20, 21]. Heme iron is mainly found in animal foods, whereas non-heme iron is present mainly in plant foods [22]. In general, heme iron is more bioavailable than other forms of iron and is the form in which iron is stored in the body [23]. Further, heme iron, but not non-heme, has been shown to have cytotoxic and hyperproliferative effects in rats [21] and to increase the formation of endogenous intestinal *N*-nitroso compounds [24], established human carcinogens. We thus focused on heme iron in addition to examining other iron-related variables in our analyses. To date, five studies [10, 11, 14, 18, 25] have examined the association between heme iron intakes and colon or colorectal cancer risk, and the results have been mixed. Two of these studies reported a stronger positive association with heme iron intake among alcohol drinkers [10, 11].

We used the dietary data collected every 2–4 years from the US female Nurses' Health Study (NHS) and the US male Health Professionals Follow-up Study (HPFS) to examine whether zinc intake is associated with a lower risk and heme iron intake is associated with an increased risk of colorectal cancer. We further tested whether these associations differ by alcohol consumption because associations with zinc and heme iron intake have been more pronounced among alcohol drinkers in some previous studies [10, 11, 18].

Materials and methods

Study population

The NHS [26] is an ongoing cohort study established in 1976 including 121,700 married women registered nurses at baseline who were 30–55 years old and resided in 11 states in the US. The HPFS [26] is an ongoing cohort study of 51,529 US male professionals who were aged 40–75 years at baseline in 1986. Questionnaires have been mailed to participants in both studies every 2 years since baseline to collect updated information on demographics, lifestyle factors, medical history, and disease outcomes. The follow-up rate has been greater than 90% in both cohort studies. These studies have been approved by the institutional review board at the Brigham and Women's Hospital, Boston, Massachusetts.

Identification of incident colorectal cancer cases

Cancer and other disease outcomes were reported by the participants in both cohorts in the biennial questionnaires. Researchers received permission from the study participants or next of kin to obtain their medical records and pathological reports on colorectal cancer and, while blinded to exposure information, abstracted the information on anatomic location, stage, and histological type of the cancer. Colon cancer was further classified into proximal colon cancers (neoplasms from the cecum to the splenic flexure) and distal colon cancers (neoplasms in the descending and sigmoid colon). Rectal cancer was defined as that occurring in the rectosigmoid or rectum [27].

Assessment of dietary factors

Information on usual dietary intake over the past year was obtained using a validated semiquantitative food frequency questionnaire (FFQ) in NHS participants in 1980, as well as in 1984, 1986, and every 4 years thereafter. A total of 61 food items were included in the 1980 FFQ; the number of food items expanded to about 130 in 1984 and in subsequent FFQs. Similar FFQs were administered for men in 1986 and repeated every 4 years thereafter. Nine possible frequency choices were available, ranging from "almost never" to "six or more times per day." Nutrient intakes were calculated by multiplying the frequency of each food consumed and the nutrient content of specified portion sizes. Zinc or iron supplement use refers to zinc or iron from multivitamins and supplements in this study. Total intake of zinc and iron was calculated by summing up the amounts from food and supplemental sources. Nutrient intakes, including total and dietary zinc and iron, were adjusted for total energy intake using the residual method [26]. Because zinc or iron supplement use was not included in the 1980 food frequency questionnaire in the NHS, we thus treated 1984 as the baseline for these analyses in NHS. The validity of the FFQs has been evaluated in 173 women from the NHS [28] and in 127 men from the HPFS [29]. The energy-adjusted Pearson correlation coefficients for

total zinc intake comparing the FFQ and the average of multiple 1-week diet records (four for women and two for men) were 0.63 for women [28, 30] and 0.71 for men [29]. The Pearson correlation coefficients between FFQ and multiple dietary records for total iron were 0.55 for women [31] and 0.54 for men [29]. Although dietary zinc and heme iron intakes were not evaluated in the validation study, we have evaluated the major contributors of dietary zinc and heme iron intakes in our validation studies [32, 33]. The top 5 foods contributing approximately 60–70% of dietary zinc and heme iron intakes included red meat (beef, pork, lamb), cold breakfast cereal, chicken without skin, skim milk, and hamburgers in both women and men. The Spearman correlation coefficients between the FFQs and the dietary records ranged from 0.38 for hamburger to 0.81 for skim milk in women [33] and ranged from 0.56 for chicken without skin to 0.88 for skim milk in men [32].

Assessment of other covariates

In the baseline and biennial questionnaires, we inquired about colorectal cancer risk factors such as body weight, physical activity, cigarette smoking, family history of colorectal cancer, and aspirin use. In women, information on menopausal status and postmenopausal hormone use was also obtained at baseline and in the subsequent follow-up questionnaires.

Statistical analyses

We excluded participants with a history of cancer (except non-melanoma skin cancer) and ulcerative colitis at baseline. In addition, we excluded participants with unreasonable baseline total energy intake (<600 or >3,500 kcal/day for women and <800 or >4,200 kcal/day for men). We calculated person-time for each participant from the date of baseline questionnaire return to the date of death, loss to follow-up, colorectal cancer diagnosis, or the end of follow-up (1 June 2006 for NHS and 1 January 2006 for HPFS), whichever came first. We used a Cox proportional hazards model [34] to calculate relative risks (RRs) and 95% confidence intervals (CIs) and adjusted simultaneously for age (in months) and year of questionnaire return using SAS PROC PHREG [35]. We observed no violation of the proportional hazard assumption based on the likelihood ratio test that compared the model with and without the interaction terms between zinc or heme iron intakes and age or follow-up time. All statistical analyses were two-sided, and a p value less than 0.05 was considered statistically significant. We conducted all analyses using the SAS software (SAS Institute, Inc., Version 9, Cary, NC).

For each cohort, energy-adjusted dietary zinc and heme iron intakes were categorized into quintiles based on the distribution in that study population. We used median values of these categories and entered these values as continuous variables into the model to conduct trend tests. In addition to age and year of questionnaire return, multivariable analyses also adjusted for established or potential risk factors for colorectal cancer (see Table 2 for the categorization of confounding variables). Given that vitamin C and phytate intakes might influence zinc and iron absorption, we conducted sensitivity analyses where we further adjusted for vitamin C intake (quintiles) and phytate intake (quintiles). Results were essentially unchanged. We modeled all covariates as time-varying variables to take into account potential changes over follow-up. To represent better long-term dietary intake [36] and to minimize the impact of random measurement errors in the dietary assessment, we analyzed cumulative average nutrient intakes. In addition to dietary zinc and heme iron intake, we further analyzed total zinc intake (quintiles), zinc from supplements (non-user, user), total iron (quintiles), dietary iron (quintiles), and iron from supplements (non-user, user). Given that there were very few cases (<20) in the category exceeding the Tolerable Upper Intake Levels (UL, 40 mg/day for zinc and 45 mg/day for iron), we used approximately half of the UL (i.e., ≥25 mg/day) to further evaluate associations with relatively high zinc or iron supplement use to detect any potential harmful effect.

