Research Article

Cancer Prevention Research

Iron Homeostasis and Distal Colorectal Adenoma Risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial

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Abstract

Red meat consumption has been positively associated with colorectal cancer; however, the biological mechanism underlying this relationship is not understood. Red meat is a major source of iron, which may play a role in colorectal carcinogenesis via increased crypt cell proliferation, cytotoxicity, and endogenous *N*-nitrosation. In a nested case–control study within the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, we prospectively evaluated multiple iron exposure parameters, including dietary intake and serum measures of iron, ferritin, transferrin, total iron binding capacity (TIBC), and unsaturated iron binding capacity (UIBC) in relation to incident colorectal adenoma in 356 cases and 396 matched polyp-free controls. We also investigated variation in eight key genes involved in iron homeostasis in relation to colorectal adenoma in an additional series totaling 1,126 cases and 1,173 matched controls. We observed a positive association between red meat intake and colorectal adenoma [OR comparing extreme quartiles ($OR_{q4\text{-}q1}$) = 1.59, 95% CI = 1.02–2.49, P_{trend} = 0.03]. Serum TIBC and UIBC were inversely associated with colorectal adenoma ($OR_{q4\text{-}q1}$ = 0.57, 95% CI = 0.37–0.88, P_{trend} = 0.03; and $OR_{q4\text{-}q1}$ = 0.62, 95% CI = 0.40–0.95, P_{trend} = 0.04, respectively). Colorectal adenoma was not associated with serum ferritin, iron, or transferrin saturation or with polymorphisms in genes involved in iron homeostasis. Serum TIBC and UIBC, parameters that have a reciprocal relationship with overall iron load, were inversely related to colorectal adenoma, suggesting that individuals with lower iron status have a reduced risk of developing colorectal adenoma. *Cancer Prev Res; 4(9); 1465–75. ©2011 AACR.*

Introduction

There is considerable epidemiologic evidence to suggest that intake of red meat, but not white meat, is positively associated with colorectal cancer (1). Red and white meat differ primarily in their content of iron; specifically, the concentration of iron in red meat is approximately 10-fold higher than that of white meat (2, 3). Heme iron, which is iron bound to a porphyrin ring such as in hemoglobin or myoglobin, is the predominant source of iron in red meat and is more readily absorbed in the intestine than non-heme iron (4).

A large body of laboratory data supports a role for iron in colorectal tumorigenesis. In animal models, for example, iron supplementation increases crypt cell proliferation in the large intestine (5), whereas rodents inoculated with colon adenocarcinoma cells have decreased tumor growth when fed an iron-deficient diet (6). Furthermore, iron supplementation enhances the rate of tumor growth (7, 8), and *in vitro* studies have shown that tumor cells have a greater ability than normal cells to grow and/or survive in the presence of high levels of iron (9). Humans consuming heme iron specifically exhibit increased endogenous formation of *N*-nitroso compounds (NOCs; ref. 10), which are known mammalian carcinogens capable of targeting many tissues, including the colorectum (11).

In contrast to the relative abundance of laboratory data supporting a role for iron in colorectal tumorigenesis, epidemiologic data on iron and colorectal neoplasia are sparse. The few published studies that have addressed questionnaire-assessed dietary iron in relation to colorectal neoplasia resulted in inconsistent findings, possibly because of the methodologic inability to assess heme iron intake specifically (12–14). In addition, although several prospective studies have evaluated the association of serologic markers of iron status with colorectal neoplasia, the

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Note: Supplementary data for this article are available at Cancer Prevention Research Online (http://cancerprevres.aacrjournals.org/).

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doi: 10.1158/1940-6207.CAPR-11-0103

results are conflicting (15). Genetic studies of iron metabolism and colorectal neoplasia are also limited. Carriage of a set of rare mutations in the *HFE* gene, which confer increased risk of developing hemochromatosis, a syndrome characterized by elevated body iron stores, have been linked to increased risk of developing colorectal cancer in one study (16), although subsequent investigations failed to replicate this finding (17–19).

To our knowledge, no single study has simultaneously investigated dietary iron intake, serum iron indices, and variation in multiple genes governing iron homeostasis in relation to colorectal neoplasia. We sought to examine whether increased iron exposure, as determined from diet, serologic markers, and genetic variation, was associated with the risk of developing colorectal adenoma, an established colorectal cancer precursor.

Methods

Study population

Our study was nested within the screening arm of the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, which is a large, randomized, controlled trial designed to test the efficacy of cancer screening and to investigate early markers and etiology of cancer (20–22). Between 1993 and 2001, approximately 155,000 men and women aged 55 to 74 years who had no history of prostate, lung, colorectal, or ovarian cancer were enrolled from 10 U.S. centers. Those randomly assigned to the screening arm ($n = 77{,}465$) were offered a flexible sigmoidoscopy examination of the distal colorectum (60 cm) at study entry, of which 83% ($n = 64{,}658$) were compliant and 89% ($n = 57{,}559$) of these were considered successful (insertion to at least 50 cm with >90% of mucosa visible or a suspect lesion identified). If neoplastic lesions were detected, participants were referred for subsequent colonoscopic examination. All available medical and pathologic reports on follow-up were obtained and coded by trained medical record abstractors. The Institutional Review Boards of the U.S. National Cancer Institute and the 10 screening centers approved the study, and all participants provided informed consent.

Study sample

We selected participants assigned to the screening arm of the PLCO Cancer Screening Trial between September 1993 and September 1999 who had undergone a successful sigmoidoscopy, completed the baseline risk factor questionnaire and the dietary questionnaire, and provided a blood sample for use in etiologic studies ($n = 42{,}037$). We excluded 4,834 individuals because of a self-reported history of any of the following: Crohn's disease, ulcerative colitis, familial polyposis, Gardner's syndrome, colorectal polyps, or cancer (except basal-cell skin cancer).

