Hemochromatosis Gene Mutations, Body Iron Stores, Dietary Iron, and Risk of Colorectal Adenoma in Women

Andrew T. Chan, Jing Ma, Gregory J. Tranah, Edward L. Giovannucci, Nader Rifai, David J. Hunter, Charles S. Fuchs

Background: **Some experimental evidence suggests that iron may play a role in colorectal carcinogenesis, but human data for this role have been conflicting, possibly because of problems related to study design or measurement of iron exposure. We assessed dietary iron intake and genetic and biochemical markers of iron status in a prospective, nested case–control study of women enrolled in the Nurses' Health Study.** ***Methods:*** **Among 32 826 women who provided a blood specimen, we identified 527 women with colorectal adenoma and 527 matched control subjects who underwent endoscopy but were not diagnosed with adenoma after blood collection. We assessed iron intake, mutations in the HFE gene that are associated with hereditary hemochromatosis (i.e., H63D and C282Y), and plasma biochemical measures of total body iron, including transferrin saturation and the ratio of the concentrations of transferrin receptors to ferritin. Logistic regression models were used to estimate relative risks (RR) and 95% confidence intervals (CI). All statistical tests were two-sided.** ***Results:*** **Women with any HFE gene mutation had higher total body iron stores, as reflected by higher transferrin saturations (P<.001) and lower levels of the ratio of transferrin receptors to ferritin (P = .02), than women with no HFE gene mutation. However, HFE gene mutations were not associated with risk of adenoma (multivariable RR = 1.08, 95% CI = 0.83 to 1.39; P = .58). Moreover, comparison of extreme categories showed no associations between adenoma and the extent of transferrin saturation (multivariable RR = 0.96, 95% CI = 0.63 to 1.47; P_{trend} = .66), the ratio of transferrin receptors to ferritin (multivariable RR = 0.98, 95% CI, 0.60 to 1.60; P_{trend} = .99), or dietary iron intake (multivariable RR = 1.04, 95% CI = 0.68 to 1.57; P_{trend} = .94).** ***Conclusions:*** **Although our study used several distinct measures of iron status (i.e., genetic mutations, biochemical markers, and dietary intake) and a nested case–control design, we did not observe a role for iron in the pathogenesis of colorectal neoplasia in women. [J Natl Cancer Inst 2005;97:917–26]**

Experimental data *(1)* have demonstrated that iron may be carcinogenic because it can catalyze the formation of free radicals, suppress the host immune system, and fuel the growth of tumor cells. However, a role for iron in the promotion of colorectal neoplasia in humans remains uncertain. Epidemiologic studies of dietary iron intake *(2–13)* or traditional biochemical markers of total body iron stores *(4,8,9,14–19)* and the risk of colorectal cancer or adenoma have been inconsistent.

Nevertheless, a genetic predisposition to colorectal cancer mediated through iron homeostasis has been hypothesized. In particular, mutations associated with hereditary hemochromatosis, an autosomal recessive disorder associated with total body iron overload, are the most likely candidates to mediate an association between iron homeostasis and colorectal cancer. Hereditary hemochromatosis is most commonly associated with polymorphisms of the HFE gene on chromosome 6. In Caucasian populations, the two most common mutations in HFE are C282Y, a guanine-to-adenine transition resulting in a cysteine-to-tyrosine change with an allele frequency of 0.06, and H63D, a cysteine-to-guanine transversion resulting in a histidine-to–aspartic acid change with an allele frequency of 0.15 *(20,21)*. Although HFE gene mutations in the heterozygous state have been found to be associated with iron overload *(20,22,23)*, studies evaluating the association between HFE gene mutation carrier status and colorectal cancer or adenoma have been conflicting *(24–29)*. Moreover, to our knowledge, no study has examined the association of HFE gene mutations with colorectal neoplasia in conjunction with other biochemical measures of iron status, such as the concentration of plasma iron, transferrin (i.e., total iron binding capacity), and ferritin.

Given these equivocal data on iron status and colorectal neoplasia, we performed an evaluation of the risk of colorectal adenoma in relation to HFE gene mutations, dietary iron intake, and biochemical markers of total body iron in a prospective, nested case–control study of women in the Nurses' Health Study. Because traditional biochemical markers such as plasma iron, transferrin, and ferritin may be perturbed by acute-phase responses and generalized inflammation, we also used soluble transferrin receptors, a novel marker of iron stores. In addition, we measured the ratio of the concentrations of transferrin receptors to ferritin; this ratio is widely considered the most reliable noninvasive measure of body iron stores *(30)*. Because we collected dietary information, plasma, and DNA for genetic analysis prior to the diagnosis of adenoma, we had the unique opportunity to prospectively evaluate each of these measures of iron status in relation to the risk of subsequent adenoma.

Subjects and Methods

Study Participants

Case subjects and control subjects were drawn from the Nurses' Health Study, which began in 1976 when 121 701 U.S.

Affiliations of authors: Gastrointestinal Unit, Massachusetts General Hospital and Harvard Medical School (ATC); Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School (ATC, JM, ELG, DJH, CSF); Cancer Epidemiology Program, Dana–Farber/Harvard Cancer Center (ELG, DJH, CSF); Departments of Epidemiology (GJT, ELG, DJH) and Nutrition (ELG, DJH), Harvard School of Public Health; Department of Laboratory Medicine, Children's Hospital (NR); Department of Medical Oncology, Dana–Farber Cancer Institute (CSF), Boston, MA.

Correspondence to: Andrew Chan, MD, MPH, Gastrointestinal Unit, Massachusetts General Hospital GRJ-722, Boston, MA 02114 (e-mail: achan@partners.org).

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female registered nurses, aged 30 to 55 years, completed a questionnaire about their health history. Questionnaires have been mailed to the participants every 2 years to update information on lifestyle factors, medication usage, examination by colonoscopy or sigmoidoscopy, and indications for these procedures. The participants also report newly diagnosed cases of colorectal polyps, cancer, and other diseases. In 1989 through 1990, we collected a blood specimen from 32 826 participants *(31)*. As previously detailed *(32)*, women who provided a blood specimen were generally similar to women who did not; however, the proportion of women who were current smokers was lower among women who gave a blood specimen (14.4%) than among women who did not (25.0%). Subsequent follow-up of this subcohort of women has been greater than 96%. The Human Research Committee at the Brigham and Women's Hospital and the Harvard School of Public Health approved this study.