We investigated whether associations differed between women and men using the Q statistic [37, 38], which follows an approximate χ^2 distribution with 1 degree of freedom. We pooled the sex-specific results using a fixed-effects model [38] .

For colorectal cancer and each subsite, we further evaluated whether the associations with intakes of dietary zinc and heme iron differed by alcohol consumption (non-drinkers, >0 to <10, ≥10 g/day), body mass index (<25, ≥25 kg/m^2), physical activity (low, high), or zinc or iron supplement use (zero, low, high). For these analyses, we categorized dietary zinc and heme iron intakes into tertiles to have a reasonable number of cases in each stratum. In addition, we further examined the potential interactions between zinc and heme iron intakes because some experimental studies [39, 40] have suggested that these two micronutrients may compete with each other due to their similar chemical properties. We used a Wald test to examine whether the beta coefficients for the cross-product terms between each of these variables and dietary zinc or heme iron intake were statistically significant.

Results

In the NHS, 1,079 incident colorectal cancer cases were documented among 69,345 women during 1,434,574

persons-years from 1984 to 2006. In HPFS, 1,035 colorectal cancer cases were identified among 45,716 men during 804,210 person-years from 1986 to 2006. Intake levels of dietary zinc and heme iron were slightly higher in men than in women. In both men and women, participants in the highest quintile of intake were generally comparable with the participants in the lowest quintile of intake of dietary zinc and heme iron with respect to age, body mass index, family history of colorectal cancer, regular aspirin use, and total vitamin D intake. Participants with low dietary zinc intake drank more alcohol but consumed less total calcium and total folate than participants with higher intakes. In contrast, men with low heme iron intake consumed more total calcium and total folate, smoked less, and were more likely to be physically active (Table 1). Use of supplements containing zinc (including both multivitamin and zinc-specific supplements) increased over study time in both men and women. For example, the proportion of individuals using of zinc-containing supplements increased from 17% in 1990 to 52% in 2004 in women and increased from 18% to 44% in men for the same time frame. Use of iron-containing supplements increased slightly from 1990 to 2004 in both women (from 20 to 26%) and men (from 14 to 20%).

Table 2 shows the associations of dietary zinc intake and total zinc intake with risk of colorectal cancer. Because the age-adjusted results were similar to the multivariable-adjusted results, we only present the multivariable-adjusted results. A suggestive inverse association with dietary zinc intake was observed in women. Comparing the highest to the lowest quintile of dietary zinc intake, multivariable RRs (95% CIs) were 0.75 (0.59, 0.95; p trend = 0.02) for colorectal cancer, 0.84 (0.64, 1.09; p trend = 0.18) for colon cancer, and 0.51 (0.29, 0.86; p trend = 0.01) for rectal cancer. Results for proximal and distal colon cancers were non-significant (multivariable RR = 0.80; 95% CI: 0.57, 1.12 for proximal colon cancer and RR = 0.84; 95% CI: 0.54, 1.30 for distal colon cancer for the extreme quintile comparison). Associations with total zinc intake were non-significant and weaker than those observed for dietary zinc intake (Table 2). For men, results of dietary and total zinc intakes were largely null (Table 2). The pooled multivariable RRs (95% CIs) comparing the highest to lowest quintile of dietary zinc intake for women and men combined were 0.86 (0.73, 1.02) for colorectal cancer, 0.92 (0.76, 1.11) for colon cancer, and 0.68 (0.47, 0.99) for rectal cancer (Table 2). Again, the significant inverse association between dietary zinc intake and risk of rectal cancer was

Table 1 Age-standardized characteristics by quintile (Q) of dietary zinc and heme iron intake in the Nurses' Health Study and Health Professionals Follow-up Study in 1990

Characteristics	Women (N = 69,345)						Men (N = 42,373)					
	Dietary zinc intake			Heme iron intake			Dietary zinc intake			Heme iron intake		
	$Q1$	$Q3$	$Q5$	$Q1$	$Q3$	$Q5$	$Q1$	$Q3$	$Q5$	$Q1$	$Q3$	$Q5$
Intake levels, medians (mg/day)	8.5	10.8	14.1	0.7	1.1	1.6	9.9	12.4	16.0	0.8	1.2	1.8
Age (years, mean)	57.2	56.5	55.4	57.6	56.4	56.2	60.3	59.3	59.3	60.1	59.5	59.1
Body mass index (kg/m^2, mean)[a]	24.2	25.4	26.2	24.3	25.4	26.3	24.8	25.6	26.0	24.7	25.5	26.3
Physical activity (MET-h/week, mean)[b]	14.2	15.2	14.8	16.5	14.9	13.2	25.6	24.7	22.9	28.7	24.3	21.0
History of colorectal cancer in a parent or sibling (%)	12.2	12.6	11.7	12.3	12.5	11.9	12.5	12.6	12.3	12.0	10.7	10.8
Former or current smokers (%)	57	56	54	52	56	57	51	51	50	46	52	56
Alcohol consumption (g/day)	8.4	6.1	4.3	5.8	6.3	5.7	14.1	10.4	7.9	10.0	11.5	9.9
Regular aspirin use (%)[c]	37	41	42	38	41	41	39	41	41	40	38	38
Multivitamin use (%)	36	38	40	42	37	35	43	42	43	44	38	35
Total vitamin D intake (IU/day)[d]	295	332	362	358	327	314	393	421	447	455	412	389
Total calcium intake (mg/day)[d]	873	996	1,059	1,087	972	880	807	923	975	1,043	888	787
Total folate intake (μg/day)[d]	375	401	449	436	404	381	471	482	548	543	482	453
Beef, pork, or lamb as a main dish (servings/week)	1.4	2.1	2.6	1.1	2.0	3.1	1.0	1.8	2.6	0.7	1.8	3.0
Processed meat intake (servings/week)	0.9	1.0	0.9	0.7	0.9	1.1	1.0	1.2	1.2	0.7	1.2	1.4