Among those who had a negative sigmoidoscopy result (i.e., no polyp or other suspect lesion detected) at the baseline screen (T0), we identified 356 incident colorectal adenoma cases at the follow-up sigmoidoscopy screening examination (administered either 3 or 5 years later, T3 or T5). The cases were matched (on age, gender, ethnicity, study center, season of blood draw, and whether their follow-up sigmoidoscopy was after 3 or 5 years) to 396 controls who were polyp free at both the baseline screen (T0) and the follow-up screen (T3 or T5). This group of cases and controls is referred to as the "serologic sample."

For analysis of variation in genes related to iron homeostasis and colorectal adenoma, we additionally selected a set of advanced colorectal adenoma cases (hereafter termed "genotyped sample" and defined as adenomas with high-grade dysplasia or villous components or $\geq$1 cm), identified at the baseline screen (T0). A full description of the selection of these study subjects has been published elsewhere (23); in brief, of the 1,234 individuals who had at least 1 distal advanced colorectal adenoma at the baseline screen, we randomly selected 772 cases and matched by gender and ethnicity to 777 of the 26,651 control participants with a negative sigmoidoscopy examination.

Dietary iron assessment

Usual diet over the 12 months prior to enrollment was assessed on the day of sigmoidoscopy by using a 137-item food frequency questionnaire (FFQ). Heme iron intake was estimated using the FFQ in conjunction with a heme iron database on the basis of measured values from various meat types cooked by different methods and to varying doneness levels (24).

Serum iron indices

Serum iron parameters were measured in the incident colorectal adenoma cases and their matched controls. To investigate whether the serum iron parameters are stable over time in a small pilot, we sampled a random subset of 50 incident cases (pilot cases) and 30 controls (pilot controls) with available blood specimens at both baseline (T0) and 5 years in to the trial (T5).

Serum ferritin, a marker of stored iron, was measured using an immunoradiometric assay kit (Coat-A-Count Ferritin IRMA; Diagnostic Products Corporation). Serum iron and unsaturated iron binding capacity (UIBC) were assessed using a standard ferrozine-based iron colormetric assay (Olympus AU 400e auto analyzer). Total iron binding capacity (TIBC), calculated as the sum of serum iron plus UIBC, is a measure of the iron binding capacity within the serum and reflects the availability of iron binding sites on transferrin. Transferrin saturation indicates the extent to which transferrin has vacant iron binding sites (i.e., low transferrin saturation indicates a high proportion of vacant iron binding sites) and was calculated as serum iron divided by TIBC. In addition, because ferritin is an acute-phase protein, and the inflammatory state is related to both body iron stores and colorectal neoplasia (25), we assessed C-reactive protein (CRP) levels as a marker of inflammation, using a latex particle enhanced immunoturbidimetric assay kit (K-ASSAY CRP Ultra; EQual Diagnostics). All serum samples were analyzed blinded to case–control status and each batch contained 10%

randomly inserted quality control serum samples that have been used in other studies (26). The coefficient of variation (CV) for all analytes was less than 6.4%.

SNP selection and genotyping

We selected 8 genes that play key roles in the uptake, absorption, and metabolism of dietary iron (see Supplementary Table S1): transferrin (*TF*), transferrin receptor (*TFRC*), heme oxygenase (*HMOX1*), solute carrier family 40-ferroportin (*SLC40A1*), solute carrier family 11-proton-coupled divalent metal ion transporters (*SLC11A2*), hepcidin antimicrobial peptide (*HAMP*), aconitase 1 (*ACO1*), and haptoglobin (*HP01*). We used dbSNP (http:/www.ncbi.nlm.nih.gov/SNP/), SNP500 (http://snp500cancer.nci.nih.gov/), and SNPper (http://snpper.chip.org/) and literature searches on iron metabolism to select single-nucleotide polymorphisms (SNP) located within exonic gene regions, putative regulatory regions, and exon–intron junctions with priority given to those linked with serum or tissue iron levels, or colorectal neoplasia, in previous studies. The selected SNPs were verified in a panel of 102 individuals of self-described Caucasian ($n = 31$), African American ($n = 24$), Hispanic ($n = 23$), and Pacific Rim ($n = 24$) ethnicity (27) by resequencing approximately 300 bp of DNA either side of the putatively polymorphic locus. SNPs with a minor allele frequency of 5% or more among Caucasians (who represent 93% of our study sample) were chosen for genotyping in the current study.

DNA was extracted from buffy coat or whole blood samples by using routine methods. Genotyping was carried out at the Core Genotyping Facility of the Division of Cancer Epidemiology and Genetics, National Cancer Institute using TaqMan (Applied Biosystems). Protocols for each specific assay are available at the following Web site: http://snp500cancer.nci.nih.gov (27). For validation purposes, TaqMan assays were initially applied to the 102 individuals with sequence information and were subsequently applied to the PLCO Cancer Screening Trial samples only if sequencing and TaqMan results were 100% concordant, otherwise, a new TaqMan assay was designed. For 6 cases and 6 controls, no genotype data were available because of insufficient DNA yields, DNA that did not amplify, or failure of unambiguous genotyping. Blinded quality control samples, comprising DNA from 40 individuals, were assayed between 2 and 4 times and these genotypes were found to be 100% concordant. All polymorphic loci analyzed in this study had genotypic distributions consistent with Hardy–Weinberg equilibrium among the controls.