Selection of Colorectal Adenoma Case Subjects and Control Subjects

When a participant reported a polyp on a biennial questionnaire, we asked for her informed consent to obtain medical records and pathology reports. With each biennial questionnaire, we obtained records on over 90% of reported polyps. Study investigators, blinded to risk factors and other medical history, reviewed all records and extracted data on histologic type, anatomic location, and size of polyps. Women were eligible for selection as either an adenoma case subject or a control subject if they were among the 14 019 women who had provided a blood specimen in 1989–1990 and reported having at least one sigmoidoscopy or colonoscopy from 1990 through 1998 after providing a blood sample. Women who had a history of inflammatory bowel disease, a familial polyposis syndrome, or diagnosed adenoma or cancer (except non-melanoma skin cancer) prior to blood draw were excluded. Case subjects among the eligible women were women who reported an incident polyp that was confirmed to be adenomatous after blinded review of medical and pathology records. We confirmed only 149 incident colorectal cancers during the study period and were therefore unable to include women with colorectal cancer in this analysis.

Consistent with other studies *(33)*, we defined advanced lesions as adenomas of at least 1 cm in diameter or any size with tubulovillous, villous, or severely dysplastic features. Early lesions were defined as adenomas with tubular histologic characteristics and of less than 1 cm in diameter. We matched one control subject (i.e., women who did not report a polyp, including hyperplastic, on endoscopy) to each case subject on date of endoscopy (i.e., had to be performed during the same 2-year period), birth year, indication for endoscopy, time period of any prior endoscopy, month and year of blood draw, and fasting status. We initially identified a total of 557 matched pairs that were eligible for analysis. We subsequently excluded five matched pairs for whom one member of the pair did not provide dietary data and 25 matched pairs for whom one member of the pair had insufficient DNA sample for genotyping. Thus, 1054 women (527 case subjects and 527 control subjects) were included in this analysis.

Laboratory Procedures

We sent a phlebotomy kit to all women willing to provide a blood specimen in 1989–1990. After receipt by overnight courier, the chilled heparinized blood was immediately centrifuged; aliquoted into plasma, erythrocytes, and buffy coat fractions; and stored in continuously monitored liquid nitrogen freezers. Over 97% of the blood samples arrived in our laboratory within 26 hours of phlebotomy. Quality control samples were routinely frozen along with study samples to monitor for changes associated with storage and to assess any assay variability.

Laboratory personnel blinded to quality control and case–control status conducted all assays. For genotyping, we extracted genomic DNA from 50 μL of buffy coat diluted with 150 μL of phosphate-buffered saline (PBS) using the QIAmp (Qiagen Inc., Chatsworth, CA) 96-spin blood protocol. Genomic DNA concentrations were calculated using PicoGreen technology (Molecular Probes, Eugene, OR). Using the 5′ nuclease assay (TaqMan) (Applied Biosystems, Foster City, CA), we performed genotyping of the HFE gene mutations C282Y (rs1800562) and H63D (rs1799945) using TaqMan primers and probes. The polymerase chain reaction amplifications were carried out on 5–20 ng of DNA using 1× TaqMan Universal PCR Mix (No AmpErase UNG). Amplification conditions on an AB9700 dual plate thermal cycle (Applied Biosystems, Foster City, CA) were as follows: 1 cycle of 95 °C for 10 minutes followed by 50 cycles of 92 °C for 15 seconds and 58 °C for 60 seconds. Following PCR amplification, end-point fluorescence of the reporter dyes was read with the Applied Biosystems 7900HT instrument, and genotypes were assigned using Allelic Discrimination Software (Applied Biosystems SDS Software v1.7a). We inserted quality-control samples equal to 10% of the total number of samples to validate genotype identification procedures; concordance for blinded samples was 100%.

Because of the substantial expense of the plasma-based assays, we were funded to conduct measurements of concentrations of iron, transferrin, ferritin, and soluble transferrin receptors among only the 759 subjects with either distal adenoma (n = 380) or a control subject matched to a woman with distal adenoma (n = 379). Moreover, we obtained similar results for genotype, dietary iron, and distal adenoma risk among these subjects. We used a Hitachi 917 analyzer (Roche Diagnostics, Indianapolis, IN) to measure iron by a colorimetric assay (Roche Diagnostics, Indianapolis, IN), transferrin by an immunoturbidimetric assay (Roche Diagnostics, Indianapolis, IN), ferritin by a particle-enhanced immunoturbidimetric assay (Kamiya Biomedical, Seattle, WA), and soluble transferrin receptors by a particle-enhanced immunoturbidimetric assay (Roche Diagnostics, Indianapolis, IN). The intra-assay coefficients of variations from the blinded quality control samples for each analyte were as follows: iron, 5.2%; transferrin, 6.4%; ferritin, 4.6%; and transferrin receptors, 7.6%. We calculated transferrin saturation as plasma iron/transferrin × 100.

Assessment of Dietary and Nutrient Intake

Every 4 years, we assessed diet in the Nurses' Health Study by using a validated semiquantitative food frequency questionnaire *(34,35)*. In the present analysis, we used data from the baseline dietary questionnaire (from 1990), which included 131 food items. For each food, a commonly used unit or portion size was specified, and the women were asked how often, on average, they had consumed that food over the past year. We computed nutrient intakes by multiplying the frequency of consumption of each food by the nutrient content of the specified portions using

composition values from US Department of Agriculture sources, supplemented with other data, including the components of specific multivitamins and breakfast cereals *(34,35)*.

Statistical Analysis

We first calculated means (± standard deviation [SD]) and proportions of baseline characteristics for the case subjects and control subjects at the time of blood draw. The chi-square (χ^2) test was used to assess whether the HFE genotypes in the control subjects were in Hardy–Weinberg equilibrium. Because of the low prevalence of homozygous mutant genotypes, we combined women with one (heterozygous) and two (homozygous) mutant alleles for analysis and used women with no (homozygous wild-type) mutant alleles as the reference group for all analyses. We evaluated mean levels of iron markers in women with no mutation in the HFE gene compared with women with any HFE gene mutation. We categorized women into quartiles according to the distribution of biochemical markers and nutrient intakes in the control participants.