[a] Body mass index was calculated as weight in kilograms divided by the square of height in meters

[b] MET denotes metabolic equivalent. MET-hours = sum of the average time/week spent in each activity × MET value of each activity. One MET, the energy spent sitting quietly, is equal to 3.5 mL of oxygen uptake per kilograms of body weight per minute for a 70 kg adult

[c] Regular aspirin user was defined as consumption of 2 or more 325-mg tablets per week. Non-regular user was defined otherwise

[d] Nutrient values for vitamin D, folate, and calcium were energy-adjusted intake

Table 2 Multivariable relative risks (RRs, 95% CIs)[a] of colorectal cancer and subsites according to quintiles (*Q*) of zinc intake[b]

Nutrients	*Q*1	*Q*2	*Q*3	*Q*4	*Q*5	*p* for heterogeneity, highest quintile	*p* for trend
Dietary zinc							
Colorectal cancer							
Women ($N = 1{,}079$)	1.00 (Reference)	0.90 (0.74,1.09)	0.99 (0.81,1.21)	0.85 (0.68,1.05)	0.75 (0.59,0.95)		0.02
Men ($N = 1{,}035$)	1.00 (Reference)	1.20 (0.98,1.47)	1.19 (0.95,1.47)	1.07 (0.84,1.35)	0.99 (0.77,1.26)		0.44
Combined[c]	1.00 (Reference)	1.03 (0.90,1.18)	1.08 (0.93,1.25)	0.94 (0.81,1.10)	0.86 (0.73,1.02)	0.11	0.18
Colon cancer							
Women ($N = 837$)	1.00 (Reference)	0.92 (0.74,1.15)	0.97 (0.77,1.22)	0.90 (0.70,1.15)	0.84 (0.64,1.09)		0.18
Men ($N = 815$)	1.00 (Reference)	1.23 (0.98,1.53)	1.25 (0.98,1.59)	1.08 (0.83,1.39)	1.02 (0.77,1.34)		0.58
Combined[c]	1.00 (Reference)	1.07 (0.91,1.25)	1.09(0.93,1.29)	1.03 (0.83,1.27)	0.92 (0.76,1.11)	0.31	0.20
Rectal cancer							
Women ($N = 242$)	1.00 (Reference)	0.86 (0.57,1.28)	1.08 (0.72,1.63)	0.72 (0.45,1.14)	0.51 (0.29,0.86)		0.01
Men ($N = 220$)	1.00 (Reference)	1.16 (0.76,1.78)	1.04 (0.66,1.65)	1.08 (0.66,1.76)	0.92 (0.54,1.57)		0.61
Combined[c]	1.00 (Reference)	0.99 (0.74,1.32)	1.06 (0.78,1.44)	0.87 (0.62,1.22)	0.68 (0.47,0.99)	0.12	0.28
Total zinc							
Colorectal cancer							
Women ($N = 1{,}079$)	1.00 (Reference)	0.96 (0.80,1.17)	0.89 (0.72,1.09)	0.88 (0.71,1.09)	0.89 (0.71,1.13)		0.31
Men ($N = 1{,}035$)	1.00 (Reference)	1.29 (1.05,1.57)	1.08 (0.87,1.35)	1.07 (0.85,1.35)	1.04 (0.82,1.31)		0.45
Combined[c]	1.00 (Reference)	1.11 (0.97,1.28)	0.97 (0.84,1.13)	0.96 (0.82,1.12)	0.96 (0.82,1.14)	0.36	0.76
Colon cancer							
Women ($N = 837$)	1.00 (Reference)	0.96 (0.77,1.20)	0.91 (0.73,1.15)	0.92 (0.72,1.17)	0.96 (0.74,1.24)		0.99
Men ($N = 815$)	1.00 (Reference)	1.27 (1.01,1.60)	1.09 (0.85,1.39)	1.14 (0.85,1.46)	1.07 (0.82,1.40)		0.70
Combined[c]	1.00 (Reference)	1.10 (0.94,1.29)	0.99 (0.84,1.17)	1.12 (0.98,1.28)	1.01 (0.84,1.22)	0.57	0.51
Rectal cancer							
Women ($N = 242$)	1.00 (Reference)	0.96 (0.65,1.41)	0.80 (0.52,1.23)	0.77 (0.48,1.22)	0.71 (0.43,1.18)		0.04
Men ($N = 220$)	1.00 (Reference)	1.34 (0.88,2.03)	1.08 (0.67,1.71)	0.84 (0.50,1.39)	0.93 (0.56,1.54)		0.42
Combined[c]	1.00 (Reference)	1.12 (0.84,1.48)	0.92 (0.67,1.26)	0.80 (0.57,1.13)	0.81 (0.57,1.16)	0.35	0.22

[a] Multivariable relative risks were adjusted for age (in months), smoking before age 30 (0, 1–4, 5–10, or >10 pack-years), history of colorectal cancer in a parent or sibling (yes or no), history of endoscopy (yes or no), regular aspirin use (yes, no), body mass index (<25, 25 to <30, ≥30 kg/m^2), physical activity (low, medium, high), alcohol consumption (0 to <5, 5 to <10, 10 to <15, or ≥15 g/day), energy-adjusted total folate intake (quintiles), total vitamin D intake (quintiles), and total calcium intake (quintiles). Postmenopausal hormone use (premenopausal, never, past, or current user) was adjusted for women only. Heme iron intake was adjusted for when modeling the associations for total zinc intake. When modeling the associations for dietary zinc intake, we further adjusted for zinc intake from supplement use and heme iron intake

[b] The cutpoints of the quintiles for total zinc intake in women were <10.0, 10.0–11.5, 11.6–13.5, 13.6–18.3, and >18.3 mg/day; the corresponding cutpoints for dietary zinc were <9.3, 9.3–10.3, 10.4–11.1, 11.2–12.3, and >12.3 mg/day. For men, the cutpoints of the quintiles for total zinc intake were <11.4, 11.4–13.2, 13.3–15.9, 16.0–24.0, and >24.0 mg/day; the cutpoints for dietary zinc intake were <10.8, 10.8–11.8, 11.9–12.9, 13.0–14.5, and >14.5 mg/day

[c] A fixed-effects model was used to calculate the pooled estimates

mainly driven by data in women, although the difference in results between men and women was not statistically significant. Further, zinc supplement use (user vs. non-user; data not shown) or relatively high dose of zinc supplement use (≥25 mg/day) was not significantly associated with risk of colorectal cancer in either men or women (data not shown).