Statistical analysis

The demographic characteristics and dietary covariates of those with and without colorectal adenoma were compared using the χ^2 test for categorical variables and the Wilcoxon rank-sum test for continuous variables. For the pilot study in which we evaluated temporal stability of iron parameters in cases and controls, we assessed differences in medians between T0 and T5 by the Wilcoxon sign-rank test. To estimate the reliability of a one-time measurement of serum iron levels, we compared iron parameter concentrations at T0 and T5 in a subset of control participants ($n = 30$) by calculating the intraclass correlation coefficient (ICC). Because the serum iron measurements were not normally distributed in the control sample, the data were logarithmically transformed to approximate normality and log values were used to derive ICCs. Intra- and interindividual variances for serologic measures were estimated using random-effects models, and the ICC was derived from these values. Unconditional logistic regression was used to estimate ORs and 95% CIs for the association between the dietary variables, as well as the serologic parameters, with colorectal adenoma risk. The dietary variables were nutrient density energy adjusted, and quartile cutoff points were based on reported intake in the controls. The serologic data were analyzed as both continuous, log-transformed variables and as categorical variables based on the distribution of quartile cutoff points in the controls. In addition to the matching factors, all models were adjusted for an *a priori* determined set of colorectal neoplasia risk factors, including the use of nonsteroidal anti-inflammatory drugs (NSAID), family history of colorectal cancer, educational attainment, the use of hormone therapy (females only), and smoking (never, former, or current smoker). Inclusion of other potential covariates such as body mass index (BMI), self-reported history of diabetes, serum CRP level, alcohol consumption, physical activity, and intake of fiber, calcium, folate, vitamin D, saturated fat, and red meat, did not change the risk estimates by more than 10% and were therefore not included in the final multivariable models. Tests for trend were calculated using a single ordinal variable corresponding to the median values for each quartile, which was entered into the multivariable model as a continuous variable.

The associations between genotypes and colorectal adenoma were estimated using unconditional logistic regression, with the most common genotype serving as a referent. All genetic analyses were adjusted for age; other colorectal adenoma risk factors were evaluated as potential covariates but did not alter the risk estimates by 10% or more; therefore, they were not included in the final models. To calculate P_{trend}, we assigned the ordinal values 0, 1, and 2 to the most prevalent genotypes in rank order of homozygous for the common allele and heterozygous and homozygous for the rare allele, respectively.

We also investigated the association between specific haplotypes and colorectal adenoma. Linkage disequilibrium between the SNPs was assessed using r^2 and D' statistics and visualized using Haploview (28). Haplotype blocks were characterized among the controls according to the method of Gabriel and colleagues (29), and haplotype distribution and frequencies were assessed using PHASE software (30). We used logistic regression to estimate the associations between each haplotype and colorectal adenoma using the most common haplotype as the reference category. Because of very high linkage disequilibrium within blocks, there was little phase ambiguity in haplotype

reconstruction and thus the related statistical uncertainty was ignored (31).

The relationship between genotypes and serum iron parameters was assessed in the controls in the serologic sample using ANOVA models adjusted for age. All reported *P* values are 2-sided and analyses were conducted using SAS software (version 9; SAS Institute).

Results

The serologic sample consisted of 356 (232 male and 124 female) incident adenoma cases, of which 143 were advanced (103 male and 40 female), and 396 (259 male and 137 female) controls. The additional genotyped sample consisted of 770 (533 male and 237 female) advanced adenoma cases and 777 (536 male and 241 female) controls. In both the serologic and genotyped samples, the cases were more likely to be current smokers, more likely to have family history of colorectal cancer, and more likely to consume alcohol, but they were less physically active and less likely to be regular users of NSAIDs; however, these differences between cases and controls were not all statistically significant in both groups (Table 1). Red meat and heme iron intake was slightly higher in the cases than in the controls in both serologic and genotyped samples, although this difference was not statistically significant. Cereals and bread accounted for 37% of the total dietary iron intake, whereas red meat accounted only for 8%; other foods contributing significantly to this variable included vegetables (15%), fruit (4%), white meat (4%), pasta (4%), orange/grapefruit juice (3%), rice (2%), coffee (2%), and milk (1%). Dietary iron was lower in the cases than in the controls in the genotyped sample ($P = 0.05$). Serum TIBC and UIBC were lower in cases than in controls ($P = 0.07$, and $P = 0.05$, respectively, Table 1). The Spearman rank correlations between the serum iron indices and dietary variables in the controls indicated that heme iron intake was moderately correlated with serum ferritin levels ($r = 0.25$), but none of the other iron indices were correlated with dietary iron intake (Table 2).

The stability of the serum iron parameters over a 6-year period was assessed in a subset of incident colorectal adenoma cases and controls (Table 3). Although serum ferritin and TIBC both decreased when comparing the serum samples collected at baseline and the serum samples collected at repeat sigmoidoscopy, these changes were not statistically significant ($P > 0.05$). The ICCs in the subgroup of controls with repeat serum measurements showed that ferritin, TIBC, and UIBC levels were stable, with all ICCs more than 0.7; however, the ICCs for serum iron and transferrin saturation were much lower (0.08 and 0.20, respectively).

In analyses investigating the association between dietary iron sources, serologic indices of iron status, and colorectal adenoma incidence, individuals in the highest compared with the lowest quartile of red meat intake had an elevated risk of developing colorectal adenoma (OR = 1.59, 95% CI = 1.02–2.49; $P_{trend} = 0.03$; Table 4). Although the risks associated with heme iron intake were elevated, they were not statistically significant, whereas the association between total iron intake and colorectal adenoma was null. Serum TIBC and UIBC were inversely associated with colorectal adenoma [OR comparing highest with the lowest quartiles of TIBC level ($OR_{q4\text{-}q1} = 0.57$, 95% CI = 0.37–0.88; $P_{trend} = 0.03$ and $OR_{q4\text{-}q1} = 0.62$, 95% CI = 0.40–0.95; $P_{trend} = 0.04$, respectively); both of these serum measures seemed to reveal a threshold effect in the second through fourth quartiles. Serum levels of ferritin, iron, and transferrin saturation were not associated with colorectal adenoma incidence (Table 4). These findings did not materially differ by gender or when the outcome was restricted to advanced adenomas (data not shown); furthermore, subsite-specific analyses revealed similar associations for colon versus rectal adenomas.