Wilcoxon signed-rank and χ^2 tests were used for comparisons of the means and proportions of the baseline characteristics. We calculated Pearson coefficients to estimate the correlation between mean levels of dietary or biochemical iron. We estimated relative risks (RR) and corresponding 95% confidence intervals (CI) for associations among genotype, quartile-specific markers, and quartile-specific nutrient intakes and colorectal adenoma using logistic regression models. We obtained similar results using conditional logistic regression models or unconditional logistic regression models adjusting for matching factors. To increase statistical power in our stratified analyses of nutrient intake, biomarkers, and genotype, we used unconditional regression models adjusting for matching factors and adenoma risk factors. Tests for trend were conducted using the median values for each quartile of iron markers or nutrient intake as a continuous variable in the regression models. To reflect participant characteristics when measures of total body iron were assessed, we used baseline data for all nutrient intake and covariate data at the time of blood draw. Details of the assessment of covariates have been previously described *(36–41)*.

Although we did not ask the participants to specify whether they had had a colonoscopy or sigmoidoscopy, based on secular trends *(42)* we assumed that a substantial portion of the procedures performed early in the study period may have been sigmoidoscopies, which encompass examination of the distal colon and rectum only. Hence, we also performed a secondary analysis in which we restricted our analyses to case subjects and their matched control subjects who received endoscopies later in the study period (1996–1998). We also assessed for the potential effect modification or statistical interaction by using a log-likelihood ratio test to compare the goodness of fit of the model with interaction terms (genotype * dietary iron intake), with the reduced model containing indicator variables of the main effects of genotype and iron intake (without interaction terms). We used the SAS version 8.2 statistical package (SAS Institute, Cary, NC) for all analyses. All *P* values are two-sided.

Results

The baseline characteristics of the study population (527 case subjects with colorectal adenoma and 527 control subjects with no adenomas found on sigmoidoscopy or colonoscopy) are presented in Table 1. The mean age of the study cohort was 57 years at the time of blood draw, and over 98% of the participants were Caucasian. Overall, women who were subsequently diagnosed with colorectal adenoma were more likely than women who did not have an adenoma to have a family history of colorectal cancer, smoke currently, have a greater body mass index, exercise less, consume more red meat, consume less calcium and folate and were less likely to use aspirin, multivitamins, and postmenopausal hormones.

The distributions of both HFE genotypes among the control subjects were in Hardy–Weinberg equilibrium, and the allele frequencies were 0.06 and 0.15 for the C282Y and H63D alleles, respectively. These frequencies were similar to those reported in other predominantly Caucasian populations *(21,43,44)*. There were no statistically significant differences between the distribution of HFE genotypes (P =.52) or of several plasma biomarkers that estimate total body iron stores in the case subjects and control subjects (Table 1). The Pearson correlation coefficients between these biomarkers and dietary intake of iron and heme iron are shown in Table 2.

Because HFE gene mutations have been associated with elevated total body iron *(45–47)*, we investigated the relationship between HFE genotype and plasma iron biomarkers of total body iron stores in 759 women within the cohort (Table 3). Women with any HFE gene mutation had higher mean plasma iron (P<.001) and lower mean transferrin levels (P<.001) and higher calculated transferrin saturation (P<.001) than women with no HFE gene mutation. Although plasma ferritin levels were not associated with the presence of an HFE gene mutation (P = .20), women with any HFE gene mutation had statistically significantly lower levels of transferrin receptors (P<.001) and of the ratio of the concentration of transferrin receptors to ferritin (P =.02) than women with no HFE gene mutation.

In view of the relationship between total body iron stores and HFE gene mutations, we examined the relationship between the presence of any HFE gene mutation and the risk of developing an adenoma. We observed no statistically significant difference in risk of adenoma in women with any HFE gene mutation and women with no HFE gene mutations, even after adjustment for adenoma risk factors (multivariable RR = 1.08; 95% CI = 0.83 to 1.39; P = .58). Moreover, there was no statistically significant increase in risk of either early or advanced adenomas (Table 4) or small (<1 cm in diameter) or large (≥1 cm in diameter) adenomas (data not shown).

We also evaluated the relationship between increased levels of dietary iron intake and risk of adenoma (Table 5). Intake of both total dietary iron and heme iron, which is purported to be a more bioavailable form of the element, was not associated with adenoma risk (P_{trend} = .94 and .23, respectively). Furthermore, adjustment for zinc intake or exclusion of the 60 participants who were iron supplement users did not materially alter these results (data not shown). However, we observed that women who consumed at least one serving of red meat per day had a higher risk of adenoma than women who consumed less than one serving per week (age-adjusted RR = 1.65; 95% CI = 1.02 to 2.67; P_{trend} = .07), confirming findings in a previous analysis of the larger cohort of women enrolled in the NHS *(48)*. In the smaller cohort analyzed in the current study, adjustment for several additional adenoma risk factors and parameters for iron status modestly attenuated