Table 3 shows the associations of heme iron intake or total iron intake with risk of colorectal cancer. A non-significant positive association between heme iron intake and risk of colorectal cancer was observed in women. Comparing the highest quintile to the lowest quintile of intake, the multivariable RRs (95% CIs) were 1.21 (0.96, 1.52; p trend = 0.10) for colorectal cancer, 1.13 (0.87, 1.47; p trend = 0.30) for colon cancer, and 1.50 (0.90, 2.49; p trend = 0.17) for rectal cancer (Table 3). For the same comparison, weaker non-significant positive associations were observed for total iron intake. For men, both heme iron and total iron intakes were not associated with risk of colorectal cancer or any subsite. Non-significant between-studies heterogeneity was observed, and the pooled RRs for women and men combined were largely

Table 3 Multivariable relative risks (RRs, 95% CIs)[a] of colorectal cancer and subsites according to quintiles (*Q*) of iron intake

Nutrients	*Q*1	*Q*2	*Q*3	*Q*4	*Q*5	*p* for heterogeneity, highest quintile	*p* for trend
Heme iron							
Women ($N = 1{,}079$)	1.00 (Reference)	1.08 (0.89,1.30)	0.99 (0.81,1.22)	1.14 (0.92,1.41)	1.21 (0.96,1.52)		0.10
Men ($N = 1{,}035$)	1.00 (Reference)	0.99 (0.81,1.21)	1.03 (0.84,1.27)	0.95 (0.76,1.18)	0.98 (0.77,1.26)		0.80
Combined[c]	1.00 (Reference)	1.04 (0.91,1.19)	1.01 (0.87,1.17)	1.05 (0.89,1.21)	1.10 (0.93,1.30)	0.22	0.51
Colon cancer							
Women ($N = 837$)	1.00 (Reference)	1.01 (0.81,1.26)	0.97 (0.78,1.23)	1.09 (0.87,1.39)	1.13 (0.87,1.47)		0.30
Men ($N = 815$)	1.00 (Reference)	0.94 (0.75,1.18)	1.05 (0.83,1.31)	0.94 (0.73,1.21)	0.99 (0.75,1.31)		0.97
Combined	1.00 (Reference)	0.95 (0.78,1.16)	1.01 (0.86,1.20)	1.02 (0.85,1.21)	1.06 (0.88,1.29)	0.57	0.51
Rectal cancer							
Women ($N = 242$)	1.00 (Reference)	1.33 (0.88,1.99)	1.03 (0.66,1.61)	1.32 (0.83,2.05)	1.50 (0.90,2.49)		0.17
Men ($N = 208$)	1.00 (Reference)	1.14 (0.75,1.73)	0.99 (0.63,1.55)	0.93 (0.57,1.53)	0.93 (0.53,1.62)		0.62
Combined[c]	1.00 (Reference)	1.23 (0.92,1.65)	1.01 (0.73,1.39)	1.13 (0.81,1.57)	1.20 (0.83,1.75)	0.16	0.73
Total iron							
Colorectal cancer							
Women ($N = 1{,}079$)	1.00 (Reference)	1.05 (0.86,1.27)	1.04 (0.84,1.29)	1.02 (0.81,1.27)	1.11 (0.88,1.41)		0.44
Men ($N = 1{,}035$)	1.00 (Reference)	1.07 (0.88,1.31)	0.98 (0.79,1.22)	0.96 (0.76,1.21)	1.08 (0.84,1.38)		0.61
Combined[c]	1.00 (Reference)	1.06 (0.92,1.22)	1.01 (0.86,1.18)	0.99 (0.85,1.16)	1.09 (0.93,1.30)	0.84	0.37
Colon cancer							
Women ($N = 837$)	1.00 (Reference)	1.09 (0.87,1.36)	1.08 (0.85,1.37)	1.04 (0.80,1.34)	1.03 (0.79,1.36)		0.87
Men ($N = 815$)	1.00 (Reference)	1.17 (0.93,1.47)	1.03 (0.81,1.32)	1.04 (0.80,1.35)	1.14 (0.85,1.51)		0.59
Combined[c]	1.00 (Reference)	1.13 (0.96,1.32)	1.06 (0.89,1.25)	1.04 (0.87,1.25)	1.08 (0.89,1.32)	0.65	0.75
Rectal cancer							
Women ($N = 242$)	1.00 (Reference)	0.93 (0.63,1.37)	0.91 (0.59,1.41)	0.98 (0.61,1.56)	1.44 (0.90,2.33)		0.05
Men ($N = 208$)	1.00 (Reference)	0.81 (0.53,1.25)	0.86 (0.55,1.36)	0.74 (0.45,1.22)	0.94 (0.55,1.60)		0.91
Combined[c]	1.00 (Reference)	0.87 (0.66,1.17)	0.89 (0.65,1.22)	0.86 (0.61,1.21)	1.19 (0.83,1.70)	0.24	0.20

[a] Multivariable relative risks were adjusted for age (in months), smoking before age 30 (0, 1–4, 5–10, or >10 pack-years), history of colorectal cancer in a parent or sibling (yes, no), history of endoscopy (yes, no), regular aspirin use (yes, no), body mass index (<25, 25 to <30, ≥30 kg/m^2), physical activity (low, medium, high), alcohol consumption (0 to <5, 5 to <10, 10 to <15, or ≥15 g/day), energy-adjusted total folate (quintiles), total vitamin D intake (quintiles), and total calcium intake (quintiles). Postmenopausal hormone use (premenopausal, never, past, or current user) was adjusted for women only. When modeling the association for total and heme iron intakes, we further adjusted for dietary zinc intake (quintiles)

[b] The cutpoints of the quintiles for total iron intake in women were <10.9, 10.9–12.7, 12.8–16.0, 16.1–22.7, and >22.7 mg/day; the corresponding cutpoints for heme iron were <0.78, 0.78–0.95, 0.96–1.10, 1.11–1.30, and >1.30 mg/day. For men, the cutpoints of the quintiles for total zinc intake were <12.6, 12.6–14.6, 14.7–17.7, 17.8–24.6, and >24.6 mg/day; the cutpoints for heme iron intake were <0.90, 0.90–1.12, 1.13–1.32, 1.33–1.60, and >1.60 mg/day