In exploratory analyses of serum iron parameter levels by genotypes among the controls in the serologic sample, serum ferritin levels varied according to genotype for 4 different SNPs in *ACO1*: IVS1+ 9689C>T ($P = 0.004$), IVS15− 752G>A ($P = 0.001$), IVS15+ 90A>G ($P = 0.003$), and IVS1+ 4665C>T ($P = 0.055$); 1 SNP in *TF*: IVS11+ 437C>T ($P = 0.002$); and 1 SNP in *SLC11A2*: IVS1−130G>A ($P = 0.022$). Serum iron was higher ($P = 0.035$) in those homozygous for the variant allele for a SNP: Ex6−98T>C in *SLC40A1*. Serum UIBC was influenced by genotype in *SLC11A2* (IVS1− 130G>A, $P = 0.026$), *TFRC* (IVS17+ 445C>T, $P = 0.018$), and *TF* (IVS8− 318T>G, $P = 0.006$) and TIBC by SNPs in *SLC11A2* (Ex16+ 1142G>A, $P = 0.045$), *TF* (IVS8− 318T>G, $P = 0.001$; IVS11+ 437C>T, $P = 0.001$), *TFRC* (IVS17+ 445C>T, $P = 0.002$), and *SLC40A1* (Ex6− 98T>C, $P = 0.032$). However, none of the genotypes or haplotypes were associated with colorectal adenoma risk (Table 5).

Discussion

This is the first prospective study to investigate the association of iron exposure with colorectal neoplasia, using a heme iron database for the assessment of dietary iron, serum iron indices, and variation in multiple genes involved in iron homeostasis. Serum TIBC and UIBC, indicators of low iron stores, were both inversely associated with colorectal adenoma, suggesting that low body iron stores are protective against colorectal adenoma and that iron may play a role in the early stages of colorectal tumorigenesis.

TIBC is commonly assessed in clinical settings as an indicator of iron load and is an indicator of levels of transferrin. Transferrin is the major iron binding and transfer protein in circulation, and its levels increase as serum iron availability decreases. The association between serum TIBC level and colorectal adenoma incidence was in the expected direction, indicating that as the concentration of transferrin increases (indicative of reduced iron levels), the risk of colorectal neoplasia decreases. Unlike other serum iron indices, such as ferritin, TIBC is not affected by the inflammatory response and does not exhibit

Table 1. Baseline characteristics of the study sample by colorectal adenoma cases and controls in the PLCO Cancer Screening Trial[a]

	Serologic sample			Genotyped sample		
Characteristic	**Cases ($n = 356$)**	**Controls ($n = 396$)**	**P[b]**	**Cases ($n = 770$)**	**Controls ($n = 777$)**	**P[b]**
Sex, % male	65.2	65.4	Matched	69.2	69.0	0.92
Race, % Caucasian	89.6	90.4	Matched	93.9	93.8	0.99
Age, y	62.0 (58.0–66.0)	62.0 (58.5–66.0)	Matched	62.0 (58.0–67.0)	60.0 (57.0–65.0)	<0.0001
BMI, kg/m^2	27.1 (24.3–30.1)	26.6 (24.3–29.2)	0.36	27.5 (24.7–30.5)	27.0 (24.6–30.1)	0.11
Smoking status, %						
Never	42.7	47.5	0.18	33.9	40.5	<0.0001
Former	43.8	39.7		47.1	47.1	
Current	8.7	6.1		14.3	6.8	
Pipe/cigar	4.8	6.8		4.7	5.5	
Physically active ≥5 h/wk, %	23.6	27.8	0.35	19.1	24.2	0.005
Education, %						
High school or less	28.9	29.8	0.92	34.0	29.2	0.001
Some college	31.7	32.3		35.8	31.8	
College graduate/postgraduate	39.3	37.9		30.1	39.0	
Regular[c] NSAID use, %	53.7	60.6	0.06	58.4	60.5	0.44
Aspirin dose ≥1/d, %	23.6	26.5	0.38	25.7	26.6	0.90
Family history of colorectal cancer, %	12.1	7.8	0.07	13.0	9.3	0.02
Diabetes, %	6.2	6.8	0.72	8.1	7.0	0.44
Current use of menopausal hormone therapy, %	13.8	14.9	0.87	14.9	16.7	0.52
Dietary intake[d]						
Total energy, kcal/d	2,101 (1,560–2,608)	2,031 (1,593–2,701)	0.94	1,976 (1,464–2,592)	2,031 (1,564–2,643)	0.15
Saturated fat, g/1,000 kcal	10.8 (9.0–12.4)	10.6 (8.8–12.5)	0.92	11.4 (9.4–13.1)	11.2 (9.4–13.1)	0.86
Fiber, g/1,000 kcal	11.1 (9.1–13.4)	11.5 (9.4–13.8)	0.26	10.5 (8.4–12.9)	10.9 (8.9–13.5)	0.008
Red meat, g/1,000 kcal	34.5 (21.5–50.4)	31.3 (20.1–46.9)	0.08	36.7 (23.6–53.1)	35.9 (21.6–51.4)	0.27
Dietary iron, mg/1,000 kcal	8.6 (7.5–9.9)	8.7 (7.4–10.3)	0.65	8.2 (7.1–9.8)	8.5 (7.3–9.9)	0.05
Supplemental iron, mg/d	0 (0–18)	0 (0–18)	0.20	0 (0–18)	0 (0–18)	0.21
Heme iron, μg/1,000 kcal	185 (118–286)	170 (108–261)	0.14	204 (128–305)	202 (114–297)	0.27
Dietary calcium, mg/1,000 kcal	419 (332–533)	426 (341–540)	0.90	416 (349–533)	429 (357–540)	0.19
Dietary folate, μg/1,000 kcal	174 (142–215)	177 (143–216)	0.84	166 (134–207)	173 (142–211)	0.01
Alcohol, g/d	2.7 (0.4–18.2)	1.9 (0.3–10.5)	0.03	2.8 (0.1–17.2)	2.4 (0.1–15.8)	0.33
Serum markers at baseline, median (IQR)						
CRP, mg/L	1.64 (0.82–3.54)	1.78 (0.82–4.09)	0.29			
Ferritin, ng/mL	140 (92–240)	161 (84–277)	0.53			
Iron, μg/dL	107 (86–132)	106 (86–131)	0.93			
Transferrin saturation, %	31 (25–39)	30 (25–37)	0.25			
TIBC, μg/dL	347 (311–386)	351 (324–390)	0.07			
UIBC, μg/dL	238 (195–279)	243 (210–280)	0.05			