Table 1. Baseline characteristics of study participants

Baseline characteristic	Case subjects (N = 527)	Control subjects (N = 527)	*P**
Demographic			
Age in years, mean (standard deviation [SD])	58.1 (6.5)	58.0 (6.5)	.81
Race†			
Nonwhite, no. (%)	7 (1)	8 (2)	.79
White, no. (%)	520 (99)	519 (98)	
Age at menarche, years, mean (SD)	12.6 (1.4)	12.6 (1.4)	.11
Postmenopausal,‡ no. (%)	455 (86)	454 (86)	.93
Age at last menses,§ years, mean (SD)	46.7 (5.9)	46.6 (6.0)	.60
Family history of colorectal cancer,‖ no. (%)	142 (27)	87 (17)	<.001
Lifestyle/clinical			
Smoking status¶			
Current, no. (%)	81 (15)	47 (9)	<.01
Former, no. (%)	228 (43)	232 (44)	
Never, no. (%)	218 (41)	245 (47)	
Body mass index,# mean (SD)	25.8 (4.6)	25.3 (4.2)	.12
Physical activity,** mean (SD)	15.6 (26.4)	18.1 (23.8)	<.01
History of diabetes mellitus, no. (%)	18 (3.4)	17 (3.2)	.86
Current use of aspirin,†† no. (%)	134 (25)	180 (34)	<.01
Current use of multivitamins, no. (%)	198 (38)	222 (42)	.13
Current use of iron supplements, no. (%)	31 (6)	29 (6)	.81
Current use of postmenopausal hormones,‡‡ no. (%)	191 (42)	231 (51)	.03
History of previous endoscopy, no. (%)	209 (40)	185 (35)	.13
Dietary intake§§			
Iron, mg/day, mean (SD)	18.1 (16.5)	18.5 (16.5)	.45
Heme iron, mg/day, mean (SD)	0.98 (0.35)	0.96 (0.39)	.24
Calcium, mg/day, mean (SD)	1059 (542)	1094 (512)	.06
Folate, mg/day, mean (SD)	434 (219)	453 (220)	.14
Red meat, servings/day, mean (SD)	0.75 (0.53)	0.69 (0.48)	.09
Alcohol, g/day, mean (SD)	5.8 (10.3)	5.7 (10.0)	.57
HFE genotype			
Wild-type/wild-type, no. (%)	329 (62)	332 (63)	.52
H63D/wild-type, no. (%)	120 (23)	123 (23)	
H63D/ H63D, no. (%)	15 (3)	14 (3)	
C282Y/wild-type, no. (%)	54 (10)	51 (10)	
C282Y/C282Y, no. (%)	1 (0.2)	4 (0.8)	
C282Y/H63D, no. (%)	8 (1.5)	3 (0.6)	
Biochemical‖‖			
Iron, μg/L, mean (SD)	92.5 (28.8)	92.7 (28.9)	.83
Transferrin, mg/dL, mean (SD)	266.1 (41.5)	266.6 (45.2)	.76
Transferrin saturation, no. (%)	35 (12)	35 (12)	.89
Soluble transferrin receptor, mg/L, mean (SD)	3.27 (0.98)	3.30 (1.3)	.93
Ferritin, ng/mL, mean (SD)	83.5 (73.7)	86.9 (86.4)	.83
Transferrin receptor/ferritin ratio	89.0 (116)	92.8 (123)	.80
Transferrin saturation > 45%, no. (%)	73 (19)	68 (18)	.29

*P values are for the hypothesis test of no difference between mean values (Wilcoxon signed rank) or categories (χ^2 test).

†Nonwhite includes African-American, Hispanic, and Asian. White includes Southern European/Mediterranean, Scandinavian, and other Caucasian.

‡Women were considered to be postmenopausal if they (*a*) reported having a natural menopause (e.g., no menstrual cycles during the previous 12 months), (*b*) had a bilateral oophorectomy, or (*c*) had a hysterectomy but had at least one ovary remaining and were at least 56 (for nonsmokers) or 54 (for smokers) years of age. These were the ages at which natural menopause occurred for 90% of the overall cohort.

§Age at last menses includes women who have reached natural menopause or who underwent oophorectomy and/or hysterectomy.

‖Colorectal cancer in a parent or sibling.

¶Control subjects do not sum to the total because three subjects did not report smoking status.

#Weight in kilograms divided by the square of the height in meters.

**Metabolic equivalent task score hours per week.

††Regular use of at least two standard tablets per week.

‡‡Postmenopausal hormones are defined as estrogen and estrogen/progesterone preparations; and the percentage of current use is among postmenopausal women only.

§§Nutrient values represent the mean of energy-adjusted intake, including supplements. Red meat intake is a composite of beef, pork, or lamb as a main dish or mixed dish/sandwich, hamburgers, hot dogs, bacon, and processed meats.

‖‖Analytes were measured in 380 case subjects with distal colorectal adenoma and 379 control subjects. Transferrin saturation is calculated as plasma iron/transferrin × 100. Not all subjects are included in all analyses because of missing data.

the relationship with red meat (multivariable RR = 1.57; 95% CI = 0.93 to 2.65; P_{trend} = .22).

In addition to examining HFE gene mutations and dietary iron intake and risk of adenoma, we examined the relationship between plasma biomarkers of total body iron and risk of adenoma. Within the cohort of 759 women with distal colorectal adenoma and matched control subjects, we did not find an association between any measures of total body iron and risk of adenoma (Table 5). We also evaluated the risk of distal adenoma associated with a transferrin saturation of more than 45%, which both the American and European Associations for the Study of Liver Disease recommend as a clinical cutpoint for initial

Table 2. Pearson correlation coefficients between dietary iron intake, heme iron, plasma iron, transferrin, transferrin saturation, ferritin, soluble transferrin receptor, and the ratio of the concentration of transferrin receptor to ferritin among control subjects*

Variable	Dietary iron	Heme iron	Plasma iron	Transferrin	Saturation	Ferritin	Receptor	Ratio
Dietary iron	1.00							
Heme iron	0.06	1.00						
Plasma iron	−0.12†	−0.02	1.00					
Transferrin	−0.17‡	−0.02	0.12†	1.00				
Transferrin saturation	−0.02	−0.02	0.84§	−0.39§	1.00			
Ferritin	0.20‖	<−0.01	0.07	−0.31§	0.21§	1.00		
Transferrin receptor	0.06	0.05	−0.15‡	0.18‖	−0.20§	<0.01	1.00	
Ratio¶	−0.01	0.04	−0.21§	0.44§	−0.35§	−0.44§	0.60§	1.00

*Among case subjects with distal colorectal adenoma and matched control subjects.
†$P<.05$.
‡$P<.01$.
§$P<.001$.
‖$P<.001$.
¶Ratio of the concentration of transferrin receptors to ferritin.

screening of participants with suspected iron overload *(49,50)*. Compared with women with a transferrin saturation of 45% or less, the 141 women with a saturation of more than 45% had a multivariable RR of 1.06 (95% CI = 0.73 to 1.56; $P = .75$) for distal adenoma.

We considered the possibility that an association of HFE mutations with adenoma risk may be restricted to women who had exposure to sufficient levels of dietary iron. However, our assessment of the joint effect of dietary iron and genotype (Table 6) revealed that there were no statistically significant interactions between genotype and either total iron intake ($P_{interaction} = .70$) or heme iron intake ($P_{interaction} = .69$). Compared with women with no HFE gene mutation in the lowest quartile of intake, women with any HFE gene mutation did not have a statistically significantly elevated risk of adenoma, even those women in the highest quartiles of total iron intake (multivariable RR = 1.14; 95% CI = 0.64 to 2.03; $P = .65$) or of heme iron intake (multivariable RR = 1.11; 95% CI = 0.64 to 1.93; $P = .71$).