[c] A fixed-effects model was used to calculate the pooled estimates

around the null value 1 (Table 3). In addition, intakes of heme iron and total iron were not associated with risk of either proximal colon or distal colon cancer (data not shown). Moreover, dietary iron intake and iron supplement use were not significantly associated with risk of colorectal cancer overall or with any of the subsites (data not shown). We did not adjust for red meat consumption in our main analysis because red meat is a major contributor to dietary zinc and heme iron in our study. However, in secondary analysis, to investigate whether the associations with dietary zinc and heme iron are independent of the constitutes in red meat, we further adjusted for red meat and processed meat, both modeled as quintiles. The results were essentially unchanged (data not shown). In addition, results were essentially the same after including dietary zinc and heme iron intake in separate models. Consistent with the results for heme iron intake observed in women, suggestive positive associations were observed for women with relatively high iron supplemental use, especially for rectal cancer (i.e., for ≥25 mg/day vs. non-user, RR = 2.54, 95% CI: 1.43, 4.50; $n = 15$ cases). For the same comparison, the associations were not statistically significant in men (RR = 0.41, 95% CI: 0.13, 1.32; $n = 3$ cases). For both zinc and iron intakes, comparing the highest with the

lowest decile, the associations for colorectal cancer and subsites were similar in magnitude as those presented in Tables 2 and 3 for the quintile analyses.

Because a previous study reported that the inverse association between zinc intake and colorectal cancer risk was markedly stronger among alcohol drinkers [10], we specifically evaluated the interaction between dietary zinc intake and alcohol consumption (Table 4). We also examined the joint associations of dietary zinc (or heme iron) intake with supplemental zinc (or iron). For zinc, we found no statistically significant interactions, though the inverse association of dietary zinc intake with risk of rectal cancer was observed mainly among non-drinkers (Table 4). Zinc supplement use did not significantly modify the association between dietary zinc intake and colorectal cancer risk. Associations of heme iron intake with colorectal cancer risk were not significantly modified by alcohol consumption or iron supplement use; however, among alcohol drinkers ($\geq$10 g/day), a small elevated risk of colorectal or colon cancer was observed for heme iron intake but the interaction test was not significant. Suggestive stronger positive associations of rectal cancer were observed for women (RR = 2.37, 95% CI: 1.33, 4.21) who were in the highest tertile of heme iron intake with high iron supplement use but not in men (RR = 0.28, 95% CI: 0.07, 1.21) but these results might be due to chance because of the smaller number of cases in these strata (Table 4). Further, associations were not significantly modified by postmenopausal hormone use (never vs. ever; in women only), menopausal status (premenopausal vs. postmenopausal in women only), body mass index ($<$25 vs. $\geq$25 kg/m^2), or physical activity (low vs. high) (data not shown). Further, no significant interaction was observed between zinc and heme iron intakes (data not shown).

Discussion

In these large prospective cohort studies of men and women, our results did not support a strong role of zinc or iron, whether from food sources only or including supplements, in colorectal carcinogenesis. However, a modest inverse association of dietary zinc intake with risk of rectal cancer in women cannot be excluded. In addition, these associations were not significantly modified by alcohol consumption or several other colorectal cancer risk factors.

Several mechanisms have been proposed to support a role of zinc in colorectal carcinogenesis. In addition to the potential effect of zinc on insulin, animal models have suggested a role of zinc to protect against chemically induced colonic proneoplastic progression [41]. Further, zinc has been shown in experimental studies to play an important role in antioxidant defense system, DNA synthesis, and immune function [42]. Despite the experimental evidence, only two epidemiological studies to date have examined the association between zinc intake and colon cancer risk, and both were in women. The Swedish study [10] reported a weak non-significant lower risk of colon cancer (highest vs. lowest quintile, RR = 0.90, 95% CI: 0.65, 1.25, p trend = 0.71). However, they had a relatively limited range in intake to examine this association ($\geq$11.1 vs. $<$9.0 mg/day). In contrast, the study from Iowa [11] had a wider range across quintiles of dietary zinc intake ($\geq$17.6 vs. $\leq$8.5 mg/day). Although associations with dietary zinc intake for colon cancer were not reported, they did observe a significantly lower risk of proximal colon cancer (highest vs. lowest quintile, RR = 0.38, 95% CI: 0.17, 0.74, p trend = 0.01) and a non-significant lower risk of distal colon cancer (highest vs. lowest quintile, RR = 0.58, 95% CI: 0.26, 1.30, p trend = 0.15). We had a reasonable contrast in zinc intake ($\geq$13.4 vs. $\leq$8.7 mg/day), but we observed a non-significant lower risk for colon cancer (highest vs. lowest quintile, RR = 0.84; 95% CI: 0.64, 1.09) with no difference by colon cancer subsite. Although results were not reported in these earlier studies for rectal cancer, we observed a significant lower risk for rectal cancer (highest vs. lowest quintile, RR = 0.51, 95% CI: 0.29, 0.86) in women. Reasons for the inconsistent results observed in these few observational studies are unclear, and the difference in ranges across quintiles of dietary zinc intake might partly explain the inconsistencies in the results. In addition, food sources of zinc differ between the United States and Sweden. In the US diet, the major food sources of dietary zinc are meat, dairy foods, and fortified cereals, while the primary sources in Sweden are mainly grains and red meat [43]. Zinc from meat is more bioavailable than zinc from plant sources [44], and plant sources of zinc such as whole grains and beans contain phytates, which can inhibit zinc absorption [44]. However, further adjustment for phytate intake did not change the results in our study. Nonetheless, future studies in different populations with a wide range of zinc intake should help elucidate the effect of zinc intake on colorectal carcinogenesis.

Zinc supplement use was not associated with colorectal cancer risk in our study, which was consistent with the study from Iowa [10]. The mean dietary zinc intake in our population was higher than the US recommended dietary allowance, which is 8 mg/day for women and 11 mg/day for men, and relatively few participants had high zinc supplement intake (i.e., $\geq$25 mg/day) or intakes less than 8 mg/day, which limited our ability to examine associations with very high or low intake. Taken together, no firm conclusion regarding zinc intake and colorectal cancer incidence can be drawn based on the current limited epidemiological evidence.