[a]All values are median (IQR) unless otherwise specified.

[b]P values derived from the Wilcoxon rank-sum test for continuous data and from the χ^2 test for categorical data.

[c]Regular use defined as ≥1/wk.

[d]Dietary data: For serologic sample, $n = 342$ cases and 384 controls, with a further 3 cases and 1 control missing data for total dietary iron and heme iron. For genotyped sample, $n = 729$ cases and 754 controls.

Table 2. Correlation matrix for serologic iron indices and dietary iron in controls only from the PLCO Cancer Screening Trial ($n = 396$)

	Serologic indices					Dietary data	
Serum variable	**Iron**	**Transferrin saturation**	**TIBC**	**UIBC**	**CRP**	**Total iron[a]**	**Heme iron[a]**
Ferritin	0.14	0.20	−0.15	−0.24	0.06	−0.07	0.25
P	0.005	<0.0001	0.002	<0.0001	0.23	0.16	<0.0001
Iron		0.87	0.29	−0.33	−0.25	−0.02	0.04
P		<0.0001	<0.0001	<0.0001	<0.0001	0.76	0.47
Transferrin saturation			−0.17	−0.72	−0.27	−0.03	0.07
P			0.0009	<0.0001	<0.0001	0.52	0.15
TIBC				0.76	−0.03	0.05	−0.04
P				<0.0001	0.55	0.31	0.38
UIBC					0.14	0.08	−0.08
P					0.004	0.13	0.13

[a]Nutrient density energy adjusted.

day-to-day variation in measurements (32). UIBC is also a measure of the availability of iron binding sites on transferrin and also increases as iron availability decreases. Prospective data on TIBC and UIBC in relation to colorectal neoplasia are limited. In contrast to our study, a nested case–control study of women reported no association between TIBC and colorectal adenoma (19), although this was a study of premenopausal women, whose serum iron indices could be affected by menstrual cycle phase. The female participants of the current study were almost all postmenopausal, limiting the potential issues of iron loss through menstruation. Another study conducted in men reported a positive association between UIBC and colon cancer (33).

In agreement with our investigation, a nested case–control study of women found no association between serum iron, ferritin, or transferrin saturation in relation to colorectal adenoma (19) whereas a nested case–control study of men found these 3 serum markers to be inversely related to colon but not rectal cancer (33). A potential limiting factor in the assessment of serum iron parameters in relation to colorectal cancer is that circulating iron levels may be affected by bleeding from subclinical lesions in the colorectum, which can deplete iron stores (34–36). Although up to 91% of colorectal cancers bleed, only 6% to 14% of small adenomas (<10 mm) bleed, increasing to 11% to 47% of 10- to 20-mm adenomas and 20% to 84% of more than 20-mm adenomas (34, 37). If iron is positively associated with colorectal neoplasia, such bleeding could bias the association toward the null or even reverse the direction. Given that most colorectal tumors can take more than 10 years to develop (38), it is possible that even prospectively collected blood specimens could be subject to such bias if premalignant subclinical lesions, such as adenomas, were present. In the current study, we sought to address this issue by investigating whether the development of colorectal adenoma is associated with significant temporal changes in iron levels. Although we observed small decreases in median levels of ferritin and TIBC over a 5-year time period, these changes were not statistically significant and were evident in both incident adenoma cases and in the controls who remained polyp-free. This finding suggests that bleeding and consequent iron losses in individuals with subclinical colorectal lesions may not be of concern.

Table 3. Medians (IQR) for serologic iron indices at 2 separate time points in a subset of incident colorectal adenoma cases ($n = 50$) and controls ($n = 30$) in the PLCO Cancer Screening Trial

	Pilot cases ($n = 50$)			Pilot controls ($n = 30$)		
	T0, median (IQR)	**T5, median (IQR)**	***P*[a]**	**T0, median (IQR)**	**T5, median (IQR)**	***P*[a]**
Ferritin	129 (76–175)	106 (68–158)	0.15	129 (67–243)	104 (61–217)	0.18
Iron	114 (100–144)	116 (91–131)	0.19	109 (90–136)	109 (73–129)	0.67
Transferrin saturation	34 (28–40)	33 (27–40)	0.72	32 (28–37)	31 (23–37)	0.82
TIBC	360 (319–393)	335 (304–383)	0.08	357 (324–397)	345 (303–368)	0.10
UIBC	235 (189–167)	233 (194–257)	0.71	242 (200–270)	232 (203–290)	0.47

[a]The Wilcoxon sign-rank test for difference between medians.