Next, we evaluated the combined influence of HFE gene mutations and high total body iron on risk of adenoma. Compared with women with no HFE gene mutation and a transferrin saturation of 45% or less, women with any HFE gene mutation and a transferrin saturation of more than 45% had a multivariable RR of 1.06 (95% CI = 0.63 to 1.80; $P = .82$) for adenoma risk. Furthermore, women with any HFE gene mutation and a ratio of the concentration of transferrin receptors to ferritin in the lowest quartile had a multivariable RR of 0.83 (95% CI = 0.43 to 1.58; $P = .56$) compared with women with no HFE gene mutation and a ratio of the concentration of transferrin receptors to ferritin in the highest quartile.

We adjusted all of the multivariable models not only for several established or purported adenoma risk factors but also for a number of potential confounding factors, including age, fasting status, indication for endoscopy, time period of endoscopy, family history of colorectal cancer, alcohol intake, age at menarche, and age at menopause or most recent menses for premenopausal women. However, to minimize the potential for residual confounding, we also conducted additional analyses restricted to those women who provided a blood specimen at least 8 hours after a meal, to women who underwent endoscopy without any occult or visible bleeding, to women with no family history of colorectal cancer, and to postmenopausal women. We found no association between HFE genotype, dietary iron, and all iron biomarkers and risk of adenoma in any subgroup (data not shown). In addition, because age, alcohol use, and menses may influence iron stores *(5,51)* and because secular patterns in use of endoscopy may have influenced adenoma risk, we stratified the study population according to categories of these characteristics. Again, there were no associations between HFE genotype, dietary iron, and iron biomarkers and risk of adenoma within strata of age,

Table 3. Plasma biomarkers of body iron stores according to HFE genotype*

Biomarker†	Participants with no HFE gene mutation (N = 475)	Participants with any HFE gene mutation (N = 284)	Difference in means	P‡
Iron, μg/L, mean (95% CI)	89.0 (86.4 to 91.5)	98.7 (95.4 to 102.0)	9.7 (5.5 to 13.9)	<.001
Transferrin, mg/dL, mean (95% CI)	270.5 (266.6 to 274.4)	259.5 (254.4 to 264.5)	11.0 (4.6 to 17.4)	.001
Transferrin saturation, %, mean (95% CI)	33.5 (32.4 to 34.6)	38.9 (37.5 to 40.2)	5.4 (3.6 to 7.1)	<.001
Soluble transferrin receptor, mg/L, mean (95% CI)	3.43 (3.32 to 3.53)	3.06 (2.92 to 3.19)	0.37 (0.20 to 0.54)	<.001
Ferritin, ng/mL, mean (95% CI)	83.0 (75.6 to 90.4)	88.8 (79.3 to 98.3)	5.8 (6.2 to 17.8)	.20
Transferrin receptor/ferritin ratio, mean (95% CI)	98.9 (87.9 to 110.0)	77.7 (63.5 to 91.8)	21.3 (3.3 to 39.2)	.02
Transferrin saturation >45%, no. (%)	70 (15)	71 (25)		<.001

*Body iron stores were measured by plasma iron, transferrin, transferrin saturation, soluble transferrin receptor, ferritin, and the ratio of the concentration of the transferrin receptor to ferritin. No HFE gene mutation represents participants without any C282Y or H63D mutations. Any HFE gene mutation represents participants with at least one C282Y or H63D mutant allele.
†Analytes were measured in case subjects with distal colorectal adenoma and their matched control subjects. Transferrin saturation was calculated as plasma iron/transferrin × 100. Not all subjects are included in all analyses because of missing data.
‡P values are for the hypothesis test of no difference between mean values (Wilcoxon signed rank test) or categories (χ^2 test).

Table 4. Relative risk of colorectal adenoma according to HFE genotype*

Subgroup†	Participants with no HFE gene mutation	Participants with any HFE gene mutation
All adenomas		
No. of case subjects	329	198
No. of control subjects	332	195
Age-adjusted RR (95% CI)	1.00 (referent)	1.02 (0.80 to 1.32)
Multivariable-adjusted RR‡ (95% CI)	1.00 (referent)	1.08 (0.83 to 1.39)
Early adenomas		
No. of case subjects	187	109
No. of control subjects	332	195
Age-adjusted RR (95% CI)	1.00 (referent)	1.00 (0.74 to 1.34)
Multivariable-adjusted RR‡ (95% CI)	1.00 (referent)	1.10 (0.81 to 1.50)
Advanced adenomas		
No. of case subjects	121	77
No. of control subjects	332	195
Age-adjusted RR (95% CI)	1.00 (referent)	1.08 (0.77 to 1.51)
Multivariable-adjusted RR‡ (95% CI)	1.00 (referent)	1.11 (0.78 to 1.57)

*CI = confidence interval; RR = relative risk. No HFE gene mutation represents participants without any C282Y or H63D mutations. Any HFE gene mutation represents participants with at least one C282Y or H63D mutant allele. Genotype analyses include 527 case subjects of colorectal adenoma and 527 matched control subjects. RRs are for any HFE gene mutation compared with no HFE gene mutation.

†All adenomas include colorectal adenomas of any size or histologic subtype; early adenomas include colorectal adenomas of <1 cm in diameter and tubular histology; advanced adenomas include colorectal adenoma of ≥1 cm in diameter and containing tubulovillous, villous, or high-grade dysplasia histologic subtypes. A total of 33 case subjects did not have either adenoma size or histologic subtype.

‡Adjusted for age, fasting status, date of blood draw, time of blood draw, time period of endoscopy, symptoms at endoscopy (e.g., screening, bleeding, or abdominal pain), time period of any prior endoscopy, family history of colorectal cancer, body mass index, pack-years of smoking, physical activity, energy-adjusted intake (including supplements) of calcium and folate, servings of red meat, alcohol consumption, regular use of multivitamins, regular use of aspirin (at least two tablets per week), menopausal status, postmenopausal hormone use, age at first menarche, and age at last menstrual period.

alcohol intake, years of menstruation, or time period of endoscopy (data not shown).