Table 4 Multivariable relative risks (RRs, 95% CIs) of colorectal cancer and subsites according to tertiles of dietary zinc and heme iron intake, stratified by alcohol consumption or zinc/iron supplement use

	Tertiles of intakes[a]						$p_{interaction}$
	T1		T2		T3		
	No. of cases	RR (95% CI)	No. of cases	RR (95% CI)	No. of cases	RR (95% CI)	
Dietary zinc							
Colorectal cancer							
Alcohol consumption							
Non-drinker	124	1.00 (Reference)	144	1.20 (0.94, 1.54)	145	0.95 (0.73, 1.23)	0.34
>0 to <10 (g/day)	334	1.30 (1.06, 1.60)	360	1.26 (1.02, 1.56)	314	1.12 (0.89, 1.40)	
10+ (g/day)	289	1.40 (1.12, 1.75)	239	1.42 (1.12, 1.80)	165	1.25 (0.96, 1.62)	
Zinc supplement use[b]							
Zero	433	1.00 (Reference)	394	1.00 (0.85, 1.15)	338	0.87 (0.72, 1.03)	0.40
Low	219	0.98 (0.82, 1.17)	248	1.07 (0.90, 1.28)	200	0.91 (0.73, 1.11)	
High	95	1.02 (0.81, 1.28)	101	0.99 (0.79, 1.26)	86	0.92 (0.70, 1.20)	
Colon cancer							
Alcohol consumption							
Non-drinker	87	1.00 (Reference)	101	1.34 (1.00, 1.78)	121	1.13 (0.84, 1.51)	0.89
>0 to <10 (g/day)	268	1.51 (1.18, 1.92)	281	1.38 (1.07, 1.78)	256	1.29 (0.99, 1.67)	
10+ (g/day)	225	1.55 (1.19, 2.01)	191	1.53 (1.16, 2.02)	149	1.43 (1.06, 1.94)	
Zinc supplement use[b]							
Zero	328	1.00 (Reference)	297	0.98 (0.82, 1.16)	271	0.90 (0.75, 1.09)	0.48
Low	174	1.00 (0.82, 1.22)	193	1.09 (0.89, 1.33)	163	0.94 (0.75, 1.18)	
High	78	1.09 (0.83, 1.42)	78	1.01 (0.76, 1.33)	70	0.93 (0.69, 1.26)	
Rectal cancer							
Alcohol consumption							
Non-drinker	37	1.00 (Reference)	33	0.91 (0.57, 1.48)	26	0.59 (0.34, 1.02)	0.73
>0 to <10 (g/day)	66	0.81 (0.53, 1.24)	85	0.96 (0.64, 1.47)	62	0.73 (0.46, 1.15)	
10+ (g/day)	64	1.01 (0.65, 1.55)	57	1.17 (0.74, 1.83)	32	0.84 (0.49, 1.43)	
Zinc supplement use[b]							
Zero	105	1.00 (Reference)	97	1.04 (0.77, 1.40)	67	0.70 (0.49, 1.01)	0.67
Low	45	0.91 (0.62, 1.32)	55	1.06 (0.73, 1.55)	37	0.75 (0.48, 1.17)	
High	17	0.81 (0.47, 1.40)	23	1.11 (0.66, 1.82)	16	0.91 (0.49, 1.70)	
Heme iron							
Colorectal cancer							
Alcohol consumption							
Non-drinker	148	1.00 (Reference)	124	0.98 (0.77, 1.26)	141	1.17 (0.91, 1.49)	0.78
>0 to <10 (g/day)	307	1.10 (0.91, 1.35)	365	1.25 (1.02, 1.52)	336	1.27 (1.03, 1.56)	
10+ (g/day)	220	1.33 (1.07, 1.66)	270	1.37 (1.10, 1.70)	203	1.25 (0.98, 1.59)	
Iron supplement use[b]							
Zero	361	1.00 (Reference)	437	1.07 (0.93, 1.25)	412	1.06 (0.90, 1.24)	0.45
Low	172	1.01 (0.84, 1.23)	199	1.16 (0.96, 1.39)	164	1.15 (0.94, 1.41)	
High	142	1.20 (0.97, 1.47)[c]	123	1.08 (0.89, 1.30)	104	1.20 (0.95, 1.53)	
Colon cancer							
Alcohol consumption							
Non-drinker	109	1.00 (Reference)	99	1.07 (0.81, 1.42)	109	1.20 (0.90, 1.59)	0.63
>0 to <10 (g/day)	244	1.18 (0.94, 1.49)	287	1.35 (1.07, 1.69)	264	1.35 (1.06, 1.72)	
10+ (g/day)	174	1.42 (1.10, 1.83)	204	1.40 (1.09, 1.80)	162	1.32 (1.01, 1.72)	
Iron supplement use[b]							
Zero	280	1.00 (Reference)	335	1.07 (0.91, 1.27)	328	1.08 (0.90, 1.30)	0.86

Table 4 continued

	Tertiles of intakes[a]						$p_{interaction}$
	T1		T2		T3		
	No. of cases	RR (95% CI)	No. of cases	RR (95% CI)	No. of cases	RR (95% CI)	
Low	142	1.05 (0.85, 1.30)	159	1.17 (0.94, 1.46)	127	0.96 (0.76, 1.21)	
High	105	1.12 (0.88, 1.42)	96	1.00 (0.76, 1.32)	80	1.07 (0.91, 1.26)	
Rectal cancer							
Alcohol consumption							
Non-drinker	39	1.00 (Reference)	25	0.74 (0.44, 1.25)	32	1.06 (0.64, 1.77)	0.71
>0 to <10 (g/day)	63	0.85 (0.56, 1.29)	78	0.94 (0.63, 1.42)	72	1.02 (0.66, 1.58)	
10+ (g/day)	46	1.07 (0.62, 1.85)	66	1.26 (0.79, 2.00)	41	0.97 (0.60, 1.58)	
Iron supplement use[b]							
Zero	81	1.00 (Reference)	102	1.07 (0.79, 1.46)	84	0.95 (0.67, 1.36)	0.67
Low	32	0.87 (0.56, 1.35)	38	1.13 (0.75, 1.70)	37	1.21 (0.78, 1.86)	
High	37	1.55 (1.04, 2.37)	27	1.11 (0.69, 1.80)	24	1.78 (1.04, 3.04)[c]	

Multivariable relative risks were adjusted for age (in months), smoking before age 30 (0, 1–4, 5–10, or >10 pack-years), history of colorectal cancer in a parent or sibling (yes, no), history of endoscopy (yes, no), regular aspirin use (yes, no), body mass index (<25, 25 to <30, ≥30 kg/m^2), physical activity (low, medium, high), energy-adjusted total folate (quintiles), total vitamin D intake quintiles), and total calcium intake (quintiles). Postmenopausal hormone use (premenopausal, never, past, or current user) was adjusted for women only. Iron supplement use analyses were further adjusted for alcohol consumption (0 to <5, 5 to <10, 10 to <15, or ≥15 g/day)