Table 4. Iron and colorectal adenoma risk in the serologic sample ($n = 356$ cases; $n = 396$ controls) from the PLCO Cancer Screening Trial

Variable	Q1	Q2	Q3	Q4	P_{trend}
Dietary variables					
Red meat (g/1,000 kcal)					
Quartile median, quartile cutoff points	13.8 (≤20.1)	26.2 (>20.1–31.3)	37.5 (>31.3–46.8)	59.8 (>46.8)	
Cases/controls	72/96	70/96	96/96	104/96	
OR[a] (95% CI)	Ref	0.97 (0.63–1.50)	1.33 (0.88–2.02)	1.45 (0.96–2.20)	0.04
OR[b] (95% CI)	Ref	1.08 (0.69–1.71)	1.42 (0.91–2.21)	1.59 (1.02–2.49)	0.03
Total dietary iron, mg/1,000 kcal					
Quartile median (quartile cutoff points)	6.6 (≤7.4)	8.1 (>7.4–8.7)	9.4 (>8.7–10.3)	11.6 (>10.3)	
Cases/controls	74/96	108/96	87/96	73/96	
OR[a] (95% CI)	Ref	1.46 (0.97–2.20)	1.18 (0.77–1.79)	0.99 (0.64–1.52)	0.54
OR[b] (95% CI)	Ref	1.49 (0.97–2.28)	1.24 (0.80–1.92)	0.98 (0.62–1.55)	0.98
Heme iron, mg/1,000 kcal					
Quartile median (quartile cutoff points)	76.3 (≤108.0)	141.4 (>108.0–170.1)	211.0 (>170.1–259.5)	355.7 (>259.5)	
Cases/controls	74/95	77/96	83/96	105/96	
OR[a] (95% CI)	Ref	1.03 (0.67–1.58)	1.11 (0.72–1.69)	1.41 (0.93–2.14)	0.07
OR[b] (95% CI)	Ref	1.08 (0.69–1.69)	1.09 (0.69–1.71)	1.46 (0.94–2.29)	0.08
Serologic indices					
Ferritin (ng/mL)					
Quartile median (quartile cutoff points)	54 (≤83)	119 (>83–161)	209 (>161–276)	419 (>276)	
Cases/controls	80/98	125/100	77/99	74/99	
OR[a] (95% CI)	Ref	1.53 (1.03–2.29)	0.96 (0.63–1.46)	0.92 (0.60–1.41)	0.15
OR[b] (95% CI)	Ref	1.60 (1.05–2.43)	0.93 (0.59–1.47)	0.86 (0.53–1.38)	0.08
Iron, μg/dL					
Quartile median (quartile cutoff points)	74 (≤86)	96 (>86–105)	118 (>105–130)	154 (>130)	
Cases/controls	95/101	77/95	88/100	96/100	
OR[a] (95% CI)	Ref	0.86 (0.57–1.30)	0.94 (0.63–1.40)	1.02 (0.69–1.52)	0.77
OR[b] (95% CI)	Ref	0.85 (0.55–1.30)	0.95 (0.63–1.45)	1.02 (0.66–1.55)	0.78
Transferrin saturation, %					
Quartile median (quartile cutoff points)	20 (≤24)	27 (>24–29)	33 (>29–37)	43 (>37)	
Cases/controls	82/98	73/95	102/107	99/96	
OR[a] (95% CI)	Ref	0.92 (0.60–1.40)	1.14 (0.76–1.70)	1.23 (0.82–1.85)	0.20
OR[b] (95% CI)	Ref	0.92 (0.59–1.43)	1.18 (0.78–1.79)	1.28 (0.83–1.98)	0.16
TIBC, μg/dL					
Quartile median (quartile cutoff points)	300 (≤323)	338 (>323–351)	369 (>351–389)	421 (>389)	
Cases/controls	119/97	72/102	82/97	83/100	
OR[a] (95% CI)	Ref	0.58 (0.38–0.86)	0.69 (0.46–1.03)	0.68 (0.45–1.01)	0.10
OR[b] (95% CI)	Ref	0.51 (0.34–0.78)	0.62 (0.41–0.95)	0.57 (0.37–0.88)	0.03
UIBC, μg/dL					
Quartile median (quartile cutoff points)	185 (≤209)	228 (>209–243)	263 (>243–280)	314 (>280)	
Cases/controls	118/98	77/101	80/99	81/98	
OR[a] (95% CI)	Ref	0.63 (0.42–0.95)	0.67 (0.45–1.00)	0.69 (0.46–1.02)	0.08
OR[b] (95% CI)	Ref	0.59 (0.39–0.90)	0.60 (0.39–0.91)	0.62 (0.40–0.95)	0.04

[a]OR adjusted for age only.

[b]Multivariate OR adjusted for age (≤59, 60–64, 65–69, ≥70 years), gender, season of blood draw (May–Sep, Oct–Apr), sigmoidoscopy interval (3 or 5 years), race (non-Hispanic white, other), study center, year of entry to study, education (≤12 years or completed high school, post–high school training other than college/some college, college graduate or postgraduate), smoking (never, former, current, cigar/pipe), regular NSAID use (yes/no), and family history of colorectal cancer (yes/no).