Because we did not ask the study participants to specify whether they had had a colonoscopy or sigmoidoscopy, a substantial portion of procedures might have been sigmoidoscopies, which encompass examination of the distal colon and rectum only. To investigate the possibility that the observed null relationships for genotype and dietary iron and risk of adenoma may have been attenuated by undiagnosed proximal adenoma among control subjects, we repeated our analyses among the subgroup of 127 case subjects and 127 control subjects who underwent endoscopy during the last few years of the study (1996–1998), when a greater proportion of the control subjects were likely to have had colonoscopies than in earlier years *(42)*. Among women who underwent endoscopy after 1996, the multivariable odds ratio (OR) for adenoma in women with any HFE gene mutation compared with women with no HFE gene mutation was 1.16 (95% CI = 0.67 to 2.01). Moreover, there was no statistically significant association between either total iron intake or heme iron intake and risk of adenoma (P_{trend} = .48 and .19, respectively).

Discussion

Given the inconsistent data on the relationship between iron and colorectal neoplasia, we evaluated the risk of colorectal adenoma in relation to several markers of iron, including HFE mutations, dietary iron intake, and biochemical markers of total body iron. Our results do not support a role for iron in the pathogenesis of colorectal neoplasia. Although we confirmed that mutations in the HFE gene are associated with higher total body iron, we did not observe any statistically significant relationship between genotype or biochemical markers and risk of adenoma. Moreover, we did not find an association between dietary iron and risk of colorectal adenoma. To our knowledge, this study is the first to directly examine three distinct measures of iron status—genetic mutations, biochemical markers, and dietary intake—and subsequent risk of neoplasia.

Although iron has been hypothesized to enhance oxidative stress *(1)*, our findings are consistent with considerable evidence *(8,17,19,25,26,28,52–55)* that iron loading does not appear to be related to colorectal carcinogenesis. Individuals with hereditary hemochromatosis at the most extreme levels of chronic total body iron overload do not appear to have a higher risk of colorectal cancer. Early cohort studies *(52,53)* of hemochromatosis subjects found that they were at greater risk of other chronic disease but not of colorectal cancer. The largest population-based study to date *(54)* found a nearly 20-fold-higher risk of liver cancer in 1847 hemochromatosis subjects and a 1.5-fold higher risk of hepatobiliary cancer in their 5973 first-degree relatives. However, the risk of colorectal cancer was not increased in either group. Finally, a study of 230 hemochromatosis subjects found an elevated risk of non-hepatic cancers but no statistically significant elevation in risk of colorectal cancer *(55)*.

This study is also consistent with several previous case–control studies *(25,26,28)* that demonstrated no statistically significant association between heterozygosity for mutations in the HFE gene and colorectal cancer. A prior study of heterozygote carriers of hereditary hemochromatosis mutations *(24)* demonstrated a modestly increased risk of colorectal cancer (in both men and women) and adenoma (in women only). However, that study was performed before wide-scale genotyping became available; consequently, risk of adenoma was based on data obtained from hemochromatosis subjects and on the medical histories of their parents, who were presumed to be heterozygote carriers of the HFE gene. In another case–control study *(27)*, HFE gene mutations were a potential risk factor only when an additional mutation in the transferrin receptor was present and only for the combined endpoint of breast and colorectal cancer.

In the North Carolina Colon Cancer Study *(29)*, individuals with an HFE gene mutation had an odds ratio for colon cancer of

Table 5. Relative risk of colorectal adenoma according to intake of dietary iron and plasma markers of body iron stores*

Variable	Quartile 1	2	3	4	P_{trend}†
Intake					
Total iron intake					
mg/day	<10.5	10.5–12.7	12.8–21.2	≥21.3	
No. of case subjects	135	146	129	117	
No. of control subjects	131	131	133	132	
Age-adjusted RR (95% CI)	1.00 (referent)	1.08 (0.77 to 1.51)	0.94 (0.66 to 1.32)	0.86 (0.61 to 1.21)	.23
Multivariable-adjusted‡ RR (95% CI)	1.00 (referent)	1.13 (0.80 to 1.61)	1.03 (0.71 to 1.48)	1.04 (0.68 to 1.57)	.94
Heme iron intake					
mg/day	< 0.70	0.70–0.80	0.90–1.0	≥ 1.1	
No. of case subjects	86	104	143	194	
No. of control subjects	92	123	144	168	
Age-adjusted RR (95% CI)	1.00 (referent)	0.91 (0.61 to 1.34)	1.07 (0.73 to 1.55)	1.24 (0.87 to 1.78)	.08
Multivariable-adjusted‡ RR (95% CI)	1.00 (referent)	0.83 (0.55 to 1.25)	0.96 (0.65 to 1.44)	1.13 (0.74 to 1.72)	.23
Red meat intake§					
Servings	≤1/ wk	2–4/wk	5–6/wk	≥1/day	
No. of case subjects	41	130	228	128	
No. of control subjects	54	124	246	103	
Age-adjusted RR (95% CI)	1.00 (referent)	1.38 (0.86 to 2.23)	1.23 (0.79 to 1.91)	1.65 (1.02 to 2.67)	.07
Multivariable-adjusted‖ RR (95% CI)	1.00 (referent)	1.38 (0.84 to 2.27)	1.19 (0.74 to 1.90)	1.57 (0.93 to 2.65)	.22
Biomarker					
Plasma iron					
Concentration (μg/L)	<72	72–90	91–112	≥113	
No. of case subjects	91	108	90	88	
No. of control subjects	93	96	93	93	
Age-adjusted RR (95% CI)	1.00 (referent)	1.15 (0.77 to 1.71)	0.99 (0.65 to 1.49)	0.97 (0.64 to 1.46)	.68
Multivariable-adjusted‡ RR (95% CI)	1.00 (referent)	1.18 (0.78 to 1.80)	1.02 (0.66 to 1.58)	1.01 (0.65 to 1.56)	.85
Plasma transferrin					
Concentration (mg/dL)	<238	238–264	265–293	≥294	
No. of case subjects	93	112	86	86	
No. of control subjects	94	92	94	92	
Age-adjusted RR (95% CI)	1.06 (0.70 to 1.60)	1.30 (0.87 to 1.95)	0.98 (0.65 to 1.48)	1.00 (referent)	.46
Multivariable-adjusted‡ RR (95% CI)	1.03 (0.66 to 1.60)	1.30 (0.85 to 1.98)	0.93 (0.60 to 1.44)	1.00 (referent)	.52
Transferrin saturation¶					
Percentage (%)	<28	28–34	35–42	≥43	
No. of case subjects	96	103	89	89	
No. of control subjects	94	92	94	92	
Age-adjusted RR (95% CI)	1.00 (referent)	1.09 (0.73 to 1.63)	0.92 (0.62 to 1.39)	0.94 (0.63 to 1.42)	.62
Multivariable-adjusted‡ RR (95% CI)	1.00 (referent)	1.12 (0.74 to 1.70)	0.93 (0.61 to 1.41)	0.96 (0.63 to 1.47)	.66
Soluble transferrin receptor					
Concentration (mg/L)	<2.6	2.6–3.0	3.1–3.6	≥3.7	
No. of case subjects	86	108	99	84	
No. of control subjects	92	94	92	93	
Age-adjusted RR (95% CI)	1.04 (0.68 to 1.57)	1.27 (0.85 to 1.91)	1.19 (0.79 to 1.80)	1.00 (referent)	.66
Multivariable-adjusted‡ RR (95% CI)	1.12 (0.71 to 1.78)	1.41 (0.92 to 2.15)	1.17 (0.76 to 1.79)	1.00 (referent)	.36
Ferritin					
Concentration (ng/mL)	<29.9	29.9–59.3	59.4–113.8	≥113.9	
No. of case subjects	81	97	104	87	
No. of control subjects	90	91	91	91	
Age-adjusted RR (95% CI)	1.00 (referent)	1.18 (0.77 to 1.81)	1.27 (0.83 to 1.94)	1.06 (0.67 to 1.66)	.95
Multivariable-adjusted‡ RR (95% CI)	1.00 (referent)	1.20 (0.76 to 1.88)	1.14 (0.72 to 1.80)	0.91 (0.55 to 1.49)	.40
Transferrin receptor/ferritin					
Ratio	<26.4	26.4–54.0	54.1–102.7	≥102.8	
No. of case subjects	83	113	90	79	
No. of control subjects	89	90	89	88	
Age-adjusted RR (95% CI)	1.04 (0.66 to 1.64)	1.40 (0.92 to 2.14)	1.13 (0.74 to 1.73)	1.00 (referent)	.36
Multivariable-adjusted‡ RR (95% CI)	0.98 (0.60 to 1.60)	1.28 (0.81 to 2.03)	1.25 (0.79 to 1.98)	1.00 (referent)	.99