[a] The cutpoints of the tertiles for dietary zinc intake were <10.1, 10.1 to 11.5, and >11.5 mg/day in women and were <11.5, 11.5 to 13.4, and >13.4 mg/day in men. The cutpoints of the tertiles for heme iron intake were <0.91, 0.91 to 1.16, >1.16 mg/day in women and were <1.06, 1.06 to 1.40, >1.40 mg/day in men

[b] Median values among supplemental users were used to define the low and high group. The median values of zinc supplemental use were 10 mg/day for women and 15 mg/day for men; the corresponding values for iron supplemental use were 7 mg/day for women and 11 mg/day for men

[c] A statistically significant heterogeneity was observed. For colorectal cancer, the RRs were 0.97 (0.73, 1.29) for women ($n = 74$ cases) and 1.52 (1.12, 2.06) for men ($n = 68$ cases); for rectal cancer, the RRs were 2.37 (1.33, 4.21) for women ($n = 22$ cases) and 0.28 (0.07, 1.21) for men ($n = 2$ cases)

In contrast to zinc, heme iron has been hypothesized to be associated with an increased risk of colorectal cancer. To date, five cohort studies have examined this hypothesis [10, 11, 14, 18, 25]. In the Iowa Women's Health Study, heme iron intake was positively associated with risk of proximal (for ≥2.05 vs. ≤0.76 mg/day, RR = 2.18, 95% CI: 1.24, 3.86, p trend = 0.01) but not with risk of distal colon cancer (RR = 0.90, 95% CI: 0.45, 1.81, p trend = 0.77) [10]. The association of proximal colon cancer with heme iron intake was particularly strong among women who consumed at least 10 grams of alcohol per day (RR = 7.20, 95% CI: 1.33, 38.91, p trend = 0.03). Similarly, a study from Sweden reported a positive association with heme iron intake among women drinking at least 20 grams of alcohol per week (≥2.06 vs. ≤0.67 mg/day, RR = 2.29, 95% CI: 1.25, 4.21, p trend = 0.007) [11]. In contrast, in a study of women and men from the Netherlands, no association between heme iron intake and colon cancer risk was observed, but there was a suggestion of a positive association among women who drank at least 5 grams of alcohol per day (tertile 3 vs. tertile 1, RR = 1.50, 95% CI: 0.95, 2.36) [18]. The NIH-AARP study of both men and women suggested a positive association between heme iron intake and colorectal cancer risk (highest vs. lowest quintile, RR = 1.13, 95% CI: 0.99–1.29, p trend = 0.02) although whether the association was modified by alcohol consumption was not reported [25]. A Canadian study of women showed null results for heme iron intake (>2.95 vs. <1.58 mg/day; RR = 0.99, 95% CI: 0.70, 1.40), and the associations did not differ by the amount of alcohol consumed [14]. A recent meta-analysis of these five studies suggested a modest positive association between heme iron intake and colon cancer risk (highest vs. lowest quintile, RR = 1.18, 95% CI: 1.06, 1.32) [20]. Our findings of non-significant associations for heme iron intake are somewhat consistent with the early two studies [14, 18] but not the others [10, 11, 25]. The narrower comparison across quintiles may partly explain the non-significant positive associations we observed. The stronger associations observed among alcohol drinkers in two of the previous studies [10, 11] may also be due to chance because of the relatively small number of cases in the highest quintile of heme iron and highest alcohol consumption categories (<50 cases). Future studies in populations with a wide range of heme iron intakes should help confirm these findings or identify any potential threshold effect of heme

iron. Notably, the significant positive associations between relatively high iron supplemental use (i.e., ≥25 mg/day vs. none) and rectal cancer risk in women were unexpected. Given that this subgroup finding is based on a relatively small number of cases, the results might be due to chance. On the other hand, we cannot rule out the possibility that iron intake may influence the rectal cancer.

Limitations of our study need consideration. Our study had relatively narrow quintile intake ranges of zinc and heme iron, which limited our ability to evaluate associations with these micronutrients for high or low intake ranges (i.e., zinc deficiency). We lacked information on cooking methods, which might impact the amount of heme iron because heme iron can be partially converted to non-heme iron depending on the type and extent of the cooking method [45]. The suggestive inverse associations between dietary zinc intake and rectal cancer risk, particularly in women, might be due to chance because of the relatively small number of rectal cancer cases. We cannot exclude the possibility that residual confounding may explain the suggestive inverse associations observed with dietary zinc intakes although we adjusted for multiple lifestyle and dietary confounders. In addition, our study population consisted of mainly Caucasians, and the results may not be generalizable to other ethnic groups with different dietary patterns. Our study has several strengths, including its large size, prospective design, long follow-up time, and the comprehensive updated measurements of zinc and iron intakes.

In summary, although a beneficial effect of zinc and a detrimental effect of heme iron on colorectal carcinogenesis have been suggested by some experimental evidence, our study did not support a strong role of zinc or heme iron intake in colorectal cancer risk. However, a potential modest inverse association between dietary zinc intake and rectal cancer risk in women requires further study.

Acknowledgments We are grateful for all participants in the Nurses' Health Study and Health Professionals Follow-up Study for their continuous contribution and support. This work was supported by the NIH grants CA87969 and CA55075.