The positive association we observed for red meat intake and colorectal adenoma is in agreement with previous studies (1); in addition, calculations of population attributable risks in the large NIH-AARP Diet and Health study cohort estimated that if individuals in the highest quintile of red meat intake adopted intake levels equivalent to those

Table 5. Distribution of iron-related genotypes and risk of colorectal adenoma ($n = 770$ cases and 777 controls) in the PLCO Cancer Screening Trial

Genotype	Cases, *n*	Controls, *n*	OR[a] (95% CI)
TF			
IVS8− 318T>G			
TT	336	341	1.0 (Ref)
GT	313	327	0.97 (0.78–1.21)
GG	98	86	1.14 (0.82–1.58)
P_{trend}			0.84
IVS11+ 437C>T			
CC	518	530	1.0 (Ref)
CT	195	193	1.04 (0.82–1.32)
TT	24	24	1.02 (0.57–1.83)
P_{trend}			0.96
IVS15+ 556T>C			
TT	443	402	1.0 (Ref)
TC	253	312	0.73 (0.59–0.91)
CC	51	44	1.05 (0.68–1.61)
P_{trend}			0.03
Ex15+ 78C>T			
CC	489	518	1.0 (Ref)
CT	209	191	1.13 (0.90–1.43)
TT	23	17	1.60 (0.84–3.05)
P_{trend}			0.42
TFRC			
IVS1+ 1014A>G			
CC	622	635	1.0 (Ref)
CA	94	92	1.03 (0.76–1.41)
AA	6	5	1.20 (0.36–3.98)
P_{trend}			0.95
Ex4− 11G>A			
GG	207	221	1.0 (Ref)
GA	375	363	1.11 (0.88–1.42)
AA	157	173	0.96 (0.72–1.29)
P_{trend}			0.24
IVS17+ 445C>T			
CC	295	306	1.0 (Ref)
CT	347	334	1.09 (0.88–1.36)
TT	99	116	0.92 (0.67–1.26)
P_{trend}			0.36
Ex19− 630A>G			
AA	344	347	1.0 (Ref)
AG	320	329	0.98 (0.79–1.22)
GG	72	73	0.98 (0.68–1.40)
P_{trend}			0.82
HMOX1			
IVS1+ 429G>A			
GG	308	309	1.0 (Ref)
GA	341	341	0.97 (0.78–1.21)
AA	92	95	0.97 (0.70–1.35)
P_{trend}			0.99
Ex1− 5G>C			
GG	663	660	1.0 (Ref)
GC	77	76	0.99 (0.71–1.39)
CC	1	5	0.23 (0.03–1.98)
P_{trend}			0.52
IVS3+ 244A>G			
AA	213	210	1.0 (Ref)
AG	368	375	0.97 (0.76–1.23)
GG	161	167	0.97 (0.72–1.30)
P_{trend}			0.95
SLC40A1			
IVS2+ 1646G>A			
GG	274	279	1.0 (Ref)
GA	349	353	0.97 (0.77–1.22)
AA	116	122	0.95 (0.70–1.29)
P_{trend}			0.66
IVS5− 2142A>G			
AA	406	399	1.0 (Ref)
AG	265	295	0.89 (0.71–1.10)
GG	56	45	1.22 (0.80–1.86)
P_{trend}			0.41
Ex6− 98T>C			
TT	281	288	1.0 (Ref)
TC	334	337	1.01 (0.81–1.27)
CC	117	121	0.96 (0.71–1.30)
P_{trend}			0.79
SLC11A2			
IVS1− 130G>A			
GG	231	228	1.0 (Ref)
GA	351	373	0.96 (0.76–1.21)
AA	161	142	1.12 (0.83–1.50)
P_{trend}			0.51
IVS15− 1125G>C			
GG	500	497	1.0 (Ref)
GC	217	229	0.96 (0.76–1.20)
CC	27	28	0.93 (0.54–1.61)
P_{trend}			0.91
Ex16− 456T>C			
TT	256	252	1.0 (Ref)
TC	341	351	0.98 (0.78–1.23)
CC	127	116	1.07 (0.78–1.46)
P_{trend}			0.68
Ex16+ 1142G>A			
GG	671	666	1.0 (Ref)
GA	69	89	0.72 (0.52–1.01)
AA	1	1	0.80 (0.05–12.91)
P_{trend}			0.18

(Continued on the following page)

Table 5. Distribution of iron-related genotypes and risk of colorectal adenoma (n = 770 cases and 777 controls) in the PLCO Cancer Screening Trial (Cont'd)

Genotype	Cases, *n*	Controls, *n*	OR[a] (95% CI)
HAMP			
1,039-bp 3′ of STP A>T			
AA	310	329	1.0 (Ref)
AT	336	336	1.04 (0.84–1.30)
TT	91	91	1.02 (0.73–1.42)
P_{trend}			0.33
IVS1− 251A>G			
AA	206	190	1.0 (Ref)
AG	387	392	0.88 (0.69–1.13)
GG	150	170	0.78 (0.58–1.05)
P_{trend}			0.41
ACO1			
IVS1− 7563C>T			
CC	402	385	1.0 (Ref)
CT	283	319	0.87 (0.70–1.08)
TT	56	47	1.17 (0.77–1.78)
P_{trend}			0.43
IVS1− 3873A>G			
AA	312	311	1.0 (Ref)
AG	346	346	0.98 (0.78–1.21)
GG	86	102	0.84 (0.61–1.17)
P_{trend}			0.45
IVS1+ 4665C>T			
CC	455	441	1.0 (Ref)
CT	248	277	0.89 (0.72–1.11)
TT	46	36	1.27 (0.80–2.00)
P_{trend}			0.46
IVS1+ 7051C>G			
CC	185	200	1.0 (Ref)
CG	364	378	1.04 (0.81–1.33)
GG	181	164	1.18 (0.88–1.58)
P_{trend}			0.65
IVS1+ 9689C>T			
CC	364	340	1.0 (Ref)
CT	305	331	0.89 (0.72–1.11)
TT	76	83	0.87 (0.61–1.23)
P_{trend}			0.67
IVS2+ 665A>T			
AA	250	285	1.0 (Ref)
AT	363	354	1.15 (0.92–1.45)
TT	121	108	1.27 (0.93–1.74)
P_{trend}			0.36
IVS4− 882C>T			
CC	379	388	1.0 (Ref)
CT	310	302	1.09 (0.88–1.35)
TT	49	65	0.78 (0.53–1.17)
P_{trend}			0.22
IVS4+ 793G>A			
GG	536	531	1.0 (Ref)
GA	179	195	0.90 (0.71–1.14)
AA	14	20	0.74 (0.37–1.50)
P_{trend}			0.39
IVS4+ 3642C>A			
CC	545	546	1.0 (Ref)
CA	188	199	0.97 (0.77–1.22)
AA	12	12	0.97 (0.43–2.18)
P_{trend}			0.88
IVS7− 505T>G			
TT	312	325	1.0 (Ref)
TG	344	350	1.00 (0.81–1.25)
GG	87	78	1.12 (0.79–1.58)
P_{trend}			0.88
IVS10− 160T>G			
TT	299	301	1.0 (Ref)
TG	334	349	0.95 (0.76–1.19)
GG	95	85	1.10 (0.78–1.54)
P_{trend}			0.86
IVS15− 752G>A			
GG	222	234	1.0 (Ref)
GA	355	374	0.99 (0.78–1.25)
AA	166	151	1.13 (0.84–1.51)
P_{trend}			0.46
IVS15+ 90A>G			
AA	321	324	1.0 (Ref)
AG	306	325	0.94 (0.75–1.17)
GG	101	90	1.10 (0.79–1.52)
P_{trend}			0.79
HP01			
IVS6+ 47C>A			
CC	534	556	1.0 (Ref)
CA	208	197	1.09 (0.87–1.37)
AA	0	1	–