*Categories of intake are quartiles based on the distribution in control subjects except for red meat intake, for which servings are based on original categories provided in semi-quantitative food frequency questionnaire. Analysis of plasma iron, plasma transferrin, transferrin saturation, soluble transferrin receptor, ferritin, and the ratio of transferrin receptor to ferritin was limited to distal adenoma (total of 759 participants: 380 women case subjects and 379 control subjects). Not all subjects are included in all analyses because of missing data. RR = relative risk; CI = confidence interval.

†Tests for trend were conducted using the median values for each quartile of nutrient intake or analyte.

‡Adjusted for age, fasting status, date of blood draw, time of blood draw, time period of endoscopy, symptoms at endoscopy (e.g., screening, bleeding, or abdominal pain), time period of any prior endoscopy, family history of colorectal cancer, body mass index, pack-years of smoking, physical activity, energy-adjusted intake (including supplements) of calcium and folate, servings of red meat, alcohol consumption, regular use of multivitamins, regular use of aspirin (at least two tablets per week), menopause status, postmenopausal hormone use, age at first menarche, and age at last menstrual period.

§Composite servings of beef, pork, or lamb as a main dish or mixed dish/sandwich, hamburger, hot dogs, bacon, hot dogs, and processed meats.

‖Adjusted for age, fasting status, date of blood draw, time of blood draw, history of previous endoscopy, time period of endoscopy, time period of prior endoscopy symptoms at endoscopy (e.g., screening, bleeding, or abdominal pain), body mass index, pack-years of smoking, physical activity, dietary intake (including supplements) of calcium and folate, alcohol consumption, regular use of multivitamins, regular use of aspirin (at least two tablets per week), menopause status, postmenopausal hormone use, age at first menarche, and age at last menstrual period.

¶Transferrin saturation was calculated as the ratio of plasma iron to transferrin × 100.

Table 6. Multivariable odds ratio of colorectal adenoma according to HFE genotype and dietary iron*

Variable	Dietary categories			
	1	2	3	4
Total iron intake				
Participants with no HFE mutation	1.00 (referent)	1.12 (0.72 to 1.76)	0.91 (0.58 to 1.43)	0.93 (0.56 to 1.53)
Participants with any HFE mutation	0.91 (0.54 to 1.52)	1.06 (0.64 to 1.75)	1.17 (0.68 to 2.01)	1.14 (0.64 to 2.03)
Heme iron intake				
Participants with no HFE mutation	1.00 (referent)	0.75 (0.45 to 1.24)	0.98 (0.60 to 1.60)	1.20 (0.73 to 1.97)
Participants with any HFE mutation	1.09 (0.58 to 2.07)	1.07 (0.60 to 1.91)	1.01 (0.58 to 1.74)	1.11 (0.64 to 1.93)

*No HFE mutation represents participants without any C282Y or H63D mutations. Any HFE mutation represents participants with at least one C282Y or H63D mutant allele. Analyses include 527 case subjects with colorectal adenoma and 527 matched control subjects; the referent category included women with no mutant alleles and the lowest iron intake. Models included indicator variables for the combination of genotype and quartile-specific dietary iron intake (based on quartile distribution in control subjects). Odds ratios were adjusted for age, fasting status, date of blood draw, time of blood draw, time period of endoscopy, symptoms at endoscopy (e.g., screening, bleeding, or abdominal pain), time period of any prior endoscopy, family history of colorectal cancer, body mass index, pack-years of smoking, physical activity, dietary intake (including supplements) of calcium and folate, servings of red meat, alcohol consumption, regular use of multivitamins, regular use of aspirin (at least two tablets per week), menopausal status, postmenopausal hormone use, age at first menarche, and age at last menstrual period.

1.4, after multivariable adjustment, compared with control subjects without HFE gene mutations. However, assessment of covariates in that study may have been prone to recall or selection bias because lifestyle and dietary factors were measured after cancer diagnosis and because the cooperation rate among control subjects was statistically significantly lower than that among case subjects. Indeed, there was no statistically significant difference in the prevalence of HFE gene mutations between case subjects and control subjects. Moreover, the collection of blood from case subjects after cancer diagnosis precluded measurements of biochemical iron markers because these values would likely be influenced by cancer symptoms (e.g., bleeding or inflammation). Retrospective analyses would also be biased if HFE gene mutations are differentially associated with survival *(56)*.