References

1. World Cancer Research Fund (2007) American Institute for Cancer Research expert panel. Food, nutrition and the prevention of cancer: a global perspective. American Institute for Cancer Research, Washington
2. Ma J, Giovannucci E, Pollak M et al (2004) A prospective study of plasma C-peptide and colorectal cancer risk in men. J Natl Cancer Inst 96:546–553
3. Pisani P (2008) Hyper-insulinaemia and cancer, meta-analyses of epidemiological studies. Arch Physiol Biochem 114:63–70
4. Giovannucci E, Harlan DM, Archer MC et al (2010) Diabetes and cancer: a consensus report. CA Cancer J Clin 60:207–221
5. Giovannucci E (2007) Metabolic syndrome, hyperinsulinemia, and colon cancer: a review. Am J Clin Nutr 86:s836–s842
6. Scott DA (1934) Crystalline insulin. Biochem J 28:1592–1602
7. Simon SF, Taylor CG (2001) Dietary zinc supplementation attenuates hyperglycemia in db/db mice. Exp Biol Med (Maywood) 226:43–51
8. Marreiro DN, Geloneze B, Tambascia MA, Lerario AC, Halpern A, Cozzolino SM (2006) Effect of zinc supplementation on serum leptin levels and insulin resistance of obese women. Biol Trace Elem Res 112:109–118
9. Sun Q, van Dam RM, Willett WC, Hu FB (2009) Prospective study of zinc intake and risk of type 2 diabetes in women. Diabetes Care 32:629–634
10. Lee DH, Anderson KE, Harnack LJ, Folsom AR, Jacobs DR Jr (2004) Heme iron, zinc, alcohol consumption, and colon cancer: Iowa Women's Health Study. J Natl Cancer Inst 96:403–407
11. Larsson SC, Adami HO, Giovannucci E, Wolk A (2005) Re: heme iron, zinc, alcohol consumption, and risk of colon cancer. J Natl Cancer Inst 97:232–233 (author reply 3–4)
12. Huang X (2003) Iron overload and its association with cancer risk in humans: evidence for iron as a carcinogenic metal. Mutat Res 533:153–171
13. Cross AJ, Gunter MJ, Wood RJ et al (2006) Iron and colorectal cancer risk in the alpha-tocopherol, beta-carotene cancer prevention study. Int J Cancer 118:3147–3152
14. Kabat GC, Miller AB, Jain M, Rohan TE (2007) A cohort study of dietary iron and heme iron intake and risk of colorectal cancer in women. Br J Cancer 97:118–122
15. Kato I, Dnistrian AM, Schwartz M et al (1999) Iron intake, body iron stores and colorectal cancer risk in women: a nested case-control study. Int J Cancer 80:693–698
16. Wurzelmann JI, Silver A, Schreinemachers DM, Sandler RS, Everson RB (1996) Iron intake and the risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev 5:503–507
17. Knekt P, Reunanen A, Takkunen H, Aromaa A, Heliovaara M, Hakulinen T (1994) Body iron stores and risk of cancer. Int J Cancer 56:379–382
18. Balder HF, Vogel J, Jansen MC et al (2006) Heme and chlorophyll intake and risk of colorectal cancer in the Netherlands cohort study. Cancer Epidemiol Biomarkers Prev 15:717–725
19. Freudenheim JL, Graham S, Marshall JR, Haughey BP, Wilkinson G (1990) A case-control study of diet and rectal cancer in western New York. Am J Epidemiol 131:612–624
20. Bastide NM, Pierre FH, Corpet DE (2011) Heme iron from meat and risk of colorectal cancer: a meta-analysis and a review of the mechanisms involved. Cancer Prev Res (Phila) 4:177–184
21. Sesink AL, Termont DS, Kleibeuker JH, Van der Meer R (1999) Red meat and colon cancer: the cytotoxic and hyperproliferative effects of dietary heme. Cancer Res 59:5704–5709
22. Hambraeus L (1999) Animal- and plant-food-based diets and iron status: benefits and costs. Proc Nutr Soc 58:235–242
23. Monsen ER (1988) Iron nutrition and absorption: dietary factors which impact iron bioavailability. J Am Diet Assoc 88:786–790
24. Cross AJ, Pollock JR, Bingham SA (2003) Haem, not protein or inorganic iron, is responsible for endogenous intestinal N-nitrosation arising from red meat. Cancer Res 63:2358–2360
25. Cross AJ, Ferrucci LM, Risch A et al (2010) A large prospective study of meat consumption and colorectal cancer risk: an investigation of potential mechanisms underlying this association. Cancer Res 70:2406–2414
26. Willett W (1998) Nutritional epidemiology, 2nd edn. Oxford University Press, New York

27. Puckett CD (1986) The educational annotation of ICD-9-CM; diseases and procedures tabular lists, report no.: vol I. Reno
28. Willett WC, Sampson L, Stampfer MJ et al (1985) Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 122:51–65
29. Rimm EB, Giovannucci EL, Stampfer MJ, Colditz GA, Litin LB, Willett WC (1992) Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. Am J Epidemiol 135:1114–1126 (discussion 27–36)
30. Cho E, Stampfer MJ, Seddon JM et al (2001) Prospective study of zinc intake and the risk of age-related macular degeneration. Ann Epidemiol 11:328–336
31. Willett WC, Sampson L, Browne ML et al (1988) The use of a self-administered questionnaire to assess diet four years in the past. Am J Epidemiol 127:188–199
32. Feskanich D, Rimm EB, Giovannucci EL et al (1993) Reproducibility and validity of food intake measurements from a semiquantitative food frequency questionnaire. J Am Diet Assoc 93:790–796
33. Salvini S, Hunter DJ, Sampson L et al (1989) Food-based validation of a dietary questionnaire: the effects of week-to-week variation in food consumption. Int J Epidemiol 18:858–867
34. Cox DR (1972) Regression models and life-tables. J R Stat Soc 34:187–220
35. Institute SAS (1991) SAS/STAT software: the PHREG procedure: preliminary documentation. SAS Institute, Cary
36. Hu FB, Stampfer MJ, Rimm E et al (1999) Dietary fat and coronary heart disease: a comparison of approaches for adjusting for total energy intake and modeling repeated dietary measurements. Am J Epidemiol 149:531–540
37. Cochran WG (1954) The combination of estimates from different experiments. Biometrics 10:101–129
38. DerSimonian R, Laird N (1986) Meta-analysis in clinical trials. Controlled Clin Trials 7:177–188
39. Solomons NW (1986) Competitive interaction of iron and zinc in the diet: consequences for human nutrition. J Nutr 116:927–935
40. Whittaker P (1998) Iron and zinc interactions in humans. Am J Clin Nutr 68:442S–446S
41. Chadha VD, Goel A, Dhawan D (2011) Regulatory role of zinc on the biokinetics and biodistribution of (65) Zn during the initiation of experimentally induced colon cancer. Nutr Cancer 63:212–217
42. Dhawan DK, Chadha VD (2010) Zinc: a promising agent in dietary chemoprevention of cancer. Indian J Med Res 132: 676–682
43. Epstein MM, Kasperzyk JL, Andren O et al (2011) Dietary zinc and prostate cancer survival in a Swedish cohort. Am J Clin Nutr 93:586–593
44. Hunt JR (2003) Bioavailability of iron, zinc, and other trace minerals from vegetarian diets. Am J Clin Nutr 78:633S–639S
45. Sinha R, Cross A, Curtin J et al (2005) Development of a food frequency questionnaire module and databases for compounds in cooked and processed meats. Mol Nutr Food Res 49:648–655