[a]Adjusted for age (≤59, 60–64, 65–69, 70–74, ≥75 years).

in the first quintile of intake, the colorectal cancer burden would be lowered by 9% (39). One of the hypothesized mechanisms relating red meat to colorectal carcinogenesis is the endogenous formation of NOCs, which is related to heme iron intake (10). Previous studies have attempted to estimate heme iron intake by applying a standard factor to total iron intake from meat. Using such estimations, 2 studies reported no association between heme iron intake and colorectal adenoma (19), nor cancer (13), whereas 2 other studies found positive associations within the

subgroups of males (12) and proximal colon cancers (14). The heme iron content of meat can vary not only by meat type but also by cooking method and doneness level (40, 41); therefore, these previous estimations of heme iron likely lead to a substantial degree of error in risk estimates. Our study utilized a unique, newly developed heme iron database to assign specific heme iron values according to meat type, cooking method, and doneness level (24). Despite this improved assessment tool, we did not identify a statistically significant association between heme iron intake and colorectal adenoma incidence. We note, however, that the serologic sample was relatively small and the power to detect modest associations may be limited.

Our study found no evidence for an association between variation in iron metabolism genes and colorectal adenoma, although we did observe significant relationships between several SNPs and serum iron indices, suggesting that circulating iron levels may be influenced by genetic variation in iron uptake and regulatory pathways. Most prior studies that have investigated iron homeostasis genes and colorectal neoplasia have focused on the *HFE* gene, which is related to the iron overload condition hereditary hemochromatosis. Although a previous study found a relation between SNPs in *HFE* and colorectal cancer risk, none of the investigations of colorectal adenoma have found such associations, including a study within the PLCO Cancer Screening Trial (18, 19). A limitation of the current study was possible low coverage of variation across the gene regions of interest and hence potential variants associated with colorectal adenoma may have been missed. However, when we subsequently investigated the extent to which our selected SNPs accounted for the total variation within these gene regions in HapMap, the coverage was more than 75% for all genes except for *HP01* and *HAMP*. In addition, we note that recent genome-wide association studies of colorectal cancer did not reveal any statistically significant relationships between SNPs in iron metabolism gene regions and colorectal cancer, suggesting that germline variation within these genes does not contribute to colorectal tumorigenesis (42).

The advantages of our study included the study of screening detected adenomas, which meant that the cases and controls were asymptomatically selected during a standardized procedure, and being precursor lesions, they were less likely to have been bleeding at the time of the baseline serum collection, which could bias the risk estimates. Furthermore, we used prospectively collected serum samples and dietary data, which included a unique heme iron database to more accurately assess intake of heme iron. We also conducted a small substudy to evaluate the reliability of a one-time measurement to capture iron parameter levels by comparing serum iron indices in repeat specimens several years apart from the same individual. We found very good agreement for TIBC, UIBC, and ferritin; however, the ICCs for iron and transferrin saturation were much lower, indicating that a one-time measurement is not likely to reflect average exposure levels, and future investigations may wish to collect data from multiple time points to more accurately assess these factors. Our study also had several caveats, including limited power for detecting associations within genetic subgroups, and the use of flexible sigmoidoscopy as the screening method meant that some of the controls could have had proximal adenomas out of reach of detection of the 60-cm sigmoidoscope.

To conclude, both TIBC and UIBC were inversely related to colorectal adenoma. These findings are consistent with laboratory data that support a role for iron in the early stages of colorectal tumorigenesis. If real, the iron metabolic pathway may offer a potential route for chemopreventive strategies against colorectal cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors thank Drs. Christine Berg and Philip Prorok, Division of Cancer Prevention, National Cancer Institute, the Screening Center Investigators, and staff of the PLCO Cancer Screening Trial; Mr. Tom Riley and staff, Information Management Services, Inc.; Ms. Barbara O'Brien and staff, Westat, Inc.; Mr. Tim Sheehy and staff, DNA Extraction and Staging Laboratory, SAIC-Frederick, Inc; and Ms. Jackie King and staff, BioReliance, Inc. Most importantly, the authors acknowledge the study participants for their contributions to making this study possible.

Grant Support

This research was supported by the Intramural Research Program of the Division of Cancer Epidemiology and Genetics and by contracts from the Division of Cancer Prevention, National Cancer Institute, NIH, Department of Health and Human Services.

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Received February 24, 2011; revised May 13, 2011; accepted June 1, 2011; published OnlineFirst June 17, 2011.

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