Our study is also consistent with prior findings that traditional markers of total body iron, including transferrin saturation, plasma iron, and ferritin, are not related to risk of either colorectal cancer *(8,17)* or adenoma *(19)*. Although our results are not consistent with an initial report *(14)* that found an elevated risk of colon cancer related to transferrin saturation after early follow-up in men participating in the National Health and Nutritional Evaluation Survey (NHANES), the finding in that study was based on only 12 cases of colon cancer and was not observed in women *(14)*; moreover, the association in men was attenuated after additional follow-up *(16)*. Analysis of a subcohort of NHANES participants that was followed through the National Health Evaluation Follow-Up Study *(4)* yielded inconsistent results according to cancer site and sex. In addition, a study of a Finnish cohort *(15)* found an elevated risk of colorectal cancer at extremely high transferrin saturations; however, the study included only 11 case subjects. Finally, two studies *(9,18)* demonstrated an association between ferritin and risk of adenoma. However, both studies measured ferritin either after or immediately before sigmoidoscopy or colonoscopy. Both also included mainly participants who were bleeding *(18)* or found a relationship only after adjustment for adenoma risk factors in a limited subgroup of participants *(9)*.

Our results are in agreement with several prior studies *(6,7, 10–13)* that also found no association between dietary iron and risk of adenoma. Although a case–control study *(9)* observed a non–statistically significant increase in the risk of adenoma with high dietary iron intake, the association disappeared when subjects who obtained most of their iron intake from supplements was excluded. In contrast, studies of dietary iron intake and invasive colorectal cancer risk have been less consistent. Two case–control studies *(3,29)* observed no association between dietary iron intake and risk of colorectal cancer, whereas other studies *(2,4,5,8)* have reported an elevated risk of colorectal using varying measures of iron intake. However, only one of the studies *(4)* also controlled for the influence of iron-rich red meat, which may independently increase the risk for colorectal cancer through carcinogenic heterocyclic amines generated in the cooking process *(29,48,57–59)*.

The strengths of our study include its prospective design, high follow-up rate, well-validated and repeated measures of exposures *(34,35)*, detailed data on potential confounders of both colorectal neoplasia and iron status, and analysis of incident adenomas. In particular, measures of plasma total body iron taken before diagnosis of adenoma minimize potential bias by symptoms associated with neoplasia. In addition, we also assessed levels of transferrin receptors that, compared with traditional biomarkers of iron stores measured in prior studies *(60–62)*, vary less within individuals *(60)*, are less affected by chronic disease *(61)*, are not statistically significantly different between healthy adult men and women, and are not associated with age *(62)*. Levels of transferrin receptors have already been shown to correlate with risk of other chronic diseases related to iron overload *(63,64)*. Finally, our study uniquely examines three measures of iron status—genetic mutations, biochemical markers of iron stores, and dietary iron intake—and their combined influence on risk of neoplasia. Unlike previous studies, we have shown that, despite an association with a variety of biochemical markers of total body iron, HFE gene mutations were not associated with risk of neoplasia.

We acknowledge several limitations to our study. First, we evaluated the risk of adenoma rather than the risk of invasive cancer. Although we also found no association between several measures of iron and high-risk advanced adenomas, we cannot exclude the possibility that iron may be associated with later-stage cancer progression. However, because adenomas rarely cause symptoms, the assessment of nutritional factors and biochemical parameters prior to adenoma diagnosis rather than invasive cancer is less likely to be biased by factors associated with symptomatic disease (e.g., inflammation or bleeding). Nonetheless, future prospective analyses of individuals with colorectal cancers are needed in which a range of biochemical and genetic markers of iron status are measured.

Second, our analysis was focused only on women, who generally experience greater lifetime iron loss through menstruation than do men. Thus, it is possible that men may experience a

differential risk of iron-related complications. However, additional analyses restricted to postmenopausal women and stratified by years of menstruation did not show any statistically significant influence of menstrual history on risk of adenoma.

Third, our study was limited largely to Caucasians. This did not substantially affect our analysis, however, because the HFE gene mutations that we studied are observed mainly in Caucasians. Nevertheless, further studies on iron and adenoma risk should examine other racial and ethnic groups, especially because the risk of colon cancer associated with HFE gene mutations in a prior study *(29)* appeared to be largely limited to African-Americans.

Fourth, our results may also have been influenced by the iron loss through bleeding that provoked the initial endoscopic examination. However, our results were unchanged when restricted to participants who underwent endoscopy for non-bleeding indications. Moreover, we observed similar results for large adenomas, which may be more likely to cause bleeding than small adenomas.

Fifth, our study included only a few participants who were homozygous for HFE gene mutations. Thus, we were unable to separately examine the influence of two gene mutations on adenoma risk. We also did not consider other polymorphisms in the HFE gene (e.g., S65C) or additional genes related to iron metabolism (e.g., transferrin receptor 2, hepcidin, and hemojuvelin); however, these mutations are considerably less common than the C282Y and H63D mutations of the HFE gene *(65)* and are of uncertain clinical significance *(66)*.

Finally, during the time period under study, many of our participants may have had only a sigmoidoscopy. Thus, case subjects may have differed from control subjects in the extent of colon examination. Although misclassification of control subjects with proximal adenoma would have biased our findings toward the null, previous data *(67)* suggest that only a small number of participants would have proximal adenoma without distal findings. Most important, our results did not change when restricting the analyses to participants who underwent endoscopy in later time periods, when the prevalence of colonoscopy was higher *(42)*. In addition, we obtained similar results for genotype, dietary iron, and distal adenoma risk for the subcohort of subjects with distal adenoma and their matched control subjects.

In conclusion, using a variety of parameters to assess iron status, we did not find a statistically significant relationship between iron and risk of colorectal adenoma in women. Our findings do not support a substantial role for iron or the HFE genotype on the pathogenesis of colorectal adenoma. Furthermore, although several studies *(29,48,57,68,69)* have suggested an association between red meat intake and the risk of colorectal neoplasia, our data suggest that the influence of red-meat consumption is mediated through mechanisms other than iron.

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Notes

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